EXAMINING DIFFERENT LEVELS OF PREVENTION OF BIRTH DEFECTS AND FETAL ALCOHOL SPECTRUM DISORDER

BY

Y. INGRID GOH, HBSc.

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Pharmaceutical Sciences,
University of Toronto

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ABSTRACT

While all women hope to deliver a healthy baby, approximately 3-5% babies are affected by birth defects. Birth defects can occur naturally or be induced by teratogens. Alcohol is a known teratogen that causes fetal alcohol spectrum disorder (FASD), the most commonly known cause of neurobehavioural and neurodevelopmental deficits. Individuals affected with FASD are likely to be involved with or require additional assistance from healthcare, education, social services, and justice sectors. Due to this immense burden, effective prevention of FASD can have a major public impact. Prevention of FASD can occur at different levels: primary prevention (preventing alcohol-induced birth defects from occurring in the first place); secondary prevention (preventing alcohol-induced birth defects from developing or progressing); tertiary prevention (improving the outcome of individuals affected with FASD); and quaternary prevention (preventing another child from being affected with FASD).
Abstract

The objective of this thesis was to explore a multilevel birth defect and FASD prevention strategy. Primary prevention by was investigated by maternal multivitamin supplementation to optimize fetal growing conditions, as alcoholics are commonly deficient in nutrients. A meta-analysis of maternal multivitamin supplementation demonstrated a decreased risk for certain congenital anomalies and pediatric cancers.

Secondary prevention was investigated by a randomized double-blinded placebo-controlled evaluating the ability of high doses of antioxidants (vitamin C and vitamin E) to mitigate the effects of prenatal alcohol exposure. The study was ceased due to safety concerns regarding high doses of vitamin C and vitamin E in preeclamptic studies.

Tertiary prevention was investigated by anonymous meconium screening of babies of Grey-Bruce, Ontario residents delivering at or transferred to St. Joseph’s Health Care in London, Ontario. A 30% prevalence of fatty acid ethyl esters (FAEE) positive meconium was observed at this high-risk unit.

Meconium screening is also a means of quaternary prevention since positive screens also identify mothers who were unable to stop consuming alcohol after 13 weeks of pregnancy, and therefore are at risk of delivering another child who is prenatally exposed to alcohol. The identification and engagement of these mothers into treatment programs constitutes primary prevention of FASD in subsequent pregnancies.
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# TABLE OF CONTENTS

ABSTRACT ...................................................................................................................... II

ACKNOWLEDGEMENTS ............................................................................................. IV

LIST OF ABBREVIATIONS ........................................................................................... XVIII

LIST OF TABLES ........................................................................................................... XXI

LIST OF APPENDICES ............................................................................................... XXIV

CHAPTER 1 ..................................................................................................................... 1

1. GENERAL INTRODUCTION .................................................................................. 2

1.1 BIRTH DEFECTS ................................................................................................. 2

1.2 TERATOGENICITY OF ETHANOL ....................................................................... 3

1.3 PREVENTION IN PREGNANCY ........................................................................ 4

1.4 LEVELS OF PREVENTION OF BIRTH DEFECTS IN PREGNANCY ............... 5

1.4.1 PRIMARY ......................................................................................................... 5

1.4.2 SECONDARY .................................................................................................. 5

1.4.3 TERTIARY ..................................................................................................... 6

1.4.4 QUATERNARY ............................................................................................... 6

1.5 NEED FOR MULTILEVEL PREVENTION IN PREGNANCY ............................. 7

1.6 LEVELS OF PREVENTION FOR FETAL ALCOHOL SPECTRUM DISORDER .... 7

1.6.1 PRIMARY ......................................................................................................... 7

1.6.2 SECONDARY .................................................................................................. 8

1.6.3 TERTIARY ..................................................................................................... 8

1.6.4 QUATERNARY ............................................................................................... 8
Table of Contents

1.7 NEED FOR MULTILEVEL PREVENTION FOR FETAL ALCOHOL SPECTRUM
DISORDER............................................................................................................................... 9

CHAPTER 2.......................................................................................................................... 10

2 OVERVIEW OF THESIS RESEARCH: RATIONALE, HYPOTHETICAL FRAMEWORK,
AND OBJECTIVES .................................................................................................................... 11

2.1 RATIONALE...................................................................................................................... 11

2.2 HYPOTHESIS .................................................................................................................. 11

2.3 OBJECTIVES OF RESEARCH .......................................................................................... 11

CHAPTER 3.......................................................................................................................... 14

3. REVIEW OF THE LITERATURE .......................................................................................... 15

3.1 PREVENTION .................................................................................................................. 15

3.1.1 CLASSIFICATION OF PREVENTION ........................................................................ 15

3.1.1.1 UNIVERSAL PREVENTION ............................................................................... 15

3.1.1.2 SELECTIVE PREVENTION ............................................................................... 17

3.1.1.3 INDICATED PREVENTION ............................................................................... 18

3.1.2 LEVELS OF PREVENTION .......................................................................................... 19

3.1.2.1 PRIMARY ............................................................................................................. 19

3.1.2.2 SECONDARY ......................................................................................................... 20

3.1.2.3 TERTIARY ............................................................................................................. 20

3.1.2.4 QUATERNARY ....................................................................................................... 21

3.2 SCREENING, DIAGNOSIS, AND TREATMENT/INTERVENTION ................................. 21

3.2.1 SCREENING ................................................................................................................. 21

3.2.2 DIAGNOSIS ................................................................................................................. 24

3.2.3 TREATMENT/INTERVENTION AND PROGNOSIS .................................................. 24
# Table of Contents

3.3  SCREENING IN PREGNANCY ........................................................................................................ 24

3.4  NUTRITION DURING PREGNANCY .................................................................................. 25

   3.4.1  RECOMMENDED DAILY INTAKE OF NUTRIENTS DURING PREGNANCY ...... 25

   3.4.2  NUTRITION AND ALCOHOL ...................................................................................... 29

3.5  MULTIVITAMINS .................................................................................................................. 30

   3.5.1  MULTIVITAMIN USE IN PREGNANCY ........................................................................ 30

3.6  FOLIC ACID AND NEURAL TUBE DEFECTS ....................................................................... 33

3.7  ALCOHOL, PREGNANCY, AND FETAL ALCOHOL SPECTRUM DISORDER .......... 35

   3.7.1  ALCOHOL USE AMONGST PREGNANT AND CHILDBEARING-AGED WOMEN .. .......................... 35

   3.7.2  DRINKING DEFINITIONS ............................................................................................... 37

   3.7.3  ALCOHOL ABUSE VS. ALCOHOL DEPENDENCE ...................................................... 38

   3.7.4  FETAL EXPOSURE TO ETHANOL .................................................................................. 39

   3.7.5  TERATOGENIC EFFECTS OF ETHANOL ..................................................................... 39

      3.7.5.1  CLINICAL MANIFESTATIONS ................................................................................. 39

      3.7.5.2  PREVALENCE/INCIDENCE OF FETAL ALCOHOL SPECTRUM DISORDER .......... 40

      3.7.5.3  SCREENING FOR FETAL ALCOHOL SPECTRUM DISORDER ............................... 40

         3.7.5.3.1  SCREENING MOTHERS ANTENATALLY/POSTNATALLY .................................... 41

         3.7.5.3.2  BIOMARKERS .................................................................................................. 42

            3.7.5.3.2.1  MECONIUM ................................................................................................. 42

            3.7.5.3.2.2  HAIR ........................................................................................................... 43

            3.7.5.3.2.3  CORD BLOOD .......................................................................................... 44

3.8  FATTY ACID ETHYL ESTERS ............................................................................................ 44

   3.8.1  SYNTHESIS OF FATTY ACID ETHYL ESTERS .............................................................. 45

   3.8.2  DEGRADATION OF FATTY ACID ETHYL ESTERS ...................................................... 48

   3.8.3  FATTY ACID ETHYL ESTERS AS BIOMARKERS OF DAMAGE ................................. 49

   3.8.4  MECONIUM AS A DEPOT FOR FATTY ACID ETHYL ESTERS ................................. 51

   3.8.5  VALIDATION OF MECONIUM AS A BIOMARKER FOR PRENATAL ALCOHOL

         EXPOSURE ................................................................................................................. 54
Table of Contents

3.8.6 FATTY ACID ETHYL ESTERS AND PREDICTION FOR FETAL ALCOHOL SPECTRUM DISORDER ................................................................. 57

CHAPTER 4 ......................................................................................................................................................... 59
4 PRELUDE .................................................................................................................................................. 60

CHAPTER 5 ......................................................................................................................................................... 61
5 PRENATAL MULTIVITAMIN SUPPLEMENTATION AND RATES OF CONGENITAL ANOMALIES: A META-ANALYSIS .......................................................... 62
5.1 ABSTRACT ............................................................................................................................................. 63
5.2 INTRODUCTION .................................................................................................................................. 65
5.3 METHODS .......................................................................................................................................... 66
5.4 RESULTS ............................................................................................................................................ 67
5.5 DISCUSSION ....................................................................................................................................... 84
5.6 CONCLUSIONS ................................................................................................................................. 85
5.7 STATEMENT OF SIGNIFICANCE AND IMPACT ................................................................................ 86

CHAPTER 6 ......................................................................................................................................................... 87
6 PRENATAL MULTIVITAMIN SUPPLEMENTATION AND RATES OF PEDIATRIC CANCERS: A META-ANALYSIS .................................................................................. 88
6.1 ABSTRACT ............................................................................................................................................. 89
6.2 INTRODUCTION .................................................................................................................................. 90
6.3 METHODS .......................................................................................................................................... 91
6.4 RESULTS ............................................................................................................................................ 93
6.5 DISCUSSION ....................................................................................................................................... 99
# Table of Contents

8.9 PHASE 1: SCIENTIFIC EVALUATION OF SCREENING METHODS .................. 129

8.9.1 EVALUATION OF SCREENING TOOLS AND METHODS ..................... 129

8.9.1.1 NEUROBEHAVIOURAL METHODS ........................................... 129

8.9.1.2 FACIAL DYSMORPHOLOGY .................................................. 132

8.9.1.3 MECONIUM TESTING FOR ETHANOL CONJUGATES .................... 134

8.9.1.4 GROWTH RETARDATION ....................................................... 135

8.9.1.5 YOUTH JUSTICE POPULATION .............................................. 136

8.9.1.6 CLINIC TOOLS ................................................................. 139

8.9.1.7 COMMUNITY TOOLS .......................................................... 141

8.9.2 PROMISING APPROACHES ...................................................... 142

8.9.3 KEY CONSIDERATIONS .......................................................... 143

8.10 PHASE 2: FEASIBILITY OF IMPLEMENTATION OF SCREENING METHODS ...... 144

8.10.1 METHODS ............................................................................. 144

8.10.2 SCREENING TOOLS ............................................................... 145

8.10.2.1 SCREENING FAEE IN MECONIUM ....................................... 145

8.10.2.1.1 EASE OF USE .............................................................. 146

8.10.2.1.2 ACCESSIBILITY ............................................................ 146

8.10.2.1.3 COST ........................................................................ 146

8.10.2.1.4 EXPERTISE ................................................................. 147

8.10.2.1.5 CULTURAL APPROPRIATENESS .................................... 147

8.10.2.1.6 FACTORS TO FACILITATE IMPLEMENTATION ..................... 147

8.10.2.1.7 BARRIERS TO IMPLEMENTATION ..................................... 148

8.10.2.2 YOUTH JUSTICE SCREENING TOOLS ................................... 148

8.10.2.2.1 EASE OF USE .............................................................. 148

8.10.2.2.2 ACCESSIBILITY ............................................................ 149

8.10.2.2.3 COST ........................................................................ 149

8.10.2.2.4 EXPERTISE ................................................................. 149

8.10.2.2.5 CULTURAL APPROPRIATENESS .................................... 149

8.10.2.2.6 FACTORS TO FACILITATE IMPLEMENTATION ..................... 150

8.10.2.2.7 BARRIERS TO IMPLEMENTATION ..................................... 150
# Table of Contents

8.10.2.3 MODIFIED CHILD BEHAVIOR CHECKLIST .......................................................... 150

8.10.2.3.1 EASE OF USE ......................................................................................... 151

8.10.2.3.2 ACCESSIBILITY ...................................................................................... 151

8.10.2.3.3 COST ........................................................................................................ 151

8.10.2.3.4 EXPERTISE ............................................................................................ 151

8.10.2.3.5 CULTURAL APPROPRIATENESS ............................................................ 152

8.10.2.3.6 FACTORS TO FACILITATE IMPLEMENTATION .................................... 152

8.10.2.3.7 BARRIERS TO IMPLEMENTATION ......................................................... 152

8.10.2.4 FACIAL DYSMORPHOLOGY .................................................................... 152

8.10.2.4.1 EASE OF USE ....................................................................................... 153

8.10.2.4.2 ACCESSIBILITY ...................................................................................... 153

8.10.2.4.3 COST ........................................................................................................ 153

8.10.2.4.4 EXPERTISE ............................................................................................ 153

8.10.2.4.5 CULTURAL APPROPRIATENESS ............................................................ 154

8.10.2.4.6 FACTORS TO FACILITATE IMPLEMENTATION .................................... 154

8.10.2.4.7 BARRIERS TO IMPLEMENTATION ......................................................... 154

8.10.2.5 MATERNAL HISTORY OF SUBSTANCE ABUSE ........................................ 154

8.10.2.5.1 EASE OF USE ....................................................................................... 155

8.10.2.5.2 ACCESSIBILITY ...................................................................................... 155

8.10.2.5.3 COST ........................................................................................................ 156

8.10.2.5.4 EXPERTISE ............................................................................................ 156

8.10.2.5.5 CULTURAL APPROPRIATENESS ............................................................ 156

8.10.2.5.6 FACTORS TO FACILITATE IMPLEMENTATION .................................... 157

8.10.2.5.7 BARRIERS TO IMPLEMENTATION ......................................................... 157

8.10.2.6 DIAGNOSTIC CLINIC – INTAKE PROCEDURE .................................... 157

8.10.2.6.1 EASE OF USE ....................................................................................... 158

8.10.2.6.2 ACCESSIBILITY ...................................................................................... 158

8.10.2.6.3 COSTS ..................................................................................................... 158

8.10.2.6.4 EXPERTISE ............................................................................................ 159

8.10.2.6.5 CULTURAL APPROPRIATENESS ............................................................ 159

8.10.2.6.6 FACTORS TO FACILITATE IMPLEMENTATION .................................... 159
Table of Contents

9.3 METHODS ............................................................................................................................. 175

9.3.1 SAMPLE COLLECTION ........................................................................................................ 175

9.3.2 SPECIMEN HANDLING ..................................................................................................... 176

9.3.3 SAMPLE PREPARATION ................................................................................................... 177

9.3.4 INSTRUMENTATION .......................................................................................................... 177

9.4 RESULTS ............................................................................................................................... 178

9.5 DISCUSSION .......................................................................................................................... 182

9.6 STATEMENT OF SIGNIFICANCE AND IMPACT .................................................................. 186

CHAPTER 10 ........................................................................................................................... 187

10. OVERALL DISCUSSION AND CONCLUSION ..................................................................... 188

10.1 SUMMARY AND DISCUSSION OF RESEARCH FINDINGS ................................................ 188

10.1.1 PRIMARY LEVEL OF PREVENTION OF BIRTH DEFECTS AND FETAL ALCOHOL
SPECTRUM DISORDER BY MEANS OF MULTIVITAMINS SUPPLEMENTATION .................... 188

10.1.1.1 NUTRIENTS AND FETAL ALCOHOL SPECTRUM DISORDER ................................. 189

10.1.2 SECONDARY LEVEL OF PREVENTION OF FETAL ALCOHOL SPECTRUM DISORDER
BY MEANS OF EARLY PREVENTION WITH ANTIOXIDANTS ................................................... 191

10.1.3 TERTIARY PREVENTION OF FETAL ALCOHOL SPECTRUM DISORDER BY MEANS
OF MECONIUM SCREENING IN HIGH-RISK POPULATIONS .............................................. 194

10.1.4 QUATERNARY PREVENTION OF FETAL ALCOHOL SPECTRUM DISORDER
BY MEANS OF MECONIUM SCREENING ................................................................................ 195

10.2 CONSIDERATIONS IN MECONIUM SCREENING ............................................................. 197

10.2.1 ETHICAL CONSIDERATION IN MECONIUM SCREENING ........................................ 197

10.2.2 ECONOMIC IMPACT OF MECONIUM SCREENING ...................................................... 200

10.2.3 IMPLEMENTATION OF MECONIUM AS A SCREENING TOOL .................................... 201
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.3</td>
<td>IMPLEMENTATION OF A MULTILEVEL PREVENTION PROGRAM FOR FETAL ALCOHOL</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>SPECTRUM DISORDER</td>
<td></td>
</tr>
<tr>
<td>10.4</td>
<td>LIMITATIONS</td>
<td>204</td>
</tr>
<tr>
<td>10.4.1</td>
<td>INTERINDIVIDUAL VARIATIONS</td>
<td>204</td>
</tr>
<tr>
<td>10.4.2</td>
<td>GENETIC VARIATIONS</td>
<td>205</td>
</tr>
<tr>
<td>10.4.3</td>
<td>ENVIRONMENTAL VARIATIONS</td>
<td>205</td>
</tr>
<tr>
<td>10.5</td>
<td>BENEFITS AND LIMITATIONS OF MULTILEVEL PREVENTION OF FETAL ALCOHOL</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>SPECTRUM DISORDER</td>
<td></td>
</tr>
<tr>
<td>10.5.1</td>
<td>UNIVERSAL</td>
<td>206</td>
</tr>
<tr>
<td>10.5.2</td>
<td>SELECTIVE</td>
<td>207</td>
</tr>
<tr>
<td>10.5.3</td>
<td>INDICATED</td>
<td>207</td>
</tr>
<tr>
<td>10.5.4</td>
<td>PRIMARY PREVENTION</td>
<td>208</td>
</tr>
<tr>
<td>10.5.5</td>
<td>SECONDARY PREVENTION</td>
<td>208</td>
</tr>
<tr>
<td>10.5.6</td>
<td>TERTIARY PREVENTION</td>
<td>209</td>
</tr>
<tr>
<td>10.5.7</td>
<td>QUATERNARY PREVENTION</td>
<td>209</td>
</tr>
<tr>
<td>10.5.8</td>
<td>MULTILEVEL PREVENTION VERSUS SINGLE LEVEL OF PREVENTION</td>
<td>210</td>
</tr>
<tr>
<td>10.6</td>
<td>SUSTAINABILITY OF PREVENTION</td>
<td>210</td>
</tr>
<tr>
<td>10.7</td>
<td>OVERALL SIGNIFICANCE AND CLINICAL IMPLICATIONS</td>
<td>211</td>
</tr>
<tr>
<td>10.7.1</td>
<td>OVERALL SIGNIFICANCE</td>
<td>211</td>
</tr>
<tr>
<td>10.7.2</td>
<td>CLINICAL IMPLICATIONS</td>
<td>212</td>
</tr>
<tr>
<td>10.7.3</td>
<td>AFFIRMATION AND ENDORSEMENT OF MULTIVITAMIN SUPPLEMENTATION</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>PRIOR TO AND DURING PREGNANCY</td>
<td></td>
</tr>
<tr>
<td>10.7.4</td>
<td>AFFIRMATION OF SCREENING MECONIUM FOR FATTY ACID ETHYL ESTERS</td>
<td>215</td>
</tr>
<tr>
<td>10.7.5</td>
<td>AFFIRMATION AND ENDORSEMENT OF MULTILEVEL PREVENTION FOR</td>
<td>216</td>
</tr>
<tr>
<td></td>
<td>FETAL ALCOHOL SPECTRUM DISORDER</td>
<td></td>
</tr>
<tr>
<td>10.8</td>
<td>FUTURE DIRECTIONS</td>
<td>216</td>
</tr>
<tr>
<td>10.9</td>
<td>AREAS FOR FURTHER DEVELOPMENT</td>
<td>217</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

[E12]  ethyl laurate
[E14]  ethyl myristate
[E16]  ethyl palmitate
[E18]  ethyl stearate
[E18:1]  ethyl oleate
[E18:2]  ethyl linoleate

ADHD  attention-deficit hyperactivity disorder
AEAT  acyl-coA o-acyl transferase
Al  adequate intake
ALL  acute lymphoblastic leukemia
ALT  alanine aminotransferase
AML  acute myeloid leukemia
ARND  alcohol related neurobehavioural deficits
AST  aspartate aminotransferase
AUDIT  alcohol use disorders identification test
BRFSS  Behavioural Risk Factor Surveillance System
BSC  Brief Screen Checklist
CADEC  Clinic for Alcohol & Drug Exposed Children
CAGE  cut-down, annoyed, guilty, eye-opener
CAPHC  Canadian Association of Paediatric Health Centres
CBCL  Child Behavior Checklist
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDBC</td>
<td>Complex Developmental Behavioural Conditions</td>
</tr>
<tr>
<td>CDT</td>
<td>carbohydrate deficient transferrin</td>
</tr>
<tr>
<td>CHD</td>
<td>congenital heart defect</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>DRI</td>
<td>dietary reference intake</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorder</td>
</tr>
<tr>
<td>EAR</td>
<td>estimated average requirement</td>
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<tr>
<td>FAE</td>
<td>fetal alcohol effect</td>
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<tr>
<td>FAEF</td>
<td>fatty acid ethyl esters</td>
</tr>
<tr>
<td>FAS</td>
<td>fetal alcohol syndrome</td>
</tr>
<tr>
<td>FASD</td>
<td>fetal alcohol spectrum disorder</td>
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<tr>
<td>GC-FID</td>
<td>gas chromatography flame ionization detection</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography mass spectrometry</td>
</tr>
<tr>
<td>GGT</td>
<td>gamma glutamyl transpeptidase</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>IQ</td>
<td>intelligence quotient</td>
</tr>
<tr>
<td>HS-SPME</td>
<td>headspace solid phase microextraction</td>
</tr>
<tr>
<td>LARGE</td>
<td>Labrador Alcohol Research Group</td>
</tr>
<tr>
<td>MAST</td>
<td>Michigan alcohol screening test</td>
</tr>
<tr>
<td>MTHFR</td>
<td>methylenetetrahydrofolate reductase</td>
</tr>
<tr>
<td>NPV</td>
<td>negative predictive value</td>
</tr>
<tr>
<td>NTD</td>
<td>neural tube defect</td>
</tr>
</tbody>
</table>
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>pFAS</td>
<td>partial fetal alcohol syndrome</td>
</tr>
<tr>
<td>PAE</td>
<td>prenatal alcohol exposure</td>
</tr>
<tr>
<td>PHAC</td>
<td>Public Health Agency of Canada</td>
</tr>
<tr>
<td>PIC</td>
<td>Personality Inventory for Children</td>
</tr>
<tr>
<td>PNET</td>
<td>primitive neuroectodermal tumours</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
</tr>
<tr>
<td>PRAMS</td>
<td>Pregnancy Risk Assessment Monitoring System</td>
</tr>
<tr>
<td>RBP</td>
<td>retinol binding protein</td>
</tr>
<tr>
<td>RDA</td>
<td>recommended dietary allowance</td>
</tr>
<tr>
<td>RNI</td>
<td>recommended nutrient intake</td>
</tr>
<tr>
<td>RR</td>
<td>risk ratio</td>
</tr>
<tr>
<td>SAMHSA</td>
<td>Substance Abuse and Mental Health Services Administration</td>
</tr>
<tr>
<td>T-ACE</td>
<td>tolerance, annoyed, cut-down, eye-opener</td>
</tr>
<tr>
<td>TLFB</td>
<td>timeline follow-back</td>
</tr>
<tr>
<td>TWEAK</td>
<td>tolerance, worry, eye-opener, amnesia, cut-down</td>
</tr>
<tr>
<td>UL</td>
<td>tolerable upper intake level</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
</tbody>
</table>
LIST OF TABLES

TABLE 1. DIETARY REFERENCE INTAKES (DRIs): RECOMMENDED INTAKE OF VITAMINS AND MINERALS FOR PREGNANT INDIVIDUALS ................................................................. 27

TABLE 2. CHARACTERISTICS OF ARTICLES INCLUDED IN META-ANALYSIS ......................... 95

TABLE 3. MATERNAL AND NEONATAL CHARACTERISTICS OF THE GREY-BRUCE PERINATAL INTENSIVE CARE GROUP ................................................................. 181
LIST OF FIGURES

FIGURE 1. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF NTD IN THEIR CHILDREN (CASE-CONTROL STUDIES) .......................................................... 69

FIGURE 2. MATERNAL MULTIVITAMIN SUPPLEMENTATION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF NTD IN THEIR CHILDREN (COHORT STUDIES AND RANDOMIZED CONTROLLED TRIALS) .............................................. 70

FIGURE 3. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF CLEFT PALATE IN THEIR CHILDREN (CASE-CONTROL STUDIES) ......................................................................................... 71

FIGURE 4. MATERNAL MULTIVITAMIN SUPPLEMENTATION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF CLEFT PALATE IN THEIR CHILDREN (COHORT STUDIES AND RANDOMIZED CONTROLLED TRIALS) .................................................. 72

FIGURE 5. MATERNAL MULTIVITAMIN SUPPLEMENTATION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF CLEFT LIP WITH/OUT PALATE IN THEIR CHILDREN (CASE-CONTROL STUDIES) ......................................................................................... 73

FIGURE 6. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF CLEFT LIP WITH/OUT PALATE IN THEIR CHILDREN (COHORT STUDIES AND RANDOMIZED CONTROLLED TRIALS) ................. 74

FIGURE 7. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF URINARY TRACT ANOMALIES IN THEIR CHILDREN (CASE-CONTROL STUDIES) ......................................................................................... 75

FIGURE 8. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OR URINARY TRACT ANOMALIES IN THEIR CHILDREN (COHORT STUDIES AND RANDOMIZED CONTROLLED TRIALS) .......... 76

FIGURE 9. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF CARDIOVASCULAR DEFECTS IN THEIR CHILDREN (CASE-CONTROL STUDIES) ......................................................................................... 77

FIGURE 10. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF CARDIOVASCULAR DEFECTS IN THEIR CHILDREN (COHORT STUDIES AND RANDOMIZED CONTROLLED TRIALS) .......... 78

FIGURE 11. MATERNAL MULTIVITAMIN SUPPLEMENTATION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF LIMB DEFECT IN THEIR CHILDREN (CASE-CONTROL STUDIES) .......................................................... 79

xxii
List of Figures

FIGURE 12. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF LIMB DEFECTS IN THEIR CHILDREN (COHORT STUDIES AND RANDOMIZED CONTROLLED TRIALS)..........................80

FIGURE 13. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF CONGENITAL HYDROCEPHALUS IN THEIR CHILDREN (CASE-CONTROL STUDIES) .................................................................81

FIGURE 14. MATERNAL MULTIVITAMIN CONSUMPTION BEFORE AND DURING THE FIRST TRIMESTER OF PREGNANCY AND THE RISK OF CONGENITAL HYDROCEPHALUS IN THEIR CHILDREN (COHORT STUDIES AND RANDOMIZED CONTROL TRIALS) ...............82

FIGURE 15. MATERNAL MULTIVITAMIN CONSUMPTION DURING THE FIRST TRIMESTER OF PREGNANCY AND RISK OF NTD IN THEIR CHILDREN (CASE-CONTROL STUDIES).....83

FIGURE 16. MATERNAL MULTIVITAMIN CONSUMPTION AND RISK FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN THEIR CHILDREN .................................................................96

FIGURE 17. MATERNAL MULTIVITAMIN CONSUMPTION AND RISK FOR NEUROBLASTOMA IN THEIR CHILDREN .................................................................................................97

FIGURE 18. MATERNAL MULTIVITAMIN CONSUMPTION AND RISK FOR PEDIATRIC BRAIN TUMOURS IN THEIR CHILDREN ..................................................................................98

FIGURE 19. MECONIUM COLLECTION AND SCREENING ACCOUNTABILITY .....................180
LIST OF APPENDICES

APPENDIX A. LIST OF PEER-REVIEWED PUBLICATIONS.................................264
APPENDIX B. LIST OF PUBLISHED ABSTRACTS...........................................266
APPENDIX C. LIST OF BOOK CHAPTERS.....................................................268
APPENDIX D. ETHICS APPROVAL DOCUMENTATION...................................269
APPENDIX E. ADDITIONAL LITERATURE REVIEW.......................................273
APPENDIX F. PEER-REVIEWED PUBLICATIONS..........................................461
Chapter 1

General Introduction
1. GENERAL INTRODUCTION

The following chapter will categorize birth defects. It will provide examples of teratogens, specifically focusing on alcohol. In addition, the chapter will highlight modes of prevention and how these modes of prevention can be applied to preventing fetal alcohol spectrum disorder.

1.1 BIRTH DEFECTS

It is estimated that 3-5% of babies are born with birth defects. Out of these, 1-3% birth defects will be identified at birth. Birth defects are the leading cause of death in babies less than one year of age.

Teratology is the study of abnormal fetal development. The word teratology arises from the Greek root teras which means monster. Teratogenic effects can be categorized into different groups. They can result in death (spontaneous abortion, stillbirth), physical malformation, reproductive effects, carcinogenesis, developmental effects, and behavioural effects. The following are examples of teratogens (agents causing birth defects) demonstrating each of the above listed teratogenic effects. Misoprostol is a non-steroidal anti-inflammatory medication that is used for the treatment of arthritis. However this drug can induce miscarriages of the fetus as it induces contractions of the uterus and causes softening of the cervix. Thalidomide, a drug for the treatment of leprosy, can cause phocomelia, a birth defect of the long limbs. Diethylstilbestrol is an estrogen that was previously used in women who were
prone to have repeated miscarriages. This drug, however, was found to be associated with reproductive teratology and carcinogenesis\textsuperscript{6,7}. There was an increased risk of infertility, vaginal or cervical cancer, and reproductive tract structural defects in females with \textit{in utero} exposure\textsuperscript{6,7}. An increased risk for hypospadias and feminization was observed in males with \textit{in utero} exposure\textsuperscript{6,7}. Alcohol is associated with both developmental and behavioural teratogenesis such as deficits in fine and gross motor skills and hyperactivity\textsuperscript{8}.

1.2 TERATOGENICITY OF ETHANOL

Maternal ethanol consumption during pregnancy can result in fetal alcohol spectrum disorder (FASD). FASD, as it is currently known, is a broad term encompassing irreversible damage that can result from alcohol consumption during pregnancy\textsuperscript{9,10}. It is further deconstructed into diagnoses including fetal alcohol syndrome (FAS), partial fetal alcohol syndrome (pFAS), fetal alcohol effects (FAE), and alcohol related neurobehavioural deficits (ARND). Children affected with FASD may exhibit pathognomonic physical changes to the face and neurobehavioural impairments which may result in secondary disabilities\textsuperscript{11,12}. As FASD has a known cause and is a major public health problem, steps should be taken towards preventing this teratogenic consequence.
1.3 PREVENTION IN PREGNANCY

Prevention in pregnancy focuses on activities that reduce the risk of a baby being born with a birth defect, thereby decreasing the morbidity and mortality. Prevention in pregnancy can occur through universal prevention programs which are targeted to the general population. For instance, both men and women can be provided information on how to reduce the risks of birth defects. An example is education regarding the teratogenic effects of drugs such as isotretinoin. Another example of universal prevention includes offering all women tests to screen for birth defects, for example, ultrasound screening for physical birth defects. Universal prevention could also take form as a campaign. For example, advertising campaigns in both print and media have been conducted warning women of the harmful effects of alcohol consumption during pregnancy.

Prevention can also occur in a selected pregnant population who share the same risk factors of having a baby with birth defects, and for whom the incidence is higher than the average population. By identifying select populations, interventions can be instituted to counter the risk. For example, mothers who take folate antagonists should be informed by their healthcare provider to take additional folic acid in order to reduce the risk of neural tube defects.

Prevention programs in pregnancy can also target mothers who have a child with (a) birth defects(s). In this indicated prevention method, pregnant women must be screened to identify that they have a child with a birth defect.
Ultrasound screening may potentially identify birth defects in utero. This may prompt earlier intervention either in utero or immediately after the delivery of the child.  

1.4 LEVELS OF PREVENTION OF BIRTH DEFECTS IN PREGNANCY

1.4.1 PRIMARY

Primary prevention in pregnancy aims to prevent or reduce the risk of birth defects before they occur. This may be accomplished by preventing the pregnancy from occurring e.g. contraception; health promotion e.g. education women regarding the teratogenic effects of certain exposures; or improving nutritional status to reduce metabolic deficiencies which may be associated with teratogenic effects.

1.4.2 SECONDARY

Secondary prevention in pregnancy is early intervention. Secondary prevention can enable medical or surgical treatment of the birth defect prior to birth of the child. This can be achieved by correcting metabolic deficiencies e.g. gestational diabetes. In addition, in utero surgical intervention may be conducted to minimize adverse consequences of certain neural tube defects and urinary tract defects. Perhaps the most controversial mode of secondary
prevention is the therapeutic abortion of the fetus after it is identified as having a birth defect.

1.4.3 TERTIARY

Tertiary prevention in pregnancy targets the individuals who have the birth defects with the goal of improving their outcome by treating their condition or preventing its progression. This can be achieved by screening individuals who may be affected by genetic disorders (e.g. phenylketonuria) and instituting necessary preventive measures. Individuals who have physically visible birth defects (e.g. oral cleft) may receive surgical interventions to correct or decrease the severity of the defect.

1.4.4 QUATERNARY

Quaternary prevention in pregnancy prevents another pregnancy from being affected by the same birth defect (essentially primary prevention for the next baby born to the same mother). If a mother is identified as having a risk factor for delivering a child with a birth defect (e.g. alcoholism), actions could be taken to treat her condition, thereby reducing the chances of her delivering a child affected with the same birth defect.
1.5 NEED FOR MULTILEVEL PREVENTION IN PREGNANCY

From the above it is observed that there are many factors that can influence the outcome of the fetus. Healthcare during the preconceptional period and during pregnancy is therefore important in decreasing the risk of birth defects. The health behaviour of mothers may also be influenced by knowledge, attitude and behaviours of the individuals. Due to the complexities of factors affecting the outcome of pregnancies, a multilevel approach is needed to prevent birth defects in pregnancy.

1.6 LEVELS OF PREVENTION FOR FETAL ALCOHOL SPECTRUM DISORDER

1.6.1 PRIMARY

Primary prevention of FASD is to provide the fetus the optimal environment for development, free from teratogenic levels of alcohol exposure. This may be accomplished by preventing the pregnancy from occurring, e.g. contraception; health promotion, e.g. educating women regarding teratogenic effects of alcohol so that they will choose not to drink while planning or during pregnancy; or improving nutritional status to reduce metabolic deficiencies due to alcohol consumption, which may increase the risk of teratogenic effects.
1.6.2 SECONDARY

Secondary prevention of FASD is early intervention during pregnancy. Ideally, an intervention would be administered to the growing fetus to reverse the damage. However, to date, there are no known *in utero* interventions that can reverse or decrease the adverse effects of prenatal alcohol exposure. The other controversial alternative is the therapeutic abortion of the alcohol-exposed fetus which will ensure that a child will not be born with FASD.

1.6.3 TERTIARY

Tertiary prevention of FASD targets the individual who has FASD to improve their quality of life. This can be achieved by screening and diagnosis of individuals. As a result they can be engaged in supportive intervention programs which will reduce their risk of developing secondary disabilities.

1.6.4 QUATERNARY

Quaternary prevention of FASD prevents another child from being born with FASD. This level of prevention targets the mother. By educating the mother about the consequences of alcohol consumption during pregnancy and treating her alcoholism, there will be a reduced risk of her delivering another child affected with FASD.
1.7 NEED FOR MULTILEVEL PREVENTION FOR FETAL ALCOHOL SPECTRUM DISORDER

Although FASD can be prevented by avoiding alcohol consumption during pregnancy, its prevention is not simple as it is complicated by maternal addiction. It is estimated that approximately 50% of individuals return to their addictive habits within several months of detoxification. Therefore, in conjunction with the multiple factors affecting pregnancy, factors that influence maternal alcohol consumption need to be considered. Due to the combined complexities, a multilevel approach is needed to prevent FASD.
Chapter 2

Overview of Thesis Research: Rationale, Hypothetical Framework, and Objectives
2 OVERVIEW OF THESIS RESEARCH: RATIONALE, HYPOTHETICAL FRAMEWORK, AND OBJECTIVES

2.1 RATIONALE

FASD is the most commonly known cause of mental disabilities and is a major public health problem which affects many service sectors\(^2\). Since the etiology of FASD is known, this birth defect is potentially 100% preventable\(^3\). However, the prevention of FASD is not simple because it is complicated by maternal addiction. Due to the multiple factors contributing to the increased risk of FASD, the need for a multilevel prevention approach is required.

2.2 HYPOTHESIS

The hypothesis underlying this thesis is that prevention of birth defects and FASD can occur at primary, secondary, tertiary, and quaternary levels.

2.3 OBJECTIVES OF RESEARCH

The objective of this thesis was to investigate the effectiveness of a multilevel approach to the prevention of birth defects and FASD. At the primary level of prevention, alcohol exposure to the gamete is prevented, which optimizes maternal health status. The secondary level of prevention is to intervene during
pregnancy to prevent the progression of alcohol-related damage. The tertiary level of prevention is intervention for individuals who are prenatally-exposed to alcohol. The quaternary level of prevention is to work with mothers to prevent their next child from being exposed to alcohol in their next pregnancy.

There are many approaches that can be investigated within each level or prevention. This thesis focuses on two strategies that involve all four levels of prevention: the use of multivitamins during pregnancy and the screening of meconium. In the primary level of prevention, one strategy to decrease the risk of FASD prior to its occurrence is to optimize the health of the mother. Alcohol contains many calories but lacks nutrients; therefore, chronic drinkers may be deficient in vitamins and minerals. Since folate deficiency is commonly observed in chronic alcohol-consuming individuals, it has been proposed that high levels of formic acid may be the cause of FASD. Supplementation of prenatal multivitamins prior to pregnancy could improve maternal nutrient status, thereby decreasing the risk of birth defects. In the secondary level of prevention, the use of high doses of vitamin C and vitamin E was investigated to evaluate the ability of antioxidants to mitigate the adverse effects or prenatal alcohol exposure. In the tertiary level of prevention, the use of screening tools to identify FASD affected children was investigated. Screening for fatty acid ethyl esters (FAEE) in meconium was used to determine the prevalence of alcoholExposed pregnancies being delivered at a high-risk birthing unit. The use of meconium screening was also used as a mode of quaternary level prevention since a positive screen identifies mothers who were unable to stop consuming alcohol
after 13 weeks of pregnancy. This mode of screening can potentially identify high-risk pregnant women and therefore provide an opportunity to engage them into programs that will prevent her subsequent pregnancy from being exposed to alcohol, thereby preventing FASD in her next child.
Chapter 3

Review of the Literature
3. REVIEW OF THE LITERATURE

3.1 PREVENTION

3.1.1 CLASSIFICATION OF PREVENTION

Prevention is a positive activity conducted within a population that will reduce the risk, burden, morbidity, or mortality of a disease or problem. Last defines prevention as “actions aimed at eradicating, eliminating, or minimizing the impact of disease and disability, or if none to these is feasible, retarding the progress of disease and disability” 25.

3.1.1.1 UNIVERSAL PREVENTION

Universal prevention programs are targeted to the general population in an attempt to prevent or delay a disease or problem. Gordon initially defined universal prevention as a measure that would result in an outcome that is “desirable for everyone in the eligible population where the benefits outweigh the costs for everyone” 26. Mrazek et al. defined universal prevention as an intervention that is “targeted to the general public or whole population group that has not been identified on the basis of individual risk” where the “intervention is desirable for everyone in the eligible population” 27.

In universal prevention, all individuals in the populations are given information and skills that are necessary to prevent the disease or problem. As
such, universal prevention is delivered to large groups without screening for any persons who are at elevated risk of developing the disease or problem. Measures in this category can be administered to both the general public and members in specific groups. Usually universal preventive measures can be implemented without professional advice or assistance. Overall, universal prevention programs may vary in size, design, and structure.

A benefit of universal prevention is that it targets members of the general population. It is expected that the entire population shares the risk, and therefore would benefit from this method of prevention. Universal prevention targets all individuals in the general population rather than select individuals. Since there is a larger audience, universal prevention has a greater potential for increasing awareness. Universal prevention is less intrusive because it does not attempt to resolve the problem, but try to prevent or delay the problem from occurring. In addition individuals are not targeted because of their risk factors. Although the total cost to run a universal prevention program may be greater, the cost per person is usually lower than other screening strategies.

Disadvantages to universal prevention include that, in reality, the risk may vary greatly amongst individuals in the population. If designed incorrectly, there may also be language and cultural barriers. The effects of universal prevention are hard to measure as it is difficult to determine the recipients of the programs. In addition, long-term effects of universal prevention are not immediately apparent. Furthermore, it is difficult to identify if the absence of disease is due to
the prevention program itself or other environmental influences. An example of a universal prevention program is infant vaccination programs.

3.1.1.2 SELECTIVE PREVENTION

Selective prevention programs are preventive strategies that target specific populations because they share the same risk factors of developing the problems or disease which is higher than average. By identifying the select population, interventions can be instituted that are designed to counter the risk factor. Gordon and Mrazek et al. define selected prevention as a desirable method for individuals who are a “member of a subgroup whose risk of being ill is above average”\cite{26,27}. The subgroups may be distinguished by age, gender, occupation, family history, or other evident characteristics, but individuals within the subgroups upon personal examination are perfectly well\cite{27}.

The advantage of selective prevention is that it targets an entire subgroup regardless of their risk. The cost is also justified because the increased risk of illness balances the benefits against risk. An individual’s personal risk is not specifically assessed or identified because it is based on an at-risk subgroup membership. The disadvantage of selective prevention is that it may result in labeling or stigma. This process is also more intensive as it requires more resources to identify the subgroup. An example of selective prevention is substance abuse prevention programs in populations with high drug-use and low-socioeconomic status.
3.1.1.3 INDICATED PREVENTION

Indicated prevention involves a population with symptoms. The population must be screened to identify these individuals with symptoms who are not yet diagnosed with a problem or disease. Gordon defines indicated prevention as prevention that is desirable for individuals who are found to have “a risk factor or condition that identifies them as being high risk for the future development of a disease” 26. Mrazek et al. defines indicated prevention as prevention that target “high-risk individuals who are identified as having minimal but detectable signs or symptoms” but “who do not meet diagnostic criterion at the current time” 27. The objective of indicated prevention is not just the reduction of the problem or disease, but also the reduction in the length and severity of the symptoms and the delaying of the onset of the problem or disease.

The advantages of indicated prevention is that it can identify individuals who have minimal but detectable/early signs, symptoms, or risk factors of the problem or disease but who do not meet diagnostic criteria at that time. This targeted approach may be less expensive to implement as a whole; however, it has a higher cost per person and requires more intensive commitment of resources to the individual. Another disadvantage is that persons may be labeled in this process. An example of indicated prevention is treatment programs and aftercare for individuals who are illicit drugs users who are not yet addicted.
3.1.2 LEVELS OF PREVENTION

Prevention can also be stratified into different levels. Introduced in 1957, the levels of prevention were initially divided into three levels. However, Jamoulle proposed a quaternary level of prevention in 1986.

Last defines primordial prevention as “actions and measures that inhibit the emergence and establishment of environmental, economic, social, and behavioural conditions, cultural patterns of livings, etc., known to increase the risk of disease” (e.g. improving housing availability, reducing child poverty).

3.1.2.1 PRIMARY

Primary prevention aims to prevent individuals from the development/occurrence of a problem/disease. In doing so, it decreases the incidence of the problem/disease. Incidence is defined as the number of newly diagnosed cases per population in a certain time period. It does not include people who already have disease. Incidence is helpful to determine if a disease is linked to new conditions/risk factors.

Primary prevention is often accomplished by health promotion and specific protection. It can be accompanied by personal or communal efforts. Last states that “protection of health by the disease in the susceptible population” can be achieved by “enhancing nutritional status, immunizing against communicable disease, and eliminating environmental risks.” An example of primary prevention is educational and health promotion campaigns.
3.1.2.2 SECONDARY

Secondary prevention is early intervention in diagnosed cases or in the presence of risk factors. By early disease detection or screening there is an increased opportunity to cure, prevent spread, progression and emergence of symptoms, adverse consequence or complications via treatment, thereby controlling disease and minimizing disability. This is also known as health maintenance. Secondary prevention can decrease the consequence of disease, thereby decreasing its prevalence in the population if it is cured. Prevalence refers to the number of established cases of the problem/disease in a population at any given time. It includes both new and existing cases. An example of secondary prevention is the treatment of hypertension so that an individual will not have a stroke or myocardial infarction.

3.1.2.3 TERTIARY

Tertiary prevention targets the person who already has symptoms of the disease. It directs efforts to recovery or rehabilitation of the disease/condition after it has developed. It looks to specifically decrease chronic effects or disability associated with a problem or disease. The objective is to “treat the patient so the disease does not worsen or reoccur, to prevent damage and pain from the disease, to prevent the disease-related complications, to improve care to individuals with the disease, and to rehabilitate people with the disease to a healthy state”. An example of tertiary prevention is the use of anticoagulants by individuals who have had a heart attack or stoke.
3.1.2.4 QUATERNARY

The objective of quaternary prevention is to prevent other persons in the community from getting a disease/problem that has been established in others in the population \(^{30}\). Quaternary prevention is usually primary prevention for another individual. Specifically, it is the “action taken to identify patients at risk of overmedicalization, to protect patients from new medical invasions, and to suggest interventions which are ethically acceptable” \(^{31}\). It is relevant in particular to chronic disease and conditions \(^{30}\). An example of quaternary prevention is travel restriction from an area with an outbreak to other uninfected areas.

3.2 SCREENING, DIAGNOSIS, AND TREATMENT/INTERVENTION

3.2.1 SCREENING

Screening is a strategy that can be used to detect a disease or problem in individuals within a population. Screening tests are administered to individuals who are usually asymptomatic. The objective of screening is early identification, leading to earlier interventions and management to reduce the morbidity and mortality associated with the disease or problem. The UK National Screening Committee defines screening as: “A public health service in which members of a defined population, who do not necessarily perceive they are at risk of, or are
already affected by a disease or its complications, are asked a question or offered a test, to identify those individuals who are more likely to be helped than harmed by further tests or treatment to reduce the risk of a disease or its complication” 32.

According to the World Health Organization, to successfully implement a screening program the following conditions should be met:

i. A suitable test should exist;

ii. The disease or condition that is being screened for should be important medically, socially, or economically;

iii. The natural history of the disease should be understood and the population at risk should be identifiable;

iv. The test should be acceptable to the population;

v. The condition should be recognizable at an early stage;

vi. There must be an accepted and effective treatment for the condition;

vii. There should be facilities for assessment, diagnosis and rehabilitation;

viii. Interventions should be acceptable to the population

ix. The cost of screening should not be disproportionate to the cost of caring for the affected individuals

x. Screening programs should be a continuing process 33.

Although screening has the potential to save or improve the quality of life through early diagnosis of serious conditions, it is not a fool-proof process. Screening can reduce the risk of developing a condition or its complications, but it cannot offer a guarantee of protection. In any screening program, there is an
irreducible minimum of false positive results (wrongly reported as having the condition) and false negative results (wrongly reported as not having the condition).

A good screening test is an inexpensive, quickly administered tool. A good screening test demonstrates both high sensitivity and high specificity. Sensitivity is the ability to correctly identify persons with the condition in the population who are positive. Specificity is the ability to correctly identify persons without the condition in the population who are truly negative. The higher the sensitivity and specificity reported, the greater the accuracy of the test. However, as this is not always achievable, one may opt for a test with high sensitivity and low specificity to ensure they are identifying all individuals who could potentially be positive. Successful screening will identify more than the possibly affected persons who have the condition. The positive predictive value (PPV) is the probability of the condition among individuals with a positive test. The negative predictive value (NPV) is the probability of no condition among those with a negative test. A reference standard, i.e. an alternative method, to determine the condition independent of the screening test is required. In most cases, individuals who screen positive are referred for further assessment and diagnosis for confirmation of the condition. A screening test has to be accepted by the population in which it is being used, otherwise it is unlikely to be implemented. In addition, there has to be evidence that there is infrastructure for appropriate treatment/intervention in the individuals to improve their outcome.
3.2.2 DIAGNOSIS

Diagnosis is the confirmation of a disease. Diagnosis should not be confused with screening. Individuals diagnosed with an ailment are actually affected, whereas screening identifies individuals who may be affected by the ailment.

3.2.3 TREATMENT/INTERVENTION AND PROGNOSIS

Treatment/interventions generally target individuals who have high symptom levels or diagnosable disorders at the current time. The objectives of interventions are to decrease the symptoms and/or severity of the disease. Prognosis is the prediction of how a disease will progress and the individual's chance of recovery.

3.3 SCREENING IN PREGNANCY

Half of all pregnancies are unplanned and many adults are not immediately aware of health and lifestyle factors that may influence the outcome of pregnancy. It is therefore important to screen during pregnancy for known reproductive risks. A report on pre-conceptional health identifies eight areas of screening: reproductive awareness, environmental toxins and teratogens, nutrition and folic acid, genetics, substance use, medical conditions and medications, infectious diseases and vaccination, and psychosocial concerns. Other methodologies of screening currently employed during pregnancy include
ultrasound and glucose tolerance tests. In addition, screens are employed after the delivery of the baby including thyroid testing and genetic testing for rare conditions.

3.4 NUTRITION DURING PREGNANCY

3.4.1 RECOMMENDED DAILY INTAKE OF NUTRIENTS DURING PREGNANCY

Increased nutrients are necessary during pregnancy to address the physiological changes in pregnancy. Inadequate nutrition may result in negative consequences for both the mother and fetus, including iron deficient anemia \(^{37}\), pre-eclampsia \(^{38}\), poor fetal growth \(^{39}\), and low birth weight \(^{40}\).

Recommended Nutrient Intake (RNI) has recently been replaced by Dietary Reference Intake (DRI) as guidelines for nutrient intake. DRIs are scientifically-based quantitative estimates of nutrient reference values for healthy populations \(^{41}\). DRIs are used to assess and plan nutrient intakes of individuals and populations. DRIs encompass Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), and Tolerable Upper Intake Level (UL). The EAR is the median usual intake value that is estimated to meet the requirement of half the healthy individuals in a life-stage and gender group. The EAR is based on criterion of adequacy from published literature. The RDA is the average daily dietary intake level that is sufficient to
meet the nutrient requirement of nearly all (97-98%) healthy individuals in a particular life-stage and gender group. The RDA is calculated based on the EAR. The AI is the recommended average approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people who are assumed to be maintaining an adequate nutritional state. The AI is expected to meet or exceed the needs of most individuals in a specific life-stage and gender group. The UL is the highest level of continuing daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals in the life-stage group for which it has been designed.

The DRI for pregnant women is the average daily dietary intake that is sufficient to meet the nutrient requirement of nearly all healthy pregnant women (97-98%). The DRI of nutrients in pregnancy is listed in Table 1.
<table>
<thead>
<tr>
<th>Vitamin</th>
<th>A (µg/day)</th>
<th>Vitamin C (mg/day)</th>
<th>D (µg/day)</th>
<th>E (mg/day)</th>
<th>K (µg/day)</th>
<th>Thiamine (mg/day)</th>
<th>Riboflavin (mg/day)</th>
<th>Niacin (mg/day)</th>
<th>B6 (µg/day)</th>
<th>Folate (µg/day)</th>
<th>B12 (µg/day)</th>
<th>Pantothenic Acid (mg/day)</th>
<th>Biotin (µg/day)</th>
<th>Choline (mg/day)</th>
</tr>
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Adapted from National Academy of Sciences
3.4.2 NUTRITION AND ALCOHOL

Nutritional deficiencies of vitamins and minerals have been reported in alcoholics \(^{43}\). These deficiencies can arise due to one or a combination of factors including decreased dietary intake due to the high caloric content of alcohol, epigastric pain resulting from gastritis, decreased absorption, abnormal metabolism, decreased storage and increased loss of nutrients, and limited finances \(^{43}\). In fact chronic severe drinking has been associated with malnutrition due to malabsorption of vitamins and fats \(^{44,45}\).

Alcohol has 7.1kcal/g energy value \(^{46}\). One study observed that women drinking one or more drinks per day weighed on average 2.3kg less than non-drinkers \(^{47}\). A study of individuals consuming more than 30% of their total calories as alcohol had decreases in proteins, fat, vitamin A, vitamin C, thiamin, calcium, and iron \(^{48,49}\). This is exacerbated by the impaired absorption of vitamins from the small intestines \(^{50}\).

Decreased storage space of vitamins within the body may occur due to the deposition of fibrous tissue and fats, as well as cellular degeneration, causing the increased release of vitamins from the liver. In addition, increased vitamins may be required to repair liver injury.

Chronic alcoholic patients are deficient in various vitamins including folate, vitamin B6, thiamine, and vitamin A \(^{51}\). Folic acid, thiamine, riboflavin, nicotinic acid, and pyridoxine are the most commonly deficient vitamins observed in alcoholics \(^{52}\). Ethanol interferes with the metabolism of folic acid, pyridoxine, and thiamine \(^{53}\).
Chronic ethanol ingestion has been associated with low hepatic retinoid levels in alcoholics\textsuperscript{54}, low vitamin C\textsuperscript{55}, vitamin D\textsuperscript{56}, vitamin K, folic acid\textsuperscript{57,58,59}, vitamin B12\textsuperscript{60,61}, riboflavin\textsuperscript{62}, thiamine\textsuperscript{63,64}, vitamin E\textsuperscript{65}, magnesium\textsuperscript{66}, zinc\textsuperscript{67}, and magnesium\textsuperscript{68}.

3.5 MULTIVITAMINS

Most multivitamin/mineral supplements contain at least 10 vitamins or minerals with a wide range of doses. There is no regulatory or generally accepted definition of a multivitamin-mineral supplement product. Many multipurpose multivitamin-mineral supplements contain several fat-soluble vitamins, seven or more water-soluble vitamins, and severe or more minerals\textsuperscript{69}. Thus for research purposes, the lack of uniformity limits the generalizability of the results of studies investigating multivitamin preparations.

3.5.1 MULTIVITAMIN USE IN PREGNANCY

Multivitamin supplements are the most widely used dietary supplements amongst American adults. The 1999-2000 National Health and Nutrition Examination Survey reported multivitamin use in 35% of adults\textsuperscript{70}. Park \textit{et al.} examined multivitamin usage in African American, Native American, Japanese American, Latino, and Caucasian population where 50% of participants used multivitamins. No substantial significant difference amongst ethnicities were observed\textsuperscript{71}.
Czeizel et al. reported that multivitamin supplementation was associated with an increased chance of multiple pregnancies \(^{72}\). Geltman et al. reported that daily supplementation with multivitamins and iron was associated with a decreased risk for anemia in high-risk infants \(^{73}\). Nilsen et al. reported that women supplementing with folic acid and/or multivitamins before or any time during the pregnancy had a 26% reduction in placental abruption (odds ratio [OR]=0.74, 95% confidence interval [CI] 0.65-0.84) and a 40% reduction of preterm labour (OR=0.60, 95% CI 0.49-0.73) \(^{74}\). A meta-analysis by Haider et al. reported that multivitamin supplementation during pregnancy was associated with reduced risk for low birth weight (risk ratio [RR]=0.83, 95% CI 0.76-0.91), small for gestational age (RR=0.92, 95% CI 0.86-0.99), and anemia (RR=0.61, 95% CI 0.52-0.71) \(^{75}\). No statistically significant difference was observed for preterm births (RR=0.92, 95% CI 0.82-1.04) and perinatal mortality (RR=1.05, 95% CI 0.90-1.23) \(^{75}\). Bodnar et al. reported that in a study of 1,835 women less than 16 weeks pregnant, regular users of prenatal multivitamins were associated with a 45% decreased risk for pre-eclampsia compared to non-users (OR=0.55, 95% CI 0.32-0.95) \(^{76}\). Rumbold et al. conducted a meta-analysis to examine the effects of multivitamin supplementation during pregnancy. Supplementation resulted in an increased chance of multiple pregnancy (OR=1.36, 95% CI 1.00-1.85) \(^{77}\). No statistically significant difference was observed for fetal loss (RR=1.09, 95% CI 0.95-1.25), early or late miscarriage (OR=1.09, 95% CI 0.94-1.26), stillbirth (OR=1.03, 95% CI 0.51-2.09), and congenital malformations (OR=1.69, 95% CI 0.81-3.53) \(^{77}\).
A meta-analysis by Lumley et al. investigating the supplementation of folate with or without multivitamins reported a decreased risk of neural tube defect (OR=0.28, 95% CI 0.13-0.58)\textsuperscript{78}. No significant difference was observed for miscarriage (OR=1.12, 95% CI 0.98-1.29), ectopic pregnancy (OR=1.09, 95% CI 0.47-2.55), stillbirth (OR=0.78, 95% CI 0.34-1.78), multiple gestation (OR=1.40, 95% CI 0.93-2.11), limb reduction (OR=0.29, 95% CI 0.04-8.34), conotruncal heart defect (OR=0.74, 95% CI 0.16-3.32), oral facial clefts (OR=0.76, 95% CI 0.24-2.37), and other birth defects (OR=0.76, 95% CI 0.38-1.51)\textsuperscript{78}. A study by Yuksiv et al. reported a higher risk for congenital anomalies in women who supplemented with multivitamins\textsuperscript{79}. However that study was limited by the fact that the majority of women were of a low socioeconomic background\textsuperscript{79}. In addition there was no consistent pattern of birth defects and the data in this study were sparse\textsuperscript{79}. Czeizel et al. reported that supplementation decreased the risk of malformations\textsuperscript{80}. Chen et al. reported that in a randomized controlled trial of women receiving periconceptional multivitamin supplementation versus no intervention, 0.35/1,000 pregnancies were affected with a neural tube defect in the supplemented group whereas 1.80/1,000 pregnancies were affected with neural tube defect in the unsupplemented group\textsuperscript{81}. Czeizel et al. reported that mothers supplementing with multivitamins in the second and third trimester of pregnancy did not demonstrate a difference in the occurrence of multiple congenital anomalies between the supplementing group and non-supplementing groups\textsuperscript{82}. 
The effects of multivitamin supplementing in pregnancy have been investigated. Fawzi et al. reported that women with HIV receiving multivitamin supplementation had a reduced risk of anemia in pregnancy and improved hemoglobin concentrations and children born to mothers had a reduced risk for anemia. Fawzi et al. also reported that multivitamin supplementation in women with HIV decreased the incidence of low birth weight (RR=0.82, 95% CI 0.70-0.95). It also reduced the risk for small gestational age (RR=0.77, 95% CI 0.80-0.97). Merchant et al. reported that multivitamin supplementation in women with HIV resulted in better mental and psychological development. Makola et al. reported that multinutrient-fortified beverages was associated with a reduced risk of anemia in pregnancy and improved hemoglobin concentrations.

3.6 FOLIC ACID AND NEURAL TUBE DEFECTS

Folic acid is essential for growth and differentiation, repair, and host defense and hence it is essential for fetal development. During embryogenesis and fetal growth, nucleic acid, and protein synthesis are reliant on the supply of folate and therefore the requirement for maternal folate increases during this period of cell formation. A deficient folate supply or problem in its metabolism may result in impaired cell formation and tissue growth. As such, nucleic acids will not be synthesized and cells will be unable to manufacture enough DNA for mitosis therefore resulting in abnormal cell division. Proteins, lipids and myelin
will also not be methylated due to the inhibition of the methylation cycle. Since cells are rapidly dividing during the fetal period, they are most susceptible to irregularities in DNA production. Folate deficiency or impairments in genetic folate metabolism are proposed mechanisms that cause congenital birth defects. It is estimated that congenital birth defects affect 5% of individuals; however, only 2-3% are recognized at birth. It has been proposed that folic acid supplementation may correct folate levels in deficient mothers or compensate for innate folate metabolism abnormalities to decrease the risk of congenital birth defects.

Neural tube defects (NTDs) comprise of malformations of the cranium, spine, and nervous system including anencephaly, spina bifida, encephalocele, and meningocele. NTDs are a major cause of mortality in newborns secondary to congenital heart defects. NTDs are estimated to affect 0.5 – 8/1,000 live births. The prevalence of NTDs varies by geographic region and ethnicity; however, it has been estimated that NTDs affect 300,000 infants worldwide. Folic acid supplementation during pregnancy has long been associated with decreasing the risk of NTDs. Studies associating folate supplementation in decreasing NTDs date back to the 1960s. The exact mechanism by which folate supplementation prevents NTDs and its recurrence remains unknown.
3.7 ALCOHOL, PREGNANCY, AND FETAL ALCOHOL SPECTRUM DISORDER

3.7.1 ALCOHOL USE AMONGST PREGNANT AND CHILDBEARING-AGED WOMEN

In 2003, Statistics Canada reported that over 51% of females over 12 years of age reported that they consumed alcohol at least once a month and 22% reported that they were occasional drinkers. Approximately 60% of women reported drinking occasionally. In 2002, the Behavioral Risk Factor Surveillance System (BRFSS), a random-digit-dialed survey, surveyed women 18-44 years of age regarding their use of alcohol during pregnancy. Participants were asked about their alcohol consumption 30 days prior to the interview.

Approximately 10% of pregnant women reported alcohol consumption during pregnancy. Approximately 2% of pregnant women engaged in binge drinking (>5 drinks per occasion) or frequent alcohol use (>7 drinks per week). Women reported they did not use birth control because they wanted a pregnancy (52.4%), did not care whether pregnancy occurred (19.1%), did not think they would become pregnant (14.3%), did not want to use birth control (5.7%), feared the side effects of birth control (4.2%), thought they were too old to become pregnant (1.8%), could not pay for birth control (1.3%), or had a lapse in use of birth control method (1.2%). The prevalence of any use of alcohol in women of childbearing age was 52.6%. The prevalence of alcohol use in women who
might become pregnant was 54.9% \(^{103}\). Pregnant women who had higher rates of alcohol consumption tended to be single and older in age \(^{104}\).

The Youth Risk Behavior Surveillance System is an anonymous voluntary survey conducted in grade 9-12 students in public and private schools \(^{105}\). Approximately 35% reported they were sexually active and 16% reported that either they or their partner used birth control pills to prevent pregnancy \(^{105}\). The reported prevalence of birth control use prior to last sexual intercourse was 24.0% in Caucasian, 21.1% in African American, and 9.1% in Hispanic female students \(^{105}\). Approximately 22.5% of the sexually active students reported drinking alcohol or using drugs prior to intercourse \(^{105}\). Approximately 17.7% of the female sexually active students reported drinking alcohol or using drugs prior to intercourse \(^{105}\). The reported prevalence of alcohol or drug use prior to sexual intercourse was 19.8% in Caucasian, 12.9% in African American, and 16.5% in Hispanic students \(^{105}\).

Pregnant women 18-44 years were interviewed during 1995-1999 about their alcohol consumption in the preceding month \(^{106}\). The prevalence of drinking (minimum one drink) during pregnancy deceased from 16.3% in 1995 to 11.4% in 1997 but rose to 12.8% in 1999 \(^{106}\). The rates of binge drinking (>5 drinks on one occasion) were decreased from 2.9% in 1995 to 1.8% in 1997 but rose to 2.7% in 1999 \(^{106}\). The rates of frequent drinking (>7 drinks per week or >5 drinks on one occasion) decreased from 3.5% in 1995 to 2.1% in 1997 but rose to 3.3% in 1999 \(^{106}\). Characteristics of women who reported these drinking habits during pregnancy included >30 years of age, employed, and unmarried \(^{106}\).
The Pregnancy Risk Assessment Monitoring System (PRAMS), is a standardized data collecting questionnaire issued to women delivering a live infant in participating states in the United States\textsuperscript{107}. The questionnaire is first mailed to women and if there is no response they are contacted by telephone\textsuperscript{107}. The 2001 PRAMS report observed that the prevalence of unintended pregnancy was 33.7-52\% and was more prevalent in women under 20 years of age\textsuperscript{103}. The prevalence of alcohol use in the last three months of pregnancy ranged from 3.4-9.9\%\textsuperscript{103}. The highest prevalence of alcohol use in the last three months of pregnancy was reported by older, non-Hispanic women with more than a high school education and a high income\textsuperscript{103}. Pregnant women who drank during any trimester of pregnancy were more likely to be not Caucasian, unmarried, younger, and work full-time outside of home\textsuperscript{108}. The majority of women stopped drinking or drastically reduced drinking once they discovered they were pregnant\textsuperscript{108,109}. However 20\% continued to drink during pregnancy\textsuperscript{102}.

The time to recognition of pregnancy between drinkers and non-drinkers did not differ significantly. The average number of days from the last menstrual period to pregnancy recognition was 31 days (7-227 days). The median number of days was 32.0 in non-drinkers, 31.0 in occasional drinkers, 31.0 in regular drinkers, and 30.0 in heavy drinkers\textsuperscript{110}.

3.7.2 DRINKING DEFINITIONS

A standard drink is 12 ounces (340mL) of beer, 8 ounces (227mL) of malt liquor, 5 ounces (142mL) of wine, and 1.5 ounces (43mL) of 80 proof distilled
spirits (all contain the same amount of alcohol) \(^\text{111,112}\). Abstainers are defined as individuals who do not drink alcohol. Moderate alcohol consumption is no more than one drink per day in women and two drinks per day in men \(^\text{113}\). Heavy drinking is defined as an average of more than one drink per day in women and an average of more than two drinks per day in men \(^\text{112}\). Binge drinking is defined as a pattern of alcohol consumption that results in a blood alcohol concentration that is equal to or above 0.08% which corresponds to four or more drinks in a single occasion for women and five or more drinks in a single occasion for men within two hours \(^\text{114}\).

### 3.7.3 ALCOHOL ABUSE VS. ALCOHOL DEPENDENCE

The Diagnostic and Statistical Manual of Mental Disorder (DSM-IV) defines alcohol abuse as when an individual has one of the following: continued use despite social or interpersonal problems, hazardous use, legal problems, or neglect of role obligations due to ongoing alcohol consumption \(^\text{115}\). The DSM-IV defines alcohol dependence as when an individual has three or more of the following: tolerance; withdrawal; unsuccessful attempts or persistent desire to stop use; use in longer duration or in larger amounts than intended; activities given up in favour of use; time spent in obtaining, using, or recovering from substance effects; and continued use despite physical or physiological problems due to ongoing alcohol consumption \(^\text{115}\).
3.7.4 FETAL EXPOSURE TO ETHANOL

As ethanol is a small molecule, it is readily able to cross the placenta and accumulates in the fetus at levels proportionate to maternal blood alcohol levels at approximately one hour \(^{116}\). Levels of ethanol in the amniotic fluid have been reported to reach 50% of the maternal blood levels \(^{116}\). A study following the elimination of ethanol from human amniotic fluid observed a mean rate of 0.08±0.03 mg/mL/h \(^{117}\). Thus at 3.5 hours after maternal ingestion, although there was no detectable ethanol in maternal blood, there was still detectable levels in amniotic fluid \(^{117}\).

3.7.5 TERATOGENIC EFFECTS OF ETHANOL

Since the brain begins to form from the third week of pregnancy and continues to form throughout the pregnancy, it is the organ most susceptible to ethanol damage. This is evidenced by the manifestations observed in FASD. FASD is noted to affect behaviour and other neurological functions.

3.7.5.1 CLINICAL MANIFESTATIONS

Maternal alcohol consumption during pregnancy adversely affects the fetus and can result in FASD. FASD ranges in presentation from FAS to other alcohol-related disabilities including ARND and FAE. FASD encapsulates individuals affected with behavioural changes with and/or without the presence of physical changes \(^9,^{118}\). FAS is characterized by facial dysmorphia including a smooth philtrum, thin upper lip, and small eyes \(^9,^{11}\). ARND includes deficits in
basic cognitive functioning, social, emotional, and behavioural difficulties\textsuperscript{119}. Those affected by FASD may have difficulties in planning, organization, and attention; failure to learn from consequences; memory deficit; speech/language visuospatial functioning; spatial memory; and deficits in verbal learning\textsuperscript{120,121,122,123,124,125}.

### 3.7.5.2 Prevalence/Incidence of Fetal Alcohol Spectrum Disorder

Prenatal alcohol exposure is one of the leading causes of mental retardation in the western world\textsuperscript{22}. The prevalence of FASD in the United States has been reported as 0.5-9.1/1,000 live births\textsuperscript{23,126}. The prevalence of FASD in South Africa has been reported as 39.2-46.4/1,000 births\textsuperscript{127}. To date, there is no available estimate of the prevalence of FASD in the Canadian population\textsuperscript{9}. Due to the high estimated prevalence rates observed in other countries, it is important to ascertain the prevalence of FASD in Canada.

### 3.7.5.3 Screening for Fetal Alcohol Spectrum Disorder

There is currently no standardized screening tool for FASD. This is most likely because there is no single biological marker that will quickly identify the problem unlike genetic conditions which have a single specific biomarker. Moreover, the cognitive and behavioural effects associated with FASD are not specific to prenatal alcohol exposure alone. Since FASD affects persons accessing multiple societal systems, it is important that professionals in frontline
settings including healthcare workers (including family physicians, nurses, pediatricians, psychiatrists), allied healthcare workers (physiotherapists, occupational therapist, speech pathologists), teachers, administrators, school psychologists, special education consultants, and youth justice personnel, including judges, probation officers, and corrections officers, actively screen for these individuals.

3.7.5.3.1 SCREENING MOTHERS ANTENATALLY/POSTNATALLY

Asking mothers or expectant mothers about their alcohol consumption during pregnancy is one method of screening for prenatal alcohol exposure. Ideally, if the mother answers truthfully, this screening method would be both sensitive and specific. However, this is not always the case. Mothers self-reporting may provide an incorrect assessment of alcohol use due to reasons including recall bias, fear of losing their child, embarrassment, and fear of stigma. Another method of screening is to identify mothers who have a known history of substance abuse.

Screening for FASD can be initiated as during pregnancy. Maternal self-report of alcohol consumption is the main source of information that healthcare providers rely on when assessing drinking patterns. Limitations to maternal self-report include difficulty recalling the quantity and frequency of alcohol intake, denial, embarrassment, fear of stigma, punishment, incarceration, or involuntary commitment associated with alcohol consumption during pregnancy. To overcome these obstacles, healthcare providers may consider
the use of screening questionnaires including: AUDIT (Alcohol Use Disorders Identification Test), CAGE (Cut-down, Annoyed, Guilty, Eye-opener), TWEAK (Tolerance, Worry, Eye-opener, Amnesia, Cut-down), T-ACE (Tolerance, Annoyed, Cut-down, Eye-opener), MAST (Michigan Alcohol Screening Test), and TLFB (Timeline Follow-back). The T-ACE has a reported 76% sensitivity and 89% specificity, while the TWEAK has a reported 70% sensitivity and 79% specificity. Although these screening instruments identify heavy drinking patterns, they do not measure prenatal alcohol exposure. Since screening of biological mothers is not always accurate or an available option, other tools for screening for FASD must be explored.

### 3.7.5.3.2 BIOMARKERS

#### 3.7.5.3.2.1 MECONIUM

Meconium is the first fecal matter passed in the newborn. Fatty acid ethyl esters (FAEE) are non-oxidative metabolites of ethanol that are formed in the body by esterification of ethanol with free fatty acids. FAEE do not pass the placenta. As such, the presence of FAEE in the meconium represents the true estimate of fetal ethanol exposure. Meconium begins forming at approximately 12 weeks of pregnancy. Meconium can be collected up to approximately 72 hours after birth. Commonly quantified FAEE include linoleic, palmitic, oleic, steric, and palmitoleic ethyl esters. Several FASD screening studies have been undertaken by measuring FAEE in meconium. A study
screening meconium (n=422) from November-December 1999 in a Hawaiian regional birthing center observed a prevalence of 16.7% positive FAEE. An anonymous population study collected meconium from all birthing centres in Grey-Bruce, Ontario and reported a 2.5% FAEE positive meconium. A study of meconium (n=900) collected in Montevideo, Uruguay observed a 44% positive FAEE rate.

The advantages of meconium screening are that it is an easy, non-invasive method of screening of otherwise discarded material. FAEE is thermo- and photosensitive, therefore it should be stored in opaque containers in the freezer and shipped on dry ice. The disadvantage of meconium screening is that there is a limited window to collect meconium. In addition, since meconium begins forming at approximately 12 weeks of pregnancy, it is only able to detect prenatal ethanol exposure in the second and third of pregnancy. Moreover, there has not yet been a correlation of how much FAEE correspond to the development of FASD.

3.7.5.3.2.2 HAIR

FAEE can enter the hair by capillary blood supply, sebaceous glands, and sweat glands. Neonatal hair begins forming at approximately 20 weeks of pregnancy and can be collected up to three months after birth, after which the neonatal hair typically sheds. Hydrophobic molecules can accumulate in the hair shaft. Studies in neonates have demonstrated that FAEE have been quantified in the hair of babies exposed to excessive quantities of alcohol.
The advantages to this method of screening include the fact that hair is easy to collect, that it is a non-invasive procedure and that there is a longer window for collection. The disadvantages to this method of screening include that it is only able to document exposure from the 20\textsuperscript{th} week of pregnancy. Some cultures also have sensitivities regarding cutting infant hair. This test, however, is in its developmental stages and its clinical sensitivity and specificity have not yet been determined. Further studies need to be undertaken before this tool can be used as a screening tool for FASD.

3.7.5.3.2.3 CORD BLOOD

In an attempt to identify a potential screening tool for FASD, Gallot \textit{et al.} measured aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), and carbohydrate deficient transferrin (CDT) in fetal cord blood of exposed neonates immediately after birth over a 1-year period \textsuperscript{146}. Of 870 samples, only two cases of FASD were identified and there were no significant correlations between maternal and cord blood biomarkers \textsuperscript{146}. Thus, using these parameters is not an effective means of screening for prenatal alcohol exposure.

3.8 FATTY ACID ETHYL ESTERS

Fatty acid ethyl esters (FAEE) are neutral hydrophobic non-oxidative products of ethanol metabolism that are formed by the esterification products of
ethanol and fatty acids or fatty acyl-CoA\textsuperscript{147,148,149,150}. FAEE were first identified in total body lipid extracts of rats that received intravenous ethanol\textsuperscript{151}.

FAEE have been detected in high concentrations in organs damaged by ethanol abuse and may be associated ethanol-induced damage\textsuperscript{147,148,152,153,154}. FAEE have been proposed as markers for acute and chronic ethanol consumption\textsuperscript{155}.

### 3.8.1 SYNTHESIS OF FATTY ACID ETHYL ESTERS

The formation of FAEE is catalyzed by two enzymes: microsomal acyl-coA:ethanol O-acyltransferase (AEAT) and FAEE synthase\textsuperscript{156,157,158,159}. The involvement of coenzyme A in the synthesis of FAEE was discovered by Grigor \textit{et al.} who observed this in rat microsomes\textsuperscript{158}. FAEE synthesis has also been reported to be catalyzed by cholesterol esterase, carboxylesterase, and lipoproteinlipase\textsuperscript{156,160,161,162}. FAEE synthase is found predominantly in the pancreas and liver; however, these enzymes have also been identified in rat liver and pancreas\textsuperscript{163,164}, mice brain\textsuperscript{165}, and rabbit heart\textsuperscript{157}, and rat adipose tissue\textsuperscript{160}. Four FAEE synthase enzymes have been identified in the human heart\textsuperscript{166,167,168,169}. FAEE synthase has three isoforms\textsuperscript{170}. FAEE are synthesized at high rates in the heart and other organs lacking oxidative ethanol metabolism\textsuperscript{171}.

Fatty acids are used as fuel and components of biological membranes\textsuperscript{172}. Fatty acids are supplied by dietary sources or synthesized by cytosolic enzymes in the liver\textsuperscript{172}. Non-esterified fatty acids are not present in living cells in high concentrations but are rapidly esterified for storage as triglycerides or
phospholipids. Not all cellular fatty acids are equally available for FAEE synthesis. Newly incorporated fatty acids are preferably esterified in the nuclear membrane. Fatty acids are most likely esterified in the endoplasmic reticulum where the majority of eicosanoids are synthesized. It is likely that an esterified fatty acid source provides the fatty acids for FAEE synthesis.

Grigor and Bell showed that microsomal preparations from rat liver were able to synthesize FAEE from fatty acyl-CoA and ethanol, however, unconverted free fatty acids could not be used for FAEE synthesis.

Non-esterified fatty acids in the cell cytosol of the myocyte react with ethanol in the presence of AEAT or FAEE synthase to form FAEE which bind and accumulate within the mitochondrial inner membrane. Bora et al. demonstrated that binding of ethyl oleate to mitochondria increases linearly with time.

FAEE have been also reported to accumulate in the tissues of chronic alcoholics. FAEE are delivered to tissues by low density lipoproteins (LDLs) through the blood. In response to the ingestion of ethanol, FAEE have been reported to be synthesized in the myocardium, brain, pancreas, and blood (white blood cells, especially lymphocyte-monocyte fraction), and red blood cells. FAEE have been reported to be detected in adult hair.

Significant synthesis of FAEE by human mononuclear cells has been reported after exposure to ethanol. FAEE were reported to be transported by lipoprotein particles and albumin. The majority of FAEE found in blood is
located in the plasma component. Approximately 7% is found in the red blood cell. FAEE uptake by human mononuclear cells has been reported as fast as 0.08 minutes and FAEE production increases from 10-20% over 120 minutes. FAEE have also been reported to be synthesized in the placenta, cord blood, hair, and meconium.

FAEE synthesis is rapid after ethanol exposure. Endogenous FAEE synthesis greatly exceeds uptake from exogenous sources. Kabakibi et al. observed that in an *in vitro* model using HepG2 cells, FAEE synthesis was observed after 5 minutes of incubation with ethanol and reached plateau at two hours of incubation. They observed that FAEE were synthesized by microsomal FAEE synthase and cytosolic FAEE synthase. Microsomal FAEE synthase preferentially uses fatty acyl-CoA as a substrate whereas cytosolic FAEE synthase accepts both unesterified fatty acid and fatty acyl-CoA as substrates with a slight preference for fatty acyl-CoA.

To examine the additive effect of fatty acid supplementation and ethanol consumption on the synthesis of FAEE, 100mM oleic acid was incubated in the presence of 25, 50, or 100mM ethanol. The combination of 100mM ethanol and 100mM oleic acid increased the formation of FAEE compared to 100mM ethanol alone. Dan et al. also demonstrated that the addition of 100mM of fatty acid resulted in the increase of corresponding FAEE species production in HepG2 cells.
3.8.2 DEGRADATION OF FATTY ACID ETHYL ESTERS

Most FAEE in the stomach remain intact\textsuperscript{192}. FAEE are rapidly hydrolyzed in the gastrointestinal tract in the duodenum\textsuperscript{193}. FAEE hydrolysis degrades intracellular FAEE over several hours\textsuperscript{184}.

Initial studies in blood suggested that FAEE have a reported biological half-life of 20-24 hours\textsuperscript{148}. However FAEE have been reported in blood for a minimum of 24 hours after ethanol consumption and readily concentrate in adipose tissue\textsuperscript{179}. Serum FAEE levels have been observed to correlate with ethanol levels and persist for a minimum of 24 hours following ethanol ingestion\textsuperscript{179}.

In humans, the FAEE elimination half-life was reported as 173 minutes whereas the terminal half-life was 693 minutes with no observable correlation to peak blood ethanol levels\textsuperscript{179}. Saghir \textit{et al.} reported that FAEE has a short half-life of 58 seconds in serum\textsuperscript{192}. Borucki \textit{et al.} demonstrated that in healthy humans FAEE remained elevated in serum for over 44 hours post-ethanol consumption\textsuperscript{182}. FAEE did not immediately decrease to zero\textsuperscript{182}. There was a decrease to a level that was above the level of quantitation (0.024-0.087mol/L) that plateaued for approximately 15.3 hours\textsuperscript{182}. This indicated that there is a baseline range of FAEE that are produced by natural human metabolism. They reported a sensitivity of 35\% and a specificity of 0\textsuperscript{182}. They also hypothesized that high effluxes are due to delayed elimination (reflux of FAEE out of storage compartments)\textsuperscript{182}. Borucki reported that increased FAEE levels could be observed up to 100 hours after ethanol consumption\textsuperscript{194}. A steep decline at
317nmol/h was observed in the first 17 hours. This was followed by a slow decline at 3nmol/h. Borucki et al. reported in a study of patients undergoing withdrawal that serum FAEE concentrations were not associated with prior concentrations. Alhomsi et al. reported in an experiment examining FAEE degradation and the transfer of fatty acids derived from different lipid fractions that approximately 45% of fatty acids were intact after 2 hours of exposure to FAEE, and less than 10% of FAEE were esterified in human mononuclear cells.

Compared to oxidative metabolites of ethanol, FAEE have a prolonged half-life in tissue. A reported half-life of 16 hours has been observed in rabbit adipose tissue and a reported half-life of one week has been reported in mouse placenta. Bearer et al. reported that pregnant rabbits treated on day seven with ethanol had placental accumulation of ethyl stearate whereas ethyl oleate was detected in the maternal heart and liver. The animals were sacrificed on day 14 and significant levels of FAEE were present in placental tissue, suggesting that the presence of FAEE synthase in the placenta. The placenta half-life was greater than 50-80 hours. In comparison, in rabbit adipose tissue a half-life of 16 hours was observed. Doyle reported that the half-life of FAEE in human adipose tissue is 6-24 hours.

3.8.3 FATTY ACID ETHYL ESTERS AS BIOMARKERS OF DAMAGE

In contrast to oxidative metabolites of ethanol, non-oxidative metabolites have been demonstrated to be toxic in experimental systems. FAEE have been
implicated in mediating ethanol-induced organ damage. The first suggestion of FAEE-associated damage was in an autopsy study. A study by Laposata et al. reported high concentrations of FAEE and FAEE synthase (enzyme responsible for synthesizing FAEE) in tissues obtained in postmortem autopsy of individuals with chronic ethanol abuse and who were intoxicated at death.

Alhomsi et al. reported observing cellular toxicity resulting in apoptosis and necrosis an in vitro study evaluating FAEE in human mononuclear cells. Aleryani et al. demonstrated that patients with chronic liver or pancreatic disease release FAEE synthase into their blood which causes non-oxidative ethanol metabolism in the plasma. FAEE have been associated with increasing the fragility of isolated pancreatic lysosomes. Infusion of FAEE within lipoproteins in vivo showed that they were associated with cellular damage to pancreatic acinar cells. FAEE have been associated with the disruption of oxidative phosphorylation in the mitochondria. Gubitosi et al. demonstrated that FAEE accelerate the kinetics of voltage-induced activation of the human brain delayed rectifier potassium channel Kv1.1.

Free fatty acids have been associated with inhibition/decrease of growth, decreased DNA and protein synthesis, decreased cell replication/proliferation in HepG2 cells, increased rat pancreatic lysosomal fragility in vitro, structural changes and instability of human erythrocytes, activation of the human neuronal potassium ion channel, pancreatitis-like toxicity in a rat model implicated in alcoholic pancreatitis, and decreased protein synthesis in rat pancreatic acinar cells.
Some FAEEs react with carboxylesterase enzyme and are hydrolyzed by the mitochondria to fatty acids \(^{147,148,174}\). FAEE have been reported to cause the uncoupling of oxidative phosphorylation \textit{in vitro} in isolated mitochondria \(^{176}\). The uncoupling of oxidative phosphorylation results in damage to the ATP synthesis activity of the mitochondria which can lead to cellular dysfunction and cell death \(^{176,204,205}\). This is a proposed mechanism of end-organ damage observed in adult alcoholics \(^{176,174}\). \textit{In vitro} studies in human HepG2 cells observed a decrease in protein synthesis and cell formation in response to FAEE \(^{154}\).

FAEE can hydrolyze into free fatty acids and ethanol when incubated with mitochondria or LDL particles \(^{192,148}\). FAEE and fatty acids can cause calcium dependent necrosis \(^{206}\). FAEE have been associated with impaired replication and protein synthesis in cultured HepG2 cells \(^{154}\). FAEE have toxic effects on a number of cell types and in different settings \(^{175,207}\).

### 3.8.4 MECONIUM AS A DEPOT FOR FATTY ACID ETHYL ESTERS

Meconium is expelled in the first few bowel movements of a newborn. It is commonly dark green or black in colour and is odorless. This matrix is composed of water, desquamated epithelial cells from the gastrointestinal tract and skin, lanugo (fine neonatal hair), fatty material from the vernix asosa, bile acids and salts, cholesterol and sterol precursors, blood group substances, enzymes, mucopolysaccharides, sugars, lipids, proteins, trace metals, various pancreatic and intestinal secretions, and residues of swallowed amniotic fluid \(^{208,209,210,211,212,213}\).
Meconium formation begins around the 12\textsuperscript{th}-14\textsuperscript{th} week of gestation where fetal swallowing begins\textsuperscript{211,212}. This is verified by the ability to quantify illicit drugs in the meconium of fetuses of early gestational age\textsuperscript{214}. It is believed that by fetal swallowing of amniotic fluid and expelling of urine into the amniotic fluid that fetal exposures can be concentrated into the meconium\textsuperscript{210,211}. Since ethanol is a small water-soluble molecule, it can easily cross the placenta by passive diffusion. As such, placental blood flow is a limiting factor for transport of ethanol to the fetus\textsuperscript{215}.

Meconium has been proposed as an alternative method to traditional (blood or urine) methods of testing for ethanol exposure. Meconium is a cumulative matrix where xenobiotics and endogenous substrates can accumulate during the second and third trimester of pregnancy. Meconium is an optimal matrix because it is considered metabolically inactive\textsuperscript{216}. Analysis of meconium was proposed by Callahan \textit{et al.}\textsuperscript{217,218}. Traditional tests to identify fetal ethanol exposure include neonatal and maternal urine analysis. However these test results only reflect recent exposure to ethanol prior to delivery. Meconium collection is an easy, non-invasive process that is more easily collected than urine\textsuperscript{217,219}. Urine collection may contaminate the meconium sample; however it results in a higher sensitivity of detection\textsuperscript{220}.

Limitations to meconium collection include the narrow window for sample collection. One study reported that meconium is evacuated up to 125 hours after birth; however, optimal collection occurs within the first 72 hours after birth, as a
transition period of meconium and feces follows\(^{208,220}\). This may be different for low birth weight infants or infants who have meconium stained delivery\(^{221}\).

Different technologies are employed by different laboratories in the analysis of meconium (e.g. solid-phase extraction versus silica gel chromatography and gas chromatography-flame ionization detector [GC-FID] versus gas chromatography-mass spectrometry [GC-MS]) and may contribute to the differences reported in specificity and sensitivity. Quantitative determination of FAEE by headspace solid phase microextraction (HS-SPME) and GC-MS using deuterated internal standards have been previously demonstrated to be very selective and sensitive in detecting FAEE in hair\(^{183,187}\).

Since FAEE have a longer half-life compared to ethanol and its oxidative metabolites, it has been proposed to use FAEE as a biomarker for prenatal alcohol exposure. Increased levels of FAEE have been quantified in neonates with *in utero* exposure to ethanol\(^{145,189,222}\). Bearer *et al.* reported that ethyl linoleate is detectable in meconium and is a useful marker in identifying newborns exposed to high alcohol concentrations during pregnancy\(^{223}\). Bearer *et al.* also reported that ethyl laurate (12:OEE), ethyl myristate (14:OEE), ethyl palmitate (16:OEE), ethyl stearate (18:OEE), ethyl oleate (18:1EE), ethyl linoleate (18:2EE), ethyl linolenate (18:3EE), and ethyl arachidonate (20:4EE) were detected in meconium samples\(^{222}\). The predominant forms of FAEE were ethyl linoleate>ethyl oleate>ethyl linolenate\(^{222}\). Since the placenta contains enzymes that are able to degrade FAEE, the FAEE generated by the fetus are most representative of fetal exposure\(^{137}\).
The half-life of FAEE in meconium is currently unknown. It is believed that the half-life of FAEE in meconium is extended in duration. Since meconium is metabolically inert (nothing in the normal biological function reacts with it), it is believed that drug metabolites are stable within this matrix. Other recreational drugs used in the second and third trimesters of pregnancy can be quantified in meconium. Previous studies in mice placenta (a metabolically active tissue) demonstrated that the half-life of FAEE was one week. An unpublished study by Bearer et al. reported that meconium stored under nitrogen at 37°C had no degradation of ethyl palmitate, ethyl oleate, ethyl linoleate, and ethyl arachidonate.

3.8.5 VALIDATION OF MECONIUM AS A BIOMARKER FOR PRENATAL ALCOHOL EXPOSURE

The validity of FAEE in meconium has been tested in a guinea pig model and the total FAEE concentration in guinea pigs chronically dosed with ethanol was 80-fold and 28-fold higher than a comparative sucrose and water control group, respectively.

Bearer et al. reported the sensitivity and specificity of meconium testing in women who most likely abstained from alcohol use and in women who admitted drinking during the third trimester of pregnancy. The specificity of the test was 51% in women who abstained in the last month of pregnancy. The specificity of the test was 68% in women who reported drinking in the third trimester of pregnancy. The specificity of the test was 66% in women who reported
drinking in the second trimester of pregnancy. The sensitivity of the test was 68% in women who drank in the second and third trimester of pregnancy. In comparing the sensitivity and specificity of meconium tests to screening questionnaires, meconium screening had a 74% sensitivity and a 43% specificity whereas the questionnaires had a 79% sensitivity and a 83% specificity. In another study, Bearer et al. observed a sensitivity of 84.2% and a specificity of 83.3% with ethyl oleate in meconium of babies born to South African mothers who drank an average of three or more drinks per day.

In attempts to quantify baseline levels of FAEE detected in meconium, a study collected samples of meconium from babies born to non-drinking mothers from Toronto and Jerusalem. This study compared meconium from babies born to mothers from Toronto and Jerusalem who reported social drinking (one drink on an isolated occasion to one drink per month throughout pregnancy) and heavy drinkers (two drinks/day to binge drinking [more than five drinks on occasion]). Six FAEE (ethyl laurate [E12], myristate [E14], palmitate [E16], stearate [E18], oleate [E18:1] and linoleate [E18:2]) were extracted by GC-FID. In 197 babies born to non-drinking women and in two alcohol-consuming (binge or heavy drinking) populations, a positive cut off of a cumulative sum of \( \geq 2 \text{nmol/g} \) FAEE resulted in 100% sensitivity and 98% specificity for alcohol-exposure. Longer esters were often quantified in concentrations \( > 2.0 \text{nmol/g} \) whereas shorter esters were quantified in concentrations \( < 2.0 \text{nmol/g} \). Another study reported a four-fold higher level of FAEE was detected in
meconium of infants born to alcoholic mothers compared to those who were not exposed to ethanol $^{145}$.

Bearer et al. compared the meconium of babies born to abstainers to those born to non-abstainers from Cleveland, Ohio and Amman, Jordan and statistically significant differences for ethyl linoleate and ethyl arachidonate were observed between newborns of abstainers and the non-abstainers $^{223}$. In women who drank three drinks per week in the third trimester of pregnancy, the sensitivity and specificity of ethyl linoleate was 75% and 58% $^{223}$.

The differences in the sensitivity and specificity findings reported from different authors may be due to the methodology used to conduct the analysis (e.g. solid-phase extraction versus silica gel chromatography and GC-FID versus GC-MS-MS) may contribute to the differences observed, HS-SPME GC-MS $^{183,187}$. Studies using a flame ionization detector may be limited as this method is not specific in identifying some FAEE (e.g. ethyl linoleate) and thus results should be verified using a detection system with greater specificity (e.g. mass spectrometer).

FAEE levels in meconium have correlated well with maternal self-report of ethanol consumption $^{145,222,226}$. Bearer et al. that ethyl oleate correlated well with maternal self-report from a Cape Town, South Africa population with a 84.2% sensitivity and 83.3% specificity $^{226}$. Conversely, Derauf et al. were able to quantify FAEE in meconium of mothers who denied alcohol consumption $^{228}$. This may be attributed to underreporting from the mother. Interestingly, Derauf et al. also were unable to quantify FAEE in mothers who admitted drinking during
the third trimester where two women consumed one drink daily, one woman had two drinks per week, and one woman had unspecified frequency.\textsuperscript{228}

Another limitation is that meconium analysis is based on wet weight of meconium. Meconium is approximately 70\%±10\% water content; however, the water content of the meconium varies between patients and meconium also varies in the amount of time it remains on the diaper prior to its collection.\textsuperscript{229} As such a comparison of the amount of FAEE between samples based on wet weight is difficult to achieve.

Although a cumulative FAEE (ethyl palmitate, ethyl palmitoleate, ethyl stearate, ethyl oleate, ethyl linolate, ethyl linolenate, and ethyl arachidonate) positive cutoff of 2nmol/g has been validated in a large population of non-drinking women and women with prenatal alcohol exposure, a threshold has not been established for dose-response characteristics of the cumulative meconium FAEE test (the number of drinks required to cause a positive screen).\textsuperscript{229}

### 3.8.6 FATTY ACID ETHYL ESTERS AND PREDICTION FOR FETAL ALCOHOL SPECTRUM DISORDER

Assessing the validity of FAEE as a biomarker for in utero ethanol ingestion is difficult because there is no gold standard to compare the maternal retrospective self-report to, which can be unreliable. When there is no gold standard for validity assessment, sensitivity and specificity calculations may not be possible.
The amount of drug seen by the fetus is undoubtedly a reflection of maternal consumption, metabolism, placental transfer, fetal metabolism, and elimination\textsuperscript{230}. Limitations of FAEE formation could include different diets, genetic background, and environmental exposure.

Factors resulting in a false positive include FAEE production as a result of metabolism of food (e.g. vanilla extract), and physiologic conditions. A small quantity of ethanol is synthesized in the body. As ethanol is present in a small quantity in medication and food additives, and a by-product of physiologic metabolism in the human gut, small quantities of alcohol may be detected in the meconium. Since ethanol is endogenously produced by the gut flora this can result in a baseline level of FAEE to be formed in the meconium\textsuperscript{231}. One study reported that significant alcohol exposure is reflective of a cumulative FAEE level greater than 10,000ng/g\textsuperscript{139}. Given these circumstances there has not been any correlation between the amount of FAEE present in the meconium and the ability to predict individuals affected with FASD.
Chapter 4

Prelude
4  PRELUDE

The following chapters contain studies demonstrating examples for each level of prevention. The primary level of prevention is demonstrated by maternal prenatal multivitamin supplementation and its association with decreasing the risk of congenital anomalies and certain pediatric cancers. The secondary level of prevention is demonstrated by the means of pharmacologic intervention during pregnancy to decrease the risk of FASD. The tertiary level of prevention is demonstrated by early screening of infants for FASD. The quaternary level of prevention is demonstrated by identifying at mothers who are who have a high-risk of delivering a future child affected with the same birth defect. Mothers can, thereby, be provided with the necessary interventions to decrease the risk of delivering another child affected with the same birth defect.
Chapter 5

Prenatal Multivitamin Supplementation and Rates of Congenital Anomalies: A Meta-analysis
5. Prenatal multivitamin supplementation and rates of congenital anomalies: a meta-analysis

Goh YI, Bollano E, Einarson TR, Koren G.
Prenatal multivitamin supplementation and rates of congenital anomalies: a meta-analysis.

Authors contribution:
Protocol development: YIG, TRE, GK
Literature search and abstraction: YIG, EB, TRE
Analysis: YIG, TRE
Manuscript preparation: YIG, EB, TRE, GK
5.1 ABSTRACT

Background: The use of folic acid-fortified multivitamin supplements has long been associated with decreasing the risk of neural tube defects. Several studies have also proposed the effectiveness of these supplements in preventing other birth defects; however, such effects have never been systematically examined.

Objective: We conducted a systematic review and meta-analysis to evaluate the protective effect of folic acid-fortified multivitamin supplements on other congenital anomalies.

Methods: We searched Medline, PubMed, EMBASE, Toxline, Healthstar, and Cochrane databases for studies describing the outcome of pregnancies in women using multivitamin supplements that were published in all languages from January 1966 to July 2005. The references from all collected articles were reviewed for additional articles. Two independent reviewers who were blinded to the source and identity of the articles extracted data based on predetermined inclusion and exclusion criteria. Using a random effects model, rates of congenital anomalies in babies born to women who were taking multivitamin supplements were compared with rates in the offspring of controls who were not.

Results: From the initial search, 92 studies were identified; 41 of these met the inclusion criteria. Use of multivitamin supplements provided consistent protection against neural tube defects (random effects odds ratio [OR]=0.67 95% confidence intervals [95% CI] 0.58–0.77 in case control studies; OR=0.52 95% CI 0.39–0.69 in cohort and randomized controlled studies), cardiovascular defects (OR=0.78 95% CI 0.67–0.92 in case control studies; OR=0.61 95% CI 0.40–0.92...
in cohort and randomized controlled studies), and limb defects (OR=0.57 95% CI 0.38–0.85 in case control studies; OR=0.25 95% CI 0.05–1.15 in cohort and randomized controlled studies). For cleft palate, case control studies showed OR=0.76 95% CI 0.62–0.93, and cohort and randomized controlled studies showed OR=0.42 95% CI 0.06–2.84; for oral cleft with or without cleft palate, case control studies showed OR=0.63 95% CI 0.54–0.73, and cohort and randomized controlled studies showed OR=0.58 95% CI 0.28–1.19; for urinary tract anomalies, case control studies showed OR=0.48 95% CI 0.30–0.76, and cohort and randomized controlled studies showed OR=0.68 95% CI 0.35–1.31; and for congenital hydrocephalus case control studies showed OR=0.37 95% CI 0.24–0.56, and cohort and randomized controlled studies showed OR=1.54 95% CI 0.53–4.50. No effects were shown in preventing Down syndrome, pyloric stenosis, undescended testis, or hypospadias.

Conclusion: Maternal consumption of folic acid-containing prenatal multivitamins is associated with decreased risk for several congenital anomalies, not only neural tube defects. These data have major public health implications, because until now fortification of only folic acid has been encouraged. This approach should be reconsidered.
5.2 INTRODUCTION

One in 33 children born in Canada and the United States has a birth defect\textsuperscript{232,233}. In 2005, it was estimated that about 150 000 babies are born in North America each year with a birth defect\textsuperscript{234}. The burden of illness and the economic cost of birth defects are extremely high\textsuperscript{235,236}. More than a decade ago, the preventative role of maternal folate supplementation on the occurrence and the recurrence of neural tube defects was documented in several studies\textsuperscript{237,238,239}. Subsequently, preconceptional fortification with folic acid has been shown to reduce the rates of neural tube defects in North America\textsuperscript{240}.

During the last decade, several studies have suggested that folic acid-fortified multivitamins may also prevent other congenital anomalies\textsuperscript{241}. Botto et al. suggested, on the basis of several studies, that there was a decreased risk for orofacial clefts, limb deficiencies, and cardiovascular abnormalities in babies whose mothers received multivitamin supplementation\textsuperscript{242}. To date, however, no systematic review has been conducted to examine existing evidence for the potential of folic acid-containing multivitamins to decrease the risk of congenital anomalies other than neural tube defects. The objective of the present study was to conduct a meta-analysis of studies comparing rates of congenital malformation among women taking vitamin supplements with the rates in controls.
5.3 METHODS

We conducted a search of existing studies that focused on pre- and periconceptional maternal ingestion of multivitamins and the rates of malformation in the offspring. The outcome of interest was congenital malformations. All original research articles using randomized controlled trial, case control, or cohort studies were included. All selected articles contained reports of maternal intake of multivitamins during pregnancy, a control group, and raw data describing rates of healthy and malformed children. Articles that did not report usage of multivitamins during pregnancy, articles that focused on specific vitamins, articles describing mothers exposed to other known teratogens, review articles, letters to the editor, and data reports from abstracts or meetings were excluded. Articles were searched using the terms “multivitamin,” “pregnancy,” and “malformation” in Medline (January 1966–July 2005), PubMed (1950–July 2005), EMBASE (January 1980–July 2005), Toxline (January 1960–July 2005), Healthstar (January 1966–July 2005), and Cochrane database in all languages. The references from all collected articles were reviewed to locate other original studies. Two reviewers blinded to authors’ names, institution, and journal title assessed all of the articles collected using the selection criteria described above. Data were extracted from these articles to collection forms in 2×2 tables. In cases of discrepancy between the reviewers that were not resolved by discussion, the article in question was reviewed by a third blinded reviewer. The odds ratios and 95% confidence intervals were calculated for each

Homogeneity among effects was tested by calculating chi-square.

5.4 RESULTS

Ninety-two articles were compiled from initial searches of the databases and reference review. Forty-one studies were eligible for the meta-analysis based on the inclusion and exclusion criteria. There were 27 case control studies, four randomized control trials, and 10 cohort studies.

Fifty-one articles were excluded because they did not report malformation rates, focused specifically on folic acid, did not contain a control group, were review articles, or contained data that were identical to previous studies by the same authors. The use of multivitamin supplementation by the mothers from before the time of conception was associated with a consistent protective effect against neural tube defects (OR=0.67, 95% CI 0.58–0.77 in case control studies; OR=0.52, 95% CI 0.39–0.69 in cohort and randomized controlled studies), cardiovascular defects (OR=0.78, 95% CI 0.67–0.92 in case control studies; OR=0.61, 95% CI 0.40–0.92 in cohort and randomized controlled studies), and limb defects (OR=0.57, 95% CI 0.38–0.85 in case control studies; OR=0.25, 95% CI 0.05–1.15 in cohort and randomized controlled studies).
Multivitamin supplementation beginning before pregnancy demonstrated a protective effect in case control studies against cleft palate (OR=0.76, 95% CI 0.62–0.93), oral cleft with or without cleft palate (OR=0.63, 95% CI 0.54–0.73 in case control studies), urinary tract anomalies (OR=0.48, 95% CI 0.30–0.76), and congenital hydrocephalus (OR=0.37, 95% CI 0.24–0.56), whereas no significance was observed in cohort and randomized controlled studies against cleft palate (OR=0.42, 95% CI 0.06–2.84), oral cleft with or without cleft palate (OR=0.58, 95% CI 0.28–1.19), urinary tract anomalies (OR=0.68, 95% CI 0.35–1.31), and congenital hydrocephalus (OR=1.54, 95% CI 0.53–4.50) (Figures 1–14). In addition, women who began supplementation in the first trimester after learning of the pregnancy showed a protective effect for neural tube defects (OR=0.80, 95% CI 0.72–0.89) (Figure 15). There was no heterogeneity among the studies.

In contrast, multivitamin supplementation was not associated with a protective effect for Down syndrome (OR=0.56, 95% CI 0.26–1.19 in cohort and randomized controlled studies), congenital pyloric stenosis (OR=1.10, 95% CI 0.79–1.53 in case control studies; OR=0.20, 95% CI 0.02–1.68 in cohort and randomized controlled studies), undescended testis (OR=0.81, 95% CI 0.40–1.64 in cohort studies), and hypospadias (OR=0.44, 95% CI 0.13–1.43 in cohort and randomized controlled studies).
Figure 1. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk of NTD in their children (case-control studies)

Review: Multivitamins and Birth Defects
Comparison: 03 Multivitamin before pregnancy and first trimester vs no supplementation-Case-control
Outcome: 01 Neural Tube Defects

<table>
<thead>
<tr>
<th>Study</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bower 1992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khoury 1995</td>
<td>0.72 [0.57, 0.91]</td>
<td></td>
</tr>
<tr>
<td>Mills 1999</td>
<td>0.91 [0.64, 1.29]</td>
<td></td>
</tr>
<tr>
<td>Murinare 1988</td>
<td>0.40 [0.26, 0.63]</td>
<td></td>
</tr>
<tr>
<td>Shaw 1995a</td>
<td>0.65 [0.38, 1.22]</td>
<td></td>
</tr>
<tr>
<td>Shaw 1997</td>
<td>1.28 [0.92, 1.75]</td>
<td></td>
</tr>
<tr>
<td>Thompson 2003</td>
<td>0.53 [0.26, 1.07]</td>
<td></td>
</tr>
<tr>
<td>Werler 1993</td>
<td>0.50 [0.34, 0.75]</td>
<td></td>
</tr>
</tbody>
</table>

Total (95% CI) 0.67 [0.58, 0.77]

Test for heterogeneity: Chi² = 19.31, df = 7 (P = 0.007), I² = 63.7%
Test for overall effect: Z = 5.45 (P < 0.00001)
Figure 2. Maternal multivitamin supplementation before and during the first trimester of pregnancy and risk of NTD in their children (cohort studies and randomized controlled trials)
Figure 3. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk of cleft palate in their children (case-control studies)
Figure 4. Maternal multivitamin supplementation before and during the first trimester of pregnancy and risk of cleft palate in their children (cohort studies and randomized controlled trials)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeizel 1996a</td>
<td></td>
<td>0.19 [0.01, 4.03]</td>
</tr>
<tr>
<td>Czeizel 2004</td>
<td></td>
<td>1.00 [0.06, 18.59]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>0.42 [0.06, 2.84]</td>
</tr>
</tbody>
</table>

Total events: 1 (Treatment), 3 (Control)
Test for heterogeneity: $\chi^2 = 0.63$, df = 1 (P = 0.43), I² = 0%
Test for overall effect: $I^2 = 0\%$ (P = 0.37)
Figure 5. Maternal multivitamin supplementation before and during the first trimester of pregnancy and risk of cleft lip with/out palate in their children (case-control studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Briggs 1976</td>
<td>0.33 [0.09, 1.18]</td>
<td></td>
</tr>
<tr>
<td>Czeizel 1999a</td>
<td>1.59 [0.45, 5.64]</td>
<td></td>
</tr>
<tr>
<td>Czeizel 2001a</td>
<td>1.29 [0.29, 5.77]</td>
<td></td>
</tr>
<tr>
<td>Hayes 1996</td>
<td>0.87 [0.57, 1.31]</td>
<td></td>
</tr>
<tr>
<td>Ilkka 2001</td>
<td>0.68 [0.43, 1.06]</td>
<td></td>
</tr>
<tr>
<td>Lammer 2004-1</td>
<td>0.66 [0.40, 1.08]</td>
<td></td>
</tr>
<tr>
<td>Lammer 2004-2</td>
<td>0.59 [0.21, 1.66]</td>
<td></td>
</tr>
<tr>
<td>Lammer 2004-3</td>
<td>0.54 [0.32, 0.85]</td>
<td></td>
</tr>
<tr>
<td>Shaw 2003</td>
<td>0.57 [0.41, 0.76]</td>
<td></td>
</tr>
<tr>
<td>van Rooij 2003</td>
<td>0.56 [0.40, 0.78]</td>
<td></td>
</tr>
<tr>
<td>Werler 1999</td>
<td>0.59 [0.31, 1.14]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>0.63 [0.54, 0.73]</strong></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 511 (Treatment), 673 (Control)
Test for heterogeneity: Chisq = 7.78, df = 10 (P = 0.65)
Test for overall effect: Z = 5.93 (P = 0.00001)
Figure 6. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk of cleft lip with/out palate in their children (cohort studies and randomized controlled trials)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conway 1958</td>
<td>0.39 [0.02, 8.27]</td>
<td></td>
</tr>
<tr>
<td>Czeizel 1995a</td>
<td>1.29 [0.29, 5.77]</td>
<td></td>
</tr>
<tr>
<td>Czeizel 2004</td>
<td>1.50 [0.25, 8.95]</td>
<td></td>
</tr>
<tr>
<td>Tolarova 1995</td>
<td>0.34 [0.11, 1.081]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.58 [0.28, 1.15]</td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: $\chi^2 = 3.98$, df = 3 ($P = 0.38$), $I^2 = 3.0$

Test for overall effect: $Z = 1.48$ ($P = 0.14$)
Figure 7. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk of urinary tract anomalies in their children (case-control studies)

<table>
<thead>
<tr>
<th>Study</th>
<th>OR (fixed)</th>
<th>95% CI</th>
<th>OR (fixed)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li 1995</td>
<td>0.33</td>
<td>[0.13, 0.84]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Werler 1995</td>
<td>0.53</td>
<td>[0.31, 0.96]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.48</td>
<td>[0.30, 0.76]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 65 (Treatment), 47 (Control)

Test for heterogeneity: $\chi^2 = 0.74, df = 1 (P = 0.39), I^2 = 0$

Test for overall effect: $Z = 3.16 (P = 0.002)$
Figure 8. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk or urinary tract anomalies in their children (cohort studies and randomized controlled trials)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeizel 1995a</td>
<td></td>
<td>0.32 [0.03, 3.10]</td>
</tr>
<tr>
<td>Czeizel 2004</td>
<td></td>
<td>0.74 [0.37, 1.47]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>0.68 [0.36, 1.31]</td>
</tr>
<tr>
<td>Total events: 15 (Treatment), 22 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: CHI² = 0.47, df = 1 (P = 0.49), P = 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 1.16 (P = 0.25)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Favours treatment  Favours control
Figure 9. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk of cardiovascular defects in their children (case-control studies)

Review: Multivitamins and Birth Defects
Comparison: 03 Multivitamin before pregnancy and first trimester vs no supplementation-Case-control
Outcome: 06 Cardiovascular Defects

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botto 2000</td>
<td>0.81 [0.64, 1.03]</td>
<td></td>
</tr>
<tr>
<td>Correa 2003</td>
<td>0.55 [0.37, 0.81]</td>
<td></td>
</tr>
<tr>
<td>Scanlon 1988</td>
<td>0.95 [0.63, 1.45]</td>
<td></td>
</tr>
<tr>
<td>Shaw 1995c</td>
<td>0.72 [0.47, 1.09]</td>
<td></td>
</tr>
<tr>
<td>Werler 1996</td>
<td>1.08 [0.67, 1.74]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>0.78 [0.67, 0.92]</strong></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 393 (Treatment), 843 (Control)
Test for heterogeneity: CH² = 8.13, df = 4 (P = 0.19), I² = 34.7%
Test for overall effect: Z = 3.07 (P = 0.002)
Figure 10. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk of cardiovascular defects in their children (cohort studies and randomized controlled trials)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeizel 1995a</td>
<td>0.58 [0.21, 1.60]</td>
<td></td>
</tr>
<tr>
<td>Czeizel 2004</td>
<td>0.62 [0.39, 0.97]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.61 [0.40, 0.92]</td>
<td></td>
</tr>
</tbody>
</table>

Total events: 37 (Treatment), 60 (Control)
Test for heterogeneity: CH² = 0.01, df = 1 (P = 0.91), I² = 0%
Test for overall effect: Z = 2.35 (P = 0.02)
Figure 11. Maternal multivitamin supplementation before and during the first trimester of pregnancy and risk of limb defect in their children (case-control studies)

<table>
<thead>
<tr>
<th>Study</th>
<th>OR (fixed)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shaw 1995c</td>
<td>0.64</td>
<td>[0.42, 0.99]</td>
</tr>
<tr>
<td>Werler 1995</td>
<td>0.23</td>
<td>[0.07, 0.73]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>0.57</strong></td>
<td><strong>[0.38, 0.85]</strong></td>
</tr>
<tr>
<td>Total events: 101 (Treatment), 53 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: $\chi^2 = 2.41$, df = 1 ($P = 0.12$), $I^2 = 0%$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $I^2 = 2.73$, ($P = 0.97$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 12. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk of limb defects in their children (cohort studies and randomized controlled trials)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeizel 1995a</td>
<td>0.19 [0.02, 1.65]</td>
<td></td>
</tr>
<tr>
<td>Czeizel 2004</td>
<td></td>
<td>0.33 [0.03, 3.20]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>0.25 [0.05, 1.15]</td>
</tr>
</tbody>
</table>

Total events: 2 (Treatment), 8 (Control)
Test for heterogeneity: $\chi^2 = 0.12$, df = 1 ($P = 0.73$), $I^2 = 0$
Test for overall effect: $Z = 1.78$ ($P = 0.08$)
Figure 13. Maternal multivitamin consumption before and during the first trimester of pregnancy and risk of congenital hydrocephalus in their children (case-control studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correa 2003</td>
<td></td>
<td>0.34 [0.21, 0.54]</td>
</tr>
<tr>
<td>Werler 1996</td>
<td></td>
<td>0.59 [0.21, 1.68]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>0.37 [0.24, 0.56]</td>
</tr>
</tbody>
</table>

Total events: 30 (Treatment), 176 (Control)
Test for heterogeneity: $\chi^2 = 0.90$, df = 1 ($P = 0.34$), $I^2 = 0$
Test for overall effects: $7 = 4.66$, $P < 0.9999999$
Figure 14. Maternal multivitamin consumption before and during the first trimester of pregnancy and the risk of congenital hydrocephalus in their children (cohort studies and randomized control trials)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed)</th>
<th>95% CI</th>
<th>OR (fixed)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeizel 1995a</td>
<td>0.32</td>
<td>[0.01, 7.92]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Czeizel 2004</td>
<td>2.00</td>
<td>[0.60, 6.66]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>1.54</td>
<td>[0.53, 4.50]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total events: 8 (Treatment), 5 (Control)
Test for heterogeneity: CHI² = 1.10, df = 1 (P = 0.29), I² = 9.1%
Test for overall effect: Z = 0.79 (P = 0.43)
Figure 15. Maternal multivitamin consumption during the first trimester of pregnancy and risk of NTD in their children (case-control studies)

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Control</th>
<th>OR (fixed)</th>
<th>Weight</th>
<th>OR (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>Mulinare 1988</td>
<td>33/357</td>
<td>159/1251</td>
<td>9.47</td>
<td>0.70</td>
<td>0.47 [0.47, 1.04]</td>
</tr>
<tr>
<td>Milunsky 1990</td>
<td>29/12297</td>
<td>11/3157</td>
<td>2.31</td>
<td>0.68</td>
<td>0.34 [0.31, 1.35]</td>
</tr>
<tr>
<td>Bower 1992</td>
<td>46/106</td>
<td>104/211</td>
<td>4.37</td>
<td>1.23</td>
<td>0.78 [0.72, 1.94]</td>
</tr>
<tr>
<td>Werler 1993</td>
<td>107/842</td>
<td>250/1503</td>
<td>20.71</td>
<td>0.73</td>
<td>0.57 [0.50, 0.93]</td>
</tr>
<tr>
<td>Shaw 1995a</td>
<td>243/555</td>
<td>207/356</td>
<td>19.12</td>
<td>0.54</td>
<td>0.42 [0.40, 0.71]</td>
</tr>
<tr>
<td>Khoury 1996</td>
<td>366/1349</td>
<td>399/1714</td>
<td>41.96</td>
<td>0.91</td>
<td>0.79 [0.77, 1.07]</td>
</tr>
<tr>
<td>Shaw 1997</td>
<td>56/64</td>
<td>106/164</td>
<td>3.16</td>
<td>1.09</td>
<td>0.63 [0.61, 1.51]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>15095</td>
<td>8416</td>
<td></td>
<td>100.00</td>
<td>0.80 [0.72, 0.89]</td>
</tr>
</tbody>
</table>

Total events: 300 (Treatment), 1223 (Control)
Test for heterogeneity: \( \chi^2 = 16.50, df = 6 \ (P = 0.01), I^2 = 63.2\% \)
Test for overall effect: \( Z = 4.12 \ (P < 0.0001) \)
5.5 DISCUSSION

The present meta-analysis confirms initial impressions that the use of multivitamins fortified with folic acid by women before conception and continuing through the first trimester is associated with a decrease in several serious major malformations. To our knowledge, this is the first systematic review and meta-analysis to examine and document these protective effects.

The majority of the studies included in the meta-analysis were case control studies, although there were also several randomized controlled trials and cohort studies. Not surprisingly, case control studies are more sensitive in showing significant effects for preventing specific malformations than cohort or randomized studies. The observation that all the studies for the majority of endpoints were statistically homogenous lends credibility to the documented effects.

There was heterogeneity in the case control studies and cohort studies examining neural tube defects (8 and 11 studies, respectively). Exclusion of one small case control study and one small cohort study, each of which showed no protective effect, renders the results homogeneous without changing the overall effect size. Moreover, we could not detect a publication bias by employing the funnel plot. Our study, however, is limited by the fact that multivitamin supplements in differing studies may have varied in their composition. Presently, there are widely publicized recommendations by various authorities for women to supplement with folate at daily doses of at least 0.4mg (4 mg for women at higher risk) to reduce the risk of delivering a child with neural
tube defects. In many centres women are advised to begin taking prenatal vitamin supplements when they decide to attempt to conceive, merely to allow them sufficient folate supplementation. It is currently impossible to discern whether folic acid or other vitamins are critical in the prevention of other birth defects.

Only a fraction of women currently take prenatal vitamin supplements at the time of conception, partly because one half of all pregnancies are unplanned. Serious consideration should be given to fortification of flour or other food staples with other vitamins in addition to folate. With increased surveillance of changes in malformation rates as a result of folate fortification, and subsequently larger cohorts, it will be possible to determine whether folate fortification itself is capable of protecting against birth defects other than neural tube defects.

5.6 CONCLUSIONS

The results of the present meta-analysis support the use of prenatal multivitamin preparations containing folic acid to reduce the incidence of several congenital anomalies, including neural tube defects, cardiovascular anomalies, oral cleft, urinary tract anomalies, congenital hydrocephalus, and limb defects. Randomized trials will be necessary to prove which specific vitamin(s) render protective effects.
5.7 STATEMENT OF SIGNIFICANCE AND IMPACT

To our knowledge this is the first meta-analysis to investigate the effects of multivitamins supplementation in pregnancy and the occurrence of birth defects other than neural tube defects. Since women who are chronic alcohol consumers are at increased risk for being nutrient deficient, there is a potential increased risk for the child to develop other birth defects in addition to FASD. Thus the following results reaffirm the importance of multivitamin supplementation during pregnancy, especially in high-risk populations.
Chapter 6

Prenatal Multivitamin Supplementation and Rates of Pediatric Cancers: A Meta-analysis
6. PRENATAL MULTIVITAMIN SUPPLEMENTATION AND RATES OF PEDIATRIC CANCERS: A META-ANALYSIS

Goh YI, Bollano E, Einarson TR, Koren G.

Prenatal multivitamin supplementation and rates of pediatric cancers: a meta-analysis.


Authors contribution:

Protocol development: YIG, TRE, GK

Literature search and abstraction: YIG, EB, TRE

Analysis: YIG, TRE

Manuscript preparation: YIG, EB, TRE, GK
6.1 ABSTRACT

Prenatal supplementation of folic acid has been shown to decrease the risk of several congenital malformations. Several studies have recently suggested a potential protective effect of folic acid on certain pediatric cancers. The protective role of prenatal multivitamins has not been elucidated. We conducted a systematic review and meta-analysis to assess the potential protective effect of prenatal multivitamins on several pediatric cancers. Medline, PubMed, EMBASE, Toxline, Healthstar, and Cochrane databases were searched for studies published in all languages from 1960 to July 2005 on multivitamin supplementation and pediatric cancers. References from all articles collected were reviewed for additional articles. Two blinded independent reviewers assessed the articles for inclusion and exclusion. Rates of cancers in women supplemented with multivitamins were compared with unsupplemented women using a random effects model.

Sixty-one articles were identified in the initial search, of which, seven articles met the inclusion criteria. There was an apparent protective effect for leukemia (odds ratio [OR]=0.61, 95% confidence interval [CI] 0.50–0.74), pediatric brain tumors (OR=0.73, 95% CI 0.60–0.88) and neuroblastoma (OR=0.53, 95% CI 0.42–0.68). In conclusion, maternal ingestion of prenatal multivitamins is associated with a decreased risk for pediatric brain tumors, neuroblastoma, and leukemia. Presently, it is not known which constituent(s) among the multivitamins confer this protective effect.
6.2 INTRODUCTION

It is estimated that 9,510 children in the United States under the age of 15 were diagnosed with cancer in 2005\textsuperscript{281}. The most prevalent forms of childhood cancer are leukemia, malignant brain and spinal cord tumors, and neuroblastoma\textsuperscript{282,283}. Leukemia is estimated to account for 25–35\% of pediatric cancers\textsuperscript{284}.

The two major morphological types of blood-borne cancers are acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML). The American Cancer Society estimates that 2,670 and 1,196 children were diagnosed with ALL and AML in 2005, respectively\textsuperscript{283}. Malignant brain and spinal cord tumors occur in 2,200 (17\%) of pediatric cancers\textsuperscript{283}. These cancers include astrocytoma, primitive neuroectodermal tumors, and medulloblastoma\textsuperscript{282}. Neuroblastoma is diagnosed in approximately 650 American children annually\textsuperscript{283}. An estimated 463 children die from ALL in the United States each year\textsuperscript{283} and only about half of children with brain tumors will survive more than five years\textsuperscript{283}.

A large number of investigations into the epidemiology of these pediatric cancers have been undertaken in an attempt to identify risk factors and protective agents. Investigators have looked for relationships between genes and environmental exposures such as radiation\textsuperscript{285,286}, N-nitroso compounds\textsuperscript{287}, pesticides\textsuperscript{288}, tobacco\textsuperscript{289,290}, electromagnetic frequencies\textsuperscript{291}, infectious agents\textsuperscript{285,292,293}, parental occupation\textsuperscript{294}, drugs\textsuperscript{295,296}, alcohol\textsuperscript{297}, infant feeding\textsuperscript{298}, multivitamins\textsuperscript{299,300}, and cancer.

It is now generally accepted that women of childbearing potential should supplement with folic acid before pregnancy and in early pregnancy to decrease
the proven risk of neural tube defects. This suggested relationship was proven by the British and Hungarian randomized studies of supplementation with folic acid before pregnancy and in early pregnant women \(^{252,264}\). Folic acid fortification of flour has subsequently resulted in decreasing rates of neural tube defects \(^{301,302}\). In addition, it has most recently been suggested that folic-acid containing multivitamins may also be beneficial in preventing congenital anomalies other than neural tube defects \(^{242,303}\). Botto et al. \(^{242}\) noted that there was an apparent decreased risk for orofacial clefts, limb deficiencies, and cardiovascular abnormalities with multivitamin supplementation. In addition, Bailey et al. \(^{303}\) reported a decrease of cardiovascular abnormalities and orofacial clefts. A recent study conducted by our group followed the prevalence of neuroblastoma rates after folic acid fortification of flour in Ontario, Canada, showing an apparent protective effect \(^{302}\). To date, several studies have investigated the potential effect of multivitamin use before and in early pregnancy on rates of common pediatric tumors. The objective of this study was to conduct a systematic review and meta-analysis of prenatal multivitamin use before and in early pregnancy and the risk of pediatric cancers.

6.3 METHODS

A search of the existing literature regarding pre- and periconceptional ingestion of multivitamins and the rates of cancer in offspring was undertaken. The outcome of interest was pediatric cancer. All original research articles using
case–control or cohort study design were included. Included articles must have contained a control group of healthy children with accounts of maternal intake of multivitamins during pregnancy. In addition, all included articles must have contained raw data of number of cases and controls using multivitamins. We excluded articles that did not involve women taking multivitamins during pregnancy or focused on specific vitamins, mothers exposed to other known teratogens, review articles, or data reported in abstracts or meetings.

Articles were searched using the terms multivitamin, pregnancy, cancer, and neoplasms in Medline (1966–July 2005), PubMed (–July 2005), EMBASE (1980–July 2005), Toxline (1960–July 2005), Healthstar (–July 2005), and the Cochrane database in all languages. References from all collected articles were reviewed for additional original studies of interest.

All of the articles were reviewed using the above selection criteria by two reviewers who were blinded to the study outcome, names, and institutions of authors. Data from the articles were extracted by the two reviewers onto collection forms. In cases of discrepancies, discussions were undertaken and if unresolved, the article was reviewed by a third blinded reviewer who served as a tiebreaker. All data were entered into 2×2 tables. OR and 95% CI were calculated for each case–control study using Review Manager 4.2.7 (2004, The Cochrane Collaboration). Homogeneity among effects was tested by calculating \( \chi^2 \). A funnel plot was used to assess publication bias, following which the Begg–Mazumdar test was executed to calculate Kendall’s \( t \); a test that evaluates the agreement between the effect and variances.
6.4 RESULTS

Sixty-one articles were compiled from initial searches and reference review. Using our exclusion criterion, seven articles were eligible for inclusion: two articles addressing brain tumors, two addressing neuroblastoma, and two articles addressing leukemia, and one article addressing both brain tumors and leukemia \(^{287,296,305,306,307,308,309,310,311}\) (Table 2). All studies were of case–control design. Thirty-eight articles were excluded because they did not contain information on maternal multivitamin use during pregnancy \(^{299,312,313,314,315,316,317,318,319,320,321,322,323,324,325,326,327,328,329,330,314,331,332,333,334,335,336,337,338,339,340,341,342,343,344,345,346}\). Three studies were excluded because they dealt with vitamin ingestion by children \(^{294,337,347}\). Two studies were excluded because they combined multivitamin and iron supplementation into one category \(^{348,349}\). One article reported on folic acid fortification in food \(^{302}\). Two articles were excluded because they focused only on folate \(^{348,349}\). Three studies were rejected because they did not provide complete raw data to allow analysis \(^{310,311,350}\). Two papers were rejected because they were duplicates of articles published in different journals: \(^{351,352}\) one contained data that were previously published \(^{287}\), and one was rejected because it was a news report \(^{353}\).

Use of prenatal multivitamins by the pregnant mothers was associated with a protective effect for childhood leukemia (OR=0.64, 95% CI 0.53–0.78). As pediatric leukemia has different origins, ALL and AML were analyzed separately. The ingestion of prenatal multivitamins was associated with a protective effect for
ALL (OR=0.61, 95% CI 0.50–0.74) (Figure 16). There was no significant heterogeneity among the ALL studies ($X^2=1.27, I^2=0\%$). There was only one study that reported information regarding AML therefore it could not be meta-analyzed; nevertheless it suggested a protective effect as well. The use of multivitamins by pregnant mothers was associated with a protective effect for several solid tumors. Supplementation with prenatal vitamins was associated with a decreased risk for neuroblastoma (OR=0.53, 95% CI 0.42–0.68) (Figure 17). Prenatal supplementation was also associated with decreased risk for pediatric brain tumors (OR=0.73, 95% CI 0.60–0.88) (Figure 18). A funnel plot did not show significant publication bias.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year Published</th>
<th>Country</th>
<th>Dates of study</th>
<th>Age of children (years)</th>
<th>Language</th>
<th>Matching Controls</th>
<th>Primary Outcome</th>
<th>Matching variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarasua et al.</td>
<td>1994</td>
<td>United States (Denver)</td>
<td>January 1, 1976-December 31, 1983</td>
<td>0-14</td>
<td>English</td>
<td>random digit dialing</td>
<td>brain tumours, ALL</td>
<td>+/-3 years of age; telephone exchange area</td>
</tr>
<tr>
<td>Bunin et al.</td>
<td>1994</td>
<td>United States, Canada</td>
<td>1986-1989</td>
<td>&lt;6</td>
<td>not specified</td>
<td>random digit dialing*</td>
<td>astrocytic gloma</td>
<td>matching area code and next 5 digits of phone number; date of birth +/-1 year and race; black and non-black</td>
</tr>
<tr>
<td>Michaelek et al.</td>
<td>1996</td>
<td>United States (New York)</td>
<td>January 1, 1976-December 1, 1987</td>
<td>&lt;15</td>
<td>English</td>
<td>birth certificate registry</td>
<td>neuroblastoma</td>
<td>birth year; race</td>
</tr>
<tr>
<td>Preston-Martin et al.</td>
<td>1996</td>
<td>United States (West Coast)</td>
<td>1984-1991</td>
<td>&lt;20</td>
<td>English, Spanish</td>
<td>random digit dialing</td>
<td>brain tumours</td>
<td>age; gender; area code</td>
</tr>
<tr>
<td>Preston-Martin et al.</td>
<td>1998</td>
<td>United States, France, Italy, Israel, Canada, Australia</td>
<td>1976-1994 varied by location</td>
<td>not specified</td>
<td>varied by location</td>
<td>brain tumours</td>
<td>varied by location</td>
<td></td>
</tr>
<tr>
<td>Olshan et al.</td>
<td>2002</td>
<td>United States, Canada</td>
<td>May 1, 1992-April 30, 1994</td>
<td>&lt;19</td>
<td>English, Spanish</td>
<td>random digit dialing</td>
<td>neuroblastoma</td>
<td>date of birth +/-6 months for cases &lt;3 years OR date of birth +/-1 year for cases &gt;3 years</td>
</tr>
<tr>
<td>Wen et al.</td>
<td>2002</td>
<td>United States, Canada, Australia</td>
<td>January 1, 1989-June 15, 1993</td>
<td>&lt;15</td>
<td>English</td>
<td>random digit dialing*</td>
<td>ALL</td>
<td>+/-2 years of age; race; telephone area code and exchange</td>
</tr>
</tbody>
</table>

*=random digit dialing criterion relaxed in order to find a match
Figure 16. Maternal multivitamin consumption and risk for acute lymphoblastic leukemia (ALL) in their children

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ross 2005</td>
<td>42/148</td>
<td>55/122</td>
<td>15.15</td>
<td>0.48 [0.29, 0.80]</td>
<td></td>
</tr>
<tr>
<td>Sarasua 1994</td>
<td>46/231</td>
<td>10/30</td>
<td>5.30</td>
<td>0.30 [0.22, 1.13]</td>
<td></td>
</tr>
<tr>
<td>Vwen 2002</td>
<td>1522/3449</td>
<td>220/379</td>
<td>78.55</td>
<td>0.64 [0.52, 0.80]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>3823</td>
<td>531</td>
<td></td>
<td>100.00</td>
<td>0.61 [0.50, 0.74]</td>
</tr>
</tbody>
</table>

Total events: 1710 (Treatment), 265 (Control)
Test for heterogeneity: Chi² = 1.27, df = 2 (P = 0.53), I² = 0%
Test for overall effect: Z = 5.07 (P < 0.000001)
**Figure 17. Maternal multivitamin consumption and risk for neuroblastoma in their children**

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment n/N</th>
<th>Control n/N</th>
<th>OR (fixed) 95% CI</th>
<th>Weight %</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michalek 1996</td>
<td>61/272</td>
<td>122/283</td>
<td>0.38 [0.26, 0.55]</td>
<td>49.54</td>
<td></td>
</tr>
<tr>
<td>Olshan 2002</td>
<td>265/525</td>
<td>137/229</td>
<td>0.68 [0.50, 0.94]</td>
<td>50.46</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>797</strong></td>
<td><strong>512</strong></td>
<td></td>
<td><strong>100.00</strong></td>
<td><strong>0.53 [0.42, 0.66]</strong></td>
</tr>
</tbody>
</table>

Total events: 326 (Treatment), 255 (Control)

Test for heterogeneity: Chi² = 5.57, df = 1 (P = 0.02), I² = 82.0%

Test for overall effect: Z = 5.15 (P < 0.00001)
Figure 18. Maternal multivitamin consumption and risk for pediatric brain tumours in their children

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Control</th>
<th>OR (fixed)</th>
<th>Weight</th>
<th>OR (fixed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>Bunić 1994</td>
<td>128/260</td>
<td>27/50</td>
<td>6.39</td>
<td>83.03</td>
<td>0.83 [0.45, 1.52]</td>
</tr>
<tr>
<td>Preston-Martin 1996</td>
<td>420/1117</td>
<td>120/224</td>
<td>34.67</td>
<td>52.05</td>
<td>0.52 [0.39, 0.70]</td>
</tr>
<tr>
<td>Preston-Martin 1998</td>
<td>142/531</td>
<td>579/1686</td>
<td>56.47</td>
<td>70.00</td>
<td>0.70 [0.56, 0.87]</td>
</tr>
<tr>
<td>Sarasue 1994</td>
<td>39/224</td>
<td>6/26</td>
<td>2.47</td>
<td>70.00</td>
<td>0.70 [0.26, 1.86]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>2132</td>
<td>1986</td>
<td>100.00</td>
<td>65.00</td>
<td>0.65 [0.55, 0.76]</td>
</tr>
</tbody>
</table>

Test for heterogeneity: $\chi^2 = 3.22$, df = 3 ($P = 0.35$), $I^2 = 7.0\%$

Test for overall effect: $Z = 5.21$ ($P < 0.00001$)
6.5 DISCUSSION

There is a large body of evidence supporting the protective effect of folic acid in decreasing the effect of neural tube defects\textsuperscript{252,264}. In addition, folic-acid containing multivitamins have also been associated with prevention of other congenital anomalies other than neural tube defects\textsuperscript{303}. The data from the present meta-analysis suggest that prenatal supplementation of multivitamins containing folic acid is associated with an overall 18\% protective decreased risk for pediatric brain tumors, 47\% for neuroblastoma, and 36\% protective effect for leukemia. To our knowledge, this is the first systematic review and meta-analysis examining such protective effect. Based on these data, one can estimate that maternal multivitamin supplementation may prevent 900 cases of pediatric leukemia and 300–400 cases of pediatric brain tumors annually in the United States.

The most apparent limitation of all studies considered in this meta-analysis is their retrospective design and the potential for recall or reporting bias. The bias may stem from parents of the case group wishing to attribute their child’s cancer to a cause. On the other hand, recall bias may alter the exposure rate they report. Hence, reporting bias may result in over-reporting or lack of multivitamin use. In addition, some studies specifically asked about multivitamin use, whereas other studies posed an open-ended question on whether women took any medications.

Second, the composition of the multivitamins probably varied, as did the timing and duration of exposure. This may be a limitation because different
components within the multivitamin may be responsible for these protective effects. However, as different brands of multivitamins contain different amounts of vitamins and minerals, it is difficult to ascertain which component is responsible for the protective effect. In addition, women who began supplementing before pregnancy and continue throughout pregnancy may have a different risk of delivering a child with pediatric cancer compared to women who began supplementing after discovering they were pregnant. Considering that, different vitamins and minerals are important in the production and replication of DNA and cells, mothers who began supplementation prior to pregnancy may theoretically have a lower risk of delivering a child with pediatric cancer.

Third, the selection of control groups varied between studies. The majority of the investigators utilized a random-digit telephone-dialing method; however, their inclusion criterion varied in certain circumstances. In cases where matches could not be found to meet the original criteria, investigators loosened different criteria in order to find a match. In addition, as random dialing was used, some households with multiple phone lines had a greater chance of selection. In terms of identifying controls, one study matched by birth certificate registry, whereas another matched by physician’s patient roster. Most studies matched based on ±1 years of age; however, there was a study that matched on ±2 years of age or ±3 years of age. In addition, a fair number of studies did not match for ethnicity or matched on a black/non-black basis. Moreover, social class differences were not considered. Mothers of lower social class may not have had sufficient
resources to afford a well-balanced diet. Maternal medical history and medication intake were also not reported in all articles. Confounding effects such as absorption problems and drug interactions involving multivitamins were therefore not addressed.

In addition to the articles included in this meta-analysis, one rejected article, which did not present raw data, also reported an apparent protective effect. A study conducted by Bunin et al.\textsuperscript{310} of the Children’s Cancer Group from 1986 to 1989 found that the use of multivitamins during the first 6 weeks of pregnancy decreased the risk of primitive neuroectodermal brain tumors (OR=0.56, P=0.02). In addition, a later case–control study, which also did not report raw data, used data from the Children’s Cancer Study Group to compare children who were diagnosed with retinoblastoma between 1982 and 1985, and found a decreased occurrence of retinoblastoma with prenatal multivitamin supplementation in both sporadic and heritable tumors (OR=0.4, P=0.03, OR=0.2, P=0.02, respectively)\textsuperscript{311}. Although our conclusions suggest that folic-acid containing multivitamins are associated with a decreased risk for certain pediatric cancers, the available data do not allow determination of which of the constituent(s) may cause these protective effects. The papers included in the study were based on maternal reports of multivitamin use. As such, the components and the quantity of each vitamin contained within the multivitamins were not available in some of the papers included.

It may be possible that the observed effect may be related to folic acid. It has been hypothesized that the potential association of folate deficiencies and
pediatric cancer is due to partially altered DNA methylation and impaired DNA synthesis and repair\textsuperscript{354}. This transformation may be the source for the primitive neuroectodermal tumors\textsuperscript{354}. In addition, polymorphism of the MTHFR gene may also be an important etiologic factor\textsuperscript{355,356,357}. Polymorphism of C677T and A1298C may reduce the risk of ALL\textsuperscript{358}. In contrast, the same authors hypothesized that folate may also enhance the development and progression of already existing, undiagnosed premalignant or malignant lesions\textsuperscript{359,360}. Conversely, other researchers reported that folate supplementation may prevent breast cancer\textsuperscript{361,362,363,364,365}, colorectal adenoma, and carcinoma in adults\textsuperscript{359}, and folate receptor overexpression has been noted in ependymomas\textsuperscript{366}.

Several other vitamins have also been investigated regarding their ability to prevent cancer. The antioxidant mechanism of vitamins C and E has been investigated in the reduction of nitrosation process in cured meats and the formation of carcinogens\textsuperscript{287}.

To date, there have been no experimental data establishing a direct relationship between multivitamins and the pathogenesis of pediatric brain tumors. Because folic acid is the standard prophylactic therapy to reduce the risk of neural tube defects in pregnancy, there is no way to ethically conduct a randomized-control trial to separate these effects. The only possible method to elucidate whether the observed protective effect is due to folic acid itself or other vitamins would be to conduct a head-to-head comparison of folic acid versus folic acid containing multivitamins. This, however, is not feasible given the large sample size that would need to be followed for a long period of time. Presently,
many women actively planning pregnancy commence prenatal multivitamins before conception, and hence it is not likely that such comparison is presently feasible.

In conclusion, prenatal multivitamins containing folic acid appear to be associated with a significant protective effect on three common pediatric cancers. Given that women who are considering pregnancy are generally advised to supplement with folic acid, the results from this study suggest that supplementation with a folic acid-containing multivitamin may be a preferred method.

6.6 STATEMENT OF SIGNIFICANCE AND IMPACT

To our knowledge this is the first meta-analysis to investigate the effects of multivitamins supplementation in pregnancy and the occurrence of pediatric cancers. Since women who are chronic alcohol consumers are at increased risk for being nutrient deficient, there is a potential increased risk for the child to develop certain pediatric cancers. Thus the following results suggest an additional incentive for multivitamin supplementation during pregnancy.
Chapter 7

The Use of Antioxidants in the Treatment of Fetal Alcohol Spectrum Disorder
7. THE USE OF ANTIOXIDANTS IN THE TREATMENT OF FASD

7.1 THE ANTIOXIDANT EFFECT: CAN WE MITIGATE FETAL ALCOHOL SPECTRUM DISORDER WITH ANTIOXIDANTS?

Goh YI, Rovet J, Ungar WJ, Koren G.

The Antioxidant Effect: Can We Mitigate Fetal Alcohol Spectrum Disorder With Antioxidants?

JFAS Int 2005;3:e10 - April 2005

Authors contribution:

Protocol development: YIG, JR, WJU, GK

Manuscript preparation: YIG, JR, WJU, GK
It is estimated that 46% of women consume alcohol\textsuperscript{367}. Given that approximately half of pregnancies are unplanned there is a potential of 23% of babies being unknowingly exposed to alcohol. It is known that alcohol consumption during pregnancy can potentially result in a child having fetal alcohol spectrum disorder. In the full presentation, fetal alcohol syndrome may be the clinical result. FAS alone, affects 1-4 of 1,000 live births\textsuperscript{126,368}. Children with FASD are less likely to be diagnosed than children with FAS because they rely on confirmation of the mother drinking in pregnancy and these children do not exhibit the pathognomonic facial changes.

The majority of studies on FASD have focused on the mechanism of damage. One such mechanism is oxidative stress which is a result in the production of reactive oxidative species that are generated by the metabolism of ethanol.

It is known that the primary prevention of FASD is avoiding alcohol consumption during pregnancy. This is usually hard for women who have unplanned pregnancy, and a still more challenging goal for women who are addicted to alcohol. To date there is no known treatment for women who have consumed alcohol during pregnancy to increase the chances for having a healthy child.

\textit{In vitro} studies have shown that antioxidant treatment can attenuate ethanol-damaged neural cells\textsuperscript{369}. In pregnant diabetic women, antioxidants were shown to be beneficial by preventing pre-eclampsia\textsuperscript{370}. The above evidence has led us to design a trial to evaluate the effectiveness of antioxidants in mitigating
fetal damage from alcohol exposure in pregnancy. We present this protocol with the hope it will facilitate similar research in other countries. As importantly, other centers may consider joining us in this protocol.

The primary objective of the study is to evaluate whether antioxidants in combination with prenatal multivitamin supplementation will impact the outcome of alcohol exposed pregnancies. The secondary objective is to evaluate the cost-effectiveness of implementing this treatment. It is hypothesized that together these treatments will be beneficial to improving the fetus' health and will result in savings to the health care system and society.

The study is a randomized, three-arm, double-blinded, placebo-controlled trial. One hundred and eighty nine women will be asked to participate in this trial where they will be randomized into one of three possible groups. The first group will receive study medication and prenatal multivitamins. The second group will receive placebo and prenatal multivitamins. The third group will not receive any medications but will be advised to obtain prenatal multivitamins containing at least 0.4mg of folic acid as recommended by Health Canada and the FDA. All groups will receive information and counseling from research staff and if need be, referred to specialists or other social services. All women participating will be advised to discontinue drinking alcohol.

Participants included in the trial must be 0-24 weeks pregnant with a TWEAK score of 3 or greater, a history of binge drinking (5 or more drinks) during pregnancy. Participants will be excluded if they have any co-morbid condition(s) that prevents them from providing meaningful consent.
are currently being enrolled into the study through the Motherisk Alcohol and Substance Use Helpline 1-877-327-4636. However, recruitment will be extended to hospitals, treatment programs for addicted women, hostels, shelters, food banks, and community centers.

Pregnant women will be prescreened and informed about the study. If they elect to participate an appointment will be set up either at The Hospital for Sick Children or in their homes. At the first visit, subjects will be asked to provide written informed consent to participate in the study. The medical and obstetrical history as well as social circumstances will be documented. In addition, participants will complete a series of questionnaires and be seen by a study physician for a physical assessment. Blood and urine will be collected to assess for any underlying maternal medical conditions and measure baseline antioxidant levels.

The participant’s family physician will be notified of her participation in the study and will be contacted to verify medical history and to access results of tests pertaining to the pregnancy. In addition, results from the blood and urine tests will be sent to participant’s doctors. Participants who do not have a family physician will be referred to a family physician by the study staff.

All participants will be asked to take a daily multivitamin and participants in the treatment arms will be asked to take one of each tablet daily. All subjects will receive harm reduction counseling throughout the study. Diaries will be provided to participants to monitor compliance, adverse events, nutrient intake and any problems that may be experienced through the study. The frequency of
contact is required to keep the trial participants engaged. Participants randomized into the drug or placebo arm will be contacted at least two times a week. Participants randomized into the counseling arm will be contacted once every two weeks. During this contact, participants will be asked about their general health, pregnancy complications, adverse effects, drinking and drug use pattern and compliance to study.

Due to the vulnerability and high-risk nature of our patient population, measures have been built into the study to encourage retention and compliance with the study and its interventions. These may include the use of outreach workers, food vouchers, transportation assistance, home help and other strategies.

Women will return to the study clinic every two months to have a medical examination, return diaries and remaining study drug, complete questionnaires, give blood and urine samples, monitor compliance and medical status. Subjects will be issued new diaries and study drug at this time. This pattern will continue until the birth of the child.

Study staff will visit the mother within 24 hours of giving birth. At this time, each mother will be interviewed about the outcome of their pregnancy and any birth complications. The baby will be assessed by study physicians for any dysmorphologies and APGAR scores will be recorded. Meconium and hair will be collected from the baby and analyzed for fatty acid ethyl esters as a biomarker for prenatal ethanol exposure $^{145,189,371}$. 

145,189,371
The child will be assessed at 1, 3, 6, and 14 months of life. Each visit will consist of a physical examination, a full-scale psychometric test, a health resource use, economic, resource utilization and time loss questionnaire.

Participants who complete the study will have the option of having their child followed yearly at the Motherisk clinic. This is important since the full adverse effects of \textit{in utero} alcohol exposure may take years to unmask. Following children as they develop will enable close monitoring of learning, intelligence, behavioural change, and physical functions. Healthcare resource utilization will be measured as well as use of community and educational services. From this information, modeling of the impact of long-term cost and consequences will be performed.

To the best of our knowledge, this is the first study to examine the effects of drug treatment to attenuate the onset of FAS and ARND. If successful, it will have tremendous implications for the reduction of the most prevalent and preventable type of mental deficiency. Potential confounders in the study include, age, maternal illness and co-morbidities, other maternal exposures in pregnancy, nutrition, socio-economic status, and education. The randomized design aims to balance these potential confounders among the groups. In addition, although this is a double-blinded, placebo-controlled randomized trial, the third arm receiving only counseling will not be blinded. As such, there may be bias in some outcome measurements. The two treatment arms, however, will be blinded to the participants and to the investigators conducting the
assessments. The results will therefore accurately reflect the potential of antioxidants to attenuate alcohol-related problems in fetuses exposed in utero.
7.2 MEGA-DOSE VITAMIN C AND E IN PREVENTING FASD: THE DECISION TO TERMINATE THE STUDY PREMATURELY

Goh YI, Ungar WJ, Rovet J, Koren G.

Mega-dose Vitamin C and E in Preventing FASD: The Decision to Terminate the Study Prematurely


Authors contribution:

Protocol development: YIG, JR, WJU, GK

Manuscript preparation: YIG, JR, WJU, GK
7.2.1 ABSTRACT
Since 2004 we have been conducting a randomized control double-blind trial on the favourable effect of mega-vitamin C and E in pregnant women with heavy alcohol exposure. The study was terminated in the summer of 2006 due to new evidence showing intrauterine growth restriction caused by such treatment in women with pre-eclampsia.

7.2.2 ARTICLE
FASD affects 1 of 100 children\textsuperscript{126}. As FASD results from alcohol exposure during pregnancy mothers are advised to discontinue drinking when planning pregnancy. However, a large number of pregnancies are unplanned or unrecognized until fairly late in gestation. In the 1988 National Maternal and Infant Health Survey on drinking by pregnant women Floyd \textit{et al.} reported that 45\% women surveyed reported consuming alcohol during the three months before finding out they were pregnant\textsuperscript{372}. As such, providing a means of ameliorating the damage inflicted by maternal use of alcohol is of important interest.

One of the mechanisms believed to cause FASD is oxidative damage generated by oxidative stress which occurs during alcohol exposure. Previous experimental studies have demonstrated that ethanol damage to neural cells can be attenuated by treatment with antioxidants\textsuperscript{369,373}. In addition, previous studies have also documented the potential benefits of mega-dose antioxidant vitamin supplementation with vitamin C and E in diabetic and pre-eclamptic women.
As a result of these previous studies, we designed the EViCE (Effectiveness of Vitamin C and E in alcohol exposed pregnancies) study to examine the effectiveness of mega-dosing of Vitamin C and E in mitigating the effects of ethanol in alcohol exposed women. This was a randomized control study initiated in the fall of 2004. In the study, women with alcohol-exposed pregnancies were randomized into three groups: vitamin, placebo, and counseling only. Women assigned to the vitamin group received a 1000mg vitamin C, 400IU vitamin E, and a prenatal multivitamin containing folic acid. Women assigned to the placebo group received two placebos and a prenatal multivitamin containing folic acid. Women assigned to the counseling group did not receive any vitamin therapy but were advised to take prenatal multivitamins containing folic acid. The rationale for including a counseling group was to examine the effect of usual care.

As part of the ethicality of any human study, it is critical to follow up the world experience to see whether new knowledge may affect the conduct of the trial. The turning point of the EViCE trial arose with the publication of pre-eclampsia trials using vitamin C and E. The trial of vitamin C and E by Rumbold et al. suggested that supplementation with these vitamins did not reduce the risk of pre-eclampsia. More important, however, was the randomized control trial published by Poston et al. where women randomized to receive antioxidants delivered more low birth weight babies compared to the placebo arm. The study by Poston et al. investigated the effects of 1000mg vitamin C and 400IU RRR-vitamin E. Of the 2,395 patients analyzed, despite...
similar incidences of pre-eclampsia (RR=0.97, 95% CI 0.80-1.17), babies born to women who took antioxidants had lower birth weight (RR=1.15, 96% CI 1.02-1.30) \(^{376}\). This difference could not be accounted for by gestational age. These findings have resulted in major changes in the research involving pregnant women receiving mega-doses for vitamin C and E and led us to assess the viability of our trial. In August 2006, a safety committee meeting was convened to review the justification of continuing the trial. The committee was comprised of the study investigator as an observer, neurologist, toxicologist, and two obstetricians, one of whom coordinated a vitamin C and E study for pre-eclampsia. Data regarding birth outcomes of the EViCE study were presented to the safety committee. There were no cases of low birth weight babies observed in our trial. The committee reviewed the decision on a Toronto-based vitamin C and vitamin E study in pre-eclampsia which was also terminated. The committee concluded that recruitment into the trial should be discontinued and that the ongoing participants be followed to the designated study end points.

A previous review of vitamin C supplementation during pregnancy did not reveal any adverse fetal effects \(^{377}\). Similarly a review of vitamin E supplementation did not report any adverse effects \(^{378,379}\). However, a recent prospective observational study conducted by our group in pregnant women supplementing with mega-doses of vitamin E, detected an apparent decrease in mean birth weight that could not be explained by other variables including maternal age, gestational age, and smoking \(^{380}\). The safety committee concluded that the principle of equipoise was violated with the results of a new
randomized study showing vitamin C and vitamin E to be associated with a clinically significant risk for intrauterine growth retardation in pre-eclampsia.

We subsequently encountered a previous trial in pregnancy that was suspended prior to its completion—a placebo-controlled trial of women receiving nicotine patches. One mother reported excess symptoms associated with withdrawal and excess fetal movements when she used her study medication. It was revealed that she had been randomly assigned to placebo, hence exposing the fetus to the risk of nicotine withdrawal.

Our study is being renewed with a different design omitting the placebo arm and adding a dose-escalating strategy. Together these trials demonstrate the importance of continuous monitoring of studies for unseen adverse effects, especially in a population as vulnerable as pregnant alcoholic women.

### 7.3 STATEMENT OF SIGNIFICANCE AND IMPACT

To our knowledge this is the first attempt to investigate the use of high doses of vitamin C and vitamin E in alcohol exposed pregnancies in the attempt to mitigate alcohol-related damage. During the course of this study, over 2,000 women were screened for the study, of which eight women were randomized and completed the study. All eight babies delivered to the eight women were not low birth weight (<2500 grams). Due to the premature discontinuation of the study we are unable to evaluate the overall effects due to small sample size. Due to
the possible association of high doses of vitamin C and vitamin E and low birth weight, other alternatives of antioxidant therapy should be considered.
Chapter 8

Development of Canadian Screening Tools for Fetal Alcohol Spectrum Disorder
8. DEVELOPMENT OF CANADIAN SCREENING TOOLS FOR FETAL ALCOHOL SPECTRUM DISORDER

Goh YI, Chudley AE, Clarren SK, Koren G, Orrbine E, Rosales T, Rosenbaum C;
Taskforce for Development of FASD Screening Tools.
Development of Canadian screening tools for fetal alcohol spectrum disorder.

Authors contribution:
Protocol development: YIG, AEC, SKC, GK, EO, TR, CR
Literature search, clinic contact, evaluative processes: YIG, AEC, SKC, GK, EO,
TR, CR
Manuscript preparation: YIG, AEC, SKC, GK, EO, TR, CR
8.1 STRUCTURED ABSTRACT

FASD is the most common cause of neurobehavioural handicap in North America. Screening for FASD may facilitate diagnosis and hence management of these children. We present a variety of screening tools for the identification of children at risk for FASD.

We critically reviewed and evaluated published and practiced methods for their potential of screening suspected cases, their epidemiological characteristics (sensitivity, specificity, positive and negative predictive values) [Phase I], as well as their feasibility [Phase II].

The following five tools were selected for the FASD screening toolkit: screening fatty acid ethyl esters in neonatal meconium, the modified Child Behavior Checklist, Medicine Wheel tool, Asante Centre Probation Officer Tool, and maternal history of drinking and drug use.

The toolkit for FASD screening aims at screening different populations, from the newborns to youth and at-risk mothers. It is anticipated that the toolkit will facilitate diagnosis of FASD.
8.2. PROJECT OVERVIEW

8.2.1 PROJECT RATIONALE

On March 1, 2005, Fetal Alcohol Spectrum Disorder: Canadian guidelines for diagnosis were published in the Canadian Medical Association Journal. The development of the guidelines was facilitated by the Public Health Agency of Canada and Health Canada. The current capacity of diagnostic clinics, however, is low compared to the number of patients referred for diagnosis. In addition, the validity and reliability of FASD screening tools have not been verified. As a result, healthcare and frontline professionals are inconsistent in the criteria in which they use to screen and refer children for assessment and diagnosis of FASD.

The Canadian Association of Paediatric Health Centres (CAPHC), funded by the Public Health Agency of Canada (PHAC), facilitated a national initiative entitled: “Developing a National Screening Tool Kit for Those Identified and Potentially Affected by FASD”. This project brought together many FASD experts and organizations to critically evaluate and recommend FASD screening tools for a national screening toolkit. The objectives of this initiative were three-fold: a) to survey and critically evaluate FASD screening tools and methods in the published literature and used by clinics in Canada for referral to or acceptance into diagnostic clinics; b) to evaluate epidemiological characteristics (sensitivity, specificity, and predictive values) of these tools; and c) to develop practical guidelines (toolkit), based on the identified and evaluated tools.
8.2.2 METHOD

Professionals from diverse backgrounds, including FASD experts were asked to critically review the literature related to the screening and identification of FASD and to present their own research and findings. Nine panels were created and focused on the following areas: impact of screening, population variability, growth retardation, facial dysmorphology, neurobehavioural characteristics (two panels), biomarkers in meconium, clinic tools and youth justice population (A full listing of articles reviewed and workshop presentations can be found on the CAPHC website at www.caphc.org/documents_programs/fasd/).

8.3 ESSENTIAL COMPONENTS OF SCREENING

The UK National Screening Committee definition of screening was adopted for the purposes of this task. It defines screening as: “A public health service in which members of a defined population, who do not necessarily perceive they are at risk of, or are already affected by a disease or its complications, are asked a question or offered a test, to identify those individuals who are more likely to be helped than harmed by further tests or treatment to reduce the risk of a disease or its complications.”

Although screening has the potential to save or improve the quality of life through early diagnosis of serious conditions, it is not a fool-proof process.
Screening can reduce the risk of developing a condition or its complications, but it cannot offer a guarantee of protection because there is always an irreducible minimum of false positive cases (wrongly reported as having the condition) and false negative cases (wrongly reported as not having the condition).

An effective screening tool is cost-effective and quickly administered. Successful screening will identify a greater number of individuals than the true number who are affected by the condition. In most cases, these individuals are referred for further assessment and diagnosis for confirmation of the condition.

A good screening test should demonstrate both high sensitivity and high specificity. Sensitivity is the ability to correctly identify persons with the condition in the population who screen positive. Specificity is the ability to correctly identify persons without the condition in the population who screen negative. The higher the sensitivity and specificity reported, the greater the accuracy of the test. The positive predictive value (PPV) is the probability of the condition among individuals with a positive test. The negative predictive value is the probability of no condition among those with a negative test. A reference standard (an alternative method to determine the condition independent of the screening test) is required. At present, such a standard is not available for FASD other than a full diagnostic work-up.
8.4 SCREENING CRITERIA IN THE CONTEXT OF FASD

According to the World Health Organization, to successfully implement a screening program the following conditions should be met:

i. A suitable test should exist;

ii. The disease or condition that is being screened for should be important medically, socially, or economically;

iii. The natural history of the disease should be understood and the population at risk should be identifiable;

iv. The test should be acceptable to the population;

v. The condition should be recognizable at an early stage;

vi. There must be an accepted and effective treatment for the condition;

vii. There should be facilities for assessment, diagnosis and rehabilitation;

viii. Interventions should be acceptable to the population;

ix. The cost of screening should not be disproportionate to the cost of caring for the affected individuals; and

x. Screening programs should be a continuing process.

The rationale for FASD screening meets most, but not all of the above screening criteria.

Universal screening for FASD using these criteria can result in beneficial outcomes. FASD results from maternal prenatal alcohol exposure. The prevalence of FASD is estimated at 9.1 per 1000 live births, with an estimated
lifetime cost for one individual exceeding $1 million\textsuperscript{382,383,384,385}. The screening options for FASD are non-invasive and can result in earlier interventions such as early diagnosis, special education, increased resources and environmental modifications that have been shown to reduce the effects of developmental disabilities in children with FASD. As a result there would be a reduction of secondary disabilities, leading to societal savings that can offset screening costs.

A drawback to universal FASD screening is that there is currently no widely validated, generally accepted screening test. In addition the acceptability of various test methods has not been fully explored. Although the tests are non-invasive, there may be ethical and stigma issues for children and their family as well as time and cost issues for providers. In some areas of the country, FASD is not universally recognized or is believed to occur only in aboriginal populations only. Although facilities for diagnosis and assessment exist in Canada, they are overwhelmed; current average wait times in diagnostic clinics are six months to two years.

\section{8.5 Comparisons with Other Universal Screening Programs for Children}

Despite affecting an estimated 1/100 or over 330,000 Canadians, there is currently no accepted standardized screening test for FASD in Canada\textsuperscript{126,382,384,385}. In contrast, rarer conditions such as phenylketouria and congenital hypothyroidism are universally screened for at birth\textsuperscript{386}. These conditions,
however, have specific and effective biomedical treatments. HIV, cord blood testing, and testing for other rare genetic disorders have also become routine in maternal and perinatal care \(^{387,388}\).

Not only can a positively screened child be closely monitored for developmental disabilities and receive earlier interventions that can decrease or mitigate against FASD related secondary disabilities, but the mother can also receive necessary interventions to reduce or prevent ethanol consumption. Animal studies have demonstrated that ethanol exposed pups receiving earlier intervention had better outcomes because of brain plasticity \(^{389}\).

A diagnosis of FAS or ARND would enable increased access to services and supports and more appropriate sentencing or conditions of probation after sentencing of affected youth in the youth justice system. Detecting a FASD affected child also identifies the addicted mother and possibly other children who may be at risk. Helping mothers could potentially result in reduced alcohol exposure in subsequent pregnancies \(^{390}\).

### 8.6 IMPACT OF SCREENING

While FASD screening may facilitate early diagnosis and intervention, the potential negative impact of screening on children’s and families’ lives must be carefully considered. Families and communities may suffer from stigmatization and screening may cause additional burden to already stressed families. The overall system capacity to consistently provide interventions, supports and
resources throughout the life stages for these children must be assessed and will require political will and commitment for the long term.

Screening on a national level should be evaluated by careful cost-benefit analysis, and compared with the benefits of screening for identified high risk groups. The ability to reach the highest risk populations and the likelihood of compliance with treatment must also be addressed.

8.7 POPULATION VARIABILITY

The epidemiology of FASD is quite variable. Overall, the prevalence of full-blown FAS is estimated to be somewhere between 1-3/1000 live births. Rates can vary ethnically, culturally, and regionally dependent. Published Canadian prevalence studies tend to focus on aboriginal populations, with prevalence rates varying from 1.5 to 9.1 per 1,000 for FASD. Approximately 12.5% of women of childbearing age are at-risk drinkers (>7drinks/week – 4 or more drinks per occasion), suggesting the importance of prevention efforts.

8.8 POPULATION VARIABILITY AND KEY SCREENING DOMAINS

Variability amongst the population may result in varied FASD effect and may limit the ability to screen. Research has shown that ethnic group/genetic factors, cultural/environment factors and age-related factors varied to such a
significant degree that population-specific norms need to be developed. Alcohol damage is affected by genetic factors, maternal drinking history and pattern of drinking, mother’s nutrition and weight, and other risk factors, e.g. smoking and drug use.

The most important risk factor is high blood alcohol concentration, and associated variables such as: timing of exposure during fetal development, the pattern of consumption, i.e. binge drinking and the frequency of use. The Canadian Diagnostic Guidelines emphasize the importance of confirmed alcohol exposure, rather than hearsay, lifestyle, other drug use or a history of alcohol exposure used solely to indicate maternal alcohol consumption for a specific pregnancy. Psychometric screening norms: functional norms on standardized assessments vary across cultures. In addition behavioural expression of disability can be affected by environment. A child’s genetic make-up may vary from standardized screening norms, e.g. birth weight and growth; head circumference; and facial features, e.g. palpebral fissure measurements. In addition, facial features modify with age; some key psychometric assessments are difficult before age five or six; and risks factors may vary depending on child’s stage of development, e.g. behaviours such as lying, cheating, and stealing.
8.9 PHASE 1: SCIENTIFIC EVALUATION OF SCREENING METHODS

8.9.1 EVALUATION OF SCREENING TOOLS AND METHODS

Panelists presented and critically reviewed research related to tools and methods for FASD screening. Information was provided from critical review of the literature as well as unpublished research findings and practical application of clinic tools and methods in Canada. Benefits and limitations of tools and methods were discussed in detail and are summarized in this section.

8.9.1.1 NEUROBEHAVIOURAL METHODS

The neurobehavioural profile for FASD is complex. Neurobehavioural deficits/problems must be closely examined to distinguish between those caused by FASD brain damage and those attributable to other causes or conditions. The literature was critically reviewed to determine which specific neurobehavioural deficit(s) constitute effective screening methods.

In practice, screening for FASD occurs frequently in children exhibiting problem behaviour. It is typically initiated by non-clinicians e.g. teachers, foster parents, and youth court workers. The checklists presently used are not scientifically validated and clinic intake procedures may screen for a variety of neurobehavioural deficits.

Confirmed alcohol exposure is required for referral for FASD assessment. Clinic data has shown that First Nations children are more likely to be screened
for FASD while non-aboriginal children are more likely to be considered ADHD. A concise, validated neurobehavioural checklist would be a valuable tool.

At present there is no single, consistent neurobehavioural profile of FASD in children. The literature search identified a number of cognitive, academic and behavioural factors that are associated with FASD. Broad-based indicators for screening from multiple sources are required, for example:

- alcohol exposure, without which a diagnosis cannot be made
- attention deficit disorder
- academic school performance problems
- behavioural school performance problems
- screening of specific high risk groups which may have built-in markers (e.g. youth justice, Neonatal Abstinence Syndrome in infants)

In an attempt to develop a screening tool, Streissguth et al. proposed the Fetal Alcohol Behaviour Scale (FABS) \(^ {394} \). This tool was unable to discriminate between FASD and other clinical groups. The Personality Inventory for Children (PIC) has also been considered as screening tools, but it can only be administered by psychologists \(^ {395} \). The Child Behavior Checklist (CBCL), a well-established tool for evaluating children’s behavioural problems is administered by psychologists. Research from the Hospital for Sick Children in Toronto has demonstrated the utility of items from the CBCL as a possible screening tool for FASD behavioural phenotype which can be administered by non-clinicians \(^ {396} \). Children with FASD were found to exhibit seven specific behavioural
characteristics that were highly sensitive and specific for distinguishing them from children with attention-deficit hyperactivity disorder (ADHD):

- acts too young for his/her age
- can’t concentrate/poor attention
- can’t sit still/restless/hyperactive
- disobedient at home
- no guilt after misbehaving
- impulsive/acts without thinking
- lying or cheating

This information was used to create a screening tool for referral for FASD diagnosis. The modified CBCL test was further validated for children (6-16 years of age) with or without hyperactivity and poor attention.

A systems approach to FASD screening has been proposed. This is based on the premise that FASD is not a behavioural disorder, but a neurological deficit (brain damage) resulting from prenatal alcohol exposure. The damage can manifest differently depending on age and environmental factors. Screening for FASD is strongly influenced by social system and the professionals working within these systems. A systems approach includes a staged screening process that examines problems in multiple profile domains that interfere with development, investigate developmental history-risk factors, e.g. prenatal alcohol and drug history, screening in communities with high prevalence, and collaboration of professionals from various systems.
The benefits of screening using the modified CBCL include its quick and straightforward administration; it can be administered by trained non-clinicians or a parent/caregiver who knows the child; it can be administered to all children; it uses scientifically objective measures; standardized tools exist for assessing cognitive and academic functioning; this tool may be able to differentiate between non-FASD ADHD children and FASD affected children.

The limitations of screening using the modified CBCL include that although the findings have been replicated in another cohort, the research has not been replicated in a large population. It is currently being repeated in a larger sample, being evaluated in persons with opposition defiant disorder and conduct disorder, and potential confounders such as age, gender, socioeconomic status, home situation and IQ effects are being examined. There may be rater bias by the users. Users of the tool must have background in normal child development to assess age appropriate behaviour. There are many overlaps with other neurobehavioural deficits e.g. ADHD. The behaviours being screened can also arise due to prenatal/genetic factors or environmental factors or experience. Although a statistical significant difference was observed, this does not mean that there are clinically significant differences. Finally, there is a circularity of diagnosis—an individual has FASD and therefore has neurobehavioural deficits.

8.9.1.2 FACIAL DYSMORPHOLOGY

Children affected with FAS are characterized by three dysmorphic facial features: a poorly formed philtrum, thin vermillion border of the upper lip and a
short palpebral fissure length. The majority of children affected with FASD do not exhibit these facial characteristics. Assessment of facial dysmorphology, using a tool for diagnostic purposes, was considered for its applicability as a screening method. Measurements could be obtained manually by using a ruler. Alternatively digital photography coupled with measurement software could be used to obtain measurements. A study in Seattle screened 2,000 children in foster care, demonstrating a prevalence of 1/100 \(^{397}\).

The benefits of facial screening are it is a safe, non-invasive methodology that is relatively low cost. Screening for facial dysmorphology has been demonstrated to have very high sensitivity, specificity, and positive predictive value \(^ {398} \). Digital cameras and software allow non-clinicians to interpret results with high interobserver reliability. Screening using this tool will decrease duties of diagnostic clinics.

The limitations of screening for facial dysmorphology include that a vast majority of children with FASD do not present with facial dysmorphology. In addition, the face changes with age. Facial features can be affected by genetics and ethnicity and there are no specific ethnic norms available. Thus it is hard to screen in ethnically diverse and mixed populations. Accurate measurements are dependent on well-taken photograph. There is a need to distinguish between “statistically significant” and “clinically relevant”.
8.9.1.3 MECONIUM TESTING FOR ETHANOL CONJUGATES

Meconium begins forming at approximately 12th–14th week of pregnancy. Prenatal exposures to chemicals can be quantified in meconium. As the fetus swallows amniotic fluid, prenatal exposure to chemicals can be quantified in meconium. Meconium measurement of fatty acid esters (FAEEs) (fatty acids synthesized with ethanol) is a unique biological marker for fetal exposure to ethanol.

Studies of fatty acid ethyl esters (FAEE) in meconium have been conducted in Canada, the United States, Europe and South Africa. FAEE are unique biological markers of fetal exposures to excessive maternal drinking. Meconium levels of FAEEs above 2nmol/g identify heavy fetal alcohol exposure from light exposure at very high specificity and sensitivity.

The benefit of screening meconium is that it is an objective, non-invasive, sensitive and specific method that collects a natural waste product. Meconium screening identifies both mother and child. Positive FAEE results have been associated with lower Apgar scores, low birth weight and lower executive functioning. Animal studies have also demonstrated a relationship between FAEE levels and growth retardation as well as brain weight. Screening meconium can demonstrate prenatal exposure when maternal self-report is not reliable. Meconium screening has been demonstrated to be cost-effective.

A limitation of meconium screening is that it must be collected in the first 72 hours after delivery. Also, exposure during the first trimester of pregnancy is
not captured in this screen. There are ethical concerns regarding informed consent of the mothers and reporting positive screens to child protective services.

8.9.1.4 GROWTH RETARDATION

Intrauterine growth can be influenced by a number of factors including genetics, ethnicity, and diabetes. Growth retardation is considered as part of the diagnosis process because alcohol is associated with impaired fetal growth. Growth retardation as a screening mechanism may have merit in combination with other biomarkers such as meconium screening.

The limitations of growth restrictions included that growth standards differ for various populations. Only a small percentage of infants who are small for gestational age are associated with prenatal alcohol exposure of more than two drinks per day. The consequences of small for gestation age are significant e.g. high fetal and higher infant mortality, short-term metabolic problems, and deficits in growth and neuro-cognitive delays. However the sensitivity is 10-30%, therefore a majority of cases are missed.

It was generally agreed that growth retardation on its own is not useful as a screening method, but may have merit in combination with other perinatal screens, such as meconium screening.
8.9.1.5 YOUTH JUSTICE POPULATION

The youth justice population poses unique challenges for screening of FASD. There is evidence in the literature that individuals affected with FASD represent a disproportionately large number of youth and adults in the criminal justice system. In youth corrections, behavioural characteristics may drive interventions. Failure to feel remorse or understand consequences of actions has been described as neurobehavioural characteristics of some of those affected by FASD. This presents a challenge to the youth justice system to find appropriate deterrents/incentives for this population.

The FASD Youth Justice Project Manitoba screens youth 12-18 years of age with no prior FASD diagnosis and confirmed prenatal alcohol exposure who are undergoing pre-sentencing in Winnipeg. Items included on the screening questionnaire include:

- repeated failure to comply
- lacking empathy
- poor school experiences
- difficulties within institutions: compliance, peers, academics
- unable to connect actions with consequences
- not affected by past punishment
- followers, rather than leaders, in crime
- crimes involving risky behaviour for little gain

Screening was effective in this selective population and resulted in 50/178 individuals receiving diagnostic assessment which resulted in 30 being
diagnosed with FASD, 29 with ARND. The screener had a 60% positive predictive value (personal communication, Manitoba FASD Youth Justice Program).

In the Youth Justice system in Saskatchewan, judges are instructed to screen based on the criteria used in the FASD Youth Justice Project in Manitoba. If the judge observes these characteristics, the youth court worker collects alcohol history through interview with the mother or a reliable source.

The FASD Screening Tool Project in Saskatchewan has reviewed a number of screening tools and conducted a research study to validate a screening tool for use with offenders. In a collaborative research approach, agreement was reached on 28 risk factor items. The screening tool had a high inter-rater reliability 0.82, with a high validity 76% (personal communication, FASD Screening Tool Project).

A study conducted at Stony Mountain Institution near Winnipeg, Manitoba screened all offenders undergoing preliminary assessment. A Brief Screen Checklist (BSC) that included behavioural and historical indicators and maternal alcohol consumption was used to identify individuals for further assessment. Information was collected from the offender, parole officers and collateral sources. The study concluded that the incidence of FASD was ten times greater in the study sample compared to the North American population. The BSC items were highly correlated with a diagnosis of FASD. In addition, there was a high rate of neuropsychological impairments found in the study sample (personal communication, Stony Mountain Institution).
The Asante Centre for Fetal Alcohol Syndrome Probation Officer Screening & Referral Form is completed for all youth adjudicated on probation orders who reside in the Vancouver Coastal and Fraser Regions who are suspected of having fetal alcohol spectrum disorder. The tool is a pre-coded questionnaire which collects information on social and neuro-developmental history of the youth as well as the probation officer’s knowledge of the youth and FASD. The survey tool was designed to screen youth for referral based on environmental factors and personal (neurobehavioural) factors. Referrals were made based on the combination of either one social/environmental factor plus two personal factors or no environmental/social factors but at least three personal factors. 26.5% of youth met the FASD criteria for further assessment (personal communication, Asante Centre for Fetal Alcohol Syndrome).

Subsequent to the Workshop, the Steering Committee members reviewed the Saskatchewan Fetal Alcohol Spectrum Disorders Functional Screening Tool. This comprehensive functional screening tool, which is still in development, was not considered appropriate for inclusion as a recommended tool at this time. The benefits for screening within the youth justice system is that by investing in the youth justice system by screening for FASD individuals it will enable the correct services of the needs and services for individuals to reduce the risk of re-offending, thereby resulting in long-term cost- and time-savings. A further benefit is that the tool can also be administered by frontline workers.

The limitations of screening within the youth justice system include that there is no validated screening tool for young offenders. It may also be difficult to
collect information regarding maternal alcohol exposure. Frontline workers may be resistant to use screening techniques and there are issues regarding obtaining consent and maintaining the confidentiality of the information. On the other hand, the family and/or youth may not agree to the assessment.

### 8.9.1.6 CLINIC TOOLS

Screening tool currently used in diagnostic clinics across Canada show promise for broader application for different populations. However, all the tools require further validation.

The Complex Developmental Behavioural Conditions (CDBC) Referral Form is used by the CDBC Network in British Columbia which provides screening and referral for provincial and regional developmental paediatric services. The Program diagnostic assessment services are intended for children and youth who have significant difficulties in multiple areas of function including those with known or suspected history of exposure to substances with neurodevelopmental effects. The referral form has been developed with guidelines that reflect the diagnostic assessment process, i.e. development and learning, mental health/behaviour, adaptive and social skills and biomarkers. Within the CDBC Network, referrals are taken from paediatricians or child psychiatrists with exceptions in remote areas where a family physician or nurse practitioner can make a referral. This tool has the potential to be developed and used as a screening tool by a wider range of providers, e.g. teachers, day care workers.
The Clinic for Alcohol & Drug Exposed Children (CADEC) in Manitoba does not use a screening checklist per se but provides the following criteria for referral:

- Consent
- Age 9 months to 12 years
- Confirmation of prenatal alcohol exposure
- Readiness for the assessment — child and parents’ stability
- Behavioural and developmental concerns
- Realistic expectations of assessment

In addition, background information on the child’s birth history, medical history, family history, school records, psychological assessment, and social history is obtained. The diagnostic rate using these intake criteria was approximately 50%. The specificity of this tool was 24.5%. The sensitivity was 100% but there are an unknown number of false negatives. These criteria have not been validated (personal communication, CADEC).

The Labrador Alcohol Research Group (LARGE) is a primary health care approach in Labrador to address FASD in the population (personal communication, LARGE). Referral information includes family/household information, family history, foster home involvement, neurobehavioural indicators, school support, public health reports and medical information.

The benefits for these clinic screening tools are they screen for all complex developmental behavioural conditions. They also reflect the diagnostic criteria/domain used in the assessment process.
These tools are limited because they must be completed by a specialist physician. These tools also require further validation.

8.9.1.7 COMMUNITY TOOLS

The Medicine Wheel Tools were developed for the Elsipogtog Mi’gmag First Nations community in New Brunswick (personal communication Elsipogtog Mi’gmag First Nations). The tools employ the Medicine Wheel framework which draws from traditional medicine in combination with scientific measures and indicators. A set of tools has been designed for a staged approach to screening and assessment in the school environment. The first stage of screening involves the Medicine Wheel Student Index which is administered by the child’s teacher. It explores mental, emotional, physical and social indicators along with spheres of learning and special services received. The tool takes approximately 15 minutes to administer. Significant problems in cognitive sub-domains or problems in one cognitive area coupled with problems in one or more of the conduct, social sub-domains or physical domain suggest the need for the second stage of screening involving the Medicine Wheel Developmental History. This is a semi-structured parent interview—administered by a professional (other than teacher) in collaboration with the parent. Children who have screened positive proceed to diagnosis and assessment. A study screening 237 results in 29 referrals to diagnostic clinics where 67% were diagnosed with FASD.

The benefit of this screening tool includes that it is quick to administer. It can be administered by properly trained teachers and relies on their judgment.
The tools incorporate a First Nation’s worldview and framework providing cultural context and relevance. Parents are engaged in the second step of the process as such there is a feeling of contribution.

Limitations include that the tool has not been validated and assessed in other populations.

8.9.2 PROMISING APPROACHES

It was recognized that there is not one screening tool or method that would be suitable for all ages, cultures and environments. Several tools show promise for universal and/or targeted screening and may be included in the toolkit. Universal screening methods include meconium screening, and the modified CBCL. The targeted screening methods include the Medicine Wheel screening tools, screening for facial dysmorphology, and all presented youth justice screening tools.

While challenged by attempts to identify effective screening methods, the Steering Committee strongly felt that one should not disregard that maternal history of drug or alcohol abuse has been shown to correlate strongly with problem drinking in the index pregnancy. Hence it is important to consider children of these mothers as being an at-risk group that needs careful follow-up. These children can be considered for diagnostic assessment if concerns regarding their appearance, growth, behaviour or development become evident.
8.9.3 KEY CONSIDERATIONS

National screening initiatives must be considered within the context of providers’ and health and educational/social services’ capacity to diagnosis, treat and support families, children and youth with FASD throughout life stages. Screening tools should be assessed for cultural appropriateness, age/stage of development, and environment or genetic factors which may influence their outcomes. Effective screening by non-clinicians will require training, as well as their support and commitment. The screening tools will need to be given consideration in terms of cost-effectiveness, public acceptance, and potential stigmatization. The screening tools will need further validation. In addition to screening, primary prevention plans should be developed.

Screening for FASD may be a challenging task as most screening methods employ clinical and laboratory markers which are not part of the sought condition itself, but rather strongly correlate with it. In the context of FASD, most proposed screening methods constitute one or more signs of the syndrome itself. Second, many of the proposed FASD screening methods have not been validated for their epidemiological properties of sensitivity, specificity and predictive values. Third, due to the limited diagnostic capacity there is a fear that screening may result in a “positive screen” becoming a false “de facto diagnosis”. There was a consensus in the Steering Committee that “screening” is not “diagnosis” and should never be used as such. The Steering Committee felt strongly that wide screening will empower a change in climate toward more support for diagnosis and management of children and adults affected by FASD,
as governments and other decision makers will realize the scope of this epidemic in their jurisdictions. Fourth, given the paucity of validated screening methods, it is apparent that different approaches and methods are required for screening depending upon the life stage of the child — from infant to young adult.

8.10 PHASE 2: FEASIBILITY OF IMPLEMENTATION OF SCREENING METHODS

A workshop with frontline providers of various disciplines and sectors (e.g. health, education, social services, and youth justice) was hosted to assess the feasibility of implementing select screening tools across the country.

In addition, a half-day session was held with First Nations and Inuit organizations to assess the Medicine Wheel tool and identify issues affecting the implementation of screening methods in their respective communities.

8.10.1 METHODS

Workshop participants were pre-assigned to small discussion groups to review selected screening tools. Participants were assigned to groups based on their professional background and likelihood of using the tool in their work. Each group evaluated one of the six screening tools: FAEE screening in meconium, Youth Justice screening tools, modified CBCL, facial dysmorphology, maternal history of substance abuse, and the Clinic for Alcohol & Drug Exposed Children intake process.
Each group was led by a Steering Committee member. Participants first received a review of the screening tool, following which, they were asked to comment on the practical application of the screening tool and rate the screening tool on a scale of 1-5. Tools were assessed on ease of use (1=very difficult, 5=very easy); accessibility (1=inaccessible, 5=very accessible); cost (1=very expensive; 5=inexpensive); expertise (1=high level of expertise, 5=minimal expertise); cultural appropriateness (1=very inappropriate, 5=very appropriate). They were also asked to comment on factors to facilitate implementation and barriers to implementation.

Subsequent to review of the screening tools, participants discussed gaps and opportunities for screening and made recommendations on how to build capacity.

8.10.2 SCREENING TOOLS

8.10.2.1 SCREENING FAEE IN MECONIUM

Screening for biomarkers in meconium is an objective non-invasive method of universal screening. This screening method identifies both the mother and child; therefore, systems should be in place for the management of both persons prior to the implementation of screening. This method could provide prevalence data. Obtaining consent for the collection must be carefully considered amongst different languages and cultures.
8.10.2.1.1 EASE OF USE

Participants were given a hands-on demonstration of meconium collection. Meconium collection was given an average rating of very easy (average=5). Collection was deemed much easier than collecting cord blood, asking screening questionnaires or venipuncture. Due to light and temperature sensitivities, there needs to be protective collection techniques. There may also be an issue for specimens coming from remote communities requiring multiple transitions or are delayed during transport. The analysis can be conducted in a laboratory with a gas chromatograph.

8.10.2.1.2 ACCESSIBILITY

Meconium collection was deemed slight inaccessible (average=2). Currently, only one lab in Canada processes meconium samples. Early hospital discharge should not limit the accessibility of the test because infants are not sent home before they pass their first stool. Issues were also identified regarding follow-up, turn-around time to receive results, and sensitivity of the providers review screening results with mothers. When conducted anonymously, there is no opportunity to follow up with the mothers and the data can only be used to assess the prevalence.

8.10.2.1.3 COST

Testing meconium was considered affordable (average=4). Costs related to shipping and training staff were not included in this consideration.
8.10.2.1.4 EXPERTISE

Meconium testing received an average rating for expertise required to perform the test (average=3). Persons obtaining the sample would have to receive training regarding obtaining consent for the screening test. Lab technicians would also require training on conducting the analysis. Persons disclosing screening results would have to be able to communicate the difference between screening and diagnosis. They would also need training on substance abuse issues.

8.10.2.1.5 CULTURAL APPROPRIATENESS

Meconium testing was deemed very culturally appropriate (average=5). It is an objective test and with universal implementation there is little risk of stigmatization of cultural/ethnic groups or communities. Although the test, itself, is straightforward, language and cultural issues should be considered when describing the screening test and communicating the test results.

8.10.2.1.6 FACTORS TO FACILITATE IMPLEMENTATION

Identifying a larger amount of prenatal alcohol exposure than self-reported may increase awareness of this issue. This screen identifies two patients (i.e. the mother and the child) who can potentially be helped.
8.10.2.1.7 BARRIERS TO IMPLEMENTATION

Barriers to implementation of meconium screening as a universal screener include that it requires the support from provinces and territories. In addition, healthcare providers and organizations are already overworked and understaffed, thus this may increase their workload. There needs to be a method of support for both the child and mother who are identified as at risk.

The test is limited to detecting alcohol exposure after the first trimester; therefore there may be a false sense of security when the meconium screens negative. In addition there is a question as to who ‘owns’ the results. This may be more relevant in custody cases.

Setting up a national laboratory requires further consideration. There needs to be additional support from the public, healthcare professionals, hospitals, and government for wide-scale implementation.

8.10.2.2 YOUTH JUSTICE SCREENING TOOLS

The youth justice screening tools focused on the Asante Centre for Fetal Alcohol Syndrome Probation Officer Screening & Referral Form. This is a quick and easily administered tool in trained workers. The validity of the tool needs to be established prior to wider implementation.

8.10.2.2.1 EASE OF USE

The form was deemed very easy to use (average=5). The tool can be administered within two to three minutes by trained frontline personnel.
8.10.2.2.2 ACCESSIBILITY

The form was deemed accessible (average=4). To date, the tool has only been used for pre-sentence referrals. The language of the form needs to be simplified. Confirmation of prenatal alcohol exposure may be difficult to obtain in this age group.

8.10.2.2.3 COST

Screening using this form is slightly inexpensive (average=4). Although there is little cost associated with the form, there is a cost for training providers regarding the administration of the form. Testing of the form in other organizations will be able to confirm its validity.

8.10.2.2.4 EXPERTISE

The form was considered user-friendly and required minimal expertise (average=4).

8.10.2.2.5 CULTURAL APPROPRIATENESS

The form was considered appropriate across many cultures (average=4). Currently, the form is only available in English. It will require translation into other languages; however, it may be difficult to translate certain concepts to other languages.
8.10.2.6 FACTORS TO FACILITATE IMPLEMENTATION

The Substance Abuse and Mental Health Services Administration (SAMHSA) FASD Center of Excellence (US) has developed and validated a tool and produced training manual for this population which may be able to assist in the validation of this tool. The tool may be improved when combined with the Saskatchewan Youth Justice Screening Project and FASD Functional Screening Tool. This tool has contributed to the results of the recent National Round Table on Youth Justice.

8.10.2.7 BARRIERS TO IMPLEMENTATION

Staff may be reluctant to ask about maternal alcohol consumption; and validation of mother’s alcohol consumption may be difficult. The tool is currently undergoing validation test. Securing on-going funding to provide training and support, particularly in isolated communities, is a chronic problem. The tool may need to be adapted for different languages and cultures. Providers would need to be trained on the administration of this tool.

8.10.2.3 MODIFIED CHILD BEHAVIOR CHECKLIST

The tool is not inclusive of other brain domains such as executive functioning, memory, abstract thinking. The objectivity of the assessor may be influenced by differences in cultures, values and settings.
8.10.2.3.1 EASE OF USE

The modified CBCL is a short and easy to use questionnaire (average=4). An interviewer’s manual would be beneficial for administrators to clarify the language. Some of the questions are open to interpretation and may elicit different responses from the respondent.

8.10.2.3.2 ACCESSIBILITY

The modified CBCL was deemed accessible (average=4). It requires a consent process when the information is being provided by someone other than the legal guardian. Educators are most likely to be the first people to be a source of information. Administering the tool in person would overcome literacy issues or problems with interpretation of questions. The tool is straightforward to translate.

8.10.2.3.3 COST

The modified CBCL was deemed inexpensive to administer (average=5). There are low material costs and it takes a short time to complete.

8.10.2.3.4 EXPERTISE

Moderate level of expertise would be required to administer the modified CBCL (average=3). The tool could be administered by trained frontline providers
8.10.2.3.5 CULTURAL APPROPRIATENESS

The modified CBCL was deemed culturally appropriate (average=3). Different/modified tools may be required for various age groups and populations as there is an acceptance of certain behaviours varies by culture and environment.

8.10.2.3.6 FACTORS TO FACILITATE IMPLEMENTATION

The modified CBCL integrates well with the diagnostic process. It can be improved with a procedural manual to clarify the role of the referrer and the role of the screener.

8.10.2.3.7 BARRIERS TO IMPLEMENTATION

It is not clear how the modified CBCL will account for social and environmental influences on behaviour. The tool needs to undergo validation testing in different cultural groups and ages/stages of development.

8.10.2.4 FACIAL DYSMORPHOLOGY

Screening for facial dysmorphology is only suitable for target populations. It only screens for individuals with FAS, which is a small percentage of the FASD population. Measurements can be challenging to obtain. Dysmorphology is not feasible to use as a screening tool and would be more appropriately used in the diagnostic process.
8.10.2.4.1 EASE OF USE

The facial screening tool was deemed neither difficult nor easy to use. Most professionals considered learning to take accurate photographs relatively easy. Manual facial measurement, on the other hand, was seen as considerably more difficult. Obtaining measurements require good hand and eye coordination.

8.10.2.4.2 ACCESSIBILITY

Facial measurement is objective; however it is limited by its lack of ethnic norms. This is an accessible screen (average=3) and digital photographs can be transmitted over distances. Interpretation of the results requires training.

8.10.2.4.3 COST

Opinions varied on the cost associated with facial screening. On average it was deemed slightly inexpensive (average=4). The measurement software and ruler were inexpensive; however the equipment and time commitment was considered expensive.

8.10.2.4.4 EXPERTISE

Facial measurements would require a fair amount of expertise to administer (average=2). Considerable training and hand and eye coordination would be required to accurately measure, interpret, and deliver results.
8.10.2.4.5 CULTURAL APPROPRIATENESS

Facial screening was considered culturally inappropriate (average=2). The measures are based on Caucasian norms and it is difficult to assess the effects of genetic influences and cultural differences which may affect interpretation of results.

8.10.2.4.6 FACTORS TO FACILITATE IMPLEMENTATION

Facial screening could be useful in a targeted population. If the child has these dimorphic features, maternal alcohol use does not have to be confirmed.

8.10.2.4.7 BARRIERS TO IMPLEMENTATION

Facial screening does not identify the majority of persons affected with FASD. Establishing facial norms for all cultures and persons of mixed heritage would be difficult (require considerable resources). Facial features also fade with age. Judgment on facial features could be used to label individuals. It is difficult to accurately measure facial features. This tool does not screen for the majority of individuals affected by FASD. This tool cannot be used on its own and is typically used during the diagnostic process. As such the tool is not considered to be appropriate as a general population screening tool.

8.10.2.5 MATERNAL HISTORY OF SUBSTANCE ABUSE

Screening for maternal history of substance (alcohol and drug) abuse assumes that if the mother has a substance abuse problem, she is likely to have
abused alcohol. If she has an alcohol abuse problem then it is likely there was prenatal alcohol exposure. Substance abuse is defined as misuse or abuse. A positive screen can result in children receiving necessary supports but may result in stigmatization and isolation. Families have to be prepared to receive a positive result.

8.10.2.5.1 EASE OF USE

Screening mothers was deemed neither difficult nor easy (average=3). It would depend on who provided the information (e.g. friend, family member, neighbour) as different people have varying judgment of what constitutes alcohol and drug abuse. Service providers may be uncomfortable screening women.

8.10.2.5.2 ACCESSIBILITY

Screening for maternal history of alcohol use was deemed neither accessible nor inaccessible (average=3). Although it would be easier to obtain information from smaller community, there may an increased risk of stigmatization. However fear of repercussions for the mother and child may influence the response. Confirmation from multiple sources can assist in the validation of information. The legalities of the collection of the information must be considered.
8.10.2.5.3 COST

Screening for maternal alcohol consumption is a slightly inexpensive method (average=4) where the question can be added to existing questionnaires. Professionals need to be trained on how to ask the question without alienating the mother. There are costs associated with the treatment services provided to the mother.

8.10.2.5.4 EXPERTISE

Maternal screening requires expertise to obtain reliable information (average=2). This may include training on substance abuse and training on how to approach the subject. With appropriate training any frontline worker or compassionate community member could be able to screen mothers.

8.10.2.5.5 CULTURAL APPROPRIATENESS

Screening mothers was deemed culturally appropriate (average=3). Community readiness to address prenatal alcohol exposure is essential. Some physicians still promote alcohol consumption during pregnancy. The question can be adapted into many languages and cultures. However care must be taken to avoid stereotyping within cultures.
8.10.2.5.6 FACTORS TO FACILITATE IMPLEMENTATION

A reporting body needs to track all screened individuals who are waiting for diagnosis. In addition, screening provides an opportunity to assist the mother in a supportive environment.

8.10.2.5.7 BARRIERS TO IMPLEMENTATION

Information received may be biased. Maintaining confidentiality in smaller communities may be a challenge. In cases where there is a question of custody, disclosure may be required. There must be capacity to support identified mothers and children.

8.10.2.6 DIAGNOSTIC CLINIC – INTAKE PROCEDURE

The Clinic for Alcohol & Drug Exposed Children (CADEC) Intake Procedure was selected to illustrate the strengths and challenges which are encountered by both referring providers and diagnostic clinics in identifying, referring and assessing children at risk for FASD. CADEC is a FASD diagnostic clinic in Manitoba that receives referrals from a range of sources. The persons making the referrals for CADEC are experts who are sensitive to family conditions. There currently exists extensive wait lists for diagnosis. The identification of children necessitates immediate case management and family support. Since information is collected from multiple sources, ownership of information is challenging.
8.10.2.6.1 EASE OF USE

The CADEC intake procedure was deemed straightforward and neither difficult nor easy to use (average=3). The overall process of collecting information from multiple sources was considered time consuming and requires an experienced and knowledgeable individual. Supports need to be available to children waiting assessment.

8.10.2.6.2 ACCESSIBILITY

The CADEC intake procedure was deemed slightly inaccessible as it requires culturally competent individuals and interpreters (average=2). Healthcare providers need access to both electronic and paper documents. In addition, it may be difficult to acquire information on prenatal alcohol exposure. This information could impact the reunification of a child with their family. Moreover, long wait times could have negative effects on the child and their family.

8.10.2.6.3 COSTS

The CADEC intake procedure was deemed slightly inexpensive (average=2). However with increased screening follows an increased need for diagnostic capabilities and resources for follow up. Society must recognize that the cost of early identification and treatment will result in long-term cost-savings.
8.10.2.6.4  EXPERTISE

The CADEC intake procedure requires a fairly high level of expertise in child growth and development, women's health, and family counseling (average=2). The family must be prepared to accept the potential consequences of a positive screen.

8.10.2.6.5  CULTURAL APPROPRIATENESS

The CADEC screening form was deemed neither culturally appropriate nor inappropriate (average=3). This process requires screeners who are culturally competent and interpreters who can assist the family through the process.

8.10.2.6.6  FACTORS TO FACILITATE IMPLEMENTATION

There should be clarity around who ‘owns’ the screening and diagnostic results. In addition, support and follow-up are best offered by a case management approach where all services are coordinated with the family by a designated provider.

8.10.2.6.7  BARRIERS TO IMPLEMENTATION

The interface between community referral agents and diagnostic clinics is crucial as part of a comprehensive and seamless approach for children and families with FASD. Before implementing screening tools, capacity of diagnostic clinics, as well as ‘fit’ and compatibility of screening tools with clinic intake procedures should be assessed. The screening process adds to an already
overworked system. In addition diagnosis will result in the need for increased resources of supports and services. Despite the benefits of early screening and diagnosis, family readiness must also be considered.

8.10.2.7 MEDICINE WHEEL TOOLS

The Medicine Wheel tools present a holistic approach to screening involving a community approach. A video demonstrating the use of the Medicine Wheel tools in a Mi’gmaq community school was shown. A number of interventions were provided to increase school performance and address behaviour issues. As part of this process, a comprehensive screening program was initiated in the school — all children were screened using the Medicine Wheel tools. The school did not wait for a diagnosis to act. Supports were provided to the parents to help them realize their personal goals.

8.10.2.7.1 EASE OF USE

The Medicine Wheel screening tools appear easy to use and are relevant to First Nations and Inuit cultures (average=4). The tools are adaptable therefore it is possible to use components that are suitable for individuals or communities.

8.10.2.7.2 ACCESSIBILITY

The Medicine Wheel screening too was deemed accessible but would require translation (average=4). The tool is completed by teachers who knows the child well. Unfortunately the tool is not applicable to high school students
(young adults). The tool could be tailored to individual communities but this will require additional work.

### 8.10.2.7.3 COST

The cost of the Medicine Wheel screening tool was deemed slightly inexpensive (average=4). The cost of ignoring the child who may have FASD is greater than the cost delivering services. The materials are inexpensive, however the training required to correctly administer the tool and the high teacher turnover may result in high costs. Thus measures should be taken to retain teaching staff and also train community members. The implementation of this tool on a national level would be complicated and potentially expensive.

### 8.10.2.7.4 EXPERTISE

The Medicine Wheel screening tool was deemed to require a fair amount of expertise (average=2). There is limited professional expertise available to First Nation and Inuit communities thus it is important to develop experts within the community. Experts or leaders would be required to coordinate or sustain the program. The tool provides an opportunity for the parents to contribute to the screening but requires a psychologist/social worker. The implementation of the tool is dependent on the philosophy of individuals. For examples some professionals may not embrace the holistic approach.
8.10.2.7.5 CULTURAL APPROPRIATENESS

The Medicine Wheel screening tools was deemed culturally appropriate for First Nations and Inuit communities (average=4). The tools would require translation, which may be difficult. Since these tools are pre-developed, some communities may feel they have a lack of input. Some communities may resist this screening approach because of the bias/prejudice that may be associated.

8.10.2.7.6 FACTORS TO FACILITATE IMPLEMENTATION

The Medicine Wheel tools are holistic and based on the framework of aboriginal teaching. The tool can be administered by teachers and is a systems approach to screening that includes the family. It also evaluates various determinants of health. The tool is able to evaluate the child’s progress while emphasizing the child’s positive attributes. There are opportunities to adapt the tool to different First Nations and Inuit communities. This should be done with the input of community workers and parents. The Medicine Wheel is able to be applied in any school setting therefore it can be generalized to the Canadian population.

8.10.2.7.7 BARRIERS TO IMPLEMENTATION

Barriers to implementation of this screening include that First Nations communities have limited access to assessment and diagnostic services. In fact diagnostic capacity is not existent in some Inuit communities.
The tools may have to be adapted. The academic curriculum varies across the country. In addition, social and cultural norms also vary across communities therefore receiving a formal education at school may not be as important as learning traditional skills.

It may be difficult to achieve support from different levels of governments as some governments may not wish to address FASD and related issues. There also must be a method of establishing collaborations between health and education sectors.

Within First Nations communities there is a legacy of psychological inferiority. Some highly skilled personnel from First Nations communities feel a lack of respect from mainstream counterparts and are reluctant to engage with mainstream organizations and providers. This can be a barrier to information sharing, referrals and program development and participation.

The tool needs to be adapted/translated and validated before its use.

8.10.3 OPPORTUNITIES

8.10.3.1 INFRASTRUCTURE IMPROVEMENTS

The establishment of a secretariat at an international level would centralize efforts. Standardized national screening would enable the collection of data and statistics. The data would demonstrate that there is a greater need for diagnostic centres. Personnel in the field are too overworked to evaluate their
efforts and publish their data. There needs to be an ICD-10 code for FASD and/or alcohol-exposure.

8.10.3.2 WORKING WITHIN LIMITS AND CONSTRAINTS

In northern First nations and Inuit communities there is lack of and time-limited funding available for FASD awareness and programming. There is a negative impact on communities when programs and initiatives are suspended due to the lack of funding. There are a lack of FASD coordinators and an absence of screening and diagnostic services within the region and limited support services.

There is a lack of consistent messaging from the medical establishments in Eastern Canada regarding FASD awareness and prevention. Conversely in Western Canada there are more education and training opportunities for professionals and FASD education is included in the school curriculum.

FASD awareness must be addressed within the context of overall quality of life improvements and efforts to address the root causes of substance abuse. Raising awareness requires different strategies and approaches depending on who is being targeted.

8.10.3.3 RAISING AWARENESS AND KNOWLEDGE

A community strategy for prevention should be developed and there should be improved primary prevention strategies. Community leaders and champions in each sector should be identified. There should be political support
for research within different sectors. Local media outlets should be used to engage public dialogue around FASD. Persons affected by FASD should also communicate with the public.

Education of professionals within different sectors would result in better support for affected individuals. Delivering presentations at conferences will increase awareness of professionals e.g. doctors, lawyers, and justice workers. Promoting the importance of screening across the community is also important. Educating and providing professionals with screening checklists will also heighten their awareness. Training frontline providers on how to approach women who may be drinking during pregnancy and harm reduction would decrease the “shame and blame” mentality.

Education of children and youth would also improve awareness. Awareness should be increased amongst vendors and servers of alcoholic beverages. Perhaps placing warning labels on beverages would be effective. Hosting a workshop or focus group twice a year for persons involved in FASD prevention and intervention will provide opportunities to brainstorm community awareness strategies.

8.10.3.4 CAPACITY

Screening will result in an estimate of the prevalence FASD, which would advocate for the establishment of more diagnostic clinics and associated services. In addition, there needs to be an evaluation of what is needed to
support families as well as criteria for a waiting list for screening and assessment should be developed.

**8.10.3.5 TARGETING**

As FASD is a society-wide issue, education should target both women and men from all cultures and economic backgrounds. Conducting outreach to sexually active youths is also important.

**8.10.4 GAPS**

**8.10.4.1 READINESS TO ACCEPT FASD SCREENING**

There currently exists a regional difference in the level of readiness to accept FASD screening.

**8.10.4.2 FUNDING AND DIAGNOSTIC CAPACITY**

FASD diagnosis is a multifaceted process that requires the expertise of a multidisciplinary team. Current funding for FASD diagnostic efforts is limited. Intermittent, time-limited funding allows initiatives to begin while sustainability remains an ongoing challenge. Due to the limited diagnostic capacity and long waiting lists, screening may run the risk of being a diagnostic tool. Thus frontline workers must be educated on how to interpret the screening information. Interim supports for individuals between screening and diagnosis must be established.
8.10.4.2.1 STRATEGIES FOR OBTAINING FUNDING SUPPORT

Engage community leaders and a national champion to raise awareness and advocate for resources and sustained funding. Raise awareness within governments by providing evidence with regard to lifelong costs for those affected with FASD. Develop partnerships with medical organizations such as hospitals and universities to elicit support. Promote opportunities to continue professional education. Analyze existing data from clinics to provide further evidence.

8.10.4.3 AWARENESS AND IMPLEMENTATION STRATEGIES

The participants of the workshop proposed many methods of increasing FASD awareness and the implementation of the screening toolkit across the country. It was proposed that a secretariat be developed to coordinate FASD-related activities at the national/provincial/territorial level. A national champion should be identified to advocate and raise awareness of FASD. FASD screening should be advocated within the context of other child health initiatives for sustainable long-term funding.

By using standardized screening tools across the country, screening initiatives could be coordinated on a national level and included in PHAC's strategic plan. Screening and diagnostic capacity and supports could be fostered with a continuum of care framework. The use of a standardized screening method would also result in the collection of vital statistics, providing prevalence data and estimates of life time costs.
As a first step, it would be necessary to assess the readiness of the community to address FASD. Increased awareness of FASD amongst the community and its leaders could be used to elicit support. Awareness could focus on prevention and education regarding the effects of alcohol consumption during pregnancy. Public dialogue could be initiated through local media. Efforts should be directed to the entire community to avoid singling out or stigmatizing specific groups within the community. Community forums could be assembled to review and adapt the screening tools and methods.

It was recognized that a cross-sectoral approach must be employed to engage professionals in health, social services, education, and recreation. Frontline providers would need training on approaching women who may be drinking in order to implement harm reduction strategies. Physicians would need to improve their understanding and ability to understand FASD. Medical training and awareness could be improved by partnerships with medical organizations, hospitals and medical schools. Medical researchers should be informed of the links between alcohol and brain damage and initiatives should be undertaken to engage graduate students in FASD data aggregation and analysis. Awareness should be raised amongst lawyers and other youth justice professionals. Perhaps this could be modeled after other existing projects e.g. British Columbia Probation Officer snapshot survey and professional development support. Professionals should convene in forums to share knowledge and lessons learned. In addition the gaps between mainstream and First Nations and Inuit professionals need to be addressed.
8.10.5 RECOMMENDATIONS

After reviewing and evaluating information collected from the literature review, diagnostic clinic surveys, and the workshops that were held with the scientific experts and frontline provider, the Steering Committee recommended that screening tools be included in the toolkit based on criterion including sensitivity, specificity, positive and negative predictive values, and practical applicability (ease of use, accessibility, cost, expertise required, and cultural appropriateness). Since it was recognized that there is no “one size fits all” screening tool, multiple tools were recommended to screen for FASD amongst children and youth of different ages, stages, and within diverse settings. Universal screening of all mothers for prenatal alcohol exposure was also recommended. Universal screening of FAEE in meconium could be conducted in newborns. Screening using the modified CBCL could be conducted in children 6-18 years of age. Screening using the Medicine Wheel tool could be conducted in children 4-14 years of age. Screening using the Asante Centre Probation Officer Tool could be conducted in the youth population. Facial dysmorphology was eliminated by consensus as a screening tool.

8.10.6 FUTURE DIRECTIONS — IMPLEMENTATION

The workshop identified challenges and opportunities for implementation of FASD screening. In order to effectively implement screening, community, provincial, and professional participation and cooperation will be required. A
A toolkit will now be assembled containing manuals and tools required for screening and the interpretation of results. Focus groups and workshops will be conducted with policy makers and stakeholders to garner support, assess readiness and identify methods of improving knowledge transfer and the adoption of the toolkit into practice. In addition, assessment of resource requirements will occur prior to the implementation of this screening program.

8.11 STATEMENT OF SIGNIFICANCE AND IMPACT

To our knowledge this is the first attempts to systematically evaluate FASD screening tools for children. The following results will be used in the development of a national FASD screening toolkit, thereby standardizing FASD screening across Canada.
Chapter 9

Rates of Fetal Alcohol Exposure Among Newborns in a High-Risk Obstetric Unit
9. RATES OF FETAL ALCOHOL EXPOSURE AMONG NEWBORNS IN A HIGH-RISK OBSTETRIC UNIT

Goh YI, Hutson JR, Lum L, Roukema H, Gareri J, Lynn H, Koren G

Rates of fetal alcohol exposure among newborns in a high-risk obstetric unit.

(in review)

Authors contribution:

Protocol development: YIG, JRH, LL, HR, JG, HL, GK

Data collection: YIG, LL, HR

Analysis: YIG, JRH

Manuscript preparation: YIG, JRH, LL, HR, JG, HL, GK
9.1 ABSTRACT

Meconium FAEE are sensitive and specific biomarkers for prenatal alcohol exposure (PAE) in pregnancy. We recently reported a 2.5% rate of FAEE positive meconium in a general population sample of infants born in the region of Grey-Bruce, Ontario. Women in this region with high-risk pregnancies are transferred to a tertiary-care facility in London, Ontario. The objective of this study was to determine, in a population-based sample, whether high-risk pregnancies are associated with an increased risk of in utero alcohol exposure. Grey-Bruce residents transferred to the high-risk obstetric unit of St. Joseph's Health Care in London, Ontario were identified and consented to this anonymous prevalence study. Meconium was collected and analyzed for FAEE using GC-MS. The prevalence of FAEE positive meconium was compared to the population-based prevalence in the Grey-Bruce. Fifty meconium specimens were collected from eighty-eight infants born from August 1, 2006 to July 1, 2007. Fifteen (30%) tested positive for FAEE conferring a 12-fold increased risk as compared to the general incidence in Grey-Bruce (RR=12.04, 95% CI=6.40-22.65, p<0.0001). Referral to a high-risk tertiary unit conferred a 12-fold increased risk for in utero alcohol exposure. These results suggest that high-risk pregnancies should be screened for PAE and followed-up for potential diagnosis of fetal alcohol spectrum disorder.
9.2 INTRODUCTION

Fetal alcohol spectrum disorder (FASD) is estimated to affect up to 1/100 North American births \(^{126}\). This disorder is characterized by physical characteristics and/or a pervasive pattern of neurobehavioural deficits. The diagnosis of FASD, which in the majority of cases requires evidence of maternal alcohol consumption, has proven to be difficult as maternal reporting may be hampered by fear, embarrassment and guilt.

The use of biological markers has emerged as a viable method for the identification of in utero alcohol exposure \(^{223,403}\). The use of an objective laboratory screening method can potentially eliminate the bias associated with maternal self-reporting. Meconium begins forming at approximately 12 weeks of gestation and serves as a reservoir of fetal chemical exposures during the second and third trimesters of pregnancy. Ethanol undergoes non-oxidative metabolism and conjugates to fatty acids to form fatty acid ethyl esters (FAEE). FAEE in meconium have been demonstrated as biomarkers of in utero prenatal alcohol exposure \(^{226,230}\). Once formed, FAEE do not cross the human placenta, therefore FAEE detected in meconium represent fetal exposure to ethanol \(^{137}\).

In an attempt to assess the feasibility of meconium as a universal neonatal screening tool, our group recently set out to anonymously screen all babies born in the Grey-Bruce region of Ontario \(^{404}\). While this study determined the rate of fetal alcohol exposure in the general population, in Grey Bruce, all cases of high-risk maternal (e.g. pre-eclampsia) and/or fetal (e.g. intrauterine growth retardation) conditions are transferred out of the region to St. Joseph’s Health
Care, a tertiary healthcare centre in London, Ontario. The National Institute of Child Health and Human Development defines high-risk pregnancy as a pregnancy that is affected by one or more of the following factors: young or old maternal age, overweight or underweight, having problems in previous pregnancies, or preexisting health conditions which can result in complications including pre-eclampsia and preterm labour.\(^{405}\)

The objective of our study was to compare the prevalence of FAEE positive meconium in the general Grey-Bruce population to the high-risk Grey-Bruce population referred to a tertiary healthcare setting in London, Ontario. We hypothesized that a higher rate of FAEE positive meconium would be observed in tertiary versus primary healthcare settings as alcohol consumption during pregnancy may result in, or be associated with high-risk pregnancies.

9.3 METHODS

9.3.1 SAMPLE COLLECTION

The study was approved by the Research Ethics Boards of the Hospital for Sick Children, Toronto, Ontario; Grey-Bruce Health Services Ethics Committee; the University of Western Ontario, and St. Joseph’s Health Care, London, Ontario. In all of these institutions the study was exempt from written informed consent due to the study’s anonymous nature. The meconium was collected anonymously and therefore investigators were unable to trace the
identity of the patient. Individuals agreed to participate in the study by oral consent which could be withdrawn at any time. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 1983.

Babies born to Grey-Bruce residents delivering or transferred to St. Joseph’s Health Care over a one year period were screened by nurses or the onsite research coordinator. Mothers who were residents of Grey-Bruce were informed of the anonymous meconium collection and were offered the option of participating in the study. They received specimen bags and instructions for meconium collection. Mothers declining to participate were instructed to mark a designated box on the specimen bag instead of depositing a specimen. Mothers agreeing to participate in the study were instructed to deposit their baby’s meconium-containing diaper into the specimen bag and inform the nurse that the specimen was collected. At the end of the one-year period, charts of all pregnant women from the region of Grey-Bruce admitted to St. Joseph’s Health Care were reviewed to collect information on maternal characteristics.

9.3.2 SPECIMEN HANDLING

All specimen bags were stored at -20°C freezer until transported on dry ice to The Hospital for Sick Children in Toronto, Ontario. Upon arrival, each meconium specimen was transferred into a 50mL labeled Sarstedt® screw cap
conical polypropylene tube and stored in an opaque container in a -20°C freezer until analysis.

9.3.3 SAMPLE PREPARATION

The FAEE were extracted using a modified method including head space solid microextraction (MS-SPME) followed by gas chromatography with mass spectrometry (GC-MS) as proposed by Pragst. Meconium samples were thawed and approximately 50 mg meconium was aliquoted for analysis. Deuterated standards of ethyl palmitate, ethyl oleate, ethyl linoleate, and ethyl stearate were added to serve as internal standards. Samples were vortexed in phosphate buffer (0.1M, pH 7.6). The mixture was transferred to glass SPME vial (10 ml, 22.5x46mm) (Supelco, Bellefonte, PA) and capped with a 18mm, 35 Shore A Screw Cap with PTFE/Silicone Septa (Supelco, Bellefonte, PA). A standard curve was prepared using blank meconium and the four FAEE at concentrations of 2, 5, 10, 20, 50, and 100ng.

9.3.4 INSTRUMENTATION

HS-SPME was carried out using an auto-injector (AOC-5000 Shimadzu, Kyoto, Japan) with a polydimethylsiloxane/divinylbenzene fiber (PDMS/DVB) from Supelco (Bellefonte, PA). The fibre was injected into a gas chromatograph (GC-2010 Shimadzu, Kyoto, Japan) [FactorFour Capillary Column (30mx0.25µm, Varian, Lake Forest, CA)] with a mass spectrometer (GCMS-QP2010; Shimadzu, Kyoto, Japan) operated in electron ionization mode and scanned from m/z 80-
350. The detection limits of the FAEE were 15ng/g for ethyl palmitate, ethyl stearate, and ethyl linolate and 50ng/g for ethyl oleate. The assay precision was between 3.64-13.15% depending on the FAEE and the concentration, which is consistent with other SPME methods. Samples were quantified by comparing the ratio of FAEE to the deuterated internal standards. The resulting chromatograms were analyzed using Varian Star Chromatography workstation software v.4.5 (Varian Associates Inc. ©1989-1996). Meconium with cumulative FAEE concentrations greater than 2nmol/g were considered positive for significant prenatal alcohol exposure in accordance with published guidelines.

The rate of positive FAEE meconium results in the high-risk Grey-Bruce population delivering at a tertiary care center was compared to the previously established rate of the general low-risk Grey-Bruce population.

9.4 RESULTS

Meconium collection occurred over a one-year period. The study collection commenced August 1, 2006 and concluded July 31, 2007. During this period a total of 3,129 infants were born at St. Joseph’s Health Care. Eighty-three mothers from the Grey-Bruce area delivered at St. Joseph’s Health Care. Only 64 mothers from Grey-Bruce were identified by the screening procedure. A total of fifty meconium specimens were collected (Figure 19). Fifteen specimens (30%) tested positive (>2nmol/g) for FAEE. The characteristics of maternal clinical conditions and fetal outcomes in this population are listed in Table 3.
In the Grey Bruce general population, a total of 682 meconium samples were collected and analyzed where there were no documented cases of refusal of participation in meconium testing\textsuperscript{404}. Of these 682 samples, 17 specimens (2.5\%) yielded a positive FAEE result\textsuperscript{404}. Comparison of the prevalence in the general population of Grey-Bruce (2.5\%) to that of infants born in the high-risk unit (30\%) yielded a 12-fold higher risk (RR=12.04, 95\% CI=6.40-22.65, p<0.0001).
Figure 19. Meconium collection and screening accountability

3129 total number of babies delivered at St. Joseph’s Health Care

892 low-risk births

2237 high-risk births

83 mothers/babies referred from Grey-Bruce area

64 Grey-Bruce mothers identified by screeners

- 3 mothers declined to participate in the study
- 4 mothers consented to participate but baby was discharged before meconium came
- 43 mothers orally consented to participate in the study and meconium was collected*

19 Grey-Bruce mothers not screened

- 2 mothers consented to participate but meconium was not collected by staff
- 5 samples not collected, no specified reason
- 5 unknown
- 2 life support was removed, mother not asked regarding the study

50 meconium specimens were collected

*discrepancy due to multiple deliveries
Table 3. Maternal and neonatal characteristics of the Grey-Bruce perinatal intensive care group

<table>
<thead>
<tr>
<th>Maternal Characteristics</th>
<th>Number of Cases</th>
<th>Percent of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical uterine anomalies</td>
<td>7</td>
<td>15.22%</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>7</td>
<td>15.22%</td>
</tr>
<tr>
<td>Previous caesarian section</td>
<td>7</td>
<td>15.22%</td>
</tr>
<tr>
<td>Advanced maternal age</td>
<td>5</td>
<td>10.87%</td>
</tr>
<tr>
<td>Postdated labour</td>
<td>5</td>
<td>10.87%</td>
</tr>
<tr>
<td>Previous fetal demise</td>
<td>5</td>
<td>10.87%</td>
</tr>
<tr>
<td>Previous chromosomal and other congenital anomalies</td>
<td>5</td>
<td>10.87%</td>
</tr>
<tr>
<td>Intrauterine growth retardation</td>
<td>4</td>
<td>8.70%</td>
</tr>
<tr>
<td>Placenta previa</td>
<td>3</td>
<td>6.52%</td>
</tr>
<tr>
<td>Breech present</td>
<td>3</td>
<td>6.52%</td>
</tr>
<tr>
<td>Previous postpartum hemorrhage</td>
<td>3</td>
<td>6.52%</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>3</td>
<td>6.52%</td>
</tr>
<tr>
<td>Preecclampsia</td>
<td>3</td>
<td>6.52%</td>
</tr>
<tr>
<td>Oligohydramnios</td>
<td>2</td>
<td>4.35%</td>
</tr>
<tr>
<td>Previous neonatal death</td>
<td>2</td>
<td>4.35%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chronic Maternal Conditions</th>
<th>Number of Cases</th>
<th>Percent of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor IV Leiden</td>
<td>2</td>
<td>4.35%</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>1</td>
<td>2.17%</td>
</tr>
<tr>
<td>S. Lupus Erythmatosus</td>
<td>1</td>
<td>2.17%</td>
</tr>
<tr>
<td>Type II diabetes</td>
<td>1</td>
<td>2.17%</td>
</tr>
<tr>
<td>Depression</td>
<td>1</td>
<td>2.17%</td>
</tr>
<tr>
<td>Rh sensitization</td>
<td>1</td>
<td>2.17%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fetal/Neonatal Characteristics</th>
<th>Number of Cases</th>
<th>Percent of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prematurity</td>
<td>16</td>
<td>34.78%</td>
</tr>
<tr>
<td>Meconium aspiration</td>
<td>5</td>
<td>10.87%</td>
</tr>
<tr>
<td>Congenital malformation</td>
<td>4</td>
<td>8.70%</td>
</tr>
<tr>
<td>Dilated ventricles</td>
<td>2</td>
<td>4.35%</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>2</td>
<td>4.35%</td>
</tr>
<tr>
<td>Fetal arrhythmia</td>
<td>2</td>
<td>4.35%</td>
</tr>
<tr>
<td>Intracranial hemorrhage</td>
<td>1</td>
<td>2.17%</td>
</tr>
<tr>
<td>Hypoxic ischemia</td>
<td>1</td>
<td>2.17%</td>
</tr>
</tbody>
</table>
In the present study, higher rates of FAEE positive meconium were documented in a high-risk tertiary healthcare setting compared to primary healthcare settings serving the same regional population. Identical research design was applied to both studies. The general population captured babies born within the region, whereas the high risk subset aimed at recruiting cases leaving the region to give birth in a tertiary-care unit. Of importance, there were no refusals to this anonymous study among the general population in Grey Bruce, while there were three refusals among the high risk cohort, suggesting that the true relative risk may be even higher. Although some mothers were missed during the screening procedure, we do not believe that this created a recruitment bias, because the reasons for omission in the general population were technical (e.g. insufficient meconium). A chart review of all of the women transferred the high-risk unit demonstrated that history of substance abuse was not identified as the primary reason for the referral in any case. Since the meconium collection was anonymous, there is no way to identify which positive FAEE results were associated with particular maternal or fetal outcomes.

Previous studies have shown that mild, sporadic drinking beyond the first trimester of pregnancy does not translate to positive FAEE test (i.e. levels above 2nmol/g)\textsuperscript{230}. Our study detected a 12-fold higher rate of FAEE positive meconium (30% vs. 2.5%) among babies born in a high-risk obstetric unit as compared to the general population of Grey-Bruce\textsuperscript{404}. The true rate of fetal alcohol exposure in our study could have been higher, as we did encounter three
refusals among the high-risk group, whereas no refusals occurred in the primary care groups.

The sensitivity and specificity of FAEE detection in meconium have been reported by several groups. Although Bearer and Chan report a high sensitivity and specificity of FAEE (Bearer reported a sensitivity of 88% and specificity of 64%), Ostrea reported a sensitivity of 26.9% and specificity of 96.8%. Although Ostrea et al. report ethyl linoleate having a low specificity, it had an extremely high positive predictive value, indicating that if ethyl linoleate was detected in the meconium it is highly likely due to fetal alcohol exposure. The variability of reported values of specificity and sensitivity are likely due to differences in the analytical methods used to establish them as well as differences in the sensitivity of maternal reporting. Unlike the previous studies that used solid phase extraction followed by GC-FID or silica gel chromatography, the present study used GC-HS-SPME. Analysis of FAEE using GC-HS-SPME has been demonstrated to be highly sensitive and specific in hair. In addition, because FAEE do not cross the placenta once they are formed, it is reflective of fetal alcohol exposure. Importantly, to date there have not been any documented cases of false positives with meconium testing. In fact, elevated levels of FAEE in meconium have been associated with FASD deficits including growth restriction, decreased executive function, and poorer neurodevelopmental outcome. A limitation is that FAEE in meconium only detects second and third trimester exposure, hence the test is unable to detect prenatal alcohol exposure in the first trimester. Maternal nutrition may
theoretically be a confounding factor as poor nutrition may result in less fatty acid content, which may result in less FAEE production in nutritionally-deficient individuals\textsuperscript{408,409}. The positive cut-off rate utilized by our laboratory of $>2\text{nmol/g}$ FAEE was determined in a previous study quantifying the amount of FAEE in meconium of babies born to women who did not consume any ethanol (abstainers), consumed very little quantities (social drinkers), and women who self-reported heavy prenatal alcohol consumption\textsuperscript{227}. The cut-off limit had a sensitivity of 100\% and a specificity of 98.4\%.

Studies evaluating the stability of FAEE in meconium demonstrated that FAEE are sensitive to temperature and light\textsuperscript{139}. Approximately 86\% of the FAEE in meconium was degraded within 24 hours when specimens were stored at room temperature in light whereas 60\% of the FAEE in the meconium was degraded within 24 hours when specimens were stored in the dark\textsuperscript{139}. In our study diapers were immediately placed in the -20°C freezer once collected. Moreover the opaque diaper reduced the likelihood of degradation due to light. It is currently unknown whether conditions other than alcohol use could result in a positive meconium result in women of high-risk status.

Affecting 1/100 individuals, FASD is the leading preventable cause of developmental disability in North America\textsuperscript{103}. In comparison to other screened neonatal disease, such as phenylketouria and congenital hypothyroidism, the prevalence of FASD is much higher (1\% vs. 0.008\%, 0.016-0.022\%, respectively)\textsuperscript{404,410,411}. Moreover, economic studies have estimated that the lifetime cost for FASD exceeds $1\text{ million CAD}\textsuperscript{383}. Another economic study estimated $4.0
billion was spent in the United States. Due to the population health and economic impact, screening neonates who may possibly be affected with FASD is of great public health importance. Persons diagnosed with FASD at a later stage in life are at a higher risk of having secondary disabilities. Screening for in utero alcohol exposure at birth can prompt referral for diagnosis at an early point in time, thereby allowing early intervention for both mother and child. Implementation of a universal screening program must be complemented with a structured follow-up program for both mothers and infants, meeting their psychological and physiological needs, as well as providing the necessary social supports.

The high prevalence of positive meconium FAEE among newborns in a high-risk obstetric unit may stem from two overlapping mechanisms:

1) Ethanol-induced perinatal risks, such as prematurity and intrauterine growth retardation,

2) Chronic alcohol using women tend to exhibit higher rates of perinatal comorbidities due to neglect, suboptimal healthcare, higher rates of gestational diabetes, hypertension and psychiatric conditions.

In conclusion, the present findings suggest that screening neonates in high-risk perinatal units for prenatal alcohol exposure may have merit as a routine practice to identify and follow-up neonates at risk for FASD, as well as identifying and mobilizing resources to alcohol-dependent mothers.
9.6 STATEMENT OF SIGNIFICANCE AND IMPACT

To our knowledge this is the first attempt to screen the meconium of a selected high-risk population. The following results suggest that selective screening in high-risk population may be an effective method of identifying babies with prenatal alcohol exposure.
Chapter 10

Overall Discussion and Conclusion
10. OVERALL DISCUSSION AND CONCLUSION

10.1 SUMMARY AND DISCUSSION OF RESEARCH FINDINGS

In this thesis a multilevel approach to birth defect and FASD prevention has been examined using specific examples in each level of prevention. The primary level of prevention is demonstrated by the means of maternal multivitamin supplementation. The secondary level of prevention is demonstrated by the means of pharmacologic intervention during pregnancy. The tertiary level of prevention is demonstrated by early screening of the infant. Finally, the quaternary level of prevention is demonstrated by identifying at mothers who are who have a high-risk of delivering a future child affected with the same birth defect.

10.1.1 PRIMARY LEVEL OF PREVENTION OF BIRTH DEFECTS AND FETAL ALCOHOL SPECTRUM DISORDER BY MEANS OF MULTIVITAMINS SUPPLEMENTATION

Folic acid supplementation has long been associated with reducing the risk of NTD. The results from the meta-analyses suggests that prenatal multivitamin supplementation is associated with a decreased risk for certain congenital birth defects and pediatric cancers\(^{416,417}\).

Maternal health optimization by multivitamin supplementation is one example of primary level prevention of birth defects. Alcohol dependent individuals have been shown to be deficient in nutrients due to the empty calories
consumed with ethanol. As such, mothers are likely to be deficient in nutrients. With the deficiency of nutrients, mothers are likely to have an increased risk of delivering a child with birth defects. Therefore with maternal ethanol consumption, not only is a child at risk of developing FASD, their risk for having other birth defects is also increased. Prenatal multivitamin supplementation has been associated with a decreased risk for oral clefts. FAS is characterized by facial dysmorphology. As such supplementation with multivitamins may potentially decrease the risk of adverse facial dysmorphology related to FAS.

This example of primary level prevention of FASD through multivitamin supplementation is a model that should be implemented universally. Currently all Canadian pregnant women are recommended to supplement with 0.4mg folic acid to decrease the risk of neural tube defects. Given the results from the meta-analyses suggesting that prenatal multivitamin supplementation is associated with the decrease of other birth defects and certain pediatric cancers it appears beneficial for all women to supplement with multivitamins.

10.1.1.1 NUTRIENTS AND FETAL ALCOHOL SPECTRUM DISORDER

Due to the differing composition of the multivitamins contained in studies, it is difficult to determine which component(s) is/are responsible for these protective effects. In addition, it is difficult to ascertain whether these effects are due to a single component within the multivitamin or if it results from a synergistic effect. Furthermore, the mechanisms underlying the associated protective effect
have yet to be elucidated. Below are examples of selected components within multivitamins and how may protect against FASD.

Since ethanol metabolism results in the generation of oxidative stress the use of antioxidants may be beneficial in decreasing the generated reactive oxidative species. In pregnancy, one highly studied antioxidant is folic acid (vitamin B19). Folic acid requirements increase during pregnancy due to the increased synthesis of DNA and one-carbon transfer reactions such as nucleotide synthesis and cell division. As such, pregnant women are at a higher risk of developing folate deficiency than non-pregnant women. Moreover, chronic alcohol consumers have been demonstrated to have folate deficiency. As such, women who are chronic consumers of alcohol are usually folate deficient prior to pregnancy and thus have a higher risk of delivering a child with a birth defect. The combination of folate deficiency and the increased need for folate during pregnancy may synergistically result in negative outcomes for the fetus.

Methanol is a chemical that is endogenously produced in the body but also present in most alcoholic beverages. Since ethanol is preferentially metabolized by alcohol dehydrogenase compared to methanol, methanol and its metabolite, formic acid, may be detected in higher levels in blood of individuals who consume alcohol. Formic acid is a toxic substance that is known to cross the placenta and cause cellular damage. Since folate is a cofactor for the elimination of formic acid, folate deficiency may result in the accumulation of formic acid in the mother. Formic acid generates oxygen radicals which are
responsible for cellular damage. This has previously been demonstrated in studies where animals with high formic acid levels and decreased formic acid elimination rates were observed to be folate deficient. Moreover, *in vitro* studies have observed that pretreatment of brain cultures with folic acid decreased formic acid-induced neuronal death. The reduction of antioxidant capacity is observed in addition to the decreased presence of folate. Together the above factors may explain the mechanism of damage resulting in FASD.

Another proposed mechanism of how ethanol causes FASD involves retinol. Retinol is bound to retinol binding protein (RBP) and oxidized from retinal to retinoic acid in a similar oxidation pathway as ethanol. This conversion is catalyzed by alcohol dehydrogenase, aldehyde dehydrogenase, and cytochrome P450. The involvement of these enzymes suggests that fetal alcohol syndrome may arise due to inhibition of aldehyde-catalyzed retinoic acid synthesis.

### 10.1.2 SECONDARY LEVEL OF PREVENTION OF FETAL ALCOHOL SPECTRUM DISORDER BY MEANS OF EARLY PREVENTION WITH ANTIOXIDANTS

In attempts to investigate the secondary level of FASD prevention, a double-blinded randomized controlled trial was conducted to examine the potential protective abilities of antioxidants to mitigate ethanol related damage. The rationale for the use of antioxidants is based on the proposed
mechanism that FASD results from oxidative stress generated by the metabolism of ethanol\textsuperscript{438,439,440}. Since oxidative stress has been implicated in the formation of FASD in animal studies, trials of antioxidants have been undertaken\textsuperscript{369}. Vitamin C is important in the antioxidant defense as it acts as a free radical scavenger that protects tissue from free radical damage (reactive oxygen molecules), thereby preventing oxidative stress\textsuperscript{441}. Vitamin C also acts synergistically with vitamin E, another antioxidant which protects phospholipid fatty acids (components of the cell membrane) from free radical oxidation, as it converts the oxidized form of vitamin E to the active form of vitamin E\textsuperscript{441}.

A systematic review of the literature investigated the role of antioxidants in the prevention of experimental FASD, demonstrating that antioxidants can attenuate damage induced by ethanol\textsuperscript{369}. Informed by this systematic review we conducted a study to examine whether the combination of vitamin C (1mg), vitamin E (400IU), and multivitamin supplementation would be able to mitigate the adverse effects of fetal alcohol exposure at a secondary prevention level\textsuperscript{437}. As previously stated, during the course of this trial, several studies evaluating the use of antioxidants in pre-eclampsia observed that the combination of vitamin C and vitamin E had no protective effect for pre-eclampsia and, in fact, was associated with an increased risk of low birth weight which could not be explained by any other cause\textsuperscript{376}. Many trials involving the supplementation of vitamin C and vitamin E in pregnant women were suspended due to these findings.
Since low birth weight is a common adverse effects observed in alcohol-exposed pregnancies, this study was cancelled during its active recruitment phase to ensure that the study was not putting the fetuses or their mothers at increased health risk. This is especially important in high risk, alcohol dependent and vulnerable women. In this case of this study, at its outset, both animal research and clinical trials regarding pre-eclampsia suggested that high dose vitamin C and vitamin E were safe. However, new information became available during a trial resulting in its discontinuation. This highlights the need to continually monitor safety during clinical trials. At the Hospital for Sick Children, similar to all institutions following the Tricouncil Code of Ethics, continuous evaluation of emerging data is demanded and has to be reported to the Research Ethics Board. Should new information suggesting increased risks arise, studies should be reevaluated by regulatory bodies to decide whether or not to proceed.

Had this example of secondary level prevention demonstrated effectiveness, it would have ideally been targeted to a selected population of women who had alcohol-exposure during pregnancy. Unfortunately, there is currently no known in utero intervention to prevent FASD. There are several animal studies suggesting the possibility of other nutrients to consider for in utero intervention. Given the findings with folic acid as previously discussed, perhaps high doses of folic acid may be beneficial in mitigating alcohol-induced damage associated with FASD. Another area being investigated is the use of choline. Studies by Thomas et al. have demonstrated that the
administration of choline into animals reduced the behavioural detriment and improved the body weight of alcohol-exposed 442,443.

10.1.3 TERTIARY PREVENTION OF FETAL ALCOHOL SPECTRUM DISORDER BY MEANS OF MECONIUM SCREENING IN HIGH-RISK POPULATIONS

The anonymous screening of meconium of Grey-Bruce babies delivered or transferred to St. Joseph’s Hospital in London, Ontario revealed a 30% positive rate for prenatal alcohol exposure as determined by the presence of FAEE. The results of this study suggest that infants of high-risk pregnancies of the residents from Grey-Bruce delivering at St. Joseph’s Health Care in London, Ontario are at higher risk of testing positive for FAEE. Certainly this is a highly selected population; however, the high proportion of FAEE positives suggests that there is a significant increased risk of ethanol exposure in the second and third trimester of pregnancy in this group. High amounts of ethanol exposure and high FAEE levels have been associated with a higher risk of developing FASD 444.

FASD has been associated with high costs both to healthcare and social system 445. Streissguth et al. demonstrated that early identification and appropriate intervention decreased the risk of developing secondary co-morbidities associated with FASD 413. Since earlier screening and diagnosis results in an improved prognosis, there is likely to be reduced costs expended on services that would otherwise be used by these individuals. In fact, a study by
Hopkins et al. evaluating the costs associated with universal and targeted meconium screening calculated a cost-savings associated with both universal and targeted screening methods. The advantage of screening FAEE in meconium is that it is a non-invasive test, as such, samples are easily collected. In addition, it is an unbiased test that is able to demonstrate prenatal exposure when maternal self-report is unreliable. The limitations of meconium screening include that meconium commences forming at approximately the thirteenth week of pregnancy. As such, any exposures prior to this period will not be represented in the meconium. The period of meconium collection is also time-sensitive, with a small window of approximately 72 hours to obtain the sample. In addition, premature babies may have a small sample of meconium which is delayed in excretion time.

It is unknown whether there are elements that would result in false positive tests of meconium. For example, increased sugar levels may result in the increased generation of endogenous alcohol, which in turn may result in a false positive. If increased sugar levels do result in a false positive FAEE screening may be less specific for identifying prenatal ethanol exposure.

### 10.1.4 QUATERNARY PREVENTION OF FETAL ALCOHOL SPECTRUM DISORDER BY MEANS OF MECONIUM SCREENING

Meconium was demonstrated by our study as an effective means of screening for mothers who are unable to discontinue drinking in the second and third trimester of pregnancy. Maternal self-reporting of alcohol consumption was
significantly lower than the prevalence of prenatal alcohol exposure determined by meconium screening. Maternal underreporting may be due to maternal guilt, fear, or shame. However this may also be confounded by the method in which the question was administered to the mother. The framing of the question plays an important role in determining how mothers will report. For instance, if mothers were asked in a manner that construes alcohol consumption in a negative light, it is likely that they will respond that they had no exposure. On the other hand, if mothers were asked in a non-accusatory manner, she would be more likely to answer more truthfully.

Meconium screening provides an unbiased method of screening for maternal alcohol exposure during the latter portion of pregnancy. If mothers are screened positive by their baby’s meconium, it suggests that there may have been significant alcohol consumption since the positive screen cut-off level was established in women who drank two or more drinks per day or were binge drinkers. By identifying mothers, they can be engaged to participate in programs to change their alcohol consumption habits, thereby decreasing their chances of delivering another child who may be affected by FASD. As such, meconium screening constitutes both a means of tertiary prevention by enabling early intervention in exposed babies, as well as quaternary prevention by identifying mother who are at risk for having future alcohol exposed pregnancies. The results for the study suggests that meconium should be considered in high-risk environments and perhaps screening babies in neonatal intensive care units is a god area to begin screening. Overall, screening meconium to identify babies
and mothers at risk should be considered to be implemented on a universal level in order to increase its acceptance and avoid marginalization of certain populations. However, with the universal implementation of this screening process, there are many ethical issues to consider.

10.2 CONSIDERATIONS IN MECONIUM SCREENING

10.2.1 ETHICAL CONSIDERATION IN MECONIUM SCREENING

There are important ethical considerations associated with meconium screening. Generally speaking, the objective of screening is to identify a population which is at a higher risk of developing a disease/problem. This involves screening individuals who do not exhibit symptoms and identify them as being at risk. Ethical screening needs to maintain and preserve individual’s autonomy, beneficence, non-maleficence, and justice.\textsuperscript{446,447}

Screening should involve informed consent. Informed consent is the right of the individual to receive information in order to evaluate treatment options with risk and benefits prior to making a decision. There is a fundamental right to refuse to participate in screening without fear of any consequence of refusal. In Canada there is no specific law specifying that written informed consent is required prior to screening; however, medical practitioners, under ethical guidelines, usually obtain verbal consent prior to screening.\textsuperscript{448} The American Academy of Pediatrics Newborn Screening Task Force recommends parents to
be informed prior to screening of their infant. In the United States, the United States Health Insurance Portability and Accountability Act states that individuals have the right to control their personal information.

One of the dilemmas that arise with neonatal meconium screening is the issue of autonomy. Autonomy is the ability of competent persons to make their own decisions. While the baby is not capable to make decisions, the decision rests on its caregiver. Ideally, the caregiver would act in the best interest of the child; however, this may be conflicted if the caregiver is at risk of being identified as being the contributor to the child’s problem (i.e. the mother drank during pregnancy). The autonomy of this process is therefore complex. This dilemma is commonly overcome by the authorities (e.g. children’s protective services) taking over the role of guardian.

A second dilemma arising from neonatal meconium screening is the issue of beneficence and non-maleficence. Beneficence is the ability to demonstrate the benefits over the risks of the screening program and non-maleficence is the ability to avoid causation of harm. The benefit of neonatal meconium screening is that it identifies babies who may be at greater risk for developing FASD and their alcohol dependent mothers. As such, the child could benefit by early diagnosis which could lead to early intervention and better outcome, preventing the onset of secondary disabilities. Conversely, screening may lead to stigmatization and labeling of the child and at times, removal of child from the custody of the mother. Importantly, in areas where there is limited diagnostic capacity, screening may wrongly become a de facto diagnosis. Thus prior to the
implementation of a screening program there must be a means of diagnosis and treatment.

A third dilemma is justice. Justice is the fairness of risk and benefits. Justice may depend on numerous variables such as whether the child will have access to diagnosis, access to service, and whether parents can afford the service. Furthermore since limited resources exist to implement interventions, there is a dilemma of whether it is right to screen when limited diagnosis and treatment options are available.

The process of screening may result in negative outcomes for the family. First there is a potential for misuse and abuse of information collected through the screening process. The misuse of screening information may result in the denial of healthcare coverage for the family. Second, there may be social stigmatization of the family within the community. This effect may be more profoundly observed if the community is small. There is great tangible risk for the parent of the babies, especially the mother, as the screening may bring to light alcohol consumption during pregnancy, implying the inability to stop drinking. This may result in the involvement of child welfare. Third, the screening process may impinge on reproductive autonomy (a woman’s ability to control reproductive choices). Mothers may feel pressured to terminate future pregnancies. In addition, parents may be ostracized by professionals who are not respectful of their situation. As such parents may be weary of healthcare professionals and may refuse support services. Fourth, the child may be removed from the birth family and placed in foster care. Together these factors including fear, guilt and
stigmatization may cause women to decline participating in the screening process. Women who are already weary of the healthcare system may go underground to avoid the hostile environments.

There is always the question of who owns the test results. Meconium is an excrement of the child, who is themselves, is an individual; however, the results are also reflective of maternal exposure. Similar issues were raised with neonatal screening for HIV, as the identification of antibodies in the infant translates to the identification of an infected mother. However, over the years HIV testing in pregnancy has gained acceptance.

In Indiana a prescreening process occurs prior to meconium screening. The prescreen identifies risk factors such as maternal confirmation of substance use during present or past pregnancies, lack of prenatal care, low birth weight, unexplained abruption placenta, and the presence of withdrawal symptoms in the newborn is conducted prior to screening meconium \(^{450}\).

### 10.2.2 ECONOMIC IMPACT OF MECONIUM SCREENING

In 2005, the Ontario Government established a state-of-the-art screening program at The Children’s Hospital of Eastern Ontario. This $18 million newborn screening program screens for 27 rare genetic diseases including 20 inherited metabolic disorders, 4 endocrine disorders and 3 blood disorders \(^{451}\). All of the disorders tested within this program are rarer than the prevalence of FASD. If the screening program for FASD is implemented and is successful, it will result in the decrease of alcohol consumption in future pregnancies which would
eventually lead to decrease in FASD. A decrease in FASD will result in a
decrease of associated costs.

Lupton et al. reported that in 1998 the economic burden associated with
caring for individuals affected with FAS was estimated to exceed $4 billion US
with an average lifetime cost of $2 million US for individuals. Stade et al.
reported that in 2003, individuals up to 65 years of age with FASD would have a
total average lifetime costs of $1 million CAN. FASD affects not only the
health costs but also education, community services and the corrections sector
as well.

A cost-utility analysis was conducted by Hopkins et al. in 2004 comparing
universal and targeted meconium screening in newborns to no screening. An
incremental cost of $65,875 CAN per QALY (quality-adjusted life year) was
gained from the societal perspective. Screening a targeted group of infants
whose older sibling diagnosed with FASD was less costly than universal
screening and more effective than no screening. Targeted screening in high-
risk populations may be an attractive option in terms of economic benefit and
establishing policy objectives and then expanding screening to a larger level.

10.2.3 IMPLEMENTATION OF MECONIUM AS A SCREENING TOOL

An important question with regards to the implementation of meconium
screening is on which level it should be implemented: universal versus selected
and mandatory versus voluntary. Universal mandatory screening violates the
principles of autonomy and justice. No choice is offered in this process and
there is no consideration of the risks and benefits in the program. Universal voluntary screening will satisfy the criterion of ethical screening; however, may result in decreased compliance and may construe unwillingness to participate in the screening process as guilt. Selected mandatory screening may result in greater identification, but it not only does it violate the principles of autonomy and justice; it also ostracizes the targeted population. Targeted voluntary screening may result in the least participation because the population is being ostracized, therefore given a choice individuals may decline to participate in this form of screening. The drawback of screening in a targeted approach is that individuals who are not in the population will be missed in this screening process. Many researchers recommend to not screen universally because of the potentially high costs involved in the process. However, a recent report evaluating costs associated with universal or targeted meconium screening suggested that there would be cost-savings associated with both types of levels of screening compared to no screening at all. 401

Prior to its implementation as a screening tool, a follow-up procedure must be established. When an infant screens positive, a referral path for diagnosis and treatment should be available in the community. Mothers need to be informed that their child may have the potential to be affected. Informing the mother of the positive screen is a delicate procedure that will require the experience of a skilled individual. Mothers need to be informed that a positive screen does not necessarily imply that their infant is affected with FASD; however it highlights the need to follow the infant closely as they age to
immediately source assistance if their child is not achieving their age-expected goals. In addition, mothers who are screened positive by meconium must receive counseling, evaluation and intervention of their own illness. Identifying and treating women who have alcohol dependence would enhance their quality of life, improve their ability to parent their children and decrease the risk of having another child that is affected with FASD. Without such pre-requisites, screening per se will become futile, if not counter productive.

10.3 IMPLEMENTATION OF A MULTILEVEL PREVENTION PROGRAM FOR FETAL ALCOHOL SPECTRUM DISORDER

Since a multilevel prevention program is a big undertaking, care should be used in the designing and implementation of such a complex program. Ideally prevention programs should be developed to best suit the prevention of FASD as well as the needs of the target population. As such a rigorous planning process is required to identify interventions that would be acceptable and effective in its target population. A conceptual framework that may be helpful in the design and implementation of a multilevel FASD prevention program is the PRECEDE-PROCEED model. The PRECEDE-PROCEED model is based on the premise that lasting behaviour change will occur when individuals are provided with education, motivation, and tools that are necessary to make a voluntary change in their behaviour. It is a nine phase model that identifies predisposing factors, reinforcing factors, enabling causes in educational diagnosis, evaluation
and policy, and regulatory and organizational constructs in educational and environmental development.  

A limitation of using this model is that it is an expensive, complex process that will require long planning and expansive personnel to execute. In the case of a multilevel prevention strategy for FASD, programs will have to be designed so they integrate together to prevent redundancy and therefore additional costs.

10.4 LIMITATIONS

Despite multiple efforts to reduce teratogenic risks of alcohol during pregnancy there are factors which affect the subgroup risks within the population.

10.4.1 INTERINDIVIDUAL VARIATIONS

Food and energy intakes vary amongst individuals due to different body sizes and lifestyles. As such, well-nourished women may have different risks compared to malnourished women. Socioeconomic and cultural factors can also impact nutrient levels. Mothers who are unable to afford food have a higher risk of being nutrient deficient. In addition, different cultures may have different dietary practices which may alter risk factors. Furthermore, access to necessities such as food and clean water varies amongst countries which may also alter the risks to the fetus.

Maternal health status and age may be a contributing factor to the development of birth defects. The standard of healthcare varies worldwide. As
such there may be differing status of health as well as management of medical conditions from country to country. Medications or procedures used in the management of medical conditions may alter the risk for birth defects. Increase maternal age may increase the risk of genetic related birth defects.

### 10.4.2 Genetic Variations

Genetic variations in the mother may alter her susceptibility to deliver a child affected with birth defects. For instance, genetic differences between the absorption and processing of vitamins and minerals may impact the formation of the fetus. For example, women with the MTHFR gene may be at increased risk of delivering a child with a neural tube defect.

### 10.4.3 Environmental Variations

Environmental variation may contribute to the outcome of the pregnancy. Maternal illness during pregnancy may affect the development of the fetus. Exposures to medications prior and during pregnancy may impact the formation of birth defects. Exposures to recreational drugs during pregnancy may also impact the formation of birth defects. Occupational exposures or other environmental exposures may also be a confounder.
10.5 BENEFITS AND LIMITATIONS OF MULTILEVEL PREVENTION OF FETAL ALCOHOL SPECTRUM DISORDER

10.5.1 UNIVERSAL

The benefit of a universal FASD prevention strategy is that it targets all members of the general population and therefore has desirable outcomes for the entire population. This prevention strategy assumes that all women of reproductive age can become pregnant and are at risk for alcohol exposure and will therefore benefit from the prevention effort. Since universal campaigns are aimed large audiences, it has a greater potential for success. Universal prevention is also less intrusive because it does not target individual risk factors or label individuals as alcohol consumers or at risk for FASD. An example of universal FASD prevention includes educational campaigns. Although this type of prevention attempts to educate the population in general, it requires a lot of effort to ensure that all individuals in the population are educated. As such it may not be possible to reach all of the individuals in the population.

The disadvantages of universal FASD prevention include the fact that, in reality, the risk may vary greatly amongst individuals in population. If prevention programs are designed incorrectly there may be language and cultural barriers. Since the effects of universal prevention are hard to measure as it is difficult to determine if the program itself is responsible for decreasing the incidence of FASD or if it is a combination of factors such as other environmental influences.
10.5.2 SELECTIVE

The advantage of selective prevention is that it targets an entire subgroup regardless of their risk for FASD. The cost is justified because the increased risk of FASD balances the benefits against risk. In selective prevention, an individual’s risk of FASD is not specifically assessed or identified as it is based on an at-risk subgroup membership. Selective prevention has a higher in sensitivity; as such there will be cost-savings associates with this model. The disadvantage of selective prevention of FASD is that it may result in labeling or stigma. In addition, this process is more intensive as it requires more man power to identify the subgroup. Although identification of individuals may be quicker in selected populations, it will produce a false sense of security.

10.5.3 INDICATED

The advantage of indicated prevention is that it identifies individuals who have symptoms of FASD who have not yet been diagnosed. The benefit of indicated prevention is that there would be highest in sensitivity of all three. This approach may be less expensive to implement as a whole; however, it has a high cost per person and requires a greater commitment of time and resources to the individual. The disadvantage is that persons may be labeled in this process and it also may result in a false sense of security in the population for which the prevention is not indicated.
10.5.4 PRIMARY PREVENTION

The benefit of primary prevention is that FASD is being prevented prior to its occurrence. As such, this method would be the ideal method of prevention. Women of reproductive age would avoid exposing their fetus to alcohol and therefore avoid any worry associated with prenatal alcohol exposure. The disadvantage of primary prevention of FASD is that although educating individuals may increase awareness, they may not necessarily use this information. For example, women who drink may not necessarily use contraception and may become pregnant. In addition women who are dependent on alcohol may not easily be able to voluntarily discontinue drinking.

10.5.5 SECONDARY PREVENTION

The benefit of preventing FASD at the secondary level is that the majority of women discover that they are pregnant after consuming alcohol. As such they would benefit if there was something that they could do to decrease the risk of FASD after recognizing that they are pregnant. The disadvantage is that currently the only fail-proof method of preventing FASD is medical abortion. Until a means of mitigating in utero alcohol damage is discovered, immediate cessation of alcohol consumption will reduce the risk of the development of FASD in the infant but will not address any damage that has already occurred.
10.5.6 TERTIARY PREVENTION

The benefit of preventing FASD at the tertiary level is that individuals can be engaged and may receive necessary interventions to decrease the occurrence of secondary disabilities that are observed such as poor achievement in school, dependent living, socioeconomic problems, and legal problems. Tertiary prevention may be an important option for adoptive parents. Adoptive parents are eager to provide a nurturing environment to their adoptive children and as such are keen to know about their child’s health status. Tertiary prevention by means of screening after birth will enable adoptive parents to prepare to potential issues which may later arise thereby prompting them to seek immediate action should they notice any developmental delays or abnormal behavioural.

10.5.7 QUATERNARY PREVENTION

The benefit of preventing FASD at the quaternary level is that by treating the mother for her alcoholism, quaternary prevention is essentially primary prevention for her next child. The disadvantage of quaternary FASD prevention is that the mother must be willing to engage in treatment programs in order to decrease her alcohol exposure. In addition if treatment of the mother is unsuccessful then the risk of her second child developing FASD remains the same.

In cases where the child is removed from the custody of the mother the mother may or may not be motivated to be engaged into treatment. Some
mothers may be motivated to receive treatment in order to obtain custody of their child. On the other hand, some mothers may self-medicate with alcohol to deal with her grief of the child being removed. As such they may be unlikely candidates to be engaged into treatment programs and therefore run the risk of delivering another child who is affected by FASD.

10.5.8 MULTILEVEL PREVENTION VERSUS SINGLE LEVEL OF PREVENTION

The above examples illustrate multiple levels of FASD prevention. Each level of prevention has its advantages and disadvantages. In addition, each level of prevention targets a different audience. If prevention is to occur only on a single level, it is evident that there will be individuals who will be overlooked. In such cases, the prevention may not reach its intended audience. By using a multilevel paradigm, there are more opportunities to prevent FASD i.e. if an individual is not engaged at one level; there is an opportunity to engage the individual when they are at the next level. Because the multilevel prevention model is cyclical, there will always be an opportunity to engage individuals.

10.6 SUSTAINABILITY OF PREVENTION

In the development with prevention methods, the sustainability of these preventive efforts should be considered. Sustainability should be considered at the onset of program development. In addition to sustained funding, there also
needs to be sustained involvement from the entire community including community agencies, schools, and institutions. The emergence of new professions may arise with the creation of these multilevel prevention programs. In addition there may be opportunities for professional development.

10.7 OVERALL SIGNIFICANCE AND CLINICAL IMPLICATIONS

10.7.1 OVERALL SIGNIFICANCE

Public health promotion in pregnancy is important for maintaining both the mother’s health status and providing optimal growing conditions for the fetus. By providing an ideal environment, the risks of birth defects can be decreased. The benefit of using a multilevel strategy is that it has the potential to reach a vast audience. In doing so it does not single out one population. The limitation to a multilevel strategy is that it is an expensive endeavour. Although a multilevel strategy is most likely to reach the majority of the population, it may not reach all individuals. In addition to become generally accepted, a prevention strategy needs to garner the acceptance of the general population.

Supplementation will decrease the risk for congenital anomalies and pediatric cancers. With the event that supplementation can reduce these problems; supplementation with multivitamins is a cost-effective method of preventing congenital anomalies and pediatric cancers. It is likely that there would be cost savings associated with this process. Moreover, not all persons in
the world have accessibility to nutrition. Thus supplementation will enable them to reach protective levels.

Currently many individuals affected with FASD are not identified. Meconium screening is a method to screen for mother and children who are at risk. This is the first study to record the prevalence of FAEE meconium in a high-risk population.

10.7.2 CLINICAL IMPLICATIONS

With the implementation of multilevel approach it is likely that it will result in a decrease in FASD. Early interventions have been demonstrated to be associated with better prognosis in individuals affected with FASD \(^{413}\). Health Canada recommends that pregnant women supplement with 0.4mg of folic acid \(^{420}\). The Institute of Medicine recommends that pregnant women supplement with 0.6mg of folic acid \(^{42}\). The results from these multivitamin studies have changed the recommendations from the Society of Obstetricians and Gynecologists of Canada \(^{454}\). They now currently recommend using supplementing with a multivitamin containing 1mg of folic acid \(^{454}\). Recent studies from Canada and the United States suggest that the majority of women of reproductive age do not have protective amount of folate (900nmol) stores as previously calculated by Daly et al. \(^{455}\).

As such it was suggested that mothers who do not supplement with folic acid prior to pregnancy and may have high risk of folic acid depletion due to the increase folic acid demands during pregnancy. This may be confounded with
other conditions which may cause folate deficiency. These women should consider supplementing with a multivitamin containing 5mg of folic acid in order to quickly increase their folate status to the protective level \(^{454}\).

The clinical utility of meconium screening has been demonstrated to identify individuals at risk of being diagnosed with FASD. As such screening for this prevalent problem should be considered. Perhaps meconium screening should commence in high-risk units of tertiary healthcare centers and then later expand to the entire population to ensure greater acceptance since the test will not target a specific population. If meconium screening does occur, a greater capacity of FASD diagnosis needs to be developed. Currently wait lists span from 6 months to 2 years \(^{456}\). In addition to the need increased diagnostic capacity, training of healthcare professionals needs to also occur. Healthcare professionals need to be educated regarding the collection and shipment of the meconium. Moreover, they have to be capable of obtaining informed consent and interpreting the results of the screen to the mother. Despite high initial costs associated with the implementation of a meconium screening program, it will result in long-term cost savings. In addition by identifying a child affected with FASD we also identify a mother who may not be aware of the impact of prenatal alcohol exposure or a mother who is not able to discontinue drinking during pregnancy. As such the mother can receive the necessary education or intervention to decrease the risk of her delivering another child affected by FASD.
10.7.3 AFFIRMATION AND ENDORSEMENT OF MULTIVITAMIN SUPPLEMENTATION PRIOR TO AND DURING PREGNANCY

These findings from the study reaffirm the need for supplementation prior and during pregnancy. The current recommendations are for pregnant women to supplement with folic acid; however, these results suggest not only supplementing with folic acid alone, but supplementing with multivitamins containing higher levels of folic acid. Unfortunately, multivitamin supplementation does not occur universally around the world as it is not routine practice of the country that or persons within the country cannot afford to supplement with multivitamins. First world countries such as Canada and the United States recommend supplementing with folic acid and have incorporated folic acid into food by enriching flour. On the other hand, some first world countries such as the United Kingdom and France do not fortify their flour with folic acid. In contrast, third world countries that may not have access to multivitamins may benefit the most by providing multivitamins to mothers who may not receive adequate nutrient intake. Maternal supplementation with prenatal multivitamins can decrease the risk for congenital anomalies and pediatric cancer. A decrease in these birth defects would eventually result in cost-savings to the healthcare system and society. Studies have already demonstrated that folic acid fortification of food has resulted in cost-benefit in Canada, United States, South Africa, Netherlands, and Chile. Waitzman et al. estimated that the lifetime medical costs for spina bifida was over $635,000. Wald et al.
estimated that with an investment of 1 cent per person per year to fortify flour, $1,000 in savings in neural tube defects are realized.\(^7^0\).

Education of the benefits of multivitamin supplementation should commence as early as in school when students are taught about human reproduction. Physicians should consider discussing the importance of multivitamin supplementation during pregnancy with their patients as part of their yearly physical so that patients are reminded of the benefits. The benefits of multivitamin supplementation can also be promoted across the general population.

### 10.7.4 AFFIRMATION OF SCREENING MECONIUM FOR FATTY ACID ETHYL ESTERS

The results from the meconium screening study endorse the use of screening of meconium for fatty acid ethyl esters. Screening identifies children who are at risk of being diagnosed with FASD. With the prevalence of FASD being estimated at 1/100, and the costs associated estimated at $4.0 billion there is a great potential for cost-savings.\(^4^1^2\). The initial launch of a meconium screening program may commence in a selected or indicated population; however, the overall goal is to implement universal screening on a national level similar to other newborn screening programs.
10.7.5 AFFIRMATION AND ENDORSEMENT OF MULTILEVEL PREVENTION FOR FETAL ALCOHOL SPECTRUM DISORDER

A multilevel and multi-targeted approach is needed to decrease the risk of developing FASD. Since the population varies widely in risk factors, uptake capacity, comprehension, cultures, and socioeconomics this multilevel approach may be the best method of reaching all individuals compared to a single level of approach. Through the use of a multilevel approach a wider audience can be reached therefore there is a greater chance of preventing FASD before it occurs and there will be lesser chance of missing individuals who are affected with FASD.

10.8 FUTURE DIRECTIONS

The most important step to the implementation of a multilevel strategy during for FASD is the need to engagement professionals from many disciplines. By engaging individuals across multiple fields, identical messages can be reinforced by multiple individuals on the target population. In addition, multidisciplinary teams can be formed which can collaborate in creating prevention strategies as well as diagnosis and treatment. Moreover prevention strategies should be implemented across sectors such as healthcare, education, and social services.
An infrastructure must be built to support the prevention strategy. Whether it is a centre where individuals can receive answers to their questions or a clinic that can provide diagnosis or a program to assist in the quality of life of individuals affected with FASD. The infrastructure needs to be built with support from stable funding sources. Many initiatives are ineffective because of the limited funding they receive. For instance funding may be limited to a 5-year term where the first few years may be focused on the development of the initiative so that when a model is implemented for use in the community, it only lasts for a short duration after which, communities are left helpless.

There is a need to increase awareness of FASD. Without increasing awareness, there will be no drive to screen for this prevalent problem.

10.9 AREAS FOR FURTHER DEVELOPMENT

With regards to multivitamin supplementation to optimize maternal health, further investigation should be conducted determine what are the optimal levels of each vitamin and mineral within each prenatal multivitamin. There are currently no regulations with regards to the proportion of components within multivitamins.

In addition given the fact that it is recognized that certain vitamins are depleted with alcohol consumption, fortification of alcoholic beverages with these vitamins may improve nutrient status. However, fortifying alcoholic beverages
may endorse alcohol consumption because individuals may use faulty reasoning that they contain nutrients to justify their drinking.

With regards to meconium screening, the next step following the results of the anonymous screening study is to conduct a study where specimens will be screened with the identification of mother and child. Mothers will have to provide written informed consent to participate in the study. By participating in this study, children will be followed as they age and have access to necessary interventions they require. This study will enable investigators to determine the participation rate in this type of screening program. Results from this study can also assist in the creation of policy and recommend what type of infrastructure is necessary with this type of screening process. The following step would be the widespread implementation of meconium as a screening tool which is included with the other types of genetic screens that are routinely conducted in newborns.

Further studies that need to be conducted with regard to meconium screening include ascertaining whether dietary consumption or lack of consumption of fatty acid have an impact on the overall rate and quantity of formation of FAEE. It is unknown whether FAEE degrades in utero and how much of an effect this degradation would contribute to the overall quantification in meconium. Factors resulting in false positive screens have yet to be identified. In addition the exact dose-response correlation between the number of drinks and the quantity of FAEE produced has yet to be determined. Furthermore the exact dose-response correlation between the quantity of FAEE and the prognosis of being diagnosed with FASD is unknown.
The development and validation of FASD screening tools needs to continue. Identification of other screening tools should be undertaken. A study to determine the prevalence of FASD of Canada needs to be undertaken.

With the implementation of these preventive measures, a re-evaluation of costs should be conducted to recognize how much cost-savings have been achieved with multilevel intervention.

10.10 CONCLUSION

Pregnancy is a sensitive time for fetal development as such it is important to reduce any avoidable risk to the fetus. Since ethanol is the known cause of FASD steps should be taken to prevent the occurrence of this avertable birth defect. Due to the complexity of alcoholism, instituting prevention in a single level may only address a small proportion of the population. This thesis demonstrates that a multilevel prevention strategy should be implemented to prevent birth defects and FASD. It identifies two main examples which cover all four levels of prevention: the use of multivitamins and neonatal meconium screening. The use of multivitamins can be beneficial in the primary and secondary level of preventing FASD. In the primary level all women should receive prenatal multivitamin supplementation prior to pregnancy to increase her nutrient status prior to pregnancy. This is especially important in nutrient deficient populations such as chronic alcoholics. By optimizing maternal health status, there will be a decreased risk of delivering a child affected with congenital
anomalies and pediatric cancers. In the secondary level women can take steps towards improving the outcome of her baby once she recognizes that she is pregnant. For instance commencing multivitamin supplementation once discovering pregnancy will attempt to increase maternal nutrient levels to protective levels. In addition in an attempt to evaluate a pharmacologic intervention for alcohol exposed pregnancies a randomized controlled trial was undertaken to establish the effectiveness of high-dose vitamins during pregnancy. In the tertiary level once a baby that has prenatal exposure to alcohol is born steps should be taken to reduce secondary effects and improve the overall quality of life. Screening babies’ meconium for FAEE will identify babies who are prenatally exposed to alcohol. This will enable the referral to a follow-up program that will be able to institute the necessary interventions if needed. Moreover, by screening and identifying babies we are also identifying the mother. In the quaternary level of prevention, identifying the mother allows for the implementation of interventions which will decrease the likelihood of her delivering another child affect with FASD. Due to the multiple factors which can contribute to FASD, the use of a multilevel prevention strategy would be able to reach a wider audience and therefore be more effective in overcoming this devastating problem.
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Appendices
APPENDIX A. LIST OF PEER-REVIEWED PUBLICATIONS

Koren G, Goh YI, Klieger C.
Folic acid: the right dose.

Development of Canadian screening tools for fetal alcohol spectrum disorder.

Goh YI, Koren G.
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Goh YI, Koren G.
Folic acid in pregnancy and fetal outcomes.

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Koren G, Goh I.
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Goh YI, Ungar WJ, Rovet J, Koren G.
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Goh YI, Rovet J, Ungar WJ, Koren G.
The antioxidant effect: can we mitigate fetal alcohol spectrum disorder with antioxidants?

Goh YI.
Knowledge is the key to prevention.
APPENDIX B. LIST OF PUBLISHED ABSTRACTS

Goh YI, Hutson JR, Lum L, Roukema H, Koren G.
Quantifying the rates of prenatal alcohol exposure amongst newborns in a high-risk obstetric unit.

Goh YI, Hutson JR, Roukema H, Koren G.
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Goh, YI, Ungar, WJ, Koren, G.
Literature review of economic evaluation of multivitamin supplementation during pregnancy and congenital malformations.

Goh YI, Velazquez-Armeta EY, Nava-Ocampo AA, Koren G.

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APPENDIX C. LIST OF BOOK CHAPTERS

Goh YI, Koren G, Ungar WJ.
Economic Evaluations in Child Protection, in Economic Evaluation in Child Health

Goh, YI, Chapter 23: Mineral and vitamin supplementation before, during and
after conception, in Maternal-Fetal Nutrition during Pregnancy and Lactation

Goh, YI, Chapter 20: Folate Acid and Congenital Birth Defects: A Review,
Maternal Fetal Toxicology, in Medication Safety in Pregnancy and Breastfeeding
THE HOSPITAL FOR SICK CHILDREN (HSC)  
RESEARCH ETHICS BOARD (REB)

The HSC REB aims to adhere to the principles and practices stated in the Declaration of Helsinki, the World Health Organization and the Canadian Tri-Council Policy Statement (1998)

Approval & Terms of Agreement  

APPLICANTS: Drs. Gideon Koren, J. Rovet, W. Ungar  

PROJECT TITLE: Effectiveness and Cost-Effectiveness of Prenatal Antioxidant Therapy  

Protocol Version Date: June 3, 2003  

Consent Form Version Date: August 5, 2003  

FILE NUMBER: 1000003111  

MEMBERS OF THE BOARD*: Dr. Melvin Freedman, Chair  

Dr. D. Bagli  
Ms. S. Binetsbaum  
Ms. L. Brisbois  
Mr. O. Browne  
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Dr. M. Rossi  
Mr. R. Sugarman  
Dr. J. Vajjar  
Dr. R. Zlotnik Shaul  

*Meeting may not have been attended by all members.

I agree to carry out the proposed research involving human subjects in accordance with the protocol approved by the REB using the approved consent form/s. I shall notify the division/department head and the REB prior to implementing any modifications in the protocol and of any adverse or unexpected events as soon as possible. I certify that the research contract and corresponding protocol are consistent (where applicable).

SIGNATURE OF INVESTIGATOR  

[Signature]  

DATE: August 13/03

I agree to monitor the protocol on an ongoing basis, and to notify the Research Ethics Board as appropriate (see attached letter).

SIGNATURE OF (DIVISION/DEPARTMENT HEAD)**  

[Signature]  

DATE: August 13/03

The REB of the Hospital for Sick Children has reviewed and approved the above-named project.

Chair, Research Ethics Board: [Signature]

DATE: August 16/03
Appendix D

Office of Research Ethics
The University of Western Ontario
Room 00045 Dental Sciences Building, London, ON, Canada N6A 5C1
Telephone: (519) 651-3036 Fax: (519) 850-2456 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. G. Koren
Review Number: 11098E
Protocol Title: Prevalence of Fetal Alcohol Exposure in the Region of Grey Bruce, Ontario.
Department and Institution: Medicine and Dentistry, University of Western Ontario
Sponsor: CIHR FETAL ALCOHOL SYNDROME NEW EMERGING TEAM GRANT
Ethics Approval Date: April 22, 2005
Expiry Date: October 31, 2005
Documents Received for Information: Study Protocol (dated 25/11/04)

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research
Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement and
the Health Canada/ACCP Directives and Practices, Consolidated Guidelines, and the applicable laws and
regulations of Ontario has reviewed and granted expedited approval to the above named research study on the approval
date noted above. The membership of this REB also complies with the membership requirements for REBs as defined in
Division 5 of the Food and Drug Regulations.

This approval shall remain valid until the expiry date noted above assuming timely and acceptable responses to the
HSREB's periodic requests for surveillance and monitoring information. If you receive an updated approval notice prior to
that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without
prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the
change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number).
Expected review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed
information/consent documentation.

Investigators must promptly also report to the HSREB:
a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation and/or recruitment
advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to the
office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not
participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Paul Harding
Deputy Chair: Susan Hodkinson

Karen Kueneman

Ethics Officer to Contact for Further Information
Karen Kueneman, Janice Sutherland, Susan Underhill, Jennifer McEwen

This is an official document. Please retain the original in your files.

UWO HSREB Ethics Approval
2005-11-02 (E-99)

Fax received:
Date: 27/04/2005
Page 1 of 1
Appendix D

Office of Research Ethics
The University of Western Ontario
Room 00545 Dental Sciences Building, London, ON, Canada N6A 5C1
Telephone: (519) 661-3036 Fax (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/researchethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. G. Koren
Review Number: 11080E
Revision Number: 1
Protocol Title: Prevalence of Fetal Alcohol Exposure in the Region of Grey Bruce, Ontario.
Department and Institution: Medicine and Dentistry, University of Western Ontario
Sponsor:
Ethics Approval Date: December 5, 2005
Expiry Date: December 31, 2008
Documents Reviewed and Approved: Added Study Site, Parent Information Sheet, Revised Informed Consent Procedure, Revised Study End Date

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research (HSREB) which is organized and operates according to the Tri-Council Policy Statement and the Health Canada/ICH Good Clinical Practice Practice Consolidated Guidelines, and the applicable laws and regulations of Ontario has reviewed and granted expedited approval to the above named research study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

This approval shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB’s periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g., change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

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- a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to the office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. John W. McDonald
Deputy Chair: Susan Hodkinson

Ethics Officer to Contact for Further Information
Karen Kueneman
Janice Sutherland
Jennifer McGowan

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APPENDIX E. ADDITIONAL LITERATURE REVIEW

1. FETAL DEVELOPMENT

Following the fertilization of an egg and its implantation into the uterine wall, the embryo undergoes gastrulation. The epiblast cells form the primitive streak and mesoderm, which later develop into the notochord and somites. The somites differentiate into myotomes (precursors of skeletal muscle), sclerotomes (precursors of vertebrae), and dermatomes (precursors of dermal components). During the fourth week of pregnancy, the notochord forms the neural plate and undergoes neurulation to become the neural tube. Neural crest cells from the neural tube form the neural folds resulting in the formation of the head and neck. The neuroepithelial layer of the neural canal form glioblasts, which differentiate into astrocytes, oligodendrocytes and ependymal cells, and produce cerebral spinal fluid. The neural crest cells also migrate to form the peripheral nervous system. Neural crest cells differentiate into three main sections: foregut, midgut, and hindgut, from which the organs develop. The following table lists the period of formation of selected organs within in the fetus.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Begins Formation</th>
<th>Ends Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>19 days</td>
<td>9 weeks</td>
</tr>
<tr>
<td>Digestive tract</td>
<td>26 days</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Lungs</td>
<td>26 days</td>
<td>birth</td>
</tr>
<tr>
<td>Urogenital system</td>
<td>19 days</td>
<td>38 weeks</td>
</tr>
<tr>
<td>Limb buds</td>
<td>4 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Head and neck</td>
<td>4 weeks</td>
<td>20 weeks</td>
</tr>
<tr>
<td>Eyes</td>
<td>3 weeks</td>
<td>20 weeks</td>
</tr>
<tr>
<td>Ears</td>
<td>3 weeks</td>
<td>24 weeks</td>
</tr>
<tr>
<td>Brain and cranial nerves</td>
<td>3 weeks</td>
<td>birth</td>
</tr>
</tbody>
</table>
The period where fetal organs are most susceptible to teratogens is during their time of development. Since different organs develop at different stages of pregnancy, there is a window during which teratogens may exert their effect (Figure A1).
Figure A1. Development of the human fetus.

http://www.motherisk.org
2. CONGENITAL ANOMALIES

2.1 NEURAL TUBE DEFECT

The formation of the neural tube during the third and fourth week of pregnancy, neurulation, is an important process fetal development. It results in the formation of the brain and spinal cord, the basis of the nervous system. Neural tube defects are birth defects of the brain and spinal cord which result from the failure of the neural tube to close. This can be due to the abnormal induction of sclerotomes and the neural tube. The most common types of neural tube defects are anencephaly and myelomeningocele. These are both open neural tube defects where the spinal cord is exposed to the external environment as opposed to be protected by bones, muscle, and skin.

Anencephaly originates from the failure of the rostral end of the neural tube to close, resulting in the incomplete formation or absence of the cranial vault and cerebral hemisphere, which is lethal combination. Anencephaly comprises 50-65% of all neural tube defects.

Myelomeningocele is the most common form of neural tube defect which includes spina bifida cystica and open spina bifida. It results from the improper closure of the neural tube in the vertebral column. This open lesion in the caudal spine contains dysplastic spinal cord which may lack in neural function below the level of the defect. Myelomeningocele can vary in size and location, and can result in a range of consequences—from no physical handicap to lifelong disabilities. Affected patients usually have reduced ability to walk, or require
the use of a wheelchair, have little or no bowel and/or bladder control, and require frequent surgical interventions to minimize the effects of hydrocephalus.

2.1.1 PREVALENCE

The prevalence of neural tube defects varies geographically. Neural tube defects have been estimated to affect 0.6-1/1,000 (3,000-4,000) pregnancies in the United States. In Canada the prevalence of neural tube defects is estimated to affect 0.86-1.58/1,000 births. An incidence of 1.23/1,000 was reported in United Arab Emirates. A prevalence of 10.73/10,000 was reported in France. In the United Kingdom, a study reported a 2.8/1,000 prevalence. A Northern Ireland study reported a prevalence of 7.1/1,000. In China a prevalence of neural tube defects was reported at 138.7/10,000 births. The prevalence was 17.03/10,000 in Chile.

There also appears to be cultural differences amongst the occurrence of neural tube defects. The prevalence of neural tube defects amongst Hispanic women has been reported to be three time higher than the Caucasian population. A 1990-1994 study from California observed that the rate of neural tube defects was highest in the Hispanic population (odds ratio [OR]=1.12, 95% confidence interval [CI] 1.04–1.21), followed by Caucasians (OR=0.96, 95% CI 0.89–1.04), African Americans (OR=0.75, 95% CI 0.59–0.91), and Asians (OR=0.75, 95% CI 0.60–0.90).
2.1.2 EPIDEMIOLOGY

Neural tube defects are associated with genetic syndromes including Meckel-Gruber syndrome (an autosomal recessive lethal malformation involving the defects of the kidney, liver and central nervous system), trisomies 13 and 18 and other chromosomal rearrangements. Chromosome abnormalities are observed in 5-17% of neural tube defects. Genes in the region of 13q33-34 associated with 13q deletion syndrome has been associated with neural tube defects. Spina bifida and anencephaly can occur within the same family thus there may be a common genetic linkage. The recurrence risk for siblings is approximately 2-5%.

Alterations in genes involved in folic acid metabolism including genes encoding folate receptors, 5,10-methylenetetrahydrofolate reductase (MTHFR), and cystathionine β-synthase, have been associated with an increased risk for neural tube defects. Homozygosity for the C677T thermolabile variant of the MTHFR gene is also a risk for neural tube defects. Maternal periconceptional folic acid supplementation has been reported to reduce the risk for neural tube defects (50-70%) However the mechanism of how folic acid reduces the risk of neural tube defects is unclear and may be altered by these genetic effects.

Folate acts as a cofactor for an enzyme involved in DNA and RNA biosynthesis, and is also a supplier of methyl groups to the methylation cycles. Folate deficiency results in the up-regulation of folate receptors. Some mothers who deliver children with neural tube defects have autoantibodies that
bind to folate receptors on the placental membrane, thus blocking the binding of folic acid \(^{39}\). Since folate has a higher affinity for the folate receptor it may be able to displace these autoantibodies if administered in high doses \(^{39}\).

Maternal medical conditions (diabetes and obesity) and medication use (anti-convulsant medications and folate antagonists) are associated with an increased risk for neural tube defects \(^{40,41,42,43}\). Environmental exposure to chemicals (e.g. chlorine, cadmium) have been associated with an increased risk of neural tube defects \(^{44}\).

### 2.1.3 TREATMENT AND PROGNOSIS

A higher mortality rate for children with spina bifida in the first year of life has been observed compared to the general population \(^{1}\). Severe cases of neural tube defects are incompatible with life. Surgical correction may be undertaken to correct neural tube defects. Conversely, minor cases of neural tube defects do not require any intervention.

Neural tube defects are one of the most costly birth defects that requires multiple surgeries and result in a lifetime of disability \(^{45}\). There are many healthcare costs associated with severe neural tube defects. Costs associated with medical care for myelomeningocele have been estimated at over $70,000 (adjusted to 2001 dollars) annually for the first 20 years of life, which include the costs associated with an average of five surgeries per year in the first five years of life (20 year lifetime cost is $1.4 million/case) \(^{1,46}\). The highest cost per case was observed in individuals who also had conditions that limited their activity.
(e.g., cerebral palsy [$503,000], Down syndrome [$451,000], and spina bifida [$294,000]) \(^{47}\). In addition, these conditions had among the highest total lifetime costs ($2.4 billion, $1.8 billion, and $489 million, respectively), reflecting their relatively high incidences \(^{47}\). Neural tube defects are estimated to have a total cost of $489 million and the cost per new case is estimated at $294,000 \(^{47}\).

2.2 CARDIOVASCULAR DEFECTS

Congenital cardiac defects are the most common type of congenital anomalies and account for 20% of all congenital defects. Congenital cardiac defects are the leading cause of death in infants under one year of age \(^{48}\).

2.2.1 PREVALENCE

The estimated prevalence of moderate and severe forms of congenital heart defects is 5-8/1,000 live births \(^{49,50}\). If functionally unimportant cardiovascular abnormalities and small muscular ventricular septal defects are included, the prevalence can be as high as 75/1,000 live births \(^{49}\). The prevalence of severe forms of heart defects is 2.5/1,000 live births \(^{49}\). Approximately 74% of all congenital heart defects are recognized in the first year of life, 18% between 1-4 years of age, and 8% is between 5-13 years of age \(^{51}\).

The most common cardiovascular birth defects are ventricular septal defect, patent ductus arteriosus, atrial septal defect, pulmonary stenosis, atrial stenosis, coarctation of aorta \(^{49}\). Less common cardiovascular births defects
include pulmonary atresia, hypoplastic left heart syndrome, total anomalous pulmonary venous connection, and transposition of great arteries.\(^4^9\)

Ventricular septal defects occur in approximately 2-5% live births and occur as an isolated defect in 12/10,000 live births.\(^4^8,4^9\) They are the most common form of congenital cardiac defect. Ventricular septal defect is characterized by a hole in the interventricular septum resulting from the deficient development of the proximal truncoconal swellings causing the failure of the muscular and membranous ventricular septa to fuse. This may be accompanied by an endocardial cushion defect and excessive perforation of the muscular ventricular septum. Overall, this results in massive left to right shunting of the blood and pulmonary hypertension.

Dextrocardia is where the primitive heart tube loops to the right instead of left, resulting in situs inversus (reversal of organs).

Atrial septal defects occur in approximately 29-56/100,000 live births.\(^4^9,5^2\) It is characterized by a hole in the atrial septum resulting from the septum secundum being too short to cover the ostium secundum. This results in the left to right flow of blood causing the enlargement of right ventricle and pulmonary trunk and heart failure.

Atrioventricular septal defects occurs in approximately 27-34/100,000 live births.\(^4^9,5^2\) It is a malformation of the inlet of the heart resulting from the failure of the superior and inferior cushions to fuse, causing the incomplete closure of the septum premium and the ventricular septum. This affects the atrial septum,
ventricular septum and atrioventricular valve. Pulmonary hypertension can result from left to right shunting of blood and can lead to congestive heart failure.

Persistent truncus arteriosus occurs in approximately 1/10,000 live births. It results from the failure of the truncoconal septa and ventricular membranous septum to form. Thus blood from both sides of the heart to mix causing the body and lungs to receive partially oxygenated blood.

Patent ductus arteriosus occurs in 50-57/100,000 live births. It results from the failure of the ductus arteriosus to close between the proximal descending aorta to the pulmonary artery after birth. It comprises 5-10% of congenital cardiac defects. Patent ductus arteriosus is three times more common in girls than boys.

Transposition of the great arteries occurs in approximately 30/100,000 infants. It results from the failure of the truncoconal septal to spiral within the outflow tracts, resulting in the pulmonary artery arising left ventricle which empties into the pulmonary circulation and the aorta arising from the right ventricle which empties into the systemic circulation. It is the most common type of malformation that causes cyanosis in the newborn.

Tetralogy of Fallot occurs in approximately 31-35/100,000 live births. It results from the misalignment of the muscular outlet septum resulting in pulmonary stenosis. In addition, there is a ventricular septal defect and right displacement of aorta resulting in right ventricular hypertrophy due to outflow obstruction. This defect results in cyanosis of the newborn.
Coarctation of aorta occurs in approximately 35-36/100,000 live births\textsuperscript{49,50}. It results from abnormal proliferation of ectopic ductal tissue within the aortic arch causing a narrowing of the distal aortic arch and hypoplasia of the proximal aorta or aortic arch. Approximately 40% of cases present with other cardiac malformations such as such as ventral septal defect, bicuspid aortic valve, atrial septal defect, and mitral valve problems\textsuperscript{48}. This defect is occurs twice as often in males than females\textsuperscript{54}.

Interruption of the aortic arch occurs in approximately 6.6-8/100,000 live births\textsuperscript{50,55}. They are a result of the failure of a portion of the aorta to develop. The interruption may be distal to the left subclavian artery (type A) or between the left carotid and the left subclavian arteries (type B). Interruption of the aortic arch associated with a major cardiac abnormality including ventricular septal defect, aortopulmonary window, truncus arteriosus (truncus), or other complex malformations. Type B interruption is associated with 22q11 deletion (Di George syndrome)\textsuperscript{48}.

Aortic valve stenosis occurs in approximately 20-26/100,000 live births\textsuperscript{49,50}. It results from the restriction of blood flow through the aortic valve.

Hypoplastic left heart syndrome occurs in approximately 23-34/100,000 live births\textsuperscript{49,50}. It is characterized by a small and imperforate aortic valve, underdeveloped left ventricle and hypoplastic or atretic mitral valve, which results in no forward flow of blood through the left heart. The pulmonary venous return enters the right atrium from the left atrium and the pulmonary artery is the only
outlet from the heart. The hypoplastic aortic arch and very small ascending aorta act as a conduit for flow into the coronary arteries.

Pulmonary atresia occurs in 13-21/100,000 live births\textsuperscript{49,50}. This malformation it is characterized by the absence of direct connection between the heart and the lungs. The two major forms are pulmonary atresia with intact ventricular septum and pulmonary atresia with ventricular septal defect (tetralogy of Fallot with pulmonary atresia). Pulmonary atresia with intact ventricular septum is characterized by a severely hypoplastic right ventricle and tricuspid valve with well-developed pulmonary arteries supplied by a patent ductus. Pulmonary atresia with ventricular septal defect is characterized by two ventricles with a single subaortic ventricle septal defect and variable pulmonary artery blood supply.

Pulmonary stenosis occurs in 53-65/100,000 live births\textsuperscript{49,50}. It is an isolated abnormality that results from the incomplete opening of the pulmonary valve due to fusion of the valve cusps.

Total anomalous pulmonary venous connection occurs in 9/100,000 live births\textsuperscript{49,50}. It is an isolated malformation resulting from the pulmonary veins to make a direct connection with the left atrium causing pulmonary venous blood to enter the systemic circulation.

Ebstein's anomaly occurs in 1/200,000 live births\textsuperscript{56}. It is characterized by cardiac enlargement, advanced echocardiographic severity score, cyanosis, and severe regurgitation of the tricuspid valve\textsuperscript{57,58}. 
Malformation of the tricuspid valve and right ventricle which can cause adherence of the septal posterior leaflets to the myocardium, displacement of the functional annulus, dilation of the right ventricle, structural changes to the anterior leaflet e.g. redundancy or fenestrations, and dilation of the right atrioventricular junction.\textsuperscript{59,60}

Conotruncal anomalies and atrioventricular canal defects are the most prevalent lesions among those with severe lesions, whereas atrial septal defect, ventricle septal defect, and patent ductus arteriosus were the most common lesions among those with other congenital heart defects in children.\textsuperscript{61} Approximately 52% of children with congenital heart defects are female.\textsuperscript{61} Shunt lesions (atrial septal defect, patent ductus arteriosus, atrioventricular canal defects) are more common in females than males (9.95/1000 vs. 7.92/1000) whereas transposition complexes and coarctations are more common in males (0.31/1000 vs. 0.22/1000 and 0.30/1000 vs. 0.19/1000, respectively).\textsuperscript{61}

The rates of congenital heart defect may vary amongst cultures. One article reported that atrial septal defect and coarctation of aorta occurs more often in the Caucasian population than in African American or Hispanic populations.\textsuperscript{49} Another study reported an excess of complex congenital heart defect in Asian infants whereas there was an excess of coarctation of aorta was observed amongst non-Asian infants.\textsuperscript{62}
2.2.2 EPIDEMIOLOGY

Congenital cardiac defects may due to multifactorial circumstances including disturbance to normal developmental mechanisms, single-gene mutations, chromosomal aberrations, and teratogens. Congenital heart defects can be due to genetic factors. The most often chromosomal effect associated with congenital heart disease is trisomy 21 and deletion in chromosome band 22q11.

Congenital cardiac defects have been associated with maternal diabetes. One study reported a five-fold increased risk of congenital heart disease in infants born to mothers with pre-existing diabetes. Another study reported that paternal occupations of jewellery making, welding, lead soldering, ionizing radiation and paint stripping were associated with a higher risk of congenital heart defects in the infant.

2.2.3 TREATMENT AND PROGNOSIS

Congenital heart disease is the most common form of birth defect with a high risk of mortality. The most prevalent life-threatening defects include coarctation of the aorta, aortic stenosis, and ventricular septal defect. Congenital heart defects account for 3% of deaths in infancy. A 1985-1994 study in the Northern region of the United Kingdom reported that the 1-year mortality for infants born with congenital heart defects was 18%. The 1-year mortality of infants born in Scotland from 1988-1994 was 25%. The 5-year
mortality of infants with congenital heart defects born in Glasgow from 1980-1997 was also 25%.

Approximately three-quarters of infants who have congenital heart defects will be diagnosed by one year of age. Approximately 4% will die by 16 years of age. Approximately 50% of infants with a congenital heart defect will require an invasive diagnostic procedure and 40% will need cardiac surgery.

Severe aortic valve stenosis presents with heart failure, cardiovascular collapse or early death. It can be surgically corrected or a balloon catheter may be inserted. The majority of infants with aortic valve stenosis are discharged without being diagnosed. There is a 21% mortality in infants who are not identified in the first year of life.

Atrial septal defects are usually asymptomatic at birth and 59% are diagnosed after one year of age. They may be heard as a murmur and are identified by echocardiography. Atrial septal defects can be surgically corrected with a good prognosis and quality of life. Left untreated, symptoms usually do not manifest until adulthood where chronic volume in the right side of the heart may cause pulmonary hypertension, atrial fibrillation, or congestive heart failure.

Coarctation of the aorta presents with heart failure, cardiovascular collapse or death. Infants are managed with prostaglandin infusion prior to surgical intervention. Surgical intervention has been associated with a decreased mortality.
Atrioventricular septal defect presents with heart failure and premature death will occur if left untreated. Atrioventricular septal defects can be surgically corrected. Infants with high pulmonary resistance will not be identified until surgical correction is not possible. Hypoplastic left heart syndrome can present with heart failure and are usually diagnosed two days postnatally. However approximately 40% of cases are unrecognized at six weeks of age. Left untreated, infants will die of heart failure or irreversible pulmonary vascular disease. Infants are managed with prostaglandin infusion prior to surgical intervention. Surgical repair involves the closure of the ventricular septal defect, division of the single atrioventricular valve into left and right valves, and closure of atrial septal defect. Survival rates have been reported from 25%-50% with surgery.

Interruption of the aortic arch can present with rapid deterioration to heart failure or death upon closure of the duct. Infants are managed with prostaglandin infusion prior to surgical repair of the aortic arch. Surgical mortality rates have been reported from 35-57%.

Pulmonary atresia has a wide presentation. Infants with pulmonary atresia with intact ventricular septum will die without treatment; however, infants with pulmonary atresia with ventricular septal defect may survive into adulthood without intervention. A 56% mortality rate has been reported in untreated infants. Infants with pulmonary atresia with intact ventricular septum may receive a shunt to replace the ductus, following which a cavopulmonary connection (right heart bypass) is created. Infants with pulmonary atresia with ventricular septal
defect may receive surgery to close the ventricular septal defect and a conduit may be inserted to connect the right ventricle to the pulmonary arteries.

Pulmonary stenosis has a wide presentation. Mild cases will be asymptomatic, whereas severe cases present with congestive heart failure. Most children are not diagnosed prior to their discharge from the hospital. Mild stenosis is usually left untreated, whereas moderate to severe stenosis require a valvotomy which is usually accomplished with a transvenous balloon dilation and is associated with a good prognosis.

Tetralogy of Fallot presents with cyanosis. The median age of death in an untreated patient is two to seven years. Treatment usually involves an aortopulmonary shunt to increase pulmonary artery flow. Surgical correction of the ventricular septal defect and correction of the pulmonary outflow obstruction are associated with a good prognosis and a 1% mortality rate.

Total anomalous pulmonary venous connection usually results in death prior to one year of age if left untreated. Surgical correction to reconnect the pulmonary vein to the left atrium decreases the mortality rate to 8-35%. 

Transposition of the great arteries results in death without treatment. Infants are usually managed with prostaglandin infusion to maintain ductal patency. A balloon atrial septostomy may be conducted prior to surgical arterial switch operation. The mortality associated with surgical arterial switch is approximately 6%.
Ventricular septal defects occur in 2-5% of infants but usually close spontaneously. Larger ventricular septal defects which do not naturally close may require surgical correction.\textsuperscript{49}

Patent ductus arteriosus usually close spontaneously. However some may have to be surgically closed by transcatheter closure. Closure of the ductus is associated with good prognosis.\textsuperscript{48}

Ebstein's anomaly has a poor prognosis without surgical intervention.\textsuperscript{81} Surgical repair with pacing by a tricuspid valve prosthesis insertion has resulted in 85% of children reaching adulthood.\textsuperscript{82,83,84,85}

Congenital cardiovascular defects account for 3% hospital costs in children.\textsuperscript{86} A 1992 study reported a total cost of $360 million US spent annually on congenital cardiovascular defects with a cost of $262,000 per new case.\textsuperscript{47} A 1997 study estimated that $3.4 billion US was spent on pediatric cardiovascular care in California.\textsuperscript{86}

\subsection{2.3 Oral Cleft and Cleft Palate}

Complete or partial failure of the fusion of facial swellings can result in facial cleft. The most common types are cleft lip and cleft palate.\textsuperscript{87} These two conditions can occur together. Cleft lip is due to the underdevelopment of the mesenchyme of the maxillary swellings that result in inadequate contact of the maxillary swelling with the medial nasal process and intermaxillary process. This may occur due to inadequate migration of the neural crest or excessive cell death during modeling of the nasal placode and maxillary swelling.\textsuperscript{87} Cleft palate can
also result from the failure from the palatine shelves to fuse during the seventh to tenth week of gestation. It may also be caused by inadequate growth of the palatine shelves, failure of the shelves to elevate at the proper time, an excessively wide head, failure of the shelves to fuse, and secondary rupture after fusion. An oral cleft can be a minor notch in the vermillion border to completely separate lips from the lateral philtrum.

2.3.1 INCIDENCE

The incidence of oral cleft and cleft palate vary from race, gender and geographical location. The incidence was reported as 0.21-0.41/1,000 live births in African-Americans, 1.14-2.13/1,000 live births in Orientals, and 0.77-1.40/1,000 live births in Caucasians \(^88\).

Oral clefts are reported to occur more often in boys than girls \(^89\). In addition, boys are reported to have more severe clefts than girls \(^90\). Girls are reported to have more isolated cleft palates than boys \(^90,91\). The incidence across different countries has been reported as 1.0/1,000 South America, 1.12/1,000 United States, 1.33/1,000 Italy, 1.47/1,000 Belgium and Netherlands, 1.69/1,000 Denmark, 1.74/1,000 Finland, 1.75/1,000 France, 1.81/1,000 Czechoslovakia and 13.4-30.7/10,000 China \(^90,92,93,94,95,96,97,98,99\).

2.3.2 EPIDEMIOLOGY

Oral cleft or cleft palate may be of genetic origin or associated with teratogens. Genes that have been associated with oral cleft or cleft palate
include SKI/MTHFR, TGFβ2, TGFα, MSX1, PVRL1, TGFβ3, GABRβ3, RARα, and BCL3. Maternal cigarette smoking has been associated with increasing the risk or oral clefts which may possibly be related to the increase serum carbon monoxide levels which effect cytochrome oxidase. Folate deficiency has also been associated with an increased risk for oral clefts. Maternal corticosteroid and anticonvulsant use has also been associated with an increased risk of oral clefts. It is postulated that the mechanism may be related to the inhibition of enzymes in the nicotinamide adenine dinucleotide (NADH) dehydrogenase electron transport chain.

2.3.3 TREATMENT AND PROGNOSIS

Oral cleft and cleft palate can be surgically corrected. The majority of children who have the correction prior to 18 months will have normal speech. Speech therapy can also potentially improve speech quality. In addition, dental and orthodontic treatment may be required to maintain good dentition.

2.4 LIMB DEFECTS

Limb malformations are divided into three major groups: a/hypoplasia, supernumerary structures, and fusion/separation defects. These include amelia (complete absence of a limb/failure of formation), rudimentary limb (severe hypoplasia of a limb), terminal transverse (absence or hypoplasia of distal segments of axial sides of the limb), longitudinal preaxial (absence or hypoplasia of preaxial structures), longitudinal postaxial (absence or hypoplasia of postaxial
structures), intercalary (absence or hypoplasia of the proximal part of the limb), split hand/foot (absence or hypoplasia of central axis involving digits), and digital deficiencies (asymmetric deficiencies of the digits, transverse arrest, longitudinal arrest, failure of differentiation, duplication, overgrowth, undergrowth, congenital constriction band syndrome, or generalized skeletal abnormalities)\textsuperscript{111}.

\subsection*{2.4.1 PREVALENCE}

Limb deficiency has been reported to occur with other major congenital anomalies 12-33\% of the time\textsuperscript{111,112,113}. The most common defect is polydactyly (presence of supernumerary digits) which occurs in 5-19/10,000 live births\textsuperscript{114,115,116}.

In Canada the prevalence of limb defects from 1966-1984 was 6.0/10,000 births\textsuperscript{117}. Evans \textit{et al.} reported from 1975-1984 the overall prevalence of limb defects was 0.55/1,000 births or 1/1,816 in Hungary\textsuperscript{111}. A Swedish registry of malformations reported 81\% infants affected with upper limb defects and 42\% infants affected with lower limb defects\textsuperscript{118}. From 1964-1965 the Swedish national registry reported the prevalence of single limb malformation as 4.5/10,000 births and multiple malformations as 8.3/10,000 births\textsuperscript{118}. A United States study report the prevalence of limb deficiency as 3.8-5.3/10,000 births\textsuperscript{119,120}. A registry from 1983-1993 of 11 nations reported that the prevalence of limb deficiency associated with major congenital anomalies was 1.3/10,000 births, 0.7/10,000 live births, and 0.6/10,000 stillbirths\textsuperscript{121}. 
The absence of limbs or severe hypoplasia of limb skeletal structures has been reported in 3-8/10,000 live births. Upper limbs are two times likely to be affected than lower limbs. Unilateral defects are four times likely to occur than bilateral defects. Limb defects are more likely to occur on the right side. The incidence of upper limb malformations has been estimated as 22.91/10,000 births; polydactyly as 9.5/10,000 births; transverse failure of formation at hand/finger level as 5.8/10,000 births; syndactyly as 1.5/10,000 births; hypoplasia as 1.3/10,000 births; radial club hand as 0.8/10,000 births; ring constriction as 0.6/10,000 births; and amelia as 0.2/10,000 births.

2.4.2 EPIDEMIOLOGY

The cause of most limb defects are unknown. Congenital limb deficiency may be due to genetic variation, environmental teratogen, or gene-environment interaction. Polydactyly and ectrodactyly tend to run in families with autosomal dominant, autosomal recessive or x-linked pattern of inheritance. Drugs known to cause limb defects include aspirin, dimethadione, retinoic acid, thalidomide.

2.4.3 TREATMENT AND PROGNOSIS

Surgery is performed to achieve maximum limb function and prosthesis may be used to improve cosmetic appearance. Physiotherapy and occupational therapy can be undertaken to improve limb function. Limb defects have been
estimated to result in a total cost of $167-$170 million\textsuperscript{47}. The cost per new case is estimated as $199 and $99,000, for lower-limb and upper-limb respectively\textsuperscript{47}.

\subsection{2.5 URINARY TRACT DEFECTS}

Urinary tract defects include abnormalities of the kidney and urinary tract. Abnormalities of the kidney include unilateral or bilateral renal agenesis (absence of kidney), horseshoe kidney (fusion of the poles of 2 kidneys), duplex kidneys (kidneys with 2 pelvic structures and 2 ureters), multiple ureters, renal hypoplasia (reduced kidney size), and dysplasia (kidney with abnormal structures).

Abnormalities of the tract include pyelectasis (dilation of the renal pelvis to 10mm), hydronephrosis (dilation of the renal pelvis more than 10mm), posterior urethral valves, megaureter, vesicoureteral reflux (retrograde flow of urine into the upper tracts), pelviureteric junction stenosis (obstruction between the renal pelvis and ureter), and congenital urethral obstruction\textsuperscript{127}.

Congenital urethral obstruction of the posterior urethral valves can result in a variety of problems that vary in severity depending on the timing and degree of obstruction as well as the anatomy of the upper tracts\textsuperscript{127}. Urethral obstructions include bladder agenesis (absence of bladder), bladder extrophy (incomplete closure of interior abdominal wall), bladder obstruction, and urachal sinus or vesicourachal diverticulum (urachus remains open and communicates with the bladder). When fetal renal tissue persists amongst matured renal tissue it is called a nephrogenic rest. Nephrogenic rests are observed in 1\% of infant
kidneys at autopsy and less than 1% of these nephrogenic rests will develop into Wilms’ tumor \(^{128}\).

### 2.5.1 Incidence

Approximately 10% of newborns have developmental abnormalities of the urinary tract; however, the majority do not result in clinical problems \(^{127}\). Congenital abnormalities of the kidney and urinary tract occur in 1/500 live births \(^{129}\). Unilateral renal agenesis occurs in 1/1,000 live births, whereas bilateral agenesis occurs in 1-3/10,000 live births \(^{130}\). Renal agenesis has been reported to occur more often in males and is also more often observed on the left side \(^{130}\). Renal dysplasia occurs in 2-4/1,000 births \(^{130}\). Kidney abnormalities of shape and position are the most common \(^{131}\). Horseshoe kidney is the most common renal fusion abnormality that occurs in 1/500 live births \(^{130}\). It is observed more often in boys than girls \(^{131}\). Fetal pelvic dilation is observed in approximately 1-5% of pregnancies \(^{132}\). Posterior urethral values occurs in 1/5,000-8000 births and is often observed in boys \(^{130}\). Bladder extrophy occurs in 1/10,000-40,000 births \(^{130}\). Bladder agenesis is rare and incompatible with life \(^{130}\).

### 2.5.2 Epidemiology

Approximately half of all cases of childhood renal failure result from anomalous development of ureteric bud or metanephros \(^{130}\). An important process of kidney formation is the signaling process that results in the outgrowth of the ureter from the mesonephric duct \(^{129}\). Defects during this induction period
can result in the loss or gain of function, resulting in renal agenesis or the formation of multiple ureters \(^{129}\). Agenesis occurs before the fifth week of gestation and can be due to either genetic or environmental effects \(^{130}\). Renal agenesis has been reported to be associated with failure of GDNF-RET signaling \(^{133}\). There is a 5% recurrence in affected families \(^{130}\). Malformations of the ureter are the most common abnormalities in kidney formation.

Nephron precursor cells transition from mesenchyme to epithelial cells, forming the renal vesicle. Defects in nephron induction of the mesonephric duct or metanephric mesenchyme can result in nephrogenic rests which are believed to be precursors of Wilms’ tumours \(^{129}\). Wilms’ tumor affects 8/1,000,000 individuals \(^{129}\).

### 2.5.3 TREATMENT AND PROGNOSIS

Congenital abnormalities of the kidney and urinary tract can result in renal failure or result in increased risk for hypertension or cardiovascular disease later in life \(^{129}\). Defects in nephron formation can increase the risk for Wilms’ tumour \(^{129}\). Urethral obstruction is usually treated with ablation in the first day of life because it may result in death in infancy \(^{134}\). Duplex kidneys usually have no renal impairment; however some may result in the presence of vesicoureteral reflux or obstruction \(^{132}\). Vesicoureteral reflux is associated with urinary tract infection and renal scarring \(^{127}\). It usually disappears with growth; however there is an increased risk of urinary infection, hypertension, functional deterioration of the kidney, pyelonephritis, and renal scarring \(^{127,135}\). Bladder agenesis is rare.
and incompatible with life. Bladder extrophy is surgically correctable; however patients with extrophy have a 700 times higher risk of bladder carcinoma\\textsuperscript{130}.

### 3 PEDIATRIC CANCERS

Cancer is the second leading cause of death in children 1-14 years old in the United States (U.S.)\\textsuperscript{136}. In 2008, it is estimated that 10,730 U.S. children 0-14 years will develop cancer\\textsuperscript{137}. Approximately 10% of all cancers diagnosed in children less than 15 years of age are during infancy\\textsuperscript{138}. Infantile cancers are more often diagnosed during infancy than in adulthood\\textsuperscript{138}. Common pediatric cancers include embryonal tumors e.g. neuroblastoma; brain tumors e.g. medulloblastoma; hepatoblastoma; retinoblastoma; and Wilms’ tumor\\textsuperscript{138}. The Canadian Cancer Society reports that the overall incidence of childhood cancers have remained relatively stable from 1985-2008 and the mortality rates have decreased\\textsuperscript{139}.

Causes that have been implicated in neonatal and infantile cancers include genetic, intrauterine, environmental, and transplacental sources\\textsuperscript{140}. The Surveillance Epidemiology and End Results (SEER) program reported that the incidence of pediatric cancers was 183.4/1,000,000 in 1970 to 189/1,000,000 in 1980 and 220/1,000,000 in 1990\\textsuperscript{141}. Of the 220 cases diagnosed in 1990, approximately 17% were neonatal tumors\\textsuperscript{141}. Congenital cancers have an estimated prevalence of 1/12,500 to 13,700 total births\\textsuperscript{142}. It is estimated that
7,000 children are diagnosed in the United States annually with 10% diagnosed in the first year of life, 2% during the first month, and 1% on the first day of life.

### 3.1 LEUKEMIA

Childhood leukemia is a cancer of the hematopoietic system. The majority involve malignant transformation of lymphoid progenitor cells. The major morphological subtypes of leukemia include acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML). Acute leukemia is the most common childhood cancer. It accounts for approximately 35% of all childhood cancers under 15 years of age and 12% of all childhood cancers in children 15-19 years of age. Of the estimated 2,500 annual cases, 80% are acute lymphoblastic leukemia, 15% are acute myelogenous leukemia, and 5% are chronic leukemia. Leukemia occurs twice as frequently as solid tumors in children 1-14 years of age, whereas in infants solid tumors occur five times as frequently.

The word leukemia is derived from the Greek word meaning “white blood”. These cancers are of the blood-forming or hematopoietic tissues. In the hematopoietic system, undifferentiated pluripotent stem cells in the bone marrow proliferate, differentiate, and mature in the myeloid and lymphoid cell lines. Myeloid cell mature to form red blood cells, monocytes, granulocytes and platelets, while lymphoid cells mature to form B cells and T cells. Altered hematopoiesis occurs in leukemia resulting in arrested cell development.
Leukemia can occur at any stage of the hematopoietic system. It can spread from the bone marrow into blood, lymph nodes, spleen, liver, central nervous system, and other organs. Leukemia arises from a neoplastic transformation of a single cell which is expanded by cell division. The most likely cell to undergo transformation is a pluripotent stem cell or a progenitor cell\textsuperscript{148,148}.

Congenital and neonatal leukemia (in infants under one month of age) occurs in 4.7/1,000,000 live births per year, representing less than 1\% of all childhood leukemia\textsuperscript{149}. Leukemia is the second most common congenital malignancy after neuroblastoma\textsuperscript{149,150,151}. Congenital leukemia has a higher mortality rate than any other congenital cancer\textsuperscript{149,150,151,152,152}. Congenital leukemia can be distinguished from leukemia that occurs in older children as it presents with profound leukocytosis, central nervous system involvement, unfavorable morphology, leukemia cutis, and abnormal karyotypes, all of which are associated with poor prognosis\textsuperscript{147}.

### 3.1.1 ACUTE LYMPHOBLASTIC LEUKEMIA

Acute lymphoblastic leukemia (ALL) is a rapidly proliferative cancer that is characterized by a large number of immature lymphocytes\textsuperscript{153}. The rapid multiplication of lymphoblasts results in the replacement of normal cells. In addition it can take over normal parts of the bone marrow, resulting in bone marrow failure\textsuperscript{153}.
3.1.1.1 INCIDENCE

ALL is the most common childhood malignancy \(^{154}\). It comprises of one-fourth of all childhood cancers and three-fourths of all newly diagnosed leukemia \(^{155}\). Approximately 60% of cases are diagnosed in the first five years of life \(^{156}\). Approximately 2,500-4,000 cases of ALL are diagnosed annually in the United States, of which two-thirds are diagnosed in children and adolescents \(^{157,158,159}\).

In the United States an incidence of 3-4/100,000 children under 15 years of age have been reported \(^{154,160}\). ALL is the most frequent form of cancer in Canadian children under 20 years of age \(^{161}\). Approximately 250 cases are diagnosed per year \(^{161}\). This accounts for 20% of all childhood cancers diagnosed in Canada \(^{161}\). It is estimated that 400-450 new cases of ALL are diagnosed in the United Kingdom each year \(^{162}\).

ALL has a distinctive age distribution \(^{163}\). An increased incidence is observed after birth which peaks between two and five years of age followed by a decline to a steady rate and then an increase incidence later in life is observed \(^{163,164}\). This is mainly due to pre B-ALL cases (referred to as "common ALL") in this age range \(^{164}\). Infant ALL accounts for 2.5-5% of all childhood leukemia \(^{165,166,167,168,169}\).

Leukemia is more prevalent in boys compared to girls \(^{145}\). This is especially marked in adolescent boys with T-cell ALL \(^{170}\). ALL is more common in the Caucasian population compared to African Americans \(^{171}\). Frequencies are reported ranging from 13%-18% in Asian populations \(^{172,173}\).
3.1.1.2 ETIOLOGY AND PATHOLOGY

The etiology of ALL is unknown for the majority of cases. Relationships have been drawn to ionizing radiation and some rare genetic abnormalities. Genetic syndromes including Down syndrome, Bloom's syndrome, Fanconi's anemia, Neurofibromatosis, Klinefelter's syndrome, Immuno-deficiency and Ataxia telangiectasia, trisomy G, and Schwachmann's syndrome, have been associated with increased risk for ALL. Down syndrome, alone, is associated with a 10-20 fold increased risk of both ALL and acute myeloid leukemia (AML). Studies investigating in utero and postnatal environmental factors have suggested that leukemia is related to ionizing radiation, some antineoplastic agents, and certain toxic chemicals. The only confirmed cause associated with ALL is ionizing radiation.

Epidemiologic studies have failed to identify associations with other environmental factors including electromagnetic fields, radon exposure, pesticides, and maternal smoking.

Leukemia results from genetic mutation and transformation of a single hematopoietic progenitor cell during maturation. Approximately 85% ALL are of B-cell lineage, of which 2-3% are mature B-cell ALL expressing surface immunoglobulin and CD20+. Approximately 20-30% have pre-B-cell phenotype with presence of cytoplasmic but no surface immunoglobulin—an intermediate stage of B-cell differentiation. The remaining cases are early pre B-cell type (B-precursor ALL) which lack immunoglobulin expression and are identified by
common ALL antigen (CD10) and terminal deoxynucleotidyl transferase (CD19). Approximately 15% of ALL are of T-cell lineage (the stage of thymic differentiation). The majority of the cases have an intermediate or mature phenotype. T-cell ALL is often diagnosed in teenage males with leukocytosis, meningeal involvement, and mediastinal lymphadenopathy and has similar prognosis to B-cell ALL.\textsuperscript{184}

Leukemic cells often express markers of their hematopoietic lineage. Approximately 7-25% express myeloid markers.\textsuperscript{185} Non-random genetic abnormalities have been identified in childhood ALL.\textsuperscript{186}

Cytogenic chromosomal abnormalities have been observed in ALL patients. Due to the chromosomal abnormalities exhibited in congenital and neonatal ALL it is postulated that leukemia may arise due to chromosomal fragility. Leukemia are characterized by chromosomal translocations that involve over 200 genes.\textsuperscript{147} Approximately 80% of all infant acute leukemia is observed with chromosomal translocations of the MLL gene at 11q23.\textsuperscript{147} Abnormalities involving chromosome band 11q23, close to topoisomerase II binding sites suggest that \textit{in utero} exposure to topoisomerase II inhibitors may be associated with fetal chromosome damage, thus increasing the risk of leukemia.\textsuperscript{187} Infant ALL is characterized by a high frequency of 11q23 rearrangements, in particular that of t(4;11)(q21;23).\textsuperscript{165,168,188,189,190,191} The t(4;11) is the best characterized translocation among those leukemia involving the MLL gene. Infants with the t(4;11) have a significantly worse prognosis than older children with the same translocation.\textsuperscript{192,193} Translocation t(12;22), the most common cytogenetic
abnormality in childhood ALL but is not detectable by routine karyotyping analysis \(^{194}\). In contrast to pediatric leukemia, few cases of infant ALL have a hyperdiploid karyotype, the \(t(1;19)(q23;p13)\) or the Philadelphia chromosome. In addition, blast cells of infants are less likely to express the CD10 and CD 34 antigens \(^{165,168,188,190,196,197,189,190}\).

The leukemic process may originate \textit{in utero} \(^{147}\). Evidence of leukemic cells at birth in autopsies of stillborns with leukemia and diagnosis of leukemia in monozygotic twins with karyotypic abnormalities support \textit{in utero} leukemogenesis \(^{198}\). There is a high concordance rate of leukemia in identical twins—if one develops leukemia, there is a 25% risk that the twin will develop leukemia as well \(^{199}\). The concordance in genotype of in monozygotic twins diagnosed with leukemia has been as great as 100% in children under one year of age \(^{199}\). Characteristics of the leukemic blasts of each pair of twins have been found to be identical in twin pairs who have presented with leukemia at time periods ranging from birth to 15 months of age. One plausible explanation for this phenomenon is that an \textit{in utero} leukemogenic event occurred in one twin and shared placental circulation allowed the transfer of the leukemic clone and its establishment in the second twin \(^{200,201,202}\).

There is evidence suggesting that childhood ALL can be initiated prenatally, demonstrated by characteristic chromosomal translocations that are present at birth \(^{203,204,205,206}\). Evidence of the prenatal origin of ALL was demonstrated by Gale \textit{et al.} in a retrospective study where they backtracked MLL/AF4 fusion gene, a leukemo-specific clonal marker, to Guthrie cards (dried
blood spots on filter-paper obtained by heel prick) of three children who developed ALL at the ages of five months to two years \(^{204}\). Guthrie cards positive for the TEL/AML1 (ETV6/RUNX1) were observed in more than ten ALL patients with this fusion gene \(^{205,207,208,209}\). The TEL-AML fusion occurs in approximately 25\% of common ALL in children diagnosed between two and ten years of age \(^{210}\). Congenital leukemia has also been associated with chromosomal abnormalities including Down syndrome, trisomy 9 and trisomy 13 \(^{147,211,212,213}\).

An alternative explanation for the origin of congenital leukemia involves the "two-hit" hypothesis \(^{214,215}\). The two-hit hypothesis suggests that a germline or somatic mutation in an allele of a gene occurring at an early stage of embryonic development would result in increased susceptibility such that a subsequent mutation or exposure to another agent would result in the loss of heterozygosity, resulting in the development of leukemia.

Genes involved in leukemia include mixed lineage leukemia (MLL) (gene on chromosome 11), translocation-ETS-leukemia (TEL)(gene on chromosome 12) and AML1 \(^{147}\). There is strong evidence supporting that the rearrangements of MLL gene or TEL-AML1 gene fusion occurs \textit{in utero} \(^{147}\). TEL-AML1 is a fusion protein that inhibits normal AML1 transcriptional activity resulting in the alteration of self-renewal capacity and differentiation capacity of hematopoietic stem cells \(^{216}\). Infants with ALL often have acquired ALL1/MLL/HRX gene fusions as the major consistent genetic abnormality \(^{213,179,217}\). The frequency of the ALL1/MLL/HRX gene fusion in infants can be as high as 75\%.
ALL in infancy and in older children appear to be clinically distinct. ALL of infancy is associated with a high leukocyte count at presentation, hepatosplenomegaly and central nervous system involvement. Taken together, all these aspects taken together suggest that the classic form of infant ALL originates in a stem cell that has not fully committed to lymphoid differentiation.

Little is known about the etiology of childhood leukemia. The risk of childhood ALL is higher among white males, children aged two to five years, and in children with certain congenital disorders. Only exposure to in utero ionizing radiation and some genetic syndromes, such as Down, are considered causal and, together, are estimated to account for only 5–6% of childhood ALL cases.

It is hypothesized that the link between folate deficiency and cancer risk is due to the impairment of DNA synthesis and repair, and alteration of DNA methylation. Usually, folate, in the form of 5,10-methylenetetrahydrofolate, acts as a methyl donor for the conversion of deoxyuridylate to thymidylate. However, in conditions where folate levels are diminished then this process is impaired and may result in the misincorporation of uridine into DNA, leading to chromosomal aberrations and malignant transformations. In addition to folate levels, polymorphisms in folate dependent enzymes have been linked to various types of cancer including ALL in children.
There has been a dramatic increase in the survival rates of children affected with ALL in the last 40 years. Previously associated with a poor prognosis (15%), childhood T-cell ALL or mature B-cell ALL with the improvement of treatment, has a cure rate of approximately 80% in diagnosed children. On the other hand children living in disadvantaged countries without adequate treatment have a cure rate of less than 35%. African-American children have the same cure rates as Caucasian children when given equal access to effective treatment. Male children have also been shown to better prognosis when receiving treatment.

Age and leukocyte count at diagnosis are strong indicators of ALL prognosis. Prognosis decreases with increased age and increased leukocyte count. Congenital ALL has a 20-30% survival rate whereas older children have an 80% survival rate. Event-free survival rates in adolescents 15-20 years of age have improved.

Poor prognosis has been attributed to unfavorable features at presentation (high white count and extramedullary involvement, including frequent CNS disease), but there may be other characteristics which may affect prognosis, such as mechanisms of drug resistance. Children between 1-9 years of age with a leukocyte count of <50x10^9/L at standard risk.
Genetic abnormalities of leukemia have an effect on the prognosis. Different biologic subgroups have different event-free survival rates from 20% in cases with the BCR-ABL1 or MLL-AF42 oncogene fusions to 90% in those with the TEL-AML1 fusion. Cytogenetic subtypes of ALL are often observed in children 2-9 years of age and are associated with low leukocyte count. These are associated with a good prognosis and high cure rate. Hypodiploidy with less than 45 chromosomes and 24-28 chromosomes (near-haploidy) are associated with increased risk factors. Hypodiploidy (<2% pediatric cases) (<45 chromosomes per leukemia cell) confers a poor outcome. Prognosis is worse for low hypodiploidy (33-39 chromosomes) or near-haploidy (23-29 chromosomes). B-cell precursor ALL with hyperdiploidy (>50 chromosomes per leukemia cell) and t(12:21) with the TEL-AML1 (a fusion gene that includes the 5 portion of TEL (translocation-ETS-leukemia) gene on chromosome 12, which encodes a nuclear phosphoprotein that is a member of the ETS family of transcription factors, the majority of the coding region of AML1, and another transcription-factor gene that encodes the alpha subunit of core-binding factor which regulates the formation of definitive hematopoietic stem cells) fusion gene comprises of 50% of childhood cases and has a favourable prognosis. Translocation t(12:22), the most common cytogenetic abnormality in childhood ALL is not detectable by routine karyotyping analysis. However the translocation t(12:22) is associated with a good prognosis.

Hyperdiploidy with 51-65 chromosomes is also associated with a good prognosis. The increase in chromosomes are often associated with
Intracellular accumulation of methotrexate polyglutamates is increased in these leukemic cells which results in prolonged retention of antifolate activity of methotrexate.  

Approximately <20% children with favourable genetic features (>50 chromosomes hyperdiploidy and TEL-AML1 fusion) and relapse will occur in 1/3 of individuals with high-risk abnormalities (Philadelphia chromosome with BCR-ABL fusion t(4:11) with MLLAF4 fusion) which may be cured with chemotherapy.  

Various genetic subgroups can determine prognosis of leukemia.  Age influences the prognosis of genetic lesions.  Patients with t(4:11) with favourable age or good initial treatment may have a favourable outcome than others.  The fusion of t(4:11) with the MLL-AF4 [a chimeric protein consisting of the N-terminal portion of MLL (mixed-lineage leukemia) encoded by the gene on chromosome 11 and the C-terminal portion of AF4 (ALL1 fused gene from chromosome 4)] disrupts the normal expression pattern of homeobox genes, causing a change in the self-renewal and growth of hematopoietic stem cells and committed progenitor cells.  Poor prognosis is associated with t(4:11) with the MLL-AF4 fusion gene with poor prognosis in infants compared to older children.  T-cell ALL with t(11:19) with MLL-ENL fusion and overexpression of HOX11 is associated with a good prognosis.  Structural abnormalities of chromosome band 11q23 is associated with a poorer prognosis.
Translocations involving protooncogenes on chromosome 8, 9, and 22 are also associated with a poor prognosis. Mature B-cell ALL is associated with the translocation of MYC protooncogene from chromosome 8 to an immunoglobin gene either the heavy chain on chromosome 14 or light chains on chromosome 2 and 22, respectively. The Philadelphia chromosome t(9:22)(q34:q11) translocation is in 3-5% of childhood ALL. The Philadelphia chromosome is associated with <20% disease-free survival rate with intensive conventional chemotherapy. Allogeneic hematopoietic stem cell transplantation is currently the only therapy that has altered the long-term outcome of this form of ALL. Prognosis is better in children 1-9 years of age compared to adolescents and adults. Subsets of patients with Philadelphia chromosome (presenting with low leukocyte count), 1-9 years of age, with good initial response to therapy are able to be cured with intensive chemotherapy without allogeneic stem cell transplantation.

Translocation t(1:19)(q23;p13) is the most common translocation in childhood ALL occurring in 25% of children with pre-B-cell ALL. Despite being the most common cytogenetic abnormality in this subtype, it is associated with high incidence of treatment failure. Intensive chemotherapy has improved the outcome of a subset of these patients to rates of those without cytogenetic abnormalities. Patients with B-cell ALL have poor prognosis with conventional ALL therapy but have an 80% survival rate when treated 3-6 months of rapid-sequence, intensive chemotherapy.

Treatment of ALL is directed according to phenotype, genotype, and risk.
ALL is highly responsive to chemotherapy. ALL is the only leukemic subtype that
is treated with short-term intensive chemotherapy\textsuperscript{267,268}. Total therapy process is
used to treat ALL. Total therapy for childhood leukemia was first described by
George \textit{et al.} \textsuperscript{269}. Although treatment duration may differ, it consistently includes
four stages: remission-induction therapy, followed by intensification/consolidation
therapy, central nervous system (CNS) prophylaxis or preventative therapy, and
maintenance or continuation treatment. The average duration of treatment of
ALL is 2-2 ½ years\textsuperscript{270,271}.

The objective of remission-induction therapy is to eradicate more than
99% leukemic cells and restore normal hematopoiesis and performance status.
Approximately 97-99\% of children achieve morphologic marrow remission after
four weeks of induction. Complete remission occurs in 98\% of children. When
normal hematopoiesis is restored intensification/consolidation therapy follows.
Intensification/consolidation therapy is administered after remission induction to
eradicate residual blast cells and to try to overcome drug resistance. Evidence
has suggested that this treatment procedure has improved long-term survival in
high-risk patients\textsuperscript{167,169,179,236,272}. Giving one or more courses of rotational,
multiagent chemotherapy during the first 6-8 months after diagnosis has been
demonstrated to reduce the risk of relapse\textsuperscript{272,273,274}. Reinduction treatment is a
repetition of induction therapy initially received and is administered during the
first few months of remission\textsuperscript{226,243,275}.

Maintenance/continuance therapy is done when remission is achieved.
Prolonged maintenance therapy is an integral part of ALL protocols. This
process is 2-2.5 years in duration. This period may be longer in girls than boys. Without maintenance/continuance therapy there is a greater chance for relapse within 2-4 months.

CNS preventative therapy is considered as leukemia may have subclinical CNS involvement at the time of diagnosis. The CNS can act as a sanctuary site of leukemic cells because the blood brain barrier can protect them from systemic chemotherapy. Therefore it is important to institute CNS prophylaxis at an early stage to eradicate leukemic cells which may have passed the blood brain barrier. However a problem with CNS preventative therapy is the possibility of long term neurotoxicity and development of brain tumors.

Irradiation is associated with the development of secondary neoplasms and increased risk of mortality. It is usually recommended for patients at high risk of relapse [T-cell ALL with leukocyte counts of $100 \times 10^9$ per liter / $>100,000/mm^3$ (extreme hyperleukocytosis), Philadelphia chromosome positive ALL and presence of CNS leukemia at diagnosis]. One study reported that children who did not receive radiation therapy and had 10 or more years of event-free survival. Intrathecal therapy has been proposed as an alternative method of treatment in order to avoid cranial irradiation.

Stem cell transplantation is for high-risk conditions and for relapsed cases. Stem cell transplantation has been effective for preventing further leukemia recurrence. Transplantation is beneficial for individuals with BCR-ABL+ ALL or in persons who had a poor initial response to treatment.
Adverse events resulting from treatment usually occur within first decade after diagnosis. Factors increasing the risk for relapse in the central nervous system include high-risk genetic features, T-cell immunophenotype, a large leukemia-cell burden, and the presence of leukemia cells in the cerebrospinal fluid (even from iatrogenic introduction through a traumatic lumbar puncture). A proportion of patients will die of leukemic relapse, a second cancer, or treatment-related complications during the five years.

3.2 EMBRYONAL CANCERS

3.2.1 CENTRAL NERVOUS SYSTEM MALIGNANCIES

Tumors of the central nervous system are the second most common cancer in childhood. Brain tumors are the second most common cancer in children after leukemia. Central nervous system malignancies account for 20% of childhood cancer in developed countries. Brain tumors are fifth among cancers of the neonate.

Intradural intramedullary tumors are derived from neuroepithelial tissues and divided into neuronal, glial, and primitive neuroepithelial subgroups. Neuronal tumors include gangliocytomas. Glial tumors include astrocytomas, ependymoma, and oligodendrogliomas. Primitive neuroectoderm tumours (PNETs) include medulloblastoma.

Astrocytomas and PNETs are the most common gliomas in children. PNETs are embryonal tumors composed of undifferentiated or minimally
differentiated neuroepithelial cells which have the capacity to differentiate to astrocytes, ependymal cells, melanocytes or muscle cells\textsuperscript{299,300,301}. PNETs are rare, small, malignant, blue round-celled neoplasms of the central and peripheral nervous system which are neoplastic transformations of primitive neuroepithelial cells\textsuperscript{299,302,303,304,305}. Hart et al. first used PNETs to describe undifferentiated cerebral tumors\textsuperscript{306}. Rorke and Becker et al. later defined PNETs as a central nervous system tumors predominantly composed of primitive neuroepithelial cells that are classified by their cellular differentiation\textsuperscript{307,308}.

Medulloblastoma is also known as PNET of the posterior fossa/cerebellum. Medulloblastoma and PNETs are histologically similar but they occur in different areas of the central nervous system\textsuperscript{297}.

Supratentorial primitive neuroectodermal tumors (SPNET) are rare embryonal tumors of the central nervous system that account for 2.5% of childhood brain tumors\textsuperscript{309}. They are similar to medulloblastoma; however, they have poorer response to conventional therapy\textsuperscript{309,310,311}.

They are identified based on their localization into Ewing sarcomas, lymphomas, rhabdomyosarcomas, medulloblastomas, undifferentiated small-celled carcinomas and small-celled tumors of thoracic-pulmonary origin or Askin tumors\textsuperscript{305}.

3.2.1.1 INCIDENCE

Brain and other central nervous system tumors are the most common solid tumors in children and the second most common type of pediatric cancer
According to a worldwide survey, the ten most common types of brain tumors in infancy are astrocytoma, medulloblastoma, ependymoma, choroid plexus papilloma, PNET, teratoma, sarcoma, meningioma, ganglioglioma, and neuroblastoma. Astrocytoma is the most common type of central nervous system tumor (40%), followed by medulloblastoma/PNET (22%), other gliomas (12%) and ependymomas (10%).

Brain tumors account for approximately 7.2% of all neonatal cancer. Neonatal intracranial tumors has been reported to range from 0.5-11% of childhood brain tumours. CNS tumors are highest in children under five years of age. In the first year of life they account for up to 10%, and in the first two years for up to 18%, of the total incidence of pediatric brain tumors. Central nervous system tumours occur less often in the fetus and neonate than in older children. Approximately 18% of brain tumours present during the first year of life are diagnosed in the perinatal period. PNETs most commonly occur in children than adults. The majority of childhood CNS tumors are diagnosed before five years of age, although a second peak in incidence at 13 years of age is seen for astrocytoma.

The incidence of astrocytic glioma and medulloblastoma/PNET differ in age and gender, suggesting different etiologies. Astrocytomas are more benign in young children. Approximately half of all medulloblastoma/PNET in children is diagnosed before six years of age. Medulloblastoma are more aggressive in infancy than older children. Medulloblastoma are common brain...
tumors in children but rare in adults. The incidence of brain tumours is higher in male children. Annual incidence of brain tumors vary from decades, institution, and countries. The annual incidence is approximately 34-36 per million children under 15 years of age. Approximately 40,000 children worldwide develop CNS tumors. In Canada, brain tumours account for 22% of new cases of cancers in children less than 15 years of age, translating to approximately 230 children per year. In the United States, brain tumours account for approximately 20% of all childhood cancers. The average annual incidence rate in the United States is approximately 30/1,000,000 children under 19 years of age. In the United Kingdom the incidence of primary malignant brain tumors is 2.02/100,000. A German cancer registry reported an incidence of 3.6/100,000 births. In Japan the incidence is 4.1/100,000 births. Overall the incidence of central nervous system tumors ranges between 24-27/1,000,000 children each year. Recent reports have observed an increased incidence of childhood brain tumours in developed countries.

3.2.1.2 ETIOLOGY AND PATHOLOGY

Categories of pediatric tumors include embryonal tumors, glioneuronal tumors, and others (including germ cell tumors, primitive neuroectodermal tumors, and craniopharyngiomas). Congenital or perinatal brain tumors differ from tumors presenting in later childhood and adulthood in location, clinical presentation, histological distribution of tumor types, tumor characteristics, and
prognosis. Primary intracranial tumors in older children are often located posterior fossa (brainstem and cerebellum) whereas fetal and neonatal tumors are mostly located above the tentorium. Fetal and neonatal tumors are associated with macrocephaly, dystocia, stillbirth, and intracranial teratomas. Intracranial teratomas are found in at least one-third of perinatal tumor cases. Approximately 55% brain tumors arise in the posterior fossa, or are infratentorial, and 45% are supratentorial.

Glial neoplasms (gliomas) are the most common brain tumors of children. Since these tumors display histological characteristics of their mature central nervous system glia they are named similarly: astrocytoma (astrocytes), oligodendroglioma (oligodendrocytes), and ependymoma (ependymal cells).

Astrocytomas, the most commonly observed neuroglial tumors in infancy and childhood, are composed of astrocytes with varying differentiations.

Pilocytic astrocytoma is a benign form of glioma that occurs commonly in childhood that is characterized by tightly packed piloid (hairlike) tumor cell processes. They often are located in the cerebellum. The majority of pilocytic astrocytomas have normal karyotypes; however, chromosomal alterations have been reported including deletions on the long arm of chromosome 17p and a silent p53 mutation (exon 9, codon 324).

Diffuse (fibrillary) astrocytomas are often located in white matter and often extensively invade adjacent gray matter structures. Consequently they are difficult to eradicate by surgery alone because of poor demarcation between tumor and surrounding normal tissues. Chromosome abnormalities...
in 9, 13, 17 are observed in malignant childhood astrocytomas (anaplastic astrocytoma and glioblastoma)\textsuperscript{353}. Glioblastomas have two pathways of development, approximately 95% are primary, whereas the remaining 5% are secondary, developing in patients with a diagnosis of astrocytoma\textsuperscript{356,357}.

Brainstem gliomas comprise 8-10% of all pediatric central nervous system tumors\textsuperscript{358}. Approximately 80% of brainstem gliomas are infiltrating and 50% are malignant\textsuperscript{298}. Chromosome abnormalities reported in brainstem gliomas include loss of portions of chromosome 17p, mutations of p53 and allelic losses of chromosome 10q\textsuperscript{359}.

Oligodendrogliomas are infiltrating glial neoplasms primarily composed of oligodendrocytes\textsuperscript{360}. They are often located in the cerebral hemispheres but can also be found anywhere in the neuraxis which may be associated with increased intracranial pressure and the occurrence of seizures in these patients\textsuperscript{359,361,362,363,364}. Chromosomal abnormalities reported in oligodendrogliomas include allelic deletions of chromosomes 1p and 19q\textsuperscript{365,366}.

Ependymomas comprise more than 50% of tumors found in children less than five years of age\textsuperscript{353,367}. They are often formed from remnants of the ependymal lining in the ventricular system of the posterior fossa, and often arise in the fourth ventricle but can also arise in the lateral ventricles\textsuperscript{353,368}. They can cause hydrocephalus, increased intracranial pressure, and areas of hemorrhage\textsuperscript{368}. Chromosomal abnormalities reported with ependymomas include monosomy and deletions of chromosome 22, trisomy of chromosome 7, loss of sex.
chromosomes, and structural rearrangements of chromosome 2\textsuperscript{359}. All ependymomas carry the risk of metastasis and recurrence\textsuperscript{358}.

Gangliogliomas are made of two components: a well-differentiated neoplastic astroglial element and mature neurons including large vesicular nuclei; prominent nucleoli; cytoplasmic Nissl substance; immunoreactivity for neuronal antigens, such as neurofilament protein and synaptophysin; and have little proliferative activity\textsuperscript{369}. Approximately 75% gangliogliomas are located in the temporal lobe but they can also be found in the brainstem, spinal cord, optic nerve and chiasm, pineal gland, and cerebellum\textsuperscript{353}. Calcification may occur in varying degrees and gangliogliomas tend to occur in clusters\textsuperscript{370}. No genetic abnormalities have been reported with gangliogliomas\textsuperscript{353}. Desmoplastic infantile gangliogliomas account for 0.1% of primary intracranial tumors\textsuperscript{371}. They are huge, well circumscribed, supratentorial masses that are superficially located and often involve the leptomeninges\textsuperscript{353}. They often occur in patients less than two years of age\textsuperscript{353}.

Embryonal tumors include the medulloblastoma and related primitive neuroectodermal tumors, medulloepithelioma, and CNS atypical teratoid/rhabdoid tumor\textsuperscript{353}.

Medulloblastomas arise from the vermis of the cerebellum and grow into the fourth ventricle and adjacent cerebellar hemispheres\textsuperscript{368}. As such these tumors may effect the posterior fossa\textsuperscript{358}. Chromosomal abnormalities reported with medulloblastomas include loss of portions of chromosome 17p due to deletions, breakage, unbalanced translocations, or homologous recombination.
Cmyc protein expression has also been identified in medulloblastomas and PNET tumor specimens and cell lines. PNETs are among the most common malignant solid tumor of childhood. They are malignant tumors that have glial and neuronal features. This poorly differentiated tumor has hyperchromatic nuclei and poorly defined cell borders. PNETs are usually located in the cerebellum but can also arise in other areas of the cerebrum and peripheral nerves. In rare instances, PNETs have been reported in spinal and intramedullary localizations. PNETs often result in ventricular obstruction and increase intracranial pressure. Chromosomal abnormalities commonly reported with PNET include the loss of heterozygosity of chromosome 17p (30-50%) which is associated with a poor prognosis.

The most common intracranial germ cell tumor is the germinoma. Germinomas are biphasic tumors containing a neoplastic germ cell component and a non-neoplastic small mature lymphocyte component. Approximately 5%-50% of germinomas contain syncytiotrophoblastic tumor giant cells. Other germ cell tumors include embryonal carcinoma, endodermal sinus tumor (yolk sac tumor), choriocarcinoma, and teratoma (mature and immature).

Little is known about the etiology of childhood brain tumors. Inconclusive results have suggested associations with exposures to dietary N-nitroso compounds, pesticides, farm animals, paternal smoking, and parental occupation.
There is limited evidence suggesting that a family history of cancer is more common among families of childhood brain tumor patients. A study in twins suggested that there is not a strong genetic component for childhood CNS tumors. A Swedish population-based cohort study observed an increase risk of astrocytoma development in first-degree relatives with astrocytoma. This may be due to genetic mutations such as germline mutations of the p53 gene. A study of gliomas observed a higher rate of chromosome instability (the mean number of spontaneous breaks per 1,000 peripheral blood lymphocytes) in patients compared to controls.

Brain tumors have been associated with other conditions such as Li-Fraumeni syndrome, Gardner syndrome, neurofibromatosis (von Recklinghausen disease), tuberous sclerosis, Turcot syndrome, and nevoid basal cell carcinoma syndrome (Gorlin syndrome).

Investigation into maternal consumption of cured meats and brain tumours were investigated because cured meats are a dietary source of nitrates. N-nitrosamides are formed from N-nitroso compounds. N-nitrosamides are alkylating compounds that can result in the formation of DNA adducts. Several epidemiological studies have associated maternal cured meat intake during pregnancy to be associated with elevated risk of childhood brain tumors. Conversely one study reported that maternal consumption of cured meats was not associated with medulloblastoma/PNET. Another study reported that maternal consumption of cured meats were not associated with medulloblastoma/PNET, in contrast to other childhood brain tumors. A
decreased risk for medulloblastoma/PNET was associated with maternal consumption of fruit (OR=0.5, 95% CI 0.3-0.8)\textsuperscript{314}. Vitamin C and E are nitrosation inhibitors that reduce the conversion of nitrite to nitric oxide thus blocking the formation of N-nitroso compounds\textsuperscript{401,402,403,404}.

Maternal consumption of fruits and vegetables has been associated with a decreased risk of brain tumours. One study reported that frequent maternal consumption of fruits and vegetables and high intake of vitamin C and folate during pregnancy was associated with a decrease risk for medulloblastoma/PNET\textsuperscript{398}. Another study observed that consumption of fruits and juices may decrease the risk (OR=0.06 95% CI 0.3-1.1) and vitamin C (OR=0.6 95%CI 0.4-1); however, folate and vegetables showed no association\textsuperscript{314}.

Some epidemiological studies suggested that prenatal multivitamin use is associated with reduced risk for brain tumor\textsuperscript{399,400,405}. Decreased risks were observed for PNETs\textsuperscript{398}, astrocytoma\textsuperscript{382}, and studies of all types of pediatric brain tumors combined\textsuperscript{400}. The largest study containing data from North America and Europe observed decreased risk for brain tumor (OR=0.5, 95% CI 0.3-0.8) with supplementation in all three trimesters of pregnancy\textsuperscript{405}.

Environmental factors have been analyzed in epidemiological studies to discern their association with brain tumors\textsuperscript{406}. The studies of parental occupation-related chemical exposures have yielded negative, inconclusive and contradictory results, for mothers exposed during pregnancy\textsuperscript{407}. A study of
mothers who resided on a farm during pregnancy reported a higher risk for PNET (OR=3.7 95% CI, 0.8-23.9; P=0.06)\textsuperscript{382}.

Exposure to high doses of ionizing radiation\textsuperscript{399,408} or radiotherapy for leukemia have been associated with brain tumors\textsuperscript{334,409,410}. Studies of prenatal radiation exposure have been conflicting, and those showing elevated relative risks usually lack sufficient sample size to establish significance. A study of babies born to Japanese atomic bomb survivors exposed \textit{in utero} observed no increased incidence of brain tumors; however, there were risks for other cancers\textsuperscript{411}. There has not been evidence suggesting an association with low-level electromagnetic field exposures\textsuperscript{323,324}. The role of radiofrequency exposures is currently under investigation\textsuperscript{412}.

Up to 15% of human cancers can be attributed to infections: bacterial, viral, or parasitic including Epstein–Barr virus (associated with Burkitt’s lymphoma, Hodgkin’s disease, and nasopharyngeal carcinoma); Hepatitis C (with liver carcinoma); human papilloma virus (HPV) (with cervical cancer); and human immunodeficiency virus (HIV) (with Kaposi’s sarcoma)\textsuperscript{406,413,414}. Inconclusive results have been reported with infection during pregnancy, infancy, or childhood from both self report\textsuperscript{382,415,416} and medical record data\textsuperscript{417,418,419,420}. The studies are also limited by the small number of cases\textsuperscript{421,422}. The effect of simian virus (SV40) also has conflicting results. Vaccines contaminated with SV40 were reported to be associated with increasing the risk for ependymoma and choroids plexus papilloma\textsuperscript{423}. Conversely one study reported no increased risk for brain tumors\textsuperscript{424}. Molecular studies have also had conflicting results. A
study investigating frequencies of SV40 T antigens observed higher frequencies of brain tumor compared to normal samples. Large T antigens were observed in 100% of ependymomas and choroid plexus tumors; however none were measured in oligodendroglial or pineocytoma tumors.

Maternal smoking has been proposed as a possible cause of childhood brain cancer as the carcinogenic components of cigarette smoke can cross the animal placenta. Epidemiological studies investigating the effects of cigarettes and the relation to brain cancer in children have found incongruent results. A recent meta-analysis of studies reported a summary relative risk estimate of 1.05.

Other studies suggest an association with beer (OR=4.0; 95% CI 1.1-22.1, P=0.04) and kerosene for astrocytoma (OR=8.9; 95% CI 1.1-71.1; P=0.04). Since the etiology of brain tumors probably involves the interactions of genes and environmental exposures a large sample size would be needed to discern the etiology of this multifactorial disease.

### 3.2.1.3 TREATMENT AND PROGNOSIS

Surgical removal of the tumor is usually the first step in treatment. The goal of resection is to relieve the intracranial pressure and remove as much of the tumor as possible. The extent of surgical resection of the tumor correlates with prognosis in many cases. Gross total resection is usually associated with improved overall survival. Radical resections are usually the primary method of treatment in children younger than two years of age in order to defer
radiation exposure for as long as possible\textsuperscript{352}. Adverse effects which may arise from surgical intervention include cerebellar mutism (posterior fossa syndrome)\textsuperscript{358}. This is characterized by emotional lability, mutism and high-pitched crying\textsuperscript{358}. Other symptoms may include brainstem dysfunction with dysphagia and cranial nerve palsies, hypotonia, and ataxia\textsuperscript{358}. Some tumors, due to their location and involvement with surrounding matter, are not resectable\textsuperscript{358}. Unfortunately some patients will experience tumor recurrence despite near total or gross total resection\textsuperscript{435,436}.

The majority of brain tumors respond to radiation therapy. Radiation protocols are usually one month in duration and delivered five days a week\textsuperscript{352}. The goal of radiation is to reduce or temporarily stabilize the tumor size, resulting in a better quality of life\textsuperscript{358}. Side effects of cranial radiation are variable and may occur during therapy or after radiation has been completed\textsuperscript{352}. Radiation is associated with neurocognitive effects and endocrinopathies\textsuperscript{358}. Children who receive radiotherapy have demonstrated cognitive decline\textsuperscript{437,437,438}. Advances in radiation oncology such as stereotactic radiosurgery, brachytherapy, and S-dimensional conformal radiotherapy, proton beam therapy, or conformal and intensity modulated radiation techniques have resulted in decreased adverse sequelae to surrounding normal tissue\textsuperscript{358,406}.

Chemotherapy in combination with radiation therapy has demonstrated a better prognosis than radiation alone\textsuperscript{439}. Some tumors such as ependymoma are not responsive to chemotherapy\textsuperscript{358}. The risk and benefits of dosage should be considered with chemotherapy—the dose must be high enough to cross the
blood-brain barrier to reach the target tumor; however, low enough to avoid neurotoxicity including seizures, changes in cerebral white matter, and alteration in mental status\textsuperscript{440}.

Combination therapy (surgery, radiation, and/or chemotherapy) is associated with the best chance of long-term survival\textsuperscript{352}. However, treatment is not as successful as other childhood cancers\textsuperscript{335}.

The prognosis for childhood CNS tumors are poor compared to other common childhood malignancies including, leukemia, lymphomas, and neuroblastoma\textsuperscript{154,299,302,303,304,335,441,442,443,444,445,446}.

Malignant brain tumors are the common cause of cancer mortality in children\textsuperscript{312,335,351,406}. Generally, the prognosis of congenital brain tumors is based on tumor type, size and location of the tumor, histology, stage/metastasis, degree of surgical resection, and condition of the neonate at the time of diagnosis\textsuperscript{352,406}.

Prognosis of medulloblastoma has improved over the last 80 years with the advances of treatment\textsuperscript{447}. Medulloblastoma has transformed from being uniformly fatal to having a relatively good prognosis\textsuperscript{447}. Characteristics of children with better prognosis in recurrent medulloblastoma include patients with tumors that are relatively naïve to adjuvant therapy, patients whose tumors can be completely resected at second surgery, patients who have experienced a single site recurrence, and patients with tumors that have demonstrated sensitivity to chemotherapy\textsuperscript{448,449,359,450,451,452,453}. The treatment of infants with
medulloblastoma is problematic as irradiation is associated with a high risk of intellectual, skeletal, and endocrine sequelae. A study reporting the five-year relative survival rate for children between 1975-1984 and 1985-1994 respectively were 52% and 55% for PNETs, 47% and 57% for other gliomas, and 39% and 56% for ependymomas. Brainstem glioma has a poor prognosis with the median survival ranging from 9-11 months. Only 20% survive past one year. The annual mortality rate for CNS tumors in United States children <15 years of age decreased from 1.0/100,000 deaths in 1973 to 0.78/100,000 deaths in 1994.

Brain tumor survival rates are inversely proportional to age. Prognosis for neonates with brain tumors is poor compared to older infants and children with brain tumors. Survival rates for PNET are 20% for infants diagnosed before one year of age compared to 75% for young teenagers with astrocytoma. From 1986-1994 in the United States, the reported 5-year survival rate was 45% for <1-year-olds, 59% for 1-4-year-olds, 64% for 5-9-year-olds, 70% for 10-14-year-olds, and 77% for 15-19-year-olds.

Prognosis for the fetus and neonate with PNETs, in general, remains dismal. The prognosis worsens with increasing tumor size and decreasing gestational age at diagnosis. Most infants with antenatally diagnosed intracranial teratomas die before or shortly after birth. Infants have a worse prognosis than older children, although the outcome appears to be improved by chemotherapy. A study of 250 fetal and neonatal brain tumors reported an overall survival rate was 28%.
3.2.2 NEUROBLASTOMA

Neuroblastoma is the leading malignant embryonal tumor of the perinatal period\textsuperscript{149,461,160,462}. It is the third most common pediatric cancer after acute leukemia and tumors of the central nervous system\textsuperscript{463}. It is the second most common solid tumor in childhood following brain tumors\textsuperscript{149,152,160,462}. Neuroblastoma is the most common extracranial solid tumor in children\textsuperscript{464,465,466}.

Neuroblastoma is a malignant neoplasm of the autonomic nervous system that is derived from neural crest cells\textsuperscript{461,467,468}. These neural crest cells differentiate into sympathetic neurons\textsuperscript{469}. As such, the primary site of these primitive type tumors is along the migration pathway and can often involve the adrenal glands and sympathetic chain\textsuperscript{470}. It is estimated that the primary sites of neuroblastoma are 74.5\% adrenal, 11.8\% retroperitoneum, 5.5\% posterior mediastinum, 4.4\% neck, and 1\% occurring in multiple sites\textsuperscript{462}. Since these tumors manifest commonly extradurally, it may result in neurologic damage due to compression of the spinal cord\textsuperscript{469}.

Most primary tumours (65\%) occur within the abdomen, with at least half of these arising in the adrenal medulla. Other common sites of disease include the neck, chest, and pelvis\textsuperscript{471}.

Neuroblastomas account for 30\% to 50\% of all tumors occurring in the newborn period\textsuperscript{151,316,472}. Approximately 30\% of tumors are recognized on the first day of life and an additional 20\% within the first week\textsuperscript{473}. 
3.2.2.1 PREVALENCE AND INCIDENCE

Neuroblastoma is estimated to affect 20.2 in 1,000,000 children under five years of age\textsuperscript{474}. Approximately 30\% of cases occur in the first year of life\textsuperscript{461,464,471,475}. Approximately half of the newly diagnosed patients are between 1-4 years of age\textsuperscript{464,471}. The median age of diagnosis has been reported at 22 months\textsuperscript{476}. It accounts for more than 7\% of malignancies in patients under 15 years of age\textsuperscript{463}. Neuroblastoma accounts for approximately 15\% of all pediatric oncology deaths\textsuperscript{463}.

In Canada neuroblastoma accounts for 7\% of all childhood cancers and 11\% of pediatric oncologic mortalities\textsuperscript{477}. In the United States neuroblastoma accounts for 6-10\% of all childhood cancers and 15\% of pediatric oncologic mortalities\textsuperscript{154}. In the United States the estimated incidence of neuroblastoma is 1/10,000 live births, translating to approximately 500 new cases annually\textsuperscript{154}.

Neuroblastoma rarely occur in older children and adults\textsuperscript{473}. Neuroblastoma in fetuses and neonates are different from neuroblastoma in older in children in both clinical and biologic features\textsuperscript{467,478}. The incidence is highly age-dependent, peaking during infancy and rapidly declining with older age. Children less than five years of age account for nearly 90\% of all neuroblastoma diagnoses in a given year\textsuperscript{479}.

3.2.2.2 ETIOLOGY AND PATHOLOGY

Little is known about the etiology of neuroblastoma. Its lineage stems from primordial neural crest cells. Neuroblasts, immature nerve cells of the fetus,
mature into nerve cells, fibers, and cells of the adrenal gland. Therefore tumors can develop anywhere in the sympathetic nervous system and the adrenal medulla. At birth neuroblasts mature or disappear; however, some studies have observed small quantities of neuroblasts in newborns which later mature or disappear.

It is unclear what genetic mutation results in the conversion of neuroblast to neuroblastoma. However since neuroblastoma affects young children it suggests that prenatal and perinatal factors may have an important role in its pathogenesis.

Cystic neuroblastoma is a pathologic variant of neuroblastoma in situ that occurs mostly in the perinatal period and is associated with the highest survival rate. It is characterized with small, focal nests of neuroblasts situated within an intact cyst wall (rather than diffuse infiltrates of tumor cells) and have aneuploid DNA content, lack of chromosome 1p deletion, and absence of MYCN amplification.

The literature on prenatal and perinatal factors associated with neuroblastoma is relatively limited and the results of these various studies are inconsistent. Conflicting studies with regard to neuroblastoma and the association with preterm birth (<37 weeks), post-term (>41 weeks) birth, low birth weight (<2,500 g) and high birth weight (>4,000 g) have been published. In addition there has been conflicting studies with regard to maternal reproductive history such as fetal losses or use of sex hormones.
Birth defects constitute a factor that has been most consistently positively associated with neuroblastoma \(^{495,496,497,498,499,500,501,502,503}\). A case-control study investigating the role of birth-related characteristics, birth defects and maternal reproductive history in the etiology of neuroblastoma compared 191 neuroblastoma cases and 1,681 controls \(^{461}\). A positive association between congenital malformations and neuroblastoma cases was observed (OR=2.2 95%CI 1.1–4.5) \(^{461}\). This effect was more pronounced in children less than one year of age (OR=16.8, 95%CI 3.1–90), while no association was observed in children greater than one year of age (OR=1.0, 95% CI: 0.3–2.9) \(^{461}\).

Since neuroblastoma arises from neural crest cells and is diagnosed at a very early age, it is suspected that it may have a prenatal origin \(^{504}\). Therefore the need to evaluate prenatal/perinatal interventions is warranted.

### 3.2.2.3 TREATMENT AND PROGNOSIS

Like all cancers, the prognosis of neuroblastoma is dependent on the stage of diagnosis. The prognosis for neuroblastoma, however, is also dependent on the age of diagnosis \(^{485,505}\). Fetuses and infants diagnosed before one year of age have a favourable prognosis \(^{468,506,507}\). Older children or adults diagnosed with neuroblastoma have a high mortality \(^{507}\). The five-year survival from diagnosis has been reported as 83% for infants, 55% for children 1-5 years of age, and 40% for children greater than five years of age \(^{508}\).

Genetic variants also predict the prognosis \(^{509}\). Fetal and neonatal neuroblastomas often present with normal MYCN copy number and hyperdipoid...
DNA index which are associated with a favourable prognosis\textsuperscript{510,511}. Neuroblastomas identified in older individuals often present with amplification of the MYCN oncogene (an oncogenic transcription factor which functions in the regulation of proliferation, differentiation, transformation and apoptosis and diploid tumors)\textsuperscript{512,513}. These two factors are associated with more advanced stages of the disease and a poor prognosis\textsuperscript{510,511}. Individuals with this high-risk clinical phenotype have a long-term survival of than 40\%\textsuperscript{514,515}. Improvements in diagnostic capabilities, disease management and supportive care have contributed to improved survival rates in individuals with neuroblastoma\textsuperscript{508}.

More than 85\% of neuroblastomas secrete catecholamine metabolites, homovanillic acid or vanillylmandelic acid, which are quantifiable in urine\textsuperscript{516}. These markers have been used in many screening programs to detect neuroblastoma\textsuperscript{516,517,518,519,520,521,522}. Currently all infants are screened in Japan whereas selective screening occurs in other countries in the presence of symptoms\textsuperscript{464}. A study evaluating the cost-effectiveness of screening observed that United States and Canada could have avoided $574.1 million in healthcare costs by not screening for neuroblastoma between 1989 and 2002\textsuperscript{466}.

Surgical interventions for neuroblastoma include resection of tumor, staging by examination and biopsy of advanced-stage disease\textsuperscript{464}. Resection of the tumor occurs for early stage tumors\textsuperscript{464}. In some variants surgical resection may not be required as the tumors may differentiate and spontaneously regress despite no specific treatment\textsuperscript{464}. Combination chemotherapy is successful in patients with advanced primary, refractory or metastatic neuroblastomas\textsuperscript{464}. The
most commonly used agents are cyclophosphamide, iphosphamide, vincristine, doxorubicin (adriamycin), cisplatin, carboplatin, etoposide (VP-16) and melphalan. Advanced-stage tumors are often treated with combination chemotherapy to reduce the size prior to resection. Bone marrow-ablative therapy with total body irradiation or melphalan with subsequent bone marrow transplant may be useful in patients with high-risk neuroblastomas. Radiation therapy has been successful in decreasing the local relapse rate for high-risk neuroblastoma. Radiation is often used in conjunction with chemotherapy to improve resectability of advance-stage tumors. Radiation is generally not used in early-stage tumors and is contraindicated for intraspinal tumors.

Immunotherapy with retinoids have demonstrated to sensitize neuroblastomas to cytotoxic lymphocytes. Chemotherapy in combination with anti-GD2 immunotherapy has been effective after failed surgery. Immunotherapy targeting dendritic cells in in vivo models have demonstrated tumor reduction.

4 MULTIVITAMINS AND DISEASE PREVENTION

Many individuals use multivitamin/mineral supplements for prophylactic or disease-mitigating purposes. Multivitamin-multimineral supplements are used by many individuals in hopes of reducing the risk of cancer, cardiovascular disease or other chronic disease. Inadequate intakes of multivitamins have been
associated with an increased risk of chronic diseases including coronary heart
disease, cancer and osteoporosis\textsuperscript{526}. A randomized control study of persons receiving supplementation observed no difference for death from stroke
(OR=0.85 95% CI 0.61-1.18)\textsuperscript{527}.

Multivitamin supplementation did not change alter the incidence for coronary heart disease in two studies (OR=0.87 95% CI 0.70-1.07 and OR=0.76 95% CI 0.65-0.90)\textsuperscript{528,529}. Multivitamin supplementation also did not alter the risk for cardiovascular disease (OR=1.11 0.91-1.36)\textsuperscript{530}.

Huang \textit{et al.} reviewed randomized controlled trials of multivitamin and mineral supplementation in preventing cancer, cardiovascular disease, cataracts, and age-related macular degeneration\textsuperscript{531}. No significant differences were observed for cardiovascular disease and cataracts\textsuperscript{531}. A decreased mortality rate for stroke was observed\textsuperscript{531}. A slowing of progression of macular degeneration was observed; however, these trials did not have regular multivitamin supplementation\textsuperscript{531}. They only used a combination of a few vitamins or minerals. The Clinical Trial of Nutritional Supplements 2008 reported in a randomized trial of 55-75 year olds receiving multivitamins, a decrease in total lens events was observed in participants assigned to the multivitamin/mineral formulation compared with those assigned to the placebo (hazard ratio [HR], 0.82; 95% CI, 0.68-0.98; P = 0.03)\textsuperscript{532}. A randomized placebo controlled trial of antioxidant vitamin and mineral supplementation observed no difference in the risk for cancer (OR=0.90 95% CI 0.76-1.06)\textsuperscript{533}. Ishitani \textit{et al.} reported that in a study of women over 45 years of age, supplementation of
multivitamins was not significantly associated with overall risk of breast cancer and may, in fact, decrease the risk of breast cancer in women consuming alcohol or women with estrogen receptor negative–progesterone receptor negative breast cancer 534.

Aasheim et al. reported that in a study of morbidly obese adults significantly lower concentrations of vitamin B6, vitamin C, 25-hydroxyvitamin D, and lipid-standardized vitamin E levels were observed when compared to controls (P<0.01). In addition, the status of vitamins A, vitamin B1, vitamin B2, vitamin B12 and folic acid were adequate in most of the patients (95-100%) 535.

In the Linxian General Population, multivitamin supplementation was not associated with an increased risk of cancer incidence (RR=0.84 95% CI 0.71-1.00), cancer mortality (RR=0.87 95% CI 0.75-1.00), stomach cancer mortality (RR=0.79 95% CI 0.64-0.99) and stroke mortality (RR=0.91 95% CI 0.84-0.99) 536. The SU.VI.MAX study reported no benefit with antioxidant supplementation (RR=1.04 95% CI 0.60-1.29) 537.

Ribeiro et al. reported that multivitamin supplementation in the elderly was able to reduce H₂O₂-induced DNA breakage 538. The elderly may be more susceptible as there may be an age-dependent increase of DNA damage 539.
5 AWARENESS OF THE EFFECTS OF ALCOHOL-USE DURING PREGNANCY

Most women recognize that the fetus is most at risk of being harmed in the first three months of pregnancy\textsuperscript{540}. Little \textit{et al.} reported that in a telephone survey of the general population\textsuperscript{541}. Respondents were asked what beverages would have an undesirable effect, if consumed during pregnancy\textsuperscript{541}. If they responded with alcohol then they were asked which type more harmful and asked how much a woman could safely drink during pregnancy\textsuperscript{541}. Approximately 90\% of the respondents reported that at least one alcoholic beverage was possible harmful to the unborn child\textsuperscript{541}. One-fourth said that women should abstain from pregnancy, while 16\% said that abstinence should be maintained on special occasions\textsuperscript{541}. 73\% of women who felt that alcohol was harmful were able to name at least one effect of alcohol on the fetus\textsuperscript{541}.

Fox \textit{et al.} administered a questionnaire asking the general population regarding if they thought that drinking during pregnancy affected the baby and if they were aware of FAS\textsuperscript{542}. Approximately 88\% of the female respondents reported that alcohol consumption increased the risk for birth defects and low birth weight\textsuperscript{542}. Approximately 87\% of the female respondents reported that alcohol consumption increased the risk for miscarriage and mental retardation\textsuperscript{542}. Approximately 61\% of female respondents reported that they had heard of FAS; however only 25\% were able to choose a correct description of FAS\textsuperscript{542}. MacKinnon \textit{et al.} conducted a survey of high school student to assess their
knowledge of FAS. Approximately 73.2% of the students reported hearing of FAS where 46.9% identified birth defects associated with FAS. 94.8% reported that FAS could be prevented, 50.3% of students believed that FAS could be cured and 48.5% believed that FAS was inherited. Approximately 96.3% reported that FAS was caused by too much drinking during pregnancy. Approximately 86.0% reported that physical and mental retardation could occur with prenatal alcohol exposure. 83.8% believed it would be beneficial for women to avoid alcohol and 87.3% believed that occasional alcohol use during pregnancy would increase the risk of the baby of having birth defects. On the other hand, 25.2% responded that it was okay for a woman to have 4-5 drinks in one occasion during pregnancy. Approximately 78.8% reported abstinence was the best during pregnancy whereas the remaining portion reported a higher quantity.

A study by Butters et al. administered questionnaires to women awaiting delivery. 55% of the respondents thought that alcohol should be avoided during pregnancy while 28% considered it safe to consume one drink a week. 51% of the participants thought that alcohol consumption in pregnancy would result in growth retardation and 66% thought that it would result in fetal abnormalities. No relationship between age and attitude to drinking alcohol in pregnancy was observed. Interestingly women from higher social class said that it would be safe to consume more than one alcohol drink per day in pregnancy compared to those in lower class.
Chambers et al. conducted a survey regarding alcohol use in pregnancy in Latina women. Approximately 43% women reported alcohol consumption during pregnancy and 20% reported at least one binge episode during pregnancy. Factors associated with periconceptional drinking included young age, high education, high education, English language, higher generation since immigration, higher acculturation level, lower parity, young age at first drink and smoking history. Factors associated with binge drinking during the periconceptional period included higher education, lack of religion, English language, lower parity, smoking history and ability to drink more than four drinks per occasion. Interestingly, 55% of women in the study had heard of FAS and 38% of the women could describe symptoms associated with FAS.

Blume et al. asked Hispanic women about their beliefs of alcohol consumption during pregnancy. Respondents said they believed that drinking could help their pregnancy. This was correlated with a greater number of drinking events in pregnancy.

Habbick et al. reported that despite provincial and national education campaigns there has been no change in the pattern of alcohol consumption during pregnancy in Saskatchewan. Awareness has reportedly increased over time. In 1992, the awareness rate of African American women was 80%. In 1990 after the introduction of the warning labels, 39% women age 18-29 years who were heavy drinkers were aware of the warning labels whereas only 12% women who were abstainers were aware. Kaskuta et al. reported that in a national telephone survey of the general population, 42% women 18-29...
said the saw the warning label and 33% remembered the contents of the label. Approximately 33% women 30-39 said they saw the warning label and 28% remembered the contents of the label. In 1991 44% of the less devout Mormons said they had seen the label, whereas 15% of the more devout said they had seen the label.

Hankin et al. reported that in a survey of African American women, the introduction of warning labels increased awareness from 29% to 78% 549. Hankin et al. observed that warning labels had the most effect on "light" drinkers who had little risk 553,553. Similar findings were noted in other studies 548,552,548,554. LaChausse et al. reported that in a study randomizing women to receive education or no education, knowledge of FAS increased from the pretest in the group receiving intervention 555. The study, however did not affect the attitudes of drinking during pregnancy 555.

6 METABOLISM OF ETHANOL

After ethanol consumption, 20% is absorbed through the stomach and 80% is absorbed through the small intestine into the blood 556. Ethanol is distributed throughout the body. Since the body cannot store ethanol it must be eliminated to prevent its accumulation in and destruction of cells and organs. A small fraction of ethanol is metabolized prior to reaching the peripheral circulation 557,558.
Oxidation in the liver serves as the major pathway of ethanol elimination. The major pathway of oxidation is the conversion of ethanol to acetic acid. Alcohol is first metabolized by alcohol dehydrogenase and coenzyme NAD+ to acetaldehyde. This process generates NADH. The NADH generated through this process is thought to cause several effects. NADH contributes to the conversion of pyruvic acid to lactic acid which can result in lactic acid build-up and hypoglycemia. NADH may be used to synthesize glycerol and fatty acids. In addition, NADH may be used in the electron transport chain to synthesize ATP.

Acetaldehyde is an unstable compound and forms free radical structures which can result in damage to embryonic neural crest cells. Acetaldehyde is then converted by aldehyde dehydrogenase to acetic acid. Acetic acid is converted by ACSS2 to acetyl-CoA. Acetyl-CoA is used in lipid synthesis and energy generation. Acetyl-CoA enters the citric acid cycle to produce water and carbon dioxide.

Alcohol is also metabolized in the liver by cytochrome P450IIIE1 (CYP2E1). The metabolism of ethanol by CYP2E1 generates oxidative stress and reactive oxygen species. In the oxidative pathway, alcohol dehydrogenase is thought to comprise of 60% of hepatic elimination whereas CYP2E1 is thought to comprise the other 40%. Catalase also oxidizes ethanol in the presence of H₂O₂, however this contributes to a clinically insignificant amount of ethanol metabolism. Other mechanisms of ethanol
metabolism include non-oxidative metabolism (see Chapter on fatty acid ethyl esters).

Overall, the elimination of ethanol ranges from 10 mg/dL/hr to 25 mg/dL/hr (2.2 to 5.4 mmol/L/hr)\(^{565}\). It is believed that 90-95% of ethanol is metabolized by liver ADH\(^{565}\), <5% metabolized my microsomal P450 enzymes CYP2E1 (CYP2A1 and CYP3A4 are involved), and 2-5% are excreted unchanged through breath, urine and sweat\(^{566,565}\).

The rate of alcohol metabolism in the liver varies amongst individuals. Alteration in metabolism may be genetically related. Enzymatic polymorphisms in alcohol dehydrogenase can alter elimination as well as CYP2E1\(^{567}\). It can also be altered by the presence of inducers or inhibitors of enzymes. Aspirin, cigarette smoking and sugar have been reported to slow gastric emptying\(^{568,569,570}\). Conversely gastric bypass surgery, erythromycin, and ranitidine increase gastric emptying\(^{571,572,573}\).

Gastric emptying plays a major role in the absorption of alcohol in the intestine. Food high in fat content slows the emptying rate, thus prolonging the alcohol absorption process\(^{569,574}\). Alcohol absorption is slower after having a meal compared to on an empty stomach thus individuals have decreased peak serum concentrations and oral bioavailability\(^{569,570}\).

### 6.1 FACTORS AFFECTING ETHANOL METABOLISM IN WOMEN

Ethanol metabolism can also be influenced by gender. Many studies have suggested a difference of ethanol elimination between men and women.
575,576,577,578,579,580,581,582,583,584,585,586,587,588,588, whereas other studies have observed no difference 584,589,589,590.

Some studies report higher elimination rates in women 575,576,577,579,580,581,582,583,585,586,587,588,591,592,593. One study observed differences in blood alcohol concentration 594. Conversely, some studies did not observe any differences in elimination kinetics between genders 589,594,595,584,596,590. Women have a greater ethanol bioavailability because they have less gastric alcohol dehydrogenase activity 558. In addition, since women have a smaller amount of total body water than men, they have a higher blood alcohol content (serum concentration) 573,597.

Sex hormones can influence the metabolism of ethanol. Estrogen is reported to influence the activity of alcohol dehydrogenase in the liver, whereas testosterone is said to inhibit the activity 585,586,587,589,591,593,595,598,599,600. A study by Dettling observed that women with high progesterone levels had an elimination rate of 0.2044±0.0414 g/kg/h whereas women with low progesterone had an elimination rate of 0.1850±0.0276 g/kg/h 601. There are conflicting studies as to whether menstrual cycles alter pharmacokinetics 558,602,603,604.

7 MECHANISMS OF ETHANOL-INDUCED FETAL INJURY

The mechanism of how ethanol induces its teratogenic effect remains unknown. Hypotheses of possible mechanisms include altered placental nutrient
transport, umbilical artery constriction due to fetal hypoxia, and increase oxygen consumption secondary to ethanol, abnormal muscle organogenesis, prostaglandin and fetal cAMP effects, and altered hormone metabolism. One hypothesis of ethanol induced damage is the oxidative stress generated by ethanol metabolism.

Non-oxidative metabolism of ethanol results in the generation of FAEE. FAEE causes the release of intracellular calcium from the endoplasmic reticulum. Elevated calcium levels in the cytosol can result in the conversion of FAEE into FAEE in the mitochondria. The presence of fatty acids inhibits the synthesis of mitochondrial adenosine triphosphate (ATP) which affects calcium-activated ATPases. In addition elevated calcium concentration results in mitochondrial depolarization.

Prenatal ethanol consumption is responsible for FASD. However it is currently unknown how much ethanol exposure is responsible for causing FASD. This may be due to a combination of factors including timing of exposure, duration of exposure, quantity of exposure, maternal history, concomitant medications, genetics, etc. It is unknown if more damage is acquired by multiple exposures versus longer exposure. It has also been postulated that multiple exposures to high quantities may result in more damage since there would be increased fluxes of oxidative stress. Since the organogenesis begin in the first trimester, it is commonly believed by the general public that exposures to external chemicals are dangerous during this period. However since the brain is
the most targeted organ by ethanol this is not the case. In fact damage can
occur the whole duration of the pregnancy.

8 SCREENING FOR FETAL ALCOHOL SPECTRUM DISORDER

8.1 SCREENING MOTHERS ANTENATALLY/POSTNATALLY

Asking mothers about their alcohol consumption during pregnancy is one
method of screening for prenatal alcohol exposure. Ideally, if the mother
answers truthfully, this screening method would be both sensitive and specific.
However, this is not always the case. Mothers may provide an incorrect
assessment of alcohol use due to reasons including recall bias, fear of losing
their child, embarrassment, and fear of stigma. Another method of
screening is to identify mothers who have a known history of substance abuse.

Screening for FASD can be initiated as early as during pregnancy.
Maternal self-report of alcohol consumption is the main source of information that
healthcare providers rely on when assessing for drinking patterns. Limitations to maternal self-report include difficulty recalling the quantity and
frequency of alcohol intake, denial, embarrassment, fear of stigma, punishment,
incarceration or involuntary commitment associated with alcohol consumption
during pregnancy. To overcome these obstacles healthcare providers may
consider the use of screening questionnaires including: AUDIT (Alcohol Use
Disorders Identification Test), CAGE (Cut-down, Annoyed, Guilty, Eye-opener),
TWEAK (Tolerance, Worry, Eye-opener, Amnesia, Cut-down), T-ACE (Tolerance, Annoyed, Cut-down, Eye-opener), MAST (Michigan Alcoholism Screening Test), and TLFB (Timeline Follow-back). The T-ACE has a reported 76% sensitivity and 89% specificity while the TWEAK has a reported 70% specificity and 79% specificity. Although these screening instruments identify heavy drinking patterns they do not measure prenatal alcohol exposure. Since screening of biological mothers is not always accurate or an available option, other tools for screening must also be explored.

8.2 PHYSICAL SCREENING TOOLS

8.2.1 FACIAL PHENOTYPE

FAS is characterized by facial dysmorphia: small palpebral fissures, smooth philtrum and thin upper lip. Astley et al. derived a quantitative case definition of the FAS facial phenotype by evaluating children 0-10 years of age between January 1993-January 1995. They demonstrated that hypoplastic midface, smooth philtrum and thin upper lip are best differentiated in children with and without FAS. D-scores were 100% sensitive, 87.2% specific (3-point Likert scale). From their facial studies, Astley et al. developed a photographic screening tool for the FAS facial phenotype. This tool employs the 4-digit diagnostic code (growth deficiency, FAS face phenotype, central nervous system (CNS) damage/dysfunction, and gestational alcohol exposure and D-score to measure the magnitude of expression of FAS facial phenotype and D-
score to measure the magnitude of expression of FAS facial phenotype. To evaluate this screening tool, they recruited 42 subjects with FAS and compared them to 84 controls without FAS. Photographs were obtained aligned to the frontal plane and phenotypic expressions were recorded on a 5-point Likert scale. Facial measurements had 99% sensitivity, 95% specificity, and 98% accuracy. Sensitivity and specificity were not affected by race, gender, and age.

Using the facial screening tool in the foster care population of the Region 4 Foster Care Passport Program, they screened children age 0-12 years between March 1999-September 2001. Facial features were ranked using the Lip-Philtrum Guide in their Fetal Alcohol Syndrome Facial Photographic Analysis Software (Version 1.0.0.). The prevalence of FAS in this population was 10/1,000. The screening tool performed with 100% sensitivity, 99.8% specificity, 85.7% predictive value positive, and 100% predictive value negative. Astley et al. proposed that the face not only could be a screening tool but a method of diagnosing FAS since craniofacial anthropometry coupled with multivariate analysis can identify individuals with FAS.

Avner et al. validated the facial photographic method proposed by Astley et al. using their software. A study of 40 children resulted in four false positive cases and no false negative cases—100% sensitivity, 64% specificity. They observed that the computer-assisted measurement tended to underestimate the true length of the palpebral fissure, especially in children under four years old. As such, this method may serve as a useful FAS screening tool since it will
identify more individuals rather than miss children potentially affected with FAS.

To examine ethnic differences, a study of South African students was conducted to assess the suitability of the reference values for facial phenotype. Facial measurements: palpebral fissure length (PFL), interpupillary distance (IPD), inner canthal distance (ICD) and outer canthal distance (OCD) were obtained in a group of black South African boys (n=17) and girls (n=17) of 7 years of age. Eye distance measurements in the study did not reflect published measurements. Another study examining unique facial features amongst ethnic populations analyzed facial differences between Cape Coloured, Finnish Caucasian, African American, and North American Caucasian. Reduced size of the eye orbit was a consistent feature discriminating FAS. However, each population had unique, overlapping variables—demonstrating that ethnic differences in the presentation of FAS do exist. Another facial landmark study observed that adding mid-face hypoplasia with palpebral fissure length, upper lip thickness, and philtrum smoothness was able to reveal differences between FAS and normal subjects of subjects of different ethnic variations.

The advantages of using facial phenotype screening are that it is standardized, objective, reproducible, and can diagnosis with the presence of CNS dysfunction/facial anomaly. It is also an inexpensive screen that does not require professional expertise during photograph collection. Photographs can be cropped if needed and transferred to a centralized facility for interpretation.
A disadvantage is that the photographs need to be taken by a trained person to obtain the correct image: the camera must be aligned horizontally, and facial expression must have eyes opened and lips gently closed. Another disadvantage is that it will only screen for FAS, therefore persons affected with FASD (not exhibiting the facial features) will screen negative. It is also important to screen at an early age as facial features associated with prenatal alcohol exposure change and become less distinctive as individuals age. Ethnic difference may affect the expression of facial phenotype. Normative ranges for all ethnicities have not yet been determined. Moreover, it may be difficult to derive normal values for interracial children.

8.2.2 INTRAUTERINE GROWTH RESTRICTION

Burd et al. published a checklist they use to screen for FAS. Poitra et al. published their experience using this 32-item FAS screening test in kindergarten students. Burd et al. reported in the normal sample this screening tool had a 100% sensitivity, 94% sensitivity, with a positive predictive value of 92% and accuracy of 94%. Staff received four hours of training on how to use the screening tool and a 10-minute screening was conducted by the school after obtaining consent. 1,384 students were screened over a 9-year period during which time 69 (5%) screened positive. After referral to diagnostic centres for diagnosis, 7 (10%) were found to have FAS or partial FAS. The screening tool was 100% sensitive, 95.43% specific, and 95% accurate. As such it is efficient for community-based screenings. The advantage of
this screening method is that it is a low cost screening program that schools should be able to complete without additional financial, logistical, or technical support that screens for FASD. The disadvantage is that no neurobehavioural considerations are considered in this screening method. Although studies have demonstrated that ultrasound screening for small for gestation age have demonstrated 80-90% sensitivity intrauterine growth restriction has a low specificity as there are many conditions which may result in intrauterine growth restriction \textsuperscript{632,633}.

8.3 NEUROIMAGING

8.3.1 ULTRASOUND

A study by Bookstein \textit{et al.} assessed 18 children 5-16 weeks after birth using ultrasound imaging of 50 freeze-frame midsaggital sections \textsuperscript{634}. They observed that the midline corpus callosum of infants exposed prenatally to alcohol exhibited abnormality of the splenium \textsuperscript{634}. Despite the use of ultrasonography, a non-invasive and relatively inexpensive test, this study was limited by its small sample size and has not been reproduced to demonstrate its validity and reproducibility. Ultrasound also requires a skilled technologist and radiologist to interpret the results.
8.3.2 ELECTROENCEPHALOGRAPHY (EEG)

A literature search of studies using electroencephalography (EEG) techniques identified 17 papers in infants exposed to alcohol. Disturbances in sleep cycles and arousals were apparent depending on trimester of exposure. Also observed were sensory impairment suggestive of atypical brain maturation and impairments in attention and cognitive functions. The advantage of EEG testing is that it is noninvasive, generally available and inexpensive, and does not require active response. The disadvantages are that there is a lack of control factors in studies, there is questionable reliability, and there is difficult comparing studies comparing different measures.

8.3.3 MAGNETIC RESONANCE IMAGING (MRI)

Magnetic resonance has been used to detect chemical elements e.g. markers of neuronal integrity and cell death. Functional MRI (fMRI) has also been utilized to identify differences. fMRI may help characterize the neural underpinnings of abnormalities. Magnetic resonance spectroscopy (MRS) is used to quantify metabolites in body tissues. MRI studies have demonstrated that persons with FASD have reduction in size of the cranial vault, reduced brain size, alteration in size and shape of corpus callosum, displacement of corpus callosum, reduction of basal ganglia size, reduction of cerebellum size, reduction in hippocampus, reduction in white matter in cerebrum, altered corpus callosum, frontal and parietal lobe anomalies, reduced
surface area and volume of cerebellum\textsuperscript{655}, altered frontal-striatal response\textsuperscript{656}, abnormal cortical thickness\textsuperscript{657}, and reduced volume of basal ganglia\textsuperscript{647}. Greater inferior-middle frontal lobe activity was observed in FASD in children and adults\textsuperscript{658}. MRS studies demonstrated altered N-acetylaspartate/choline metabolite ratios in persons affected with FASD compared to controls\textsuperscript{659,660}.

The advantage to screening using MRI is that it is a non-invasive test that has no age restriction. The disadvantage, however, include the cost. It is an expensive piece of medical equipment that is not readily available in all areas. This immobile machine also requires a skilled operator and radiologist for interpretation. Therefore persons in remote communities are unable to obtain easy access. Furthermore MRI has neither been validated as a screening tool nor has its specificity and sensitivity been determined. Perhaps MRI may be more effective in the diagnostic process to confirm neurological irregularities and the development of interventions.

\textbf{8.3.4 DIFFUSION TENSOR IMAGING (DTI)}

Diffusion tensor imaging (DTI) uses the diffusion of water molecules to measure the tissue microstructural integrity that is useful in characterizing white matter\textsuperscript{661}. Diffusion tensor imaging may be useful in studying neurodevelopmental disorders, including FASD, because it is sensitive to abnormalities against the “background” changes of normal development\textsuperscript{662}. Microstructural abnormalities have been observed in patients with FAS\textsuperscript{661}. One study used DTI to examine the corpus callosum in adults with FASD and
observed lower fractional anisotrophy and higher mean diffusivity were observed in splenium and genu of the corpus callosum in the alcohol-exposed group versus controls. No associations were found between the DTI measures and dysmorphia score, IQ, or processing speed. Another study of 14 children with FASD aged 10-13 years and matched controls exhibited a trend toward smaller total cerebral volume in the FASD group (p=0.057). There was also a greater mean diffusivity observed in the isthmus of the corpus callosum of FASD subjects (p=0.013), suggesting micro-structural abnormalities in this region.

The advantages and disadvantages of DTI are identical to those of MRI.

### 8.4 PSYCHOLOGICAL/NEUROBEHAVIOURAL

Children with prenatal alcohol exposure may present with a complex diagnostic picture and a wide variety of psychological symptoms. Persons affected with FASD have deficits in cognitive and academic functioning, psychological disorders, behavioural problems, and difficulties with independent living. Neuropsychological sequelae including executive functioning difficulties have been observed. Social skills deficits including poor social judgment, failure to learn from experience, difficulty understanding consequences of actions, aggression, inappropriate sexual behaviour, delinquency, lack of understanding of social cues, and communicating in social contexts have also been observed. Hyperactivity and attention problems are some of the most frequently reported symptoms associated with prenatal alcohol exposure and reported in the research literature.
Individuals with FASD also often demonstrate impulsivity, poor judgment, and great difficulty learning from consequences $^{670,675,676}$. Exposure to alcohol in the first and second trimesters has been associated with lower overall academic achievement. Lower reading scores, spatial and verbal memory and learning were associated with second trimester binge drinking as were problems in processing and arithmetic $^{675,677,678}$. Mattson et al. reviewed IQ in many studies with children diagnosed with FAS and found a mean of 65.73 (20-120) $^{677}$. The mean IQ for FASD was 72.26 (47.4-98.2) $^{677}$. They concluded that high levels of prenatal alcohol exposure are related to increased deficits in intellectual functioning $^{677}$.

There is limited literature regarding using psychological evaluations to screen for FASD. The majority of literature reports on psychological testing as a process used in of FASD diagnoses. These testing methods include the use of Bayley Scales, Wechsler Intelligence Scale for Children (WISC-III), Griffiths Mental Developmental Scales, Wechsler Preschool and Primary Scale of Intelligence (WPPSI-R), Fagan Test of Infant Intelligence, Children’s Memory Scale (CMS), Behavioral Rating Inventory of Executive Function (BRIEF), Parent and Teachers Conners’ Ratings Scales-Revised (CRS-R), and Child Behavioral Checklist (CBCL) $^{679,680,681,682,680,683,684}$.

Streissguth et al. set out to develop a scale that would describe the behaviours of FASD $^{685}$. They assembled a list of 68 short descriptors “Personal Behaviors Checklist” that were answered by someone familiar with the child’s behavior and condensed the checklist into 36-item scale called the Fetal Alcohol
Behavior Scale (FABS). A screening study was conducted to evaluate its ability to detect persons affected with FASD and a normative study to evaluate the sensitivity of the test in children. 186 caregivers completed the five minute questionnaire for their children. The Cronbach’s coefficient was 0.89, indicating high reliability. FABS scores appear to be correlated with maternal alcohol problems and reflect the behavioral phenotype of fetal alcohol fairly specifically rather than being raised in an alcoholic environment. Further studies are needed to clarify its utility in a diagnosis or screening context. Instruments like the FABS should not be used clinically for diagnosis without additional evidence of prenatal alcohol exposure.

Nash et al. evaluated the CBCL with a sample of children diagnosed with FASD and ADHD. Greenbaum had previously shown that the CBCL was able to successfully distinguish between FASD and normal children. Parents of 54 children (11 FAS, 43 ARND) completed the CBCL. The CBCL has open-ended questions and a rating scale of 113 behavioural descriptors. Greenbaum had previously demonstrated in a sample of 35 children affected with ARND that there were significant differences in 62 items when compared to a control group of 35 matched for age, gender, and socioeconomic status. Twelve items were significantly different \( p<0.001 \). These were ‘acts too young for age’, ‘argues’, ‘can’t concentrate=poor attention’, ‘can’t sit still=restless=hyperactive’, ‘cruelty, bullying or meanness to others’, ‘disobedient at home’, ‘no guilt after misbehaving’, ‘impulsive=acts without thinking’, ‘lying or cheating’, ‘showing off=clowning’, ‘steals from home’, and ‘steals outside’. In this study the 12 items
were scored. Seven of the 12 items strongly differentiated FASD children from ADHD and normal controls (p<0.001). They were “no guilt”, “lying or cheating”, “can’t concentrate”, “restless”, “impulsive”, “disobedient”, and “acts young”. Six items differentiated the FAS/ARND from ADHD group (P<0.001). They were “no guilt”, “cruelty”, “acts young”, “steals from home”, “steals outside”, and “lying or cheating”. A 86% sensitivity and 82% specificity were observed with 6 of the 7 items when comparing FASD, ADHD, and controls. A 81% sensitivity and 72% specificity were observed with three of six items when comparing FASD vs. ADHD group.

From these observations Nash et al. proposed that a FASD screening tool should be considered involving a two-step approach: first identify behaviours suggesting FASD and then discriminate FASD from ADHD. The advantage of using this screening tool is that it specifically differentiates between FASD and ADHD. The limitation is that these are primary results which have not been replicated in a large sample size. In addition it has not been validated in different ethnicities or languages.

8.5 JUSTICE SYSTEM

The neurological impairments observed in persons affected by FASD including learning disabilities, poor judgment, impulsivity, increase the susceptibility to criminal behaviour and victimization. These individuals socialize with maladaptive or socially deviant individuals who are more accepting of their behaviours. As such there is a higher chance of persons affected by
FASD to get into trouble with the law and become involved with the justice system. Identifying individuals in the corrections system affected with FASD is very important. In fact they often are involved with nonviolent crimes with repeated offences or failed compliance to parole conditions. Inherent disabilities may pose as a barrier to programs, skills learned, and/or treatment received while incarcerated. These include substance abuse treatment, anger management, and vocational training. By identifying and understanding the neurobehavioural problems, programs can be developed that would result in altered sentencing or probation plans for offenders. Programs may be custom designed to the individuals’ ability thereby improving the overall impact of the intervention. Identification is important in youth juvenile systems because early identification can prevent secondary disabilities. This could result in future cost-savings to the corrections system and a decrease in future criminal activity.

Systematic screening followed by diagnosis would assist in identifying affected individuals, thus enabling the tailoring of interventions during their incarceration and follow-up. A sensitive and specific screening procedure would only need to screen individuals once. Information required for screening would include access to medical records, childhood pictures, and past and current psycho-educational testing. Corrections systems staff would need to be trained to recognize characteristics of individuals affected with FASD and trained on management strategies. Screening strategies include collecting height, weight, head circumference, analysis of facial photographs, exposure data, cognitive
testing, educational assessments, behavioral data, sensory impairments, IQ or achievement testing.

Fast et al. reported their experiences with the ALARM in the criminal justice system. ALARM is a mnemonic that stands for adaptive behaviour, language, reasoning and memory. Impairment in these characteristics may be observed in a person with FASD. In a sample of 253 adolescents and adults affected with FASD, 60% had some contact with the law and 35% were incarcerated. Juvenile offenders alone comprised of 23% of the incarcerated individuals. The mean age of trouble with the law was reported at 12.8 years.

Conry et al. published the first prevalence estimate in the correction population in British Columbia. Over a 1-year period, youth undergoing forensic psychiatric inpatient assessment were assessed for FASD. 23.3% were diagnosed with FAE and 1% had a diagnosis of FAS.

Burd et al. reported that of 43 states or major city correctional facilities in the United States, only Minnesota reported a FASD screening program. Only three states’ correction facilities and one city’s correction facility have access to diagnostic services for offenders who have FAS. To overcome this disparity they proposed four potential screening strategies for FASD in the correction system. They used the FAS Indicator tool as well as data from their records (IQ, data, height, weight, face) to screen offenders for FAS. The FAS Indicator tool is currently not validated and as such more research has to be undertaken. Option one is to screen five inmates who are most likely to have
FAS. Option two is to screen 20-30 inmate records per day. Option three is to have a prospective screening program by a designated date where all person entering the system will be screened. Option four is to screen all offenders in the facility using the tool. The advantages of conducting these investigations are the ability to determine the prevalence of FASD within correctional facilities, identify costs associated with the screening process, provide those who screen positive access to diagnosis, assess the sensitivity, specificity, and accuracy of the screening tool as well as the positive and negative predictive values. The major barrier to this form of screening is that there the lack of infrastructure and resources currently available to conduct the screen, diagnose and treat the individuals who are identified by this screening method. Implementation of this screening tool requires cooperation of the staff from receiving training to an increase in staff to receive training and an increase in staff workload. Incomplete records will also pose as a barrier to the screening program. Once there are positive screens there are limited diagnostic facilities available. The other major barrier of screening is difficulty in obtaining the maternal history of alcohol exposure. As such, persons with FAS are more likely to be identified compared to persons with ARND.
9 DIAGNOSIS OF FETAL ALCOHOL SPECTRUM DISORDER

Initially identified by Lemoine et al. in the late 1960s. FAS was not internationally recognized until Jones et al. reported this anomaly and coined the term fetal alcohol syndrome. Diagnostic guidelines for FASD have been published by the United States and Canada. These guidelines both address the physical and neurological changes that are observed in persons affected with FASD. Physical changes include the characteristic pattern of facial abnormalities short palpebral fissures, smooth philtrum, thin vermilion border of upper lip; evidence of growth retardation; and evidence of central nervous system abnormalities. Both guidelines emphasize the need for a multidisciplinary team for the diagnosis of FASD. This team should be comprised of a physician experienced in evaluating a broad range of developmental disabilities, psychologist and/or neuropsychologist, educational specialist, skilled maternal interviewer, physical occupational and speech and language therapist. In addition, consideration of other developmental disabilities should be ruled out prior to the designation of FASD.

9.1 DIAGNOSTIC CAPACITY

There is currently limited capacity to diagnose FASD affected individuals. Diagnosis is also hampered by the fact that there are a lack of available clinics. A minimum of 3-4 month waiting list for clinics currently exist at some FASD diagnostic clinics. Given that clinics are not readily accessible in rural
regions, residents also have to travel to access clinics. The advances in technology have enabled the commencement of tele-diagnosis; however, there are limited professionals available to perform this service. Difficulty obtaining stable funding has been a dilemma for many diagnostic clinics and additional funding would be required from the education ministry to fund adapted school programs. Given the current limited resources, we need to utilize resources available in community and enhance support to individuals and families living in community.

Before the broad implementation of a screening tool, it is essential that the diagnostic capacity for FASD is available. Currently there is at least a 3-4 month wait time at some FASD diagnostic clinics. Goulden reported that by adopting the Canadian diagnostic guidelines for FASD, there would be an estimated 50% increase in the clinic workload. To our knowledge there has not been any investigation of this hypothesis. However, if a nationwide screening tool is implemented it is possible for diagnostic wait times to increase if the number of clinics do not increase since screening currently occurs in a small proportion of the population. Accessing diagnostic services could be difficult due to the lack of availability of clinics. In addition, rural residents need to travel to access clinics. The advances in technology have enabled the commencement of tele-diagnosis; however, there are limited professionals available. Difficulty obtaining stable funding has been a dilemma for many diagnostic clinics and additional funding would be required from the education ministry to fund school programs. Given the current limited resources, we need to utilize resources
available in community and enhance support to individuals and families living in community. 

10 INTERVENTIONS FOR FETAL ALCOHOL SPECTRUM DISORDER

Streissguth et al. demonstrated that with early intervention of children affected with FASD have better outcomes. In doing so, their risk for developing secondary disabilities is reduced. Interventions that have been demonstrated to be successful in the treatment of FASD affected individuals include individualized planning, structured teaching, and cognitive-behavioural methods.

10.1 EDUCATIONAL INTERVENTIONS

Individuals affected with FASD have individual learning profiles. Schools need to tailor academic and functional support for each child to develop their skills to function as an adult. Individuals with FASD have varied IQ and deficits. In fact some individuals are ineligible to receive services because they have average intellectual abilities. Due to difficulties in executive function, individuals have differences in learning sequences, retaining information in memory for later, following directions, organizing information, inhibiting emotional responses and may be overly active.

Assessment of necessary supports and modifications should be done to create individualized goals and objectives. Developing structured routines
results in predictability and understanding. Adapting the environment decreases
distractions and helps with organization and structure.

10.2 JUSTICE INTERVENTIONS

Due to the neurological impairments associated with FASD affected
individuals have a greater chance of getting into trouble with the law and become
involved with the justice system\textsuperscript{689}. Identifying individuals in the corrections
system affected with FASD is important because they are often repeat offenders
and also fail to comply with parole conditions\textsuperscript{690}. These inherent disabilities can
also pose as a barrier to programs, skills learned, and/or treatment received
while incarcerated\textsuperscript{689}. By identifying and understanding the neurobehavioural
problems, programs can be individually tailored that would result in effective
sentencing or probation plans for offenders. Programs may be custom designed
to the individuals’ ability thereby improving the overall impact of the intervention.
Moreover educating persons involved in the corrections system including judges,
lawyers, justice workers about the complexity of FASD will also assist with the
management strategies.
11 IMPACT OF FETAL ALCOHOL SPECTRUM DISORDER

11.1 ECONOMIC

There is a high cost associated with FASD. Abel et al. estimated that the annual cost of FASD to the healthcare system was $74.6 million US annually in 1991. Harwood et al. estimated societal costs of FASD to be as high as $9.69 billion US. A cross-sectional survey by Stade et al. reported that the total adjusted annual expenditure per FASD person aged 1-21 years was $14,342 (95%CI $12,986-$15,698). The average cost of FASD annually was $344,208,000 (95%CI $311,664,000-$376,752,000) in 2007. Cumulative costs of healthcare expenditures related to FAS have been estimated between $75 million and $9.7 billion annually.

11.2 SOCIETAL

Previous studies have suggested that earlier intervention in FASD affected individuals will decrease the risk of developing secondary disabilities. As such the potential economic costs may decrease with earlier identification of these individuals. Thus it is important to develop a screening program that will enable individuals to be referred to diagnostic centres to receive full evaluation. In doing so, those who are diagnosed with a FASD condition can receive the necessary support or interventions required at an earlier point in life to reduce secondary disabilities and the use of resources associated with these secondary disabilities.
The PRECEDE-PROCEED model was designed by Green and Kreuter in 1970’s and provides a theoretical framework for developing a health promotion program. The PRECEDE-PROCEED framework is a model that provides structure so that the most appropriate intervention strategies can be identified. The model is based on the premise that lasting behaviour change will occur when individuals are provided with education, motivation, and tools that are necessary to make a voluntary change in their behaviour. The model assists in the development of health education in different situations. Identifying these factors make it possible to adapt the intervention to different types of situations.

The PRECEDE-PROCEED model is used in public health as its framework considers multiple factors that "shape health status and help the planner arrive at a highly focused subset of those factors as targets for intervention." The PRECEDE-PROCEED model has been used in a variety of processes including asthma, work-related injury, and public health promotion. The PRECEDE-PROCEED model allows for planning for a variety of situations.

The PRECEDE-PROCEED model is a nine phase model. The PRECEDE phase consists of social assessment to identify and evaluate the social problems
which impact the quality of life of a target population; epidemiological
assessment defined as program objectives which define the target population,
the desired outcome, and how much benefit the target population should benefit,
and by when that benefit should occur; behavioral/environmental assessment
focusing on systematic identification of health practices and other factors which
seem to be linked to health problems; educational/ecological assessment to
determine which factors should be modified to result in behavior change; and
administrative/policy assessment to identify areas that could hinder or facilitate
development of the program including policy and resources. The compatibility of
the program with the goals and objectives should be evaluated. Overall the first
five stages is to identify the health problems and their determinants 711.

The PROCEED phase consists of implementation of the program; process
evaluation to evaluate how the program is being implemented; impact evaluation
to measure the program effectives in terms of intermediate objectives and
changes in predisposing, enabling, and reinforcing factors; and outcome
evaluation which measure the program’s ability to meet the overall objectives and
changes in health and social benefits 708.

The acronym PRECEDE-PROCEED stands for predisposing, reinforcing,
and enabling causes in educational diagnosis and evaluation and policy,
regulatory and organizational constructs in educational and environmental
development. The predisposing stage identifies epidemiological factors of the
problem as well as characteristics of individuals who are considered at risk of
developing the problem. Predisposing factors also include individuals’
knowledge, attitudes, beliefs, and perceived abilities that influence their health behaviours. Predisposing factors must be changed before enabling factors and reinforcing factors can be addressed.

Reinforcing factors are incentives to continue behaviours. This can include support received from professionals, peers, or social groups. In addition there may be physical or social benefits. Reinforcement can be either positive or negative depending on the attitudes and behaviours of the individuals. For instance knowledge received by individuals can positive influence motivation in individuals seeking help and increase the vigilance of persons involved (family or friends). On the other hand social stigma can be a negative reinforcing factor. Stigmatization may contribute to delays in treatment. In addition there may be confusion, anger, isolation and shame.

Enabling factors are determinants/conditions that facilitate change. These can include availability, accessibility and affordability or resources, environmental conditions, supportive policies, and necessary skills.

It is important to identify which factors present as barriers to the introduction of change. Both behaviour and environmental factors influence the above three (predisposing factors, enabling factors, and reinforcing) factors. Understanding the beliefs and values of individuals in the communities may assist in the introduction of change into the community.

The evaluation process of this model allows for evaluates safety and efficacy of treatment; establishment of causation; large scale surveillance; health assessments; and determines if prevention and intervention is effective.
The advantages of this model are that it assists in the characterization of the resources, identifies educational and behavioural barriers, and organizational factors in the community. As such, interventions and learning opportunities are tailored to the needs and cultural values of the community \(^7\). Because of the rigorous planning involved with this model it is likely that the interventions will be appropriate for the population. The orderly and sequential nature of this model can be useful in organizing research and serve as a framework for planning and implementation of intervention strategies \(^\)\(^1\). Clear measurable goals enable better evaluation of the program. The use of the PRECEDE-PROCEED model forces the planner to justify why the problem is selected for intervention using qualitative and quantitative data to identify priorities. The model also considers that problems are influenced by multiple, interrelated factors.

Limitations to the model include that the planning process is complex due to the interaction of a multitude of variables. In addition, the recipients of the intervention have different risk factors and thus there may be many subgroups that need to be targeted within the population.

13 META-ANALYTICAL METHOD

13.1 SYSTEMATIC REVIEWS

A systematic review is a quantitative or qualitative comprehensive search for studies on a specific topic that are appraised and synthesized according
clearly defined predetermined method and criteria \cite{715,716}. Systematic reviews have been reported to be the highest level of research design evaluating effectiveness of interventions \cite{717}. When conducted properly, this method is able to systematically critically appraise and synthesize studies and reports while avoiding bias \cite{717,718}. Systematic review can include appraisal of single trials, appraisals of sources of evidence and meta-analysis \cite{719}.

### 13.2 META-ANALYSIS

Meta-analysis is a systematic method that uses quantitative statistical procedures to combine, synthesize, and integrate information across several independent studies \cite{720,721}. This systematic process enables the analysis of data from separate studies (randomized controlled trial, observational or cross design) in a rigorous structured manner \cite{721,715}. Meta-analysis can be conducted using the results of two or more primary studies evaluating healthcare interventions \cite{716}. Data are systematically are pooled from individual studies, weighed, and reanalyzed using statistical methods to produce generalizable conclusions of treatment effect with high statistical power \cite{721,722}. Meta-analysis is useful in overcoming divergent results and can assist in overcoming the discordant interpretations and inferences made by some reviews \cite{716,721}.

#### 13.2.1 HISTORY

The world meta-analysis stems from the Greek word “meta” for “after” and “analysis” for “break up”. The process of meta-analysis dates back to the 17th
century. In 1904, Karl Pearson was the first medical researcher to use this technique to combine data from different studies to examine the preventive effect of serum inoculations against enteric fever. The first meta-analysis was published in 1955 on the effects of therapeutic intervention versus placebo. The term meta-analysis was coined by psychologist, Gene Glass, in 1976. The process of meta-analysis became popular in the 1980s. The Cochrane Collaboration was formed in the 1990s to prepare, maintain and disseminate comprehensive and systemic reviews of the effects of healthcare.

13.2.2 META-ANALYTIC OBJECTIVES

The objective of conducting a meta-analysis is to provide a stable and precise estimate of a treatment effect and explain heterogeneity between results of studies. Due to the limited amount of strong evidence available in reviews as well as small populations in studies, it is difficult to form definitive conclusions about interventions/treatment. Meta-analysis is able to overcome this by combining trials and is evaluating the same intervention in a number of smaller but comparable trials. Meta-analysis will also increase the power of the outcomes enabling subgroup analysis. Combining the individual studies will increase the sample size, thereby augmenting the statistical power of the analysis and precision of the treatment effect or epidemiologic association. In addition studies with results lacking in statistical significance or demonstrating contradictory results are hard to decipher. Pooling independent studies in a well-conducted meta-analysis,
allows for an objective appraisal of the evidence would resolve uncertainties or disagreements\textsuperscript{720,715}. Meta-analysis may provide a solution when results disagree in magnitude or direction of effect, a large single trial is too costly and time-consuming to perform\textsuperscript{721}.

Meta-analysis improves the overall reliability and accuracy of a conclusion\textsuperscript{718}. It improves the overall reliability and accuracy of the conclusions as well as the precision of the estimated measure of treatment effect or epidemiologic association by narrowing the effect size\textsuperscript{720,721,715}.

Using a large number of individual studies or patient data enables for testing of \textit{a priori} hypotheses regarding treatment effects in subgroups of patients e.g. male vs. female, old vs. younger, disease states\textsuperscript{718,734}. The combination of all relevant findings results in a consistent and generalizable finding across populations\textsuperscript{721}. It assists in the generalizability of the results\textsuperscript{729}. It may also highlight areas where there is a lack of adequate evidence\textsuperscript{715}. It can also assist in the calculation of sample sizes\textsuperscript{715}. Therefore it can potentially prevent delays in introduction of effective treatments into clinical practice\textsuperscript{721,721}. It is also able to answer new research questions\textsuperscript{721}. It may also counterbalance excess enthusiasm accompanying the release of a new drug\textsuperscript{721}. Qualitative information can be evaluated by weighing various studies according to methodological quality\textsuperscript{721}. Quantitative information can be evaluated by pooling results of different studies to get high statistical power\textsuperscript{720}. Meta-analysis allows quantification of epidemiologic association between a risk factor treatment effect and an outcome\textsuperscript{720}. 
13.2.3 META-ANALYTIC METHODOLOGY

Meta-analysis is a systematic process. In brief, a clear objective should be stated with a protocol prior to the comprehensive search of primary studies including objectives, rules of inclusion/exclusion, plan to evaluate quality of study, summary descriptive measures, description of methods used to extract, detailed statistical analysis describing the procedures to analyze the data. Studies are selected for inclusion using clearly defined and reproducible eligibility criteria, appraised for their quality, the data pooled, the results synthesized statistically, and sensitivity analysis conducted. The objectives and all the components of meta-analysis should be stated before obtaining data to guard against fishing expedition (data dredging). It is important to have a transparent review process described in sufficient detail where readers are able to replicate the quantitative component of the argument. The meta-analysis should be designed, conducted and reported by researchers who do not have conflicts of interest.

13.2.4 LITERATURE SEARCH STRATEGY

The quality and completeness of a meta-analysis depends on the comprehensive extent of the literature search and trials included. The meta-analysis literature search should be conducted using a clearly delineated, and exhaustive search strategy. A meta-analysis should report the search strategies used to identify studies, the sources (and their description) used in the
search, the name and number of sources, the date on which they were searched, and the period covered by the search \textsuperscript{737}. The breath and depth of the search can be examined by observing the strategy used to search each source \textsuperscript{738}. The methods should list the terms used to search bibliographic databases \textsuperscript{738}. Terms including terms related to the condition, intervention and method of trials may be used to conduct the search \textsuperscript{738}.

In manual searches, reports should include the number of searchers and their background. In addition, a description of the procedure of the hand searches should be explained \textsuperscript{715,738}. Together this will enable readers to judge the comprehensiveness of the literature search \textsuperscript{718}. Articles should be searched in a variety of languages to avoid language bias \textsuperscript{739,740}.

It is challenging to include all of the relevant trials available in a meta-analysis \textsuperscript{718}. A search of medical literature may result in many articles and these article may vary in quality and conclusions \textsuperscript{721}.

A literature search should employ multiple methods including manual or computer search \textsuperscript{721}. Furthermore, consulting reference sections of papers, books, manuals, workshop proceedings may further identify primary studies to include to the meta-analysis \textsuperscript{741}. Additional articles may be obtained by scanning all records available in a bibliographic database, hand searching journals, theses, textbooks, and looking for unpublished information \textsuperscript{742}. Searching electronic bibliographic databases is the most cost-effective source of identifying articles followed by hand searching and direct contact with investigators and sponsoring
organizations \(^{718,721,743}\). Employing only one search method decreases sensitivity as relevant articles may be missed \(^{716}\).

Bibliographic databases vary in scope, currency, accessibility and cost \(^{744}\). The most popular electronic bibliographic database is MEDLINE. MEDLINE is the most widely used database containing over 400 indexed journals from Jan 1, 1966-present \(^{745}\). EMBASE is a European database which was constructed in 1980 \(^{745}\). A search should occur across multiple databases as unindexed studies may introduce bias \(^{744}\).

The advantage of using an electronic database for searches is that it can quickly search for numerous concepts in many references simultaneously \(^{719,727}\). On the other hand, it is limited by inadequate indexing as the coding system may not include all possible coding terms. Authors may not list relevant search terms and due to poor quality of original trials they may be missed in a search. In addition there may be inconsistencies between indexers which may decrease the identification of articles \(^{746}\). A study by Dickersin et al. demonstrated that only 30-80\% of known randomized controlled trials are identifiable using the MedLine search engine \(^{747}\). Moreover, articles published before the creation of the database may not be indexed. In addition there may be a delay of article indexing after its publication \(^{745}\).

A search of indexed material that are not captured in database searches should be identified manually \(^{745}\). Manual searches by hand are advantageous as it is more accurate in identifying articles. In doing so, it increases the yield of search retrievals of trials which may have been missed in database searches.
The disadvantage of manually searching by hand is that it is time-consuming\textsuperscript{745}. Another method of manual searching is to examine the reference list of all eligible trials identified by other methods\textsuperscript{748}. Scanning reference lists of published reports may result in reference bias as authors are more likely to cite trials with positive outcomes for the intervention\textsuperscript{718}. Retrieving articles may also be a problem if one does not have access to the journals in which they were published\textsuperscript{721,749,750,751}.

Searching for only published articles will result in publication bias, as journals are more likely to publish studies with positive results than negative results\textsuperscript{752}. There is a decreased propensity of investigators to write and submit or reviewers and editors to accept manuscripts of trials with negative outcomes\textsuperscript{745}. This may result in the failure to identify and include unpublished trials, thereby overestimating the effect of the interventions\textsuperscript{745}. Identifying unpublished trials are more challenging than identifying published trials there was previously no compulsory registration of trials therefore it is difficult to estimate the total number of trials that existed\textsuperscript{753}. If unpublished studies are identified journals may be reluctant to publish reviews with unpublished data\textsuperscript{721,754}. The introduction of clinical trial registries has been helpful in decreasing publication bias\textsuperscript{745}.

Contacting experts in the field or corresponding authors to identify additional published or other unpublished sources of research is also a time-consuming effort\textsuperscript{745}. Often there is a varied response rate and multiple reminders are necessary\textsuperscript{755}. Contacting pharmaceutical companies regarding
sponsored trials may result in identifying trials that favour using their product.  

Electronic databases enable fair ease of searching for published data, however, unpublished studies are difficult to obtain. To avoid publication bias, a search should be conducted for both published and unpublished data searches. This may be due to publication bias as negative studies may be harder to publish and therefore not available in the literature. To overcome this dilemma, a database of unpublished studies may be created. In light of the recent creation of registries of clinical trials this may be a source of information for future meta-analysis and help overcome publication bias. This process is, however, limited by the fact that there are multiple registries. If there was an international registry it would overcome this hurdle.

Most meta-analysis reports do not include a detailed description of the searching process. However lack of or poor description does not necessarily infer a poor search as authors may have been unaware of the importance of reporting search methods or they could have been constrained by journal space.

13.2.5 INCLUSION/EXCLUSION

Standardized criteria for inclusion/exclusion of studies in meta-analysis do not exist. A priori definition of eligibility criteria for studies included and excluded with straightforward selection criteria should be defined.
Inclusion criterion bias occurs when the investigator creates a set of selection criteria based on primary review of literature\textsuperscript{737}. Reviewers need to define and justify selection criteria. Clear/concise selection criteria should be defined to minimize bias, and errors when reviewers are selecting studies to be included in systemic review\textsuperscript{718,737}. Selection criteria should describe which studies are eligible for inclusion on basis of description of population, intervention, outcome measures, study design, languages, published/unpublished, and quality of study\textsuperscript{715,760,720}. Care needs to be exercised to prevent the exclusion of relevant studies or inclusion of inadequate studies\textsuperscript{721,761}.

In the meta-analysis evaluating drug treatments, some believe that only randomized studies should be included because of the potential bias in non-randomized studies\textsuperscript{721}. The determination of whether studies are similar is subjective\textsuperscript{721,759,762}. To reduce the subjectivity, protocols have been developed for the selection of trials\textsuperscript{762}. For example Gelber and Goldhirsch proposes identifying articles by a very specific and objective process\textsuperscript{721,759}. On the other hand, Sacks proposes reading the methodology to avoid bias arising from the study results\textsuperscript{718}.

Regardless, the types of studies that have been included in the meta-analysis must be explicitly stated so that readers can decide whether the trials were appropriately incorporated into the meta-analysis and contain adequate power to answer the clinical question\textsuperscript{721}. By including all studies in the meta-
analysis, it would increase the representativeness of the conclusions; however, it would decrease the statistical validity because it is less rigorous \textsuperscript{763}.

\subsection*{13.2.6 QUALITY ASSESSMENT}

Quality assessment of studies for inclusion and exclusion criterion is important in assessing the quality of the studies \textsuperscript{764,765}. Quality has different meanings—from methodological aspect from design to reporting, and generalizability of the results or a combination \textsuperscript{764,765}. Quality evaluation could consider literary style of the report, clinical relevance of research question, likelihood of producing biased results, prevision and extent to which it is possible to generalize result, appropriateness of statistical analysis, presentation of data, and ethical implications of intervention evaluation \textsuperscript{738,764}. Internal validity is the confidence that the trial design, conduct, analysis and presentation have minimized or avoided biased comparisons of the interventions under evaluation \textsuperscript{738}. A description of the methods/tool used to do the quality assessment should be included \textsuperscript{745}. Authors should describe the circumstances in which assessments were generated \textsuperscript{738}. If the reviewers did not assess the quality of the trials included they should explain why \textsuperscript{721}.

Prior to quality assessment of the article, the articles must be cleaned for identification \textsuperscript{764,765,766,767}. The assessment should be conducted without knowledge of authors, institution, sponsorship, publication year, journal, and study results. Published empirical studies addressing these issues showed that the assessment under these conditions yielded lower and more consistent scores
compared to assessments under open conditions. This implies that bias could be introduced by open assessments; however, there are also data showing that abstraction in masked conditions does not affect the overall results of systematic reviews. Overall, masking the conditions reduces the likelihood of bias but increases the resources required to conduct the reviews.

Quality assessment of a trial could provide information to the reviewers during meta-analysis but could also lead to bias or inaccurate conclusions. As such, meta-analysis should describe the tools and methods to conduct quality assessment and provide the reader enough information to allow for the recalculation of effect estimates. Tools used to conduct quality assessment include the Jadad scale and Downs and Black scores.

At least two reviewers should independently assess the quality of the included studies in order to minimize the risk of selection bias. The use of two independent reviewers have been able to reduce the possibility that relevant reports will be discarded. Reviewers are asked to review the methods and results section of studies and rate the quality of the study from 0.0-1.0 with the author, institution, information withheld. Quality scores may be used to exclude studies. Consensus will be required to determine the quality score of the article. The scores are based on the presence or absence of information reported by the study. Individual components are quick and easy to score but provide minimal information about the overall quality of trials. A checklist or scale of several components can overcome this problem. Checklist differ from scales because checklist component are evaluated separately and don't have
numeral scores whereas in a scale each item is scored numerically and an overall quality score is generated. Jadad et al. report that there are at least nine checklist and 25 scales available in the literature for evaluating quality of literature. However only 1 scale has been developed following established methodological procedures. Other instruments that have been extensively validated and produce reliable scores even in individuals have not been trained to use it. Only one validated assessment tool for measuring quality. The more valid the systemic review, the more confidence readers have in its results for use in clinical practice.

The ideal reports contain information on the number and background of individuals who generated the assessments, extent of agreement of assessments, methods used to reconcile discrepant assessments, whether assessment were generated under masked conditions.

Quality assessment will inform reader about the credibility of the evidence in the literature. Some considerations based on quality may include or exclude trials, conduct sensitivity analyses allowing comparison between the results of trials with different quality, display graphically the results of each of the trial according to their quality, perform cumulative meta-analyses using quality assessments as the input sequence, weight trials according to their quality. Ideally reviewers would deal with perfect trials, thereby avoiding worry of the quality of the included trials.

Trials conducted in real-life are imperfect. Therefore the reviewer is reliant on the reported information to evaluate the quality of the trial. This may result
in a well-reported but poorly designed trial judged to be of high quality, while a well-designed trial poorly reported would be judged as bad quality. This may prompt some meta-analytic reviewers to not assess the trial quality. The assessment of trial quality, may limit the inclusion of trials into the study.

13.2.7 DATA ABSTRACTION

Data from published articles should be abstracted by at least two independent reviewers and the results compared. Data abstraction forms should be generated. Abstractors should be blinded to names of authors, institutions, journals, source of funding, and acknowledgements. This can be accomplished by photocopying papers, removing title page, concealing journal identifications, blacking out with marker, scanning text into computers and preparing standardized formats.

Data extracted from the original study should include all summary statistics, estimates of treatment effects or epidemiologic association (differences between groups, odds ratios, relative risks) are computed separately for each study. Extraction of raw data is best. Investigators must be certain that subjects are not counted more than once when there are multiple publications reporting results of a single study.

13.2.8 POOLING RESULTS AND ASSESSING HETEROGENEITY

It is important to determine whether it is rational to pool (combine) results of trials if the population, intervention, comparison group or outcomes measures
differed \(^{718}\). The homogeneity of the outcomes of the trials can be used to assess the statistical validity \(^{718}\). The forest plot can be used to assess the heterogeneity or homogeneity \(^{718}\). Results in similar directions suggest homogeneity whereas results in different directions suggests significant heterogeneity is present \(^{718}\). If significant heterogeneity is present, authors should identify potential sources contributing to the heterogeneity \(^{777,778,779,780}\). Pooling techniques can assess the treatment effect of homogenous studies. If the treatment effect is not homogenous (same/similar results are obtained from the studies) then a decision has to be made whether to analyze subsets that do display homogeneity or to analyze all studies together and try to account for the lack of homogeneity using random effects \(^{721}\). Increased power due to pooling should be carefully interpreted \(^{721}\). Subgroups analysis can result in problems including multiple comparison, wrong interpretation of differences, interactions \(^{715}\).

The meta-analysis of randomized controlled trials and the meta-analysis of epidemiological studies should be made distinct. Randomized controlled trials provide an unbiased estimate of the underlying treatment effect as such variability could be attributed to random variation. In epidemiological studies, e.g. case-control, cross-sectional or cohort studies, variability may be due to effects such as confounders and bias. As such estimates of association may deviate from the true causal effects that occur due to chance alone. Combining epidemiological studies may result in falsely precise and biased estimates of association \(^{777,778}\).
To determine if the treatment effects are similar, a test of homogeneity is conducted to appraise the consistency across studies\(^ {777,779,780}\). If the treatment effects are not homogenous, a decision must be made whether to analyze subsets that does display homogeneity or to analyze all the studies jointly and attempt to take into account the lack of homogeneity\(^ {721,781}\). Using the random effects method, the treatment effect of homogenous studies can be derived using pooling techniques and calculating significance or 95% CIs.

The qualitative approach involves weighing various studies according to methodological quality, evaluating scientific validity, pooling results of different studies to create a large study with and large statistical power\(^ {721}\). However heterogeneity may occur because baseline characteristics are different\(^ {721}\). Graphical analysis can be used to detect homogeneity including Mantel-Haenszel Chi-square test for homogeneity and regression techniques\(^ {782,783,784}\). Heterogeneity may occur due to inappropriate use of metric; small study numbers, different baseline risks within the patient population; correlated data from studies; predictable outcome and stratified randomization; manufactured data; and duplicate publications\(^ {782,785,786,787}\). Large between-study heterogeneity due to genuine diversity or bias is more common in epidemiological studies compared to randomized trials\(^ {716}\).

### 13.2.9 Discordance

Discordance among meta-analysis can occur when there are differing population of patients, intervention, outcome measure, selection criteria,
application of selection criteria, strategies to search literature, method to measure outcomes, end points, human error (random/systematic), methods to assess quality, interpretations of quality assessments, methods to incorporate quality assessment in review and statistical methods. Discordance may be confusing to clinicians and patients. Differences may lead to different healthcare decisions with implications for patient outcomes, costs of treatment, or both especially if one review suggests clinically important benefits while the other review suggests lack of benefit/harm.

13.2.10 SENSITIVITY ANALYSIS

Sensitivity analysis is used to assess the robustness of the findings. After the overall effect is calculated by fixed and random effects model, a separate meta-analysis is performed on a subset of the studies that have common features. The sensitivity analysis will yield similar results and justify the conclusion of the original analysis. If there is a difference then the cause needs to be identified.

13.2.11 ANALYSIS

Analysis can be conducted using the fixed effects model which considers variability that is due to random variation, or the random effects model which assumes that each study has its own source of variation which results in wider confidence intervals than the fixed effects model. Effects are randomly distributed and the central point of distribution is the focus of the combined effect
estimate\textsuperscript{780}. Significant differences between the combined calculated effect of the fixed and random effects models be observed if the studies are markedly heterogeneous\textsuperscript{718,715}.

### 13.2.12 META-ANALYTICAL RESULTS

### 13.2.13 ODDS RATIO

The odds is the number of the subjects in a group who achieve the endpoint divided by the number of patients who do not\textsuperscript{718}. The odds ratio (OR) is the ratio of two odds: the ratio of the odds of the treatment group to the odds of the control group\textsuperscript{718,715}. The risk is the number of subjects in a group who achieve the endpoint divided by the total number of patients in the group\textsuperscript{718}. The risk ratio/relative risk (RR) is represented by the risk of ratio of the risk of the treatment group to the risk of the control group\textsuperscript{718}. If the odds ratios or relative risk is greater than 1 then there is an increased risk for the outcome being present in the intervention/treatment group\textsuperscript{718}. If the odds ratio or relative risk is less than 1 then there is a decreased risk for the outcome being present in the intervention/treatment group\textsuperscript{718}. If the odds ratio or relative risk is equal to 1 then there is no difference observed between the intervention/treatment group—the outcome is just as likely to be present in both groups\textsuperscript{718}.

The 95\% confidence interval (95\% CI) is the range where 95\% of the true population treatment will lie\textsuperscript{718}. The range of the confidence interval indicates the precision of the estimate—the wider the range, the less the precision\textsuperscript{718}. If
the interval is very long, there is less certainty of the accuracy of the study predicting the true size of the effect. If the confidence interval includes 1, it is unable to demonstrate a statistically significant difference between the two groups being compared. However if the two comparison groups does not include 1, there is a statistically significant difference.

The p-value represents the probability that the observed differences between the treatment group and control may have occurred by chance. If the p<0.05 it means implies that the observed difference may have occurred by chance 5% of the time.

13.2.14 FOREST PLOTS

Following abstraction of data, the calculation of the treatment effect with confidence intervals should be undertaken. Odds ratios or relative risk ratios and risk difference are generated. The overall treatment effect is calculated using weighted averages of individual studies as this will more likely reflect the “true effect” of the intervention. The weight of an individual study is calculated by using the inverse of the variance (the square of standard error) of the treatment. The results from the meta-analysis are displayed as a forest plot. The authors’ name of the primary studies appears on the left side of the forest plot. Each study is represented by a square on a horizontal line. The area of the black box represents the weighting of the study. The horizontal lines extending from the black box represent the 95% confidence interval (CI). The squares indicate the point estimate and the 95% CI of the odds ratio of the
individual studies. The 95% CI represents where the true effect in 95% of the occasions would occur if the study was conducted repeatedly. The confidence interval provides an estimate of the reliability of the results thus, representing the true effects of the study if it were to be conducted repeatedly. The vertical line in the middle of the graph where the OR=1.0 is the line of no effect of treatment (P>0.05). This indicates where the treatment/intervention has no effect. If the confidence interval includes where the OR=1.0 then the effect of the treatment/intervention and the controls are not significant at conventional levels where p=0.05. The calculated overall treatment effect/odds ratio and 95% confidence interval is represented by the diamond at the bottom of the forest plot. This is calculated by using weighted averages of individual odds ratios. The centre of the diamond represents the combined treatment effect. The horizontal tips of the diamond represent the combined 95% confidence interval. If the diamond is to the left of the vertical line (OR=1.0, line of no effect) then there are fewer occurrences of the outcomes of interest compared to the intervention/treatment group. If the diamond is to the right of the vertical line (OR=1.0, line of no effect) then there are more occurrences of the outcomes of interest compared to the intervention/treatment group. When the diamond does not touch the vertical line (OR=1.0, line of no effect) there is a statistically significant difference between the intervention/treatment and control groups.

Funnel plots are used to assess if bias is present in the studies selected for meta-analysis. It assumes that the precision of the treatment effect with the increase of sample size. Thus small studies will scatter at one end of the
graph whereas large studies will converge at the other end. In the absence of bias, the overall result is a graph resembling a symmetrical inverted funnel. It the graph is asymmetrical or skewed it indicates that there is bias present.

The advantages of conducting meta-analysis is that it estimates the size of the treatment effect. Due to its rigorous process, the method of meta-analysis lessens its subjectivity. The combination of like studies increases the statistical power of the results, thus improving the generalizability of the results. Thus meta-analysis can resolve controversies between conflicting studies and assist policy makers in making decisions. In addition, it can identify areas of insufficient research and generate new hypotheses.

The limitation of conducting meta-analysis is that the meta-analysis is only as good as the quality of the included studies. First, it is difficult to identify all studies for inclusion. Electronic searches only identify 50% of all relevant articles and searching by hand is a labor-intensive process. It is also difficult to identify unpublished articles which results in publication bias.

A meta-analysis is also limited by how well studies are conducted and reported. The data must be complete, and the information precise. The conclusions of the studies are limited by the differing characteristics of its participants, different measurements obtained, different objectives, and different duration in which the data is captured. Together these factors affect the generalizability of the results.
13.2.15 BIAS

Examination for bias is essential during the meta-analytic process. Sources that bias can be attributed to include publication bias, location bias, language bias, citation bias, multiplication bias, bias in provision of data and quality of the study. Publication bias may occur when conducting meta-analysis. This may be due to unpublished trials (~10%), trials published in languages other than English (~10%), quality of trials, and trials that are not indexed in MEDLINE (~5%) results. Published studies that are not indexed in major databases are usually missed when conducting search. Studies with significant results are more likely to get published than those without. In addition, authors are also less likely to submit negative findings for publication because they anticipate rejection. Investigators working in non-English countries and less developed counties are less likely to be published and may only publish in their local journals. These local journals are also less likely to be indexed in databases. Articles that are published repeatedly result in citation bias and multiple publication bias. The inadvertent duplication of data within a meta-analysis may result in the overestimation of treatment effect.

Inclusion bias may result in the selection of certain studies to include the meta-analysis while other studies may be excluded. To overcome this dilemma, all studies that meet the basic criteria should be included and then sensitivity analysis should be performed afterward to determine if they truly have an effect on the overall result and are a contributor to bias.
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Neural tube defects (NTDs) are malformations of the cranium, spine, and nervous system; types of NTDs include anencephaly, spina bifida, encephalocoele, and meningocoele. Neural tube defects are a major cause of mortality in newborns and have been estimated to affect 0.5 to 6 per 1000 live births. Health Canada has estimated that 155 Canadian infants are born each year with NTDs. Overall, NTDs affect approximately 300,000 infants worldwide.

Epidemiological studies that associate folate supplementation with a decreased risk of NTDs date back to the 1960s. The most definitive research addressing the benefits of folic acid supplementation in decreasing the risk of NTDs was the multicentre, randomized, double-blind trial by the Medical Research Council in the United Kingdom. The aim of this trial was to evaluate the efficacy of 4-mg doses of folic acid in preventing recurrent NTDs in women who had previously delivered children with NTDs. The trial showed that women randomized to take folic acid supplementation had a 1.5% chance of having children with NTDs (relative risk RR 0.28, 95% confidence interval [CI] 0.12 to 0.71), but women in the unsupplemented group did not show a decrease in the risk of NTDs (3.4%) (RR 0.8, 95% CI 0.37 to 1.72). Overall, supplementation with folic acid reduced the rate of recurrence of NTDs by 72% (6/903 with folate supplements vs 21/602 without). A second key trial evaluating folic acid–fortified multivitamin supplementation during pregnancy was a double-blind, randomized controlled trial, in which women were randomized to take a multivitamin supplement containing 0.8 mg of folic acid or a multivitamin containing trace-element supplementation. Five thousand women were randomized in each group: no NTDs were observed in infants from the folic acid–fortified group, whereas 6 NTDs were found in those from the trace-element group. A recent meta-analysis observed an odds ratio (OR) of 0.67 (95% CI 0.58 to 0.77) in case-control studies and an OR 0.62 (95% CI 0.39 to 0.98) in cohort and randomized controlled studies. An OR of 0.67 means 0.33 (or 33%) protective effect; an OR of 0.52 means 0.48 (or 48%) protective effect. A study investigating the relationship between serum and red blood folate concentrations and the risk of NTDs found an inverse relationship between maternal red blood cell folate and the risk of NTD. Daly et al showed that women receiving less than 150 μg and
Motherisk Update

more than 400 µg of folic acid had a 6.6/1000 and 0.8/1000 chance of having children with NTDs, respectively. Supplementation at doses of 100 µg, 200 µg, and 400 µg of folic acid resulted in a 22%, 41%, and 47% decreased risk of NTDs, respectively.4

Optimal dose of folic acid supplementation

For almost 20 years, the recommended daily dose of folate supplementation has been 0.4 mg/d. In fact, prenatal multivitamins invariably contain 0.8 to 1.1 mg of folic acid, and this had led to the assumption that daily supplementation with this dose is sufficient to prevent NTDs. However, in 2001, Wald et al systematically reviewed all reports of the correlation between ingested dose of folate and resultant serum concentrations.5 Using the data by Daly et al.,6 who correlated maternal serum folate levels with the risk of NTDs, Wald et al concluded that the current recommended daily dose of folate will render only partial protection against NTDs. According to Wald et al’s analysis, 5 mg/d of folate would be necessary to render 90% protection within the populations.4 Their analysis has been recently corroborated by our findings that in 2005 and 2006, 40% of women in Ontario did not achieve the protective 900 nmol/L red blood cell folate, despite folic fortification and the fact that more than half of pregnant women supplemented with prenatal multivitamins.7

Potential risks

Before recommending prenatal supplementation with higher doses of folic acid, one needs to consider potential health risks of such an increase.

It has been proposed that higher levels of folate can mask pernicious anemia due to B12 deficiency. Similar concerns surrounded the original North American flour folate fortification program in 1998, but were not shown following the fortification. Several recent studies have failed to show such risks.8 A recent US study suggested an association between high folate levels in older Americans and a risk of cognitive impairment.9 However, cognitive impairment is not a component of pernicious anemia, and in that study there was no increased risk for neuritis, which is a typical finding of pernicious anemia. One has to remember that the risk for the pernicious anemia is different if the whole population consumes flour with higher levels of folate, as opposed to giving 5 mg/d to pregnant women for a limited time. In fact, direct measurements of B12, or higher supplementation of B12, can further allay these concerns.

If women do not comply with the recommendation to take the currently available folate-containing preparations, it is reasonable to question whether or not they would take the preparations containing 5 mg of folate daily. In a recent controlled trial of prenatal vitamin supplementations for women who discontinued or had not started using prenatal vitamins, their compliance with 2 different brands of prenatal vitamins averaged 58% and ranged from 0 to 100%, despite the participation of self-selected, motivated women.10 Pharmacologically, administration of 5 mg of folate daily in women who have a lower compliance with taking medication should provide many more women with protective levels of folate.

Although laboratory studies have suggested that folic acid might increase the risk of certain cancers, population-based studies have repeatedly shown folic acid use to be associated with a 20% to 30% decline in incidence (Table 1). Scientists therefore refer to the potential dual effects of folate on cancer risk, with increased risk for individuals with a history of or predisposition to cancer.11 There is no question that an increased risk of cancer associated with folate use, even if it exists, is a result of long-term exposure to folate over many years, and not to several months of dosing during pregnancy.

Table 1. Associations between folate status and risk of selected cancers

<table>
<thead>
<tr>
<th>TYPE OF CANCER</th>
<th>ASSOCIATION OF FOLATE AND RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Meta-analysis shows decrease in cancer risk with high folate status</td>
</tr>
<tr>
<td></td>
<td>Majority of case-control studies show reduction in risk (30% to 35%) at the highest dietary intake of folate</td>
</tr>
<tr>
<td></td>
<td>Might increase risk postmenopausal (statistically non-significant)</td>
</tr>
<tr>
<td></td>
<td>A non-significant trend for increase in breast cancer mortality when fortified with 5 mg/d</td>
</tr>
<tr>
<td>Colorectal</td>
<td>Inverse relationship between folate status and risk of colorectal cancer in healthy people</td>
</tr>
<tr>
<td></td>
<td>Potential increased risk of adenoma</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Decreased risk with higher folate status</td>
</tr>
<tr>
<td>Ovarian</td>
<td>Statistically significant decrease in the serous subtype</td>
</tr>
<tr>
<td></td>
<td>Prospective prevention (non-significant trend)</td>
</tr>
<tr>
<td>Bladder</td>
<td>Statistically significant lower folate in cancer subjects as compared with controls</td>
</tr>
<tr>
<td>Carcinoma of head and neck</td>
<td>Protective effect</td>
</tr>
<tr>
<td>Stomach</td>
<td>No effect</td>
</tr>
<tr>
<td>Esophageal and gastric</td>
<td>Protective effect in case-control studies</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>No correlation with folate status</td>
</tr>
<tr>
<td>Cervical</td>
<td>Folate fortification not associated with the degree or pattern of global DNA methylation in cells involved in cervical carcinogenesis</td>
</tr>
</tbody>
</table>

Data from Koen and Goh.10

1546 Can Fam Physician • Le Médecin de famille canadien Vol. 54, November/Novembre 2008
Incidentally, a systematic review that assessed the association between folate status and twinning found possible but non-significant evidence of periconceptional folate intake and twinning.6

**Conclusion**

Unless prescribing clinicians can ensure that pregnant women will be appropriately compliant in using prenatal vitamin supplements containing 0.8 to 1.1 mg of folate, they should consider prenatal vitamin supplements containing 5 mg of folate daily.12 Five mg of folate should be used several months before conception until the end of the first trimester of pregnancy.

Competing interests

Motherisk received financial support from Duchesney Inc and Wyeth Inc, manufacturer of prenatal vitamins. Dr Koren has served as a consultant for Duchesney Inc.

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**MOTHERISK**

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Do you have questions about the effects of drugs, chemicals, radiation, or infections in women who are pregnant or breastfeeding? We invite you to submit them to the Motherisk Program by fax at 416 813-7502; they will be addressed in future Motherisk Updates.

Published Motherisk Updates are available on the Canadian Family Physician website (www.cfp.ca) and also on the Motherisk website (www.motherisk.org).
DEVELOPMENT OF CANADIAN SCREENING TOOLS FOR FETAL ALCOHOL SPECTRUM DISORDER

Y Ingrid Coh,1,2 Albert E Chudley2,4 Sterling K Claren3,5, Gideon Koren3,7,8, Elaine Orbins3, Ted Rosales10, Charlotte Rosenbaum, for the Taskforce for the Development of FASD Screening Tools11

1The Motherisk Program, Division of Clinical Pharmacology & Toxicology, The Hospital for Sick Children, Toronto, Ontario, 2Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, 3Department of Pediatrics & Child Health, and Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Manitoba, 4Program in Genetics & Metabolism, Winnipeg Regional Health Authority, Winnipeg, Manitoba, 5Canada Northwest FASD Research Network, Vancouver, British Columbia, 6Centre of Community Child Health Research, University of British Columbia, Vancouver, British Columbia, 7Faculty of Medicine, University of Toronto, Toronto, Ontario, 8Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario, London, Ontario, 9Canadian Association of Paediatric Health Centres (CAPHC), Ottawa, Ontario, 10Department of Pediatrics, Faculty of Medicine, Memorial University of Newfoundland, St John’s, Newfoundland and Labrador, 11Charlotte Rosenbaum Consulting Services, Kingston, Ontario

ABSTRACT

Fetal alcohol spectrum disorder (FASD) is the most common cause of neurobehavioural handicap in North America. Screening for FASD may facilitate diagnosis and hence management of these children. We present a variety of screening tools for the identification of children at risk for FASD.

Methods
We critically reviewed and evaluated published and practiced methods for their potential of screening suspected cases, their epidemiological characteristics (sensitivity, specificity, positive and negative predictive values) [Phase I], as well as their feasibility [Phase II].

Results
The following five tools were selected for the FASD screening toolkit: screening fatty acid ethyl esters in neonatal meconium, the modified Child Behaviour Checklist, Medicine Wheel tool, Asante Centre Probation Officer Tool, and maternal history of drinking and drug use.

Conclusions
The toolkit for FASD screening aims at screening different populations, from the newborns to youth and at-risk mothers. It is anticipated that the toolkit will facilitate diagnosis of FASD.

Keywords: Fetal alcohol spectrum disorder, screening

1. Project Overview

1.1 Project Rationale
On March 1, 2003, Fetal Alcohol Spectrum Disorder: Canadian guidelines for diagnosis were published in the Canadian Medical Association Journal. The development of the guidelines was facilitated by the Public Health Agency of Canada and Health Canada. The current capacity of diagnostic clinics, however, is low compared to the number of patients referred for diagnosis. In addition, the validity and reliability of FASD screening tools have not been verified. As a result, healthcare and frontline professionals are inconsistent in the criteria in which they use to screen and refer children for assessment and
diagnosis of FASD. The Canadian Association of Paediatric Health Centres (CAPHC), funded by the Public Health Agency of Canada, initiated a national initiative entitled "Developing a National Screening Tool Kit for Those Identified and Potentially Affected by FASD". This project brought together many FASD experts and organizations to critically evaluate and recommend FASD screening tools for a national screening toolkit. The objectives of this initiative were three-fold:

a) to survey and critically evaluate FASD screening tools and methods in the published literature and used by clinics in Canada for referral to or acceptance into diagnostic clinics;
b) to evaluate epidemiological characteristics (sensitivity, specificity, and predictive values) of these tools; and
c) to develop practical guidelines (toolkit), based on the identified and evaluated tools.

1.2 Method

Professionals from diverse backgrounds, including FASD experts were asked to critically review the literature related to the screening and identification of FASD and to present their own research and findings. Nine panels were created and focused on the following areas: impact of screening, population variability, growth retardation, facial dysmorphology, neurobehavioural characteristics (two panels), biomarkers in maternal, clinical tools and youth justice populations. The full listing of articles reviewed and workshop presentations can be found on the CAPHC website at www.caphc.org/documents_programs/fasd).

2. Essential Components of Screening

The UK National Screening Committee definition of screening was adopted for the purposes of this task. It defines screening as: "A public health service in which members of a defined population, who do not necessarily perceive they are at risk of, or are already affected by a disease or its complications, are asked a question or offered a test, to identify those individuals who are more likely to be helped than harmed by further tests or treatment to reduce the risk of a disease or its complications." 3

Although screening has the potential to save or improve the quality of life through early diagnosis of various conditions, it is not a foolproof process. Screening can reduce the risk of developing a condition or its complications, but it cannot offer a guarantee of protection because there is always an irreducible minimum of false positive cases (wrongly reported as having the condition) and false negative cases (wrongly reported as not having the condition). An effective screening tool is cost-effective and quickly administered. Successful screening will identify a greater number of individuals than the true number who are affected by the condition. In most cases, these individuals are referred for further assessment and diagnosis for confirmation of the condition.

A good screening test must demonstrate both high sensitivity and high specificity. Sensitivity is the ability to correctly identify persons with the condition in the population who screen positive. Specificity is the ability to correctly identify persons without the condition in the population who screen negative. The higher the sensitivity and specificity reported, the greater the accuracy of the test. The positive predictive value (PPV) is the probability of the condition among individuals with a positive test. The negative predictive value is the probability of no condition among those with a negative test. A reference standard (an alternative method to determine the condition independent of the screening test) is required. At present, such a standard is not available for FASD other than a full diagnostic work-up.

2.1 Screening Criteria in the Context of FASD

According to the World Health Organization, to successfully implement a screening program the following conditions should be met:

i. A suitable test should exist;
ii. The disease or condition that is being screened for should be important medically, socially, or economically;
iii. The natural history of the disease should be understood and the population at risk should be identifiable;
iv. The test should be acceptable to the population;
Development of Canadian screening tools for fetal alcohol spectrum disorder

v. The condition should be recognizable at an early stage;
vi. There must be an accepted and effective treatment for the condition;
vii. There should be facilities for assessment, diagnosis and rehabilitation;
viii. Interventions should be acceptable to the population;
ix. The cost of screening should not be disproportionate to the cost of caring for the affected individuals, and
x. Screening programs should be a continuing process.

The rationale for FASD screening meets most, but not all of the above screening criteria. Universal screening for FASD using these criteria can result in beneficial outcomes. FASD results from maternal prenatal alcohol exposure. The prevalence of FASD is estimated at 9.1 per 1000 live births, with an estimated lifetime cost for one individual exceeding $1 million.\(^5\)\(^6\)\(^7\)\(^8\) The screening options for FASD are non-invasive and can result in earlier interventions such as early diagnosis, special education, increased resources and environmental modifications that have been shown to reduce the effects of developmental disabilities in children with FASD. As a result, there would be a reduction of secondary disabilities, leading to societal savings that can offset screening costs.

A drawback to universal FASD screening is that there is currently no widely validated, generally accepted screening test. In addition, the acceptability of various test methods has not been fully explored. Although the tests are non-invasive, there may be ethical and stigma issues for children and their family as well as time and cost issues for providers. In some areas of the country, FASD is not universally recognized or is believed to occur only in aboriginal populations only. Although facilities for diagnosis and assessment exist in Canada, they are overwhelmed; current average wait times in diagnostic clinics are six months to two years.

2.2 Comparisons with Other Universal Screening Programs for Childhood

Despite affecting an estimated 1/100 or over 330,000 Canadians, there is currently no accepted standardized screening test for FASD in Canada.\(^5\)\(^6\)\(^7\)\(^8\) In contrast, rarer conditions such as phenylketonuria and congenital hypothyroidism are universally screened for at birth.\(^9\) These conditions, however, have specific and effective biomedical treatments. HIV, cord blood testing, and testing for other rare genetic disorders have also become routine in maternal and perinatal care.\(^10\) Not only can a positively screened child be closely monitored for developmental disabilities and receive earlier interventions that can decrease or mitigate against FASD-related secondary disabilities, the mother can also receive necessary interventions to reduce or prevent ethanol consumption. Animal studies have demonstrated that ethanol exposed pups receiving earlier intervention had better outcomes because of brain plasticity.\(^11\)

A diagnosis of FASD would enable increased access to services and supports and more appropriate sentencing or conditions of probation after sentencing of affected youth in the youth justice system. Detecting a FASD affected child also identifies the addicted mother and possibly other children who may be at risk. Helping mothers could potentially result in reduced alcohol exposure in subsequent pregnancies.\(^12\)

2.3 Impact of Screening

While FASD screening may facilitate early diagnosis and intervention, the potential negative impact of screening on children’s and families’ lives must be carefully considered. Families and communities may suffer from stigmatization and screening may cause additional burden to already stressed families. The overall system capacity to consistently provide interventional supports and resources throughout the life stages for these children must be assessed and will require political will and commitment for the long term.

Screening on a national level should be evaluated by careful cost-benefit analysis, and compared with the benefits of screening for identified high-risk groups. The ability to reach the highest risk populations and the likelihood of compliance with treatment must also be addressed.

2.4 Population Variability

The epidemiology of FASD is quite variable. Overall, the prevalence of full-blown fetal alcohol syndrome (FAS) is estimated to be
somewhere between 1-3/1000 live births. Rates can vary ethnically, culturally, and regionally dependent. Published Canadian prevalence studies tend to focus on aboriginal populations, with prevalence rates varying from 1.5 to 9.1 per 1,000 for FASD. Approximately 12.3% of women of childbearing age are at-risk drinkers (> 7 drinks/week – 4 or more drinks per occasion), suggesting the importance of prevention efforts.

2.5 Population Variability and Key Screening Domains

Variability amongst the population may result in varied FASD effect and may limit the ability to screen. Research has shown that ethnic group/genetic factors, cultural/environment factors and age-related factors varied to such a significant degree that population-specific norms need to be developed. Alcohol damage is affected by genetic factors, maternal drinking history and pattern of drinking, mother’s nutrition and weight, and other risk factors, e.g. smoking and drug use.

The most important risk factor is high blood alcohol concentration, and associated variables such as timing of exposure during fetal development, the pattern of consumption, i.e. binge drinking and the frequency of use. The Canadian Diagnostic Guidelines emphasize the importance of confirmed alcohol exposure, rather than hearsay, lifestyle, other drug use or a history of alcohol exposure used solely to indicate maternal alcohol consumption for a specific pregnancy.

Psychometric screening norms: functional norms on standardized assessments vary across cultures. In addition, behavioural expression of disability can be affected by environment. A child’s genetic make-up may vary from standardized screening norms, e.g. birth weight and growth head circumference, and facial features, e.g. palpebral fissure measurements. In addition, facial features modify with age, some key psychometric assessments are difficult before age five or six, and risk factors may vary depending on child’s stage of development, e.g. behaviours such as lying, cheating, and stealing.

3. Evaluation of Screening Tools and Methods

Panelists presented and critically reviewed research related to tools and methods for FASD screening. Information was provided from critical review of the literature as well as unpublished research findings and practical application of clinic tools and methods in Canada. Benefits and limitations of tools and methods were discussed in detail and are summarized in this section.

3.1 Neurobehavioural Methods

The neurobehavioural profile for FASD is complex. Neurobehavioural deficits/problems must be closely examined to distinguish between those caused by FASD brain damage and those attributable to other causes or conditions. The literature was critically reviewed to determine which specific neurobehavioural deficit(s) constitute effective screening methods. In practice, screening for FASD occurs frequently in children exhibiting problem behaviour. It is typically initiated by non-clinicians, e.g. teachers, foster parents, and youth court workers. The checklists presently used are not scientifically validated and clinic intake procedures may screen for a variety of neurobehavioural deficits. Confirmed alcohol exposure is required for referral for FASD assessment. Clinic data has shown that First Nations children are more likely to be screened for FASD while non-aboriginal children are more likely to be considered ADHD. A concise, validated neurobehavioural checklist would be a valuable tool.

As present there is no single, consistent neurobehavioural profile of FASD in children. The literature search identified a number of cognitive, academic and behavioural factors that are associated with FASD. Broad-based indicators for screening from multiple sources are required, for example:

- Alcohol exposure, without which a diagnosis cannot be made
- Attention deficit disorder
- Academic school performance problems
Appendix F

Development of Canadian screening tools for fetal alcohol spectrum disorder

- Behavioural school performance problems
- Screening of specific high risk groups which may have built-in markers (e.g. youth justice, Neonatal Abstinence Syndrome in infants)

In an attempt to develop a screening tool, Streissguth et al. proposed the Fetal Alcohol Behaviour Scale (FABS). This tool was not able to discriminate between FASD and other clinical groups. The Personality Inventory for Children (PIC) has also been considered as screening tools, but it can only be administered by psychologists. The Child Behaviour Checklist (CBCL), a well-established tool for evaluating children's psychological problems is administered by psychologists. Research from the Hospital for Sick Children in Toronto has demonstrated the utility of items from the CBCL as a possible screening tool for FASD behavioural phenotype which can be administered by non-clinicians. Children with FASD were found to exhibit seven specific behavioural characteristics that were highly sensitive and specific for distinguishing them from children with ADHD:

- Acts too young for his/her age
- Can't concentrate/poor attention
- Can't sit still/restless/hyperactive
- Disoriented at home
- No guilt after misbehaving
- Impulsive/acts without thinking
- Lying or cheating

This information was used to create a screening tool for referral for FASD diagnoses. The modified CBCL test was further validated for children (6-16 years of age) with or without hyperactivity and poor attention. A systems approach to FASD screening has been proposed. This is based on the premise that FASD is not a behavioural disorder, but a neurological deficit (brain damage) resulting from prenatal alcohol exposure. The damage can manifest differently depending on age and environmental factors. Screening for FASD is strongly influenced by social system and the professionals working within these systems. A systems approach includes a staged screening process that examines problems in multiple profile domains that interfere with development, investigate developmental history-risk factors, e.g. prenatal alcohol and drug history, screening in communities with high prevalence, and collaboration of professionals from various systems.

The benefits of screening using the modified CBCL include its quick and straightforward administration; it can be administered by trained non-clinicians or a parent/caregiver who knows the child; it can be administered to all children; it uses scientifically objective measures; standardized tools exist for assessing cognitive and academic functioning; this tool may be able to differentiate between non-FASD ADHD children and FASD affected children.

The limitations of screening using the modified CBCL include that although the findings have been replicated in another cohort, the research has not been replicated in a large population. It is currently being repeated in a larger sample and also investigating persons with opposition defiant disorder and conduct disorder and examining potential confounders such as age, gender, socioeconomic status, home situation and IQ effects. There may be rater bias by the users. Users of the tool must have a background in normal child development to assess age appropriate behaviour. These are many overlaps with other neurobehavioural deficits e.g. ADHD. The behaviours being screened can also arise due to prenatal/genetic factors or environmental factors or experience. Although a statistical significant difference was observed, this does not mean that there are clinically significant differences.

Finally, there is a circularity of diagnosis - an individual has FASD and therefore has neurobehavioural deficits.

3.2 Facial Dysmorphology

Children affected with FAS are characterized by three dysmorphic facial features: a poorly formed philtrum, thin vermilion border of the upper lip and a short palpebral fissure length. The majority of children affected with FASD do not exhibit these facial characteristics. Assessment of facial dysmorphology, using a tool for diagnostic purposes, was considered for its applicability as a screening method. Measurements could be obtained manually by using a ruler. Alternatively digital photography coupled with measurement
Appendix F

469

Development of Canadian screening tools for fetal alcohol spectrum disorder

software could be used to obtain measurements. A study in Seattle screened 2,000 children in foster care demonstrating a prevalence of 1/100.22 The benefits of facial screening are that it is a safe, non-invasive methodology that is relatively low cost. Screening for facial dysmorphology has been demonstrated to have very high sensitivity, specificity, and positive predictive value.23 Digital cameras and software allow non-clinicians to interpret results with high interobserver reliability. Screening using this tool will decrease duties of diagnostic clinics.

The limitations of screening for facial dysmorphology include that a vast majority of children with FASD do not present with facial dysmorphology. In addition, the face changes with age. Facial features can be affected by genetics and ethnicity and there are no specific ethnic norms available. Thus it is hard to screen in ethnically diverse and mixed populations. Accurate measurements are dependent on well-taken photograph. There is a need to distinguish between "statistically significant" and "clinically relevant".

3.3 Meconium Testing for Ethanol Conjugates

Prenatal exposures to chemicals can be quantified in meconium. Studies of fatty acid ethyl esters (FAEE) in meconium have been conducted in Canada, the United States, Europe and South Africa.24,25,26,27 FAEE are unique biological markers of fetal exposure to excessive maternal drinking. Meconium levels of FAEEs above 2mol/g indicate heavy fetal alcohol exposure from light exposure at very high specificity and sensitivity.24,25,27

Meconium begins to form at approximately the 12th -14th week of pregnancy. As the fetus swallows amniotic fluid, prenatal exposure to chemicals can be quantified in meconium. Meconium measurement of fatty acid esters (FAEEs) (fatty acids synthesized with ethanol) is an unique biological marker for fetal exposure to ethanol. The benefit of screening meconium is that it is an objective, non-invasive, sensitive and specific method that can be used to detect maternal and fetal exposure. Meconium screening identifies both mother and child. Positive FAEE results have been associated with lower Apgar scores, low birth weight and lower executive functioning.24,25 Annual studies have also demonstrated a relationship between FAEE levels and growth retardation as well as brain weight.26 Screening meconium can demonstrate prenatal exposure when maternal self-report is not reliable. Meconium screening has been demonstrated to be cost-effective.27 A limitation of meconium screening is that it must be collected in the first 72 hours after delivery. Also, exposure during the first trimester of pregnancy is not captured in this screen. There are ethical concerns regarding informed consent of the mothers and reporting positive screens to child protective services.

3.4 Growth Retardation

Intrauterine growth can be influenced by a number of factors including genetics, ethnicity, and diabetes. Growth retardation is considered as part of the diagnosis process because alcohol is associated with impaired fetal growth. Growth retardation as a screening mechanism may have merit in combination with other biomarkers such as meconium screening.

The limitations of growth restriction included that growth standards differ for various populations. Only a small percentage of infants who are small for gestational age are associated with prenatal alcohol exposure of more than two drinks per day. The consequences of small for gestational age are significant e.g. high fetal and higher infant mortality, short-term metabolic problems, and deficits in growth and neurocognitive delays. However, the sensitivity is 10-30%, therefore a majority of cases are missed. It was generally agreed that growth retardation on its own is not useful as a screening method, but may have merit in combination with other prenatal screens, such as meconium screening.

3.5 Youth Justice Population

The youth justice population poses unique challenges for screening of FASD. There is evidence in the literature that individuals affected with FASD represent a disproportionately large number of youth and adults in the criminal justice system.29 In youth corrections, behavioral characteristics may drive interventions. Failure to feel remorse or understand consequences of actions has been described as neurobehavioral characteristics of some of those affected by FASD.
Appendix F

Development of Canadian screening tools for fetal alcohol spectrum disorder

This presents a challenge to the youth justice system to find appropriate deterrents/incentives for this population.

The FASD Youth Justice Project Manitoba screens youth 12-18 years of age with no prior FASD diagnosis and confirmed prenatal alcohol exposure who are undergoing pre-sentencing in Winnipeg. Items included on the screening questionnaire include:

- Repeated failure to comply
- Lack of empathy
- Poor school experiences
- Difficulties within institutions: compliance, peers, academics
- Unable to connect actions with consequences
- Not affected by past punishment
- Followers, rather than leaders, in crime
- Crimes involving risky behaviour for little gain

Screening was effective in the select population and resulted in 50/178 individuals receiving diagnostic assessment which resulted in 30 being diagnosed with FASD, 29 with ARND. The result had a 60% positive predictive value (personal communication, Manitoba FASD Youth Justice Program).

In the Youth Justice system in Saskatchewan, judges are instructed to screen based on the criteria used in the FASD Youth Justice Project in Manitoba. If the judge observes these characteristics, the youth court worker collects alcohol history through interview with the mother or a reliable source. The FASD Screening Tool Project in Saskatchewan has reviewed a number of screening tools and conducted a research study to validate a screening tool for use with offenders. In a collaborative research approach, agreement was reached on 28 risk factor items. The screening tool had a high inter-rater reliability 0.82, with a high validity 76% (personal communication, FASD Screening Tool Project).

A study conducted at Stony Mountain Institution near Winnipeg, Manitoba screened all offenders undergoing preliminary assessment. A Brief Screen Checklist (BSC) that included behavioural and historical indicators and maternal alcohol consumption was used to identify individuals for further assessment. Information was collected from the offender, parole officers and collateral sources. The study concluded that the incidence of FASD was ten times greater in the study sample compared to the North American population. The BSC items were highly correlated with a diagnosis of FASD. In addition, there was a high rate of neuropsychological impairments found in the study sample (personal communication, Stony Mountain Institution).

The Assante Centre for Fetal Alcohol Syndrome Probation Officer Screening & Referral Form is completed for all youth adjudicated on probation orders who reside in the Vancouver Coastal and Fraser Regions who are suspected of having fetal alcohol spectrum disorder. The tool is a pre-coded questionnaire which collects information on social and neuro-developmental history of the youth as well as the probation officer's knowledge of the youth and FASD. The survey tool was designed to screen youth for referral based on environmental factors and personal (neurobehavioural) factors. Referrals were made based on the combination of either one social/environmental factor plus two personal factors or no environmental/social factors but at least three personal factors. 26.9% of youth met the FASD criteria for further assessment (personal communication, Assante Centre for Fetal Alcohol Syndrome).

Subsequent to the Workshop, the Steering Committee members reviewed the Saskatchewan Fetal Alcohol Spectrum Disorders Functional Screening Tool. This comprehensive functional screening tool, which is still in development, was not considered appropriate for inclusion as a recommended tool at this time. The benefits for screening within the youth justice system are that by investing in the youth justice system by screening for FASD individuals it will enable the correct services of the needs and services for individuals to reduce the risk of reoffending, thereby resulting in long-term cost- and time-savings. A further benefit is that the tool can also be administered by frontline workers.

The limitations of screening within the youth justice system include that there is no validated screening tool for young offenders. It may also be difficult to collect information regarding maternal alcohol exposure. Frontline workers may be resistant to use screening techniques and there are
issues regarding obtaining consent and maintaining the confidentiality of the information. On the other hand, the family and/or youth may not agree to the assessment.

3.6 Clinic Tools
Screening tool currently used in diagnostic clinics across Canada show promise for broader application for different populations. However, all the tools require further validation. The Complex Developmental Behavioural Conditions (CDBC) Referral Form is used by the CDBC Network in British Columbia which provides screening and referral for provincial and regional developmental paediatric services. The Program diagnostic assessment services are intended for children and youth who have significant difficulties in multiple areas of function including those with known or suspected history of exposure to substances with neurodevelopmental effects. The referral form has been developed with guidelines that reflect the diagnostic assessment process, i.e. development and learning, mental health/behaviour, adaptive and social skills and biomarkers. Within the CDBC Network, referrals are taken from paediatricians or child psychiatrists with exceptions in remote areas where a family physician or nurse practitioner can make a referral. This tool has the potential to be developed and used as a screening tool by a wider range of providers, e.g. teachers, day care workers.

The Clinic for Alcohol & Drug Exposed Children (CADEC) in Manitoba does not use a screening checklist per se but provides the following criteria for referral:

- Consent
- Age 9 months to 12 years
- Confirmation of prenatal alcohol exposure
- Readiness for the assessment-child and parents’ stability
- Behavioural and developmental concerns realistic expectations of assessment

In addition, background information on the child’s birth history, medical history, family history, school records, psychological assessment, and social history is obtained. The diagnostic rate using these intake criteria was approximately 90%. The specificity of this tool was 24.3%. The sensitivity was 100% but there are an unknown number of false negatives. These criteria have not been validated (personal communication, CADEC). The Labrador Alcohol Research Group (LARGE) is a primary health care approach in Labrador to address FASD in the population (personal communication, LARGE). Referral information includes family/household information, family history, foster home involvement, neurobehavioural indicators, school support, public health reports and medical information.

The benefits for these clinic screening tools are they screen for all complex developmental behavioural conditions. They also reflect the diagnostic criteria/domain used in the assessment process. These tools are limited because they must be completed by a specialist physician. These tools also require further validation.

3.7 Community Tools
The Medicine Wheel Tool was developed for the Eshuapog Mi’gmaq First Nations community in New Brunswick (personal communication Eshuapog Mi’gmaq First Nations). The tools employ the Medicine Wheel framework which draws from traditional medicine in combination with scientific measures and indicators. A set of tools has been designed for a staged approach to screening and assessment in the school environment.

The first stage of screening involves the Medicine Wheel Mat Index which is administered by the child’s teacher. It explores mental, emotional, physical and social indicators along with spheres of learning and special services received. The tool takes approximately 15 minutes to administer. Significant problems in cognitive sub-domains or problems in one cognitive area coupled with problems in one or more of the conduct, social sub-domains or physical domain suggest the need for the second stage of screening involving the Medicine Wheel Developmental History. This is a semi-structured parent interview administered by a professional (other than teacher) in collaboration with the parent. Children who have screened positive proceed to diagnosis and assessment. A study screening 237 results in 29 referrals to diagnostic clinics where 67% were diagnosed with FASD.

The benefit of this screening tool includes that it is quick to administer. It can be
administered by properly trained teachers and relies on their judgment. The tools incorporate a First Nation's worldview and framework providing cultural context and relevance. Parents are engaged in the second step of the process as such there is a feeling of contribution. Limitations include that the tool has not been validated and assessed in other populations.

4. Promising Approaches

It was recognized that there is not one screening tool or method that would be suitable for all ages, cultures and environments. Several tools show promise for universal and/or targeted screening and may be included in the toolkit. Universal screening methods include meconium screening, and the modified CBCL. The targeted screening methods include the Medicine Wheel screening tools, screening for facial dysmorphology, and all presented youth justice screening tools.

While challenged by attempts to identify effective screening methods, the Steering Committee strongly felt that one should not disregard that maternal history of drug or alcohol abuse has been shown to correlate strongly with problem drinking in the index pregnancy. Hence it is important to consider children of these mothers as being at-risk group that needs careful follow-up. These children can be considered for diagnostic assessment if concerns regarding their appearance, growth, behaviour or development become evident.

5. Key Considerations

National screening initiatives must be considered within the context of providers' and health and educational/social services' capacity to diagnose, treat and support families, children and youth with FASD throughout life stages. Screening tools should be assessed for cultural appropriateness, age/stage of development, and environment or genetic factors which may influence their outcomes. Effective screening by non-clinicians will require training, as well as their support and commitment. The screening tools will need to be given consideration in terms of cost-effectiveness, public acceptance, and potential stigmatization. The screening tools will need further validation. In addition to screening, primary prevention plans should be developed. Screening for FASD may be a challenging task as most screening methods employ clinical and laboratory markers which are not part of the sought condition itself, but rather strongly correlate with it. In the context of FASD, most proposed screening methods constitute one or more signs of the syndrome itself. Second, many of the proposed FASD screening methods have not been validated for their epidemiological properties of sensitivity, specificity and predictive values. Third, due to the limited diagnostic capacity there is a fear that screening may result in a “positive screen” becoming a false “de facto diagnosis”. There was a consensus in the Steering Committee that “screening” is not “diagnosis” and should never be used as such. The Steering Committee felt strongly that wide screening will empower a change in climate toward more support for diagnosis and management of children and adults affected by FASD, as governments and other decision makers will realize the scope of this epidemic in their jurisdictions. Fourth, given the paucity of validated screening methods, it is apparent that different approaches and methods are required for screening depending upon the life stage of the child — from infant to young adult.

**Phase 2: Feasibility of Implementation of Screening Methods**

A workshop with frontline providers of various disciplines and sectors (e.g. health, education, social services, and youth justice) was hosted to assess the feasibility of implementing select screening tools across the country. In addition, a half-day session was held with First Nations and Inuit organizations to assess the Medicine Wheel tool and identify issues affecting the implementation of screening methods in their respective communities.

**Methods**

Workshop participants were pre-assigned to small discussion groups to review selected screening tools. Participants were assigned to groups based on their professional background and likelihood of using the tool in their work. Each group evaluated one of the six screening tools: FAEE screening in meconium, Youth Justice screening tool,
modified CBCL, facial dysmorphology, maternal history of substance abuse, and the Clinic for Alcohol & DrugExposed Children intake process.

Each group was led by a Steering Committee member. Participants first received a review of the screening tool, following which, they were asked to comment on the practical application of the screening tool and rate the screening tool on a scale of 1-5: Tools were assessed on ease of use (1=very difficult, 5=very easy), accessibility (1=unaccessible, 5=very accessible); cost (1=very expensive, 5=inexpensive); expertise (1=high level of expertise, 5=minimal expertise); cultural appropriateness (1=very inappropriate, 5=very appropriate). They were also asked to comment on factors to facilitate implementation and barriers to implementation.

Subsequent to review of the screening tools, participants discussed gaps and opportunities for screening and made recommendations on how to build capacity.

6. Screening Tools

6.1 Screening FACE in Meconium

Screening for biomarkers in meconium is an objective, non-invasive method of universal screening. This screening method identifies both the mother and child; therefore, systems should be in place for the management of both persons prior to the implementation of screening. This method could provide prevalence data. Obtaining consent for the collection must be carefully considered amongst different languages and cultures.

6.1.1 Ease of Use

Participants were given a hands-on demonstration of meconium collection. Meconium collection was given an average rating of very easy (average=5). Collection was deemed much easier than collecting cord blood, as collecting questionnaires or venupuncture. Due to light and temperature sensitivities, there needs to be protective collection techniques. There may also be an issue for specimens coming from remote communities requiring multiple transit or are delayed during transport. The analysis can be conducted in a laboratory with a gas chromatograph.

6.1.2 Accessibility

Meconium collection was deemed slight inaccessible (average=2). Currently, only one lab in Canada processes meconium samples. Early hospital discharge should not limit the accessibility of the test because infants are not sent home before they pass their first stool. Issues were also identified regarding follow-up, turn-around time to receive results, and sensitivity of the providers review screening results with mothers. When conducted anonymously, there is no opportunity to follow up with the mothers and the data can only be used to assess the prevalence.

6.1.3 Cost

Testing meconium was considered affordable (average=4). Costs related to shipping and training staff were not included in this consideration.

6.1.4 Expertise

Meconium testing received an average rating for expertise required to perform the test (average=3). Persons obtaining the sample would have to receive training regarding obtaining consent for the screening test. Lab technicians would also require training on conducting the analysis. Persons disclosing screening results would have to be able to communicate the difference between screening and diagnosis. They would also need training on substance abuse issues.

6.1.5 Cultural Appropriateness

Meconium testing was deemed very culturally appropriate (average=5). It is an objective test and with universal implementation there is little risk of stigmatization of cultural/ethnic groups or communities. Although the test itself is straightforward, language and cultural issues should be considered when describing the screening test and communicating the test results.

6.1.6 Factors to Facilitate Implementation

Identifying a larger amount of prenatal alcohol exposure than self-reported may increase awareness of this issue. This screen identifies two patients (i.e. the mother and the child) who can potentially be helped.
6.1.7 Barriers to Implementation

Barriers to implementation of meconium screening as a universal screeners include that it requires the support from provinces and territories. In addition, healthcare providers and organizations are already overworked and understaffed, thus this may increase their workload. There needs to be a method of support for both the child and mother who are identified as at risk. The test is limited to detecting alcohol exposure after the first trimester; therefore there may be a false sense of security when the meconium screens negative. In addition there is a question as to who ‘owns’ the results. This may be more relevant in custody cases. Setting up a national laboratory requires further consideration. There needs to be additional support from the public, healthcare professionals, hospitals, and government for wide-scale implementation.

6.2 Youth Justice Screening Tools

The youth justice screening tools focused on the Assertive Centre for Fetal Alcohol Syndrome Probation Officer Screening & Referral Form. This is a quick and easily administered tool in trained workers. The validity of the tool needs to be established prior to wider implementation.

6.2.1 Ease of Use

The form was deemed very easy to use (average=5). The tool can be administered within two to three minutes by trained frontline personnel.

6.2.2 Accessibility

The form was deemed accessible (average=4). To date, the tool has only been used for pre-sentence referrals. The language of the form needs to be simplified. Confirmation of prenatal alcohol exposure may be difficult to obtain in this age group.

6.2.3 Cost

Screening using this form is slightly inexpensive (average=4). Although there is little cost associated with the form, there is a cost for training providers regarding the administration of the form Testing of the form in other organizations will be able to confirm its validity.

6.2.4 Expertise

The form was considered user-friendly and required minimal expertise (average=4).

6.2.5 Cultural Appropriateness

The form was considered appropriate across many cultures (average=4). Currently, this form is only available in English. It will require translation into other languages; however, it may be difficult to translate certain concepts into other languages.

6.2.6 Factors to Facilitate Implementation

The SAMHSA FASD Center of Excellence (U.S.) has developed and validated a tool and provided training manual for this population which may be able to assist in the validation of this tool. The tool may be improved when combined with the Saskatchewan Youth Justice Screening Project and FASD Functional Screening Tool. This tool has contributed to the results of the recent National Round Table on Youth Justice.

6.2.7 Barriers to Implementation

Staff may be reluctant to ask about maternal alcohol consumption, and validation of mother’s alcohol consumption may be difficult. The tool is currently undergoing validation test. Securing ongoing funding to provide training and support, particularly in isolated communities, is a chronic problem. The tool may need to be adapted for different languages and cultures. Providers would need to be trained on the administration of this tool.

6.3 Modified Child Behaviour Checklist

The tool is not inclusive of other brain domains such as executive functioning, memory, abstract thinking. The objectivity of the assessor may be influenced by differences in cultures, values and settings.

6.3.1 Ease of Use

The modified CBCL is a short and easy to use questionnaire (average=4). An interviewer’s manual would be beneficial for administrators to clarify the language. Some of the questions are open to interpretation and may elicit different responses from the respondent.
6.3.2 Accessibility
The modified CBCL was deemed accessible (average=4). It requires a consent process when the information is being provided by someone other than the legal guardian. Educators are most likely to be the first people to be a source of information. Administering the tool in person would overcome literacy issues or problems with interpretation of questions. The tool is straightforward to translate.

6.3.3 Cost
The modified CBCL was deemed inexpensive to administer (average=3). There are low material costs and it takes a short time to complete.

6.3.4 Expertise
Moderate level of expertise would be required to administer the modified CBCL (average=3). The tool could be administered by trained frontline providers.

6.3.5 Cultural Appropriateness
The modified CBCL was deemed culturally appropriate (average=5). Different modified tools may be required for various age groups and populations as there is an acceptance of certain behaviors varies by culture and environment.

6.3.6 Factors to Facilitate Implementation
The modified CBCL integrates well with the diagnostic process. It can be improved with a procedural manual to clarify the role of the referer and the role of the screener.

6.3.7 Barriers to Implementation
It is not clear how the modified CBCL will account for social and environmental influences on behavior. The tool needs to undergo validation testing in different cultural groups and ages stages of development.

6.4 Facial Dysmorphology
Screening for facial dysmorphology is only suitable for target populations. If it only screens for individuals with FAS, which is a small percentage of the FASD population. Measurements can be challenging to obtain. Dysmorphology is not feasible to use as a screening tool and would be more appropriately used in the diagnostic process.

6.4.1 Ease of Use
The facial screening tool was deemed neither difficult nor easy to use. Most professionals considered learning to take accurate photographs relatively easy. Manual facial measurement, on the other hand, was seen as considerably more difficult. Obtaining measurements require good hand and eye coordination.

6.4.2 Accessibility
Facial measurement is objective; however, it is limited by its lack of ethnic norms. This is an accessible screen (average=3) and digital photographs can be transmitted over distances. Interpretation of the results requires training.

6.4.3 Cost
Opinions varied on the cost associated with facial screening. On average it was deemed slightly inexpensive (average=4). The measurement software and ruler were inexpensive, however, the equipment and time commitment was considered expensive.

6.4.4 Expertise
Facial measurements would require a fair amount of expertise to administer (average=2). Considerable training and hand and eye coordination would be required to accurately measure, interpret, and deliver results.

6.4.5 Cultural Appropriateness
Facial screening was considered culturally inappropriate (average=2). The measures are based on Caucasian norms and it is difficult to assess the effects of genetic influences and cultural differences which may affect interpretation of results.

6.4.6 Factors to Facilitate Implementation
Facial screening could be useful in a targeted population. If the child has these dysmorphic features, maternal alcohol use does not have to be confirmed.

6.4.7 Barriers to Implementation
Facial screening does not identify the majority of persons affected with FASD. Establishing facial norms for all cultures and persons of mixed heritage would be difficult (require considerable
Development of Canadian screening tools for fetal alcohol spectrum disorder

resources). Facial features also fade with age. Judgment on facial features could be used to label individuals. It is difficult to accurately measure facial features. This tool does not screen for the majority of individuals affected by FASD. This tool cannot be used on its own and is typically used during the diagnostic process. As such, the tool is not considered to be appropriate as a general population screening tool.

6.5 Maternal History of Substance Abuse
Screening for maternal history of substance (alcohol and drug) abuse assumes that if the mother has a substance abuse problem, she is likely to have abused alcohol. If she has an alcohol abuse problem then it is likely there was prenatal alcohol exposure. Substance abuse is defined as misuse or abuse. A positive screen can result in children receiving necessary supports but may result in stigmatization and isolation. Families have to be prepared to receive a positive result.

6.5.1 Ease of Use
Screening mothers was deemed neither difficult nor easy (average=3). It would depend on who provided the information (e.g., friend, family member, neighbour) as different people have varying judgment of what constitutes alcohol and drug abuse. Service providers may be uncomfortable screening women.

6.5.2 Accessibility
Screening for maternal history of alcohol use was deemed neither accessible nor inaccessible (average=3). Although it would be easier to obtain information from smaller community, there may be increased risk of stigmatization. However, fear of repercussions for the mother and child may influence the response. Confirmation from multiple sources can assist in the validation of information. The reliability of the collection of the information must be considered.

6.5.3 Cost
Screening for maternal alcohol consumption is a slightly inexpensive method (average=4) where the question can be added to existing questionnaires. Professionals need to be trained on how to ask the question without alienating the mother. There are costs associated with the treatment services provided to the mother.

6.5.4 Expertise
Maternal screening requires expertise to obtain reliable information (average=2). This may include training on substance abuse and training on how to approach the subject. With appropriate training any frontline worker or compassionate community member could be able to screen mothers.

6.5.5 Cultural Appropriateness
Screening mothers was deemed culturally appropriate (average=3). Community readiness to address prenatal alcohol exposure is essential. Some physicians still promote alcohol consumption during pregnancy. The question can be adapted into many languages and cultures. However, care must be taken to avoid stereotyping within cultures.

6.5.6 Factors to Facilitate Implementation
A reporting body needs to track all screened individuals who are waiting for diagnosis. In addition, screening provides an opportunity to assist the mother in a supportive environment.

6.5.7 Barriers to Implementation
Information received may be biased. Maintaining confidentiality in smaller communities may be a challenge. In cases where there is a question of custody, disclosure may be required. There must be capacity to support identified mothers and children.

6.6 Diagnostic Clinic – Intake Procedure
The Clinic for Alcohol & Drug Exposed Children (CADEC) Intake Procedure was selected to illustrate the strengths and challenges which are encountered by both referring providers and diagnostic clinics in identifying, referring and assessing children at risk for FASD. CADEC is a FASD diagnostic clinic in Manitoba that receives referrals from a range of sources. The persons making the referrals for CADEC are experts who are sensitive to family conditions. There currently exists extensive wait lists for diagnosis. The identification of children necessitates immediate case management and family support. Since
information is collected from multiple sources, ownership of information is challenging.

6.6.1 Ease of Use
The CADEC intake procedure was deemed straightforward and neither difficult nor easy to use (average=3). The overall process of collecting information from multiple sources was considered time consuming and requires an experienced and knowledgeable individual. Supports need to be available to children waiting assessment.

6.6.2 Accessibility
The CADEC intake procedure was deemed slightly inaccessible as it requires culturally competent individuals and interpreters (average=2). Healthcare providers need access to both electronic and paper documents. In addition, it may be difficult to acquire information on prenatal alcohol exposure. This information could impact the reunification of a child with their family. Moreover, long wait times could have negative effects on the child and their family.

6.6.3 Costs
The CADEC intake procedure was deemed slightly expensive (average=2). However, increased screening follows an increased need for diagnostic capabilities and resources for follow up. Society must recognize that the cost of early identification and treatment will result in long-term cost savings.

6.6.4 Expertise
The CADEC intake procedure requires a fairly high level of expertise in child growth and development, women's health, and family counseling (average=2). The family must be prepared to accept the potential consequences of a positive screen.

6.6.5 Cultural Appropriateness
The CADEC screening form was deemed neither culturally appropriate nor inappropriate (average=3). This process requires screeners who are culturally competent and interpreters who can assist the family through the process.

6.6.6 Factors to Facilitate Implementation
There should be clarity around who 'owns' the screening and diagnostic results. In addition, support and follow-up are best offered by a case management approach where all services are coordinated with the family by a designated provider.

6.6.7 Barriers to Implementation
The interface between community referral agents and diagnostic clinics is crucial as part of a comprehensive and seamless approach for children and families with FASD. Before implementing screening tools, capacity of diagnostic clinics, as well as 'fit' and compatibility of screening tools with clinic intake procedures should be assessed. The screening process adds to an already overworked system. In addition, diagnosis will result in the need for increased resources of supports and services. Despite the benefits of early screening and diagnosis, family readiness must also be considered.

6.7 Medicine Wheel Tools
The Medicine Wheel tools present a holistic approach to screening involving a community approach. A video demonstrating the use of the Medicine Wheel tools in a First Nations community school was shown. A number of interventions were provided to increase school performance and address behaviour issues. As part of this process, a comprehensive screening program was initiated in the school — all children were screened using the Medicine Wheel tools. The school did not wait for a diagnosis to act. Supports were provided to the parents to help them realize their personal goals.

6.7.1 Ease of Use
The Medicine Wheel screening tools appear easy to use and are relevant to First Nations and Inuit cultures (average=4). The tools are adaptable therefore it is possible to use components that are suitable for individuals or communities.

6.7.2 Accessibility
The Medicine Wheel screening tool was deemed accessible but would require translation (average=4). The tool is completed by teachers who know the child well. Unfortunately the tool is not applicable to high school students (young adults). The tool could be tailored to individual communities but this will require additional work.
Development of Canadian screening tools for fetal alcohol spectrum disorder

6.7.3 Cost
The cost of the Medicine Wheel screening tool was deemed slightly inexpensive (average=4). The cost of ignoring the child who may have FASD is greater than the cost delivering services. The materials are inexpensive, however, the training required to administer the tool and the high teacher turnover may result in high costs. Thus measures should be taken to retain teaching staff and also train community members. The implementation of this tool on a national level would be complicated and potentially expensive.

6.7.4 Expertise
The Medicine Wheel screening tool was deemed to require a fair amount of expertise (average=3). There is limited professional expertise available to First Nation and Inuit communities thus it is important to develop experts within the community. Experts or leaders would be required to coordinate or sustain the program. The tool provides an opportunity for the parents to contribute to the screening but requires a psychologist/social worker. The implementation of the tool is dependent on the philosophy of individuals. For example some professionals may not embrace the holistic approach.

6.7.5 Cultural Appropriateness
The Medicine Wheel screening tool was deemed culturally appropriate for First Nations and Inuit communities (average=4). The tool would require translation, which may be difficult. Since these tools are predeveloped, some communities may feel they have a lack of input. Some communities may resist this screening approach because of the bias/prejudice that may be associated.

6.7.6 Factors to Facilitate Implementation
The Medicine Wheel tools are holistic and based on the framework of aboriginal teaching. The tool can be administered by teachers and is a systems approach to screening that includes the family. It also evaluates various determinants of health. The tool is able to evaluate the child’s progress while emphasizing the child’s positive attributes. There are opportunities to adapt the tool to different First Nations and Inuit communities. This should be done with the input of community workers and parents. The Medicine Wheel is able to be applied in any school setting therefore it can be generalized to the Canadian population.

6.7.7 Barriers to Implementation
Barriers to implementation of this screening include that First Nations communities have limited access to assessment and diagnostic services. In fact diagnostic capacity is not existent in some First Nations communities. The tools may have to be adapted. The academic curriculum varies across the country. In addition, social and cultural norms also vary across communities therefore receiving a formal education at school may not be as important as learning traditional skills.

It may be difficult to achieve support from different levels of governments as some governments may not wish to address FASD and related issues. There also must be a method of establishing collaborations between health and education sectors. Within First Nations communities there is a legacy of psychological inferiority. Some highly skilled personnel from First Nations communities feel a lack of respect from mainstream counterparts and are reluctant to engage with mainstream organizations and providers. This can be a barrier to information sharing, referrals and program development and participation. The tool needs to be adapted/translated and validated before it use.

7. Opportunities

7.1 Infrastructure Improvements
The establishment of a secretariat at an international level would centralize efforts. Standardized national screening would enable the collection of data and statistics. The data would demonstrate that there is a greater need for diagnostic centres. Personnel in the field are too overloaded to evaluate their efforts and publish their data. There needs to be an ICD-10 code for FASD and/or alcohol-exposure.

7.2 Working within Limits and Constraints
In northern First Nations and Inuit communities there is lack of and time-limited funding available for FASD awareness and programming. There is a negative impact on communities when programs and initiatives are suspended due to the lack of funding. There are a lack of FASD coordinators and an absence of screening and diagnostic
Development of Canadian screening tools for fetal alcohol spectrum disorder

services within the region and limited support services.

There is a lack of consistent messaging from the medical establishments in Eastern Canada regarding FASD awareness and prevention. Conversely in Western Canada there are more education and training opportunities for professionals and FASD education is included in the school curriculum. FASD awareness must be addressed within the content of overall quality of life improvements and efforts to address the root causes of substance abuse. Raising awareness requires different strategies and approaches depending on who is being targeted.

7.3 Raising Awareness and Knowledge
A community strategy for prevention should be developed and there should be improved primary prevention strategies. Community leaders and champions in each sector should be identified. There should be political support for research within different sectors. Local media outlets should be used to engage public dialogue around FASD. Persons affected by FASD should also communicate with the public.

Education of professionals within different sectors would result in better support for affected individuals. Delivering presentations at conferences will increase awareness of professionals e.g. doctors, lawyers, and justice workers. Promoting the importance of screening across the community is also important. Educating and providing professionals with screening checklists will also heighten their awareness. Training frontline providers on how to approach women who may be drinking during pregnancy and harm reduction would decrease the "shame and blame" mentality. Education of children and youth would also improve awareness. Awareness should be increased amongst vendors and servers of alcoholic beverages. Perhaps placing warning labels on beverages would be effective. Hosting a workshop or focus group twice a year for persons involved in FASD prevention and intervention will provide opportunities to brainstorm community awareness strategies.

7.4 Capacity
Screening will result in an estimate of the prevalence FASD, which would advocate for the establishment of more diagnostic clinics and associated services. In addition, there needs to be an evaluation of what is needed to support families as well as criteria for a waiting list for screening and assessment should be developed.

7.5 Targeting
As FASD is a society-wide issue, education should target both women and men from all cultures and economic backgrounds. Conducting outreach to sexually active youths is also important.

8. Gaps

8.1 Readiness to Accept FASD Screening
There currently exists a regional difference in the level of readiness to accept FASD screening.

8.2 Funding and Capacity
Current funding for FASD diagnostic efforts is limited. Intermittent, time-limited funding allows initiatives to begin while sustainability remains an ongoing challenge. Due to the limited diagnostic capacity and long waiting lists, screening may run the risk of being a diagnostic tool. Thus frontline workers must be educated on how to interpret the screening information. Interim supports for individuals between screening and diagnosis must be established.

8.2.1 Strategies for Obtaining Funding Support
Engage community leaders and a national champion to raise awareness and advocate for resources and sustained funding. Raise awareness within governments by providing evidence with regard to lifelong costs for those affected with FASD. Develop partnerships with medical organizations such as hospitals and universities to elicit support. Promote opportunities to continue professional education. Analyze existing data from clinics to provide further evidence.

8.3 Awareness and Implementation Strategies
The participants of the workshop proposed many methods of increasing FASD awareness and the implementation of the screening toolkit across the country. It was proposed that a secretariat be developed to coordinate FASD-related activities at the national/provincial/territorial level. A national champion should be identified to advocate and raise awareness of FASD.

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FASD screening should be advocated within the context of other child health initiatives for sustainable long-term funding. By using standardized screening tools across the country, screening initiatives could be coordinated on a national level and included in PHAC’s strategic plan. Screening and diagnostic capacity and supports could be fostered with a continuum of care framework. The use of a standardized screening method would also result in the collection of vital statistics, providing prevalence data and estimates of lifetime costs.

As a first step, it would be necessary to assess the readiness of the community to address FASD. Increased awareness of FASD amongst the community and its leaders could be used to elicit support. Awareness could focus on prevention and education regarding the effects of alcohol consumption during pregnancy. Public dialogue could be initiated through local media. Efforts should be directed to the entire community to avoid singling out or stigmatizing specific groups within the community. Community forums could be assembled to review and adapt the screening tools and methods.

It was recognized that a cross-sectoral approach must be employed to engage professionals in health, social services, education, and recreation. Frontline providers would need training on approaching women who may be drinking in order to implement harm reduction strategies. Physicians would need to improve their understanding and ability to understand FASD. Medical training and awareness could be improved by partnerships with medical organizations, hospitals and medical schools. Medical researchers should be informed of the links between alcohol and brain damage and initiatives should be undertaken to engage graduate students in FASD data aggregation and analysis. Awareness should be raised amongst lawyers and other youth justice professionals. Perhaps this could be modeled after other existing projects e.g. British Columbia Probation Officer snapshot survey and professional development support. Professionals should convene in forums to share knowledge and lessons learned. In addition the gaps between mainstream and First Nations and Inuit professionals need to be addressed.

RECOMMENDATIONS

After reviewing and evaluating information collected from the literature review, diagnostic clinic surveys, and the workshops that were held with the scientific experts and frontline provider, the Steering Committee recommended that screening tools be included in the toolkit based on criteria including sensitivity, specificity, positive and negative predictive values, and practical applicability (ease of use, accessibility, cost, expertise required, and cultural appropriateness).

Since it was recognized that there is no “one size fits all” screening tool, multiple tools were recommended to screen for FASD amongst children and youth of different ages, stages, and within diverse settings. Universal screening of all mothers for prenatal alcohol exposure was also recommended. Universal screening of FAEE in meconium could be conducted in newborns. Screening using the modified CBCL could be conducted in children 6-18 years of age. Screening using the Medicine Wheel tool could be conducted in children 4-14 years of age. Screening using the Asante Centre Probation Officer Tool could be conducted in the youth population. Facial dysmorphology was eliminated by consensus as a screening tool.

Future Directions — Implementation

The workshop identified challenges and opportunities for implementation of FASD screening. In order to effectively implement screening, community, provincial, and professional participation and cooperation will be required. A staged process of screening is appropriate and most effective across various settings, sectors, providers, and communities. A toolkit will now be assembled containing manuals and tools required for screening and the interpretation of results. Focus groups and workshops will be conducted with policy makers and stakeholders to garner support, assess readiness and identify methods of improving knowledge transfer and the adoption of the toolkit into practice. In addition, assessment of resource requirements will occur prior to the implementation of this screening program.
Appendix F

Development of Canadian screening tools for fetal alcohol spectrum disorder

Acknowledgments and Funding

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Corresponding Author gkoren@tuckkids.ca

TASKFORCE FOR THE DEVELOPMENT OF FASD SCREENING TOOLS

Gail Andrew
MDCM, FRCP(C), Medical Site Lead – Paediatric Rehabilitation, Medical Director FASD Clinical Services, Glenrose Rehabilitation Hospital, Edmonton, Alberta

Kwadwo Obena Asante
MSM, BSc, MBChB, FRCP, DABP, Medical Director, The Asante Centre for Fetal Alcohol Syndrome, Maple Ridge, British Columbia

Susan Astley
PhD, Professor, Epidemiology, Adjunct Professor, Pediatrics, Director, FAS Diagnostic & Prevention Network, Center on Human Development & Disability, University of Washington, Seattle, Washington

Geri Bailey
RN, Interim Manager, Maternal Child Health, Parkuitnut Inuit Women of Canada, Ottawa, Ontario

Winnie Banfield
FASD/ECD Coordinator, Health and Social Services, Government of Nunavut, Iqaluit, Nunavut

Diana Boswall
Coordinator of Reproductive Care, Government of Charlottetown, Prince Edward Island

James F. Brison
PhD, Professor, Department of Pharmacology and Toxicology, Director of Research, Faculty of Health Science, Queen’s University, Kingston, Ontario

Brian Bruskie
Custodial Youth Worker, Kilburn Hall Youth Center, Saskatoon, Saskatchewan

Teresa Brown
Project Coordinator, FASD Youth Justice Program, Manitoba Department of Justice, Winnipeg, Manitoba

Sarah Carriere
Project Coordinator, Inuit Tapiriniit Kanatami, Ottawa, Ontario

Ashley Chafe
BSW, RSW Social Worker, Child, Youth and Family Services, Corner Brook, Newfoundland

Western Health, Corner Brook, Newfoundland

Albert E. Chudley
MD, FRCP, FCCMG, Professor, Department of Pediatrics & Child Health, and Biochemistry and Medical Genetics, University of Manitoba; Head, Program in Genetics & Metabolism, Winnipeg Health Region Authority, Winnipeg, Manitoba

Sterling K. Clarren
MD, FAAP, CEO and Scientific Director, Canada Northwest FASD Research Network; Centre of Community Child Health Research, University of British Columbia, Vancouver, British Columbia

Julianne Coury
PhD, RPsych, Research Psychologist, The Asante Centre for Fetal Alcohol Syndrome, Vancouver, British Columbia

Jocelynn L. Cook
PhD, Manager, Research Coordination Unit, Health Information, Analysis & Research Division, First Nations & Inuit Health Branch, Health Canada, Ottawa, Ontario

Elizabeth Dawson
RN, Nurse Specialist Early Childhood Development, Labrador Health Secretariat, First Nations & Inuit Health, Health Canada, Happy Valley-Goose Bay, Newfoundland and Labrador
Development of Canadian screening tools for fetal alcohol spectrum disorder

Laura Elliott
FASD Outreach Worker, McMan Youth, Family and Community Services Association, Edmonton, Alberta

Ellen Fantus
DPs, Psychologist, Division of Psychology, The Hospital for Sick Children, Toronto, Ontario

Valerie Flynn
Manager, FASD Strategic Programming Unit, First Nations and Inuit Health Branch, Health Canada, Ottawa, Ontario

Y. Ingrid Goh
PhD candidate, Division of Clinical Pharmacology & Toxicology, The Hospital for Sick Children, Department of Pharmaceutical Sciences, University of Toronto

Marilyn Gosselin
BSc, BED, MEd, Registered Psychologist, Special Education Consultant & Educational Psychologist, File Hills Qu’Appelle Tribal Counsel, Fort Qu’Appelle, Saskatchewan

Phat Ha
Statistical Analyst, Public Health, Health and Social Secretariat, Assembly of First Nations, Ottawa, Ontario

Bessie Hagen
FASD Mentor Trainee, Tuktoyaktuk, North West Territories

Ray Hareley
Program Manager, PLEA Community Services of British Columbia, Vancouver, British Columbia

Mary Hutchings
Assistant Director, Child and Youth Services, Children’s Aid Society of Toronto, Toronto, Ontario

Mary Johnston
FASD Team Manager, Public Health Agency of Canada, Health Canada, Ottawa, Ontario

Michelle Keightley
PhD, C.Psych, Assistant Professor, Department of Occupational Science and Occupational Therapy,

Graduate Department of Rehabilitation Science and, Department of Psychology, University of Toronto, Toronto, Ontario

Gideon Koren
MD, FRCPC, FABMT, Professor of Paediatrics, Pharmacology, Pharmacy, Medicine and Medical Genetics, University of Toronto, Hospital for Sick Children, Professor, Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario, Toronto, Ontario

Michael Kramer
MD, Scientific Director of the Institute of Human Development, Child and Youth Health (IHDCYH); Department of Paediatrics, McGill University, Montreal, Quebec

Elana Labranche
PhD, Assistant Director of Public Health, Department Nunavut Regional Board of Health and Social Services, Nunavut, Newfoundland and Labrador

Cлавдette Launry
MSc RN, Senior Program Advisor, Office of the Chief Medical Officer of Health, New Brunswick Department of Health, Fredericton, New Brunswick

Carolyn A. Lane
MD, CCFP, FCFFP, Assistant Clinical Professor, Department of Family Medicine, University of Calgary, Calgary, Alberta

Christine Louch
MD, FCPC, DAAP, Developmental Paediatrician, BC Children’s Hospital, Associate Professor, Department of Paediatrics, University of British Columbia, Vancouver, British Columbia

Holly MacKay
Senior Program Consultant Project Monitor, Public Health Agency of Canada, Health Canada, Ottawa, Ontario

Stuart MacLeod
MD, PhD, FRCPC, Executive Director, Child & Family Research Institute, Assistant Dean (Research), University of British Columbia; Vice President, Academic Liaison & Research
Development of Canadian screening tools for fetal alcohol spectrum disorder

Elaine Orrbine
President and CEO, Canadian Association of Paediatric Health Centres (CAPHC), Ottawa, Ontario

Carla Pamak
Mental Health Worker, Nunatsiavut Government, Department of Health and Social Development, Miin, Newfoundland

Wayne Podmoroff
PhD, Psychologist, Baffin Correctional Centre Department of Justice Government of Nunavut, Iqaluit, Nunavut

Garry Prediger
Director Saskatoon Provincial Correctional Centre, Saskatoon, Saskatchewan

Luise Roberts
Director, Aboriginal Family Centre, Happy Valley-Goose Bay, Newfoundland and Labrador

Ted Rosales
MD, Pediatrician and Clinical Geneticist, Clinical Professor of Pediatrics, Faculty of Medicine, Memorial University of Newfoundland, St John's Newfoundland

Charlotte Rosenbaum
Consulting Services, FASD Screening Tool Development Project Coordinator, Kingston, Ontario

Peter Rosenbaum
BSc, MD, FRCP(C), Co-Director, CanChild Centre for Childhood Disability Research; Joint Member, School of Rehabilitation Sciences, McMaster University; Professor, Department of Pediatrics, McMaster University; Associate Member, Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, Ontario

Hazel Russell
MSW, RSW, Social worker, Child, Youth and Family Services, Western Health, Corner Brook, Newfoundland and Labrador

Irena Nulman
MD, Program Director Postgraduate Medical Training Program in Clinical Pharmacology, Division of Clinical Pharmacology & Toxicology, The Hospital for Sick Children; Associate Director, Motherisk Program; Associate Scientist, Child Health Evaluate Sciences; Associate Professor of Pediatrics, University of Toronto, Toronto, Ontario

Kelly Nash
BSc, Psychology Department, Hospital for Sick Children; Ontario Institute for Studies in Education, University of Toronto, Toronto, Ontario

Jo Nanson
PhD, Psychologist, Adjunct Professor, University of Saskatchewan, Saskatoon, Saskatchewan

Joshua McMillan
FASD Consultant/Educator/Counsellor, Hey Way Noqu Healing Circle for Addictions Society, Clearwater, British Columbia

Patricia MacPherson
Acting Senior Research Manager, Addictions Research Centre, Correctional Service Canada, Montague, Prince Edward Island

David Main
Consultant, Department of Education, Government of Newfoundland and Labrador, St. John's, Newfoundland and Labrador

Dong Maynard
RRT, MBA, Associate Director, Canadian Association of Paediatric Health Centres (CAPHC), Ottawa, Ontario

Appendix F
Development of Canadian screening tools for fetal alcohol spectrum disorder

Dave Saint-Amour  
PhD, Neuroscience, CHU Sainte-Justine Research Center, Université de Montréal, Montreal, Quebec

Gillian Saunders  
FASD Coordinator, Nunatsiavut Department of Health & Social Development, Happy Valley-Goose Bay, Newfoundland and Labrador

Debra Schleever  
Executive Assistant, Canadian Association of Paediatric Health Centres (CAPHC), Ottawa, Ontario

Dorothy Schwab  
O.T.Reg. (MB), Occupational Therapist, Clinic for Alcohol and Drug Exposed Children, Community Liaison and Follow-up Worker, Winnipeg, Manitoba

Vivi Sosikas  
MD, Associate Executive Vice President, Society of Obstetricians and Gynaecologists of Canada, Ottawa, Ontario

Renata Sharkey  
Health Policy Analyst, FASD Strategic Programming Unit First Nations and Inuit Health Branch, Health Canada, Ottawa, Ontario

Paula Stanghetta  
Paula Stanghetta & Associates Inc., Project Coordinator, Canadian Centre on Substance Abuse, Kitchener, Ontario

Eva Szczepanik  
Associate Director, Canadian Association of Paediatric Health Centres (CAPHC), Ottawa, Ontario

Nancy Taylor  
Pre-primary Program Coordinator, English Program Services, Department of Education, Nova Scotia, Halifax, Nova Scotia

Janet Thompson  
Support Teacher, The Winnipeg School Division Special Education Department, Winnipeg, Manitoba

Suzanne Tough  
PhD, Associate Professor, Institute of Maternal and Child Health, Scientific Director, Alberta Centre for Child, Family and Community Research; Associate Professor, Departments of Pediatrics and Community Health Sciences; Associate Adjunct Professor, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Calgary, Calgary, Alberta

Lori Vitale-Cox  
PhD, Educational Psychology Coordinator-Education Division, Eastern Door FASD Diagnostic Team, Elsipogtog First Nations, New Brunswick

Bev Wahl  
Principal, David Livingston Community School, Winnipeg, Manitoba

Su Ping Walther  
Senior Policy Advisor – Aboriginal Health Transition Fund, Métis National Council, Ottawa, Ontario

Sharon Wazney-Prenegard  
Social Worker, Clinic for Alcohol and Drug Exposed Children, Winnipeg, Manitoba

Betty Wiebe Hossein  
Program Counsellor, Interagency FASD Program, Winnipeg, Manitoba

Richard Willier  
Aboriginal Youth Probation Specialist for Vancouver, British Columbia Government, Ministry of Children and Family Development, Vancouver, British Columbia
Development of Canadian screening tools for fetal alcohol spectrum disorder

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Development of Canadian screening tools for fetal alcohol spectrum disorder


Prenatal Supplementation With Multivitamins and the Incidence of Pediatric Cancers: Clinical and Methodological Considerations

Y. Ingrid Goh, MD1,2,3 and Gideon Koren, MD1,3

INTRODUCTION
Cancer is the second leading cause of death in children. The American Cancer Society estimates that 9,500 children will be diagnosed in 2006 and 1,500 children will die of cancer [1]. The Canadian Cancer Society reported that, during 1997–2001, an average of 1,285 children were diagnosed and 227 died every year of cancer [2]. Using the data obtained from the Pediatric Oncology Group of Ontario (POGO), Aga et al. [3] projected that the number of pediatric cancers is expected to increase by 8% in the interval 1995–2015. Nevertheless, childhood cancers in industrialized countries are rare and the majority of children will survive with appropriate medical treatment.

Cancer in childhood has been associated with various etiologies. Research has been undertaken to identify risk factors associated with pediatric cancers, but a single cause has not been isolated [4,5]. However, one hypothesis suggests that prenatal multivitamin consumption may be associated with the prevention of cancers in children. It has been shown previously that prenatal multivitamin ingestion is associated with a decreased incidence of congenital anomalies [6]. The following review summarizes the available knowledge associating prenatal multivitamin consumption with the prevention of pediatric cancers.

BRAIN TUMORS
The first study to investigate the effect of multivitamin supplementation and its effects on pediatric cancer was by Preston-Martin et al. [7]. They conducted a case–control study, originally trying to link connection between pediatric brain tumors and maternal meat consumption. One of the factors they investigated was the effects of prenatal vitamin supplementation as a confounder for childhood brain tumors (CBT). In this study they compared 226 cases exposed to prenatal multivitamin supplementation and 209 controls identified in Los Angeles from 1972 to 1977. The OR was 0.6 with a one-sided P = 0.12.

Burin et al. [8] reported a case–control study of the Children’s Cancer Study Group from 1956 to 1989 to investigate the effects of prenatal multivitamin supplementation on the risk of primitive neuroectodermal tumors. The majority of mothers (92%) took multivitamins during their pregnancies. Multivitamin use in the first 6 weeks after the last menstrual period was associated with an OR = 0.56 (CI95% = 0.32–0.96), P = 0.02.

Lubin et al. [9] undertook a case–control study in Israel from 1984 to 1993 to investigate the effects of folate supplementation on the risk of pediatric brain tumors. In comparing 300 cases to 574 controls, they determined an OR = 1.05 (CI95% = 0.76–1.44), P = 0.76.

Burin et al. [10] conducted a case–control study in the Children’s Cancer Group from 1986 to 1989 to investigate the effects of maternal diet on the risks of astrocytic gliomas. In comparing 156 cases and 155 controls, they determined that multivitamin supplements were associated with an OR = 0.6 (CI95% = 0.29–1.5).

Sarason and Savitz [11] reported on a case–control study of residents of Denver, Colorado from 1976 to 1984 to investigate the effect of meat groups and vitamins on cancers in children. Respectively, 13% and 10% of mothers in the case and control groups reported not taking prenatal multivitamins. In comparing 223 cases (including 45 brain tumors) and 206 controls, of which 45 had brain tumors, OR = 0.70 (CI95% = 0.26–1.86), Brain tumors were more common in the absence of vitamin supplementation.

Preston-Martin et al. [12] undertook a case–control study involving children of 0–19 years of age in Los Angeles, San Francisco–Oakland, and Washington state diagnosed with a brain tumor between 1984 and 1990 to assess if maternal intake of vitamin supplements during pregnancy was associated with a decreased risk of CBT. In comparing 540 cases and 804 controls, they found that women supplementing less than two trimesters had OR = 0.98 (CI95% = 0.55–1.77) for two trimesters had OR = 0.55 (CI95% = 0.29–0.77), and for three trimesters had OR = 0.54 (CI95% = 0.39–0.75).

cases and 1,919 controls, they found that women supplementing less than two trimesters had OR = 0.8 (CI95% 0.6–1.0), for two trimesters had OR = 0.7 (CI95% 0.5–0.9), and for three trimesters had OR = 0.6 (CI95% 0.5–0.8).

We recently meta-analyzed the available literature and found that prenatal multivitamin supplementation was associated with OR = 0.73 (CI95% 0.60–0.88) for brain tumors [14].

HEPATOBLASTOMA

Buckley et al. [15] reported on their case–control study in the Children’s Cancer Study Group from 1980 to 1983 to investigate the effects of prenatal vitamin or iron supplementation on the risk of having a child with hepatoblastoma. In comparing 61 cases to 64 controls, they determined an OR = 1.4.

Buckley et al. investigated the effects of folate fortification in food using the POGO database from 1985 to 2000. They found an incidence rate IRR = 0.81 (CI95% 0.35–1.89) for hepatoblastoma [16].

LEUKEMIA

Robinson et al. [17] executed a case–control study in the Children’s Cancer Study Group from 1980 to 1984 to investigate the effects of prenatal vitamin or iron supplementation on the risk of having a child with acute myeloid leukemia (AML). In comparing 204 cases to 204 controls, they determined an OR = 1.0 (CI95% 0.51–1.96), P = 1.00.

The study by Sarason and Saveli [11] included 45 children with acute lymphocytic leukemia (ALL), OR = 0.8 (CI95% 0.22–1.13). ALL was more common in the absence of maternal vitamin ingestion.

Wen et al. [18] reported on their case–control study in the Children’s Cancer Study Group from January 1, 1989 to June 15, 1993 of children up to 15 years of age to investigate the risk of ALL with prenatal medication exposure. In comparing 1,842 cases to 1,986 controls, they determined an OR = 0.7 (CI95% 0.5–1.0). This was adjusted for mothers who supplemented before and during pregnancy or for those who only supplemented during pregnancy.

Thompson et al. [19] conducted a case–control study in Western Australia from 1984 to 1992 to investigate the effects of folate supplementation on the risk of ALL. In comparing 83 cases to 160 controls, they determined an OR = 0.4 (CI95% 0.21–0.73), P = 0.0032 in women supplementing with folate with or without iron and an OR = 0.41 (CI95% 0.22–0.75), P = 0.0404 in women supplementing with folate and iron.

Raw et al. [20] performed a case–control study in the Children’s Oncology Group from 1997 to 2002 to investigate the effects of the use of vitamins on the risk of ALL and AML. They identified children from 0 to 18 years of age, 173 cases and 178 controls.

Retinoblastoma

We recently meta-analyzed the available literature and found that prenatal multivitamin supplementation was associated with OR = 0.61 (CI95% 0.50–0.74) for ALL [14].

NEUROBLASTOMA

Michalek et al. [21] conducted a case–control study using the New York State Cancer Registry from 1976 to 1997 to investigate the risk of neuroblastoma in children born to mothers with prenatal vitamin supplementation. In comparing 183 cases and 372 controls, they determined an OR = 0.5 (CI95% 0.3–0.7). In contrast, women who reported never taking vitamins during pregnancy had an OR = 2.2 (CI95% 1.4–3.2).

Oshans et al. [22] reported on their case–control study in the Children’s Cancer Group and Pediatric Oncology Group from 1992 to 1994 to investigate the risk of neuroblastoma in children born to mothers who supplemented with multivitamins before and/or during pregnancy. In comparing 538 cases and 504 controls, they determined that mothers who began supplementing daily 2–12 months before pregnancy had an OR = 0.7 (CI95% 0.4–1.1), and 1 month before pregnancy had an OR = 0.7 (CI95% 0.5–1.1), first trimester of pregnancy had an OR = 0.7 (CI95% 0.5–1.0), second trimester had an OR = 1.0 (CI95% 0.8–1.3), and the third trimester had an OR = 0.6 (CI95% 0.4–0.9) [22]. French et al. [16] investigated the effects of folate fortification in food using the POGO database from 1985 to 2000. They found an incidence rate ratio IRR = 0.38 (CI95% 0.23–0.62) for neuroblastoma. We recently performed a meta-analysis on the available literature and found that prenatal multivitamin supplementation was associated with OR = 0.52 (CI95% 0.42–0.68) for neuroblastoma [14].

RETIINOBLASTOMA

Bunin et al. [23] conducted a case–control study in the Children’s Cancer Study Group from 1982 to 1985 to investigate the effects of prenatal vitamin supplementation on the risk of having a child with retinoblastoma. Supplementation of prenatal vitamins in the first trimester of pregnancy was associated with OR = 0.4, P = 0.03. The rates for sporadic non-heritable retinoblastoma were OR = 0.7 (CI95% 0.3–0.8) when supplementing at some point during pregnancy and OR = 0.4 (CI95% 0.2–0.9), P = 0.05 when supplementing in the first trimester of pregnancy. The rates for sporadic heritable retinoblastoma were OR = 0.2 (CI95% 0.02–0.7), P < 0.05 when supplementing at some point during pregnancy and OR = 0.3 (CI95% 0.1–0.9), P < 0.05 when supplementing in the first trimester of pregnancy.

Based on the evidence available today it is impossible to ascertain which component(s) of the prenatal multivitamin supplements are associated with these protective effects against early cancers in offspring. Nonetheless the findings suggest that not only does prenatal multivitamin supplementation prevent congenital anomalies but it may also prevent pediatric cancers. A potential flaw in all available studies is that they were not randomized. Hence it could be argued that taking prenatal vitamins may not be causal, but rather a marker of well-being, better socioeconomic class, nutrition and healthy lifestyle. Yet it is noteworthy that such potential modifiers per se (e.g., socioeconomic status, education) are not known to affect rates of pediatric tumors. With an increasing percentage of women supplementing prenatally with multivitamins,

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due to recommendations to prevent neural tube defects, it will be critical to monitor changes in population rates of pediatric tumors.

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Folic acid in pregnancy and fetal outcomes

Y. L. GOH & G. KOREN
Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Canada

Summary
Folic acid has become recognised as an important nutrient during pregnancy. The following review highlights the significant developments in recognizing folic acid importance in fetal development.

Keywords
Folic acid, multivitamin, pregnancy

History
Derived from the Latin word for leaf, folium, folic acid (folate, vitamin B9, pteroylglutamic acid, pteroylglutamate) is a water-soluble vitamin. The discovery of folic acid dates back to the early 1930s when Lucy Wills recognized that a factor in yeast was able to correct macrocytic anaemia in pregnant women (Wills and Melia 1930; Wills 1991; Hoffbrand and Weir 2001). This finding was confirmed in animal studies and as a result, supplementation of yeast or Marmite (a yeast extract) was recommended for women suffering from macrocytic anaemia in Bombay (Wills 1991; Hoffbrand and Weir 2001).

Folate was first isolated from spinach in 1941 (Mitchell et al. 1980), following which, it was synthesised by Bob Stokstad in 1943 (Hoffbrand and Weir 2001). Folic acid was found to be effective in treating megaloblastic anaemia of all types, including megaloblastic anaemia of sprue, ovicul disease, pregnancy, and malnutrition, as well as Addisonian pernicious anaemia (Moore et al. 1945; Vilter et al. 1945; Hoffbrand and Weir 2001). Victor Herbert illustrated the association of folate deficiency and anaemia when he monitored the sequence of haematological events while ingesting a folate deficient diet for four months (Herbert and Zalisly 1962). Not only was folic acid found to be useful in treating anaemia during pregnancy, Metz et al. (1961) showed that folic acid prophylaxis decreased the risk of premature births in undernourished populations.

Pharmacokinetics
The term folic acid generally refers to the fully oxidized form of the chemical compound which is not naturally available in foods. On the other hand, the term folate refers to the group of compounds that have the same vitamin activity – encompassing both folic acid and naturally occurring folates. Folates are composed of a pteridine ring, p-aminobenzoic acid, and may contain 1–6 glutamate molecules that are joined by peptide linkages (Locksmith and Duff 1998; Hoffbrand and Weir 2001). Natural folates are found in the form of polyglutamates. These are relatively heat labile molecules that may be destroyed by storage, processing, and cooking and are not readily absorbed by the body (Hall and Solomski 1998).

The absorption of dietary folate occurs in a two-step process (Van Dyk et al. 2002). Natural folates are metabolized by conjugases in the upper small intestine into monoglutamates which are then absorbed into the body by specific carriers located on the cell membrane of the proximal small intestine (Cezir et al. 1990; Hall and Solomski 1998; Bailey et al. 2001; Van Dyk et al. 2002; Sauer 2004). Conversely, since synthetic folates are relatively stable as monoglucamates, they have a better bioavailability than natural folates. As such, synthetic folates are rapidly absorbed in the unmodified or in the reduced or methylated form (Rockey et al. 1984; Cezir and Dudas 1992; Gregory 1995; Locksmith and Duff 1998; Van Dyk et al. 2002). Absorption across the enterocyte brush border membrane can occur by two means: high-capacity transporter that is pH dependent or low-capacity facilitated diffusion that is pH independent (Van Dyke et al. 2002). Of the two, the pH-dependent transporter is the prominent route of folate absorption (Malatuck et al. 1993; Van Dyke et al. 2002). This anion exchange mechanism, however, is a saturable process (Sauer 2004). After entering the cell, the transported folate monoglucamates are reconstructed into a polyglutamate chain by folypolyglutamate synthase (Sauer 2004). The majority of folate is stored in the liver (Rockey et al. 1984; Malatuck et al. 1993; Van Dyke et al. 2002).

Folate metabolism occurs in the cytoplasm and mitochondria (Sauer 2004). Polyglutamyl folates are hydrolysed to folylmonoglutamates by pteroyl-p-glutamyl-hydrodase (Sauer 2004). Those in turn are metabolised...
into 5-methyl-H4PteGlu1 within the enterocyte and transported to peripheral tissues, where it is converted to 5-methylenetetrahydrofolate by methionine synthase (Gregory 2001; Stover 2004). Its one-carbon unit metabolism synthesizes three products in the cytoplasm: 10-formyltetrahydrofolate, methylene tetrahydrofolate, and 5-methyltetrahydrofolate (Wagner 1995). These three products are required for the synthesis of purine rings; conversion of deoxyuridine monophosphate to deoxyribonucleic acid; the methylation of homocysteine to methionine, respectively (Wagner 1995). The primary form of folate found in serum is 5 methyltetrahydrofolate (Krausswitz et al. 1994). This is the chemically active form of folate which is a co-factor for a variety of biosynthetic pathways (Krausswitz et al. 1994; Buttersworth and Benson 1996).

Folic acid is required for the synthesis of methionine from homocysteine (Stover 2004). Methionine is converted to S-adenosylmethionine, which acts as a co-factor for many methylation reactions including the methylation of DNA, RNA, proteins, and neurotransmitters (Krausswitz et al. 1994; Locksmith and Duff 1996; Hall and Solcholin 1998; Clarke and Bandfield 2001). Its ability to transfer methyl groups results in the recycling of homocysteine back to methionine (Locksmith and Duff 1998). The single carbon units from this final product are used for nucleotide biosynthesis including pyrimidines, thymine, and purines, and the synthesis of DNA (Madd 1988; Rosenblatt 1995; Locksmith and Duff 1998; Ramalhelman 1999; Bailey 2001; Zhao et al. 2003). As such, all new cell formation is dependent on the adequate supply of folate. Folate also plays a role in protein metabolism by mediating the interconversion of serine and glycine and playing a role in histidine catalysis (Hibbard 1975). Therefore, folate deficiency in rapidly dividing cells may lead to alterations in DNA synthesis and chromosomal aberrations (Hench 1966; Sutherland and Ledbetter 1989). When the hypomethylation of DNA occurs, DNA strand breakage and abnormal gene expression may result (Wallan and Fother 1992; Christman et al. 1993; Pogribny et al. 1995; Pogribny et al. 1997a; Pogribny et al. 1997b). Folate is degraded through the process of irreversible oxidative cleavage for the CO-N10 bond. This results in the formation of picolinate and p-aminobenzoic acid as degradation products (Stover 2003).

Folic acid is essential for growth and differentiation, repair, and host defense (Hall and Solcholin 1998) and hence it is essential for fetal development. In order to be transported to the fetus, folate monoglutamates are transported by folate receptor, FolsR, that are highly expressed in the embryo and fetus, especially in the neural folds prior to the closure of the neural tube (de Franchis et al. 1995). During embryogenesis and fetal growth, nucleic acid, and protein synthesis are reliant on the supply of folate and therefore the requirement for maternal folate increases during this period of cell formation (Locksmith and Duff 1998). A deficient folate supply or problem in its metabolism may result in impaired cell formation and tissue growth (Hibbard 1975). As such, nucleic acids will not be synthesized and cells will be unable to manufacture enough DNA for mitosis therefore resulting in abnormal cell division (Locksmith and Duff 1998). Protein, lipids and myelin will also not be methylated due to the inhibition of the methylation cycle. Since cells are rapidly dividing during the fetal period, they are most susceptible to irregularities in DNA production. Folate deficiency or impairments in genetic folate metabolism are proposed mechanisms that cause congenital birth defects (Locksmith and Duff 1998). It is estimated that congenital birth defects affect 5% of individuals (Christman et al. 1991); however, only 2–3% are recognized at birth (Marden et al. 1964). It has been proposed that folate acid supplementation may correct folate levels in deficient mothers or compensate for innate folate metabolism abnormalities to decrease the risk of congenital birth defects (Scott 1994). The following review will highlight studies in which folate acid has been suggested to decrease the risk of birth defects.

Neural tube defects

Neural tube defects (NTDs) comprise of malformations of the cranial, spine, and nervous system including anencephaly, spina bifida, encephalocele, and meningocoele. NTDs are a major cause of mortality in newborns secondary to congenital heart defects (Fleming 2001). NTDs are estimated to affect 0.5–8,100 live births (Fleming 2001). The prevalence of NTDs varies by geographic region and ethnicity. The CDC estimates that 3,000 infants are born each year with NTDs in the USA (Centers for Disease Control and Prevention 2004). Health Canada estimates that 195 infants are born each year with NTDs (Health Canada 2002). The prevalence is highest in Celtic and the western part of the British Isles (Elwood 1976), New Brunswick (Frecher and Fraser 1987), Eastern USA (Greenberg et al. 1983) and in Sri Lanka (Rain 1983). Conversely, there is a low prevalence of NTDs in the African population (Lary and Edmonds 1996). NTDs are present at moderate incidence in the Hispanic population (Shaw et al. 1994; Lary and Edmonds 1996). Overall, it is estimated that NTDs affect 300,000 infants worldwide (Shibuya and Murray 1998). The exact mechanism by which folate supplementation prevents NTDs and its recurrence is not known (Fleming 2001). Studies associating folate supplementation in decreasing NTDs date back to the 1960s (Smithells 1968). Smithells et al. were the first to demonstrate this relationship in their case-control study where they observed that prenatal multivitamin supplementation resulted in a 71,000 incidence of NTDs, whereas unsupplemented mothers had a 221,000 incidence (Smithells 1968). This study was followed by a case-control study evaluating supplementation in English and Irish women who had previously delivered a child with NTDs (Smithells et al. 1976). Women receiving supplementation had 0.6% recurrence rate of having a child with NTD, whereas women who did not receive supplementation had a 5.0% recurrence rate (Smithells et al. 1976). Two additional prospective studies by this group suggested that these protective effects were associated with folic acid (Smithells et al. 1980; Smithells et al. 1981). In one study, folate supplementation resulted in 0.5% children with NTDs, whereas no supplementation resulted in a 6.0% incidence (Smithells et al. 1980). The second study had similar findings where 0.7% NTDs were observed in folate-supplemented women, while 4.7% NTDs were observed in unsupplemented women (Smithells et al. 1981). A cohort study of women who previously delivered a child with NTD observed that supplementation resulted in a 0.9% incidence of NTD in the following child, whereas no supplementation resulted in a 5.1% incidence.
(Smithells et al. 1983). In 1992, Holmes-Siedle et al. emulated Smithell's original protocol of a cohort study investigating the effect of multivitamin supplementation in women who previously delivered a child with NTD (Holmes-Siedle et al. 1992). They also found that supplementation reduced the risk for recurrence (Holmes-Siedle et al. 1992).

At the same time, an observational study examining diets of women by Laurence et al. (1980) concluded that poor prenatal nutrition may be related to an increased risk of delivering a child with NTD. These results prompted a small double-blind randomised controlled trial where women who had previously delivered a child with NTD were randomised to receive 4 mg folic acid or no supplementation during pregnancy (Laurence et al. 1981). Supplementation resulted in 0/44 children born with a NTD whereas no supplementation resulted in 6/61 children were born with NTDs (Laurence et al. 1981). The study was limited by the small participant size.

An observational study by Sheppard et al. (1989) investigated the effect of folate supplementation in women who had previously delivered a child with NTD. A total of 227 women who were fully supplemented during pregnancy had no recurrence of children born with NTD. Two of 213 women who were partially supplemented, delivered a second child with NTDs. An Atlanta case-control study compared 347 babies affected with NTDs with 2,829 healthy controls (Mulhern et al. 1988). Mothers who were supplemented with multivitamins during pregnancy had a protective effect for NTDs (RR = 0.44, 95% CI = 0.25 – 0.69) (Mulhern et al. 1988). Another case-control study conducted in Australia found that prenatal supplementation with folic acid consumption in early pregnancy also decreased the risk of NTD (OR = 0.7, 95% CI = 0.27 – 1.82) (Bower and Stanley 1989).

A large cohort study conducted by Milsunsky et al. (1989) investigated the importance of timing of initiation and duration of use of folic acid fortified multivitamin supplementation and its role of decreasing the risk of NTDs. Of the 5,261 women who supplemented with multivitamin before and during conception, nine were delivered a child with NTDs. On the other hand, 29 NTDs occurred in children of 12,297 women who reported taking multivitamin only in their first trimester of pregnancy. Eleven NTDs occurred in women who did not supplement during their pregnancy. Overall, the prevalence of NTDs were 0.51/1,000 and 3.5/1,000 for women with and without multivitamin supplementation in the first 6 months of their pregnancy, respectively. When the data were separately analysed by multivitamin fortification with folic acid, the prevalence for NTDs was 0.91/1,000 and 3.2/1000 for supplementation in the first 6 weeks of pregnancy and after 7 weeks of pregnancy, respectively. However, women who took multivitamins that did not contain folic acid in the first 6 weeks of pregnancy had a prevalence of 3.2/1,000 for NTDs (Milsunsky et al. 1989).

A cohort study found no recurrence of NTDs among the offspring of women who were supplementing with 5 mg of folic acid (Vergel et al. 1990). However, there was a 3% recurrence rate in unsupplemented women (Vergel et al. 1990). Contrary to these studies, a case-control study from the Atlanta Birth Defects Registry could not find a statistically significant difference in NTDs in 573 multivitamin supplemented women and 546 controls (Mills et al. 1989). This study might have been limited by recall bias. In addition, the investigators were not able to identify all of the infants; they only identified recurrent cases.

Perhaps of the most definitive research addressing the benefit of folic acid supplementation in decreasing the risks of NTDs was the multicentre randomised double-blind trial instigated by the UK Medical Research Council (MRC Vitamin Study Research Group 1991). The aim of this trial was to evaluate the efficacy of 4 mg folic acid to prevent recurrent NTD in women who had previously delivered a child with NTD. The trial covered seven countries and recruited 1,817 women who were randomised to one of four groups: folic acid (4 mg), folic acid (4 mg) and a multivitamin; multivitamin without folic acid, or no supplementation at all. Participants were asked to use their supplements 1 month prior to conception and through the first 12 weeks of their pregnancy. In this trial, Wark and colleagues (1998) showed that women randomised to the folic acid group had a 1.8% chance of having a child with a NTD (RR = 0.26, 95% CI = 0.12 – 0.71). On the other hand, women in the unsupplemented arm did not have a statistically significant chance of decrease in NTD (RR = 0.8, 95% CI = 0.57 – 1.12). Overall, supplementation with folic acid resulted in decreasing the recurrence of NTD by 72% (6/935 folic acid supplemented vs 21/602 in control) (MRC Vitamin Study Research Group 1991).

Another prominent trial evaluating folic acid fortified multivitamin supplementation during pregnancy was a double-blind randomised controlled trial where 2,104 women were randomised to 0.8 mg folic acid containing multivitamin, while 2,092 women were randomised to a multivitamin containing trace supplementation (Czeizel et al. 1992). Fortification occurred at least 1 month prior to pregnancy and continued to, at minimum, the second missed menstrual period (Czeizel and Dudas 1992). A total of 28 malformations were noted in the folic acid containing group, whereas 47 malformations were noted in the trace supplement group. No NTDs were observed in the folic acid fortified group, whereas six NTDs were in the trace element group (Czeizel and Dudas 1992).

A case-control study by Werler et al. (1999) of 436 cases of children with NTDs and matched with 2,615 healthy controls found that 57% and 48% of women did not supplement with folic acid in both the NTDS and control group, respectively. This infers that folic acid has a significant effect of decreasing the risk of NTD by 60% (RR = 0.4, 95% CI = 0.2 – 0.6). Another large case-control study by Czeizel et al. (1994) also observed 0.2/715 NTDs in folate supplemented women, whereas 0.2/391 NTDs occurred in the unsupplemented group. A case-control observational study by Ulrich et al. (1999) identified 7/8,293 children with NTDs born to women who used supplementation during their pregnancy and 0/27,412 children with NTDs born to women who did not supplement during their pregnancy. This result may be attributed to the fact that the majority of women participating in this study only began supplementing with folic acid after discovering they were pregnant. A case-control study by Berry et al. (1999) investigated the effect of folic acid supplementation on NTDs. A total of 102/135,142 children with NTDs were born to women supplementing with folic acid whereas 173/147,689 children with NTDs were born to women who were not supplementing with folic acid. An Indian randomised controlled trial found that supplementation with multivitamins decreased the recurrence of NTDS by 60%.
Appendix F

493

Reviews

The recurrence rate was 2.92% in the multivitamin group, as opposed to 7.04% in the placebo group (Central Technical Co-ordinating Unit 2000). Similarly, Thompson et al. (2003) showed a 65% risk reduction in NTDs (OR = 0.35, 95% CI = 0.17 – 0.72) in their case-control study conducted between 1992 and 1997. More recently, a cohort-controlled study by Czeizel et al. (2004), found that multivitamin supplementation decreased risk of NTDs, confirming their initial findings from their cohort study (OR = 0.11, 95% CI = 0.01 – 0.91). Our meta-analysis observed an OR = 0.67, 95% CI = 0.58 – 0.77 in case control studies and an OR = 0.52, 95% CI = 0.39 – 0.69 in cohort and randomised controlled studies (Goh et al. 2016).

To investigate whether the dosage of folic acid affects the rate of reduction, the California Birth Defects Monitoring Program conducted a case-control study comparing 338 children with NTDs with 546 controls (Shaw et al. 1995a). Women who reported any use of folic acid had an overall lower risk of having a child with NTDs (OR = 0.66, 95% CI = 0.46 – 0.79) (Shaw et al. 1995a). Women taking folic acid 0.4 – 0.9 mg had a further reduced risk. Women who supplemented with <0.4 mg did not have important reductions in risk (OR = 0.99, 95% CI = 0.92, respectively) (Shaw et al. 1995a). The only available study investigating serum folate concentrations found an inverse relation between maternal cell folate and the risk of NTD (Daly et al. 1995). Daly et al. showed in a case-control study that women receiving <150 µg, >400 µg of folic acid had a 6.61,000 and 0.81,000 chance of NTD, respectively. Supplementation at different doses of 100 µg, 200 µg, and 400 µg resulted in a 22%, 41%, and 47% decreased risk in NTD, respectively (Daly et al. 1997). Another study investigating dosing variations of folic acid corroborated this result as they noted that 100 µg, 200 µg, and 400 µg folic acid decreased NTD by 18%, 35%, and 53%, respectively (Welld et al. 1998).

Oral clefts

The risk of a child being born with a cleft lip, with or without cleft palate, is 1/1,000 (Moore and Persaud 1998; Ilicu et al. 2001). The chance of occurrence of cleft palate alone is 1/2,900 (Moore and Persaud 1998; Ilicu et al. 2001). The first studies to investigate the possibility of multivitamin's effectiveness in preventing birth defects were specifically aimed at preventing the recurrent of oral clefts with or without cleft palate (Conway 1958; Douglas 1958; Peer et al. 1964). These studies found that multivitamin supplementation resulted in a 48% decrease in oral clefts (OR = 0.52, 95% CI = 0.34 – 0.80) (Conway 1958; Douglas 1958, 1958b; Peer et al. 1964). These results were supported in an animal study by Peer et al. (1958) where Swiss albino mice receiving cortisone treatment were pretreated with vitamin B6 and folic acid or folic acid alone. A protective effect was observed in pretreated mice (oral clefts in vitamin B6 and folic acid = 321, in folic acid = 21/88), compared with unsupplemented mice (oral clefts in unsaturated = 38/40) (Peer et al. 1958).

A retrospective study of women who gave birth to children with oral clefts found that multivitamin supplementation resulted in a 3.1% incidence, whereas no supplementation resulted in a 4.8% incidence, accounting for a 30% reduction (Briggs 1976). This finding was supported by a case-control study where oral clefts occurred in children of 3/184 multivitamin supplemented women, whereas 77/4,951 occurred in unsupplemented women (Tolarav and Harris 1995). These results were challenged by the randomised controlled study of Czeizel et al. (1992), where no significant reduction of oral cleft was observed with supplementation with folate containing multivitamins (Czeizel and Dudas 1992).

A case-control study by Shaw et al. from 1987 to 1989, comparing 731 folic acid fortified multivitamin supplemented mothers to 734 controls observed a 25 – 50% decreased risk of oral cleft (Shaw et al. 1995a). A 50% decrease in rates of cleft palate with cleft lip was observed (OR = 0.5, 95% CI = 0.36 – 0.68), while cleft palate without cleft lip decreased by 27% (OR = 0.73, 95% CI = 0.46 – 1.2) (Shaw et al. 1995a). A prospective intervention study by Tolarova and Harris (1995) offered women who had a history of a child with cleft lip or without cleft palate, supplementation of 10 mg folic acid at least 2 – 3 months prior to conception and at least 3 months post-conception. This decreased the recurrence of oral cleft in high-risk families by 65.4% (OR = 0.35). An 82.6% decrease was noted in families who had a history of females affected by oral clefts or a history of unilateral clefts. In contrast, a case-control study by Hayes et al. (1996), of the Stone Epidemiology Unit Birth Defects Study, found no significant association between folate acid supplementation and oral clefts (OR = 1.2, 95% CI = 0.7 – 2.0) with or without cleft palate (OR = 0.9, 95% CI = 0.5 – 1.7).

A case-control study by Czeizel et al. (1996a) reported that women using 4 mg of folic acid during the preconception period had a significant decrease in cleft lip with or without cleft palate. In addition, a case-control study by Werler et al. (1999) found a 60% decrease in cleft palate (OR = 0.4, 95% CI = 0.2 – 0.9) and a 30% decrease in cleft palate with cleft lip (OR = 0.7, 95% CI = 0.4 – 1.1). Moreover, a case-control study in Brazil found a statistically significant inverse association between cleft palate and intake of vitamins in the first 4 months of pregnancy (RR = 0.58) (Leffredo 2000). Another challenge to the proposed protective effects of folic acid for oral clefts was a cohort study by Czeizel et al. (1999), who observed no protective effect in 5,458 supplemented, as compared with 5,821 unsupplemented women in their randomised control trial. They postulated that higher doses of folic acid was required than the quantities found in prenatal vitamins to decrease the incidence of oral clefts. Similarly a case-control study in Maryland from 1992 to 1998 found no protective effect against cleft palate (OR = 0.70, 95% CI = 0.31 – 1.56) or cleft palate with or without cleft lip (OR = 0.89, CI = 0.33 – 1.09) (Beaney et al. 2001). This may be attributed to the fact that there was a low proportion of women supplemented prior to 3 months pregnancy (Beaney et al. 2001). Our meta-analysis observed an OR = 0.76, 95% CI = 0.62 – 0.93 of cleft palate in case control studies and an OR = 0.42, 95% CI = 0.26 – 0.84 in cohort and randomised controlled studies (Goh et al. 2006). Oral cleft with or without cleft palate was OR = 0.63, 95% CI = 0.54 – 0.73 in case control studies and OR = 0.58, 95% CI = 0.28 – 1.19 for cohort and randomised controlled studies (Goh et al. 2006). These findings were supported by Werler et al. (1999) and Baidovac et al. (2007).

Congenital heart defects

Congenital heart defects (CHD) are the most prevalent major birth defects affecting an estimated 1/20 live births
Appendix F

Conotruncal heart defects specifically have a prevalence of 0.8/1000 (Botto et al. 1996). A randomized controlled study showed that women randomised to 0.8 mg folic acid containing multivitamins had 80% less children with congenital heart defects compared with women in the trace group (10 vs 20 children). These differences were especially prominent as conotruncal defects and septal defects (Czeizel and Dudás 1992).

Another intervention study found that folic acid containing multivitamins decreased the occurrence of CHDs by 32% (RR = 0.48, 95% CI = 0.23–0.83) (Czeizel et al. 1994). A later cohort study indicated that folic-fortified multivitamin supplementation decreased CHDs (OR = 0.60, 95% CI = 0.38–0.96) (Czeizel et al. 2004).

A CDC population control-based study from 1968 to 1980 observed that periconceptional multivitamin supplementation was associated with a 43% decrease in risk of conotruncal cardiovascular defects. A 52% decreased risk was observed for isolated conotruncal defects (RR = 0.48, 95% CI = 0.20–0.98) (Botto et al. 1996). In addition, a population-based case-control study of the California Birth Defects Monitoring Program from 1987 to 1998 found that women taking folic acid from 1 month prior to conception to 2 months post-conception had a 55% decreased risk for conotruncal defects compared with un-supplemented mothers (OR = 0.65, 95% CI = 0.44–0.95) (Shaw et al. 1999b). Moreover, a case-control study observed that multivitamin supplementation decreased conotruncal heart defects by 45% (OR = 0.57, 95% CI = 0.33–0.97) (Botto et al. 1996). In addition, a case-control study observed that isolated conotruncal defects had a 52% decreased risk by 64% (OR = 0.36, 95% CI = 0.15–0.86); isolated conotruncal effects by 66% (OR = 0.41, 95% CI = 0.2–0.84); isolated transposition of great arteries by 66% (OR = 0.36, 95% CI = 0.13–0.93) were observed. Differences were also observed in the ontology of Fallot, truncus arteriosus, double outlet right ventricle (Botto et al. 1996).

More studies have supported the effectiveness of folic acid to decrease the risk of CHDs. A case-control study of the Baltimore-Washington Infant Study from April 1987 to December 1989, found that women with high intakes of folic acid had a decreased risk for cardiac outflow tract defects (Scallon et al. 1998). An Atlanta case-control study from 1968 to 1990 noted that prenatal multivitamin supplementation decreased occurrence of heart defect (OR = 1.8, 95% CI = 1.4–2.3); tricuspid atresia (OR = 5.2); obstructive defects (OR = 2.7); transposition of great arteries (OR = 1.9); ventricular septal defect (OR = 1.8), compared with un-supplemented mothers (Botto et al. 2000). In a recent cohort controlled study conducted by Czeizel et al. (2004), women taking multivitamin supplementation had a protective effect against congenital heart defect (OR = 0.6, 95% CI = 0.38–0.96). There was a prominent difference in ventricular septal defects: five cases were observed in the multivitamin supplemented group vs 19 in the un-supplemented group (OR = 0.26, 95% CI = 0.09–0.72) (Czeizel et al. 2004). Our meta-analysis observed an OR = 0.78, 95% CI = 0.67–0.92 of cardiovascular defects in case control studies and OR = 0.61, 95% CI = 0.40–0.92 in cohort and randomised controlled studies (Goh et al. 2008).

Urinary tract anomalies
The prevalence of genital and urinary tract anomalies is estimated to be approximately 1/135 births in the general population (Correa-Villasenor et al. 2003). In their randomised control study, Czeizel et al. (1994) observed a decrease in the rate of congenital urinary tract anomalies (CUTA) in children born to women who took folic acid containing multivitamins (n = 2) as opposed to the trace multivitamin group (n = 9). Supplementation with folic-fortified multivitamins resulted in a 78% reduced risk in CUTA compared with the un-supplemented group (RR = 0.22, 95% CI = 0.09–0.99) (Czeizel et al. 1994). Li et al. (1993) conducted a retrospective case-control trial using the Washington State Birth Defect Registry and matched 118 cases of children born with CUTA to 369 controls from January 1990 to December 1991. Supplementation with folic acid containing multivitamins in the first trimester was associated with an 85% reduction in risk of having a child with CUTA (OR = 0.15, 95% CI = 0.05–0.43). Continued supplementation through the second and third trimester resulted in a further decrease (OR = 0.31, 95% CI = 0.09–1.02). Supplementation prior to pregnancy was not associated with the reduction of CUTA (Li et al. 1993). The most prominent decrease was noted in hydrometrocolpos (OR = 0.12, 95% CI = 0.04–0.36) compared with other UTDs (OR = 0.31, 95% CI = 0.06–1.56) (Li et al. 1995). A cohort-controlled trial conducted by Czeizel et al. (2004) observed a reduction in stenosis/ atresia of the peno-urethral junction in women with multivitamins supplementation compared to women who were not supplemented (OR = 0.19, 95% CI = 0.04–0.86). There was, however, no significant difference in UTD. Our meta-analysis observed an OR = 0.48 95% CI = 0.30–0.76 in case control studies, and OR = 0.68 95% CI = 0.33–1.31 cohort and randomised controlled studies (Goh et al. 2006).

Liab defects
The prevalence rate for all types of limb deficiency in the general population is 0.69/1,000 (McGuirk et al. 2001). In their randomised control study, Czeizel and Dudás (1992) observed one case of limb defects in the multivitamin supplemented group, whereas five cases were observed in the control group. The most significant study with respect to prenatal multivitamin supplementation and limb defects was a case-control study by Shaw et al. (1999b) showing that supplementation of folic acid containing multivitamins between 1 month prior to conception and 2 months post-conception was associated with a 39% decrease in limb defects (OR = 0.63, 95% CI = 0.40–1.0) as opposed to controls (OR = 0.71, 95% CI = 0.44–1.2). Our meta-analysis observed an OR = 0.48, 95% CI = 0.30–0.76 in case control studies; OR = 0.57, 95% CI = 0.38–0.85 in cohort and randomised controlled studies (Goh et al. 2006).

Omphalocele
Omphalocele occurs in 14/1000–16/1000 pregnancies in the general population (Caldairi et al. 1993, 1995, 1997). A case-controlled study by Botto et al. (2002) observed an incidence of 73 and 3,029 children with omphalocele born to supplemented and un-supplemented women, respectively. Overall, periconceptional use of multivitamins was associated with a 60% reduction in non-syndromic omphalocele (OR = 0.4, 95% CI = 0.2–1.0).
Appendix F

Pyeloc steatosis
In the randomised-control study conducted by Clatell and Dudas (1992), two cases of hyperpyelic steatosis were observed in the folate acid fortified multivitamin group compared with eight cases in the three multivitamin group. In contrast, a study by Correa-Vilasenor et al. (2003) did not corroborate this protective effect.

Imperforate anus
The prevalence of imperforate anus is 0.2–0.67/1,000 (Spouge and Baird 1986; Moore and Persaud 1998). A cohort study from 1984 that folate acid supplementation resulted in a 0.16/1,000 rate of imperforation, whereas unsupplemented women had a rate of 0.31/1,000. This implied a decrease of 50% associated with folate acid supplementation (Meyers et al. 2001).

Pediatric cancers
The incidence of childhood primary non-malignant and malignant brain and central nervous system tumours is 0.04/1,000 (Central Brain Tumor Registry of the United States, 2003). Neuroblastoma and medulloblastoma are the most common pediatric solid tumours (Central Brain Tumor Registry of the United States 2003; National Cancer Institute 2005). Neuroblastoma is the most common cancer in infants and the third most common form of cancer in children (American Cancer Society 2005). Medulloblastoma is a benign or malignant brain tumor that is usually located in the cerebellum (National Cancer Institute 2005). Also known as a primitive neuroectodermal tumor (PNET), medulloblastomas occur in one of five brain tumours (National Cancer Institute 2005). Despite being the leading cause of childhood cancer deaths in developed countries, little is known about the aetiology of these tumours (Bunin et al. 1994). A case-control study by Presten-Martin et al. (1992) observed that prenatal supplementation with folate acid containing multivitamins decreased the risk of brain tumours (OR = 0.6, p = 0.12). Protective trends were also observed in a case-control study of maternal multivitamin supplementation and the risk of primitive neuroectodermal tumours (PNET) of the brain in children (OR = 0.38; p = 0.005) (Bunin et al. 1993). Multivitamin use during the first 6 weeks of pregnancy was associated with decreased risk of brain tumours (OR = 0.56; p = 0.02) (Bunin et al. 1993). Another case-control study suggested a decreased risk of astrocytoma (Bunin et al. 1994). Preston-Martin et al. (1996a, 1996b) confirmed these findings in their case-control studies of prenatal multivitamin supplementation. Decreased brain tumours including the PNET and astrocytoma were noted in their initial study. In their international case-control study containing 1,951 cases and 1,919 controls, supplementation in the first two trimesters of pregnancy was shown to decrease brain tumours (OR = 0.7, 95% CI = 0.5–0.9). Greater reduction was associated with supplementation in all three trimesters (OR = 0.5, 95% CI = 0.3–0.8) (Preston-Martin et al. 1994). Multivitamin supplementation 1 month prior to conception or while breast-feeding had no effect on the overall outcome (Preston-Martin et al. 1994). A retrospective population-based study of children diagnosed with brain tumours in England observed a decrease of brain tumours reported from 1985 to 1991 (n = 16) compared with 1975 to 1984 (n = 2). This corresponds to an initial incidence of 9.6 per million decreasing to 1.7 per million. The decline of medulloblastomas may be associated with the introduction of periconceptional multivitamin supplementation introduced in the 1980s (Thorne et al. 1994). An interventional time series analysis observed a 0.157/1,000 to 0.262/1,000 decrease after the introduction of folate acid fortification of flour with an adjusted incidence (RR = 0.38, 95% CI: 0.23–0.62) (French et al. 2003). Our meta-analysis observed apparent protective effect for leukaemia (OR = 0.64, 95% CI = 0.50–0.74), paediatric brain tumours (OR = 0.73, 95% CI = 0.60–0.88) and neuroblastoma (OR = 0.33, 95% CI = 0.23–0.62) (Oh et al. 2007).

Genetics and maternal disease
Genetic polymorphisms may be responsible for variability in folate available to the fetus (Mollov et al. 1997). The most widely recognised polymorphism is the mutation of 677 C→T substitution in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene. This mutation results in a thermolabile variant C677T with decreased enzyme specific activity and elevated homocysteine concentrations, thus hindering the absorption of folate (van der Put et al. 1993; Engberg et al. 1995; Motulsky 1996; Jacques et al. 1996). The frequency of homozygous polymorphism varies in different ethnic groups (5–25%) (Whitehead et al. 1995; van der Put et al. 1999; Presto et al. 1999; Ou et al. 1996; van der Put et al. 1996, 1997). In Caucasian and Asian populations, TT homozygotes are present at approximately 12%. African-Americans have a very low prevalence, whereas Hispanics of Mexican heritage have a high prevalence (Bottor and Yang 2002). The homogeneity of the 677 TT allele variant for MTHFR doubles the risk associated with having a child with spina bifida (Bottor et al. 1990). On the other hand, the MTHFR polymorphism involving an A→C substitution at base pair 1,298 results in a benign allele unless it is present in combination with C677T (Chung et al. 2000). With both polymorphisms present, the specific activity of MTHFR is lower by two-tholds (Chung et al. 2000). MTHFR polymorphisms may also contribute to an increased incidence of Down syndrome (James et al. 1999; Hobbs et al. 2000; Al-Gazali et al. 2001; Barlak et al. 2003). Other genetic alterations may result in the alteration of folate absorption. A case-control study conducted by Lunme et al. (2004) observed that, despite taking vitamins, variations of acetyl-N-transferase 1 (NAT-1) NAT1095 resulted in a two-fold increased risk of cleft.

Folate antagonists
There are two types of folate acid antagonists: dihydrofolate reductase inhibitors and folate antagonists that affect the absorption metabolism or degradation of folate.
Appendix F

Folate antagonists inhibit dihydrofolate reductase, an essential step in reducing folic acid to tetrahydrofolate acid, therefore preventing the synthesis of DNA and cell reproduction (Lloyd et al. 1999). Folate antagonists have been associated with an increased risk of NTDs including myelomeningocele and anencephaly (Robins and Culbard 1982; Lloyd et al. 1999). A case-controlled study of women using folic acid antagonists in early pregnancy also suggested an increased risk for cardiovascular defects, oral clefts, and urinary tract defects (Herranz-Diez et al. 2000).

There has been one case report of hypersensitivity due to folic acid supplementation (Mitchell et al. 1999). Folic acid may interfere with zinc homeostasis or interact with other drugs rendering them less effective. In addition, folic acid may mask cobalamin vitamin B12 deficiency (Campbell 1996). By masking this deficiency, it may increase the neurological complications associated with the deficiency (Bailey 2001).

The above evidence suggests the benefit of folic acid supplementation in reducing the risk of NTD, oral clefts, congenital heart defects, and other congenital malformations. The US Public Health Service and Institute of Medicine recommends that women of childbearing potential should fortify their diet with 400 μg of folic acid (Centers For Disease Control and Prevention 1992; Institute of Medicine 1998). Health Canada and the European Union have identical recommendations (Government of Canada 2005; European Union 2005). The fortification of folic acid in staple foods, such as grains was mandated by the FDA in January 1998 (Food and Drug Administration 1996). This practice, however, has not been adopted by all countries. Since 50% of pregnancies are unplanned and the neural tube closes around 23–28 days post-conception, many women will have missed this crucial period for supplementation (Forrest 1994). Women who consume folic acid antagonists, have folic acid deficiencies, or have had a previous child with a NTD are recommended to ingest higher doses (5 mg) of folic acid (Crawford et al. 1999).

What is the optimal dose of folic acid supplementation?

Presently, the recommended dose of folate supplementation is 0.4 mg/day. In fact, prenatal multivitamins invariably contain 0.8–1.1 mg folic acid, and this had led to the assumption that daily supplementation with this dose is sufficient to prevent neural tube defects. However, Wald et al. (1998) systematically reviewed all reports of the correlation between ingested dose of folate and resultant serum concentrations. Using the data by Daly et al. (1995) who correlated folate levels with risk to neural tube defects, Wald and colleagues concluded that the presently recommended dose of folate will render only partial protection against neural tube defects. According to Wald’s analysis, 5 mg/day of folate will be necessary to render 60% protection in the populations (Wald et al. 1998). Wald’s analysis has been recently corroborated by us in Ontario, Canada, showing that in 2005–2006, 40% of Ontario women did not achieve the protective 900 nM red blood cell folate, despite folic acid fortification and despite the fact that more than half of the pregnant women were supplemented with prenatal multivitamins (Kapur et al. 2007).

Before moving towards supplementing women with higher doses of folic acid, one needs to consider potential health risks of such a move:

1. It has been proposed that higher levels of folate may mask pernicious anemia due to B12 deficiency. Similar concerns surrounded the original North America flour fortification programme in 1998, but were not shown following the fortification. Several recent studies have failed to show such risks. One has to remember that such risk if different in the whole population consumes flour with higher levels of folate, vs giving 5 mg/day to pregnant women. In fact, direct measurements of B12, or higher supplementa-

2. It has been argued that the present 0.8–1.1 mg/day folic acid supplementation should render sufficient protective concentrations, and if women do not take these vitamin combinations, why should one assume that they will consume 5 mg/day.

A recent study from our centre has provided the answer to this question. In a controlled trial of prenatal vitamins in pregnancy, measuring compliance carefully, we have documented that despite self selection of motivated women, their compliance with two different brands of prenatal vitamins averaged 53–58% ranging between 0 and 100%. This state of affairs may explain why 40% of Ontario women are not protected (Kapur et al. 2007). Pharmacologically, it means that 5 mg/day, combined with the impaired compliance, will give far less protection in many more women protected by 5 mg/day.

3. There are quotes of folic acid increasing the risks of certain cancers. These cancers arise mostly from laboratory studies. In contrast, case-control and some prospective studies have repeatedly shown a 20–30% decline in cancer associated with folic acid. Scientists talk therefore about the potential "dual" effects of folate on cancer. Current figures show a decrease in incidence of breast cancer in pregnancy, despite 10 years of flour fortification (Singal et al. 2006). There is no question that a risk of cancer, even if it exists, corresponds to long exposure over years, and not to several months of dosing in pregnancy. It is our view that unless the clinician can ensure excellent compliance with the 0.8–1.1 mg folate containing prenatal vitamins (which as our numbers show, is rarely the case), prenatal vitamins should be combined with 5 mg folate.

References

Reviews


Appendix F


Food and Drug Administration. 1996. Online. Available at: www.cfin.fda.gov/~brd/f695035c.html


Appendix F

12 Review


Moore CV, Bierbaum OS, Welch AD, Wright LD. 1945. The activity of synthetic Lactobacillus casei factor (folic acid) as an antipernicious anemia substance. J. Observations on four patients, two with Addisonian pernicious anemia, one with amoebic pernicious anemia, and one with pernicious anemia of pregnancy. Journal of Laboratory and Clinical Medicine 30:1059-1069.


Appendix F

Reviews


Pre-conceptional Vitamin/Folic Acid Supplementation 2007: The Use of Folic Acid in Combination With a Multivitamin Supplement for the Prevention of Neural Tube Defects and Other Congenital Anomalies

Abstract
Objective: To provide information regarding the use of folic acid in combination with a multivitamin supplement for the prevention of neural tube defects and other congenital anomalies, so that physicians, midwives, nurses, and other health care workers can assist in the education of women in the pre-conception phase of their health care.

Options: Supplementation with folic acid and vitamins is problematic, since 50% of pregnancies are unplanned, and women's health status may not be optimal when they conceive.

Outcomes: Folic acid in combination with a multivitamin supplement has been associated with a decrease in specific birth defects.

Evidence: Medline, PubMed, and Cochrane Database were searched for relevant English language articles published between 1995 and 2007. The previous Society of Obstetricians and Gynaecologists of Canada (SOGC) Policy Statement of November 1993 and statements from the American College of Obstetricians and Gynecologists and Canadian College of Medical Geneticists were also reviewed in developing this clinical practice guideline.

Values: The quality of evidence was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care.

Benefits, Harms, and Costs: Promoting the use of folic acid and a multivitamin supplement among women of reproductive age will reduce the incidence of birth defects. The costs are those of daily vitamin supplementation and eating a healthy diet.

Recommendations
1. Women in the reproductive age group should be advised about the benefits of folic acid in addition to a multivitamin supplement during well-woman visits (birth control reviews). Pap testing, yearly examination (especially if pregnancy is contemplated). (II-A)

2. Women should be advised to maintain a healthy diet, as recommended in Eating Well With Canada’s Food Guide (Health Canada). Foods containing good sources of folic acid are fortified grains, spinach, lentils, chick peas, asparagus,

Key Words: Folic acid, neural tube defect, prevention, spina bifida, risk reduction, multivitamin, preconception, birth defect
Appendix F

502

JOINT SOGC-MOTHERSK CLINICAL PRACTICE GUIDELINE

broccoli, peas, Brussels sprouts, corn, and oranges. However, it is unlikely that diet alone can provide levels similar to folic acid-multivitamin supplementation. (III-A)

3. Women taking a multivitamin containing folic acid should be advised to take more than one daily dose of vitamin supplement, as indicated on the product label. (II-2-A)

4. Folic acid and multivitamin supplements should be widely available without financial or other barriers for women planning pregnancy to ensure the extra level of supplementation. (III-B)

5. Folic acid 5 mg supplementation will not mask vitamin B12 deficiency (pernicious anemia), and investigations (examination or laboratory) are not required prior to initiating supplementation. (II-2-A)

6. The recommended strategy to prevent recurrence of a congenital anomaly (anecephaly, myelomeningocele, meningomyelocele, oral facial cleft, structural heart disease, limb defects, urinary tract anomaly, hydrocephalus) that has been reported to have a decreased incidence following preconception / first trimester folic acid +/- multivitamin oral supplementation is planned pregnancy +/- supplementation compliance. A multivitamin supplemented diet with additional daily supplementation of multivitamins with 5 mg folic acid should begin at least three months before conception and continueuntil 10 to 12 weeks post conception. From 12 weeks post-conception and continuing throughout pregnancy and the postpartum period (4–6 weeks or as long as breastfeeding continues), supplementation should consist of a multivitamin with folic acid (0.4–1.0 mg). (II-A)

7. The recommended strategy(ies) for primary prevention or to decrease the incidence of fetal congenital anomalies will include a number of options or treatment approaches depending on patient age, ethnicity, compliance, and genetic congenital anomaly risk status.

- Option A: Patients with no personal health risks, planned pregnancy, and good compliance require a good diet of folate-rich foods and daily supplementation with a multivitamin with folic acid (0.4–1.0 mg) for at least two to three months before conception and throughout pregnancy and the postpartum period (4–6 weeks and as long as breastfeeding continues). (II-2-A)

- Option B: Patients with health risks, including epilepsy, insulin dependent diabetes, obesity with BMI >35 kg/m², family history of neural tube defects, belonging to a high-risk ethnic group (e.g., 50th), require increased dietary intake of folate-rich foods and daily supplementation with multivitamins with 5 mg folic acid, beginning at least three months before conception and continuing until 10 to 12 weeks post conception. From 12 weeks post-conception and continuing throughout pregnancy and the postpartum period (4–6 weeks or as long as breastfeeding continues), supplementation should consist of a multivitamin with folic acid (0.4–1.0 mg). (II-2-A)

Option C: Patients who have a history of poor compliance with medications and additional lifestyle issues of variable diet, no consistent birth control, and possible teratogenic substance use (alcohol, tobacco, recreational non-prescription drugs) require counseling about the prevention of birth defects and health problems with folic acid and multivitamin supplementation. The higher dose folic acid strategy (5 mg) with multivitamin should be used, as it may obtain a more adequate serum red blood cell folate level with irregular vitamin folic acid intake but with a minimal additional health risk. (III-B)

5. The Canadian Federal Government could consider an evaluation process for the benefit/risk of increasing the level of national folic acid flour fortification to 500 mg/100 g (present level 140 mg/160 g). (III-B)

9. The Canadian Federal Government could consider an evaluation process for the benefit/risk of additional flour fortification with multivitamins other than folic acid. (III-B)

10. The Society of Obstetricians and Gynaecologists of Canada will explore the possibility of a Canadian Consensus conference on the use of folic acid and multivitamins for the primary prevention of specific congenital anomalies. The conference would include Health Canada/Canadian Congenital Anomalies Surveillance, Canadian College of Medical Genetics, Canadian Pediatric Society, Maternal, and pharmaceutical industry representatives.

Validation: This is a revision of a previous guideline and information from other consensus reviews from medical and government publications has been used.

Sponsor: The Society of Obstetricians and Gynaecologists of Canada.


INTRODUCTION

It is estimated that at least 2% of babies are born with some serious congenital anomaly; 2% to 3% will have anomalies that can be recognized prenatally by non-invasive screening tests, through invasive diagnostic testing, or at birth, and 2% will have developmental or functional anomalies recognized during the first year of life.1 Folic acid ingested prior to conception and during the early stages of pregnancy plays a role in preventing neural tube defects and has been associated with preventing other congenital anomalies.2 Folic acid helps produce and maintain new cells; it is important during times of rapid cell division and growth (i.e., embryonic and fetal periods). Public health initiatives to increase the awareness and prevention of birth defects have focused on folic acid intake for the prevention of NTDs, but several studies have indicated that taking multivitamins containing folic acid during the periconception period can reduce the risk of other conditions such as heart defects,3–6 urinary tract anomalies,6–8 oral facial clefts,7–9 limb defects,8 and pyloric stenosis.8 It has been estimated that as many as half of all birth defects can be prevented if women of childbearing age consume an adequate amount of folic acid, either by eating sufficient quantities of food that are fortified with folic acid or by taking vitamin supplements.8–12 The objective of this clinical practice

ABBREVIATIONS

| AD       | autosomal dominant inheritance           |
| AR       | autosomal recessive inheritance          |
| BMI      | body mass index                          |
| CI       | confidence interval                      |
| MTHFR    | 5,10-methylene tetrahydrofolate reductase |
| NTD      | neural tube defect                       |
| RBC      | red blood cell                           |
| RCT      | randomized controlled trial              |

DECEMBER 2007 DÉCEMBRE 2007 1004
Appendix F 503

Table 1. Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care

<table>
<thead>
<tr>
<th>Quality of Evidence Assessment*</th>
<th>Classification of Recommendation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: Evidence obtained from at least one properly randomized controlled trial</td>
<td>A. There is good evidence to recommend the clinical preventive action</td>
</tr>
<tr>
<td>II-1: Evidence from well-designed controlled trials without randomization</td>
<td>B. There is fair evidence to recommend the clinical preventive action</td>
</tr>
<tr>
<td>II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one center or research group</td>
<td>C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making</td>
</tr>
<tr>
<td>II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category</td>
<td>D. There is fair evidence to recommend against the clinical preventive action</td>
</tr>
<tr>
<td>III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees</td>
<td>E. There is good evidence to recommend against the clinical preventive action</td>
</tr>
</tbody>
</table>

*The quality of evidence reported in these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care. 5
†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in The Canadian Task Force on Preventive Health Care. 5

A guideline update is to give women's health care providers new data/information about the use of folic acid with a multivitamin supplement for the prevention of neural tube defects and other congenital anomalies. The quality of evidence reported in this guideline has been described using the evaluation of evidence criteria of the Canadian Task Force on Preventive Health Care (Table 1). 5

Peer-reviewed articles, government publications (Health Canada, Preconception Health 2002; 5 NIH Clinical Center, Office of Dietary Supplements 2005), 5 the 2003 Society of Obstetricians and Gynaecologists of Canada (SOGC) Policy Statement, The Use of Folic Acid for Prevention of Neural Tube Defects, 53 and statements from The American College of Obstetrics and Gynecology 54 and Canadian College of Medical Geneticians, 53 were reviewed in developing this guideline.

NEURAL TUBE DEFECTS: INCIDENCE AND INHERITANCE

Neural tube defects are severe birth anomalies that occur because of a lack of neural tube closure at either the upper or lower end in the third to fourth week after conception (day 26 to day 28 post conception). 5 The incidence (0.5–4.0/1000 births) of NTDs varies across North American regions and a decreasing incidence (1.58 per 1000 births to 0.86 per 1000 births) is shown with folic acid supplementation. 20 Recurrence risks reflect the genetic contribution in different regions, but an estimated 1% recurrence with folic acid prophylaxis is given. 2,10,12,26–27

In Canada, the birth prevalence of NTDs has declined from a rate of 10.0 per 10,000 live births in 1991 to 5.8 per 10,000 total births (live births and stillbirths) in 1999. 28 Reasons given for this decrease in the rate of NTDs include an increased use of tests (ultrasound, maternal serum screening) and subsequent pregnancy termination, the fortification of food with folic acid, 5 and increased vitamin supplementation. 28 The rate of NTDs tends to be higher in Eastern Canada than in Western Canada. 54 Women of certain ethnic groups, including Celtic 52 and Sikh, 53 as well as women from Northern China, 54 are at a higher risk of having children with NTDs. 30–38 It remains unclear whether these risks vary because of genetic predisposition, cultural dietary preferences, or a combination of these factors.

Multifactorial inheritance is the most common cause of NTDs, but monogenic, chromosomal, and teratogenic causes have specific effects that have not been studied in association with folic acid deprivation or supplementation (Table 2). 29 The prevalence of aneuploidy and additional anatomic abnormalities in fetuses with open spina bifida was reviewed using Utah Birth Defect Network data. 37 Chromosome results were known in 45 of 51 cases of open spina bifida, with six cases (13%) of aneuploidy. Additional major anatomic abnormalities were present in four of the six cases and included cardiac, renal, omphalocele, brain,
Table 2. Recognized conditions associated with neural tube defects

<table>
<thead>
<tr>
<th>Category</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Multifactorial</td>
<td>Homocysteine metabolism variants (MTHFR)</td>
</tr>
<tr>
<td>2. Monogenic</td>
<td>Alobar holoprosencephaly (acrania)</td>
</tr>
<tr>
<td></td>
<td>Cerebro-costo-mandibular syndrome</td>
</tr>
<tr>
<td></td>
<td>Fanconi's pancytopenia syndrome</td>
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<tr>
<td></td>
<td>Fraser's syndrome</td>
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<td></td>
<td>Hydrocephalus syndrome</td>
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<td></td>
<td>Joubert-Levin syndrome</td>
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<tr>
<td></td>
<td>Moldavi-Gruber syndrome</td>
</tr>
<tr>
<td>3. Chromosomal</td>
<td>Miller-Dieker syndrome (distal 17p13.3)</td>
</tr>
<tr>
<td></td>
<td>Trisomy 9 (mosaic)</td>
</tr>
<tr>
<td></td>
<td>Trisomy 13</td>
</tr>
<tr>
<td></td>
<td>Trisomy 18</td>
</tr>
<tr>
<td></td>
<td>CHILD syndrome (mutation NSDHL gene Xq 28)</td>
</tr>
<tr>
<td>4. Teratogenic</td>
<td>Fetal hyperthermia spectrum</td>
</tr>
<tr>
<td></td>
<td>Fetal alcholic syndrome</td>
</tr>
<tr>
<td></td>
<td>Fetal anhydramnios/birth defect</td>
</tr>
<tr>
<td></td>
<td>Fetal hydranencephaly</td>
</tr>
<tr>
<td></td>
<td>Fetal spinal encephalocele</td>
</tr>
<tr>
<td></td>
<td>Maternal pre-existing diabetes (pre-conception)</td>
</tr>
<tr>
<td>5. Unknown Etiology</td>
<td>Caudal dysplasia sequence</td>
</tr>
<tr>
<td></td>
<td>Extrophy of cloaca sequence</td>
</tr>
<tr>
<td></td>
<td>Laterality sequence</td>
</tr>
<tr>
<td></td>
<td>Limb-body wall complex</td>
</tr>
</tbody>
</table>

and bilateral oral clefting. There was a 4% risk of aneuploidy in sonographically isolated spina bifida cases within this population.32

Prenatal Diagnosis

All pregnant women should be offered routine screening for NTDs with specific and appropriate timing. Folic acid supplementation will not eliminate but will reduce the risk of NTDs. Women with an increased risk for a pregnancy complicated by NTDs often have a history of

- a previous fetus or child with an NTD
- a first-, second-, or third-degree relative with an NTD
- pre-existing maternal diabetes as well as insulin-dependent (type 1) diabetes
- epilepsy and the ingestion of valproic acid or carbamazepine for seizure control
- use of folic acid antagonists (amnioplatin, methotrexate)

Non-invasive prenatal diagnostic testing by ultrasound and maternal serum screening, which should be offered at 16 to 20 weeks' gestation and 15 to 20 weeks' gestation, respectively, will identify 95% to 100% of NTDs (anecephaly, 100%; spina bifida, 95%). Ultrasound imaging of the cranium and the identification of cranial scalping (lemon sign) and cerebellar crowding (banana sign) in association with mild ventricularomegaly is diagnostic of an open myelomeningocele if, even with improved ultrasound technique and resolution, a defect is not easily identifiable in the spine because of the level of the spinal defect, fetal position, or maternal habitus. After 15 weeks of pregnancy, invasive prenatal diagnostic testing with ultrasound-guided amniocentesis can evaluate the fetal karyotype and measure amniotic fluid alpha fetoprotein and acetycholinesterase to assist in differentiating between open or closed lesions.
FOLIC ACID AND PREVENTION

A Health Canada document,14 *Preconception Health: Folic Acid for Primary Prevention of Neural Tube Defects—A Resource Document for Health Professionals* 2002, states that, from the human data, it is clear that periconceptional use of supplements containing folic acid substantially reduces the risk of occurrence (first affected pregnancy) and recurrence (additional affected pregnancies) of neural tube defects. Similar summary information is available from the National Institutes of Health Clinical Center document, * Dietary Supplement Fact Sheet: Folic Acid* 2005.15

Women should be advised to maintain a healthy diet, as recommended in *Eating Well With Canada's Food Guide* (Health Canada).16 Good sources of folic acid include broccoli, spinach, peas, Brussels sprouts, corn, lentils, and oranges.

A randomized trial18 for the prevention of primary occurrence found periconceptional vitamin supplementation (12 vitamins including 0.8 mg of folic acid, 4 minerals, 3 trace elements) decreased the incidence of a first occurrence of NTD. Previous case-control studies had provided supportive or equivocal evidence that pregnant women using multivitamins containing folic acid or dietary folic acid had a lower risk of occurrence NTDs than women not taking supplements.19-24

With respect to prevention of recurrence of NTDs, a randomized double-blind clinical trial19 involving 1195 completed high-risk pregnancies women from 33 centers reported 72% fewer cases of NTDs among the offspring of the folic acid supplementation group than among the offspring of controls who did not take folic acid supplementation.19 The recurrence rate decreased from 3.5% to 1% for women randomized to receive 4 mg folic acid supplementation prior to pregnancy and throughout the first six weeks of pregnancy. The results in the group taking vitamins without folic acid were similar to the results in the group not taking vitamin supplementation, with recurrence risks of 3.5%.

Wald et al.14 evaluated the dose of folic acid required to maximize the already known benefit of folic acid in preventing NTDs. The study analyzed published data from 13 studies of folic acid supplementation on serum folate concentrations, as well as results from a large cohort study on the risk of NTDs according to serum folate.

Such results predict that the preventive effect is greater in women with low serum folate than in those with higher concentrations. The results have also been used to predict direct observations from large randomized trials and the effect of food fortification. From a typical western background serum folate of 5 mg/mL, about 0.2 mg/d (the US level of folic acid fortification) would be expected to reduce NTDs by about 20%; a similar effect can be expected from the current British recommendation (0.24 mg/d). An increase of 0.4 mg/d would reduce risk by about 36%, of 1 mg/d by 57%, and taking a 5 mg tablet daily would reduce risk by about 85%.56

Wald et al.14 concluded that folic acid fortification levels should be increased accordingly and that women planning a pregnancy should take 5 mg folic acid tablets daily instead of the 0.4 mg dose currently recommended. Some of the subsequent letters to the editor showed support57,58 for the concept, although others recommended caution.59 This increased dosage of folic acid has not yet been widely implemented for preconception populations.

Folic acid supplementation reduces NTDs,60-66 but new data (2006) for Ontario analyzed by Motherisk indicated that 40% of females in the reproductive age had HBC folate below 900 nmol/L and half of these (20%) were below 700 nmol/L, with 900 nmol/L or greater being necessary for maximum protection against NTDs. On the basis of this information, it can be estimated that 200 000 pregnant Canadian women are suboptimally protected against NTD each year.67 Other investigators have indicated that women attempting pregnancy will achieve a level of 900 nmol/L with a supplementation dosage of 0.4 mg folic acid.68 Additional information indicates that only 28% of Canadian women took folic acid or a multivitamin containing folic acid and that supplementation was not used the same way to the same extent in all ethnic groups.69 Other strategies have been proposed to influence and improve folic acid supplementation.70-72

FOLIC ACID AND VITAMIN SUPPLEMENTATION AND BIRTH DEFECTS OTHER THAN NEURAL TUBE DEFECTS

Folic acid in combination with multivitamin supplements has been shown to reduce other congenital anomalies, such as heart defects,2-5 urinary tract anomalies,2-6 oral facial clefts,20-26,73,74 limb defects,2 and pyloric stenosis.3 A recent review has analyzed the published literature regarding the prevention of congenital anomalies with periconceptional folic acid supplementation.75 Meta-analysis of prenatal multivitamin supplementation containing folic acid and the rates of congenital anomalies has shown decreased risks for the following:

- NTD (OR case-control 0.67; [0.58-0.77] cohort/RCT 0.52[0.49-0.69])
- Cardiovascular defects (OR case-control 0.78; [0.67-0.92] cohort/RCT 0.61[0.40-0.92])
- Limb defects (OR case-control 0.48; [0.30-0.76] cohort/RCT 0.57[0.38-0.85])
Table 3. Interactions: Drugs and folic acid[46-57,48,20,84]

<table>
<thead>
<tr>
<th>Drug</th>
<th>DX</th>
<th>Effect</th>
<th>Mechanism</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>cancer</td>
<td>reduced folic acid effect</td>
<td>Interference with erythrocyte</td>
<td>caution</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>cancer</td>
<td>reduced folic acid effect</td>
<td>maturation</td>
<td></td>
</tr>
<tr>
<td>Phenobarbital, phenytoin, primidone</td>
<td>epilepsy</td>
<td>reduced folic acid levels</td>
<td>Increased folic acid metabolism</td>
<td>caution</td>
</tr>
<tr>
<td>Phenobarbital, phenytoin, primidone</td>
<td>epilepsy</td>
<td>loss of seizure control, decreased phenytoin levels</td>
<td>Increased phenytoin metabolism</td>
<td>monitor phenytoin levels</td>
</tr>
<tr>
<td>Sulphasalazine</td>
<td>Crohn's disease</td>
<td>decreased folic acid levels</td>
<td>Impaired absorption</td>
<td></td>
</tr>
<tr>
<td>Sulphasalazine</td>
<td>ulcerative colitis</td>
<td>decreased folic acid levels</td>
<td>Impaired absorption</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>type II diabetes</td>
<td>reduced effect</td>
<td>Increased glucose</td>
<td>caution</td>
</tr>
<tr>
<td>Triamterene</td>
<td>diuretic</td>
<td>---</td>
<td>---</td>
<td>NIH caution</td>
</tr>
<tr>
<td>Lithium</td>
<td>sedation</td>
<td>---</td>
<td>---</td>
<td>NIH caution</td>
</tr>
</tbody>
</table>

- Cleft palate (OR case-control 0.76; [0.62–0.93] cohort/RCT 0.42 [0.36–2.84])
- Oral clefts with or without cleft palate (OR case-control 0.65; [0.54–0.75] cohort/RCT 0.56 [0.28–1.19])
- Urinary tract anomalies (OR case-control 0.46; [0.30–0.76] cohort/RCT 0.68 [0.35–1.31])
- Congenital hydrocephalus (OR case-control 0.37; [0.24–0.56] cohort/RCT 1.54 [0.53–4.50])

No effects were shown in preventing Down syndrome, pyloric stenosis, undescended testis, or hypoplasia. This meta-analysis is limited to studies with the combined multivitamin-folic acid treatment and excludes studies that did not report malformation rates, focused on folic acid alone, and did not contain a control group. Additional studies support these associations.

Other pediatric benefits have been identified following prenatal multivitamin supplementation before and in early pregnancy. Maternal use of prenatal multivitamins is associated with a decreased risk for pediatric brain tumors (OR 0.73; [0.60–0.88]), neuroblastoma (0.53; [0.42–0.68]), and leukemia (ALL) (OR 0.61; [0.50–0.74]). It was stated that it is not known which constituent(s) among the multivitamins confers this protective effect.

MATERNAL ISSUE WITH FOLIC ACID FORTIFICATION

A debate entitled Should Folic Acid Be Mandatory? was recently published. The "yes" opinion states that further investigation of the potential cancer-promoting effects of exposure to folic acid in susceptible people is desirable before mandatory fortification starts. Folic acid has not been shown to promote breast cancer or to prevent[58] it. Ovarian cancer studies[59] suggest (but not with statistical significance) that relatively high dietary folic acid intake may be associated with a reduction in ovarian cancer risk among women with high alcohol and methionine intakes.

FOLIC ACID METABOLISM

The risk of toxicity from folic acid intake from supplements and/or fortified foods is low. It is a water-soluble vitamin, so any excess intake is usually excreted in urine.

Medical conditions that increase the need for folic acid or result in increased excretion of folic acid include pregnancy/lactation, alcohol abuse, malabsorption (gastric bypass patients may be at risk), renal dialysis, liver disease, and certain anemias.

Serum folic acid levels may be affected by the metabolism of other medications, including antimetabolite agents, antiepileptic medications, and other medications (Table 3).[14,17,40,44]

OTHER VITAMIN ISSUES

Multivitamins should have vitamin A as beta-carotene rather than as retinol. Excess retinol (10 000 IU; 5000 RE) on a daily basis may cause birth defects.[58] For this reason, women should not take more than one daily dose, as indicated on the product label.
FOLIC ACID FOOD FORTIFICATION

In Canada since 1998, in an effort to try and reduce the rate of NTDs, there has been mandatory folic acid fortification of white flour, enriched pasta, and cornmeal. The overall benefit of fortification in reducing NTDs has been determined (105,104,79). The most recent Canadian data have shown that the prevalence of neural tube defects decreased from 1.58 per 1000 births before fortification to 0.86 per 1000 births during the full fortification period (1998–2002), a 46% reduction (95% CI, 40–51). The decrease was greater for spina bifida than for anencephaly and encephalocele.50

POTENTIAL HARM OF FOLIC ACID INTAKE

Folic acid, in a 0.4 to 1.0 mg daily dose, is not known to cause demonstrable harm to the developing fetus or the pregnant woman. Folic acid is water soluble and excess is excreted through the urinary tract. Patients aged 50 years or over are at greater risk for vitamin B12 deficiency than younger women, but this is not the age group in which pregnancy usually occurs. A recent Australian study found that high serum folate did not mask the macrocytosis of cobalamin deficiency of pernicious anemia. Macrocytosis appears to retain its value as a marker of cobalamin deficiency in people with serum folate concentrations above the population average. The folate acid dose of 5 mg has not been reported to have maternal or fetal risks.88–90

Folic acid and multivitamin supplementation is possibly associated with an increased incidence of twins.120,92

There are some concerns about folic acid supplementation being associated with an increased risk of neoplasia or possible exacerbation of pre-existing colorectal cancer. Increased rates for colorectal cancer have been observed since food fortification was introduced in Canada and United States. This effect has not been proven but needs to be acknowledged.93

This guideline recommends the use of folic acid in the perinatal period; the use of folic acid is therefore limited to usually recurrent 6- to 12-month time periods. Other long-term uses for folic acid in the clinical context (alcoholism, anemia, liver disease, kidney disease, malabsorption, cardiac disease, cancer treatment) are not discussed. Allergic responses to folic acid are rare, but may include rashes, itch, swelling, and bronchospasm.94

CURRENT SITUATION IN CANADA

In Canada, flour is fortified with folic acid. Its introduction coincided with an observed decrease in NTDs in liveborns,205 but this may be related to prenatal diagnosis/termination rather than fortification alone.967 Women who are motivated may be able to reach appropriate RBC folate levels with a selected diet, supplemented foods, and compliant daily oral folic acid supplementation (0.4–1.0 mg), but this situation may represent less than 15% to 20% of the pregnant population.

The combination of multivitamin and folic acid can be taken as oral supplementation or single combined pill (multivitamin with 0.4–1.0 mg or 5 mg) or as a multiple tablet option (multivitamin with 0.4–1.0 mg; for higher folic acid doses, add single 1 mg folic acid tablets as necessary). Oral supplementation may be variable because of compliance issues with daily oral tablet use (nausea, “forgets,” “don’t like to take pills”).103,104

Conception data indicate that 50% of pregnancies are unplanned with no additional oral supplement (multivitamin with folic acid) being used. The options described below take this into consideration.

TREATMENT OPTIONS

Option A: Patients with no personal health risks, planned pregnancy, and good compliance require a good diet of folate-rich foods and daily supplementation with a multivitamin with folic acid (0.4–1.0 mg) for at least two to three months before concepion and throughout pregnancy and the postpartum period (4–6 weeks and as long as breastfeeding continues). (II-2-A)

Option B: Patients with health risks, including epilepsy, insulin-dependent diabetes, obesity with BMI > 35 kg/m², family history of neural tube defect, belonging to a high-risk ethnic group (e.g., Asian) require increased dietary intake of folate-rich foods and daily supplementation, with multivitamins with 5 mg folic acid, beginning at least three months before conception and continuing until 10 to 12 weeks post conception. From 12 weeks post-conception and continuing throughout pregnancy and the postpartum period (4–6 weeks or as long as breastfeeding continues), supplementation should consist of a multivitamin with folic acid (0.4–1.0 mg). (II-2-A)

Option C: Patients who have a history of poor compliance with medications and additional lifestyle issues of variable diet, no consistent birth control, and possible teratogenic substance use (alcohol, tobacco, recreational non-prescription drugs) require counseling about the prevention of birth defects and health problems with folic acid and multivitamin supplementation. The higher dose folic acid strategy (5 mg) with multivitamin should be used, as it may obtain a more adequate serum red blood cell folate level with irregular vitamin / folic acid intake but with a minimal additional health risk. (III-B)
Appendix F 508

SUMMARY

Folic acid (in the diet and/or in a supplement) with a multivitamin has been proven to decrease or minimize specific birth defects including neural tube defects, congenital heart disease, urinary tract anomalies, oral facial defects with or without cleft palate, limb defects, and hydrocephalus, as well as some pediatric cancers. The public health flour fortification initiative has been very beneficial with respect to primary prevention of birth defects. The recent comprehensive Canadian analysis of neural tube reduction after folic acid flour fortification has reported a 46% reduction. The observed reduction was greater for spina bifida (53%) than for anencephaly (38%) and encephalocele (31%). Further reductions in the incidence of congenital anomalies sensitive to folic acid and multivitamin should be possible with the participation of key stakeholders.

Recommendations

1. Women in the reproductive age group should be advised about the benefits of folic acid in addition to a multivitamin supplement during wellness visits (birth control renewal, Pap testing, yearly examination) especially if pregnancy is contemplated. (III-A)

2. Women should be advised to maintain a healthy diet, as recommended in Eating Well With Canada’s Food Guide (Health Canada). Foods containing excellent to good sources of folic acid are fortified grains, spinach, lentils, chick peas, asparagus, broccoli, peas, Brussels sprouts, corn, and oranges. However, it is unlikely that diet alone can provide levels similar to folic-acid multivitamin supplementation. (III-A)

3. Women taking a multivitamin containing folic acid should be advised not to take more than one daily dose of vitamin supplement, as indicated on the product label. (II-2-A)

4. Folic acid and multivitamin supplements should be widely available without financial or other barriers for women planning pregnancy to ensure the extra level of supplementation. (III-B)

5. Folic acid 5 mg supplementation will not mask vitamin B12 deficiency (pernicious anemia), and investigations (examination or laboratory) are not required prior to initiating supplementation. (II-2-A)

6. The recommended strategy to prevent neural tube defects is to take folic acid 5 mg daily, starting at least three months before conception and continuing throughout pregnancy. Supplementation should consist of a multivitamin with folic acid 0.4 mg/day. (III-B)

The recommended strategy (ies) for primary prevention or to decrease the incidence of neural tube defects will include a number of options or treatment approaches depending on patient age, ethnicity, compliance, and genetic congenital anomaly risk status.

• Option A: Patients with no personal health risks, planned pregnancy, and good compliance require a good diet of folate-rich foods and daily supplementation with a multivitamin with folic acid 5 mg/day for at least two to three months before conception and throughout pregnancy. (II-2-A)

• Option B: Patients with health risks, including obesity with BMI > 35 kg/m², family history of neural tube defect, belonging to a high-risk ethnic group (e.g., Sikh) require increased dietary intake of folate-rich foods and daily supplementation, with multivitamins with 5 mg folic acid, beginning at least three months before conception and continuing until 10 to 12 weeks post conception. From 12 weeks post-conception and continuing throughout pregnancy and the postpartum period (4-6 weeks or as long as breastfeeding continues), supplementation should consist of a multivitamin with folic acid 0.4 mg/day. (II-2-A)

• Option C: Patients who have a history of poor compliance with medications and additional lifestyle issues of variable diet, no consistent birth control, and possible teratogenic substance use (alcohol, tobacco, recreational non-prescription drugs) require counseling about the prevention of birth defects and health problems with folic acid and multivitamin supplementation. The higher dose folic acid strategy (5 mg) with multivitamin should be used, as it may obtain a more adequate serum red blood cell folate level with irregular vitamin / folic acid intake but with a minimal additional health risk. (III-B)

The Canadian Federal Government could consider an evaluation process for the benefit/risk of increasing the level of national folic acid flour fortification to 500 mg/100 g (present level 140 mg/100 g). (III-B)
9. The Canadian Federal Government could consider an evaluation process for the benefit-risk of additional flour fortification with multivitamins other than folic acid. (III-B)

10. The Society of Obstetricians and Gynaecologists of Canada will explore the possibility of a Canadian Consensus conference on the use of folic acid and multivitamins for the primary prevention of specific congenital anomalies. The conference would include Health Canada/Con genital Anomalies Surveillance, Canadian College of Medical Genetists, Canadian Paediatric Society, Motherisk, and pharmaceutical industry representatives.

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Appendix F

Pre-conceptional Vitamin/Folic Acid Supplementation 2007


Increasing Folate Supplementation for Selected Groups of Canadian Women

Gideon Koren, MD, FRCP, Y. Ingrid Goh, HBSc
Motherisk Program, The Hospital for Sick Children, Toronto ON

Abstract
After review of current evidence related to the potential risks and benefits of folate acid supplementation, we conclude that unless clinicians can be assured that pregnant women will reliably use prenatal vitamin supplements containing 0.8-1.1 mg of folate, the prenatal vitamin supplements should be combined with 5 mg of folate.

Résumé
À la suite de l'analyse des données actuelles quant aux risques et aux avantages potentiels de la supplémentation en acide folique, nous en sommes venus à la conclusion suivante : si les cliniciens peuvent être assurés que les femmes enceintes utiliseront de façon fiable des suppléments vitaminiques prénataux contenant 0,8-1,1 mg de folate, les suppléments vitaminiques prénataux devraient être utilisés conjointement avec 5 mg de folate.


BACKGROUND
Folic acid is essential for growth and differentiation, repair, and host defence and hence it is essential for fetal development. The term “folate” generally refers to the fully oxidized form of the chemical compound, which is not naturally available in foods. The term “folate” refers to the group of compounds that have the same vitamin activity, encompassing both folic acid and naturally occurring folates. Folates are composed of a pteridine ring and p-aminobenzolic acid, and may contain one to six glutamate molecules that are joined by peptide linkages. Natural folates are found in the form of polyglutamates. These are relatively heat labile molecules that may be destroyed by storage, processing, and cooking and that are not readily absorbed by the body.

Key Words: Folic acid, pregnancy, multivitamin

Folic acid and Neural Tube Defects
NTDs comprise malformations of the cranial, spine, and nervous system, including anencephaly, spina bifida, encephalocoele, and meningoecele. These malformations are a major cause of mortality in newborns, second only to congenital heart defects. They have been estimated to affect 0.5 to 8 newborns per 1000 live births.

Health Canada has estimated that 195 Canadian infants are born each year with NTDs, and it has been estimated that NTDs annually affect 300,000 infants worldwide. Epidemiological studies associating folate supplementation with a decreasing incidence of NTDs date back to the 1960s.10,11 The most definitive research addressing the benefits of folic acid supplementation in decreasing the risk of NTDs was a
Appendix F 513

Increasing Folate Supplementation for Selected Groups of Canadian Women

multicentre randomized double-blind trial instigated by the United Kingdom Medical Research Council. The aim of this trial was to evaluate the efficacy of folic acid 4 mg daily in preventing an NTD in subsequent offspring of women who had previously delivered a child with an NTD. The trial took place in seven countries and recruited 1817 women who were randomized to one of four daily treatment groups: folic acid (4 mg), folic acid (4 mg) with a multivitamin, multivitamin supplementation without folic acid, or no supplementation at all. Participants were asked to use their supplements for one month prior to conception and throughout the first 12 weeks of pregnancy. The authors showed that women randomized to take folic acid supplementation had a 1.0% chance of having a child with an NTD (RR 0.28; 95% CI 0.12-0.71), but women in the unsupplemented arm did not show a decrease in the risk of NTD (RR 0.8; 95% CI 0.37-1.72). Overall, supplementation with folic acid reduced the rate of recurrence of NTD by 72% (6/593 with folic supplements vs. 21/602 without).12

A second key trial evaluating folic acid-fortified multivitamin supplementation during pregnancy was a double-blind, randomized controlled trial in which 204 women were randomized to take a 0.8 mg folic acid-containing multivitamin supplement, while 2052 women were randomized to take a multivitamin containing trace supplementation.13 Supplementation began at least one month prior to pregnancy and continued at least the time of the second missed menstrual period. Twenty-eight malformations were noted in the newborns in the folic acid-supplemented group, whereas 47 malformations were noted in the newborns in the trace supplement group.13 No NTDs were observed in the babies in the folic acid-fortified group, whereas six NTDs were observed in the babies in the trace element group.13

The meta-analysis conducted by Motherisk found that multivitamin supplementation significantly reduced the risk of NTD, both in case-control studies (OR 0.67; 95% CI 0.58-0.77) and in cohort and randomized controlled studies (OR 0.52; 95% CI 0.30-0.90).14 The only available study investigating the relationship between serum and RBC folate concentrations and the risk of neural tube defects found an inverse relationship between maternal RBC folate and the risk of NTD.15 Daly et al. showed in a case-control study that women receiving < 150 µg of folic acid daily had a risk of NTD of 6.6/1000 live births, and that women receiving > 400 µg daily had an NTD risk of 0.8/1000 live births. Supplementation at doses of 100 µg and 400 µg daily resulted in a decrease in risk of NTD of 22%, 41%, and 47%, respectively.16 Another study investigating dosing variations of folic acid corroborated this result, noting that daily folic acid doses of 100µg, 200 µg, and 400µg decreased the NTD risk by 18%, 35%, and 53%, respectively.17

Folic Acid and Other Malformations

In the Motherisk meta-analysis, we have shown that folic acid-containing prenatal vitamin supplements are also associated with a reduction in risk of oral clefts, heart defects, urinary tract anomalies, and limb anomalies in newborns.14 It could be argued that women who supplement with prenatal vitamins may have a lower risk of having children with malformations because they have better health and health-related behaviours than women not taking vitamins. However, in the case of neural tube defects15 and oral clefts,14 population-based studies have shown that the folic acid fortification program was associated with decreased risk, thus corroborating the associations shown in the meta-analysis.16

Early Pediatric Cancer

In a recent meta-analysis, we observed an apparent protective effect for early leukemia (OR 0.61; 95% CI 0.50-0.74), pediatric brain tumors (OR 0.73; 95% CI 0.60-0.88), and neuroblastoma (OR 0.53; 95% CI 0.42-0.68) associated with use of prenatal vitamin supplements containing folic acid.19

THE OPTIMAL DOSE OF FOLIC ACID SUPPLEMENTATION

For almost 20 years, the recommended daily dose of folic acid supplementation has been 0.4 mg. In fact, prenatal multivitamins invariably contain 0.8 to 1.1 mg of folic acid, and this has led to the assumption that daily supplementation with this dose is sufficient to prevent neural tube defects. However, in 2001, Wald systematically reviewed all reports of the correlation between ingested dose of folic acid and resultant serum concentrations.10 Using the data of Daly et al.,15 who correlated maternal serum folate levels with the risk of neural tube defects, Wald concluded that the currently recommended daily dose of folic acid would render only partial protection against neural tube defects. According to Wald’s analysis, 5 mg per day of folic acid would be necessary to render 90% protection against NTD in the prenatal population.15 Wald’s analysis has been recently corroborated by our

ABBREVIATIONS

CI confidence interval
NTD neural tube defect
OR odds ratio
RBC red blood cell
RR relative risk
### Associations between folate status and risk of selected cancers, and folate status and risk of twinning

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>- Meta-analysis: decreased cancer risk with high folate status</td>
</tr>
<tr>
<td></td>
<td>- Majority of case-control studies: reduction in risk (30-36%) at the highest dietary intake of folate status.</td>
</tr>
<tr>
<td></td>
<td>- May increase risk in post-menopausal women (non-significant statistically)</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>- Inverse relationship between folate status and risk of colorectal cancer in healthy people</td>
</tr>
<tr>
<td></td>
<td>- Potential increased risk of adenoma</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>- Decrease in risk with higher folate status</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>- Significant decrease in the clear cell subtype</td>
</tr>
<tr>
<td></td>
<td>- Prospective prevention (non-significant trend)</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>- Significantly lower folate in cancer cases compared with controls</td>
</tr>
<tr>
<td>Carcinoma of head and neck</td>
<td>- Protective effect</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>- No effect</td>
</tr>
<tr>
<td>Esophageal, gastric, and pancreatic cancer</td>
<td>- Protective effect in case-control studies</td>
</tr>
<tr>
<td>Non-Hodgkin's lymphoma</td>
<td>- No correlation with folate status</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>- Folate fortification not associated in the degree or pattern of global DNA methylation in cells involved in cervical carcinogenesis</td>
</tr>
<tr>
<td>Twinning</td>
<td>- Systematic review: possible but non-significant evidence of preconceptional folate intake and twinning</td>
</tr>
</tbody>
</table>

Finding that in 2005–2006, 40% of Ontario women did not achieve the protective red blood cell folate level of 900 mmol/L, despite flour fortification and despite the fact that more than 50% of pregnant women took prenatal multivitamin supplements.25

Before recommending prenatal supplementation with higher doses of folic acid, we need to consider the potential health risks of such a move.

It has been proposed that higher levels of folate intake may mask pernicious anemia arising from vitamin B12 deficiency. Similar concerns surrounded the original North American flour folate fortification program in 1998 but several recent studies have failed to show such a risk.30 A recent US study suggested an association between high folate levels in older Americans and a risk of cognitive impairment.21 However, cognitive impairment is not a component of pernicious anemia, and the study showed no increased risk for neuritis, which is a typical finding in patients with pernicious anemia. The risk for pernicious anemia will be different if the general population is consuming flour with higher levels of folate, rather than having pregnant women supplement their intake by 5 mg/day for a limited time. In fact, direct measurements of serum levels of vitamin B12, or higher dose supplementation of vitamin B12, may further allay these concerns.

If women do not comply with the recommendation to take the currently available folate-containing preparations, it is reasonable to question whether they would take the preparations containing 5 mg/day. In a recent controlled trial of prenatal vitamin supplements in women who discontinued or had not started taking prenatal vitamins, their compliance with two different brands of prenatal vitamins averaged 58% and ranged from 0 to 100% despite the participation of self-selected, motivated women.22 Pharmacologically, administration of 5 mg of folic acid per day in women who have a lower compliance with taking medication should provide many more women with protective levels of folate.

Although laboratory studies have suggested that folic acid might increase the risk of certain cancers, population-based studies have repeatedly shown folic acid use to be associated with a 15-20% decline in incidence.23-25 This trend towards reduction in cancer risk associated with folic acid use is shown in the Table. Scientists therefore refer to the potential dual effects of folate on cancer risk, with increased risk for individuals with a history of or predisposition to cancer.23 There is no question that an increased risk of cancer associated with folate use, even if it exists, is a result of long exposure to folate over years, and not to several months of dosing during pregnancy.

### CONCLUSION

Unless the prescribing clinician can ensure that pregnant women will be perfectly compliant in using prenatal vitamin supplements containing 0.8 to 1.1 mg of folate, the prenatal...
vitamin supplements should be combined with 5 mg of folate.

ACKNOWLEDGEMENTS
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DECEMBER 2007 995
DRUGS IN PREGNANCY

COMMUNIQUÉ

THE INAUGURAL MEETING OF ONTARIO FASD CLINICS
MARCH 15, 2007, TORONTO

Y Ingrid Goh*, Gideon Koren†, Judy Kay‡
†The Hospital for Sick Children, Motherisk Program, Toronto, Canada;
‡Healthy Generations Family Support, Sioux Lookout, Canada

ABSTRACT

In the recent inaugural meeting of all Ontario FASD Diagnostic Clinics, each clinic identified their strengths and weaknesses. The group agreed to develop a FASD diagnostic clinic website which would list all of the clinics available in Ontario. This would enable people to identify clinics near their area. In addition, it would increase public awareness of FASD and the shortage of such facilities.

With sponsorship of the federal Public Health Agency of Canada, the Ontario FASD group meeting recently convened individuals with a vested interest in FASD diagnostic clinics. Stakeholders in attendance informed the forum on their state of FASD clinics. They identified their patient population; adults, child or both. Attendees were encouraged to identify strengths and challenges in their clinic operations. A common theme among clinics was the need to urgently identify sources of funding for operations and staffing. The need for psychologists was also stressed.

Northwestern Ontario FASD Clinic - Kenora and Sioux Lookout
The Northwestern Ontario FASD (diagnostic) Clinic with clinic sites in Sioux Lookout and Kenora suspended operations in July 2006 due to lack of funding. Initially established as a demonstration project in 2005, this clinic received over 250 referrals in an 18 month period. At the time of their closing they had 62 patients on their waiting list. The Winnipeg FASD diagnostic clinic (Clinic for Alcohol and Drug Exposed Children) began to refuse referrals of patients from Northwestern Ontario since the opening of the Northwestern Ontario FASD clinic. As such patients residing in Northwestern Ontario have been left without access to diagnosis. This group is actively seeking funding to reinstate the clinic.

Peel Infants Development - Peel Region
The Peel region received funding to provide FASD diagnostic training for a multidisciplinary team. After receiving the training, they established a FASD diagnostic team that diagnoses children between the ages of 0-6 years. Since there is no dedicated funding for the team, this is a virtual team. To achieve the necessary funding required for psychological assessment, they access funding from service resolution and extended healthcare benefits. Service resolution alters funding used to access services that are not readily available in certain areas. Extended healthcare benefits (private insurance) are often the source of funding for families who have these benefits.

Breaking the Cycle FASD Clinic, Mothercraft - Toronto
Mothercraft’s Breaking the Cycle (BTC) program in Toronto primarily operates with the funding support of the Public Health Agency of Canada and the in-kind contributions from partner organizations. The FASD Assessment and Diagnostic Clinic operate in the context of the comprehensive
services offered at BTC. Motherisk contributes the psychologist on the assessment/diagnostic team, as well as the clinic coordinator, case managers, and early intervention staff; the pediatrician on the team is contributed by the Motherisk Program, a partner in BTC. Unlike most FASD diagnostic clinics, their strength lies in their ability to engage and work directly with the biological mother and child. They also have experience in how to support caregivers to decrease placement breakdowns. This is one of the very few clinics that also offer FASD diagnosis for adult mothers.

**FASD Durham - Durham Region**

The Durham FASD diagnostic clinic is a virtual team. This team received training in Seattle. Durham has a preschool and a school age team. The Grandview Team assess children ages 0 to 6 years, and a school age team conducts their intake and coordination through Resources for Exceptional Children. Together they wish to expand their FASD diagnostic team into the community and therefore expand their ability to diagnose by integrating diagnosis into the medical service. A logistical problem they have overcome is documentation storage, which has been resolved by storing the files in the team physician’s office.

**St. Michael FASD Clinic, St. Michaels Hospital - Toronto**

St. Michael’s Hospital FASD diagnostic clinic is a multidisciplinary team that offers diagnoses and plans of care to clients of all ages (birth throughout adulthood). St. Michael’s has identified an urgent need for training of psychologists and designated funds for psychological services. St. Michael’s is currently initiating Telediagnosis to provide diagnosis to Northern Communities. St. Michael’s conducts research and education to improve outcomes of those with FASD, and is willing to train emerging teams.

**Anishnawbe’s Health FASD Clinic - Toronto**

Anishnawbe’s diagnostic team was established in collaboration with St. Joseph’s Health Centre. This clinic provides an Aboriginal traditional holistic approach. Their strength lies in directly serving the aboriginal populations.

**Motherisk FASD Clinic, Hospital for Sick Children - Toronto**

The Motherisk FASD diagnostic clinic is a multidisciplinary team at the Hospital for Sick Children that works in collaboration with agencies including Children’s Aid Services and other ministries. In addition to diagnosis, Motherisk conducts large-scale research in various aspects of FASD. Motherisk is currently providing training in FASD diagnosis throughout Canada and is willing to train emerging FASD clinics. Motherisk is able to provide video conferencing to assist other teams to provide a complete diagnosis.

**Surrey Place - Toronto**

Surrey Place Centre offers clinical and diagnostic services to individuals with developmental disabilities of all ages. Currently, they are designated as an emerging team for FASD diagnosis for adults. They have psychologists, nurses, speech pathologists and behaviour therapists on their diagnostic team. Surrey Place also works with various Associations for Community Living in northern Ontario on a videoconferencing program. They hope to use this technology to perform diagnostic assessments in remote areas including psychological and other forms of assessment. Facilitators need to be trained and testing takes longer; however using videoconferencing overcomes the problem of no service in some areas.

**Peterborough**

Peterborough needs to identify source of funding for psychologists. Their strength lies in their speaker’s bureau.

**Norwest Health Centre - Thunder Bay**

Although this clinic does not offer diagnostic services at this time they are considered an emerging clinic. Presently and on a regular basis, they make referrals to diagnostic clinics, prepare, complete and send out the FASD Referral package, arrange travel/accommodation if necessary, assist with finding travel subsidy, and follow up with the family after the diagnostic assessment. The Norwest Health Centre at Thunder Bay provides training and education of individuals regarding FASD diagnosis.
Appendix F

Inaugural meeting of Ontario FASD clinics – March 2007

Hamilton
Hamilton is an emerging FASD diagnosis team. McMaster University currently has several physicians who diagnose children 0-6 years using the 4-digit code; however, this does not include complete evaluation (including psychometric assessment). They have been able to secure funding for a coordinator from the Trillium Foundation for 2 years. They will be seeking additional funding to sustain the clinic.

Kingston
Kingston is also an emerging FASD diagnosis team who hopes to establish a clinic in the near future. They need to be trained in FASD diagnosis. Their sources of funding include Trillium Grant, Children and Youth Services Fiscal dollars.

Need for Training
It was suggested that healthcare providers may be interested in attending FASD diagnostic workshops and trained to diagnose using the 4-digit code if they receive CME points. Healthcare professionals working with persons affected by FASD should also be trained on how to adapt their therapy as persons affected with FASD may learn in different manners. Therefore trying to teach in a standard manner may not be effective. Ontario also needs to develop a protocol on how to diagnose adults with FASD. Moreover, it was suggested that a parent be part of the FASD diagnosis team. The parent would be able to offer peer support to parents of newly diagnosed children and educate them on how to get past legal hurdles. The diagnosis team in Seattle, Washington currently includes parents on their FASD diagnostic team and has found this approach successful. A standardized summary tool for Canadian psychiatrists which summarize psychiatric findings in the patient chart is needed to make it easier for health care providers to quickly review charts prior to seeing patients for their appointment.

Potential Sources of Funding
Funding in existing clinics is largely done through donations, in kind and grants. This is not a long-term sustainable solution. As FASD is an acquired brain injury resulting from alcohol exposure, the Province of Ontario must recognize the need to fund FASD as western Canadian provinces have done.

Next Steps
The group agreed to develop a FASD Diagnostic Clinic website which would list all of the FASD clinics available in Ontario. This would enable caregivers to locate the closest diagnostic centre where they can have their child assessed for FASD. The FASD Stakeholders for Ontario Diagnostic Working Group is committed to continue to strengthen a network for FASD Diagnostic Clinics in Ontario.

Corresponding Author: Judy Kay
healthy.silvael.on.ca
Prenatal Multivitamin Supplementation and Rates of Pediatric Cancers: A Meta-Analysis

YI Goh1,2, E Bollano3, TR Einanson1,2, and G Koren1,2,3,4,5

Prenatal supplementation of folic acid has been shown to decrease the risk of several congenital malformations. Several studies have recently suggested a potential protective effect of folic acid on certain pediatric cancers. The protective role of prenatal multivitamins has not been elucidated. We conducted a systematic review and meta-analysis to assess the potential protective effect of prenatal multivitamins on several pediatric cancers. Medline, PubMed, EMBASE, Toxline, Healthstar, and Cochrane databases were searched for studies published in all languages from 1960 to July 2005 on multivitamin supplementation and pediatric cancers. References from all articles collected were reviewed for additional articles. Two blinded independent reviewers assessed the articles for inclusion and exclusion. Rates of cancers in women supplemented with multivitamins were compared with unsupplemented women using a random effects model.

Sixty-one articles were identified in the initial search, of which, seven articles met the inclusion criteria. There was an apparent protective effect for leukemia (odds ratio [OR] = 0.61, 95% confidence interval [CI] = 0.50-0.74), pediatric brain tumors (OR = 0.73, 95% CI = 0.60-0.88) and neuroblastoma (OR = 0.53, 95% CI = 0.42-0.68). In conclusion, maternal ingestion of prenatal multivitamins is associated with a decreased risk for pediatric brain tumors, neuroblastoma, and leukemia. Presently, it is not known which constituent(s) among the multivitamins confer this protective effect.

It is estimated that 9,540 children in the United States under the age of 15 were diagnosed with cancer in 2005.3 The most prevalent forms of childhood cancer are leukemia, malignant brain and spinal cord tumors, and neuroblastoma.2,3 Leukemia is estimated to account for 25-35% of pediatric cancers.4 The two major morphological types of blood-borne cancers are acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML). The American Cancer Society estimates that 2,870 and 1,196 children were diagnosed with ALL and AML in 2005, respectively.5-6 Malignant brain and spinal cord tumors occur in 2,200 (17%) of pediatric cancers.7 These cancers include astrocytoma, primitive neuroectodermal tumors, and medulloblastoma.2 Neuroblastoma is diagnosed in approximately 600 American children annually.7 An estimated 163 children die from ALL in the United States each year,8 and only about half of children with brain tumors will survive more than five years.8

A large number of investigations into the epidemiology of these pediatric cancers have been undertaken in an attempt to identify risk factors and protective agents. Investigators have looked for relationships between genes and environmental exposures such as radiation,9-10 N-nitroso compounds,11 pesticides,12-13 tobacco14-15 electromagnetic frequencies,16 infectious agents,17-19 parental occupation,17 drugs,18-19 alcohol,20 infant feeding,21 multivitamins,22-23 and cancer.

It is now generally accepted that women of childbearing potential should supplement with folic acid before pregnancy and in early pregnancy to decrease the proven risk of neural tube defects. This suggested relationship was proven by the British and Hungarian randomized studies of supplementation with folic acid before pregnancy and in early pregnant women.24-25 Folic acid fortification of flour has subsequently resulted in decreasing rates of neural tube defects.26-27 In addition, it has most recently been suggested that folic acid containing multivitamins may also be beneficial in preventing congenital anomalies other than neural tube defects.26,27 Botto et al.26 noted that there was an apparent decreased risk for orofacial clefts, limb deficiencies, and cardiovascular abnormalities with multivitamin supplementation. In addition, Bailey et al.27 reported a decrease of cardiovascular

1Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada. 2The Mofolk Group, Division of Clinical Pharmacology/Toxicology, The Hospital for Sick Children, Toronto, Ontario, Canada. 3Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada. 4Ivey Chair in Molecular Toxicology, University of Western Ontario, London, Ontario, Canada. 5Department of Medicine, University of Western Ontario, London, Ontario, Canada. Correspondence to G Koren (glemen@uhn.on.ca)

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ARTICLES

abnormalities and orofacial clefts. A recent study conducted by our group followed the prevalence of neuroblastoma rates after folic acid fortification of flour in Ontario, Canada, showing an apparent protective effect. To date, several studies have investigated the potential effect of multivitamin use before and in early pregnancy on rates of common pediatric tumors. The objective of this study was to conduct a systematic review and meta-analysis of prenatal multivitamin use before and in early pregnancy and the risk of pediatric cancers.

RESULTS

Sixty-one articles were compiled from initial searches and reference review. Using our exclusion criterion, seven articles were eligible for inclusion: two articles addressing brain tumors, two addressing neuroblastoma, and two articles addressing leukemia, and one article addressing both brain tumors and leukemia (Table 1). All studies were of case-control design. Thirty-eight articles were excluded because they did not contain information on maternal multivitamin use during pregnancy. Three studies were excluded because they dealt with vitamin ingestion by children. Two studies were excluded because they combined multivitamin and iron supplementation into one category. One article reported on folic acid fortification in food. Two articles were excluded because they focused only on folate. Three studies were rejected because they did not provide complete raw data to allow analysis. Two papers were rejected because they were duplicates of articles published in different journals. One contained data that were previously published, and one was rejected because it was a news report. Use of prenatal multivitamins by the pregnant mothers was associated with a protective effect for childhood leukemia (odds ratio (OR) = 0.64, 95% confidence interval (CI) = 0.53-0.78). As pediatric leukemia has different origins, ALL and AML were analyzed separately. The ingestion of prenatal multivitamins was associated with a protective effect for ALL (OR = 0.61, 95% CI = 0.50-0.74) (Figure 1). There was no significant heterogeneity among

Table 1 Characteristics of articles included in meta-analysis:

<table>
<thead>
<tr>
<th>Author</th>
<th>Year published</th>
<th>Country</th>
<th>Dates of study</th>
<th>Age of children (years)</th>
<th>Language</th>
<th>Matching controls</th>
<th>Primary outcome</th>
<th>Matching variables</th>
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<tr>
<td>Buxin et al.</td>
<td>1994</td>
<td>United States, Canada</td>
<td>1986-1989</td>
<td>&lt;6</td>
<td>Not specified</td>
<td>Random-digit dialing*</td>
<td>Astrocytic glioma</td>
<td>Matching area code and next five digits of phone number; date of birth: ±1 year and race; black and non-black</td>
</tr>
<tr>
<td>Preston et al.</td>
<td>1998</td>
<td>United States, France, Italy, Israel, Canada, Australia</td>
<td>1976-1994, Varies by location</td>
<td>Not specified</td>
<td>Not specified</td>
<td>Varies by location</td>
<td>Brain tumor</td>
<td>Varies by location</td>
</tr>
<tr>
<td>Okhan et al.</td>
<td>2002</td>
<td>United States, Canada</td>
<td>May 1, 1950-April 30, 1994</td>
<td>&lt;19</td>
<td>English, Spanish</td>
<td>Random-digit dialing</td>
<td>Neuroblastoma</td>
<td>Date of birth ±6 months for cases &lt;3 years or date of birth ±1 year for cases &gt;3 years</td>
</tr>
<tr>
<td>Wen et al.</td>
<td>2002</td>
<td>United States, Canada, Australia</td>
<td>January 1, 1989-June 15, 1993</td>
<td>&lt;15</td>
<td>English</td>
<td>Random-digit dialing*</td>
<td>ALL</td>
<td>±2 years of age: race, telephone area code and exchange</td>
</tr>
</tbody>
</table>

ALL, acute lymphocytic leukemia; Random-digit dialing criteria relaxed in order to find a match.
the ALL studies ($\chi^2 = 1.27, p = 0.09$). There was only one study that reported information regarding AML, therefore it could not be meta-analyzed; nevertheless, it suggested a protective effect as well. The use of multivitamins by pregnant mothers was associated with a protective effect for several solid tumors. Supplementation with prenatal vitamins was associated with a decreased risk for neuroblastoma (OR = 0.53, 95% CI = 0.42-0.68) (Figure 2). Prenatal supplementation was also associated with decreased risk for pediatric brain tumors (OR = 0.73, 95% CI = 0.60-0.88) (Figure 3). A funnel plot did not show significant publication bias.

**DISCUSSION**

There is a large body of evidence supporting the protective effect of folic acid in decreasing the effect of neural tube defects.\textsuperscript{2,25} In addition, folic-acid-containing multivitamins have also been associated with prevention of other congenital anomalies other than neural tube defects.\textsuperscript{26} The data from the present meta-analysis suggest that prenatal supplementation of multivitamins containing folic acid is associated with an overall 18% protective decreased risk for pediatric brain tumors, 47% for neuroblastoma, and 36% protective effect for leukemia. To our knowledge, this is the first systematic review and meta-analysis examining such protective effect. Based on these data, one can estimate that maternal multivitamin supplementation may prevent 500 cases of pediatric leukemia and 200-400 cases of pediatric brain tumors annually in the United States.

The most apparent limitation of all studies considered in this meta-analysis is their retrospective design and the potential for recall or reporting bias. The bias may stem from parents of the case group wishing to attribute their child's cancer to a cause. On the other hand, recall bias may alter the exposure rate they report. Hence, reporting bias may result in over-reporting or lack of multivitamin use. In addition, some studies specifically asked about multivitamin use, whereas other studies posed an open-ended question on whether women took any medications.

Second, the composition of the multivitamins probably varied, as did the timing and duration of exposure. This may be a limitation because different components within the multivitamin may be responsible for these protective effects. However, as different brands of multivitamins contain different amounts of vitamins and minerals, it is difficult to ascertain which component is responsible for the protective effect. In addition, women who began supplementing before pregnancy and continued throughout pregnancy may have a different risk of delivering a child with pediatric cancer compared to women who began supplementing after discovering they were pregnant. Considering that, different vitamins and minerals are important in the production and replication of DNA and cells, mothers who began supplementation prior to pregnancy may theoretically have a lower risk of delivering a child with pediatric cancer.

Third, the selection of control groups varied between studies. The majority of the investigators utilized a random-digit telephone-dialing method; however, their inclusion criterion varied in certain circumstances.\textsuperscript{29,30,32,33} In cases where matches could not be found to meet the original criteria, investigators loosened different criteria in order to find a match.\textsuperscript{24} In addition, as random dialing is being used, some

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Maternal multivitamin consumption and risk for ALL in their children.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Maternal multivitamin consumption and risk for neuroblastoma in their children.}
\end{figure}

CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 81 | NUMBER 5 | MAY 2002
ARTICLES

<table>
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<th>OR (random) 95% CI</th>
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<td></td>
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<tr>
<td>Preterm birth (1995)</td>
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</tr>
<tr>
<td>Gestation (1964)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total random (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity</td>
<td>$\chi^2 = 0.01$, df = 2 ($P = 0.95$), $I^2 = 0$%</td>
<td></td>
</tr>
<tr>
<td>Test for overall effect</td>
<td>$\chi^2 = 0.01$, df = 2 ($P = 0.95$)</td>
<td></td>
</tr>
</tbody>
</table>

0.2 0.5 1 2 5 10

Figure 3. Maternal multivitamin consumption and risk for pediatric brain tumors in their children.

households with multiple phone lines have a greater chance of selection. In terms of identifying controls, one study matched by birth certificate registry, whereas another matched by physician's patient roster. Most studies matched based on ±1 years of age; however, there was a study that matched on ±2 years of age or ±3 years of age. In addition, a fair number of studies did not match for ethnicity, or matched on a black/non-black basis. Moreover, social class differences were not considered. Mothers of lower social class may not have had sufficient resources to afford a well-balanced diet. Maternal medical history and medication intake were also not reported in all articles. Confounding effects such as absorption problems and drug interactions involving multivitamins were therefore not addressed.

In addition to the articles included in this meta-analysis, one rejected article, which did not present raw data, also reported an apparent protective effect. A study conducted by Buskin et al. of the Children's Cancer Group from 1986 to 1989 found that the use of multivitamins during the first 6 weeks of pregnancy decreased the risk of primitive neuroectodermal brain tumors (OR = 0.56, $P = 0.02$). In addition, a later case-control study, which also did not report raw data, used the Children's Cancer Study Group to compare children who were diagnosed with retinoblastoma between 1982 and 1985, and found a decreased occurrence of retinoblastoma with prenatal multivitamin supplementation in both sporadic and heritable tumors (OR = 0.4, $P = 0.03$, OR = 0.2, $P = 0.02$, respectively).

Although our conclusions suggest that folic acid containing multivitamins are associated with a decrease in certain pediatric cancers, the available data do not allow determination of which of the constituent(s) may cause the protective effects. The papers included in the study were based on neonatal reports of multivitamin use. As such, the component and the quantity of each vitamin contained within the multivitamins, were not available in some of the papers included.

It may be possible that the observed effect may be related to folic acid. It has been hypothesized that the potential association of folic acid and pediatric cancer is due to partially altered DNA methylation and impaired DNA synthesis and repair. This transformation may be the source for the primitive neuroectodermal tumors. In addition, polymorphism of the MTHFR gene may also be an important etiologic factor. Polygenomic of C677T and A1298C may reduce the risk of ALL. In contrast, the same authors hypothesized that folate may also enhance the development and progression of already existing, undiagnosed premalignant or malignant lesions. Conversely, other researchers reported that folic acid supplementation may prevent breast cancer, colorectal adenoma, and carcinoma in adults, and folic acid receptor overexpression has been noted in epidermoidomas.

Several other vitamins have also been investigated regarding their ability to prevent cancer. The antioxidant mechanism of vitamins C and E has been investigated in the reduction of nitrosation process in cured meats and the formation of carcinogens.

To date, there have been no experimental data establishing a direct relationship between multivitamins and the pathogenesis of pediatric brain tumors. Because folic acid is the standard prophylactic therapy to reduce the risk of neural tube defects in pregnancy, there is no way to ethically conduct a randomized-control trial to separate these effects. The only possible method to elucidate whether the observed protective effect is due to folic acid itself or other vitamins would be to conduct a head-to-head comparison of folic acid versus folic acid containing multivitamins. This, however, is not feasible given the large sample size that would need to be followed for a long period of time. Presently, many women actively planning pregnancy commence prenatal multivitamins before conception, and hence it is not likely that such comparison is presently feasible.

In conclusion, prenatal multivitamins containing folic acid appear to be associated with a significant protective effect on three common pediatric cancers. Given that women who are considering pregnancy are generally advised to supplement with folic acid, the results from this study suggest that supplementation with a folic acid-containing multivitamin may be a preferred method.

METHODS

A search of the existing literature regarding pre- and periconceptional ingestion of multivitamins and the rates of cancer in offspring was undertaken. The outcome of interest was pediatric cancer.
All original research articles using case-control or cohort study design were included. Included articles must have contained a control group of healthy children with accounts of maternal intake of multivitamins during pregnancy. In addition, all included articles must have contained raw data of number of cases and controls using multivitamins. We excluded articles that did not involve women taking multivitamins during pregnancy or focused on specific vitamins, mothers exposed to other known teratogens, review articles, or data reported in abstracts or meetings.

Articles were searched using the terms multivitamin, pregnancy, cancer, and neoplasms in Medline (1966-July 2005), PubMed (July 2005), EMBASE (1980-July 2005), TOXLINE (1980-July 2005), Healthstar (July 2005), and the Cochrane database in all languages. References from all collected articles were reviewed for additional original studies of interest.

All the articles were reviewed using the above selection criteria by two reviewers who were blinded to the study outcome, names, and institutions of authors. Data from the articles were extracted into two reviewers using data collection forms. In cases of discrepancies, discussions were undertaken and if unresolved, the article was reviewed by a third blinded reviewer who served as a tiebreaker. All data were entered into 2 × 2 tables. ORs and 95% CI were calculated for each case-control study using Review Manager 4.2.7 (2004, The Cochrane Collaboration). Homogeneity among effects was tested by calculating Z2. A funnel plot was used to assess publication bias, following which the Egger-Mazandarani test was executed to calculate Kendall’s c test that evaluates the agreement between the effect and variances.

CONFLICT OF INTEREST

W. Goih, Dr. D. Beilona, and Dr. TR Elsmore state no conflict of interest. Dr. G. Karon received research funding from the Canadian Institutes of Health Research and the Research Leadership for Better Pharmacotherapy Learning and Leadership. He also received funding from Duchesney Inc., producer of one prenatal vitamin ( progyn) which was not on the market during the time covered by this study.

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Appendix F

MEGA-DOSE VITAMIN C AND E IN PREVENTING FASD: THE DECISION TO TERMINATE THE STUDY PREMATURELY

Y Ingrid Gold, Wendy Ungar, Joanne Ravel, Gideon Koren

1Maternal Program, Division of Clinical Pharmacology & Toxicology, Hospital for Sick Children, Toronto, Ontario. 2Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario. 3Department of Child Health Evaluative Studies, Hospital for Sick Children, Toronto, Ontario. 4Department of Psychology, Hospital for Sick Children, Toronto, Ontario. 5Department of Medicine, University of Western Ontario, London, Ontario.

ABSTRACT

Since 2004 we have been conducting a randomized control double-blind trial on the favourable effect of mega-dose vitamin C and E in pregnant women with heavy alcohol exposure. The study was terminated in the summer of 2006 due to new evidence showing intrauterine growth restriction caused by such treatment in women with pre-eclampsia.

FASD (fetal alcohol spectrum disorder) affects 1 of 100 children. As FASD results from alcohol exposure during pregnancy, mothers are advised to discontinue drinking when planning pregnancy. However, a large number of pregnancies are unplanned or unrecognized until fairly late in gestation. In the 1988 National Maternal and Infant Health Survey on drinking by pregnant women, Floyd et al. reported that 45% of women surveyed reported consuming alcohol during the three months before finding out they were pregnant. As such, providing a means of ameliorating the damage inflicted by maternal use of alcohol is of important interest. One of the mechanisms believed to cause FASD is oxidative damage generated by oxidative stress which occurs during alcohol exposure. Previous experimental studies have demonstrated that ethanol damage to neural cells can be attenuated by treatment with antioxidants. In addition, previous studies have also documented the potential benefits of mega-dose antioxidant vitamin supplementation with vitamin C and E in diabetic and pre-eclamptic women. As a result of these previous studies, we designed the EViCE (Effectiveness of Vitamin C and E in alcohol exposed pregnancies) study to examine the effectiveness of mega-dosing of Vitamin C and E in mitigating the effects of ethanol in alcohol exposed women. This was a randomized control study initiated in the fall of 2004. In the study, women with alcohol-exposed pregnancies were randomized into three groups: vitamin, placebo, and counseling only. Women assigned to the vitamin group received a 1000mg vitamin C, 400IU vitamin E, and a prenatal multivitamin containing folic acid. Women assigned to the placebo group received two placebos and a prenatal multivitamin containing folic acid. Women assigned to the counseling group did not receive any vitamin therapy but were advised to take prenatal multivitamins containing folic acid. The rationale for including a counseling group was to examine the effect of usual care.

As part of the ethicality of any human study, it is critical to follow up the world experience to see whether new knowledge may affect the conduct of the trial. The turning point of the EViCE trial arose with the publication of the previous FASD trials using vitamin C and E. More important, however, was the randomized control trial published by Poston et al. where women randomized to receive antioxidants delivered more low birth weight babies compared to the placebo arm. The study by Poston et al. investigated the effects of 1000mg vitamin C and 400IU RRR-vitamin E. Of the 2,395 patients analyzed, despite similar incidences of pre-eclampsia (RR: 0.97 [95% CI 0.80-1.17]), babies born to women who took antioxidants had lower birth weight (RR: 1.15 [96% CI 1.02-1.30]). This difference could not be accounted for by gestational age. These findings have resulted in
Mega-dose vitamin C and E in preventing FASD: the decision to terminate the study prematurely

major changes in the research involving pregnant women receiving mega-doses for vitamin C and E and led us to assess the viability of our trial. In August 2006, a safety committee meeting was convened to review the justification of continuing the trial. The committee was comprised of the study investigator as an observer, neonatologist, toxicologist, and two obstetricians, one of whom coordinated a vitamin C and E study for pre-eclampsia. Data regarding birth outcomes of the EVICE study were presented to the safety committee. There were no cases of low birth weight babies observed in our trial. The committee reviewed the decision on a Toronto-based vitamin C and vitamin E study in pre-eclampsia which was also terminated. The committee concluded that recruitment into the trial should be discontinued and that the ongoing participants be followed to the designated study end points.

A previous review of vitamin C supplementation during pregnancy did not reveal any adverse fetal effects. Similarly a review of vitamin E supplementation did not report any adverse effects. However, a recent prospective observational study conducted by our group in pregnant women supplementing with mega-doses of vitamin E, detected an apparent decrease in mean birth weight that could not be explained by other variables including maternal age, gestational age, and smoking. The safety committee concluded that the principle of equipoise was violated with the results of a new randomized study showing vitamin C and vitamin E to be associated with a clinically-significant intraventricular growth retardation in pre-eclampsia.

We subsequently encountered a previous trial in pregnancy that was suspended prior to its completion—a placebo-controlled trial of women receiving nicotine patches. One mother reported excess symptoms associated with withdrawal and excess fetal movements when she used her study medication. It was revealed that she had been randomly assigned to placebo, irrevocably exposing the fetus to the risk of nicotine withdrawal.

Our study is being renewed with a different design omitting the placebo arm and adding a dose-escalating strategy. Together these trials demonstrate the importance of continuous monitoring of studies for unseen adverse effects, especially in a population as vulnerable as pregnant alcoholic women.

Corresponding Author: ggobin@rickhills.ca

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Mega-dose vitamin C and E in preventing FASD: the decision to terminate the study prematurely


Prenatal Multivitamin Supplementation and Rates of Congenital Anomalies: A Meta-Analysis

Y. Ingrid Goh, HBSc,1,2 Enkelejd Bollano, MD,3 Thomas R. Elmarson, PhD,1,2 Gideon Koren, MD,4,5

1Department of Pharmaceutical Sciences, University of Toronto, Toronto ON
2The Motherisk Program, Division of Clinical Pharmacology/Toxicology, The Hospital for Sick Children, Toronto ON
3Faculty of Medicine, University of Toronto, Toronto ON
4Ivey Chair in Molecular Toxicology, University of Western Ontario, London ON
5Department of Medicine, University of Western Ontario, London ON

Abstract

Background: The use of folic acid fortified multivitamin supplements has long been associated with decreasing the risk of neural tube defects. Several studies have also proposed the effectiveness of these supplements in preventing other birth defects; however, such effects have never been systematically examined.

Objective: We conducted a systematic review and meta-analysis to evaluate the protective effect of folic acid fortified multivitamin supplements on other congenital anomalies.

Methods: We searched Medline, PubMed, EMBASE, Toxicline, Healthstar, and Cochrane databases for studies describing the outcome of pregnancies in women using multivitamin supplements that were published in all languages from January 1985 to July 2005. The references from all collected articles were reviewed for additional articles. Two independent reviewers who were blinded to the source and identity of the articles extracted data based on predetermined inclusion and exclusion criteria. Using a random effects model, rates of congenital anomalies in babies born to women who were taking multivitamin supplements were compared with rates in the offspring of controls who were not.

Results: From the initial search, 92 studies were identified; 41 of these met the inclusion criteria. Use of multivitamin supplements provided consistent protection against neural tube defects (random effects odds ratio [OR] 0.87, 95% confidence interval [CI] 0.75–0.99 in cohort and randomized controlled studies), cardiovascular defects (OR 0.78, 95% CI 0.67–0.92 in case control studies; OR 0.81, 95% CI 0.40–1.72 in cohort and randomized controlled studies), and limb defects (OR 0.45, 95% CI 0.30–0.76 in case control studies; OR 0.57, 95% CI 0.39–0.86 in cohort and randomized controlled studies). For certain malformations, case control studies showed OR 0.76 (95% CI 0.20–2.90); and cohort and randomized controlled studies showed OR 0.42 (95% CI 0.09–1.84), for oral cleft with or without cleft palate, case control studies showed OR 0.85 (95% CI 0.54–1.33). For oral cleft deformities, case control studies showed OR 0.49 (95% CI 0.30–0.76), and cohort and randomized controlled studies showed OR 0.68 (95% CI 0.35–1.31), and for congenital hydrocephalus case control studies showed OR 0.37 (95% CI 0.24–0.59), and cohort and randomized controlled studies showed OR 1.54 (95% CI 0.53–4.60). No effects were shown in preventing Down syndrome, pyloric stenosis, undescended testes, or hypospadias.

Conclusion: Maternal consumption of folic acid-containing prenatal multivitamins is associated with decreased risk for several congenital anomalies, not only neural tube defects. These data have major public health implications, because until now fortification of only folic acid has been encouraged. This approach should be reconsidered.

Résumé

Contexte : L'utilisation de suppléments multivitaminiques enrichis à l’acide folique est depuis longtemps associée à une baisse du risque d’anomalies du tube neural. Plusieurs études ont également proposé l’efficacité de ces suppléments pour la prévention d’autres anomalies congénitales, cependant, ces effets n’ont jamais fait l’objet d’un examen systématisé.

Objectif : Nous avons mené un examen et une méta-analyse systématiques afin d’évaluer l’effet protecteur des suppléments multivitaminiques enrichis à l’acide folique sur d’autres anomalies congénitales.


Résultats : La recherche initiale a permis l’identification de 92 études ; 41 d’entre elles ont satisfait aux critères d’inclusion. L’utilisation de suppléments multivitaminiques a offert une protection uniforme contre les anomalies du tube neural (rapport [IC] à 95 %, 0.50–0.77), dans les études cas-témoins ; RC à 95 %, 0.30–0.69, dans les études de cohorte et les essais...
comparatifs randomisés, les anomalies cardiovasculaires (RC: 0.78, IC à 95 %: 0.67–0.92, dans les études cas-témoins; RC: 0.81, IC à 95 %: 0.46–1.52, dans les études de cohortes et les essais comparatifs randomisés) et les anomalies affectant les membres (RC: 0.48, IC à 95 %: 0.30–0.76, dans les études cas-témoins; RC: 0.51, IC à 95 %: 0.39–0.95, dans les études de cohorte et les essais comparatifs randomisés). Dans le cas de la ferme patellaire, les études cas-témoins ont indiqué un RC de 0.76 (IC à 95 %: 0.62–0.93) et les études de cohorte et les essais comparatifs randomisés ont indiqué un RC de 0.42 (IC à 95 %: 0.06–2.94). Dans le cas de la ferme orbitale avec ou sans ferme patellaire, les études cas-témoins ont indiqué un RC de 3.63 (IC à 95 %: 0.54–27.3) et les études de cohorte et les essais comparatifs randomisés ont indiqué un RC de 0.58 (IC à 95 %: 0.29–1.19). Dans le cas des anomalies du tractus urinaire, les études cas-témoins ont indiqué un RC de 0.48 (IC à 95 %: 0.30–0.76) et les études de cohorte et les essais comparatifs randomisés ont indiqué un RC de 0.68 (IC à 95 %: 0.35–1.31) et dans le cas de l’hydrochalasie congénitale, les études cas-témoins ont indiqué un RC de 0.37 (IC à 95 %: 0.24–0.56) et les études de cohorte et les essais comparatifs randomisés ont indiqué un RC de 1.54 (IC à 95 %: 0.53–4.53). Aucun effet préventif n’a été constaté en ce qui concerne le syndrome de Down, la sténose du pylore, la cryptorchidie ou l’hypoglycémie.

Conclusion : La consommation de multivitaminés prénatales contenant de l’acide folique par la mère est associée à une baisse non seulement du nombre d’anomalies du tube nerveux, mais également de celui d’anomalies congénitales graves. Ces données entraînent d’importantes conséquences en matière de santé publique publique, jusqu’à présent, seule la fortification de l’acide folique a été favorisée. Cette approche devrait être examinée en question.


INTRODUCTION

One in 33 children born in Canada and the United States has a birth defect.1,2 In 2005, it was estimated that about 150,000 babies are born in North America each year with a birth defect.3 The burden of illness and the economic cost of birth defects are extremely high.4 More than a decade ago, the preventative role of maternal folic acid supplementation on the occurrence and the recurrence of neural tube defects was documented in several studies.5,6 Subsequently, preconceptional fortification with folic acid has been shown to reduce the rates of neural tube defects in North America.5

During the last decade, several studies have suggested that folic acid-fortified multivitamins may also prevent other congenital anomalies.5–8 Botto et al. suggested, on the basis of several studies, that there was a decreased risk for otoacoustic emissions, limb deficiencies, and cardiovascular abnormalities in babies whose mothers received multivitamin supplementation.9 To date, however, no systematic review has been conducted to examine existing evidence for the potential of folic acid-containing multivitamins to decrease the risk of congenital anomalies other than neural tube defects.

The objective of the present study was to conduct a meta-analysis of studies comparing rates of congenital malformation among women taking vitamin supplements with the rates in controls.

METHODS

We conducted a search of existing studies that focused on pre- and periconceptual maternal ingestion of multivitamins and the rates of malformation in the offspring. The outcome of interest was congenital malformations. All original research articles using randomized controlled trial, case control, or cohort studies were included. All selected articles contained reports of maternal intake of multivitamins during pregnancy, a control group, and raw data describing rates of healthy and malformed children. Articles that did not report usage of multivitamins during pregnancy, articles that focused on specific vitamins, articles describing mothers exposed to other known teratogens, review articles, letters to the editor, and data reports from abstracts or meetings were excluded.

Articles were searched using the terms “multivitamin,” “pregnancy,” and “malformation” in Medline (January 1966–July 2005), PubMed (1950–July 2005), EMBASE (January 1980–July 2005), TOXLINE (January 1960–July 2005), Healthstar (January 1966–July 2005), and Cochrane database in all languages. The references from all collected articles were reviewed to locate other original studies.

Two reviewers blinded to authors’ names, institution, and journal title assessed all of the articles collected using the selection criteria described above. Data were extracted from these articles to collection forms in 2 × 2 tables. In cases of discrepancy between the reviewers that were not resolved by discussion, the article in question was reviewed by a third blinded reviewer. The odds ratios and 95% confidence intervals were calculated for each study using Review Manager 4.2.7 (2004, The Cochrane Collaboration). Homogeneity among effects was tested by calculating chi-square.

RESULTS

Ninety-two articles were compiled from initial searches of the databases and reference review. Forty-one studies were eligible for the meta-analysis based on the inclusion and exclusion criteria. 1,2,5,9 There were 27 case control studies, four randomized control trials, and 10 cohort studies. Fifty-one articles were excluded because they did not report malformation rates, focused specifically on folic acid, did not contain a control group, were review articles, or contained data that were identical to previous studies by the same authors. The use of multivitamin supplementation by the mothers from before the time of conception was associated with a consistent protective effect against neural tube defects (odds ratio [OR] 0.67, 95% confidence interval [CI] 0.58–0.77 in case control studies; OR 0.52, 95% CI 0.39–0.69.
Appendix F

**Figure 1. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of NTD in their children (case control studies)**

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (random) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 1982</td>
<td>1.80 (0.69, 9.62)</td>
<td></td>
</tr>
<tr>
<td>History 1986</td>
<td>0.70 (0.27, 0.91)</td>
<td></td>
</tr>
<tr>
<td>MBM 1993</td>
<td>0.31 (0.60, 1.25)</td>
<td></td>
</tr>
<tr>
<td>Maternal 1996</td>
<td>0.40 (0.25, 0.64)</td>
<td></td>
</tr>
<tr>
<td>Shenv 1998</td>
<td>1.16 (0.47, 2.87)</td>
<td></td>
</tr>
<tr>
<td>Shtrim 2000</td>
<td>0.32 (0.10, 1.01)</td>
<td></td>
</tr>
<tr>
<td>Yerden 1992</td>
<td>0.50 (0.13, 1.79)</td>
<td></td>
</tr>
<tr>
<td>Total (89%) CI</td>
<td>0.67 (0.49, 0.93)</td>
<td></td>
</tr>
<tr>
<td>Total events: 527 (Treatment), 149 (Control)</td>
<td>Test for heterogeneity: X^2 = 12.91, df = 7 (P = 0.030), I^2 = 63.7%</td>
<td></td>
</tr>
</tbody>
</table>

NTD: neural tube defects; OR: odds ratio; CI: confidence interval.

**Figure 2. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of NTD in their children (cohort and RCT studies)**

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (random) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1984</td>
<td>0.07 (0.01, 0.61)</td>
<td></td>
</tr>
<tr>
<td>Cohort 2014</td>
<td>0.30 (0.13, 0.67)</td>
<td></td>
</tr>
<tr>
<td>IEFP 2003</td>
<td>0.56 (0.24, 1.31)</td>
<td></td>
</tr>
<tr>
<td>MCP 1986</td>
<td>0.21 (0.10, 0.44)</td>
<td></td>
</tr>
<tr>
<td>Shriver 1996</td>
<td>0.49 (0.24, 1.03)</td>
<td></td>
</tr>
<tr>
<td>Smith 1996</td>
<td>0.14 (0.04, 0.52)</td>
<td></td>
</tr>
<tr>
<td>Shechter 1981</td>
<td>0.50 (0.18, 1.39)</td>
<td></td>
</tr>
<tr>
<td>Shriver 1994</td>
<td>0.57 (0.29, 1.11)</td>
<td></td>
</tr>
<tr>
<td>Total (90%) CI</td>
<td>0.57 (0.39, 0.90)</td>
<td></td>
</tr>
</tbody>
</table>

NTD: neural tube defects; RCT: randomized controlled trial; OR: odds ratio; CI: confidence interval.

**Figure 3. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of cleft palate in their children (case control studies)**

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (random) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 1982</td>
<td>1.74 (0.48, 2.20)</td>
<td></td>
</tr>
<tr>
<td>Cohort 1998</td>
<td>0.36 (0.15, 0.83)</td>
<td></td>
</tr>
<tr>
<td>Cohort 2001</td>
<td>0.20 (0.04, 0.93)</td>
<td></td>
</tr>
<tr>
<td>Merid 1998</td>
<td>0.31 (0.60, 1.22)</td>
<td></td>
</tr>
<tr>
<td>Mulan 2007</td>
<td>0.24 (0.44, 1.08)</td>
<td></td>
</tr>
<tr>
<td>Lander 2001</td>
<td>0.70 (0.41, 1.11)</td>
<td></td>
</tr>
<tr>
<td>Lander 2002-3</td>
<td>0.50 (0.31, 0.79)</td>
<td></td>
</tr>
<tr>
<td>Shriver 1982</td>
<td>0.67 (0.40, 1.13)</td>
<td></td>
</tr>
<tr>
<td>Weaver 1998</td>
<td></td>
<td>0.62 (0.27, 1.43)</td>
</tr>
<tr>
<td>Total (90%) CI</td>
<td>0.76 (0.62, 0.92)</td>
<td></td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval.
Figure 4. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of cleft palate in their children (cohort and RCT studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeckel 1995a</td>
<td>0.19 (0.04, 0.40)</td>
<td></td>
</tr>
<tr>
<td>Czeckel 2004</td>
<td>1.00 (0.66, 1.50)</td>
<td></td>
</tr>
<tr>
<td><strong>Total (35%) CI</strong></td>
<td><strong>0.42 (0.06, 2.64)</strong></td>
<td></td>
</tr>
<tr>
<td>Total events: 1 (Treatment), 3 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi² = 0.03, df = 1 (P = 0.90), r² = 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 0.09 (P = 0.37)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RCT: randomized controlled trial; OR: odds ratio; CI: confidence interval.

Figure 5. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of cleft lip with or without palate in their children (case control studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duggal 1979</td>
<td>0.23 (0.09, 0.55)</td>
<td></td>
</tr>
<tr>
<td>Czeckel 1993a</td>
<td>1.69 (0.42, 6.79)</td>
<td></td>
</tr>
<tr>
<td>Czeckel 2004</td>
<td>1.75 (1.25, 2.47)</td>
<td></td>
</tr>
<tr>
<td>Hayman 1993</td>
<td>0.39 (0.07, 1.93)</td>
<td></td>
</tr>
<tr>
<td>Italy 2001</td>
<td>0.73 (0.46, 1.17)</td>
<td></td>
</tr>
<tr>
<td>Larsson 2004-1</td>
<td>0.39 (0.10, 1.53)</td>
<td></td>
</tr>
<tr>
<td>Larsson 2004-2</td>
<td>0.09 (0.01, 0.62)</td>
<td></td>
</tr>
<tr>
<td>Larsson 2004-3</td>
<td>0.34 (0.12, 0.99)</td>
<td></td>
</tr>
<tr>
<td>Godfrey 2003</td>
<td>0.56 (0.41, 0.81)</td>
<td></td>
</tr>
<tr>
<td>Wirkow 2003</td>
<td>0.63 (0.41, 0.94)</td>
<td></td>
</tr>
<tr>
<td><strong>Total (35%) CI</strong></td>
<td><strong>0.69 (0.04, 1.32)</strong></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi² = 7.77, df = 1 (P = 0.05), r² = 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 5.34 (P = 0.0001)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval.

Figure 6. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of cleft lip with or without palate in their children (cohort and RCT studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conway 1958</td>
<td>0.39 (0.02, 0.82)</td>
<td></td>
</tr>
<tr>
<td>Czeckel 1993a</td>
<td>1.29 (0.29, 5.77)</td>
<td></td>
</tr>
<tr>
<td>Czeckel 2004</td>
<td>1.50 (0.25, 8.99)</td>
<td></td>
</tr>
<tr>
<td>Tolosa 1995</td>
<td>0.34 (0.11, 1.08)</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95%) CI</strong></td>
<td><strong>0.58 (0.28, 1.19)</strong></td>
<td></td>
</tr>
<tr>
<td>Total events: 10 (Treatment), 64 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi² = 3.09, df = 3 (P = 0.30), r² = 3.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 1.48 (P = 0.14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RCT: randomized controlled trial; OR: odds ratio; CI: confidence interval.
OBSTETRICS

Figure 7. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of urinary tract anomalies in their children (case control studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li 1995</td>
<td>0.98 (0.63, 1.51)</td>
<td>0.86 (0.31, 0.90)</td>
</tr>
<tr>
<td>Werler 1999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>0.48 (0.30, 0.75)</td>
</tr>
<tr>
<td>Total events: 88 (Treatment), 47 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chisq = 4.74, df = 2 (P = 0.095), I² = 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 3.10 (P = 0.002)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0.1 0.2 0.5 1 2 5 10
Favours treatment Favours control

Figure 8. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of urinary tract anomalies in their children (cohort and RCT studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeizel 1996a</td>
<td>0.92 (0.62, 1.37)</td>
<td>0.74 (0.37, 1.47)</td>
</tr>
<tr>
<td>Czeizel 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>0.69 (0.35, 1.39)</td>
</tr>
<tr>
<td>Total events: 15 (Treatment), 22 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chisq = 4.74, df = 3 (P = 0.095), I² = 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 3.10 (P = 0.002)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0.1 0.2 0.5 1 2 5 10
Favours treatment Favours control

Figure 9. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of cardiovascular defects in their children (case control studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Botto 2000</td>
<td>0.81 (0.64, 1.03)</td>
<td></td>
</tr>
<tr>
<td>Correa 2003</td>
<td>0.95 (0.63, 1.45)</td>
<td></td>
</tr>
<tr>
<td>Scantlon 1998</td>
<td>0.79 (0.47, 1.34)</td>
<td></td>
</tr>
<tr>
<td>Sher 1995</td>
<td>0.66 (0.35, 1.26)</td>
<td></td>
</tr>
<tr>
<td>Werler 1999</td>
<td>1.08 (0.67, 1.74)</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>0.78 (0.57, 1.08)</td>
</tr>
<tr>
<td>Total events: 893 (Treatment), 643 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chisq = 6.31, df = 2 (P = 0.044), I² = 34%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 3.07 (P = 0.002)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0.1 0.2 0.5 1 2 5 10
Favours treatment Favours control

OR: odds ratio; CI: confidence interval.
Appendix F 535

Figure 10. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of cardiovascular defects in their children (cohort and RCT studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeizel 1996a</td>
<td>0.58 [0.21, 1.60]</td>
<td></td>
</tr>
<tr>
<td>Czeizel 2004</td>
<td>0.62 [0.39, 0.97]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.61 [0.40, 0.92]</td>
<td></td>
</tr>
<tr>
<td>Total events: 37 (Treatment), 60 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: χ² = 0.01, df = 1 (P = 0.91), I² = 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 2.35 (P = 0.02)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Favours treatment Favours control

RCT: randomized controlled trial; OR: odds ratio; CI: confidence interval.

Figure 11. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of limb defects in their children (case control studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li 1995</td>
<td>0.39 [0.15, 0.64]</td>
<td></td>
</tr>
<tr>
<td>Weiler 1999</td>
<td>0.53 [0.31, 0.90]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.48 [0.30, 0.76]</td>
<td></td>
</tr>
<tr>
<td>Total events: 85 (Treatment), 47 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: χ² = 0.74, df = 1 (P = 0.39), I² = 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 3.16 (P = 0.002)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Favours treatment Favours control

OR: odds ratio; CI: confidence interval.

Figure 12. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of limb defects in their children (cohort and RCT studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shaw 1995c</td>
<td>0.64 [0.42, 0.99]</td>
<td></td>
</tr>
<tr>
<td>Weiler 1999</td>
<td>0.23 [0.07, 0.75]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.57 [0.38, 0.85]</td>
<td></td>
</tr>
<tr>
<td>Total events: 101 (Treatment), 53 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: χ² = 2.41, df = 1 (P = 0.12), I² = 50.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 2.73 (P = 0.006)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Favours treatment Favours control

RCT: randomized controlled trial; OR: odds ratio; CI: confidence interval.
Appendix F

Figure 13. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of congenital hydrocephalus in their children (case control studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correa 2003</td>
<td>0.94 [0.21, 0.54]</td>
<td></td>
</tr>
<tr>
<td>Yerlei 1999</td>
<td>0.59 [0.21, 1.65]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.37 [0.24, 0.56]</td>
<td></td>
</tr>
<tr>
<td>Total events: 30 (Treatment), 178 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi² = 0.90, df = 1 (P = 0.34), I² = 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 4.66 (P &lt; 0.00001)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval.

Figure 14. Maternal multivitamin consumption before and in first trimester of pregnancy and risk of congenital hydrocephalus in their children (cohort and RCT studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czeliez 1995a</td>
<td>0.32 [0.04, 2.92]</td>
<td></td>
</tr>
<tr>
<td>Czeliez 2004</td>
<td>2.00 [0.60, 6.66]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>1.54 [0.53, 4.80]</td>
<td></td>
</tr>
<tr>
<td>Total events: 8 (Treatment), 5 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi² = 1.16, df = 1 (P = 0.29), I² = 9.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 0.73 (P = 0.43)</td>
<td></td>
<td></td>
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</tbody>
</table>

RCT: randomized controlled trial; OR: odds ratio; CI: confidence interval.

Figure 15. Maternal multivitamin consumption in first trimester of pregnancy and risk of NTD in their children (case control studies)

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>OR (fixed) 95% CI</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowser 1992</td>
<td>1.23 [0.78, 1.94]</td>
<td></td>
</tr>
<tr>
<td>Khoury 1996</td>
<td>0.91 [0.77, 1.07]</td>
<td></td>
</tr>
<tr>
<td>Mulhern 1996</td>
<td>0.70 [0.47, 1.04]</td>
<td></td>
</tr>
<tr>
<td>Shaw 1996a</td>
<td>0.54 [0.42, 0.71]</td>
<td></td>
</tr>
<tr>
<td>Shaw 1997</td>
<td>1.09 [0.67, 1.91]</td>
<td></td>
</tr>
<tr>
<td>Yerlei 1993</td>
<td>0.73 [0.57, 0.93]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.80 [0.72, 0.89]</td>
<td></td>
</tr>
<tr>
<td>Total events: 871 (Treatment), 1212 (Control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: Chi² = 16.00, df = 5 (P = 0.000), I² = 69.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 4.01 (P &lt; 0.0001)</td>
<td></td>
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</tr>
</tbody>
</table>

NTD: neural tube defects; OR: odds ratio; CI: confidence interval.
Appendix F

Prenatal Multivitamin Supplementation and Rates of Congenital Anomalies: A Meta-Analysis

in cohort and randomized controlled studies), cardiovascular defects (OR 0.78, 95% CI 0.67–0.92 in case control studies; OR 0.64, 95% CI 0.48–0.92 in cohort and randomized controlled studies), and limb defects (OR 0.48, 95% CI 0.30–0.76 in case control studies; OR 0.57, 95% CI 0.38–0.85 in cohort and randomized controlled studies).

Multivitamin supplementation beginning before pregnancy showed a less consistent protective effect against cleft palate (OR 0.76, 95% CI 0.62–0.93 in case control studies; OR 0.42, 95% CI 0.06–2.84 in cohort and randomized controlled studies), oral cleft with or without cleft palate (OR 0.63, 95% CI 0.54–0.73 in case control studies; OR 0.58, 95% CI 0.28–1.19 in cohort and randomized controlled studies), urinary tract anomalies (OR 0.48, 95% CI 0.30–0.76 in case control studies; OR 0.68, 95% CI 0.35–1.31 in cohort and randomized controlled studies), and congenital hydrocephalus (OR 0.37, 95% CI 0.24–0.56 in case control studies; OR 1.54, 95% CI 0.53–4.50 in cohort and randomized controlled studies) (Figures 1–14).

In addition, women who began supplementation in the first trimester after learning of the pregnancy showed a protective effect for neural tube defects (OR 0.80, 95% CI 0.72–0.89) (Figure 15). There was no heterogeneity among the studies.

In contrast, multivitamin supplementation was not associated with a protective effect for Down syndrome (OR 0.56, 95% CI 0.26–1.19 in cohort and randomized controlled studies), congenital pyloric stenosis (OR 1.10, 95% CI 0.79–1.53 in case control studies; OR 0.20, 95% CI 0.02–1.68 in cohort and randomized controlled studies), undescended testis (OR 0.81, 95% CI 0.40–1.64 in cohort studies), and hypospadias (OR 0.44, 95% CI 0.13–1.43 in cohort and randomized controlled studies).

**DISCUSSION**

The present meta-analysis confirms initial impressions that the use of multivitamins fortified with folic acid by women before conception and continuing through the first trimester is associated with a decrease in several serious major malformations. To our knowledge, this is the first systematic review and meta-analysis to examine and document these protective effects.

The majority of the studies included in the meta-analysis were case control studies, although there were also several randomized controlled trials and cohort studies. Not surprisingly, case control studies are more sensitive in showing significant effects for preventing specific malformations than cohort or randomized studies. The observation that all the studies for the majority of endpoints were statistically homogenous lends credibility to the documented effects. There was heterogeneity in the case control studies and cohort studies examining neural tube defects (8 and 11 studies, respectively). Exclusion of one small case control study (Bower and Stanley9) and one small cohort study (Sheppard et al.4), each of which showed no protective effect, renders the results homogeneous without changing the overall effect size. Moreover, we could not detect a publication bias by employing the funnel plot. Our study, however, is limited by the fact that multivitamin supplements in differing studies may have varied in their composition.

Presently, there are widely publicized recommendations by various authorities for women to supplement with folate at daily doses of at least 0.4 mg (4 mg for women at higher risk) to reduce the risk of delivering a child with neural tube defects. In many centres women are advised to begin taking prenatal vitamin supplements when they decide to attempt to conceive, merely to allow them sufficient folate supplementation. It is currently impossible to discern whether folic acid or other vitamins are critical in the prevention of other birth defects.

Only a fraction of women currently take prenatal vitamin supplements at the time of conception, partly because one half of all pregnancies are unplanned. Serious consideration should be given to fortification of flour or other food staples with other vitamins in addition to folate. With increased surveillance of changes in malformation rates as a result of folate fortification, and subsequently larger cohorts, it will be possible to determine whether folate fortification itself is capable of protecting against birth defects other than neural tube defects.

**CONCLUSION**

The results of the present meta-analysis support the use of prenatal multivitamin preparations containing folic acid to reduce the incidence of several congenital anomalies, including neural tube defects, cardiovascular anomalies, oral cleft, urinary tract anomalies, congenital hydrocephalus, and limb defects. Randomized trials will be necessary to prove which specific vitamin(s) renders protective effects.

**ACKNOWLEDGEMENTS**

Supported by grants from the Canadian Institutes of Health Research and the Research Leadership for Better Pharmacotherapy During Pregnancy and Lactation.

**REFERENCES**

Appendix F

539

Prenatal Multivitamin Supplementation and Rates of Congenital Anomalies: A Meta-Analysis


Appendix F

SCIENTIFIC NEWS

FASTRAC: THE RIGHT TRACK BUT WRONG ENGINE: A CRITICAL REVIEW OF THE FETAL ALCOHOL TEACHING AND RESEARCH AWARENESS CAMPAIGN

Y. Ingrid Goh
Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Department of Pharmaceutical Sciences, University of Toronto, Canada

A CRITICAL REVIEW of “The Effectiveness of a Multimedia Program to Prevent Fetal Alcohol Syndrome” LaChausse RG. Health Promot Pract 2006; 7(6):1-3.

Affecting 1 in 100 children, Fetal Alcohol Spectrum Disorder (FASD) can be prevented by the abstinence of alcohol by pregnant women. As many pregnancies are unplanned, it is important to educate women of reproductive age regarding the consequences of alcohol consumption during pregnancy. A proposed method of targeting women of reproductive age is by educating in schools.

LaChausse reports on findings from the Fetal Alcohol Spectrum Teaching and Research Awareness Campaign (FASTRAC). This peer-delivered educational program was administered to 114 students in a Southern California high school from grades 9-12. Teen peer-educators were trained to present a 35-slide PowerPoint presentation that was based on an educational module for resident physicians. The content of the presentation included the history of FASD, information on Fetal Alcohol Syndrome (FAS) and other Fetal Alcohol Effects, identification of alcohol as a teratogen, explaining how it causes fetal damage including brain development, specific characteristics of FAS, results from animal experiments, the role of partner’s alcohol use during pregnancy, and information on the social and cost impact.

The objective of the study was fourfold: to determine whether FASTRAC was effective in increasing knowledge regarding FAS, whether FASTRAC could decrease trends toward alcohol consumption during pregnancy, whether the program could increase the perceived severity of alcohol consumption during pregnancy, and to determine whether FASTRAC could increase the intention not to drink during pregnancy. Parents were provided with informed consent forms and students provided verbal assent to participate in the study. Students were administered the questionnaire one week prior and two weeks following the peer-education. The questionnaire contained questions adapted from the FAS Knowledge Attitudes Beliefs and Behaviors from Alaska,3 Intention to abstain from alcohol and drug use, and two additional questions: how do you feel about another student your age drinking alcohol while she is pregnant and how severe do you think it might be for your baby if you use alcohol during pregnancy? Students were divided into intervention and comparison groups.

$9 male and $9 female students completed the study. Although the students were ethnically diverse, the majority (69%) were of Latino descent. Increase of knowledge was observed in both groups. However, there was no observed change in attitudes towards drinking during pregnancy, likelihood to drink during pregnancy, and the perceived severity of alcohol’s effect during pregnancy.

LaChausse reports on an interesting concept of educating students regarding FAS. Previous studies have investigated other in-school interventions for topics such as smoking, drugs and health in classroom environments. LaChausse noted that peer-led interventions resulted in better results than teacher-led interventions. Although this may be true in some conditions, the peer person may have some influence on the outcomes themselves. If it is a peer member who is younger than the teacher but foreign to students, perhaps students may relate better because of the younger age. However, because they are strangers, they may not be as influential. On the other hand, if the peer member is a student from their own class, that peer’s popularity and influence may affect the outcome.

In a similar way, if the student is socially accepted perhaps the message would be better conveyed than from a student who is socially outcast.
Previous studies on peer-educators have suggested that they can be more effective than teacher-led education, however this may vary on the age of the audience. Another limitation that arises in the peer-education methodology is that the knowledge that is imparted may not be standardized. Yet, since peer-educators are using standardized PowerPoint presentations, there are fewer chances of variation.

The author noted that although the intervention increased knowledge of FAS, it did not change attitudes towards drinking during pregnancy. However, if the students' initial attitudes towards drinking during pregnancy were not generally acceptable, it would make sense that there was no change. The author also stated that participants were not less likely to use alcohol during pregnancy than when first questioned and that they did not perceive an increased severity of alcohol effect during pregnancy. Although the study reported that there was a change in the initial change in knowledge this could have been confounded as students from the intervention group could have passed on information to students who were in the comparison group.

The Ontario Student Drug Use Survey reported that in grade 7 already 31.4% of students had experienced alcohol exposure. Perhaps targeting a grade 9.12 audience may be too late and the educational intervention may be more effective earlier.

The author stated that the presentation took 45 minutes. It is widely accepted that students’ attention span lasts only for approximately 20 minutes. Perhaps this format of presentation as well as the length of presentation resulted in poor knowledge translation. In addition, the slides that were presented were initially geared to the medical profession. High school students may not have the knowledge to follow such a presentation.

As such adapting a presentation that is more suitable to high school students may be more beneficial. Interaction with students may also promote learning.

A useful element that should be incorporated in such programs are alternatives to drinking. They should be provided with approaches that will support pregnant individuals to abstain from alcohol consumption, for example using mocktails as alternatives. In addition, one should discuss

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2. LaChausse RG. "The Effectiveness of a Multimedia Program to Prevent Fetal Alcohol Syndrome". Health Promot Pract 2006; 7(2):1-5.
Appendix F


DOES SPORADIC ALCOHOL CONSUMPTION AFFECT THE FETUS?
Y. Ingrid Goh, H.B.Sc., Division of Clinical Pharmacology and Toxicology,
The Hospital for Sick Children, Department of Pharmaceutical Sciences,
University of Toronto, Canada


Alcohol consumption by the pregnant woman can result in negative effects in the unborn child. This is known as Fetal Alcohol Spectrum Disorder (FASD), which includes the full-blown Fetal Alcohol Syndrome (FAS). These effects can manifest both physically and mentally in the child and have major impact on quality of life. 2 Given the paucity of information regarding the exact quantity of alcohol consumption that can result in these detrimental effects, the Surgeon General of the United States as well as Health Canada advise women to refrain from consuming any alcohol-containing beverages during pregnancy. 3,4

While problem drinking is associated with adverse fetal outcome, the threshold of this phenomenon has not been established. In efforts to address this lack of knowledge, Martinez-Frias et al. conducted a case-control study aimed at examining the association of sporadic and daily consumption of alcohol. In this case control study, data from the Spanish Collaborative Study of Congenital Malformations (ECEMC) were used.

The ECEMC surveillance system facilitates the performance of case-control studies. This unique system comprises of 86 hospitals in Spain that collect case and control infants. Infants in these facilities with major or minor congenital defects are diagnosed in the first three days of life. The next gender-matched non-malformed infant in the same facility serves as the control for each case. Test results of the infants as well as information regarding maternal exposures are sent to a coordinating center in Madrid.

Infants were grouped into five categories based on their mother’s drinking reports. Level 1 consisted of mothers who reported drinking no more than one or two glasses of wine or beer (10-20 gm absolute alcohol) sporadically during gestation. Level 2 consisted of mothers who reported drinking several glasses of wine and/or beer coupled with distilled spirits (at least 90 gm absolute alcohol). Level 3 consisted of mothers who reported 250-500 mL of wine or 500-1000 mL of beer (16-48 gm) daily throughout the pregnancy. Level 4 consisted of mothers who reported drinking either 250-500 mL of wine or 500–1000 mL of beer, plus two glasses of distilled spirits, or 500–1000 mL of wine or 1000–2000 mL of beer, and no distilled spirits (56-88 gm). Level 5 consisted of mothers who reported drinking more than 500 mL of wine or more than 1000 mL of beer, plus several glasses of distilled spirits daily (greater than 92 gm absolute alcohol) and those who reported that they were alcoholic. The non-exposed group consisted of mothers who denied any drinking during their pregnancy.

The authors selected 11 groups of common effect associated with prenatal alcohol exposure to analyze: all types of central nervous system (CNS) defects; all types of eye anomalies; microphthalmia; facial anomalies; cardiovascular defects; oral clefts; genital defects; limb deficiencies; intestinal atresias; renal defects; and spina bifida defects. After excluding infants with chromosomal abnormalities and known teratogenic exposures, the odds ratios, 95% confidence interval and Fisher’s p-value were calculated.

From January 1977 to June 2001, ECEMC assessed 1,820,862 liveborn infants, of which 30,836 infants were diagnosed as having major
Appendix F

Does sporadic alcohol consumption affect the fetus?

and/or minor abnormalities. The matching control infants were collected. After excluding infants with chromosomal abnormalities and known exposure to other teratogens, 26,354 malformed and 25,836 control infants remained for analysis. 4,705 mothers of the malformed infants and 4,329 mothers of the control infants reported alcohol consumption. Women who were not able to specify the quantity and frequency of alcohol consumption were excluded.

The Level 1 group consisted of 1,737 cases and 1,633 controls. Prominent risks noted with 10-20g of alcohol exposure included eye anomalies OR=1.62 (95% CI=97-2.62; p=0.051). The odds ratio for microphthalmia was 2.50 (95% CI=0.87-7.52; p=0.06). The Level 2 group consisted of 101 cases and 101 controls. Prominent risks noted with a maximum of 90g alcohol and sporadic binge included limb deficiencies (excluding hypoplastic phalanges) OR=7.16 (95% CI=0.89-155.3; p=0.03). The risk of oral clefts was OR=3.49 (95% CI=0.67-24.29; p=0.097). The Level 3 group consisted of 2,267 cases and 2,141 controls. Prominent risks noted with 16-48 g of alcohol exposure included facial anomalies OR=1.15 (95% CI=1.17-2.06; p=0.001). The Level 4 groups consisted of 109 cases and 86 controls. Prominent risks noted with 56-88 g of alcohol exposure included eye anomalies OR=1.01 (95% CI=0.62-1.63; p=0.007). The Level 5 group consisted of 67 cases and 20 controls. Prominent risks noted with over 92 g alcohol exposure included hypoplastic nose, flat facial anomalies, central nervous system defects, eye anomalies, microcephaly, congenital heart defects, vascular anomalies, and oral clefts. The risk for microphthalmia and limb deficiencies was statistically significant. However, the sample size was too small to reach statistical significance for the other anomalies investigated.

In both Level 4 and Level 5, differences were observed in birth weight length, and occipital-frontal circumference (OFC). In addition increased anomalies were observed with increased alcohol consumption. In uncorrected infants, the mean birth weight was 3,174 g (SD=2.24; n=21,488); gestational age was 39.09 weeks (SD=2.24; n=20,339); birth length was 49.08 cm (SD=3.10; n=20,383); OFC was 34.01 cm (SD=1.97; n=21,793).

A difference was found in both birth length and OFC in the Level 4 group (47.15 cm (SD=4.07; n=13) and 32.93 cm (SD=2.52; n=14), respectively). Additional differences were noted in the Level 5 group. The mean birth weight was 2273.97 g (SD=669.30; n=67); mean birth length was 42.00 cm (SD=4.22; n=21); mean OFC was 30.57 cm (SD=2.46; n=21).

The authors concluded that even very low sporadic doses of alcohol during gestation may increase eye anomalies, and that higher doses would increase the risk of congenital defects associated with the timing of the exposure. This is one of the first epidemiological case-control studies investigating the association of congenital anomalies arising from prenatal alcohol exposure. Other authors conducting case-control studies have also reported a relationship between prenatal alcohol exposure and congenital defect.5,6 The association of facial anomalies with increased alcohol consumption supports the characteristics commonly associated with FAS.7 The results are also consistent with that of Shaw and Lammer 1999 who found an increased risk of cleft lip with or without cleft palate in women who ingested low quantities of alcohol during pregnancy.8 On the other hand, the findings of this study refute those of Lundeberg, et al. who reported no effect of small amounts of alcohol consumption during pregnancy.9

The main limitation of this study is that with low level drinking (Level 1), none of the associations was statistically significant, including the two "more likely", yet the authors interpret their results as if an association was proven. Moreover, it is quite possible that women who drink in pregnancy are very different from those choosing not to drink in important determinants of fetal health, and hence mild alcohol use may be marker rather than a cause.

A second limitation in the study is the fact that the data were based on self-reported alcohol consumption. Bias associated with retrospective self-reporting may be a major limitation as women may not want to be associated with a negative stigma.10 Because of the decision of the mother to under report or deny the use of alcohol...
Does sporadic alcohol consumption affect the fetus?

some infants classified as non-exposed, may in fact be prenatally exposed to alcohol. In cases where mothers had malformed infants, they are likely not to answer all questions in detail.1

A potential limitation is that there are 86 hospitals participating in this registry. In total, Spain has 747 hospitals.11 Participants in this registry may be biased toward tertiary health care centers or a skewed population and not accurately reflect that of Spain as a whole.

Another potential limitation is that pregnancies that are therapeutically aborted are not evaluated for congenital anomalies. Another matter to consider is that therapeutic abortions were illegal in Spain until 1985. As such, there may have been an increased rate of termination after that period, thus, skewing the overall results.

Other factors that may not be controlled include the use of other medications, the mother’s socioeconomic condition, and education level.

It is currently not known how much alcohol is required to cause adverse fetal effects. Although the American and Canadian guidelines advise women abstain from alcohol consumption during pregnancy, the British Royal College of Obstetricians and Gynaecologists states that it is recommended that women should be careful about alcohol consumption in pregnancy and limit this to no more than one standard drink (120g0) per day.11 The present study claims to show evidence that mild drinking is teratogenic, a claim not supported by the author’s data.

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Y. Ingrid Goh is funded by the CIHR FAS-NET grant.
THE ANTIOXIDANT EFFECT: CAN WE MITIGATE FETAL ALCOHOL SPECTRUM DISORDER WITH ANTIOXIDANTS?

Y. Ingrid Goh, HBSc., Joanne Royer, PhD., Wendy J. Unger, PhD., Gideon Koren MD.
The Hospital for Sick Children, Toronto, Ontario, Canada

It is estimated that 46% of women consume alcohol. Given that approximately half of pregnancies are unplanned there is a potential of 23% of babies being unknowingly exposed to alcohol. It is known that alcohol consumption during pregnancy can potentially result in a child having fetal alcohol spectrum disorder (FASD). In the full presentation, fetal alcohol syndrome (FAS) may be the clinical result. FAS alone affects 1-4 of 1000 live births. Children with FASD are less likely to be diagnosed than children with FAS because they rely on confirmation of the mother drinking in pregnancy and these children do not exhibit the pathognomonic facial changes.

The majority of studies on FASD have focused on the mechanism of damage. One such mechanism is oxidative stress which is a result in the production of reactive oxidative species that are generated by the metabolism of ethanol.

It is known that the primary prevention of FASD is avoiding alcohol consumption during pregnancy. This is usually hard for women who have unplanned pregnancy, and a still more challenging goal for women who are addicted to alcohol. To date there is no known treatment for women who have consumed alcohol during pregnancy to increase the chances for having a healthy child.

In vitro studies have shown that antioxidant treatment can attenuate ethanol-damaged neural cells. In pregnant diabetic women, antioxidants were shown to be beneficial by preventing pre-eclampsia. The above evidence has led us to design a trial to evaluate the effectiveness of antioxidants in mitigating fetal damage from alcohol exposure in pregnancy. We present this protocol with the hope it will facilitate similar research in other countries. As importantly, other centers may consider joining us in this protocol.

The primary objective of the study is to evaluate whether antioxidants in combination with prenatal multivitamin supplementation will impact the outcome of alcohol exposed pregnancies. The secondary objective is to evaluate the cost-effectiveness of implementing this treatment. It is hypothesized that together these treatments will be beneficial to improving the fetuses' health and will result in savings to the health care system and society.

The study is a randomized, three-arm, double-blinded, placebo-controlled trial. One hundred and eighty-nine women will be asked to participate in this trial where they will be randomized into one of three possible groups. The first group will receive study medication and prenatal multivitamins. The second group will receive placebo and prenatal multivitamins. The third group will not receive any medications but will be advised to obtain prenatal multivitamins containing at least 0.4mg of folic acid as recommended by Health Canada and the FDA. All groups will receive information and counseling from research staff and if need be, referred to specialists or other social services. All women participating will be advised to discontinue drinking alcohol.

Participants included in the trial must be 0-24 weeks pregnant with a TWEAK score of 3 or greater, a history of binge drinking (3 or more drinks) during pregnancy. Participants will be excluded if they have any co-morbid condition(s) that prevents them from providing meaningful consent. Participants are currently being enrolled into the study through the Motherisk Alcohol and Substance Use Helpline 1-877-337-4636. However, recruitment will be extended to hospitals, treatment programs for addicted women, hostels, shelters, food banks, and community centers.
The antioxidant effect can we mitigate fetal alcohol spectrum disorder with antioxidants?

Pregnant women will be prescreened and informed about the study. If they elect to participate an appointment will be set up either at The Hospital for Sick Children or in their homes. At the first visit, subjects will be asked to provide written informed consent to participate in the study. The medical and obstetrical history as well as social circumstances will be documented. In addition, participants will complete a series of questionnaires and be seen by a study physician for a physical assessment. Blood and urine will be collected to assess for any underlying maternal medical conditions and measure baseline antioxidant levels.

The participant's family physician will be notified of her participation in the study and will be contacted to verify medical history and to access results of tests pertaining to the pregnancy. In addition, results from the blood and urine tests will be sent to participant's doctors. Participants who do not have a family physician will be referred to a family physician by the study staff.

All participants will be asked to take a daily multivitamin and participants in the treatment arms will be asked to take one of each tablet daily. All subjects will receive harm reduction counseling throughout the study. Diaries will be provided to participants to monitor compliance, adverse events, nutrient intake and any problems that may be experienced through the study. The frequency of contact is required to keep the trial participants engaged. Participants randomized into the drug or placebo arm will be contacted at least two times a week. Participants randomized into the counseling arm will be contacted once every two weeks. During this contact, participants will be asked about their general health, pregnancy complications, adverse effects, drinking and drug use pattern and compliance to study.

Due to the vulnerability and high-risk nature of our patient population, measures have been built into the study to encourage retention and compliance with the study and its interventions. These may include the use of outreach workers, food vouchers, transportation assistance, home help and other strategies.

Women will return to the study clinic every two months to have a medical examination, return diaries and remaining study drug, complete questionnaires, give blood and urine samples and monitor compliance and medical status. Subjects will be issued new diaries and study drug at this time. This pattern will continue until the birth of the child.

Study staff will visit the mother within 24 hours of giving birth. At this time, each mother will be interviewed about the outcome of their pregnancy and any birth complications. The baby will be assessed by study physicians for any dysmorphologies and APGAR scores will be recorded. Meconium and hair will be collected from the baby and analyzed for fatty acid ethyl esters as a biomarker for prenatal ethanol exposure.

The child will be assessed at 1, 3, 6, and 14 months of life. Each visit will consist of a physical examination, a full-scale psychometric test, a health resource use, economic, resource utilization and time loss questionnaire.

Participants who complete the study will have the option of having their child followed yearly at the Motherisk clinic. This is important since the full adverse effects of in utero alcohol exposure may take years to unmask. Following children as they develop will enable close monitoring of learning, intelligence, behavioral change, and physical functions. Health care resource utilization will be measured as well as use of community and educational services. From this information, modeling of the impact of long-term cost and consequences will be performed.

To the best of our knowledge, this is the first study to examine the effects of drug treatment to attenuate the onset of FAS and ARND. If successful, it will have tremendous implications for the reduction of the most prevalent and preventable type of mental deficiency. Potential confounders in the study include, age, maternal illness and co-morbidities, other maternal exposures in pregnancy, nutrition, socio-economic status, and education. The randomized design aims to balance those potential confounders among the groups. In addition, although this is a double-blinded, placebo-controlled randomized trial, the third arm receiving only counseling will not be blinded. As such, there may be bias in some outcome measurements. The two treatment arms, however, will be blinded to the participants and to the investigators conducting the assessments. The results will therefore accurately reflect the potential of antioxidants to attenuate...
The antioxidant effect: can we mitigate fetal alcohol spectrum disorder with antioxidants?

alcohol-related problems in fetuses exposed in
utero.

Support
The study is supported by the FAS-NET CIHR grant and Pharmavite, Apotex Inc., and RU Communicating Inc.

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REFERENCES
Appendix F

SCIENTIFIC NEWS

KNOWLEDGE IS THE KEY TO PREVENTION: REDUCTION OF ALCOHOL-EXPOSED PREGNANCIES THROUGH MOTIVATIONAL INTERVENTION

Y. Ingrid Oah, B.Sc(Hons), The Hospital for Sick Children, Toronto, Ontario, Canada


Prenatal alcohol exposure during pregnancy is the leading known cause of mental retardation.2 It is estimated that up to 10 of 1,000 live births in industrialized countries are affected by Fetal Alcohol Syndrome (FAS) or Fetal Alcohol Spectrum Disorder (FASD).3 Consequences of alcohol-exposed pregnancies include physical and mental disabilities, leading to poor academic performance, legal and employment difficulties, thus resulting in a poor quality of life.4-6

Since the consumption of alcohol is a potentially preventable source of birth defects, it is essential to strive to educate women of childbearing age about preventive strategies. “Motivational intervention to reduce alcohol-exposed pregnancies-Florida, Texas, and Virginia 1997-2001” reviews the effectiveness of counseling sexually active women to decrease their alcohol intake and implementing effective contraception.7 This commentary evaluates a study conducted by the CHOICES intervention research group.7

Women of reproductive age who reported drinking at least seven drinks per week, or had at least one binge-drinking episode three months prior to recruitment, were sexually active and using ineffective contraception, were recruited into this single-arm study. Subjects were assessed on their demographics, alcohol and substance use history, AUDIT (Alcohol Use Disorders Identification Test) scores, and mental health treatment. Four motivational intervention sessions and one session of contraceptive counseling followed. The women were followed-up for 6 months to evaluate changes in their practices.

Participants were compensated $20.00 to $35.00 for each session.

Of the 2,384 screened women, 190 consented to participate and 143 (ages 19-44) completed the 6-month follow-up. The majority of subjects were ethnic minorities with high school education and reported annual incomes of less than $20,000. These women were recruited from 6 community-based settings: primary practice health care in Florida and Virginia; urban jail in Texas; and two alcohol treatment centers in Texas. The results indicate that 68.5% of the women were no longer at risk of having an alcohol-exposed pregnancy:

- 12.6% reduced drinking only.
- 23.1% used effective contraception only.
- 32.9% reported contraceptive use and decreased drinking.

The women scored an average AUDIT score of 17. Women at higher risk of having an alcohol-exposed pregnancy had lower success in reducing their risk. Women who scored low AUDIT scores were less likely to reduce drinking than women with medium and high scores. They were, however, more likely to institute effective contraception. Overall, the lower scores were most likely to reduce their risk for alcohol-exposed pregnancy than those with higher scores. The authors concluded that the baseline AUDIT scores were the strongest predictor for reduced risk of alcohol-exposed pregnancy.

This study is the first to investigate the effects of offering both motivational and contraceptive counseling to women who are at risk of having an alcohol-exposed pregnancy. These findings are significant since women who are unaware of their pregnancy will continue their usual practices in their early stages. Previous studies have indicated that motivational interventions alone can significantly decrease the consumption of alcohol during pregnancy because of the woman’s desire...
to have a healthy baby. The success of these studies relied on sensitive screening of prenatal clinics, motivational interviews, treatment referral, monitoring of care, and compassionate medical care.

Although 143 women completed the CHOICES study, the sample size is fairly small considering the involvement of three states. A larger sample would have provided results with more power. Another design aspect is the lack of a randomized control group in this study and the short (6-months) duration of the study. The short length may have resulted in increasing the subjects' compliance with regard to the use of contraception and reduced alcohol use. In the long run it is hard to tell whether they would continue this practice or return back to their initial habits. The participation of a diverse ethnicity of women increases the generalizability of these findings. However, the inconsistency among their surrounding environments (i.e. jail vs. housing) may predispose them to limited accessibility of materials and social circumstances, therefore altering their compliance.

The data collected in this study were based on the women's self-reported alcohol use and change in practice. There is debate about the reliability of self-reported alcohol consumption and practices since the drinkers may report a lower value because they wish to appear at a low risk. A method to corroborate subjects' reporting is to obtain biological samples or ask a family member or partner to confirm their reports. Another motivational and confounding factor may have been the compensation that women received per session. Subjects may have unconsciously altered their responses to please the interviewers.

AUDIT is a screening tool that was developed by the World Health Organization in the late 1980's to detect early hazardous or harmful drinking. The test is comprised of ten questions that are highly correlated with hazardous alcohol consumption and reliably identified high-risk drinkers in a six-nation study. In terms of sensitivity and specificity, AUDIT has been analyzed and found valid across different cultures and age groups. However, it has been debated whether the cut off scores for AUDIT (low=1-7, medium=8-18, high=19-40) should be set at different levels for males and females. A study investigating the test reliability of AUDIT indicated a higher reliability for males, young adults, and moderate consumers. An alternative scale, TWEAK, which focuses on identifying the risk of drinking by pregnant women could be employed as a screening instrument.

It is currently not known how much alcohol is required to cause FAS/FAE. In fact, although the Surgeon General advises women not to consume any alcoholic beverage during pregnancy, a recent survey of physicians reported that 41% of physicians placed the threshold for FAS at one to three drinks per day, while 38% placed the threshold at one or fewer drinks per day. Another important issue is training professionals to counsel high-risk drinkers. A randomized control trial of videotaped alcohol counseling and motivational training enabled professionals to express greater empathy, minimize patient defensiveness and increase support in the woman's belief in the ability to change.

A study reporting alcohol use among adolescents indicated that alcohol use was prevalent in 23.9%, 37.9%, and 48.9% of students in grade 8, 10, and 12, respectively. A prevention trial that informed women of alcohol's effect in pregnancy showed that the majority of women reduced or stopped drinking after learning of FAS/FAE. Although the majority of women in the study reported high school education, none had knowledge about FAS/FAE. Therefore, it is important to educate women about FAS/FAE when they become sexually active and start drinking. While this study addresses an important aspect of primary prevention of FAS, longer studies are needed to see whether this short-term effect will eventually persist into these women's future pregnancies.

REFERENCES

Appendix F


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