THE USE OF COW MILK, MEAT AND CEREAL TO PREVENT
IRON DEPLETION IN INFANTS FROM LOW-INCOME HOUSEHOLDS

by

George Stuart Yeung

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

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Doctor of Philosophy, 1999
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ABSTRACT

The hypothesis of this thesis was that there would be a reduction of iron depletion and/or anaemia in six to 12 month old cow milk-fed infants from low-income households consuming infant cereals and pureed meats (n=43) when compared to infants from low-income households with no dietary intervention (n=54). Unfortunately the incidence of end-point (haemoglobin<110 g/L or ferritin<10 μg/L confirmed in a second sample) was very low in the control group (9.3%) much below the 30% anticipated; thus, the hypothesis could not be adequately tested. However, the three objectives of the study were achieved. Firstly the data obtained indicate that the control group followed proper feeding patterns which explained the low incidence of iron depletion. Most of the treatment infants complied with the dietary restrictions of the protocol and thus had low rates of iron depletion. Secondly three lines of evidence show that the iron from cereal and meat was well utilised and prevented iron deficiency in cow milk-fed infants: (i) The incidence of iron depletion/anaemia in the treatment group (7.0%) was low compared to past studies of infants in Canada and not different from control infants who followed proper infant feeding patterns (p=0.66). (ii) Infants using cow milk but not consuming infant cereal or meat,
were at great risk of reaching end-point \( p=0.0002 \) which may explain why feeding cow milk at ten months of age was associated with iron depletion/anaemia. (iii) The rate of fall in plasma ferritin (Ferr) with age was the same in both groups, suggesting that the iron status of the two groups were equivalent \( p=0.49 \). Thirdly transferrin receptor (TfR) was evaluated as a diagnostic measure of iron deficiency. The sensitivity was calculated to be 0.75 and the specificity to be 0.94. It is concluded that the consumption of adequate amounts of infant cereals and pureed meats prevented iron depletion and anaemia in infants fed whole cow milk after six month of age and that, as a measure of iron deficiency, TfR appears to be an accurate, relatively sensitive and specific diagnostic measure compared to traditional measures of iron status.
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# TABLE OF CONTENTS

**ACKNOWLEDGEMENTS** ........................................................................................................ iv

**TABLE OF CONTENTS** ................................................................................................... vi

**LIST OF TABLES** ........................................................................................................... ix

**LIST OF FIGURES** ............................................................................................................ x

**PUBLISHED MATERIAL** ................................................................................................... xiii

**LIST OF ABBREVIATIONS** ............................................................................................... xiv

1. **INTRODUCTION** ........................................................................................................... 1

2. **REVIEW OF LITERATURE** .......................................................................................... 3
   2.1 Iron Deficiency ............................................................................................................. 3
      2.1.1 Stages of Iron Deficiency ....................................................................................... 4
      2.1.2 Assessment of Iron Status ..................................................................................... 5
         2.1.2.1 Ferritin ........................................................................................................... 7
         2.1.2.2 Serum Iron, Total-Iron-Binding Capacity, Transferrin Saturation, Free Erythrocyte Protoporphyribin ................................................................. 8
      2.1.3 Transferrin Receptor ............................................................................................. 11
         2.1.3.1 Definition and Mechanism ............................................................................. 11
         2.1.3.2 Clinical Usefulness of TfR ............................................................................ 13
      2.1.4 Prevalence of Iron Deficiency in Canadian Infants ............................................... 15
      2.1.5 Morbidity Associated with Iron Deficiency ......................................................... 17
      2.1.6 Risk Factors ......................................................................................................... 19
         2.1.6.1 Premature Birth ............................................................................................ 20
         2.1.6.2 Prolonged Breast Feeding ............................................................................. 20
         2.1.6.3 Early Introduction to Cow Milk ...................................................................... 22
         2.1.6.4 Low Socio-economic Status ....................................................................... 24
      2.1.7 Preventing Iron Deficiency in Infants .................................................................. 25
         2.1.7.1 Screening and Supplementation ................................................................... 25
         2.1.7.2 Iron-Fortified Formula ............................................................................... 27
         2.1.7.3 Iron-Fortified Infant Cereal ......................................................................... 28
         2.1.7.4 Meat .............................................................................................................. 29
      2.1.8 Infant Feeding Practices in Canada ...................................................................... 31
      2.1.9 Infant Feeding Guidelines .................................................................................... 33
6. CONSUMPTION OF CEREAL, MEAT AND MILK IN THE PREVENTION OF IRON DEPLETION

6.1 Introduction................................................................. 93
6.2 Subjects and Protocol........................................................ 95
  6.2.1 Data Generated.......................................................... 95
  6.2.2 Statistical Analysis.................................................... 95
6.3 Results............................................................................. 96
  6.3.1 Change in Haemoglobin and Ferritin Levels with Age .......... 96
  6.3.2 Achievement of End-Points.......................................... 99
  6.3.3 Other Factors Related to End-point and Compliance ........... 104
6.4 Discussion....................................................................... 109

7. USE OF TRANSFERRIN RECEPTOR AS AN INDEX OF IRON DEFICIENCY

7.1 Introduction......................................................................... 118
7.2 Population used to Establish Normative Percentile Estimates ...... 120
7.3 Statistical Analysis................................................................ 121
7.4 Results.............................................................................. 122
  7.4.1 Percentile Estimates...................................................... 122
  7.4.2 Infant Iron Study.......................................................... 132
7.5 Discussion........................................................................... 135
  7.5.1 Percentile Estimates Derived from the Iron Prevalence Study .... 135
  7.5.2 Percentile Estimates Applied to the Infant Iron Study........... 137

8. GENERAL DISCUSSION .......................................................... 142
  8.1 Future Directions............................................................. 153

9. SUMMARY AND CONCLUSIONS.............................................. 155
  9.1 Summary.......................................................................... 155
  9.2 Conclusions....................................................................... 157

REFERENCES..................................................................... 158

LIST OF APPENDICES............................................................ 172
LIST OF TABLES

Table 2.1 Biochemical Measurements in Stages of Iron Deficiency ........................................ 5
Table 2.2 Changes in Feeding Practice from 1977-78 to 1984-85 ........................................ 31
Table 5.1 Disposition of Subjects ......................................................................................... 59
Table 5.2 Characteristics of All Subjects Who Completed the Study ................................. 60
Table 5.3 Family Background for Infants Who Completed the Study ............................... 61
Table 5.4 Specific Food Use Related to Low Ferr or HgB ................................................... 69
Table 5.5 Mean Height for Age Z-score by Age for Treatment Versus Control Group ... 81
Table 5.6 Average Reported Infant Cereal and Meat Intake of Treatment Infants .......... 83
Table 6.1 Effect of Treatment in Avoidance of Confirmed End-Points for all Subjects ................................................................................................................................. 101
Table 6.2 Effect of Treatment in Avoidance of Confirmed End-Points (Non-compliant infants excluded from treatment group) ..................................................... 101
Table 6.3 Effect of Treatment in Avoidance of Single End-Points for all Subjects .......... 102
Table 6.4 Effect of Treatment in Avoidance of Single End-Points (Non-compliant infants were excluded from the treatment group) .................................................. 102
Table 6.5 Effect of Compliance to Treatment on Confirmed End-points .......................... 103
Table 6.6 Effect of Compliance to Treatment on Single End-points ................................. 103
Table 6.7 Socio-demographic Background of Infants Who Reached Confirmed End-point (Excluding non-compliers) ......................................................... 105
Table 6.8 Socio-demographic Background of Non-Compliers who Reached Confirmed End-point ................................................................. 106
Table 6.9 Effect of Socio-economic Status (SES) on Compliance .................................. 106
Table 6.10 Age When Confirmed End-point was Reached (non-compliers excluded) . 107
Table 6.11 Age When Putative End-point was Reached (non-compliers excluded) ..... 107

Table 6.12 Prevalence of Iron Deficiency (based on confirmed end-points, non-compliers excluded)........................................................................................................ 108

Table 7.1 Description of the sample used to generate percentile estimates (from the Iron Prevalence Study) .................................................................................................................. 121

Table 7.2 Mean HgB, FEP, Ferr and TfR for infants in the Iron Prevalence Study. ..... 127

Table 7.3 Observed prevalence of low HgB, high FEP and low Ferr Among Infants in the Iron Prevalence Study .......................................................... 127

Table 7.4 TfR and Log TfR:Ferr Values in Subjects with Low HgB, High FEP and Low Ferr from the Iron Prevalence Study .................................................. 128

Table 7.5 TfR and Log TfR:Ferr values in subjects with HgB<110g/L and Ferr<15µg/L from the Infant Iron Study (Chapter 6). .................................................. 133

Table 7.6 Overall, biologic and analytic variation of TfR and Ferr in blood samples from the Infant Iron Study (Chapter 6). .................................................. 133

Table 7.7 Diagnostic Sensitivity and Specificity of TfR from data generated from the Infant Iron Study (Chapter 6).......................................................... 134
LIST OF FIGURES

Figure 4.1 Experimental Protocol ................................................................. 45
Figure 5.1 Proportion of Infants Breast-Feeding: Birth-6 Months .................. 64
Figure 5.2 Proportion of Infants Fed Iron-Fortified Formula: Birth-6 Months ...... 64
Figure 5.3 Proportion of Infants Fed Non-Fortified Formula: Birth-6 Months ...... 65
Figure 5.4 Proportion of Infants Fed Whole Cow Milk: Birth-6 Months .......... 65
Figure 5.5 Proportion of Infants Fed Infant Cereal: Birth-6 Months .................. 66
Figure 5.6 Proportion of Infants Fed Meat: Birth-Six Months ......................... 66
Figure 5.7 Proportion of Infants Fed Fruits/Vegetables: Birth-Six Months ........ 67
Figure 5.8 Proportion of Infants Fed Juice: Birth-Six Months .......................... 67
Figure 5.9 Proportion of Infants Consuming Breast Milk: 8-12 Months .......... 71
Figure 5.10 Proportion of Infants Consuming Iron-Fortified Formula: 8-12 Months .... 71
Figure 5.11 Proportion of Infants Consuming Non-Fortified Formula: 8-12 Months .... 72
Figure 5.12 Proportion of Infants Consuming Whole Cow Milk: 8-12 Months ....... 72
Figure 5.13 Proportion of Infants Consuming Infant Cereal: 8-12 Months ......... 73
Figure 5.14 Proportion of Infants Consuming Meat: 8-12 Months .................... 73
Figure 5.15 Proportion of Infants Consuming Iron Supplements: 8-12 Months .... 74
Figure 5.16 Weight for Age z-score (WAZ) .................................................. 76
Figure 5.17 Height for Age z-score (HAZ) .................................................... 77
Figure 5.18 Weight for Height z-score (WHZ) ............................................... 78
Figure 5.19  Head Circumference by Age (males) ................................................................. 79
Figure 5.20  Head Circumference by Age (females) .............................................................. 80
Figure 5.21  Recorded Morbidity by Treatment ................................................................. 82
Figure 7.1  Subjects Used to Derive Percentile Estimates by Age and Gender from the Iron Prevalence Study ......................................................................................... 125

Figure 7.2
a. Distribution of Transferrin Receptor Concentration for all Subjects Studied in the Iron Prevalence Study................................................................. 126
b. Distribution of Transferrin Receptor Concentration for subjects in the Infant Iron Study. ................................................................. 126

Figure 7.3  Percentile estimates of transferrin receptor concentration for all infants with Hgb ≥ 100 g/L and FEP ≤ 100 µg/L from the Iron Prevalence Study. .................... 128

Figure 7.4  Distribution of transferrin receptor concentration for infants with Hgb>100g/L, FEP<100mg/L and Ferr>10µg/L from the Iron Prevalence Study. 129

Figure 7.5
a. Distribution of log (TfR:Ferr) for infants with Hgb>100 g/L, FEP<100 µg/L and Ferr>0 µg/L from the Iron Prevalence Study ................................................................. 130
b. Distribution of log (TfR:Ferr) for infants from the Infant Iron Study. ......................... 130

Figure 7.6  Percentile estimates of log (TfR:Ferr) for infants with Hgb>100 g/L, FEP<100 µg/L and Ferr>10 µg/L from the Iron Prevalence Study ......................... 131
The following publications are based on Chapter 6:


GS Yeung and SH Zlotkin. Providing infants with the iron they need. Agri-Food Research in Ontario Magazine. 1998; 21(1).

The following publications are based on Chapter 7:


LIST OF ABREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>CPS</td>
<td>Canadian Paediatric Society</td>
</tr>
<tr>
<td>Ferr</td>
<td>Ferritin</td>
</tr>
<tr>
<td>HgB</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>SI</td>
<td>Serum iron</td>
</tr>
<tr>
<td>TIBC</td>
<td>Total iron binding capacity</td>
</tr>
<tr>
<td>FEP</td>
<td>Free erythrocyte protoporphyrin</td>
</tr>
<tr>
<td>TfR</td>
<td>Transferrin receptor</td>
</tr>
<tr>
<td>Log TfR:Ferr</td>
<td>Log of transferrin receptor to ferritin ratio</td>
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1. INTRODUCTION

Iron deficiency is the most prevalent nutritional problem in the world, affecting two thirds of children in most developing nations. It is not only a problem in developing countries but affects up to one third of Canadian infants from disadvantaged households. It has been demonstrated both in developed and developing countries that moderate iron deficiency anaemia (haemoglobin <100 g/L) depresses mental and motor functions in affected infants. Despite controversy regarding the effectiveness of treatment with iron to reverse these effects there is universal consensus that iron deficiency anaemia is associated with developmental cognitive and motor deficits, and that its prevention is essential for every infant.

During the period between six and 12 months the infant is particularly vulnerable for iron depletion since foetal reserves have been used and the increase in blood volume is rapid. The Canadian Paediatric Society (CPS) recently proclaimed that prolonged exclusive breast-feeding or the use of iron-fortified formula will prevent iron deficiency anaemia; however, a large segment of the Canadian population either cannot or will choose not to breast-feed or feed iron-fortified formula during the first year of life. Two commonly suggested means to prevent iron deficiency anaemia in disadvantaged infants are the routine use of iron drops or to blood test all infants and to treat only those with the condition. Neither of these interventions has proven effective.

Thus, the aim of this research was to determine whether a practical, inexpensive intervention (the use of iron-fortified cereal and meat) is efficacious in the prevention of
iron depletion. The incidence of iron depletion and/or iron deficiency anaemia was compared between two groups of infants from low income households, one receiving no intervention and the other receiving cow milk, cereal and meat.
2. REVIEW OF LITERATURE

Iron deficiency is the most prevalent nutritional problem in the world, affecting a large number of children and infants (Scrimshaw 1991). Even in developed countries such as Canada, children and infants from disadvantaged households are at risk. Despite the acknowledgement that iron deficiency anaemia is associated with psychomotor and cognitive abnormalities in affected infants, current Canadian recommendations for the prevention of this nutritional disorder are unlikely to be successful in the population at highest risk (disadvantaged infants). The promotion of health in 'disadvantaged Canadian groups' has been declared a major policy thrust by Health and Welfare Canada. In the following, a discussion of iron deficiency in infants, its stages, diagnosis, consequences, prevalence in Canada and methods of prevention are presented. This discussion is intended to give a general background on some of the important factors in preventing iron deficiency in 'disadvantaged infants' in Canada, especially nutrition.

2.1 Iron Deficiency

Iron deficiency generally refers to any impairment in the production of essential iron compounds, due to lack of iron. When essential iron compounds are present in limited concentrations, impaired physiological function can occur even though such an impairment may not be apparent (Dallman et al. 1981). The following section describes each stage of iron deficiency.
2.1.1 **Stages of Iron Deficiency**

Iron deficiency can be separated into three distinct stages: iron depletion, iron deficiency without anaemia (functional iron deficiency) and iron deficiency anaemia. The initial or iron depletion stage of deficiency results from an inadequate supply of dietary iron or from an increased need for iron during periods of growth such as infancy, adolescence, and pregnancy. During this stage, iron stores are released to ensure that the synthesis of haemoglobin (HgB) and other iron containing compounds takes place (Zlotkin et al. 1992). It is marked by depressed serum ferritin (Ferr) concentrations, due to a declining concentration of Ferr in the liver, spleen, and bone marrow with no change in HgB or any of the indices of erythropoiesis (Dallman and Reeves 1984; Zlotkin et al. 1992). The second stage, or iron deficiency without anaemia, is likely transient and consists of a decrease in transport iron (Dallman and Reeves 1984). During this stage, serum iron decreases resulting in the iron transport protein transferrin no longer being fully saturated. Erythroid precursors are not provided with enough iron to mature properly, however mature red blood cells remain unaffected. The third stage, or iron deficiency anaemia, develops when the supply of transport iron decreases sufficiently to restrict HgB production (Dallman and Reeves 1984). This results in the development of microcytic cells due to insufficient iron during formation, rather than normal mature red blood cells.

Thus, iron deficiency is a general term encompassing these three stages. It is important not to confuse the general term iron deficiency with the functional iron
deficiency stage. Similarly, anaemia is a general term for a condition marked by low haemoglobin concentration and has many causes other than iron deficiency (Yip 1990). Iron deficiency anaemia is a more severe form of iron deficiency. Iron depletion (reduced or low iron stores) and functional iron deficiency should not be confused since physiological difficulties are not associated with depleted iron stores as they are with functional iron deficiency. Thus, depleted iron stores are a risk factor for functional iron deficiency but should not be confused with true iron deficiency (Beaton et al. 1989).

2.1.2 Assessment of Iron Status

The different stages of iron deficiency are characterised by number of laboratory tests and are summarised in Table 2.1.

Table 2.1 Biochemical Measurements in Stages of Iron Deficiency

<table>
<thead>
<tr>
<th></th>
<th>Ferr</th>
<th>HgB</th>
<th>SI</th>
<th>TIBC</th>
<th>FEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depleted iron Stores</td>
<td>Low</td>
<td>Normal</td>
<td>Normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Functional Iron Deficiency</td>
<td>Low</td>
<td>Normal</td>
<td>Low</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Iron Deficiency Anaemia</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>high</td>
<td>high</td>
</tr>
</tbody>
</table>

Ferr (ferritin), HgB (haemoglobin), SI (serum iron), TIBC (total iron-binding capacity), FEP (free erythrocyte protoporphyrin).
Adapted from Cook and Finch (Cook and Finch 1979)
No single biochemical indicator of iron status currently in use provides a high degree of accuracy or reliability. By the early 1980's, laboratory measurements had been refined not only to identify iron deficiency readily but also to estimate the magnitude of the deficit and to monitor its correction with iron therapy (Cook and Skikne 1982; Cook and Skikne 1989). Until 30 years ago, the diagnosis of iron deficiency was considered a simple matter primarily pertaining to hospitalised patients with iron deficiency anaemia (Dallman and Reeves 1984). In the last two decades, attention has shifted to the more common milder cases of iron deficiency that are typically seen in the outpatient setting. However, there is no single measure of iron deficiency that is not either too costly, too cumbersome or not specific enough to be used for screening (Cook et al. 1992). Therefore, high sensitivity and specificity are of special importance for a test of iron status, because further identification of the cause of depletion of iron stores would require tedious clinical and laboratory investigations (Punnonen et al. 1994). Currently, the use of several indicators of iron status provides the best assessment for diagnosis. The optimal set of methods is different for each population group. For example, the detection of mild iron deficiency in an otherwise healthy individual requires a different approach than the identification in the hospitalised patient with severe anaemia or advanced malignancy (Cook and Skikne 1982). With mild iron deficiency, laboratory tests may be less reliable, with the values of iron-deficient and iron-sufficient persons overlapping (Oski 1993). The following sections describe methods commonly used to assess the different stages of iron deficiency.
2.1.2.1 **Ferritin.** Ferritin is the major iron-storage protein found in reticuloendothelial cells. It contains approximately 23% iron and apoferritin (the protein moiety free of iron). It functions to store iron in the liver, spleen and bone marrow and normally appears in small quantities in serum. Serum Ferr as a measure of iron storage in the body is commonly used as an indicator of iron depletion (Cook and Skikne 1982; Dallman 1989). It is recognised as a useful survey measurement for the initial assessment and monitoring of iron stores in a normal population (Cook et al. 1974). Ferr in combination with Hgb measurements distinguish between iron deficiency anaemia and the anaemia of chronic disease (Cook and Finch 1979; Cook and Skikne 1982; Pilon et al. 1981) if the Ferr value is <15-25 ug/L (Ferguson et al. 1992).

However, the utility of serum Ferr as a test for iron depletion has been questioned because of high biologic variation and its reactivity to infection or inflammation (Dallman and Reeves 1984; Lipschitz et al. 1974). Studies indicate that the day-to-day coefficient of variation for serum Ferr is as high as 23.2% and anywhere between three to ten separate blood samples are required to estimate an individual’s true serum Ferr with 20% accuracy (Ahluwalia et al. 1993; Borel et al. 1991; Cooper and Zlotkin 1996). Drawing this many blood samples is very impractical both for screening and in clinical situations, especially for infants. Thus single Ferr samples are most often the norm in both situations, despite the high variation. Current data on the prevalence of iron depletion is therefore subject to this high biologic variation and may be suspect.

Another major problem with this marker as a sole indirect measure of iron stores is
that it is an acute-phase protein and therefore increases in concentration during inflammation (Cook and Skikne 1982). This would mask low iron stores in an individual suffering from infection or inflammation (Cook and Skikne 1982). However, low serum Ferr values (below 10 μg/L) are highly specific for iron depletion since no other conditions drive serum Ferr down, but this gives no indication of severity of the iron deficit (Cook et al. 1992; Cook and Skikne 1982). Iron depletion in itself is not associated with insufficient supply of iron to tissues (Hallberg and al 1993). Therefore the absence of iron stores is simply a risk factor for the development of iron deficiency anaemia.

2.1.2.2 Serum Iron, Total-Iron-Binding Capacity, Transferrin Saturation, Free Erythrocyte Protoporphyrin. A number of laboratory measures are used to determine iron deficiency including serum iron, total-iron-binding capacity, transferrin saturation, and erythrocyte protoporphyrin. Transferrin is a glycoprotein formed in the liver that transports iron obtained from dietary sources and from the breakdown of red blood cells by reticuloendothelial cells. Most of this iron is transported to the bone marrow for use in haemoglobin synthesis while some is stored in the form of ferritin and haemosiderin. Thus, the measurement of transferrin saturation or iron levels can be useful for screening for iron deficiency but their values are affected by infection and inflammation (Cook and Skikne 1989). Infection, inflammation and malignancy typically produce low serum iron and low total iron-binding levels, and a transferrin saturation which tends towards the low end of the normal range (Gibson 1990). These changes arise from a failure to release iron to serum transferrin from the reticuloendothelial cells of the bone marrow, spleen, and
liver. Thus, the values of the iron measurements appear low when iron stores are sufficient.

Another serious limitation of serum iron is its large biological variation (Ahluwalia et al. 1993; Borel et al. 1991; Romslo and Talstad 1988). Furthermore, measurement of serum iron requires freshly separated plasma, has relatively poor reproducibility and necessitates a relatively large volume of blood. (Cook and Skikne 1989). Total-iron-binding capacity appears to have a considerably lower biological variation than that of serum iron. Statland and Winkel (1977) reported an average intra-individual day-to-day variation for serum iron of 29.3% and for iron-binding capacity of 8.8%. Since transferrin saturation is the ratio of serum iron and total-iron-binding capacity expressed as a percentage, transferrin saturation will reflect the biological and analytical variability of both measures, reducing its reliability (Dallman and Reeves 1984).

As a measure of functional iron deficiency, free erythrocyte protoporphyrin has greater stability than transferrin saturation. Beaton, Corey and Steele (1989) (Beaton et al. 1989) determined, using data from the Hispanic Health and Nutrition Examination Survey (HHANES), that a correlation of 0.99 occurred between three repeated measurements of free erythrocyte protoporphyrin taken within-person across days. However, chronic disease states, such as infection, inflammation, and certain neoplastic diseases are associated with elevated protoporphyrin levels (Dallman and Reeves 1984; Gibson 1990). Thus, erythrocyte protoporphyrin like the above parameters cannot be
used to distinguish between iron deficiency caused by total iron depletion or resulting from chronic disease.

2.1.2.3 **Haemoglobin.** HgB has been shown to be a convenient screening tool for anaemia but it has low specificity and lacks sensitivity. Using HgB alone, it is not possible to distinguish between iron deficiency anaemia and anaemia due to chronic infection. It is relatively non-specific in that low HgB values arise with chronic infections and inflammation, haemorrhage, protein-energy malnutrition, thalassaemia minor, vitamin B-12 or folate deficiency, haemoglobinopathies, pregnancy, and other states in which there is over-hydration or acute plasma volume expansion (Gibson 1990). Furthermore, HgB values overlap between normal and deficient populations, with a wide spread of values in a normal population. For example, Garby, Irnell and Werner (1969) (Garby et al. 1969) demonstrated by a response or lack of response to oral iron therapy that about 20% of either normal or anaemic women were classified incorrectly on the basis of their initial HgB concentration. However, in conjunction with a second, more specific index of iron deficiency the utility of HgB values can be enhanced.

2.1.2.4 **Biochemical Definitions of Iron Deficiency.** Different cut-off values have been employed to define iron deficiency which affects estimation of its prevalence. A well accepted criteria, used in the 2nd National Health and Nutrition Examination Survey (NHANES II), defined iron deficiency anaemia as a HgB <110 µg/L and one of low serum Ferr, high transferrin or high FEP. The NHANES II study also suggested that two or three abnormal indicators of iron status were more indicative of an iron deficiency of
biologically significant severity than was a single indicator (Life Science Research Office, 1984). Iron deficiency without anaemia has been defined as HgB $\geq 110$ μg/L and any two of Ferr $< 10$ μg/L, transferrin $> 3.36$ g/L or FEP $> 550$ nm/L. Iron depletion without anaemia has been defined as a Ferr $< 10$ μg/L with all other haematologic parameters within the normal range. However, testing for three or four of these indices is costly and requires a fairly large blood volume, which is often impractical to collect, especially from infants.

2.1.3. **Transferrin Receptor.**

Recently, there has been growing interest in a new potential marker of functional iron deficiency, the transferrin receptor (TfR). Increases in circulating TfR have been associated with iron deficiency. The following sections describe the development of this promising method for identifying functional iron deficiency.

2.1.3.1 **Definition and Mechanism.** TfR is integral in the utilisation of iron from the external environment and controlling its distribution internally. Iron-chelating transport proteins, such as serum transferrin, chelate iron in its most soluble and stable form for the biosynthesis of essential iron enzymes and glycoproteins (Irie and Tavassoli 1987). However, distinct tissues require selective amounts of iron under different circumstances implying control over receptivity to iron-loaded transferrin. This requirement is fulfilled by an integral membrane glycoprotein, the transferrin receptor (Turkewitz et al. 1988). Thus, iron transport in plasma is carried out by transferrin,
which donates iron to cells through interaction with a specific membrane receptor, the transferrin receptor (Beguin 1992).

TfR membrane protein is a 760-amino acid disulphide-linked transmembrane glycoprotein, composed of two monomers of approximately 95,000 Daltons binding one or two molecules of transferrin each (Beguin 1992; Huebers and Finch 1987). TfR on the cell surface functions to control the movement of transferrin iron through the plasma into cells (Trowbridge 1989) and the number of receptors on the cell surface reflects the iron requirement of the cell. Thus the highest concentration of TfR is in rapidly proliferating cells, haemoglobin synthesising tissues, and the placenta (Cook et al. 1993).

The intracellular cycle of transferrin and TfR has been well characterised. In an energy and temperature dependant process, diferric and monoferric transferrin molecules bind to TfRs on the cell surface (Huebers and Finch 1987). Intracellular delivery of transferrin bound iron is facilitated by receptor mediated endocytosis. This is the process by which transferrin bound iron binds to specific cell-surface receptors at a pH of approximately seven and are internalised within intracellular vesicles (Beguin 1992; Dautry-Varsat et al. 1983; Huebers and Finch 1987; Irie and Tavassoli 1987; May et al. 1985). Due to the acidification of the endosome (pH 5.0), iron is released from the iron-transferrin complex into the cytoplasm. Receptor returns to the cell membrane and apotransferrin to the plasma where more iron can be secured (Beguin 1992; Huebers and Finch 1987; Irie and Tavassoli 1987).

In the mid-1980's, detection of the receptor in body fluids such as serum was
recognised as a potentially useful index of iron need because of its role in cellular iron uptake and its reflection of cell proliferation potential (Kohgo et al. 1986). Kohgo et al. (1986), using a two-site sandwich radioimmunoassay with B3/25 and OKT9 monoclonal antibodies against the human TfR, were able to detect circulating TfRs in normal human serum at concentrations ranging between 104 and 410 ng/ml. Flowers et al. (1989) later developed an enzyme-linked immunoassay that detected normal values nearly 20-fold higher at 5.63 ± 1.42 mg/L. This method could reproducibly detect free receptor or receptor-transferrin complexes in serum with equal sensitivity. The discrepancy in the calculated values is accounted for by the inability of the monoclonal antibodies in the radioimmunoassay to detect transferrin-TfR complexes as efficiently as free receptor.

Subsequent research identified the exact physicochemical and immunochemical properties of the TfR (Shih et al. 1990). Serum receptor exhibited an apparent molecular weight of 85,000 Daltons compared with 190,000 Daltons for placental TfR. Furthermore amino acid residues 1-19 of serum receptor were identical to residues 101-119 of the intact tissue receptor. Thus, TfR in human serum is a truncated form lacking the cytoplasmic and transmembrane domains (residues 1-100) of the intact receptor.

2.1.3.2 Clinical Usefulness of TfR. Research on TfR has shown it to be more sensitive, more specific and more stable than traditional iron status markers. Increases in TfR concentration occur earlier in the development of functional iron deficiency than do changes in other indices such as FEP and it has been shown to be a quantitative measure of functional iron deficiency (Cook et al. 1993). Measurements of TfR along with
haemoglobin have been shown to distinguish between iron deficiency and the anaemia of chronic disease in adults. Ferguson et al. (1992) tested whether serum TfR could distinguish true iron deficiency from the anaemia of chronic disease and iron deficiency in patients with acute infection or chronic liver disease. Mean serum receptor levels were elevated in subjects with true iron deficiency but remained normal in all patients with acute infection and in all but four out of 41 patients with anaemia of chronic disease. This was confirmed by a later study that showed patients with anaemia of chronic disease had normal serum TfR concentrations while those with true iron deficiency had elevated levels (Punnonen et al. 1994). Unlike serum Ferr, serum TfR does not appear to be affected by these disorders and can be regarded as a specific laboratory measure for the development of iron deficiency. Day-to-day variation in TfR in the elderly (Ahluwalia et al. 1993) and adults (Cooper and Zlotkin 1996) is relatively low compared to other measures such as Ferr, serum iron and transferrin saturation. Measurements of TfR in blood, along with HgB, make it possible to distinguish iron deficiency anaemia from the anaemia of chronic disease in adults (Ferguson et al. 1992; Punnonen et al. 1994). Furthermore, the combination of measurements of serum Ferr and serum TfR has been proposed as a reliable method for assessing iron status and that log (TfR:Ferr) may be useful as a single parameter to estimate body iron in population studies (Anttila and Cook 1997; Cook and Skikne 1989; Punnonen and Irjala 1997). Thus, unlike other methods of assessing iron availability to the developing erythrocyte, increases in TfR concentration are sensitive and specific to iron deficiency.
Although TfR has been shown to be sensitive and specific to changes in body iron, it cannot be used to diagnose iron deficiency until a 'cut-off' point, above which is indicative of iron deficiency, is established. Then, the sensitivity and specificity of TfR as a diagnostic test can be evaluated. Furthermore, evaluations of TfR’s variability and specificity have been confined to adults despite the fact that infants after six months of age are at particular risk for iron deficiency. Thus, further evaluation of TfR’s utility as a means of diagnosis for iron deficiency requires the development of a ‘cut-off’ separating normal and iron deficient values.

2.1.4 Prevalence of Iron Deficiency in Canadian Infants

In general, the prevalence of iron deficiency anaemia is quite low in Canadian infants. In a group of Canadian infants, six to 18 months of age, iron depletion and iron deficiency anaemia were found in 10.5% and 3.5% respectively (Greene-Finestone et al. 1989). In Montreal, iron deficiency anaemia among infants of ‘upper middle class’ parents enrolled in a longitudinal study of growth was very low (Brault-Dubuc et al. 1983). In Vancouver, iron deficiency anaemia (Hgb \leq 110 \text{ g/L} and two or three of Ferr \leq 10 \mu{\text{g/L}}, TIBC > 60 \mu{\text{mol/L}}, zinc erythrocyte protoporphyrin > 70 \mu{\text{mol/mol}}) was found in 6.9% of 434 nine-month-old infants of various backgrounds. Low iron stores (Ferr \leq 10 \mu{\text{g/L}}) were observed in 24.4% of the infants (Innis et al. 1997). Recently a cross-sectional survey was undertaken to estimate the prevalence of iron depletion, iron deficiency anaemia, and factors affecting the prevalence, in healthy infants living in Edmonton, Toronto, Montreal,
and Halifax. It was found that among the 428 evaluable subjects, 4.3% of the subjects were anaemic (HgB ≤ 100g/L and Ferr ≤ 10 µg/L or FEP ≥ 100) and 33.9% of the infants were iron deplete (Ferr ≤ 10 µg/L) (Zlotkin et al. 1996).

Despite the generally low rates of iron deficiency anaemia in Canadian infants, those from low-income households are still at risk. In 1992, Lehmann et al. examined the iron status of close to 300, 1-year old infants from 'disadvantaged families' in Montreal. Iron deficiency anaemia (Ferr < 10 µg/L and Mean Corpuscular Volume < 72 fL or HgB < 115 g/L) was found in 25% of the infants; iron depletion (Ferr) in 37%. The investigators concluded that "...socio-economically disadvantaged infants in Montreal are at risk. Preventative measures must be taken to ensure adequate iron status in the first year of life".

Native Canadian infants are also at great risk for iron deficiency and anaemia. A review of statistics from the Sioux Lookout Zone Hospital (Ontario) from 1990 to 1992 revealed a prevalence of anaemia (HgB < 110 g/L) in infants of greater than 52% (Whalen et al. 1997). Longstaffe, Moffatt et al. (1993) described a high incidence of iron deficiency anaemia in 'high risk' urban Winnipeg infants (90% Aboriginal) receiving non iron-fortified formula (at age 12 mo., 18% with low HgB, 53% low ferritin) in a double-blind < 10 µg/L randomised controlled trial. Thus the consequences of iron deficiency are a concern for a significant portion of the Canadian population, infants from socio-economically disadvantaged households.
2.1.5 **Morbidity Associated with Iron Deficiency**

A number of negative health outcomes are associated with both iron deficiency and iron deficiency anaemia due to the insufficient supply of iron in the body. Most research addressing the consequences of iron deficiency has focused on the more severe clinical cases of anaemia. However, the impairment of many bodily functions, including the activity of some iron-dependent enzymes, occurs long before anaemia sets in (Scrimshaw 1991). These include, loss of energy and appetite, decreased immune function, gastrointestinal changes, reduced work capacity, impaired cognitive function, defects in thermoregulation, and resistance to infections (Dallman 1990; Prasad and Prasad 1991). This section will focus on the infant population although pregnant women and the growing foetus, infants and young children and women of childbearing years are all at higher risk for iron deficiency (Dallman 1989).

Healthy, term, breast-fed infants are unlikely to become iron deficient before six months of age since, at birth, they have a generous supply of transplacentally transmitted storage iron which lasts until about four months of age (Dallman 1988). Body iron needs increase about 70% due to rapid growth and basal losses between four to 12 months of age (Dallman 1988). At this age infants depend primarily on exogenous sources of iron to meet their needs. It is at this stage of infancy that there is most concern over iron deficiency.

Of particular concern is the effect of iron deficiency on cognitive and motor development. At birth, only 10% of adult brain iron levels are present, increasing to 50% at about the age of 10 years. Brain iron content continues to increase up to the age of 20-30
years (Hallberg and al 1993). Since brain development is the most rapid in the first two years of life, there is concern that inadequate iron during this period would have a deleterious effect on enzymes important in brain growth (Larkin 1993).

This concern has been supported by a number of studies in at least four different cultures which have shown an association between iron deficiency anaemia during infancy and early childhood with deficits in development (Aukett et al. 1986; Lozoff et al. 1987; Lozoff et al. 1991; Walter et al. 1988; Walter 1992). In all of these studies, the iron status of the subjects was well defined both before and after therapy. All reported lower scores on the Bayley Index in the infants with iron deficiency anaemia. Changes in cognitive and motor function at 12 months of age have been associated with even moderate iron deficiency anaemia (HgB $<100$ g/L). Such changes may begin even when the HgB concentration is between 100 and 109 g/L ($<110$ g/L is the lower limit of the normal 95% reference range at this age).

Changes in cognitive and motor function are not affected by depleted iron stores (marked by low Ferr concentrations) if the HgB concentration is within the normal range (Idjradinata and Pollit 1993; Lozoff et al. 1991; Walter 1992). Depleted stores alone do not significantly alter developmental status, however, iron depletion is a risk factor for iron deficiency and severe anaemia.

In the studies by Walter et al. (1992) and Lozoff et al. (1991), the reversal of the anaemia and iron deficiency did not produce improvements in the test scores, suggesting that iron deficiency anaemia at a critical period of brain growth and development may
produce irreversible abnormalities. Furthermore, it was also shown that schoolchildren with iron deficiency anaemia had poorer cognitive function than controls, which was not totally improved by iron treatment (Pollitt et al. 1989). This is further supported by a recently published double-blind controlled trial showing that lower mental scores persisted in infants with iron deficiency anaemia, despite extended oral iron therapy and an excellent haematologic response (Lozoff et al. 1996). However, a blinded, iron versus placebo treatment trial (Idjradinata and Pollit 1993) showed different results. Complete reversal of the abnormalities in Bayley Index in infants with iron deficiency anaemia after iron treatment was observed. Furthermore, Bruner et al. (1996) showed that adolescents with iron deficiency, when treated with iron, performed better on a test of verbal learning and memory when compared to girls with iron deficiency who were not treated (controls) (Bruner et al. 1996). Thus, there is strong reproducible evidence to support the relation between iron deficiency anaemia and impaired motor/cognitive performance in infants and children, but there remains some controversy about the reversibility of the effects with iron treatment. However, there is universal agreement that the prevention of iron deficiency anaemia is essential.

2.1.6 Risk Factors

Any situations in which iron intake is compromised, loss of body iron or iron need is excessive or a combination of these, increases the risk of iron deficiency. For example, blood loss due to trauma or parasitic infection can cause iron deficiency. In
developing countries high infection rates, particularly hookworm, schistosomiasis, malaria as well as other infectious diseases is the second leading cause of iron deficiency anaemia, behind inadequate intake and absorption of iron (Florentino and Guirriec 1984; Hershko 1992; Stoltzfus et al. 1997; WHO 1995). Although parasitic infection is not a major cause in Canada, certain segments of the infant population are still at risk for iron deficiency. The following sections describe some of these risk factors, including: premature birth, prolonged breast feeding, early introduction to cow milk and low socio-economic status

2.1.6.1 **Premature Birth.** Preterm infants can become iron deficient more rapidly than term infants. Body iron stores increase in proportion to body weight during foetal life (Widdowson and Spray 1951). Thus, preterm infants are born with the same iron stores per kilogram as term infants. However, preterm infants have a faster growth rate than their term counterparts, thus increased iron need. This is reflected in earlier depletion of iron stores as shown by a more rapid postnatal decrease in serum Ferr in preterm infants (Haga 1980). Furthermore preterm infants become iron deficient more rapidly than do term infants as shown by earlier postnatal decline in HgB and red cell mass (Dallman 1981; Lundstrom 1980; Siimes 1982). Other studies show that iron deficiency may develop after two (Jansson et al. 1979) or three months (Halliday et al. 1985; Lundstrom 1980) of age in preterm infants if iron intake is inadequate.

2.1.6.2 **Prolonged Breast Feeding.** There is general consensus that breast milk is the ideal food for full-term infants (Canadian Paediatric Society Nutrition Committee,
1979; Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998). Some of its many benefits include the unique physiochemical and immunological properties of human milk, the psychosocial benefits of bonding due to close physical contact, nutritional benefits and the fact that it is inexpensive. Infants primarily or exclusively breast-fed have adequate iron intakes until around six months of age. The actual iron content of breast milk is quite low, ranging from 0.2 to 0.7 μg/ml but is highly bioavailable. Approximately 50% of iron from breast milk is absorbed (Fomon 1982; Saarinen et al. 1977) compared to 20% from cow milk (Saarinen et al. 1977). Iron provided by breast milk appears to be adequate to prevent iron deficiency anaemia for at least six months of life (Lonnerdal 1984). Thus, iron deficiency anaemia is rarely observed in otherwise healthy, breast-fed infants under the age of six months (Duncan and al 1985; Saarinen and Siimes 1979; Saarinen et al. 1977).

Beyond six months of age there is concern that iron intakes are inadequate in exclusively breast-fed infants. A study conducted in Chile found that 26.95% of infants breast-fed for nine months were iron deficient and 14.7% had iron deficiency anaemia (Pizarro et al. 1991). In Argentina, 27.8% of infants exclusively breast-fed for six months had iron deficiency anaemia by nine months of age (Calvo et al. 1992). In Japan, 22% of exclusively breast-fed infants at nine months of age had iron deficiency anaemia (Hokama 1993). Recently, a survey in Vancouver found that 15.2% of nine-month-old breast-fed infants compared to 6.4% of those breast-fed for less than eight months had iron deficiency anaemia (Innis et al. 1997). In this study, all instances of iron deficiency
anaemia were confirmed in a second test. This result could not be attributed to delayed introduction of iron-fortified infant cereal since 83% of those with iron deficiency anaemia were fed infant cereal by four to six months of age. The authors suggest that the amount of cereal consumed was not enough to prevent iron deficiency. Thus, there is compelling evidence that breast milk alone does not provide adequate iron intake for the second six months of life.

2.1.6.3 Early Introduction to Cow Milk. Early introduction to cow milk may lead to iron deficiency since it is a poor source of iron and may increase endogenous iron loss through occult bleeding. Like human milk, cow milk has approximately 0.5 mg/L of iron (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998). However, cow milk is not nearly as well absorbed as human milk. A study using a radio-labelled iron (FeSO₄) tracer showed that as much as 48.8% of iron from human milk is absorbed while 19.5% of iron from cow milk is absorbed (Saarinen et al. 1977). Thus the low concentration and relatively poor bioavailability make cow milk a poor source of iron.

Early cow milk feeding can lead to intestinal blood loss in infants which in turn can cause iron deficiency anaemia (Hoag et al. 1961; Rasch et al. 1960). A prospective study in which either cow milk or non-iron-fortified formula was given at two months of age was the first to show this in nonanaemic infants (Woodruff et al. 1972). A sensitive guaiac test was used to detect the presence, but not quantity, of blood in the stools. The investigators found that the percentage of guaiac-positive stools observed in the cow
milk-fed group was much higher than in the formula-fed group. However, it was not possible to judge the nutritional significance of the blood loss. In a similar study, Fomon et al. (1981) found that the number of infants with guaiac-positive stools and the total number of guaiac-positive stools was significantly higher in infants fed cow milk compared to those fed formula at four months of age, but found no difference at later ages. This study clearly demonstrated that cow milk-feeding caused occult blood loss in four month old infants, however did not do so for older infants.

Occult blood loss due to cow milk-feeding may not be a concern in infants six months of age and older. Fuchs and colleagues recently completed a prospective randomised study of gastrointestinal blood loss in 104 infants in the second 6 months of life (Fuchs et al. 1993). Infants were assigned to receive either whole cow milk or one of three formulas beginning at four to six months of age. Stools were tested for blood until the infants reached 12 months of age. There were no differences in stool blood losses between any of the four groups. When the authors looked for the expected correlation between the volume of whole cow milk consumed and faecal haemoglobin, it was not present. Although 27% of the cow milk fed infants developed biochemical evidence of iron depletion (apparently none were anaemic), the authors make a strong argument that the cause was related to inadequate iron intake or absorption, not excessive blood loss. These results are consistent with studies which also show no difference in faecal haemoglobin or guaiac-positive stools between cow milk and formula fed infants older than six months (Thomas et al. 1986; Woodruff et al. 1972). In contrast, a study by
Ziegler et al. (1990) concluded that older infants fed cow milk had a significantly higher proportion of guaiac-positive stools and greater excretion of faecal haemoglobin (Ziegler et al. 1990). However, conclusions regarding faecal blood loss were based largely on analysis of the number of guaiac-positive stools, rather than the number of infants with positive guaiac tests. Upon re-analysis of the data at each time interval, no significant differences between cow milk-fed and formula fed infants was detected (p=0.07). A recent study used stable isotopes to measure endogenous iron losses in healthy infants (Belsten et al. 1997). The investigators concluded that occult bleeding does not make a significant contribution to iron loss in healthy infants six to 12 months of age, irrespective of the main milk source.

2.1.6.4 Low Socio-economic Status. In Canada, infants from families with low socio-economic status are at higher risk of iron deficiency than the general population. Greene-Finestone et al. (1989) studied infant feeding practices and socio-demographic factors in infants from the Ottawa-Carleton region. She demonstrated that the prevalence of mild iron deficiency anaemia (HgB 101 - 110 g/L) and moderate-severe iron deficiency anaemia (HgB < 100 g/L) was 3 and 5 times higher respectively among those from lower socio-economic households. Other studies confirm the predisposition of disadvantaged infants to high risk of iron deficiency and anaemia (Egbuonu and Starfield 1982). In Canada, the prevalence of iron deficiency is much higher in infants from low socio-economic than compared to the general population (See section 2.1.4). Low socio-economic status per se is not responsible for iron deficiency; but rather, specific practices
associated with poverty may be contributing factors. Economic constraints, lower education and certain cultural background affect the types of foods consumed and are often associated with lower breast-feeding rates and the earlier introduction of solids and unmodified cow milk (Czajka-Nairns et al. 1978; Greene-Finestone et al. 1989; Lehmann et al. 1992; Matthews et al. 1995; Williams et al. 1996). This data supports the rationale for specifically targeting disadvantaged infants as a group at high risk for the development of iron deficiency anaemia.

In the United States, the problem of iron deficiency in low-income populations has been alleviated through the Special Supplemental Food Program for Woman Infants and Children (WIC). Coupons for the purchase of iron-fortified formula are given to families deemed eligible for financial support. This is an effective but very expensive program. Given the current economic climate in Canada, it is very unlikely that a similar program would be started. An alternate approach, therefore, must be found for infants at risk of anaemia.

2.1.7 Preventing Iron Deficiency in Infants

A number of strategies have been tested and shown to be efficacious; however, have only limited effectiveness in preventing iron deficiency in high-risk populations such as infants from low-income households. The following sections will describe some of these strategies.

2.1.7.1. Screening and Supplementation. The two most commonly suggested
means to prevent iron deficiency anaemia are the routine use of ferrous sulphate drops in all infants receiving cow milk prior to nine months of age or to screen all infants at age nine months and to treat only those with the condition. The latter was suggested by the Periodic Health Examination Task force but has not been practised routinely (Canadian Task Force on the Periodic Health Examination, 1979). For screening to be successful, deficiency would have to be detected early and the identified individuals would be treated. This implies an effective screening program with adequate access to the whole population, or at least to the vulnerable group, a good screening test that is reliable and not too expensive and a high acceptance of the test by the population at risk. Treatment would then be given to a limited number of infants. Screening programs have shown limited success in England (Bristol and Nottingham) (Wharton 1992), but only with great cost and effort. It is unlikely that blood testing would be a cost-effective approach to preventing iron deficiency in Canada. It is unlikely that such programs would have access to individuals at highest risk since those in the lowest socio-economic situations may not have homes or telephones. Furthermore, as discussed in sections 2.1.2 to 2.1.2.4, currently there is a lack of a reliable and inexpensive screening test. Even if effective screening could be carried out, this intervention would not prevent anaemia but would detect those in need of treatment.

As an alternative to screening, the routine use of elemental iron supplements in all infants at risk of iron deficiency anaemia would be efficacious in preventing anaemia, but would likely be an ineffective public health intervention because of cost, inconvenience
and poor compliance. Ferrous sulphate and other soluble ferrous salts are relatively inexpensive and well absorbed (Brise and Hallberg 1962). However, providing daily doses for all infants can be very costly over time. Furthermore, despite good tolerance to oral iron supplements in infants (Burman 1972), compliance remains very poor. This was illustrated in a prospective study of 130 preterm infants (Stekel 1984). From age three months to age 12 months, half were provided iron-fortified milk while the other non-fortified milk and iron drops. By 12 months, 4% of those receiving iron-fortified milk had iron deficiency anaemia while 24% of those receiving iron drops did. The investigators felt that the most likely explanation for this result was poor compliance in the iron drops group, despite the very high motivation of mothers and careful supervision by professional personnel.

2.1.7.2 Iron-Fortified Formula. Numerous studies have shown that infants fed adequate amounts of iron-fortified formula, in the second six months, maintain adequate iron stores and are not at risk of iron deficiency anaemia (Fuchs and al 1993; Longstaffe et al. 1993; Moffatt et al. 1993a; Penrod et al. 1990; Tunnessen and Oski 1987; Yip et al. 1987). However, it is very expensive costing almost three times more than cow milk. This has resulted in criticism of current infant feeding guidelines, which emphasise iron-fortified formula as the only alternative to breast-feeding in the second six months of life (Canadian Paediatric Society Nutrition Committee, 1991). Such guidelines ignore the majority of economically disadvantaged children whose parents cannot afford to purchase this relatively expensive type of food (Shears 1991).
2.1.7.3 **Iron-Fortified Infant Cereal.** The role of cereals in the prevention of iron deficiency anaemia also remains controversial. In 1987 an argument was put forward (and accepted by the American Academy of Pediatrics) that the electrolytically reduced iron added to commercial infant cereals was insufficiently available for the infant to meet iron needs in the second six months (Fomon 1987; Hurrell and al 1989). Studies on iron absorption from cereal (in adults only) suggested an average absorption of about 4% (Cook and Bothwell 1984). Fuchs et al. (1993) found that iron fortified cereal was not a reliable means of preventing iron depletion in infants fed cow milk in the second six months of life. They argue that this was due to poor availability of the electrolytically reduced iron from the cereal.

In contrast, two relatively recent studies have shown that the use of iron-fortified infant cereals may contribute substantially to the prevention of iron deficiency anaemia. Walter et al compared iron-fortified cereal (iron content 55 mg/g of cereal) to unfortified cereal in 515 Chilean infants in a double-blind randomised design (Walter et al. 1993). In this rather complex design, infants received cereal, plus, either iron fortified formula, unfortified formula or were breast fed. Infants consumed on average 30 g of cereal/day. There was no difference in the prevalence of iron deficiency anaemia (HgB<105 g/L) between the group of infants receiving 'iron-fortified cereal and unfortified formula' versus 'non-fortified cereal and iron-fortified formula'. The authors concluded that "iron-fortified infant cereal can contribute substantially to preventing iron deficiency anaemia".

Beaton, Tanaka, Zlotkin et al. completed a study which examined the efficacy of
electrolytically reduced iron fortification of infant cereals (NHRDP Project 6606 4104 61).

The objective of this study was to determine whether the iron added to infant cereals in Canada (35 mg/g of cereal) is available for utilisation by infants six to 12 month of age. The hypothesis that such iron in cereal could not be utilised was tested in a double-blind controlled trial of iron supplementation in 114 infants. Infants were provided either with iron-fortified cereals (regular commercial infant cereals) or the identical cereals, but non-iron fortified. The study concluded that the iron added to commercial infant cereals in Canada was available for utilisation. The conclusion was based on three independent outcome measures. The proportion of infants reaching an end-point suggestive of an early stage of iron depletion was lower in the infants receiving the iron fortified cereal (20%) versus in the controls (48%) (p=0.002). There was a significantly larger fall in slope of serum Ferr with increasing age (p=0.032). Furthermore, for infants in the iron fortified group, there was a significantly smaller serum Ferr response to the administration of ferrous sulphate solution (Fer-in-Sol) after discharge from the fortification trial (p=0.004). They estimated that as much as 13% of the iron in fortified infant cereal is absorbed. The study concluded that "existing iron fortification practice in Canada provides iron that is available for utilisation by the infant ..." and that "reasonable usage of iron fortified cereals should be effective in preventing problems of iron deficiency".

2.1.7.4 Meat. There are two broad types of dietary iron, haeme and non-haeme. Most iron in the diet of infants is in the form of iron salts and is referred to as non-haeme
iron (e.g., cereal). The extent to which this iron is absorbed is highly variable and depends both on the person's iron status and on the other components of the diet (Cook et al. 1969). For example, the greater the iron need, the better non-haeme iron is absorbed. Other components of the diet such as ascorbic acid can enhance the absorption of non-haeme iron by forming soluble iron complexes. For example, orange juice (containing ascorbic acid) doubles the absorption of non-haeme iron from the entire meal. Inhibitors of iron absorption form insoluble complexes with iron. Such inhibitors include bran, phytates, the tannins in tea, and phosphates.

In contrast, haeme iron is derived primarily from the haemoglobin and myoglobin of meat. It is well absorbed and its absorption is less strongly influenced by the person's iron status or the other constituents of the diet. Haeme iron itself promotes the absorption of non-haeme iron, thus meat fish and poultry when ingested with iron salts, enhance their absorption (Cook and Monsen 1976). For example, adults can absorb as much as four times more non-haeme iron from a mixed meal when the principle protein source is meat, fish, or chicken than when it is milk, cheese, other dairy products or eggs.

Few studies have examined the use of meat as a source of iron for infants in the second six months, although it has long been recognised that meat is a good potential source of bioavailable iron. Haschke et al. examined iron status in 88 full-term infants randomly chosen to receive 'iron-fortified meat, cereal and iron-fortified formula' versus 'iron-fortified meat, cereal and unfortified formula' in the second six months of life (Haschke et al. 1988). HgB, haematocrit, free erythrocyte protoporphyrin and ferritin
were similar in both groups with the exception of lower ferritin at age 365 d (p<0.05) in the group fed the unfortified formula. No infant had a haemoglobin concentration <100 g/L. These authors concluded that regular consumption of commercially prepared iron-fortified cereal with iron fortified meat prevented iron deficiency even if iron intake were substantially below the recommended intake.

2.1.8 Infant Feeding Practices in Canada

The evolution of attitudes and guidelines on infant feeding is reflected in the changing trends in Canadian infant feeding throughout the years. For example, Tanaka et al. (Tanaka et al. 1987) compared infant feeding practices in 1977-78 (Montreal and Toronto) versus 1984-85 (Toronto). They found that mothers, in 1984-85 compared to 1977-78, breast-fed more and for longer, fed cow milk prior to six months of age less often and were introducing solids later (Table 2.2). The investigators note that changes closely correspond to infant feeding guidelines.

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<th>Table 2.2 Changes in Feeding Practice from 1977-78 to 1984-85</th>
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<td>% initiated breast-feeding</td>
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<td>% breast-feeding at 3 months</td>
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<td>% introduced to cow milk prior to 6 months</td>
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<td>% feeding solids at 3 months</td>
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Similarly, cultural attitudes may be reflected in regional differences in infant feeding practices across Canada. A study of infant feeding practices in Newfoundland and Labrador found relatively low breast-feeding rates (31.3%-51.3% at birth, 27% at four months) and a high usage of evaporated cow milk by four months (27%) (Matthews et al. 1995). Cereal was fed to 98.4% and meat was fed to 27.5% of the infants. A comparable group of infants was studied in Vancouver (Williams et al. 1996). Their breast-feeding rates were much higher (89.2% at birth) and cow milk was used by less than 1% of infants before six months of age. The use of cereal and meat was similar to Newfoundland and Labrador (85% and 27% respectively). Similar results were seen across Quebec where cereal was introduced at an average age of 3.5 ± 1 months and meat was introduced at 5.7 ± 0.6 months (Carceller et al. 1995).

Socio-economic level also has an effect on feeding patterns. A survey of one year olds from low socio-economic households in Montreal showed they had low breast-feeding rates (17% at birth and 6% by four months of age) and a high proportion were fed cow milk before six months of age (28%) (Lehmann et al. 1992). This data support the findings of a study of socio-demographic factors in Ottawa-Carleton (Greene-Finestone et al. 1989) and the above mentioned survey in Vancouver (Williams et al. 1996). Both of these studies also found that lower incidence and duration of breast-feeding and earlier introduction to solids was seen in families that were non-Caucasian, with lower income and lower education.
Infant feeding guidelines are established to ensure healthy growth and development. There is little doubt that such guidelines affect feeding choices, as suggested by Tanaka et. al (1987). However, to be effective, feeding guidelines must also consider the factors that affect compliance in the population. As shown above, feeding practices across Canada are affected by a number of factors.

2.1.9 Infant Feeding Guidelines

In recognition of the importance of the prevention of iron deficiency anaemia, the Nutrition Committee of the Canadian Paediatric Society (CPS) recently published guidelines for the prevention of iron deficiency anaemia in Canadian infants (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998). A summary of those guidelines follows: Exclusive breast-feeding until 6 months of age should be encouraged. For breast-fed infants, after 6 months, extra iron should be introduced in the form of iron-fortified infant cereals and other iron-rich foods. After weaning from breast milk, and for all formula fed infants, use iron-fortified formulas. Defer the introduction of cow milk until nine to 12 months of age.

Recommendations previously made that are important to this discussion include introduction of solid foods by six months of age emphasising good iron sources such as meat and infant cereals (Canadian Paediatric Society Nutrition Committee, 1979). The most profound changes from previous recommendations on the topic were the recommendations to use iron-fortified formula and the delayed introduction of cow milk.
Recent American Academy of Pediatrics (AAP) guidelines on the topic recommend that cow milk should not be fed to infants until after their first birthday (American Academy of Pediatrics 1992).

Guidelines such as the ones described here must fulfil certain criteria: the guideline must be efficacious, safe and effective within the target population. With respect to the first two criteria, the CPS guidelines are likely to be efficacious in preventing iron deficiency and safe if followed, although, the prohibition of cow milk until nine to 12 months of age may be overzealous. After six months of age, cow milk may be acceptable if other iron-rich foods are included in the diet (section 2.1.6.3). The guidelines are also relatively effective for much of the population since the foods advocated are available and acceptable infant foods. However, since publication of the CPS guidelines, there has been significant criticism and comment that a large segment of the Canadian population either cannot or will choose not to feed iron-fortified formula to their infants throughout the first year of life or after weaning from breast-feeding. The criticism emphasised that the CPS guidelines ignored the group at greatest risk, economically disadvantaged Canadian children whose parents could not purchase iron-fortified formula, a relatively expensive type of food (Shears 1991). The higher incidence of iron deficiency and earlier use of cow milk within this population support this criticism. Thus there remains a need to find an alternative approach to preventing iron deficiency in large groups of disadvantaged Canadian infants who are unlikely to follow CPS guidelines.
2.1.10 **Alternative Solution**

Although untested, iron-fortified infant cereal in combination with nonfortified meat can also meet iron needs of infants in the second six months of life. One to two jars per day of (70 to 140 g/day respectively) of commercial infant meat products will provide an intake of about 0.85-1.70 mg of iron/day. At an assumed bioavailability of 25%, the meat would provide 0.21-0.43 mg of absorbable iron per day, which is about 20%-54% of the estimated median need of the 6-12 month old infant. In addition iron-fortified infant cereals (regular commercial cereal) at an intake of 30 g/d (2/3 of a cup of dry cereal) will provide an iron intake of 10.2 mg/day. At a conservative assumed bioavailability of 4%, the cereal would provide 0.41 mg of absorbable iron per day. This is about 50% of the estimated median need of the 6-12 month old infant (Oski 1993). At this level of meat and cereal intake, total absorbable iron intake will be between 0.61 mg -0.84 mg/day, which is about 76% - 105% of the estimated median need of the 6 to 12 month old infant.

This amount may be further increased if cereal is consumed with meat since haeme iron enhances the absorption of iron from non-haeme sources by a factor of three or four. Meat and infant cereal are commonly used by infants by six months of age across Canada (see section 10.8). Thus, these iron-rich foods may prevent iron deficiency even in infants who are not breast- or formula-feeding, but are feeding whole cow milk. The efficacy of this low cost strategy to prevent iron deficiency must be tested before we can evaluate if it is effective in the segment of the Canadian population not following the CPS
guidelines.

2.2 Summary

There are three distinct phases in the development of iron deficiency marked by different laboratory measures. Presently, there is no single measure that is both sensitive and specific to functional iron deficiency. Quantification of circulating TfR appears to be a promising index of functional iron deficiency.

The most severe stage of iron deficiency, iron deficiency anaemia, is associated with impairments in mental and motor development of infants. It is unclear if these impairments can be reversed by treatment with iron but there is universal consensus that prevention of iron deficiency anaemia is essential. During the period between six and 12 months the infant is particularly vulnerable for iron deficiency since foetal reserves have been used and the increase in blood volume is rapid. Thus, the Canadian Paediatric Society (CPS) recently proclaimed that prolonged exclusive breast-feeding or the use of iron-fortified formula will prevent iron deficiency anaemia. Such feeding practices have been highly effective in preventing iron deficiency in Canada's general infant population. However, a large segment of the Canadian population either cannot or will choose not to breast-feed or feed iron-fortified formula during the first year of life.

It is clear that Canadian infants from low socio-economic households are at risk of iron deficiency. Two commonly suggested means to prevent iron deficiency anaemia in disadvantaged infants are the routine use of iron drops or to blood test all infants and to
treat only those with the condition. Neither of these interventions has proven effective. Thus an acceptable, low cost alternative remains to be found.

The use of iron-rich weaning foods such as meat and iron-fortified infant cereal in combination with whole cow milk, during the second six months of life, is one potential solution. Whole cow milk is widely accepted in Canada and is about one third the cost of iron-fortified infant formula. There is concern that cow milk causes occult blood loss in young infants and increases the risk for iron deficiency. This is indeed the case for infants younger than four months of age, but evidence indicates that there is little concern in infants six months of age and older.

In North America, infant cereal is iron fortified and despite low bioavailability, is a good source of iron. Meat is also a very good source of highly bioavailable iron (haeme-iron). In Canada, both of these weaning foods are widely used in the second six months of life. If consumed in adequate amounts, the combination of meat, infant cereal and cow milk may be a relatively inexpensive method of providing infants with the iron they need in the second six months of life.
3. INTRODUCTION TO EXPERIMENTATION

3.1 Hypothesis

There is a significant reduction in the incidence of iron depletion and/or anaemia in six to 12 month old cow milk-fed infants from low-income households consuming infant cereals (30g/day) and pureed meats (70g/day) when compared to infants from low-income households with no dietary intervention.

3.2 Objectives

(i) To examine the patterns of food usage within the study population and identify those that may be implicated in the cause or prevention of iron depletion in low-income families in Toronto (Chapter 5).

(ii) To determine the effect of the consumption of infant cereal and pureed meat in six to 12 month old cow milk-fed infants from low-income households compared to similar infants receiving no dietary intervention on the incidence of iron depletion and/or anaemia (Chapter 6).

(iii) To evaluate the use of transferrin receptor as a diagnostic measure of iron deficiency (Chapter 7).

The following chapter, Materials and Methods, details sample criteria, recruitment, protocol and data collection used to investigate the hypothesis. The rationale, description and results for each objective in the study are presented in Chapters
Patterns of food use are presented in Chapter 5 since the information they provide is important in understanding the outcome of Chapter 6. A general discussion of the findings is found in Chapter 8 and major conclusions of the thesis are presented in Chapter 9.
4. MATERIALS AND METHODS

4.1 Sample

4.1.1 Target Population

The target population consisted of healthy infants recruited before six months of age within or around the Metropolitan Toronto area. Included were 103 male and female infants fulfilling the following criteria:

- from low income households (defined by the low-income cut-offs established by Statistics Canada in 1991 plus $3500 for an estimated increase in the threshold for (96/97),
- full-term birth (38-42 weeks gestation),
- normal birth weight (above 2.5 kg),
- free from major illness or thalassaemia minor,
- ability of a parent to communicate in English.
- HgB > 110 g/L (to exclude anaemia and possible undiagnosed disease or thalassaemia minor) and serum Ferr > 10 μg/L (to exclude iron depletion)

During initial contact, the nature of the study was explained and any questions were answered. A Screening/History questionnaire (see Appendix A) was administered to establish eligibility. During the first visit (at six months of age), after informed consent was received (see Appendix B for consent and clinical information forms), a capillary blood sample was taken to determine the haematologic status of the infant.
4.1.2 **Subject Recruitment**

Initially, our target was to recruit 140 subjects, expecting that 110 would complete the study. Recruitment started in August of 1995 and ended in February 1997. Three different methods were used to contact potential subjects; however, only one proved to be successful:

Infants were recruited from community centres whose staff had agreed to distribute information letters (see Appendix B) and encourage eligible clients to contact us. These centres included:

- York Community Services
- Broadview Community Centre
- East End Community Services
- Regent Park Community Services
- Kingston Community Services

Despite constant effort in encouraging recruitment, this strategy had limited success and was discontinued in September 1996. Only seven subjects were recruited by this method.

Infants of teen mothers were recruited from the Adolescent Clinic (Tots and Teens Program) at the Hospital for Sick Children. Of the 15 subjects recruited by this method, ten were unable to complete the study either because of loss to follow up or lack of compliance to treatment. This strategy was discontinued in September 1997.

Primarily, infants were recruited by direct mailings using mailing lists supplied by Carnation Canada Ltd. Information letters and consent to contact forms (see Appendix
B) were mailed to families living in postal code regions where the median household income was below the 1991 low-income cut-offs defined by Statistics Canada. In all cases the initial invitation (mailing) asked the parents to contact the study personnel for information about the study using pre-addressed stamped postcards or by telephone. Consent was not solicited until there had been an opportunity to explain the nature of the study and the expected degree of involvement of the household. New mailing lists were generated every third month at the beginning of the study; however, by October 1996 lists were generated every month.

4.1.3 Randomisation

Stratified randomisation was used to assign infants, six months ± one week of age to a treatment or control group. Stratification was by Negroid ancestry to avoid difference in haemoglobin between whites and blacks. A one week window around scheduled dates was intended to encourage retention of subjects and provide for summer vacations and illness.

4.1.4 Haematologic End-point Definition

An end-point is a haematologic value, below which, anaemia or iron deficiency may be present. An end-point was declared on evidence of early iron deficiency anaemia (HgB < 110 g/L) or iron depletion (Ferr < 10 µg/L). If a putative end-point was detected, a second blood sample was drawn for HgB and Ferr. If any one of the same
indices of iron status was low in both the original and repeat examination, an end-point was declared, the child was discharged from the study and the parents and physician were advised. If the level in the second sample was above the end-points, the infant continued in the study.

4.2 Protocol

Figure 4.1 describes the overall study protocol. The protocol was followed until the infant reached the age of 12 months ± one week or until a confirmed haematologic end-point was reached. Infants eligible to enter the study were randomised to one of two groups:

The treatment group consisted of infants receiving cereal and meat along with whole cow milk (ad libitum amounts) as their sole source of milk intake. Parents received instructions to use meat at an intake of at least 70 g/day (1 jar) to 140 g/day (2 jars). A wide selection of commercial infant meat products (including beef, chicken, ham, lamb, turkey, veal and meat-vegetable combinations) were provided to the parents free of charge (see Appendix D for product list). Parents also received instructions on use of iron-fortified infant cereals (regular commercial cereal) at an intake of at least 30 g/d (2/3 of a cup of dry cereal). A choice of four infant cereals (containing iron at 0.34 mg/g cereal) were provided at no charge to the parents.

One of the main purposes of this research was to test an alternative to past approaches used to prevent iron deficiency that, although efficacious, have not proven
effective in the population under study. Thus, the use of vitamin/mineral supplements containing iron and other milk sources such as iron-fortified formulas were restricted since their use by treatment infants would confound the results of the study. Other than these exclusions, parents were free to feed their infants whatever other foods they deemed appropriate, including vegetables, fruit, etc.

The control group was not provided with any feeding instructions or food products. They were free to feed their infants whatever foods they deemed appropriate. To minimise differences between groups due to financial advantages of being in one group or the other, families were provided with laundry detergent and infant clothing worth an equivalent dollar value to the food provided to the treatment group (see Appendix D for product schedule).

The study protocol was explained and detailed instruction booklets were provided during the first visit (see Appendix B). Furthermore, parents were encouraged to call if they had any questions or concerns throughout the study period.
Low iron = hemoglobin < 110 g/L or ferritin < 10 μg/L.

164 eligible subjects randomized

Treatment n=79
- meat, milk, cereal
- no other milk, no Fe-supp

6, 8, 10, 12 months
- blood, dietary, anhemo

Rejected by low baseline blood n=6
- withdrawn n=14
- lost to follow-up n=14
Income too high/halassaemia n=2

Completed Trial
n=45

Control n=77
- no dietary intervention

6, 8, 10, 12 months
- blood, dietary, anhemo

Rejected by low baseline blood n=2
- withdrawn n=5
- lost to follow-up n=13
Income too high/halassaemia n=5

Completed Trial
n=54

Low iron = hemoglobin < 110 g/L or ferritin < 10 μg/L.

Recheck blood
Normal on recheck
Low iron

Exclude (if at initial visit) or declare end-point

Treat subject

Figure 4.1 Experimental Protocol
4.2.1 End-point Protocol

Restrictions on use of iron containing vitamin/mineral supplements and other milk sources, such as iron-fortified formula, were removed for infants in the treatment group who reached end-point. Infants reaching end-point, in either group, due to iron depletion before the age of nine months were supplied with iron-fortified Carnation follow-on formula (Nestle Canada Inc.) until they reached nine months of age, in accordance with CPS guidelines (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998). Infants reaching end-point due to iron deficiency anaemia before nine months of age were supplied with Carnation follow-on formula and with a one-month supply of Fer-in-Sol (Mead Johnson Canada) providing the equivalent of 15mg elemental iron per day. Infants nine months or older who had reached end-point, by either criteria, were not supplied with formula but were provided with Fer-in-Sol drops. Formula was not provided at this age since many infants in both groups were expected to be regularly consuming or soon to be consuming cow milk. Infants were followed for one month after declaration of end-point or left to the care of their doctor to ensure that their iron status had recovered.

4.2.2 Blinding

Although it was not possible to have the parents or study nurses blinded, the technician performing the laboratory analyses and the investigator responsible for data analysis were blinded with regard to group randomisation.

46
4.2.3 **Sample Size Calculation**

The hypothesis tested was that the 'cereal and meat' group would yield a clinically efficacious result compared to the control group. In the recent study by Beaton et al. (NHRDP Project 6606 4104 61), 20% of infants in the iron-fortified cereal group had evidence of iron depletion (rates of iron deficiency were not determined). There is no data available on the expected end point rate from a 'meat alone' group. The only data available on the expected end point rate from a 'meat + cereal' group are that of Haschke (1988) who described a 0 - 8 % rate of iron depletion, however, Haschke's meat was iron fortified. Thus, for the purposes of calculating the sample size, an end point rate of ≤ 10% in the 'cereal + meat' group was considered to be clinically significant.

As noted in section 2.1.4, in Montreal, iron deficiency anaemia was detected in 25% of socio-economically disadvantaged children and iron depletion in 37% (Lehmann et al. 1992). These rates are unacceptably high. In a prevalence study across Canada, iron deficiency anaemia was detected in 4.3% of the children and iron depletion in 33.9% (Zlotkin et al. 1996). Beaton et al. (NHRDP Project 6606 4104 61) found a 20% rate of iron depletion in infants, receiving iron-fortified cereal and non iron-fortified formula or breast milk, based on a low Ferr confirmed in a second sample. Thus, for the purposes of calculating the sample size, it is estimated that an end-point rate of 30% (a confirmed end-point) would be expected in a disadvantaged population in Canada. Comparing estimated end-point rates from the treatment to the control group, with a type I error of
5%; type II error of 20%; and a two-tailed test, conventional sample size estimates yields about 55 infants per group (see Appendix E for detailed calculation).

4.3 Data Collection

4.3.1 Food Intake and Socio-demographic Information

Socio-demographic information and retrospective dietary patterns were obtained from parents of infants in both groups using a Background and an Initial Dietary History questionnaire during the first visit (at six months). Current feeding patterns were obtained using questionnaires during the eight, ten and 12 month visits (Compliance Check questionnaire for treatment and Criteria Check for control). Three-day food records were obtained at 8.5 and 10.5 months of age to compute energy, iron and macronutrient intakes (reprints of questionnaires appear in Appendix A).

4.3.2 Anthropometric Measures

Nude body-weight, recumbent length and head circumference were measured in triplicate during each home visit (six, eight, ten and 12 months). Infants were weighed by trained nurses using accurate portable infant scales (Health o meter model 386). A five-pound standard weight was used to zero all scales at each visit. Recumbent length was measured, with the assistance of the parent, using an infantometer constructed by the engineering department of the Hospital for Sick Children (Toronto, Canada). Head circumference was measured, with the assistance of the parent, using a flexible plastic
tape measure. Details of these methods appear in the Nurse’s Guide Booklet (see Appendix C).

4.3.3 **Health Status and Compliance**

Daily records of morbidity were recorded in both groups during the study period. Daily meat and cereal consumption was also recorded as a measure of compliance, in the treatment group, on calendars provided to the families (see Appendix B). Treatment families were asked to feed at least one jar of meat and 2/3 cup cereal per day. This recommendation was based on theoretical calculations of iron utilisation in section 2.1.10 and were meant to act as a guideline for the parents. The utilisation of dietary iron can vary by more than 100% depending on the true absorption (which largely depends on iron need, the type of iron in the food and the presence or absence of iron-inhibitors and enhancers). We arbitrarily defined compliance as infants who were consistently using meat and cereal throughout the study period. Each month, mothers answered the monthly compliance check questionnaire (Appendix A) that asked, among other questions, if their infants consumed any meat and if they consumed any cereal in the last month. Six mothers consistently said ‘no’ to one of these questions each month they were asked. In each of these cases, the lack of meat or cereal intake was confirmed by the daily meat and cereal use calendars (Table 5.6, p.83). Infants from these families were treated as non-compliers.

Six mothers from the treatment group reported that their infants did not consume
cereal and/or meat during the first month of the study when they were first trying to introduce these foods. These infants were not excluded as non-compliers because their meat and cereal intakes increased as the study went on. All other families reported that their infants did consume both meat and cereal each month they were asked.

4.3.4 Blood Samples

Samples were collected at six, eight, ten and 12 months of age (± one week) in capillary tubes, via finger prick, by trained nurses during home visits (see Appendix C for details). If the infant was sick on the scheduled sampling day, the visit was rescheduled. Haemoglobin and ferritin were measured on each occasion. Transferrin receptor was measured in batch at the end of the study, since it was not used to define end-points. A total volume of 250 μL of blood was collected at each visit.

4.4 Laboratory Analyses

Blood samples, collected in anticoagulant treated tubes from infants in their homes, were kept cool until transferred to the laboratory of Dr. S. Zlotkin at the Hospital for Sick Children. The cyanmethemoglobin method was used to assay HgB (Drabkin and Austin 1932). A standard haemoglobin solution was used to control accuracy for all samples. Plasma Ferr was assayed by an immunoradiometric assay using ‘Fer-Iron’ kits (Mills et al. 1974). This kit utilised the Lyphochek Anaemia Control as an external reference standard. HgB and Ferr samples were analysed on weekly basis upon receipt. Plasma TfR were measured in triplicate by an indirect enzyme-linked immunosorbent
assay (ELISA) method (Cooper and Zlotkin 1996). The standard used in the assay was transferrin receptor isolated from human placental tissue using the technique of Turkewitz et al. (Turkewitz et al. 1988). Primary monoclonal antibody against human transferrin receptor was supplied by Cetus/Chiron (Emeryville, CA) (clone 454A12); secondary goat antibody to mouse enzyme conjugate against primary antibody (alkaline phosphatase) was supplied by Calbiochem (San Diego, CA). The typical working range of the assay as developed is 0-30 g/ml, with a sensitivity of 1 mg/L. Over this range of concentration the standard curve has a correlation coefficient of typically 0.95. The dose response curve for this assay shows a recovery (% actual/expected TfR concentration) of 96% at dilutions up to 15 times normal dilution.

4.5 Data Management, Statistical Analysis and Quality Control

All data, except dietary intakes, were entered in the computer within seven days of collection. Screening/history, identification (core), blood and anthropometric data were entered in applications written in SAS/FSP. These applications were linked by 'family number' and 'subject code' so that only the core data set identified subjects and randomisation assignments. All other data were entered by code. Blood and anthropometric entry applications had range checks and re-entry screens to insure quality control. Dietary and demographic data were entered in applications created in Microsoft Access with specific validation rules and scroll down menu choices to aid in accurate data entry.
Weight and height were transformed to z-scores using the Epi Nut module of the US Centers for Disease Control (CDC) Epi Info 6.04 software package. This used the National Center for Health Statistics (NCHS) reference data as a standard, as is recommended for international reference by the World Health Organisation (WHO). Head circumference was not transformed since there is no representative reference standard available. Slope of change in anthropometrics with age was compared between treatment and control groups.

The primary analysis compared the occurrence of end-points (iron depletion or mild anaemia) between the two groups using Mantel-Haenszel Chi-square analysis (Czajka-Nairns et al. 1978). Secondary analysis compared slope of change, for HgB, Ferr and TfR with age, between treatment and control groups.

All data analysis was conducted with SAS version 6.12 (SAS Institute, Inc., Care, NC). The acceptable level of statistical significance for all tests was p<0.05.
5. FOOD USE

5.1 Introduction

Nutrition in infants and young children can affect growth and morbidity since it is the period of most rapid growth and development and highest nutrient requirements in human life. For example, iron deficiency is a great concern in the second six months of life since body iron needs increase about 70% due to rapid growth (and therefore rapid expansion of blood volume) and basal losses (Dallman 1988). Thus, guidelines, such as the CPS guidelines have been published (Canadian Paediatric Society Nutrition Committee, 1979; Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998).

In general, it appears that Canadian parents adhere to the CPS recommendations (Tanaka et al. 1987; Williams et al. 1996; Zlotkin et al. 1996) with the exception of those from low socio-economic households and specific cultural groups (Lehmann et al. 1992; Williams et al. 1996; Yeung 1993). There has been significant criticism that the CPS guidelines ignore the majority of economically disadvantaged families who either cannot or will choose not to feed iron-fortified formula throughout the first year of life or after weaning from breast milk (Shears 1991). These families tend not to breast-feed or use iron-fortified formula after six months; but instead, they often introduce cow milk at an early age (Greene-Finestone et al. 1989; Lehmann et al. 1992). This is likely the explanation for the higher incidence of iron deficiency in this population (Greene-Finestone et al. 1989; Lehmann et al. 1992).
Thus, the purpose of this study was to examine the patterns of food usage within the study population and identify those that may be implicated in the cause or prevention of iron depletion in low-income families in Toronto. This chapter also examined differences in the backgrounds and feeding patterns between the control and treatment groups.

5.2 Data Generated

For this phase of the project, socio-demographic, food introduction/termination, current food use and anthropometric data were used. Food introduction/termination data, from birth to six months, were based on retrospective Initial Dietary History questionnaires, which were administered by the nurse during the first home visit (at six months of age). Current food use was measured at each visit (six, eight, ten and 12 months of age) for all infants studied. Data obtained from the control group provided information on normal feeding patterns of the sample surveyed. Data obtained from the treatment group gave an indication of compliance to the intervention and overall feeding pattern.

All anthropometric measurements were done in triplicate to insure accuracy. For weight and height, the mean of these replicates was converted to z-scores using the median values from the NCHS reference standards. Weight for age, height for age and weight for height z-scores were generated. Head circumferences were not transformed because a representative standard does not currently exist.
5.3 Statistical Analysis

Chi-square tests were performed to determine potentially significant differences in human milk, formula and cow milk feeding at birth and six months with respect to parental marital status, education, ethnicity and income expressed as a percentage of low-income cut-offs. Level of education was categorised as having primary to high school, college or university education. Ethnicity was categorised as Caucasian and non-Caucasian (predominantly of Negroid ancestry, Oriental, East Indian and Hispanic). Income as a percentage of low-income cut-offs based on definitions from Statistics Canada, 1996 was broken down into tertiles (less than 30%, between 30 and 80%, greater than 80%)

Chi-square tests were performed to determine potential differences in feeding practices between the two study groups. Use of specific foods such as breast milk and iron-fortified formula at birth and at six months of age were compared between the treatment and control groups. This analysis was used to look for differences in non-fortified formula use and use of cow milk with respect to study groups, at six months of age only, since very few infants were consuming these at birth and for cereal use at three months (representing the first solid food introduced).

Chi-square tests were performed to determine potential differences in feeding practices between those infants who had normal and low iron indices (Ferr < 10 μg/L or Hgb < 110 g/L). Differences in the use of specific foods such as breast milk, iron-
fortified and non-fortified formula, cow milk, cereal and meat and iron supplements at eight, ten and 12 months with respect to infants who had any low iron indicator during the study and those who did not were compared.

The General Linear Models (GLM) procedure was used to examine change in anthropometry over time/age, taking advantage of the repeated measures on individuals. This made it possible to estimate the coefficients for the slope of change with age and to contrast the estimates for differential slope in the two groups. Treatment group and family identification number were used as class variables with family number nested within treatment group. Analysis of head circumference was carried out for each gender separately, since there was no suitable standard by which to transform the data to z-scores.

5.4 Estimates of Morbidity and Compliance

Perceived morbidity was recorded daily by the care-giver on the calendars provided (see Appendix B). The information was categorised as “well”, “mild” illness, “diarrhoea” and “severe” illness. “Mild” illness included minor cold, cough, teething or ‘colic’. “Diarrhoea” was defined as having more than four loose stools in a day. “Severe” illness included high fever or clinical sickness requiring medical attention. The data were summarised as the proportion of recorded days that were identified as falling into each category.

Compliance was assessed each month, using the monthly compliance check
questionnaire (Appendix A) that asked, among other questions, if their infants consumed any meat and if they consumed any cereal in the last month. Six mothers consistently said ‘no’ to one of these questions each month they were asked. In each of these cases, the lack of meat or cereal intake was confirmed by the daily meat and cereal use calendars (Table 5.6, p.83). These calendars were filled out by mothers in the treatment group who were asked to recall and report how many jars of meat and how many cups of dry cereal were fed each day. Infants from these families were treated as non-compliers.

5.5 Results

5.5.1 Recruitment and Fate of Subjects Recruited

Table 5.1 presents a summation of counts of subjects deemed eligible for admission into the controlled trial and the disposition of those subjects in the trial. Families were screened for income prior to enrolment, however, a number were later found having too high an income. Two infants were also excluded on suspicion of thalassaemia minor based on HgB<100 g/L and Ferr>40 μg/L. The majority of “treatment” families that were included in the “withdrew” category were unable or unwilling to exclusively feed cow milk. Included in the “other” category are families that we lost contact with. Many of these families were recruited from the Teen Clinic at the Hospital for Sick Children.

Table 5.2 presents the characteristics of subjects included in the start of the study. All characteristics were statistically comparable between the two groups. One subject was
not included in this table because she had missed the first visit and had started at eight months of age.

The family background of subjects included in the trial are presented in Table 5.3. The mean household income expressed as percentage of low-income cut-off based on definitions from Statistics Canada, 1996 for the entire study population was 79%. Although not statistically different, mean income was higher in the control group while household size was not different. Mean income was below the low-income cut-off for both groups when expressed as percentage of low-income cut-off (Statistics Canada, 1996). Slightly more than half of the fathers and mothers surveyed had primary to secondary school education. More fathers in the control compared to treatment group had reached university and college. Overall, education level was not significantly different. An approximately equal proportion of mothers, between treatment and control groups, had reached only primary to secondary school. Although more mothers from the treatment group had attended college more mothers from the control group had attended university. Overall, mothers in the control group had a higher level of education ($p=0.05$) and no mother in either group has less than grade 11 education.

Infants were primarily of Caucasian ancestry followed by Negroid. Stratification of infants by Negroid ancestry was successful since both groups had roughly equal ethnic mixes.
Table 5.1 Disposition of Subjects

<table>
<thead>
<tr>
<th>Disposition of Subject(^1)</th>
<th>Total Group</th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admitted to screen</td>
<td>164</td>
<td>85</td>
<td>79</td>
</tr>
<tr>
<td>Rejected by blood values</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Actually Entered Study</td>
<td>156 (100%)</td>
<td>79 (100%)</td>
<td>77 (100%)</td>
</tr>
<tr>
<td>Withdrew from study*</td>
<td>19</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Moved</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Too many missed visits</td>
<td>14</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Income later found to be too high</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Suspected Thalassaemia Minor</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other reasons for discharge</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Completed</td>
<td>103 (66.0%)</td>
<td>49 (62.0%)</td>
<td>54 (70.0%)</td>
</tr>
</tbody>
</table>

\(^1\)Subjects admitted to screen appeared to have met all eligibility criteria except the initial blood values.
* more infants withdrew from the treatment group, n=156, Chi-square:4.596, 1 degree of freedom, p<0.032
Table 5.2 Characteristics of All Subjects Who Completed the Study

<table>
<thead>
<tr>
<th>Measure</th>
<th>Total Group</th>
<th>Treatment Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>54 (52.4%) male 49 (47.6%) female</td>
<td>24 (49.0%) male 25 (51.0%) female</td>
<td>30 (55.5%) male 24 (44.4%) female</td>
</tr>
<tr>
<td>Birth Order</td>
<td>1.7 ± 1.0</td>
<td>1.7 ± 1.1</td>
<td>1.6 ± 0.9</td>
</tr>
<tr>
<td>Birth Weight (g)</td>
<td>3487 ± 535.0</td>
<td>3500 ± 518</td>
<td>3459 ± 554</td>
</tr>
<tr>
<td>Age at First Visit (mo)</td>
<td>6.0 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>7.89 ± 1.0</td>
<td>7.8 ± 1.0</td>
<td>7.9 ± 1.1</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>67.4 ± 3.0</td>
<td>67.3 ± 3.0</td>
<td>67.5 ± 3.0</td>
</tr>
<tr>
<td>Head Circumference (cm)</td>
<td>44.3 ± 1.5</td>
<td>44.3 ± 1.5</td>
<td>44.4 ± 1.6</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>128.5 ± 11.0</td>
<td>126.6 ± 9.6</td>
<td>130.3 ± 11.9</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>39.1 ± 25.0</td>
<td>42.7 ± 30.8</td>
<td>35.8 ± 17.9</td>
</tr>
</tbody>
</table>

Means expressed as mean ± SD.
Means were compared between groups and differences were not significant p>0.05
Table 5.3 Family Background for Infants Who Completed the Study

<table>
<thead>
<tr>
<th>Measure</th>
<th>Total Group</th>
<th>Treatment Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Income</strong></td>
<td>$23,372 ± 8,941</td>
<td>$20,189 ± 8,620</td>
<td>$24,960 ± 8,942</td>
</tr>
<tr>
<td>% Low-Income cut-offf</td>
<td>79%</td>
<td>69%</td>
<td>83%</td>
</tr>
<tr>
<td><strong>Number of Family Members</strong></td>
<td>3.8 ± 1.3</td>
<td>3.6 ± 1.2</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td>Single parent families</td>
<td>35 (35%)</td>
<td>18 (38.3%)</td>
<td>17 (32.1%)</td>
</tr>
<tr>
<td>No. of Fathers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 years of age</td>
<td>2 (2%)</td>
<td>1 (2.1%)</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td>20-29 years</td>
<td>22 (22.2%)</td>
<td>15 (31.9%)</td>
<td>7 (13.5%)</td>
</tr>
<tr>
<td>30-39 years</td>
<td>39 (39.4%)</td>
<td>14 (29.8)</td>
<td>25 (48.1%)</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>6 (6.1%)</td>
<td>3 (6.4%)</td>
<td>3 (5.8%)</td>
</tr>
<tr>
<td>Father's Education:</td>
<td>% reaching this level</td>
<td>% reaching this level</td>
<td>% reaching this level</td>
</tr>
<tr>
<td>Primary to secondary</td>
<td>54.0% (11.5 ± 1.0)</td>
<td>66.7% (11.4 ± 1.1)</td>
<td>42.4% (11.7 ± 1.0)</td>
</tr>
<tr>
<td>College</td>
<td>19.0% (2.5 ± 1.0)</td>
<td>16.7% (2.2 ± 0.8)</td>
<td>21.2% (2.6 ± 1.2)</td>
</tr>
<tr>
<td>University</td>
<td>27.0% (4.8 ± 2.4)</td>
<td>16.7% (3.8 ± 1.1)</td>
<td>36.4% (5.2 ± 2.7)</td>
</tr>
<tr>
<td>No. of Mothers:</td>
<td>% reaching this level</td>
<td>% reaching this level</td>
<td>% reaching this level</td>
</tr>
<tr>
<td>&lt;20 years of age</td>
<td>7 (7%)</td>
<td>2 (4.3%)</td>
<td>5 (9.4%)</td>
</tr>
<tr>
<td>20-29 years</td>
<td>56 (56%)</td>
<td>26 (40.4%)</td>
<td>30 (56.6%)</td>
</tr>
<tr>
<td>30-39 years</td>
<td>36 (36%)</td>
<td>19 (40.4%)</td>
<td>17 (32.1%)</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>1 (1%)</td>
<td>0</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td>Mother's Education:</td>
<td>% reaching this level</td>
<td>% reaching this level</td>
<td>% reaching this level</td>
</tr>
<tr>
<td>Primary to secondary</td>
<td>51.1% (11.2 ± 1.2)</td>
<td>52.2% (11.2 ± 1.3)</td>
<td>50% (11.2 ± 1.2)</td>
</tr>
<tr>
<td>College</td>
<td>28.7% (1.9 ± 0.9)</td>
<td>37.4% (1.7 ± 1.0)</td>
<td>20.8% (2.2 ± 0.6)</td>
</tr>
<tr>
<td>University</td>
<td>20.2% (3.4 ± 1.4)</td>
<td>10.1% (3.0 ± 1.0)</td>
<td>29.2% (3.6 ± 1.5)</td>
</tr>
<tr>
<td>Ethnic Background</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>55 (55.0%)</td>
<td>27 (57.4%)</td>
<td>28 (52.8%)</td>
</tr>
<tr>
<td>Oriental</td>
<td>4 (4.0%)</td>
<td>1 (2.1%)</td>
<td>3 (5.7%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>5 (5.0%)</td>
<td>2 (4.3%)</td>
<td>3 (5.7%)</td>
</tr>
<tr>
<td>Negroid</td>
<td>25 (25.0%)</td>
<td>13 (27.7%)</td>
<td>12 (22.6%)</td>
</tr>
<tr>
<td>East Indian</td>
<td>9 (9.0%)</td>
<td>3 (6.4.5%)</td>
<td>6 (11.3%)</td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>2 (2.0%)</td>
<td>1 (2.1%)</td>
<td>1 (1.9%)</td>
</tr>
</tbody>
</table>

Means expressed as mean ± SD.

1Low-income cut-offs defined by Statistics Canada, 1996

*Level of education reached and group assignment are related, n=94, Chi-square:6.04,
1 degree of freedom, p<0.05, education data for mothers was missing for 9 subjects.
5.5.2 Diet History: Birth to Six Months

5.5.2.1 Milk Use. Dietary data was collected for 100 of the infants surveyed. The overall rate for initiation of breast-feeding was 83%, which dropped to 30% by six months of age. The overall incidence of breast-feeding was not different at birth but was higher at six months for families whose mothers had higher education (n=90, Chi-square: 3.79, 1 degree of freedom, p=0.05; n=68). There were no differences in breast-feeding incidence at birth or six months with respect to parental marital status, ethnicity and income expressed as a percentage of low-income cut-offs.

A smaller proportion of mothers initiated breast-feeding in the treatment group at birth (75% of treatment, 90.7% of control, Chi-square=4.53, 1 degree of freedom, p=0.03). This difference continued (Chi-square=4.96, 1 degree of freedom, p=0.03) as breast-feeding declined steadily in both groups to 18.7% of treatment and 38.8% of control by six months of age (Figure 5.1).

Iron-fortified formula was fed to 19% of the infants surveyed at birth increasing to 77% by six month of age. There were no differences in iron-fortified formula-feeding incidence at birth or six months with respect to parental education, marital status, ethnicity and income expressed as a percentage of low-income cut-offs. At birth, more infants from the treatment group were fed iron-fortified formula than from the control (28.3% of treatment, 11.1% of control, Chi-square=4.75, 1 degree of freedom, p=0.03). By six months, the proportion increased to 62.6% of treatment and 60.7% of control and there was no difference between groups (Figure 5.2).
Non-iron-fortified formula was generally not fed to the surveyed infants at birth and only fed to 25.6% of the infants by six months. There were no significant differences in incidence between groups at any age prior to six months (Figure 5.3). Very few infants were fed cow milk prior to six months (10% of treatment compared to less than 4% of control infants) (Figure 5.4). This was not significantly different.

5.4.2.2 Cereal and Meat Use. Generally, infant cereal was the first solid food fed to infants at around three months to five months of age. By three months, more treatment infants were consuming cereal than control (47.9% and 24.5% respectively, Chi-square=6.01, 1 degree of freedom, p=0.01). By five months, virtually all of the infants were eating cereal in both groups (Figure 5.5). Meat was not introduced to infants until after six months in the majority of infants. By six months of age, meat was being consumed by 25% treatment and 24.5% of the control group (Figure 5.6).

5.4.2.3 Other Foods. Vegetables and fruits were introduced to the majority of infants surveyed between four to five months of age. There were no significant differences between groups (Figure 5.7). Fruit juices were introduced to the majority of infants between four to six months of age. There were not significant differences between study groups (Figure 5.8). In general, foods or supplements, other than those already described, were not used by the families surveyed.
Figure 5.1 Proportion of Infants Breast-Feeding: Birth-6 Months

Figure 5.2 Proportion of Infants Fed Iron-Fortified Formula: Birth-6 Months
Figure 5.3 Proportion of Infants Fed Non-Fortified Formula: Birth-6 Months

Figure 5.4 Proportion of Infants Fed Whole Cow Milk: Birth-6 Months
Figure 5.5 Proportion of Infants Fed Infant Cereal: Birth-6 Months

Figure 5.6 Proportion of Infants Fed Meat: Birth-Six Months
Figure 5.7 Proportion of Infants Fed Fruits/Vegetables: Birth-Six Months

Figure 5.8 Proportion of Infants Fed Juice: Birth-Six Months

67
5.5.3 Feeding Practices During the Study Period: Eight to 12 Months of Age

Associations between specific food intake (regardless of amount) and low iron indices (Ferr < 10 μg/L or HgB < 110 g/L) were examined (Table 5.4). The only significant association was with cow milk use at ten months of age. The Relative Risk for an infant ingesting cow milk and having a low Ferr or HgB was 1.37 with 95% confidence intervals of 1.007 to 1.859. Infants who were still being breast-fed at 12 months tended to have low iron indices (p=0.063). No other foods were associated with an increased risk for low iron indices.
Table 5.4 Specific Food Use Related to Low Ferr or HgB

<table>
<thead>
<tr>
<th>Food Use</th>
<th>N</th>
<th>Chi-square</th>
<th>Degrees of freedom</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast milk: 8 months of age</td>
<td>95</td>
<td>0.19</td>
<td>1</td>
<td>0.77*</td>
</tr>
<tr>
<td>10 months of age</td>
<td>93</td>
<td>0.12</td>
<td>1</td>
<td>0.51*</td>
</tr>
<tr>
<td>12 months of age</td>
<td>92</td>
<td>3.70</td>
<td>1</td>
<td>0.063*</td>
</tr>
<tr>
<td>Iron-fortified formula: 8 months of age</td>
<td>91</td>
<td>0.008</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td>10 months of age</td>
<td>91</td>
<td>0.008</td>
<td>1</td>
<td>1.0*</td>
</tr>
<tr>
<td>12 months of age</td>
<td>93</td>
<td>0.875</td>
<td>1</td>
<td>0.097*</td>
</tr>
<tr>
<td>Non-fortified formula: 8 months of age</td>
<td>91</td>
<td>3.58</td>
<td>1</td>
<td>0.11*</td>
</tr>
<tr>
<td>10 months of age</td>
<td>91</td>
<td>3.37</td>
<td>1</td>
<td>0.11*</td>
</tr>
<tr>
<td>12 months of age</td>
<td>93</td>
<td>0.21</td>
<td>1</td>
<td>0.54*</td>
</tr>
<tr>
<td>Cow milk: 8 months of age</td>
<td>94</td>
<td>1.36</td>
<td>1</td>
<td>0.24</td>
</tr>
<tr>
<td>10 months of age</td>
<td>92</td>
<td>4.41</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>12 months of age</td>
<td>93</td>
<td>0.36</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Cereal: 8 months of age</td>
<td>96</td>
<td>0.002</td>
<td>1</td>
<td>1.0*</td>
</tr>
<tr>
<td>10 months of age</td>
<td>93</td>
<td>2.25</td>
<td>1</td>
<td>0.18*</td>
</tr>
<tr>
<td>12 months of age</td>
<td>94</td>
<td>0.43</td>
<td>1</td>
<td>0.51</td>
</tr>
<tr>
<td>Meat: 8 months of age</td>
<td>96</td>
<td>0.48</td>
<td>1</td>
<td>0.49</td>
</tr>
<tr>
<td>10 months of age</td>
<td>92</td>
<td>1.07</td>
<td>1</td>
<td>0.38*</td>
</tr>
<tr>
<td>12 months of age</td>
<td>94</td>
<td>0.017</td>
<td>1</td>
<td>1.0*</td>
</tr>
<tr>
<td>Iron supplements: 8 months of age</td>
<td>51</td>
<td>0.29</td>
<td>1</td>
<td>0.68*</td>
</tr>
<tr>
<td>10 months of age</td>
<td>49</td>
<td>0.33</td>
<td>1</td>
<td>0.62*</td>
</tr>
<tr>
<td>12 months of age</td>
<td>94</td>
<td>0.21</td>
<td>1</td>
<td>0.76*</td>
</tr>
</tbody>
</table>

*Fisher's Exact Test used because 25% of cells have 5 or less counts

5.5.3.1 Milk Use. During the study period, control infants were primarily fed iron-fortified infant formula while treatment infants were fed whole cow milk as their only milk source, in accordance to the study protocol. Approximately 20% of control infants were consuming breast milk throughout the study period. Treatment infants did not consume breast milk in accordance to the study protocol (Figure 5.9). The proportion of control infants fed iron-fortified formula decreased from 61.2% at eight months to
49.0% at 12 months of age. Infants in the treatment group did not consume iron-fortified formula during the study period (Figure 5.10). Use of non-fortified formula by control infants dropped from 18.4% at eight months to 6.1% at 12 months. Treatment infants did not use non-fortified formula (Figure 5.11). In contrast, all treatment infants consumed cow milk. Few control infants used cow milk until 12 months when 50% did so (Figure 5.12).

5.5.3.2 Cereal and Meat Use. At eight months of age, iron-fortified infant cereal was fed to nearly all of the infants in both groups. By the end of the study, this declined to approximately 75.5% (Figure 5.13). Meat was used by 63.5% of control infants at eight months of age. This increased to 95.5% by the time the infants were 12 months of age. Nearly all infants in the treatment group consumed meat throughout the study period (Figure 5.14).

5.5.3.3 Use of Supplements Containing Iron. About 25% of control infants were using iron supplements throughout the study period. Infants in the treatment group did not use supplements containing iron as dictated by the study protocol (Figure 5.15).

5.5.3.4 Other Food Use. Vegetables, fruits and fruit juices were used by almost all of the infants throughout the study period. Foods or supplements other than those described were generally not consumed by the study infants.
Figure 5.9 Proportion of Infants Consuming Breast Milk: 8-12 Months

Figure 5.10 Proportion of Infants Consuming Iron-Fortified Formula: 8-12 Months
Figure 5.11 Proportion of Infants Consuming Non-Fortified Formula: 8-12 Months

Figure 5.12 Proportion of Infants Consuming Whole Cow Milk: 8-12 Months
Figure 5.13  Proportion of Infants Consuming Infant Cereal: 8-12 Months

Figure 5.14  Proportion of Infants Consuming Meat: 8-12 Months
Figure 5.15 Proportion of Infants Consuming Iron Supplements: 8-12 Months
5.4.4 Anthropometry

Weight for age z-score (WAZ) fell progressively with age in the control but not the treatment group (slope = -0.05 ± 0.02, p=0.003; slope = -0.03 ± 0.02, p=0.08 respectively). However, there was no difference in the rate of change between groups (p=0.42) (Figure 5.16). There was a moderate but significant increase in height for age z-score (HAZ) across age in the treatment (slope=0.04 ± 0.01, p=0.0006) but not control group (slope = -0.003 ± 0.01, p=0.77). The rate of change between the two groups was significantly different (p=0.007) (Figure 5.17); however, mean HAZ was not different between groups at any age (Table 5.5). Weight for height z-score (WHZ) did not change for either group over age (slope = -0.03 ± 0.02, p=0.11 for treatment; slope = -0.03 ± 0.02, p=0.15 for control) and there was no difference between groups (p=0.96) (Figure 5.18). Overall, infants were growing around the NCHS growth standard median in terms of weight, height and weight for height.

Head circumference increased with age for treatment and control infants for both males (slope=0.46 ± 0.02, p=0.0001; slope=0.48 ± 0.02, p=0.0001 respectively) (Figure 5.19) and females (slope=0.47 ± 0.02, p=0.0001; slope=0.48 ± 0.02, p=0.0001 respectively) (Figure 5.20). The rates of change were not different between study groups for either male (p=0.30) or females (p=0.78).
Figure 5.16 Weight for Age z-score (WAZ). Differences from ‘0’ in slopes of change for Control (---) and Treatment (-----) groups: Control: slope = -0.03 ± 0.02, p=0.08; Treatment: slope = -0.05 ± 0.02, p=0.003
Differences in slopes of change between study groups F= 0.64, 1, 285 degrees of freedom, p=0.42
Figure 5.17 Height for Age z-score (HAZ). Differences from '0' in slopes of change for Control (—) and Treatment (-----) groups: Control: slope = -0.003 ± 0.01, p=0.77; Treatment: slope=0.04 ± 0.01, p=0.0006
Differences in slopes of change between study groups F= 7.38, 1, 290 degrees of freedom, p=0.007
Figure 5.18 Weight for Height z-score (WHZ). Differences from '0' in slopes of change for Control (—) and Treatment (-----) groups: Control: slope = -0.03 ± 0.02, p=0.15; Treatment: slope = -0.03 ± 0.02, p=0.11
Differences in slopes of change between study groups F<0.001, 1, 284 degrees of freedom, p=0.96
Figure 5.19  Head Circumference by Age (males). Differences from '0' in slopes of change for Control (---) and Treatment (-----) groups: Control: slope = 0.48 ± 0.02, 
p=0.0001; Treatment: slope=0.46 ± 0.02, p=0.0001
Differences in slopes of change between study groups F= 1.07, 1, 172 degrees of freedom, p=0.30
Figure 5.20 Head Circumference by Age (females). Differences from ‘0’ in slopes of change for Control (—) and Treatment (-----) groups: Control: slope = $0.48 \pm 0.02$, $p=0.0001$; Treatment: slope=$0.47 \pm 0.02$, $p=0.0001$

Differences in slopes of change between study groups $F=0.08$, 1, 15 degrees of freedom, $p=0.78$
Table 5.5 Mean Height for Age Z-score by Age for Treatment Versus Control Group

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Treatment</th>
<th>N</th>
<th>Mean ± SD (HAZ)</th>
<th>T</th>
<th>Degrees of freedom</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Control</td>
<td>52</td>
<td>0.17 ± 0.13</td>
<td>0.41</td>
<td>98</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>48</td>
<td>0.16 ± 1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>52</td>
<td>0.12 ± 0.95</td>
<td>0.37</td>
<td>98</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>48</td>
<td>0.19 ± 1.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>50</td>
<td>0.11 ± 1.03</td>
<td>0.52</td>
<td>94</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>46</td>
<td>0.22 ± 1.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>52</td>
<td>0.15 ± 1.03</td>
<td>0.91</td>
<td>95</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>45</td>
<td>0.35 ± 1.10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Age rounded to nearest whole number

5.5.5 Morbidity and Compliance

Perceived morbidity was similar between study groups (Figure 5.19). In both groups infants were “well” about 80% of the time, “mildly” ill less than 20% of the time and rarely suffered from diarrhoea or “severe” illness. For definitions, please refer to section 5.3.

Table 5.6 shows the average daily consumption of iron-fortified infant cereal and meat consumed by infants in the treatment group. The level of intake recommended to the families was 2/3 cup (30 g) or more of dry cereal and between one to two jars of meat (70-140 g). Based on our definition of compliance (section 4.3.3) six of 43 (12%) were non-compliant. Four consistently failed to consume meat and two consistently failed to consume infant cereal.
Figure 5.21 Recorded Morbidity by Treatment
Table 5.6  Average Reported Infant Cereal and Meat Intake of Treatment Infants

<table>
<thead>
<tr>
<th>Subject</th>
<th>Infant Cereal (cups/day) (45g/cup)</th>
<th>Meat (jars/day) (70g/jar)</th>
<th># of days Recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>&lt;1/3</td>
<td>1</td>
<td>112</td>
</tr>
<tr>
<td>2</td>
<td>1/2</td>
<td>2</td>
<td>183</td>
</tr>
<tr>
<td>3*</td>
<td>&gt;2/3</td>
<td>&lt;1</td>
<td>177</td>
</tr>
<tr>
<td>4*</td>
<td>1/3</td>
<td>&lt;1</td>
<td>176</td>
</tr>
<tr>
<td>5*</td>
<td>1/2</td>
<td>&lt;1</td>
<td>119</td>
</tr>
<tr>
<td>6</td>
<td>&gt;2/3</td>
<td>&gt;2</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>&gt;2/3</td>
<td>&gt;2</td>
<td>96</td>
</tr>
<tr>
<td>8</td>
<td>1/3</td>
<td>1½</td>
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</tr>
<tr>
<td>9</td>
<td>&gt;2/3</td>
<td>1½</td>
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<tr>
<td>10</td>
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<td>1½</td>
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<tr>
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<tr>
<td>39</td>
<td>1/3</td>
<td>1</td>
<td>160</td>
</tr>
</tbody>
</table>
Records missing for six subjects.
*Subjects identified as non-compliers by monthly compliance check (see section 4.3.3 for definition of non-complier).
Infant cereal and meat intakes are expressed as the mean intake/day based on the number of days filled out on the daily compliance calendars (Appendix B). Mothers were asked to mark: C3 ate more than 2/3 of a cup
C2 ate 2/3 of a cup
C1 ate 1/3 of a cup
C0 ate less than 1/3 of a cup
M3 ate more than 2 jars of meat
M2 ate 2 jars of meat
M1 ate 1 jar of meat
M0 ate less than 1 jar of meat

For the purposes of calculating average intakes the mean of the reported categories (0, 1, 2, 3) was calculated and rounded to the nearest 1/2. Thus a mean reported category for cereal of:
‘0-0.5’ represented an average intake of less than 1/3 cup/day
‘1’ represented an average intake of 1/3 cup/day
‘1.5’ represented an average intake of 1/2 cup/day
‘2’ represented an average intake of 2/3 cup/day
‘2.5-3’ represented an average intake of more than 2/3 cup/day

A mean reported category for meat of:
‘0-0.5’ represented an average intake of less than 1 jar/day
‘1’ represented an average of 1 jar/day
‘1.5’ represented an average of 1.5 jar/day
‘2’ represented an average of 2 jars/day
‘2.5-3’ represented an average of more than 2 jars/day
5.6 Discussion

This study examined patterns of food use within the study population that may have been implicated in the cause or prevention of iron depletion in low-income families in Toronto. Patterns of food use refers to whether specific foods were consumed or not and does not consider the amount or frequency. Prior to the study period (six months of age) feeding patterns were adequate to maintain iron stores. During the study period, with the exception of cow milk, no specific foods were associated with iron depletion.

By design, all of the infants who were entered into the study were iron replete which implies that their pre-study dietary intakes were adequate to maintain iron stores. In general, the low-income households surveyed in this study were following CPS guidelines for the infant’s first six months of life except with respect to the duration of breast-feeding. Retrospective data show that a relatively large proportion initiated breast-feeding in both groups although this level declined steadily with age to between 20% to 40% (Figure 5.1). This low prevalence rate indicates that the majority of mothers did not meet the current CPS recommendation that infants should be breast-fed until nine to 12 months of life and exclusively so for the first four to six months (Canadian Paediatric Society, 1998). However, these rates are similar to those observed in average income families in other parts of Canada (Carceller et al. 1995; Greene-Finestone et al. 1989; Matthews et al. 1995; Tanaka et al. 1987; Williams et al. 1996) and much higher than those of other low-income families (Greene-Finestone et al. 1989; Lehmann et al. 1992; Longstaffe et al. 1993). If not breast-fed, the infants surveyed were generally fed iron-fortified formula, in accordance
with CPS guidelines (Figure 5.2). Furthermore, cow milk was generally avoided in the first six months and solid foods were delayed until the third or fourth month, usually starting with infant cereal (Figures 5.4 and 5.5 respectively) unlike other low-income families previously studied (Greene-Finestone et al. 1989; Lehmann et al. 1992; Longstaffe et al. 1993). Thus, the feeding patterns of families surveyed were similar to those of average income families in Toronto and other areas of Canada but unlike those of other low-income families.

There were differences in the pre-study feeding patterns between study groups. Control families breast-fed more often and for longer, used less formula just after birth and introduced solids later. Despite these differences in early feeding patterns there were no observed differences in anthropometric indices, haemoglobin or ferritin at six months of age (Table 5.2).

Many of the families surveyed were highly educated which may explain why the feeding patterns in this group of infants were different from other infants from low-income families surveyed in the past. Firstly, low family income was an inclusion criteria for this study but those of any education level were included. The result was that almost half of the parents had higher education and none had achieved below grade 11 level. The inadvertent inclusion of highly educated yet economically deprived parents was unlike other studies, for example, Lehmann et al. (1992) where none of the mothers had higher than grade 11 education and Longstaffe et al. (1993) where families were low-income and were less well educated.
Secondly, differences in education level may explain differences in pre-study feeding patterns between groups. The initial recruitment of 164 subjects who passed the screening questionnaire exceeded our goal of 140. However, fewer than the anticipated 55 subjects per group (49 treatment, 54 control) completed the study. More families “withdrew” from the treatment group (p=0.032) (Table 5.1). Fourteen of the families in the treatment group withdrew because they were aware of current infant feeding guidelines and were unwilling to use whole cow milk as the only milk source throughout the study. This was not a problem with the control group since there was no restriction on feeding. Although undocumented, during initial telephone contact, a number of families that were deemed ineligible, because their income was too high, did express a willingness to participate only if they were in the control group. The reason for this was, generally, that they were unwilling to feed cow milk or give up breast-feeding at six months, in accordance to the CPS guidelines (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998). Similarly, there were eight families who passed the initial screen but dropped out of the study as soon as they became aware that they were assigned to the treatment group. Four of these eight families did not want to discontinue breast-feeding and the other four did not want to feed cow milk at six months. All of these families dropped out before any data about education was collected. However, five of these families did provide information on their income. The mean income expressed as percent of low-income cut-off was 92%. This was much higher than that of the treatment group in general (69% of low-income cut-off)
or that of the control group (83% of low-income cut-off). These families generally had a higher income and may have been more informed about current infant feeding guidelines.

The result was that, at six months of age, treatment and control groups were similar in iron status, anthropometrics, birth weight, birth order and ethnicity; however, more mothers in the control group had higher education levels than in the treatment group (p=0.05) (Table 5.3). The mean income was slightly higher, but not significantly so, for control families (p=0.06), although both groups fell below the low-income cut-offs (69% of cut-off for treatment, 83% for control) defined by Statistics Canada in 1996. Consistent with the results of previous work (Greene-Finestone et al. 1989; Lehmann et al. 1992; Matthews et al. 1995; Williams et al. 1996), in this study, breast-feeding was positively associated with education; but unlike other studies, breast-feeding was not associated with other variables such as marital status, ethnicity and income. These variables are interrelated and are often associated together. The current study population may have been too uniform in income to associate with differences in breast-feeding patterns since the subjects were by definition low-income. However, within this stratum of income, education was the most important variable associated with choice to breast-feed (section 5.4.2.1.).

During the study period, patterns of food use were not associated with low iron status (Ferr < 10 μg/L or Hgb < 110 g/L) except for feeding cow milk at ten months of age which was the only feeding practice associated (p=0.04) (Table 5.4). This finding is consistent with other studies that have found associations between use of cow milk and low
iron status (Greene-Finestone et al. 1989; Lehmann et al. 1992; Matthews et al. 1995; Williams et al. 1996).

Throughout the study most of the control families continued to follow CPS recommendations while treatment families followed the feeding plan prescribed in the study protocol. Sixty to 70% of control mothers fed iron-fortified infant formula throughout the study period and a 20-30% continued to breast-feed (Figures 5.10 and 5.9). Cow milk was generally delayed until ten months of age (Figure 5.12) in accordance with CPS recommendations (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998). Furthermore, iron supplements were used by about 25% of control families (Figure 5.15).

Families in the treatment group were not regularly feeding iron supplements, any type of formula or breast milk (Figures 5.9-5.11, 5.15) but were feeding whole cow milk (5.12). However, one mother continued to give a single feed of human milk each day throughout the study period. This amount was deemed negligible to the study outcome and the infant remained in the study. Similarly, infants were not excluded if, on the advice of their paediatricians, they used formula instead of cow milk during periods of illness which lasted less than one week. As dictated by the study protocol, treatment families were not using iron-containing supplements. However, once infants reached 12 months of age (the end to the study period), 16% of the treatment families fed vitamin/mineral supplements containing iron.

Most of the infants in both study groups were regularly consuming infant cereal
(Figure 5.13), meat (Figure 5.14) and fruits, vegetables or juices. However, the intake may have been higher in the treatment group since meat and cereal were provided at no cost. Daily compliance calendars provided an estimate of meat and cereal intake for treatment infants; however, there was no quantitative measure for control infants. Mothers of treatment infants were asked to recall and report how many jars of meat and how many cups of dry cereal were fed each day. Most of the mothers filled out the calendars; however, there were some missing values in most cases and some mothers did not use them at all.

Attempts to collect more quantitative data in the form of three-day food records were unsuccessful in both groups. Families were given detailed instruction, examples and blank food records (see Appendix A). Reminder calls were made and letters were sent each time a food record was to be taken. Despite all of this effort, many families did not complete the records. Most of those that were filled out did not provide precise quantities of food items, especially if a home recipe was used. Personal observations suggest that mothers felt that they were too busy to put in the time and effort to measure how much of each food was consumed by their babies. Because the quantitative data were so unreliable and imprecise, they were not included in this thesis. However, current food use and compliance checks questionnaires (Appendix A) provided a general feeding pattern for families in each study group and a measure of compliance in the treatment group.

Despite the differences in feeding patterns between study groups, growth rates were similar. There was no difference in the change in slope of WAZ between groups,
but WAZ did fall with age for control infants but not treatment (Figure 5.16). HAZ increased with age in the treatment group but not control and this difference was significant (p=0.007) (Figure 5.17). Although the slopes of change were statistically different between groups the mean HAZ were not different at any age (Table 5.5). The largest mean difference in height between groups was only 0.46 cm at 12 months of age (T=0.91, 95 degrees of freedom, p=0.36). The statistical power of this analysis was approximately 0.16 which means that a sample size of approximately 900 subjects would be required to detect a difference this small. This suggests that although slopes of change may have been statistically different, the difference in achieved HAZ at each age was negligible between groups.

Mean WAZ, HAZ and WHZ were around the NCHS median. Head circumference increased with age for males and females in both groups at a similar rate. Thus, the dietary intervention may have resulted in a small increase in growth rate in terms of height, but generally did not have an appreciable influence on growth rate or achievement of size. There was certainly no negative affect on growth associated with cow milk-feeding.

In summary, pre-study feeding patterns of this sample sufficed to prevent iron deficiency until six months of age. The majority fed according to CPS recommendations unlike other low-income families surveyed in the past, likely because the current sample of families were generally better educated. Lower education levels in the treatment group may explain why treatment families tended to breast-feed less and introduce solids earlier, prior
to the study period. During the study period, only cow milk feeding at ten months of age was associated with poor iron status. The majority of infants in the treatment group fed in accordance with the dietary restrictions of the protocol while the majority of control families continued to feed in accordance to CPS guidelines. Differences in feeding patterns between the two groups did not have an appreciable influence on growth rate or achieved size.
6. CONSUMPTION OF CEREAL, MEAT AND MILK IN THE PREVENTION OF IRON DEPLETION

6.1 Introduction

Iron deficiency is the most prevalent nutritional problem in the world, affecting a large number of children and infants in both developed and developing countries (Scrimshaw 1991). It is not only a problem in developing countries but affects up to one third of Canadian infants from disadvantaged households (Lehmann et al. 1992; Longstaffe et al. 1993). The most severe form, iron deficiency anaemia, is associated with depressed mental and motor function in infants and children (Aukett et al. 1986; Bruner et al. 1996; Idjradinata and Pollit 1993; Lozoff et al. 1987; Lozoff et al. 1991; Walter et al. 1988; Walter 1992). Despite controversy regarding the effectiveness of treatment with iron to reverse these effects, there is universal consensus that the prevention of iron deficiency anaemia is essential for every infant. Two commonly suggested means to prevent iron deficiency anaemia in disadvantaged infants are the routine use of iron drops or to screen for and treat only those with the condition. Neither of these interventions has proven effective (Stekel 1984). In light of this, the Canadian Paediatric Society (CPS) recently proclaimed that prolonged exclusive breast-feeding or the use of iron-fortified formula will prevent iron deficiency anaemia (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998).

A large segment of the Canadian population, primarily from low-income households, either cannot or will choose not to breast-feed throughout the first year of life
because of the inconvenience and effort involved or feed iron-fortified formula because of the high cost (Shears 1991). Instead they often use cow milk as an inexpensive alternative. However, cow milk is strongly associated with iron depletion and the development of iron deficiency anaemia in infants in the first year of life (Penrod et al. 1990). Because of the risk of anaemia associated with cow milk, the CPS recommends delaying introduction to cow milk until nine to 12 months (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998) while the AAP recommends delaying its introduction until one year of age (American Academy of Pediatrics 1992). Ingestion of cow milk can lead to iron deficiency in infants because it is a poor source of iron and can cause occult blood loss in the stool (Belsten et al. 1997; Fuchs and al 1993; Thomas et al. 1986; Woodruff et al. 1972; Ziegler et al. 1990). However, occult blood loss is common before six months of age (Hoag et al. 1961; Rasch et al. 1960; Woodruff et al. 1972, Fomon et al. 1981) but not after (Belsten et al. 1997; Fuchs et al. 1993; Thomas et al. 1986; Woodruff et al. 1972). Thus, whole cow milk use after six months may be an acceptable lower cost alternative to iron fortified formula if consumed along with iron-rich foods such as iron-fortified cereal and meat.

Thus, the purpose of this study was to determine the effect of the consumption of infant cereal and pureed meat in six to 12 month old cow milk-fed infants from low-income households compared to similar infants receiving no dietary intervention on the incidence of iron depletion and/or anaemia.
6.2 Subjects and Protocol

See sections 4.1 to 4.2.3.

6.2.1 Data Generated

For each blood sample measured, HgB was assayed in triplicate and Ferr was assayed in duplicate. A mean of these was calculated and used to determine haematologic status. A mean HgB less than 110 g/L or a mean Ferr of less than 10 μg/L was considered a putative end-point. If these low values were confirmed in a second blood sample, a true end-point was declared (ie. the infant was excluded from the study).

For infants in the treatment group, compliance was assessed using daily records of meat and cereal consumption (see section 4.3.3 for definition of compliance). Parents were asked to estimate amount of cereal and meat consumed at the end of each day and record it on the calendars provided (see section 4.3.3). Data were expressed as average cereal and average meat intake per day over the period for which records were collected.

6.2.2 Statistical Analysis

To examine changes in HgB and Ferr stores across age, the General Linear Models (GLM) procedure of SAS was used taking advantage of the repeated measures on individuals. This made it possible to estimate the coefficients for the slope of change with age and to contrast the estimates for differential slope in the two groups. Serum Ferr values were log transformed for this analysis to normalise data. Data from second blood samples drawn to confirm putative end-points were not included in these analyses since their inclusion would weight these low values. The primary analysis compared the occurrence of
end-points (iron depletion or mild anaemia) between the two groups using Mantel-Haenszel Chi-square analysis (Czajka-Nairns et al. 1978). During analysis, if counts less than five (less than five infants reaching end-points) were encountered in both groups, Fisher's Exact Test was used. Relative Risk for developing iron depletion or anaemia when following treatment diet versus control was also calculated. These analyses were performed including and excluding non-compliers in the treatment group using true end-points (confirmed in a second blood sample) and using putative end-points (any single end-point whether or not it was confirmed in the second blood sample). Non-compliers were defined in Section 4.3.4.

6.3 Results

6.3.1 Change in Haemoglobin and Ferritin Levels with Age

Log ferritin levels fell progressively with age in both the treatment and control groups (slope = -0.08 ± 0.02, p=0.0001; slope = -0.07 ± 0.02, p=0.0001 respectively). There was no difference in the rate of change between groups (p=0.49) (Figure 6.1). In contrast, haemoglobin levels did not change significantly across age (slope = 0.02 ± 0.31; p=0.95; slope = -0.31 ± 0.30; p=0.30 respectively). Again, there was no difference between groups (p=0.44) (Figure 6.2).
Figure 6.1 Change in Ferritin (Ferr) Across Age. Differences from '0' line in slopes of change for Log Control (---) and LogTreatment (-----) groups: Control: slope = -0.07 ± 0.02, p=0.0001; Treatment: slope = -0.08 ± 0.02, p=0.0001.

Data points not transformed, trend lines are Log transformed.

Differences in slopes of change between study groups $F= 0.49, 1, 292$ degrees of freedom, $p=0.49$
Figure 6.2 Change in Haemoglobin (HgB) Across Age. Differences from ‘0’ line in slopes of change for Control (---) and Treatment (-----) groups: Control: slope = 0.02 ± 0.31, p=0.95; Treatment: slope = -0.31 ± 0.30, p=0.30.
Differences in slopes of change between study groups F= 0.59, 1, 290 degrees of freedom, p=0.44
6.3.2 Achievement of End-Points

Table 6.1 shows confirmed end-points for all infants who completed the study (including those who did not consume prescribed amounts of infant cereal or meat). In the treatment group, 16.3% of treatment infants reached end-point compared to 9.3% of control infants; however, the difference was not significant.

Table 6.2 shows confirmed end-points when non-compliant infants were excluded from the treatment group (refer to section 4.3.3 for definition of non-compliance). The proportion of infants in the treatment group reaching end-point was not significantly higher than in the control group. Fisher’s exact test was included in this analysis since half of the cells had five or fewer counts.

Table 6.3 shows the proportion of single (putative) end-points (i.e. end-points not confirmed after a second blood sample was taken) for all subjects who completed the study (including those infants in the treatment group who did not consume any meat or cereal). The proportion of end-points in the treatment group (36.7%) was significantly higher than in the control group (18.5%). The relative risk was 1.56, with a confidence interval of 1.02-2.37.

Table 6.4 shows single end-points for infants when non-compliant infants were excluded from the treatment group. Single end-point rates were not different between the two groups (treatment vs. control, 27.9 vs 18.5%, p=0.28).

Table 6.5 shows the effect of compliance to treatment on achievement of confirmed end-point. Three out of 43 (7.0%) infants who complied with the treatment
reached confirmed end-point compared to five out of six (83.3%) of infants who did not comply (p=0.0002).

Table 6.6 shows the effect of compliance to treatment on achievement of single end-point. Twelve out of 43 (24.5%) infants who complied with the treatment reached putative end-point compared to five out of six (83.3%) of infants who did not comply (p=0.015).
Table 6.1 Effect of Treatment in Avoidance of Confirmed End-Points for all Subjects

<table>
<thead>
<tr>
<th>Status</th>
<th>Treatment Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-Point Achieved</td>
<td>8 (16.3%)</td>
<td>5 (9.3%)</td>
</tr>
<tr>
<td>No End-Point by 12 months</td>
<td>41 (83.7%)</td>
<td>49 (90.7%)</td>
</tr>
</tbody>
</table>

Total number of subjects: 103
Mantel-Haenszel Chi square: 1.15, 1 degree of freedom, p=0.28
Mantel-Haenszel Relative Risk: 1.35 (95% confidence interval 0.78-2.14)

Table 6.2 Effect of Treatment in Avoidance of Confirmed End-Points (Non-compliant infants excluded from treatment group)

<table>
<thead>
<tr>
<th>Status</th>
<th>Treatment Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-Point Achieved</td>
<td>3 (7.0%)</td>
<td>5 (9.3%)</td>
</tr>
<tr>
<td>No End-Point by 12 months</td>
<td>40 (93.0%)</td>
<td>49 (90.7%)</td>
</tr>
</tbody>
</table>

Total number of subjects: 97 (6 non-compliers excluded)
Mantel-Haenszel Chi square: 0.16, 1 degree of freedom, p=0.69
Fisher's Exact Test (2-tail): p=0.78
Mantel-Haenszel Relative Risk: 0.83 (95% confidence interval 0.35-2.0)
Table 6.3 Effect of Treatment in Avoidance of Single End-Points for all Subjects

<table>
<thead>
<tr>
<th>Status</th>
<th>Treatment Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single End Point</td>
<td>18 (36.7%)</td>
<td>10 (18.5%)</td>
</tr>
<tr>
<td>No End Point by 12 months</td>
<td>31 (63.3%)</td>
<td>44 (81.5%)</td>
</tr>
</tbody>
</table>

Total number of subjects: 103
Mantel-Haenszel Chi square: 4.27, 1 degree of freedom, p=0.034
Mantel-Haenszel Relative Risk: 1.56 (95% confidence interval 1.02-2.37)

Table 6.4 Effect of Treatment in Avoidance of Single End-Points (Non-compliant infants were excluded from the treatment group)

<table>
<thead>
<tr>
<th>Status</th>
<th>Treatment Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single End Point</td>
<td>12 (27.9%)</td>
<td>10 (18.5%)</td>
</tr>
<tr>
<td>No End Point by 12 months</td>
<td>31 (72.1%)</td>
<td>44 (81.5%)</td>
</tr>
</tbody>
</table>

Total number of subjects: 97 (6 non-compliers excluded)
Mantel-Haenszel Chi square: 1.91, 1 degree of freedom, p=0.28
Mantel-Haenszel Relative Risk: 1.32 (95% confidence interval 0.80-2.17)
Table 6.5 Effect of Compliance to Treatment on Confirmed End-points

<table>
<thead>
<tr>
<th>Status</th>
<th>Compliers</th>
<th>Non-compliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-Point Achieved</td>
<td>3 (7.0%)</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>No End-Point by 12 months</td>
<td>40 (93.0%)</td>
<td>1 (16.7%)</td>
</tr>
</tbody>
</table>

Number of Subjects: 49
Mantel-Haenszel Chi square: 22.5, 1 degree of freedom, p=0.001
Fisher's Exact Test (2-tail): p=0.0002
Mantel-Haenszel Relative Risk: 25.6 (95% confidence interval 6.6-99.3)

Table 6.6 Effect of Compliance to Treatment on Single End-points

<table>
<thead>
<tr>
<th>Status</th>
<th>Compliers</th>
<th>Non-compliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-Point Achieved</td>
<td>12 (27.9%)</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>No End-Point by 12 months</td>
<td>31 (72.1%)</td>
<td>1 (16.7%)</td>
</tr>
</tbody>
</table>

Number of Subjects: 49
Mantel-Haenszel Chi square: 7.14, 1 degree of freedom, p=0.008
Fisher's Exact Test (2-tail): p=0.015
Mantel-Haenszel Relative Risk: 9.4 (95% confidence interval 1.8-49.6)
6.3.3 Other Factors Related to End-point and Compliance

Table 6.7 shows that four of five infants in the control group and two of three infants in the treatment group (excluding non-compliers) who reached confirmed end-point were below the group means for income and most had only primary to secondary school education (Table 5.3). Socio-demographic data was not obtained for one of the treatment families who reached confirmed end-point (non-compliers were not included).

Of the six non-compliant infants, five reached confirmed end-point. The sixth infant reached putative end-point at eight months of age that was not confirmed in a second blood sample. Verbal reports and daily compliance records indicated that this infant consumed adequate amounts of meat but would not eat cereal.

Table 6.8 shows the socio-economic background of four of these infants (data was not available for one family). Despite the observation that the mean income of this group was below the mean of the entire treatment group, the effect of socio-economic status on compliance was not significant (p=0.11) (Table 6.9).

There was no difference in the rate of confirmed (Table 6.10) or putative end-points (Table 6.11) between groups at any age during the study period. Three (7.0%) of the treatment infants (non-compliers excluded) reached confirmed end-point because their Ferr < 10 μg/L and none because their HgB < 110 g/L. Three (5.6%) of the control infants reached end-point because their Ferr < 10 μg/L and two (3.7%) because their HgB < 110 g/L. None of the infants had both low Ferr and HgB (Table 6.12).
Table 6.7 Socio-demographic Background of Infants Who Reached Confirmed End-point (Excluding non-compliers)

<table>
<thead>
<tr>
<th>Group</th>
<th>% Low-income Cut-off</th>
<th>Number of Parents in Household</th>
<th>Mother’s Education</th>
<th>Father’s Education</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1*</td>
<td>1</td>
<td>College</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>1</td>
<td>Primary to Secondary</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>63</td>
<td>2</td>
<td>Primary to Secondary</td>
<td>College</td>
</tr>
<tr>
<td>Control</td>
<td>113</td>
<td>2</td>
<td>Primary to Secondary</td>
<td>College</td>
</tr>
<tr>
<td>Control</td>
<td>45</td>
<td>1</td>
<td>Primary to Secondary</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>56</td>
<td>2</td>
<td>College</td>
<td>Primary to Secondary</td>
</tr>
<tr>
<td>Treatment</td>
<td>45</td>
<td>1</td>
<td>Primary to Secondary</td>
<td></td>
</tr>
</tbody>
</table>

Data missing for one subject
*This mother had no independent income and relied on periodic assistance from different friends and family members at the time income was assessed.
Table 6.8 Socio-demographic Background of Non-Compliers who Reached Confirmed End-point

<table>
<thead>
<tr>
<th>% Low-income Cut-off</th>
<th>Number of Parents in Household</th>
<th>Mother’s Education</th>
<th>Father’s Education</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>1</td>
<td>Primary to secondary</td>
<td>Primary to secondary</td>
</tr>
<tr>
<td>45</td>
<td>2</td>
<td>Primary to secondary</td>
<td>Primary to secondary</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>Primary to secondary</td>
<td>Primary to secondary</td>
</tr>
<tr>
<td>78</td>
<td>2</td>
<td>College</td>
<td>Primary to secondary</td>
</tr>
</tbody>
</table>

Data missing from two non-compliers

Table 6.9 Effect of Socio-economic Status (SES) on Compliance

<table>
<thead>
<tr>
<th>Status</th>
<th>Non-Compliers</th>
<th>Compliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower SES</td>
<td>3 (20.0%)</td>
<td>12 (80.0%)</td>
</tr>
<tr>
<td>Higher SES</td>
<td>1 (3.4%)</td>
<td>28 (96.6%)</td>
</tr>
</tbody>
</table>

Lower SES defined as the mother having no higher than secondary school education and income less than 69% of low-income cut-off (less than the mean % of low-income cut-off in the treatment group).

Number of Subjects: 44
Chi-square: 3.28, 1 degree of freedom, p=0.07
Fisher’s Exact Test: p=0.11
Table 6.10 Age When Confirmed End-point was Reached (non-compliers excluded)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>End-point (treatment group)</th>
<th>End-point (control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1 (2.3%)</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td></td>
<td>n=43</td>
<td>n=54</td>
</tr>
<tr>
<td>10</td>
<td>0 (0%)</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td></td>
<td>n=42</td>
<td>n=53</td>
</tr>
<tr>
<td>12</td>
<td>2 (4.7%)</td>
<td>3 (5.8%)</td>
</tr>
<tr>
<td></td>
<td>n=42</td>
<td>n=52</td>
</tr>
<tr>
<td>Total</td>
<td>3 (7.0%)</td>
<td>5 (9.3%)</td>
</tr>
<tr>
<td></td>
<td>n=43</td>
<td>n=54</td>
</tr>
</tbody>
</table>

No differences were observed between groups at any age (T-test, p>0.05)

Table 6.11 Age When Putative End-point was Reached (non-compliers excluded)

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>End-point (treatment group)</th>
<th>End-point (control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>6 (13.9%)</td>
<td>3 (5.6%)</td>
</tr>
<tr>
<td></td>
<td>n=43</td>
<td>n=54</td>
</tr>
<tr>
<td>10</td>
<td>2 (4.8%)</td>
<td>1 (1.9%)</td>
</tr>
<tr>
<td></td>
<td>n=42</td>
<td>n=53</td>
</tr>
<tr>
<td>12</td>
<td>4 (9.5%)</td>
<td>6 (11.5%)</td>
</tr>
<tr>
<td></td>
<td>n=42</td>
<td>n=52</td>
</tr>
<tr>
<td>Total</td>
<td>12 (27.9%)</td>
<td>10 (18.5%)</td>
</tr>
<tr>
<td></td>
<td>n=43</td>
<td>n=54</td>
</tr>
</tbody>
</table>

No differences were observed between groups at any age (T-test, p>0.05)
Table 6.12 Prevalence of Iron Deficiency (based on confirmed end-points, non-compliers excluded)

<table>
<thead>
<tr>
<th></th>
<th>Treatment (n=43)</th>
<th>Control (n=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgB &lt; 110 g/L</td>
<td>0</td>
<td>2 (3.7%)</td>
</tr>
<tr>
<td>Ferr &lt; 10 µg/L</td>
<td>3 (7.0%)</td>
<td>3 (5.6%)</td>
</tr>
<tr>
<td>HgB &lt; 110 g/L and Ferr &lt; 10 µg/L</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
6.4 Discussion

The objective of this study was to determine the effect of the consumption of infant cereal and pureed meat in six to 12 month old cow milk-fed infants from low-income households compared to similar infants receiving no dietary intervention on the incidence of iron depletion and/or anaemia. Based on data from previous Canadian studies, we had assumed in generating the hypothesis and determining the sample size that the incidence of iron depletion in the control group (low-income families without dietary intervention) would be 30% or higher and that the intervention would reduce this to an incidence of 10% or less. This assumption was incorrect. Unexpectedly, the families in the control group were fed in accordance with CPS recommendations. These recommendations were established to act as guidelines for parents to meet the nutritional needs and prevent iron deficiency in their infants (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998). The result was that the incidence of iron depletion in the control group was much lower than anticipated. Thus, it was not possible to test the original hypothesis as written. Because the control infants were generally fed according to CPS guidelines and were at low risk for iron deficiency, the study actually turned out to be an equivalence study. Thus the hypothesis tested was that there would be no difference in the incidence of iron depletion and/or anaemia in six to 12 month old cow milk-fed infants from low-income households consuming infant cereals (30g/day) and pureed meats (70g/day) when compared to infants feeding in accordance to CPS guidelines.

If purposefully designed as an equivalence study, the sample size would have been
around 500 infants per group. This estimate is based on $\alpha=0.05$ and $\beta=0.20$, assuming the incidence of end-point in the control group was 9% (as observed) and that the incidence in the treatment group would have been less than 15%. Despite the small sample size in the current study, three lines of evidence from our results suggest that the iron from cereal and meat was well utilised and prevented iron deficiency in cow milk-fed infants. Firstly, the incidence of end-point (low HgB or low Ferr) in the treatment group was low compared to past surveys of infants from low and normal socio-economic households and not statistically different from control infants who followed CPS infant feeding guidelines. Secondly, almost all of the treatment infants who consumed cow milk but did not consume either meat or cereal reached end-point. Finally, the rate of fall in plasma Ferr with age was the same in both groups, suggesting that the iron status of the two groups were equivalent.

Why were the low-income families in the control group different from the other low-income families described in the literature? The answer, quite clearly, is related to the educational background of the families and their choice of infant foods. Despite having incomes below the defined Canadian poverty level (Table 5.3), the majority of families were relatively well educated. As such, they generally followed current Canadian infant feeding guidelines (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998) and were consuming adequate amounts of iron. In the first six months of life, most had been breast-fed (Figure 5.1) or fed commercial formula (figure 5.2). During the second six months of life (ie. during the study), most infants in the control group continued to be breast-fed (Figure 5.9) or were fed iron-fortified formula (Figure
5.10). Most received iron-fortified cereal and meat at the appropriate age, despite not having received any advice from study personnel (Figures 5.5, 5.6, 5.13, 5.14). As a result, few infants (9.3%) in the control group had evidence of iron depletion.

Despite having very different dietary intakes, the proportion of infants in the treatment group (excluding non-compliers) that had evidence of iron depletion was not different than that in the control group. The feeding patterns of the treatment group generally followed the dictates of the study protocol. Whole cow milk was the only source of milk used throughout the study period (Figure 5.12) and iron supplements were not used by any infants (Figure 5.15). The only major sources of iron for infants that complied with the treatment diet were the meat and iron-fortified cereal, which were generally consumed in amounts prescribed in the study protocol (Table 5.6). Consumed at this level, the cereal and meat theoretically should provide adequate levels of absorbable iron (see section 2.1.10). Although the individual contributions of infant cereal versus meat could not be separated, the results of this study are consistent with others showing that infant cereal (NHRDP Project 6606 4104 61; Walter et al., 1993) and iron-fortified meat and cereal (Haschke et al. 1988) contribute substantially to preventing iron deficiency. Only 7.0% (three infants) reached end-point when following this diet. This value was not different from the control group (9.3%) who were generally feeding according to CPS recommendations (p=0.69) (Table 6.2). This is the first line of evidence that suggests that the iron from cereal and meat was well utilised and prevented iron deficiency in cow milk-fed infants. If, as had originally been anticipated, 30% of the control infants were iron
deficient, the reduction to 7.0% in intervention group would have been both statistically and clinically significant.

To put the results of the current study into context, rates of putative end-point can be compared to past studies of infants from low-income and less educated families. The only other Canadian study that included a longitudinal design was that of Longstaffe et al (1993) in Winnipeg. Longstaffe documented a 53% rate of iron depletion in six to 15 month old socio-economically disadvantaged infants fed non iron-fortified formula. Another study of socio-economically disadvantaged infants in Montreal (Lehmann et al. 1992) used a cross-sectional design. To allow for more meaningful comparisons with this cross-sectional study, the proportion of end-points from the current study was calculated at each age (Tables 6.10 and 6.11). Lehmann et al. (1992) found that 37% of one year old infants from socio-economically disadvantaged households had Ferr < 10 μg/L. This was much higher than the proportion of putative end-points at 12 months of age in the control (11.1%) and treatment group (9.3%) (Table 6.11). Furthermore, putative end-point rates in the both groups (ranging from 2.0% to 13.9%) were lower than the rates observed in cross-sectional surveys of infants from average income families in Vancouver (24.4%) (Innis et al. 1997) and in Toronto, Halifax, Montreal and Edmonton (33.9%) (Zlotkin et al. 1996) who are not at particular risk for iron deficiency.

The rate of putative end-points when non-compliers were excluded from the treatment group (27.9%) was not significantly higher than in the control group (18.5%) (p=0.28) (Table 6.4). When the end-point rate was analysed at different ages, there were
still no differences between groups (Table 6.1). However, six of the infants from the treatment group that reached putative end-point at eight months did not consume cereal and/or meat during the first month of the study. They were not excluded in the original analysis as non-compliers because their intakes increased to acceptable levels as the study went on. Although the difference was not significant, this may explain why 16.3% of the treatment infants reached putative end-points compared to 5.6% in the control at eight months of age (Fisher’s exact test, p=0.10).

Putative rather than confirmed end-points were used in the current study to make direct comparisons with the prevalence rates of past studies of iron depletion. The current study declared end-point only after they had been confirmed in a second blood sample so as to reduce the number of infants falsely identified as being iron depleted. It has been estimated that Ferr must be measured in six separate blood samples over a period of days to get an accurate measure because of the high biological variation associated with Ferr measures (Cooper and Zlotkin 1996; Ahluwalia et al. 1993; Borel 1990). We chose only to re-test low samples because of the cost, inconvenience and stress to the infant associated with taking multiple blood samples. In addition, it would not have been ethically unacceptable to routinely re-test ‘normal’ values. Putative end-point was confirmed in a second sample only 43% of the time. The other 57% of the time, on the second sample, the Ferr and HgB were normal. It is possible that some infants with Ferr values slightly greater than 10 μg/L were actually iron depleted but were incorrectly classified as normal (and therefore not re-tested). However, the range for normal Ferr (10 – 80 μg/L) (Wiedemann
and Jonetz-Mentzel 1993) is about eight times greater than the range for iron depletion (0 - 10 μg/L). The overall coefficient of variation (analytic and biologic) for Ferr was estimated to be 26.1% (Table 7.6). This suggests that true Ferr values below 7 and above 15 μg/L are unlikely to be misclassified. Thus, it is much more likely that a single sample yielding a low (abnormal) value would be falsely labelled as normal compared to a single sample in the normal range being falsely identified as abnormal. Unfortunately, in most published reports, only single blood samples are collected and analysed. Thus one might justifiably assume that published estimates of prevalence of iron depletion are overestimated. This implies that data from most past studies could not be meaningfully compared to confirmed end-point rates in this study; however, they could be meaningfully compared to putative end-points.

The second line of evidence that suggests that the iron from cereal and meat was well utilised and prevented iron deficiency in cow milk-fed infants was that if meat and cereal were not consumed, cow milk-fed infants were at increased risk for iron deficiency. Every effort was made to encourage use of the infant cereal and meat in this study. There were a variety of cereals and meats for parents to choose from and all foods were delivered and provided free of charge. Families were asked which foods they preferred and were free to exchange or get more at any time. Furthermore, the importance of feeding enough cereal and meat was explained to the families, nurses encouraged their use during home visits and families were encouraged to call if they had any problems or questions. Despite this effort, six (12%) mothers of infants in the
treatment group reported that their infants were ingesting cow milk but did not eat the cereal and/or meat throughout the study period and thus were labelled as non-compliers (see section 4.3.3 for criteria used to identify non-complier). The daily compliance calendar records confirmed this in each case (Table 5.6). All but one of these infants reached end-point. The other reached a putative end-point that was not confirmed in a second sample. The proportion of end-points was much higher for infants who did not comply (83.3%) compared to those who did comply (7.0%) with the treatment diet (p=0.0002) (Table 6.5). This indirectly indicates that the iron from the combination of meat and cereal prevented iron depletion in cow milk-fed infants.

The higher incidence of confirmed (83.3% to 7.0%, p=0.0002) (Table 6.5) and putative end-points in non-compliant infants (83.3% to 27.9%, p=0.015) (Table 6.6) within the treatment group was not only statistically significant but also clinically important. In the absence of iron-fortified formula, iron supplements, infant cereal and meat there are few sources of iron in the typical infant diet. Most of the non-complying infants became iron depleted (only one had low Hgb) and were at risk for developing anaemia. Anaemia was rare because all subjects started with normal iron indices and by design, infants were treated on evidence of iron depletion, before they could develop anaemia. However, if their dietary patterns were not changed, it is very likely that they would have gone on to develop iron deficiency anaemia. This observation is consistent with the literature which suggests that the early ingestion of cow milk as the principle source of nutrition for infants is a significant risk factor for the development of iron
deficiency anaemia.

Dietary data were not able to explain why some, albeit very few infants reached end-point. Within the treatment group (excluding non-compliers), all three infants reached end-point due to low Ferr (Table 6.12). Daily compliance records were not available for two of these subjects (therefore not represented in Table 5.6) but the third one was consuming at least 30 g of cereal and 70 g or more of meat per day. The other two were treated as compliers because monthly compliance checks indicated that the infants were regularly consuming meat and cereal; however, there was no indication of the amounts consumed. Within the control group, three of the five confirmed end-points (by low Ferr) were using cow milk and some formula, meat and cereal; however, there was no indication of the amounts consumed. The other two infants reached end-point by low HgB (109.3 g/L and 104.7 g/L) (Table 6.12). Both infants were using iron-fortified formula as the primary milk source. Furthermore, both had Ferr levels well within the normal range (37.0 μg/L and 19.5 μg/L). This suggests that the HgB cut-off (HgB < 110 g/L) may have been overly conservative.

The third line of evidence that suggests that the iron from cereal and meat was well utilised and prevented iron deficiency in cow milk-fed infants was that over the course of the study, changes in Ferr and HgB across age were similar between study groups, despite differences in dietary intake (Chapter 5). Ferr levels declined with age at similar rates in both groups. It is likely that this decline is a function of a physiologic change rather than iron nutriture, since iron stores are expected to decline during the second six months of
life (Dallman 1988). Haemoglobin levels did not change significantly with age in either group. These results are as expected in normal healthy infants since iron stores are generally being utilised at this age due to the rapidly expanding blood volume but functional iron should remain unaffected in the absence of iron deficiency (Dallman 1988).

In summary, three lines of evidence suggest that the iron from cereal and meat was well utilised and prevented iron deficiency in cow milk-fed infants. Firstly, the incidence of end-point in the treatment group (7.0%) was low compared to past surveys of infants from low and normal socio-economic households and not statistically different from control infants (9.3%, p=0.69) who were following CPS infant feeding guidelines. Secondly, 12% of the treatment infants who consumed cow milk did not consume either meat or cereal almost all of whom reached end-point. Finally, the rate of fall in plasma Ferr with age was the same in both groups, suggesting that the iron status of the two groups was equivalent.
7. USE OF TRANSFERRIN RECEPTOR AS AN INDEX OF IRON DEFICIENCY

7.1 Introduction

Having completed the randomised clinical trial described in chapters five and six, it became apparent that our ability to identify infants at risk for anaemia, based on serum Ferr, was inadequate. Although, serum Ferr is useful as an indicator of depletion of iron stores, there is wide intraindividual variability (Cooper and Zlotkin 1996; Ahluwalia et al. 1993; Borel et al. 1991) and it is not sensitive in detecting early tissue iron deficiency (Dallman and Reeves 1984; Lipschitz et al. 1974). In fact, on repeat sampling, only 54% of low values were confirmed. In our randomised clinical trial, the measurement of a true Ferr value was very important since infants with low values were excluded from the study. In the clinical practice of paediatricians, a low serum Ferr value is often used to determine the need for therapeutic iron supplementation. Despite the poor sensitivity (Dallman and Reeves 1984; Lipschitz et al. 1974) of this laboratory measure of iron status, it is a commonly used, important clinical and research tool. Other laboratory measures currently used to diagnose iron deficiency - including serum iron, total iron-binding capacity, transferrin saturation and free erythrocyte protoporphyrin - are unable to distinguish between iron deficiency and the hypoproliferative anaemia which accompanies infection, inflammation, or malignancy (anaemia of chronic disease).

In 1993, Cook and colleagues were among the first groups to describe a new laboratory measure of iron status, the serum transferrin receptor. The circulating soluble TfR concentration has been shown to be a quantitative measure of functional iron
deficiency (Cook et al. 1993) and has many advantages over other methods of assessing iron deficiency. Increases in TfR concentration occur earlier in the development of functional iron deficiency than do changes in erythrocyte protoporphyrin or mean corpuscular volume (Cook and Skikne 1989). Measurements of TfR in blood, along with haemoglobin, make it possible to distinguish iron deficiency anaemia from the anaemia of chronic disease (Ferguson et al. 1992; Punnonen et al. 1994). Day-to-day variation in TfR in the elderly (Ahluwalia et al. 1993) and adults (Cooper and Zlotkin 1996) is relatively low compared to other measures such as Ferr, serum iron and transferrin saturation. Furthermore, the combination of measurements of serum Ferr and serum TfR has been proposed as a reliable method for assessing iron status and that the log ratio of TfR to Ferr (log TfR:Ferr) may be useful as a single index to estimate body iron in population studies (Anttila and Cook 1997; Cook and Skikne 1989; Punnonen and Irjala 1997).

Although TfR is potentially important in the diagnosis of iron deficiency, there is a lack of normative data in infants and children. Of particular interest is the application of TfR measures in the infant population where there is high risk of iron deficiency. Within this thesis, we have studied iron status in disadvantaged infants defining endpoints in terms of primarily low Ferr confirmed in a second blood sample (The Infant Iron Study). As previously mentioned, we found that Ferr<10μg/L was only confirmed 43% of the time in a second blood sample. TfR measures are much more stable and give more clinically important information since Ferr reflects iron depletion while TfR reflects
tissue iron deficiency. The objectives of this study were two-fold: (i) To establish normative percentile estimates of TfR and log TfR:Ferr for healthy infants nine to 15 months of age using blood samples which had been previously been collected from a national iron deficiency prevalence study (Zlotkin et al., 1996). (ii) To evaluate the use of TfR as a measure of tissue iron deficiency by comparing TfR values in the samples collected in the clinical trial (Infant Iron Study) to the normative percentile estimates of TfR.

7.2 Population used to Establish Normative Percentile Estimates

Blood samples were previously collected from infants studied in four cities across Canada (Iron Prevalence Study). The current study measured the TfR levels in these samples and used them to establish normative percentile estimates. The sampling methodology has been previously described (Zlotkin et al. 1996). Briefly, healthy full-term infants, ages 8.6 and 15.5 months, were randomly chosen from lists of infants born in Toronto, Montreal, Edmonton and Halifax. Infants were excluded if they had lived outside of Canada for more than two months, had any disease, had received a blood transfusion, had major surgery or a recent infection. Signed consent was obtained from the parent or parents of the infants. At their visit to the study centre, food frequency and demographic questionnaires were completed, anthropometric measurements were made and single 250 µL capillary blood samples were obtained. A description of the sample and its derivation is shown in Table 7.1.
Table 7.1 Description of the sample used to generate percentile estimates (from the Iron Prevalence Study)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Edmonton</th>
<th>Toronto</th>
<th>Montreal</th>
<th>Halifax</th>
</tr>
</thead>
<tbody>
<tr>
<td>#on birth lists</td>
<td>5363</td>
<td>22,716</td>
<td>60,0636</td>
<td>21,100</td>
</tr>
<tr>
<td>#randomly selected</td>
<td>1000</td>
<td>772</td>
<td>1058</td>
<td>2055</td>
</tr>
<tr>
<td>Actual sampling fraction</td>
<td>1/5.4</td>
<td>1/29.4</td>
<td>1/57.4</td>
<td>1/10.3</td>
</tr>
<tr>
<td>#asked to participate</td>
<td>911</td>
<td>772</td>
<td>1058</td>
<td>2055</td>
</tr>
<tr>
<td>#who accepted (%)</td>
<td>194 (21.3)</td>
<td>61 (7.9)</td>
<td>36 (3.4)</td>
<td>233 (10.9)</td>
</tr>
<tr>
<td>#enrolled</td>
<td>189</td>
<td>46</td>
<td>29</td>
<td>124</td>
</tr>
<tr>
<td>#used in analysis</td>
<td>156</td>
<td>65</td>
<td>117</td>
<td>147</td>
</tr>
</tbody>
</table>

7.3 Statistical Analysis

Distributions of TfR concentrations were grouped in increments of 0.5. Percentiles were based on ages rounded up to the nearest whole number for both populations. Age trends and gender differences were tested using the GLM procedure. For age trends, age was treated as a continuous variable. All correlations between different iron indices used the CORR procedure (Pearson's). Percentile estimates were generated using the univariate procedure on samples provided by Zlotkin et al. (1996).

Percent coefficients of variation (CV) for TfR and Ferr were calculated using
values from the Infant Iron Study where a second blood sample was obtained to confirm end-point. Biologic variation represents the coefficient of variation for the two repeat sample means. Analytic variation represents the coefficient of variation for the triplicate values of each blood sample.

7.4 Results

7.4.1 Percentile Estimates

Three infants were excluded because they had been treated with iron prior to the study. Blood volumes were too low to analyse in another 79 samples. A total of 485 evaluable subjects were included (ages 8.6 to 15.2 months). Male infants were 51.6% of the total. Age and gender of the population are shown in Figure 7.1. Mean weight and height were as expected for infants this age (NCHS values). The average income level of the sample reflected the general population in the four cities although the educational training of the parents was somewhat higher than the general population of the four cities (Zlotkin et al. 1996).

Within the study population, there were no significant gender-related differences (F=1.66, 1, 218 degrees of freedom, p=0.20); thus, data for males and females were combined. The mean (± SD) plasma TfR concentration was 4.4±1.1 mg/L; the distribution is shown in Figure 7.2a. There was no correlation between TfR concentration and age (F=0.85, 172, 218 degrees of freedom p=0.99). There was no correlation between TfR concentration and Hgb (R=-0.06, p=0.18) or Ferr (R=-0.08,
p=0.07) but there was one with FEP (R=0.19, p=0.0001); however the correlation coefficient was low.

Mean HgB, FEP, Ferr and TfR values are shown in Table 7.2. Less than 2% of the total study population had HgB<100 g/L, less than 4% had FEP>100 μg/L while more than half had Ferr<10 μg/L (Table 7.3). Infants were considered to have iron deficiency anaemia if HgB values were below 100 g/L. Values above this were chosen as representative of normal because, in this age group, functional changes (motor and intellectual scores on Bayley Development Test) do not become apparent until below 100 g/L (Moffatt et al. 1993b; Walter 1992). A more conservative HgB value of less then 110 g/L was used to define end-points in the Infant Iron Study because, for ethical reasons, infants were identified and treated with Fer-in-Sol before functional deficits would occur. A direct measure of iron deficiency without anaemia was also measured (FEP). Percentile estimates for TfR concentration for infants with normal HgB (≥100g/L) and FEP (<100μg/L) are presented in Figure 7.3. Overall, there were only four subjects that had all iron indicators abnormal (HgB<100 g/L, Ferr<10 μg/L and FEP>100 μg/L). Their TfR and log TfR:Ferr values are shown in Table 7.4. Fifty two percent (n=251) of the total study population had Ferr <10 μg/L (Table 7.3). The mean plasma TfR concentration when these infants were excluded was 4.5±1.1 mg/L; the distribution is shown in Figure 7.4.

There was a correlation between log TfR:Ferr and HgB (R=-.26, p=0.0001) and FEP (R=0.41, p=0.0001); however, the correlation coefficients were quite low.
Excluding subjects with low HgB, low Ferr or high FEP, the mean (± SD) log TfR:Ferr was 5.4±0.5 (µg/µg); the distribution is shown in Figure 7.5a. Furthermore, there was no correlation between log TfR:Ferr and age (F=1.07, 169, 220 degrees of freedom, p=0.32) (Figure 7.6).
Figure 7.1 Subjects Used to Derive Percentile Estimates by Age and Gender from the Iron Prevalence Study
Figure 7.2
a. Distribution of Transferrin Receptor Concentration for all Subjects Studied in the Iron Prevalence Study.
Mean ± SD = 4.4 ± 1.1 mg/L. n=485.
b. Distribution of Transferrin Receptor Concentration for subjects in the Infant Iron Study.
Mean ± SD = 4.5 ± 1.2 mg/L. n=465.
Table 7.2 Mean HgB, FEP, Ferr and TfR for infants in the Iron Prevalence Study.

<table>
<thead>
<tr>
<th>Haematologic Measure</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/L)</td>
<td>126.8 ± 14.8</td>
</tr>
<tr>
<td>Erythrocyte Protoporphyrin (µg/L)</td>
<td>45.8 ± 29.3</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>12.9 ± 10.5</td>
</tr>
<tr>
<td>Transferrin Receptor (mg/L)</td>
<td>4.4 ± 1.1</td>
</tr>
</tbody>
</table>

mean ± SD; n=485

Table 7.3 Observed prevalence of low HgB, high FEP and low Ferr Among Infants in the Iron Prevalence Study

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin &lt; 100 g/L</td>
<td>8</td>
<td>1.6%</td>
</tr>
<tr>
<td>Free Erythrocyte Protoporphyrin &gt; 100 µg/L</td>
<td>17</td>
<td>3.5%</td>
</tr>
<tr>
<td>Ferritin &lt; 10 µg/L</td>
<td>251</td>
<td>51.8%</td>
</tr>
</tbody>
</table>

Total n=276
Figure 7.3 Percentile estimates of transferrin receptor concentration for all infants with HgB ≥ 100 g/L and FEP ≤ 100 μg/L from the Iron Prevalence Study. Numbers on the right side of figure represent percentiles. n=464

Table 7.4 TfR and Log TfR:Ferr Values in Subjects with Low HgB, High FEP and Low Ferr from the Iron Prevalence Study.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Haemoglobin (g/L)</th>
<th>Free Erythrocyte Protoporphyrin (μg/L)</th>
<th>Ferritin (μg/L)</th>
<th>Transferrin Receptor (mg/L)</th>
<th>Log TfR:Ferr (μg/μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65.1</td>
<td>171.1</td>
<td>&lt;0.5</td>
<td>7.0</td>
<td>15.8</td>
</tr>
<tr>
<td>2</td>
<td>79.6</td>
<td>316.3</td>
<td>&lt;0.5</td>
<td>7.2</td>
<td>15.8</td>
</tr>
<tr>
<td>3</td>
<td>70.8</td>
<td>272.2</td>
<td>0.5</td>
<td>6.6</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>85.2</td>
<td>155.2</td>
<td>2.0</td>
<td>6.95</td>
<td>8.2</td>
</tr>
</tbody>
</table>
Figure 7.4 Distribution of transferrin receptor concentration for infants with HgB>100g/L, FEP<100mg/L and Ferr>10μg/L from the Iron Prevalence Study. Mean±SD=4.5±1.1 μg/L. n=235.
Figure 7.5

a. Distribution of log (TfR:Ferr) for infants with Hgb>100 g/L, FEP<100 µg/L and Ferr>0 µg/L from the Iron Prevalence Study. Mean ± SD = 5.4 ± 0.5 (µg/µg). n=235.

b. Distribution of log (TfR:Ferr) for infants from the Infant Iron Study. Mean ± SD = 5.2 ± 0.07 (µg/µg). n=465.
Figure 7.6 Percentile estimates of log (TfR:Ferr) for infants with HgB>100 g/L, FEP<100 µg/L and Ferr>10 µg/L from the Iron Prevalence Study. Numbers on the right side of figure represent percentiles.
7.4.2 **Infant Iron Study**

In total TfR concentration was assayed in 482 blood samples from the Infant Iron Study population (included are initial blood samples from subjects excluded from the study after the first visit). Seventeen were discarded because of insufficient blood volume or problems with TfR binding to assay plates. There were no significant gender-related differences; thus, data for males and females were combined. The mean (± SD) plasma TfR concentration was 4.5±1.2 mg/L; the distribution is shown in Figure 7.2b. There was no correlation between TfR concentration and age, group, HgB or Ferr. The mean (± SD) log TfR:Ferr was 5.2±0.7 (µg/µg); the distribution is shown in Figure 7.5b. There was no correlation between log TfR:Ferr and age or HgB. However, TfR and log TfR:Ferr values were higher than the population mean for those ten blood samples where HgB was less than 110 g/L and Ferr was less than 15 µg/L. Six out of the eight had TfR values above the 95th percentile while all had elevated values for log TfR:Ferr (Table 7.5).

The overall biologic variation for the repeated blood samples (n=78) and the analytic variation for all the blood samples collected (n=465) of TfR and Ferr are shown in Table 7.6 and expressed as CV. The overall, biologic and analytic CV was higher for Ferr than TfR.

Based on the definition that samples from the Infant Iron Study with HgB below 110g/L and Ferr below 15µg/L were iron deficient, the sensitivity and specificity and positive predictive value of TfR are presented in Table 7.7.
Table 7.5 TfR and Log TfR:Ferr values in subjects with HgB<110g/L and Ferr<15μg/L from the Infant Iron Study (Chapter 6).

<table>
<thead>
<tr>
<th>Haemoglobin</th>
<th>Ferritin (μg/L)</th>
<th>Transferrin Receptor (mg/L)</th>
<th>Log TfR:Ferr (µg/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>105.1</td>
<td>13.0</td>
<td>7.1</td>
<td>6.3</td>
</tr>
<tr>
<td>95.4</td>
<td>3.0</td>
<td>6.7</td>
<td>7.7</td>
</tr>
<tr>
<td>98.4</td>
<td>8.0</td>
<td>5.6</td>
<td>6.6</td>
</tr>
<tr>
<td>99.3</td>
<td>8.5</td>
<td>6.5</td>
<td>6.6</td>
</tr>
<tr>
<td>75.0</td>
<td>4.0</td>
<td>7.2</td>
<td>7.5</td>
</tr>
<tr>
<td>80.3</td>
<td>3.0</td>
<td>5.6</td>
<td>7.5</td>
</tr>
<tr>
<td>106.8</td>
<td>8.0</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
<td>96.6</td>
<td>13.5</td>
<td>7.0</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Table 7.6 Overall, biologic and analytic variation of TfR and Ferr in blood samples from the Infant Iron Study (Chapter 6).

<table>
<thead>
<tr>
<th></th>
<th>Overall CV</th>
<th>Biologic CV</th>
<th>Analytic CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>TfR</td>
<td>16.1%</td>
<td>14.4%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Ferr</td>
<td>26.1%</td>
<td>30.4%</td>
<td>5.7%</td>
</tr>
</tbody>
</table>

Biologic CV was calculated as the standard deviation divided by the mean (expressed as a percentage) based on the means of repeated blood samples (n=78). Analytic variation was calculated the same way based on the means of the triplicate values of each blood sample (n=465). The Overall CV was based on the combined biologic and analytic standard deviation divided by the overall sample mean.
Table 7.7  Diagnostic Sensitivity and Specificity of TfR from data generated from the Infant Iron Study (Chapter 6)

<table>
<thead>
<tr>
<th></th>
<th>Iron Deficient</th>
<th>Not Iron Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>High TfR</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>Normal TfR</td>
<td>2</td>
<td>406</td>
</tr>
</tbody>
</table>

Sensitivity = $6/(6+2) = 0.75$; (95% confidence interval 0.45-1.05)
Specificity = $406/(22+406) = 0.95$; (95% confidence interval 0.94-0.96)
Positive predictive value = $6/6 + 22 = 0.21$; (95% confidence interval 0.06-0.36)
Prevalence = $(6+2)/436 = 0.02$
7.5 Discussion

7.5.1 Percentile Estimates Derived from the Iron Prevalence Study

This study is the first to establish percentile values for plasma TfR concentration in healthy infants. On the basis of the socio-economic status of the families of the infants who participated in the Iron Prevalence Study, this sample of infants represented a healthy middle-class urban population (Zlotkin et al. 1996). Plasma TfR concentrations did not change with age over the period of nine to 15 months of age and were, on average, slightly higher than published values for healthy adults when the same analytic method was used (Cooper and Zlotkin 1996). This slight elevation may reflect increased need for iron at this age due to the expanding blood volume associated with growth. As expected, there were no gender differences in TfR concentration at this age.

The observation that TfR concentrations were not associated with Hgb and Ferr and only weakly associated with FEP in normal infants is not surprising. TfR concentration increases with tissue iron deficiency but would not be expected to change when there are normal concentrations of Hgb or FEP. The majority of subjects in this study had concentrations of Hgb and FEP within the normal range (Table 7.3). There were many infants with low Ferr (Table 7.3), but there was no association with TfR in the entire population or in infants with Ferr > 10μg/L or Ferr ≤ 10μg/L. Although low Ferr does reflect iron depletion (low tissue iron stores), it does not distinguish iron deficiency from depletion and is not necessarily associated with tissue iron deficiency. The distribution for TfR of the overall population (Figure 7.2a) and those with all normal iron
indices (Figure 7.4) are skewed to the right with similar means and range, despite differences in iron stores.

For infants with normal HgB and FEP (Figure 7.3), TfR concentrations of 6.5 to 7 mg/L were at the 95th percentile while concentrations near 3 mg/L were approximately at the 5th percentile. At the 95th percentile, TfR concentrations were lowest at nine months of age. This was likely a result of the small number of subjects (n=24) in this age group.

It has been suggested that measurements of serum Ferr and serum TfR in tandem can provide a precise quantitative measure of body iron over a broad range of iron status, giving an indication of degree of tissue iron deficiency (Cook and Skikne 1989). Skikne et al. demonstrated that during phlebotomy, serum Ferr changed dramatically over the period of iron storage depletion with relatively little change once stores were fully depleted. In contrast, there was very little change in serum TfR during the period of depletion but as stores became fully exhausted there was a progressive increase (Skikne et al. 1990). Due to the reciprocal relationship between serum TfR and serum Ferr, the ratio of TfR to Ferr may be useful as a single parameter to estimate total body iron, especially in the depletion and deficiency stages. However, this measure is still subject to the high variation associated with Ferr measures (Cooper and Zlotkin 1996; Ahluwalia et al. 1993; Borel et al. 1991).

The weak association between log TfR:Ferr and HgB and FEP is not surprising. Log TfR:Ferr concentration increases with iron depletion and tissue iron deficiency but would not be expected to change with normal concentrations of HgB or FEP. The
majority of subjects in this study had a normal range of \( \text{HgB} \) and FEP (Table 7.3). The data do not provide information on how log TfR:Ferr associates with iron depletion since no external measure of iron stores other than serum Ferr was obtained in these subjects. Subjects with low Ferr were excluded from the generation of percentile estimates since log TfR:Ferr incorporates a measure of iron depletion. Thus, only those without iron depletion can be used for normative data.

On the basis of the methodology used in this study we have defined the normal range of TfR concentration as 3 to 6.5 mg/L and the normal range of log TfR:Ferr ratio as 4.5 to 6.2 (\( \mu \text{g/\mu g} \)). Thus, values above these probably reflect iron deficiency in the majority of infants; however, further studies in iron depleted and iron deficient infants are required to substantiate this conclusion.

7.5.2 Percentile Estimates Applied to the Infant Iron Study

There were no significant effects of treatment grouping, age or gender on TfR concentrations and, as a population, the subjects of the Infant Iron Study had TfR concentrations that were very similar to those used to generate 'normal' percentile estimates (the Iron Prevalence Study). The mean (± SD) TfR concentrations were virtually identical between the two studies (4.5±1.2 and 4.4±1.1 mg/L respectively) and the distributions very similar in range and shape (Figures 7.2b and 7.2a). Similarly, the mean log TfR:Ferr was similar in the two studies (5.2±0.7 vs. 5.4±0.5 respectively). Both have similarly shaped distributions except at the upper extreme. The percentile
estimates of log TfR:Ferr do not go as high in the Iron Prevalence Study because samples with low Ferr were excluded to generate normative data (Figures 7.5a and 7.5b). Other iron indices such as Hgb and Ferr were not associated with TfR concentrations or log TfR:Ferr in either study.

Transferrin receptor concentrations were not used to determine end-points during the Infant Iron Study because the diagnostic criteria (percentile estimates) had not been established at the study onset. Instead, end-points were declared primarily on evidence of iron depletion (Ferr<10μg/L). Because subjects were removed from the study and treated if they reached end-point, very few subjects went on to develop tissue iron deficiency. Thus, TfR values could not be used to confirm or refute any conclusions based on end-points determined by low Ferr or Hgb values. However, it was possible to determine that the overall CV was much lower for TfR than for Ferr, based on blood samples repeated at a second visit. The analytic CV was similar for Ferr and TfR measures but the biologic CV was about two times greater for Ferr (Table 7.6). This supports the findings of other studies estimating that anywhere between three to 10 blood samples are required to have an accurate estimate of Ferr, as compared to a single sample for TfR (Ahluwalia et al. 1993; Cooper and Zlotkin 1996).

It was also possible to estimate the sensitivity and specificity of TfR as a diagnostic measure of tissue iron deficiency. Other than taking marrow samples and staining for iron, there is no reliable ‘gold’ standard with which to determine the true sensitivity and specificity of TfR as an index of tissue iron deficiency. However, we

138
chose to define samples with HgB below 110g/L and Ferr below 15µg/L as iron deficient (Table 7.5). The more relaxed cut-off values for HgB and Ferr were chosen to include those who had iron deficiency as well as those with early iron deficiency anaemia. These subjects were likely to be truly iron deficient since both HgB and Ferr were low in all of these samples. Despite high biologic variation, low serum Ferr values are highly specific for iron depletion since no other conditions drive serum Ferr down (Cook et al. 1992; Cook and Skikne 1982). In the absence of chronic infection and in conjunction with low Ferr values, low HgB values are indicative of iron deficiency anaemia. All but one of these samples identified as being truly iron deficient came from infants excluded (based on confirmed end-point definitions) from the Infant Iron Study at the initial visit due to poor iron status. Samples with HgB≥110 g/L and Ferr≥15 µg/L were defined as not iron deficient. It is likely that these samples were correctly identified as not being iron deficient because subjects in the Infant Iron Study were iron replete to start, blood was subsequently sampled every second month and any subject reaching iron depletion was treated immediately, before iron deficiency could develop.

Based on the percentile estimates derived from the Iron Prevalence Study, a TfR greater than 6.5 is suggestive of iron deficiency. This calculation allowed for the determination of sensitivity and specificity of TfR as a diagnostic measure of iron deficiency. Thus, TfR would correctly identify those with iron deficiency 75% of the time (ie. fail to identify it 25% of the time). Furthermore, TfR would correctly identify those without iron deficiency 95% of the time (ie. produce a false positive result 5% of
the time) (Table 7.7).

It is important to note that the calculated sensitivity and specificity of TfR as a diagnostic test for iron deficiency assumed that samples were correctly identified as truly iron deficient or truly not, independent of TfR measures. Direct measures of tissue iron deficiency were not made since such tests would require larger blood volume and offered little advantage to the study. However, as argued above, there is indirect but good reason to believe that iron status was correctly identified. Another concern over these calculations is the low prevalence of iron deficiency (2%). The low prevalence has the effect of decreasing the positive predictive value estimate; however, sensitivity and specificity estimates are relatively unaffected (Lilienfeld and Stolley 1994).

The calculated sensitivity (0.75) of TfR is similar to that of other biochemical tests for iron deficiency such as transferrin saturation (0.76) but the calculated specificity (0.95) is much higher than that of transferrin saturation (0.36) (Low et al. 1997). The sensitivity and specificity of TfR as a marker of iron deficiency are higher than that of Ferr as a marker of iron depletion (0.25-0.60 and 0.30-0.60 respectively) (Balaban et al. 1993; Low et al. 1997). Thus, data from the current studies suggest that TfR is a fairly sensitive and highly specific diagnostic measure of tissue iron deficiency.

The same calculations could not be done for log TfR:Ferr since log TfR:Ferr provides a measure of body iron over a broader range of iron status than TfR alone (Cook and Skikne 1989). This measure would be sensitive to late iron depletion as well as iron deficiency. Because of this the current data do not provide a standard by which to test the
sensitivity and specificity of log TfR:Ferr. However, all subjects from the Infant Iron Study population identified as truly iron deficient (Table 7.6) had log TfR:Ferr values above 6.2 (µg/µg) (the 95th percentile). Because every subject known to be iron deficient would be identified as so by log TfR:Ferr, it is an even more sensitive index of iron deficiency than TfR alone. This is not surprising since log TfR:Ferr is affected by iron depletion. However, because it is sensitive to iron depletion, log TfR:Ferr is likely less specific for iron deficiency than TfR alone.

In summary, normative percentile estimates of TfR and log TfR:Ferr define a range above which is indicative of tissue iron deficiency. When these definitions were applied to the population studied in this thesis, TfR was shown to be a fairly sensitive and highly specific diagnostic measure of tissue iron deficiency. Log TfR:Ferr may be an even more sensitive but less specific measure of tissue iron deficiency. Furthermore, the data indicate that TfR has a much lower overall and biologic CV when compared to Ferr. Thus TfR appears to be an accurate, relatively sensitive and specific diagnostic measure of tissue iron deficiency.
8. GENERAL DISCUSSION

The hypothesis of the thesis was that there would be a significant reduction in the incidence of iron depletion and/or iron deficiency anaemia in six to 12 month old cow milk-fed infants from low-income households consuming infant cereals (30g/day) and pureed meats (70g/day) when compared to infants from low-income households with no dietary intervention. Because the incidence of end-point in the control group was so low, the hypothesis could not be tested adequately. However the three objectives of the study were met.

Inherent in the study design was the premise that because few low-income families follow CPS feeding guidelines, 30% of the infants in the control group would reach confirmed end-point and the treatment would reduce this to below 10%. This premise was incorrect. The incidence of confirmed end-points was not significantly different between the study groups (7.0% treatment vs. 9.3% control, p=0.66) since the end-point rate was lower than 10% in both groups. The reason the rate of end-point in the control group was so much lower than expected can be explained by the dietary patterns of these infants.

The first study objective was to examine the patterns of food usage within the study population and identify those that may be implicated in the cause or prevention of iron depletion in low-income families in Toronto (Chapter 5). During the study period, the majority of infants in the treatment group followed the prescribed dietary protocol. Only the use of cow milk at ten months of age was associated with low iron status. This
may reflect the 12% of treatment infants that were consuming cow milk but did not consume the meat and cereal and reached end-point.

Unexpectedly, the feeding patterns of the control group were not typical of other low-income groups where iron depletion is more prevalent. Instead the majority of these families fed in accordance to CPS guidelines (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998) which included iron-rich foods such as iron-fortified formula (Figure 5.10), iron-fortified infant cereal (Figure 5.13) and meat (Figure 5.14). Furthermore, the majority of treatment families delayed cow milk feeding until the tenth month (5.12). Thus, although these infants were from low-income families, they were not typical of infants at risk for iron deficiency.

There were many reasons that may explain why we were unable to sample our target population. The first reason was that our recruitment strategies and exclusion criteria did not target families with low education levels. For example, few families were recruited from community centres and those from the Tots and Teens program tended to drop out of the study. These families tended to have very low incomes and education levels. Thus infants at greatest risk for iron deficiency were unlikely to have completed the study. Instead, the majority of subjects were recruited from direct mailings using lists generated by Carnation Canada Ltd. based on mothers who had replied to previous mail solicitations or product offers for their infants (section 4.1.2). Although these families were low-income, they may have been more motivated to respond to programs for their infants and many of them were highly educated (Table 5.3). Thus, recruitment tended to
exclude those at higher risk for iron deficiency and was likely biased towards more motivated and more highly educated families. This may partially explain why the families studied seemed to have good knowledge of proper feeding guidelines.

A second problem was that control families tended to have higher incomes and higher education levels than treatment families (Table 5.3). The reason for this was likely related to the restrictions placed on the treatment group. These families had to be willing to use cow milk as the only milk source throughout the study period; therefore, mothers choosing to breast-feed or unwilling to feed cow milk after six months were not included.

Eight families dropped out of the study as soon as they discovered they were in the treatment group because of one of these reasons (section 5.5). These families had higher incomes (no data regarding education was collected for these families) relative to the overall study population. Furthermore, personal observations suggest that a number of more educated mothers (who did not participate in the study) were willing to participate only if they could be in the control group. This suggests that mothers unwilling to deviate from CPS guidelines tended to be excluded or drop out if randomised to the treatment group but participated if in the control. However, it is important to note that despite these biases, other baseline characteristics such as anthropometry, HgB and Ferr were similar between groups thus did not affect differences in end-point incidence (Table 5.2).

A potential third problem was that the provision of infant clothing and laundry detergent to the control group may have affected the study outcome. These items were
provided at no charge to offset the financial advantage the treatment group would have received due to the cost-free food they were provided. Non-food related items were chosen to avoid influencing the feeding choices of the control families. At each visit, clothing and detergent, with the equivalent dollar value (approximately $150 per visit) to the food provided to the treatment group, was given to the control families. This influx of products may have allowed these families to spend more money on infant foods such as iron-fortified formula. However, families tended to follow CPS guidelines before the study, particularly in the control group (Chapter 5) which suggests that this had only a small effect on feeding choices. All of these factors inherent in the research design may have resulted in a sample atypical of low-income infants, which explains why the incidence of end-point in the control group was lower than expected.

Despite not being able to adequately test the hypothesis, we were able to meet the second study objective. Three lines of evidence from our results suggest that the iron from cereal and meat was well utilised and prevented iron deficiency in cow milk-fed infants.

Firstly, the incidence of end-point (low HgB or low Ferr) in the treatment group was low compared to past surveys of infants from low and normal socio-economic households and not statistically different from control infants who followed CPS infant feeding guidelines. The proportion of infants consuming the treatment diet that reached end-point was 7.0%. This was not different from the control group (9.3%, p=0.66) who were feeding in accordance to CPS guidelines. The fact that the incidence of end-points was no higher in the treatment group (excluding non-compliers) suggests that the treatment diet did prevent
iron depletion and/or anaemia. Treatment families were only included in the study if they did not use iron-fortified formula (or any milk source other than whole cow milk) and did not use iron supplements. The only substantial sources of iron for these infants were the infant cereal and meat (Chapter 5). Although the individual contributions of infant cereal versus meat could not be separated, the results of this study are consistent with others showing that infant cereal (NHRDP Project 6606 4104 61; Walter et al., 1993) and iron-fortified meat and cereal (Haschke et al. 1988) contribute substantially to preventing iron deficiency.

Secondly, almost all of the treatment infants who consumed cow milk but did not consume either meat or cereal reached end-point. Every effort was made to encourage use of the infant cereal and meat in this study. Despite efforts to increase compliance, 12% (six) of the treatment infants ingesting cow milk did not consume any cereal and/or meat during the study period and were labelled as non-compliers (see section 4.3.3 for criteria used to define non-compliance). All but one of these infants reached end-point. The other reached a putative end-point that was not confirmed in a second sample. The proportion of end-points was much higher for infants who did not comply (83.3%) when compared to those who did comply (7.0%) with the treatment diet (p=0.0002) (Table 6.5).

Increases in end-points because of lack of compliance is of concern since it is likely that these infants would have gone on to develop iron deficiency anaemia if not treated. Cow milk is a poor source of iron (Saarinen et al. 1977) and without the cereal and meat, there were no good sources of iron in these infants’ diets (Chapter 5). Thus, it
is not surprising that almost of all of the these infants reached end-point and, if these dietary patterns were not changed, it is very likely that they would have gone on to develop iron deficiency anaemia.

The few families who did not comply were of lower socio-economic status than the overall study group. Socio-demographic data for four of the six subjects (12%) who failed to consume cereal and/or meat were available (Table 6.8). For three of the four, both parents had only primary to secondary school education and incomes well below the group mean of 69% of the low-income cut-off. It may have been that compliance to the cereal and meat may be more of a problem in infants from very low socio-economic households; however, there was insufficient power to show this statistically (p=0.11, power: 0.18) (Table 6.9).

Thirdly, the rate of fall in plasma Ferr with age was the same in both groups, suggesting that the iron status of the two groups were equivalent. It is likely that this decline is a function of a physiologic change rather than iron nutriture, since iron stores are expected to decline during the second six months of life (Dallman 1988). Haemoglobin levels did not change significantly with age in either group. These results are as expected in normal healthy infants suggesting that both groups had dietary intakes sufficient to maintain normal iron status. Thus, despite some weaknesses in the study design, this second study objective was met and indicated that consumption of infant cereal and meat prevented iron depletion/anaemia in infants consuming whole cow milk in the second six months of life.
The fact that the treatment did prevent iron depletion and/or iron deficiency anaemia implies that consuming cow milk *per se* is not an issue in the second six months of life if overall iron intake is adequate and this contradicts the CPS and AAP recommendations. The CPS recommends delaying introduction to cow milk until nine to 12 months (Canadian Paediatric Society Nutrition Committee, 1991; Canadian Paediatric Society, 1998) while the AAP recommends delaying its introduction until one year of age (American Academy of Pediatrics 1992). It should be noted however that the current study is the first direct test of these recommendations which are based primarily on concerns over the potential for high renal solute load and iron deficiency in cow milk-fed infants due to lack of absorbable iron and occult gastrointestinal blood loss. The recommendations appear to be based on indirect evidence and over-extension of the data.

Cow milk has approximately three and a half times more protein than breast milk. The concern is that this would result in a high renal solute load which would increase the risk for dehydration during periods of fever or diarrhoea (Ziegler and Fomon 1989). However, under normal circumstances this is not a concern and in extreme cases of water loss such as illness, cow milk feeding can be temporarily stopped. Occult blood loss is a concern before six months of age (Hoag et al. 1961; Rasch et al. 1960; Woodruff et al. 1972, Fomon et al. 1981) but not after (Belsten et al. 1997; Fuchs et al. 1993; Thomas et al. 1986). One study by Ziegler et al. (1990) concluded that older infants fed cow milk had a significantly higher proportion of guaiac-positive stools and greater excretion of faecal haemoglobin (Ziegler et al. 1990). However, conclusions regarding faecal blood
loss were based largely on analysis of the number of guaiac-positive stools, rather than the number of infants with positive guaiac tests. Upon re-analysis of the data at each time interval, no significant differences between cow milk-fed and formula fed infants were detected (p=0.07). Thus, the evidence suggests that the only major concern over whole cow milk use after six months is if it is not fed concurrently with adequate amounts of available iron (Saarinen et al. 1977). The current data support this argument since the use of iron-rich foods did prevent iron depletion in cow milk-fed infants. Furthermore, growth rates and morbidity were not different in infants consuming the combination of cereal, meat and cow milk when compared to controls (Figures 5.16-5.19).

Other methods of preventing iron deficiency in infants from low-income households, like feeding iron-fortified formula, are no more effective than feeding a combination of meat, infant cereal and cow milk. Iron-fortified formula does prevent iron deficiency when consumed (Fuchs and al 1993; Longstaffe et al. 1993; Moffatt et al. 1993a; Penrod et al. 1990; Tunnessen and Oski 1987; Yip et al. 1987). However, it is a relatively expensive food and not used by more than 30% of families in certain segments of the Canadian population such as lower socio-economic families (Lehmann et al. 1992; Matthews et al. 1995; Shears 1991; Tanaka et al 1987). Since most infants in Canada are fed solid foods after six months of age, choosing iron-rich solids such as meat and infant cereal and replacing iron-fortified formula with whole cow milk can provide substantial savings. The implication is that iron replete infants at six months of age, regularly consuming meat and iron-fortified infant cereal can safely use whole cow milk rather than
formula. This is a practical, culturally acceptable and lower cost alternative to iron-fortified formula for infants not breast-fed after six months of age, whether from low-income families or not.

The third objective of the study was to evaluate the use of transferrin receptor as a diagnostic measure of iron deficiency. To determine the sensitivity and specificity of TfR, we first generated percentile estimates from a group of infants previously studied (Zlotkin et al. 1996) which defined a range above which would be indicative of tissue iron deficiency. These estimates were determined using our own ‘Lab’ assay (described in section 4.4). We used our ‘Lab’ method to assay blood samples obtained in the current study (the Infant Iron Study) for TfR because the percentile estimates that were generated are specific to this method and are the only published normative data on TfR in infants to date (Yeung and Zlotkin 1997). Secondly, we calculated the sensitivity and specificity of TfR using blood samples from the current study (The Infant Iron Study). An indirect but likely reliable external measure was used to define iron deficiency (Hgb<110 g/L and Ferr<15µg/L).

We found that TfR is a relatively sensitive (0.75) and highly specific (0.94) diagnostic measure of tissue iron deficiency compared to other measures of iron deficiency, such as transferrin saturation, and iron depletion, such as Ferr. Log TfR:Ferr is likely a more sensitive but less specific measure of tissue iron deficiency than TfR alone since it gives an indication of iron depletion as well as deficiency. This implies that TfR would be much better than current biochemical markers of iron deficiency because it
is both clinically relevant and not subject to high variation.

The use of TfR would provide a more clinically relevant measure of iron deficiency in studies such as the Infant Iron Study. Due to a lack of a sensitive and specific measure for iron deficiency, the current study based end-points primarily on a marker of iron depletion (Ferr<10μg/L). Although preventing iron depletion also prevents iron deficiency anaemia, our end-point definition may have been too conservative. There are no functional consequences associated with iron depletion and it does not necessarily lead to iron deficiency anaemia (Beaton et al. 1989).

Data from the current study show that the overall and biologic CV was much lower for TfR than for Ferr, based on blood samples repeated in a second visit. This supports the findings of other studies comparing the variability of TfR to Ferr in healthy adult (Cooper and Zlotkin 1996) and elderly populations (Ahluwalia et al. 1993). Ferr measures are subject to high day-to-day variation (Ahluwalia et al. 1993; Borel 1990; Cooper and Zlotkin 1996). We tried to reduce this variance by declaring end-point only if a low value was confirmed in a second blood sample. This may have eliminated some of the false positives but did nothing to improve the number of false negatives. Putative end-point was confirmed only 43% of the time in a second blood sample. A less variable biochemical marker would have increased the statistical power in this study.

However because the calculated sensitivity and specificity were based on indirect measure of iron deficiency and the prevalence was low further studies must be conducted to confirm these findings. Furthermore, we have recently completed a study that
compared the measurement of serum TfR between two commercially available kits (Ramco and Quantikine) and the assay developed in our laboratory (included in Appendix F). The data show that all three methods were able to distinguish between ‘elevated’ and ‘normal’ serum samples. However, there was a disparity in measurements of absolute TfR levels between each of the methods for both the ‘elevated’ and ‘normal’ serum samples. We showed that the lack of agreement between methods was large enough to cause misdiagnosis. Thus, while TfR is potentially important in the diagnosis of iron deficiency, it cannot be used on a wide-scale clinical level in infants and young children until normative data based on a commercially available method of quantification is generated.
8.1 Future Directions

Based on the results of this research, a number of suggestions can be made for future endeavours.

As shown in Chapters 5 and 6, the majority of infants prior to the study onset and control infants during the study were fed in accordance to CPS recommendations and were not at particular risk for iron deficiency, despite being from low-income households. This is very different from other Canadian infants from low-income households studied in the past whose dietary patterns put them at increased risk for iron deficiency. The families in the current study were different from those studied in the past in that a much larger proportion had higher education and none had reached less than grade 11. This suggests that education may be an important risk factor in iron deficiency. Studies examining the influence of education versus income on feeding patterns may provide further insight on what affects risk for nutritional problems.

The results of Chapter 6 show that the consumption meat and iron-fortified infant cereal can maintain iron stores and prevent iron deficiency in cow milk-fed infants after six months of age. Thus, AAP and CPS guidelines with respect to the timing of cow milk introduction should be re-examined. Introduction of cow milk after six months of age may be appropriate in infants who have established a consistent pattern of meat and infant cereal intake.

The results of Chapter 7 suggest that TtR is a sensitive and specific diagnostic measure of iron deficiency when compared to traditional biochemical markers of iron
status. However, this study lacked a suitable direct standard of measuring iron deficiency to determine the true sensitivity and specificity of TfR. Furthermore, the prevalence of iron deficiency was very low in the population studied. This study should be repeated using iron stains from bone marrow as a standard in a population with a higher prevalence of iron deficiency.
9. SUMMARY AND CONCLUSIONS

9.1 Summary

1. The majority of families surveyed followed CPS recommendations prior to the study which, in these infants, maintained iron stores until six months of age.

2. The majority of families of low-income households studied in the treatment group complied with the dietary restrictions of the protocol while the majority in the control group tended to feed according to CPS recommendations. After six months of age, only feeding cow milk at ten months of age was associated with low iron depletion and/or anaemia.

3. The incidence of iron depletion/anaemia was very low in both groups and no statistical difference was detected. Proper feeding patterns led to a lower than expected incidence of iron depletion/anaemia in the control group, likely because of the fact that many mothers in this group were highly educated and were aware of proper feeding practices.

4. Twelve percent of treatment families did not feed the infant cereal or meat, almost all of whom reached end-point, despite the fact cereal and meat were provided free of charge and field workers encouraged its use. Non-compliance to the meat or cereal in the treatment group was associated with iron depletion.

5. The rate of fall in plasma Ferr with age was the same in both groups, suggesting that the iron status of the two groups were equivalent.

6. Percentile estimates of Tfr and log Tfr:Ferr define a range above which is indicative
of iron deficiency. Based on these definitions, the sensitivity (0.75) and specificity
(0.94) for TfR as a diagnostic measure of iron deficiency was calculated.
9.2 Conclusions

The hypothesis of the thesis was that there would be a significant reduction in the incidence of iron depletion and/or anaemia in six to 12 month old cow milk-fed infants from low-income households consuming infant cereals (30g/day) and pureed meats (70g/day) when compared to infants from low-income households with no dietary intervention. Inherent in the study design was the premise that because few low-income families follow CPS feeding guidelines, 30% of the infants in the control group would reach confirmed end-point and the treatment would reduce this to below 10%. This premise was incorrect. Because the incidence of end-point in the control group was so low, the hypothesis could not be tested adequately. However, the three objectives of the study were met and the following conclusions were drawn:

1. The consumption of adequate amounts of infant cereals and pureed meats prevented iron depletion and anaemia in infants fed whole cow milk after six month of age.

2. Tfr appears to be an accurate, relatively sensitive and highly specific diagnostic measure compared to traditional measures of iron status, such as transferrin saturation and serum Ferr. Log Tfr:Ferr is likely a more sensitive measure of iron deficiency than Tfr alone.
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LIST OF APPENDICES

APPENDIX A: QUESTIONNAIRES ................................................................. 173
APPENDIX B: PARENT'S WELCOME BOOKLET ............................................ 203
APPENDIX C: NURSE'S GUIDE BOOKLET .................................................... 214
APPENDIX D: PRODUCT LISTS ................................................................ 224
APPENDIX E: SAMPLE SIZE CALCULATION .............................................. 229
APPENDIX F: DISPARITY OF SERUM TRANSFERRIN RECEPTOR MEASUREMENTS AMONG DIFFERENT ASSAY METHODS .............. 231
APPENDIX A: QUESTIONNAIRES
SCREENING/HISTORY QUESTIONNAIRE

PREAMBLE:
Thank you for agreeing to join the study. First we would like to get some initial information about your baby. We'll use this information to set up your baby's file and to develop a set of date guidelines for the visits over the next 6 months. We will telephone you in the next while to set up our first appointment to come to your house. Now, I would like to obtain the following information from you:

INFORMATION ON INFANT:
1. Name of Infant: ________________________________
   (Surname) (First Name)

2. Sex: 1=male________ 2=female________

3. Birthdate: ____/____/______ **
   da mo yr

DATA ENTRY INTO IRON HISTORY:  DATA INPUT DONE:____
INFORMATION ON MOTHER

DATE: __/__/___

DA MO YR

4. __________________________ , __________________________ Ms. ___ Mrs. ___
(Surname) (First Name)

PURPOSE: To get information on mother and infant and verify that infant meets inclusion criteria. All conditions that preclude entry into study are within text of questionnaire.

PROCEDURE:
1. Ask the question that are required for Iron Core and Iron History.
2. Establish whether infant meets criteria for inclusion.
3. Enter data into Iron Core and Iron History files.

5. Address: ______________________________________________________

6. Telephone Number: ______________________________________________

7. Referring Physician:
   (Surname) (Initial)

(DETERMINE AT TIME OF DATA INPUT)

SUBJECT # _______________ STUDY GROUP # _______________

DATE __/__/___
DA MO YR

FAMILY # _____________ NAME OF INFANT ________________________
(Surname) (firstname)

BIRTH DATE ___/___/______ SEX 1=male 2=female
PREAMBLE:
We need to be sure that the babies who participate in this study are similar for certain characteristics and patterns so we need some background information about your baby and your family.
Please indicate the following:

1. Was your infant full-term (38-42 weeks gestation = full-term)?
   ( ) no **
   ( ) yes

2. What was your infant's gestation age at birth:
   ( ) 0 = between 38 - 42 weeks
   ( ) 1 = less than 38 weeks **
   ( ) 2 = greater than 42 weeks

3. Is your infant healthy, that is, is he/she not suffering from any major illnesses?
   ( ) 0 = my infant is healthy, has no major illnesses
   ( ) 1 = my infant is not healthy and is suffering from major illness **

4. Is your infant of the Caucasian, Asian, Indian, Black or Oriental race or a combination of two races?

   RACE: ( ) 0 non-black ( ) 1 = black

5. Do you use any form of daycare for your infant - for instance, does a relative help with the care of your infant? Do you have a nanny? Do you take your infant to an outside daycare centre?

   ( ) 0 = CAN BE ENROLLED IN STUDY, DAYCARE SITUATION ACCEPTABLE
   ( ) 1 = CANNOT BE ENROLLED IN STUDY, DAYCARE SITUATION UNACCEPTABLE **

176
6. Do you read and write in English?
   ____ 0 = yes, communicates in English
   ____ 1 = no, does not communicate in English**

---

CRITERIA FOR HEALTHY BABY AT BIRTH:
- birth weight $\geqslant 2500$ g
- no prolonged hospitalization (past 5 days)
- no use of antibiotics during hospitalization
- no seizures
- no extensive blood testing (involving more than heel pricking)
- cannot be a gestational diabetic baby
---

7. What was your infant's birth weight?
   __________________________ pounds   __________________________ grams
---

8. Was your infant healthy at birth?
   ____ 0 = yes, in good health at birth
   ____ 1 = no, not in good health **

---

9. Is this your first infant? (probe to establish how many live births and what birth order this infant is)
   __________________________   __________________________
   ____ birth order, based on live births
---

10. How many people live in your household? (Including all friends and relatives normally living within the household. It does not include boarders)
   __________________________
---

11. What is your estimated yearly household income? (This means the entire household income before taxes in round numbers)**
   __________________________
---

PREAMBLE: I would like some information on your current practices:
12. Are you giving your infant any formula at this time? (probe as to the type)
   ___ yes
   __________________________________________
   ___ no

NOTE: If mother is feeding her infant an iron-fortified cow’s milk or a soy-based formula (all soya-based formulas are iron-fortified) she must be reminded that for the period she is in the study, she cannot give her infant any iron-fortified formula. If this is not acceptable, she must be excluded from the study.

13. Are you giving your infant any vitamin/mineral supplements at this time? (probe as to type)
   ___ yes
   __________________________________________
   __________________________________________
   ___ no

NOTE: If mother is feeding her infant any supplements containing iron she must be reminded that for the period during the study, she cannot give her infant any iron supplements. If this is not acceptable, she must be excluded from the study.
BACKGROUNDER QUESTIONNAIRE

**Date:**

<table>
<thead>
<tr>
<th>day</th>
<th>month</th>
<th>year</th>
</tr>
</thead>
</table>

**Family Number:**

**Baby's birthdate:**

<table>
<thead>
<tr>
<th>day</th>
<th>month</th>
<th>year</th>
</tr>
</thead>
</table>

**Baby's Sex:**

- [ ] male
- [ ] female

**Baby's Doctor:**

<table>
<thead>
<tr>
<th>Surname</th>
<th>First Name</th>
<th>Initial</th>
</tr>
</thead>
</table>

**Doctor's Address:**

<table>
<thead>
<tr>
<th>Street Address</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>City</th>
<th>Province</th>
<th>Postal Code</th>
</tr>
</thead>
</table>

**Doctor's Phone #:**

( )

---

1. **Are you a single parent or is this a two-parent home?**
   (two-parent home means that there is a mother and husband/partner living together)
   - [ ] single parent
   - [ ] two parent

2. **How many people live in your household?**
   (including friends and relatives normally living with you. Do not include boarders.)
   Number of children: ______

<table>
<thead>
<tr>
<th>Child #</th>
<th>Age (years)</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>male</td>
</tr>
</tbody>
</table>

| 1       |             |      |       |
| 2       |             |      |       |
| 3       |             |      |       |
| 4       |             |      |       |
| 5       |             |      |       |

3. **Where will your child usually be during the day?**
   - [ ] in my own home
   - [ ] in private home day care
   - [ ] in public daycare.

4. **Please estimate the total household income (before taxes):**

   $
INFORMATION ON MOTHER OF INFANT (if applicable)

5. Where were you born?
   - ☐ in Canada
   - ☐ in another country
     name of country: __________________________

6. Of the following age ranges, what age range do you fall into?
   - ☐ less than 20 years old
   - ☐ 20-29 years old
   - ☐ 30-39 years old
   - ☐ 40 years or older

7. What was the highest level of education that you completed?
   (if done outside of Canada please tick off the closest category)
   - ☐ primary/secondary school (grades 1-13)
     What grade? ________
   - ☐ college (postsecondary)
     How many years? ________
     ☐ Certificate or Diploma Obtained
   - ☐ university
     How many years? ________
     ☐ Degree Obtained

8. Where did you receive your highest level of education?
   - ☐ in Canada
   - ☐ in another country
     name of country: __________________________

9. Are you currently employed?
   - ☐ no, not working outside the home
   - ☐ yes, working outside the home in a part time capacity
   - ☐ yes, working outside the home in a full time capacity
   - ☐ on maternity leave but returning to full time work
   - ☐ on maternity leave but returning to part time work

10. What is your occupation (if unemployed, what is your usual occupation when employed)?
    ________________________________
INFORMATION ON FATHER OF INFANT  (if applicable and living at the same household)

11. Where were you born?
   - in Canada
   - in another country  
     name of country: ______________________

12. Of the following age ranges, what age range do you fall into?
   - less than 20 years old
   - 20-29 years old
   - 30-39 years old
   - 40 years or older

13. What was the highest level of education that you completed?
   (if done outside of Canada please tick off the closest category)
   - primary/secondary school (grades 1-13)  
     What grade? ______
   - college (postsecondary)  
     How many years? ______
     - Certificate or Diploma Obtained
   - university  
     How many years? ______
     - Degree Obtained

14. Where did you receive your highest level of education?
   - in Canada
   - in another country  
     name of country: ______________________

15. Are you currently employed?
   - no, not working out the home
   - yes, working outside the home in a part time capacity
   - yes, working outside the home in a full time capacity

16. What is your occupation (if unemployed, what is your usual occupation when employed)?

__________________________________________________________________

181
INITIAL DIETARY HISTORY

Date: 

<table>
<thead>
<tr>
<th>Visit #:</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family #:</td>
<td></td>
</tr>
</tbody>
</table>

| Baby's birthdate: 
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>month</td>
</tr>
</tbody>
</table>

| Baby's Sex: |
|---|---|
| male |
| female |

Please answer the following. If appropriate, more than one answer may be checked per question. If you have any questions, please ask the nurse.

Also note that when a food is started we mean that the food was introduced into the diet and became a regular part of the baby's diet.

MILK and MILK PRODUCTS

BREAST-FEEDING

1. Did you ever breast-feed your baby?  
   - yes  
   - no (go to Question 5)

2. Are you breast-feeding your baby now?  
   - yes  
   - no

3. Was breast milk the first food your baby had after birth?  
   - yes  
   - no

4. How old was your baby when you stopped breast-feeding completely?  
   - less than 1 month old  
   - 1 month  
   - 2 months  
   - 3 months  
   - 4 months  
   - 5 months  
   - 6 months  
   - haven't stopped breast-feeding
5. Did you ever feed your baby formula?
   - yes
   - no (go to Question 9)

6. Was formula the first food your baby had after birth?
   - yes
   - no

7. Do you feed your baby formula now (either commercially prepared or home-prepared)?
   - yes
   - no

8. What formula(s) have you fed your baby to date?
   - Name of formula: (or mark "home-made" if applicable)
   - With iron?: □
   - Colour of label?: □
   - Age (months)?

9. Did you ever feed your baby cow's milk?
   - yes
   - no (go to Question 13)

10. Do you feed your baby cow's milk now?
    - yes □ What kind?
    - □ whole cow's milk (homogenized)
    - □ 2%
    - □ 1%
    - □ skim
    - □ evaporated
    - no

11. What kind of cow's milk have you fed your baby to date?
    (even if you only tried cow's milk for a short time and stopped, please record it here and explain in Question 12 why you stopped)

<table>
<thead>
<tr>
<th>Type of cow's milk</th>
<th>Age (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
</tr>
</tbody>
</table>

12. If you stopped feeding your baby cow's milk, then why did you stop?

________________________________________

183
OTHER TYPES OF MILK (other than breast-milk, formula or cow's milk)

13. Did you ever feed your baby any other type of milk other than breast-milk, formula or cow's milk (e.g. goat milk)?
   □ yes
   □ no (go to Question 17)

14. Do you feed your baby any other type of milk other than breast-milk, formula or cow's milk now?
   □ yes  What kind? ____________________________
          Why? ____________________________
   □ no

15. What other kind of milk has your baby had other than breast-milk, formula or cow's milk (e.g. goat milk)?

<table>
<thead>
<tr>
<th>Type of other milk (e.g. goat milk)</th>
<th>Reason for using this milk</th>
<th>Age (months)</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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</tbody>
</table>

16. If you stopped feeding your baby this other type of milk, then why did you stop?

YOGURT

17. Did you ever feed your baby yogurt?
   □ yes
   □ no (go to Question 21)

18. Do you feed your baby yogurt now?
   □ yes
   □ no  When did you stop feeding yogurt?
          □ less than 1 month old
          □ 1 month
          □ 2 months
          □ 3 months
          □ 4 months
          □ 5 months
          □ 6 months

19. How old was your baby when you started him or her on yogurt?
   □ less than 1 month old
   □ 1 month
   □ 2 months
   □ 3 months
   □ 4 months
   □ 5 months
   □ 6 months

20. If you stopped feeding your baby yogurt, then why did you stop?

__________________________________________

184
<table>
<thead>
<tr>
<th>Question</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Did you ever feed your baby any cheeses (e.g., hard cheese, cottage cheese, etc.)?</td>
</tr>
<tr>
<td></td>
<td>- yes</td>
</tr>
<tr>
<td></td>
<td>- no (go to Question 25)</td>
</tr>
<tr>
<td>22</td>
<td>Do you feed your baby any cheeses now?</td>
</tr>
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<td></td>
<td>- yes</td>
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<tr>
<td></td>
<td>- no 🟢</td>
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<td>- less than 1 month old</td>
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<td>- 2 months</td>
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<td>- 4 months</td>
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<td>- 5 months</td>
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<td></td>
<td>- 6 months</td>
</tr>
<tr>
<td>23</td>
<td>How old was your baby when you started him/her on cheese?</td>
</tr>
<tr>
<td></td>
<td>- less than 1 month old</td>
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<td>24</td>
<td>If you stopped feeding your baby cheese, then why did you stop?</td>
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<td>25</td>
<td>Did you ever feed your baby ice cream?</td>
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<td>- yes</td>
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<td></td>
<td>- no (go to Question 29)</td>
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<tr>
<td>26</td>
<td>Do you feed your baby any ice cream now?</td>
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<td>- yes</td>
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<td>- 6 months</td>
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</table>
27. How old was your baby when you started him or her on ice cream?
☐ less than 1 month old
☐ 1 month
☐ 2 months
☐ 3 months
☐ 4 months
☐ 5 months
☐ 6 months

28. If you stopped feeding your baby ice cream, then why did you stop?

FRUITS and VEGETABLES

FRUIT JUICES
29. Did you ever feed your baby fruit juices?
☐ yes
☐ no (go to Question 33)

30. Do you feed your baby any fruit juices now?
☐ yes
☐ no
   When did you stop feeding fruit juices?
☐ less than 1 month old
☐ 1 month
☐ 2 months
☐ 3 months
☐ 4 months
☐ 5 months
☐ 6 months

31. How old was your baby when you started him or her on fruit juices?
☐ less than 1 month old
☐ 1 month
☐ 2 months
☐ 3 months
☐ 4 months
☐ 5 months
☐ 6 months

32. If you stopped feeding your baby fruit juice, then why did you stop?

FRUITS
33. Did you ever feed your baby any fruits?
☐ yes
☐ no (go to Question 37)
34. Do you feed your baby any fruits now?
- yes
- no * When did you stop feeding fruits?
  - less than 1 month old
  - 1 month
  - 2 months
  - 3 months
  - 4 months
  - 5 months
  - 6 months

35. How old was your baby when you started him or her on fruits?
- less than 1 month old
- 1 month
- 2 months
- 3 months
- 4 months
- 5 months
- 6 months

36. If you stopped feeding your baby fruits, then why did you stop?

37. Did you ever feed your baby any vegetables?
- yes
- no (go to question 41)

38. Do you feed your baby any vegetables now?
- yes
- no * When did you stop feeding vegetables?
  - less than 1 month old
  - 1 month
  - 2 months
  - 3 months
  - 4 months
  - 5 months
  - 6 months

39. How old was your baby when you started him or her on vegetables?
- less than 1 month old
- 1 month
- 2 months
- 3 months
- 4 months
- 5 months
- 6 months

40. For what reason did you stop feeding your baby vegetables?
MEATS, FISH and POULTRY

MEATS

41. Did you ever feed your baby meats (beef, veal, pork, lamb, organ meats such as liver)?
   □ yes
   □ no (go to question 46)

42. How old was your baby when you started him or her on meats?
   □ less than 1 month old
   □ 1 month
   □ 2 months
   □ 3 months
   □ 4 months
   □ 5 months
   □ 6 months

43. Do you ever feed your baby meat now?
   □ yes (go to Question 46)
   □ no

44. How old was your baby when you stopped feeding him or her meats?
   □ less than 1 month old
   □ 1 month
   □ 2 months
   □ 3 months
   □ 4 months
   □ 5 months
   □ 6 months

45. For what reason did you stop feeding your baby meats?

   ____________________________

   ____________________________

FISH

46. Did you ever feed your baby fish?
   □ yes
   □ no (go to question 51)

47. How old was your baby when you started him or her on fish?
   □ less than 1 month old
   □ 1 month
   □ 2 months
   □ 3 months
   □ 4 months
   □ 5 months
   □ 6 months

48. Do you ever feed your baby fish now?
   □ yes (go to Question 51)
   □ no
49. How old was your baby when you stopped feeding him or her fish?
☐ less than 1 month old
☐ 1 month
☐ 2 months
☐ 3 months
☐ 4 months
☐ 5 months
☐ 6 months

50. For what reason did you stop feeding your baby fish?

51. Did you ever feed your baby any poultry (chicken, turkey or other fowl)?
☐ yes
☐ no (go to question 56)

52. How old was your baby when you started him or her on poultry?
☐ less than 1 month old
☐ 1 month
☐ 2 months
☐ 3 months
☐ 4 months
☐ 5 months
☐ 6 months

53. Do you ever feed your baby poultry now?
☐ yes (go to Question 56)
☐ no

54. How old was your baby when you stopped feeding him or her poultry?
☐ less than 1 month old
☐ 1 month
☐ 2 months
☐ 3 months
☐ 4 months
☐ 5 months
☐ 6 months

55. For what reason did you stop feeding your baby poultry?

CEREALS, TEETHING BISCUITS and BABY COOKIES

INFANT CEREALS
56. Did you ever feed your baby any infant cereals?
☐ yes
☐ no (go to Question 60)
57. Do you feed your baby any infant cereals now?
   - yes
   - no  
      When did you stop feeding infant cereals?
      - less than 1 month old
      - 1 month
      - 2 months
      - 3 months
      - 4 months
      - 5 months
      - 6 months

58. How old was your baby when you started him or her on infant cereals?
   - less than 1 month
   - 1 month
   - 2 months
   - 3 months
   - 4 months
   - 5 months
   - 6 months

59. If you stopped feeding your baby infant cereals, then why did you stop?
   _______________________________________________________________

OTHER CEREALS
60. Did you ever feed your baby any other cereals other than infant cereals?
   - yes
   - no (go to Question 64)

61. Do you feed your baby any other cereals other than infant cereals now?
   - yes  
      What kind? __________________________________________________
      Why? ______________________________________________________
   - no

62. What other kind of cereals has your baby had other than infant cereal?

<table>
<thead>
<tr>
<th>Other Cereal (non-infant cereals)</th>
<th>Reason for using this cereal</th>
<th>Age (months) Start</th>
<th>Stop</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

63. If you stopped feeding your baby other kinds of cereals, then why did you stop?
   _______________________________________________________________

TEETHING BISCUITS and BABY COOKIES
64. Did you ever feed your baby any teething biscuits or baby cookies?
   - yes
   - no (go to Question 68)
65. Do you feed your baby any teething biscuits or baby cookies now?
☐ yes
☐ no

When did you stop feeding these?
☐ less than 1 month old
☐ 1 month
☐ 2 months
☐ 3 months
☐ 4 months
☐ 5 months
☐ 6 months

66. How old was your baby when you started him or her on teething biscuits or baby cookies?
☐ less than 1 month old
☐ 1 month
☐ 2 months
☐ 3 months
☐ 4 months
☐ 5 months
☐ 6 months

67. If you stopped feeding your baby teething biscuits or baby cookies, then why did you stop?

VITAMIN and MINERAL SUPPLEMENTS

68. Did you ever feed your baby any vitamin or mineral supplements?
☐ yes
☐ no (Questionnaire Finished)

69. Do you feed your baby any vitamin or mineral supplements now?
☐ yes ☐ What kind?
☐ no

70. What kind of vitamin or mineral supplements do you feed your baby?

<table>
<thead>
<tr>
<th>Type and dosage of vitamin or mineral supplement</th>
<th>How often? (e.g. every day)</th>
<th>Iron fortified</th>
<th>Age (months)</th>
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QUESTIONNAIRE FINISHED. THANK YOU.
BI-MONTHLY COMPLIANCE CHECK (Treatment subjects)

Date: __/__/____  Family Number: ____________

Baby’s birthdate: __/__/____  Baby’s Sex:  □ male  
□ female

Please answer the following. If appropriate, more than one answer may be checked per question. If you have any questions, please ask the nurse.

Also note that when a food is started we mean that the food was introduced into the diet and became a regular part of the baby’s diet.

BREAST-FEEDING
71.  Are you currently breast-feeding your baby?

□ yes  □ only breast-feeding (no other milk source)
□ breast-feeding and other milk sources (e.g. formula, bottled milk)

□ no  □ discontinued at age: _____ months
□ baby was never breast-fed

FORMULA FEEDING
72.  Are you feeding your baby any formula?

□ yes  type of formula: ________________________________  
□ type of formula: ________________________________  
□ age that formula was started: _____ months  
Is the formula iron fortified?  □ yes  
□ no

□ no  □ discontinued at age: _____ months
□ baby was never formula fed
73. Are you feeding your baby cow's milk?

- ☐ yes  ☐ age when cow's milk started: _____ months
- ☐ type of cow's milk being fed now:  ☐ whole (homogenized)
  ☐ 2%
  ☐ 1%
  ☐ skim
  ☐ other: ________ (please specify)

- ☐ no  ☐ have not started yet
- ☐ discontinued at age: _____ months

74. Are you feeding your baby any other type of milk (i.e., soya milk, goat's milk)?

- ☐ yes  ☐ age when milk was started: _____ months
- ☐ type of milk: ________________________________

- ☐ no  ☐ have not started
- ☐ discontinued at age: _____ months

75. Are you feeding your baby any other milk products? (such as yogurt, cottage cheese, hard cheese or processed cheese slices)

- ☐ yes  ☐ started at age: _____ months

- ☐ no  ☐ have not started
- ☐ discontinued at age: _____ months

76. Are you feeding your baby fruits?

- ☐ yes  ☐ age when started: _____ months

- ☐ no  ☐ have not started
- ☐ discontinued (all fruits): _____ months

77. Are you feeding your baby fruit juices?

- ☐ yes  ☐ age when started: _____ months

- ☐ no  ☐ have not started yet
- ☐ discontinued at age: _____ months
78. Are you feeding your baby vegetables?

- Yes ○ age when started: _____ months
- No ○ have not started yet
  ○ discontinued at age: _____ months

79. Are you feeding your baby any meats such as beef, veal, pork, lamb, organ meats, or fish?

- Yes ○ age when started: _____ months
- No ○ have not started yet
  ○ discontinued at age: _____ months

80. Are you feeding your baby any foods such as lentils, kidney beans, white beans, pinto beans, or soya bean products such as bean curd?

- Yes ○ age when started: _____ months
- No ○ have not started yet
  ○ discontinued at age: _____ months

81. Are you feeding your baby eggs?

- Yes ○ whole egg started at: _____ months
  ○ egg white only started at: _____ months
  ○ egg yolk (yellow) only started at: _____ months
- No ○ have not started yet
  ○ discontinued whole egg at: _____ months
  ○ discontinued egg white only at: _____ months
  ○ discontinued egg yolk (yellow) only at: _____ months

82. Are you feeding your baby any vitamin/mineral supplements?

- Yes ○ type: __________________________ usage & dose: __________
  ○ type: __________________________ usage & dose: __________
- No
83. Does the supplement(s) contain iron (if applicable)?

☐ yes
☐ no

84. Are you feeding your baby Farley's biscuits?

☐ yes ☐ no

☐ yes ☐ no

☐ less than 1 biscuit/week
☐ 1 biscuit/week
☐ less than 1 biscuit/day
☐ 1 biscuit/day
☐ more than 1 biscuit/day
☐ more than 2 biscuits/day

85. Are you feeding your baby the infant cereals we provided?

☐ yes ☐ no

☐ yes ☐ no

Type: ________________________________

Frequency:

☐ less than 1 serving/week
☐ 1 serving/week
☐ less than 1 serving/day
☐ 1 serving/day
☐ more than 1 serving/day
☐ more than 2 servings/day

Serving size:

☐ less than 1/3 cup
☐ 1/3 cup
☐ more than 1/3 cup

86. How do you usually prepare the infant cereal?

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________

87. What else do you usually feed your baby at the same time as you are feeding the cereal?

__________________________________________________________________________

__________________________________________________________________________

__________________________________________________________________________
QUESTIONNAIRE FINISHED. THANK YOU.
CRITERIA CHECK (Control Subjects)

<table>
<thead>
<tr>
<th>Date:</th>
<th>Family Number: ________</th>
<th>Baby’s birthdate:</th>
<th>Baby’s Sex:</th>
<th>□ male</th>
<th>□ female</th>
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Please answer the following. If appropriate, more than one answer may be checked per question. If you have any questions, please ask the nurse.

Also note that when a food is started we mean that the food was introduced into the diet and became a regular part of the baby’s diet.

**BREAST-FEEDING**

88. Are you currently breast-feeding your baby?

- □ yes
  - □ only breast-feeding (no other milk source)
  - □ breast-feeding and other milk sources (e.g. formula, bottled milk)

- □ no
  - □ discontinued at age: _____ months
  - □ baby was never breast-fed

**FORMULA FEEDING**

89. Are you feeding your baby any formula?

- □ yes
  - □ type of formula: _____________________________
  - □ age that formula was started: ______ months
  - □ Is the formula iron fortified? □ yes □ no

- □ no
  - □ discontinued at age: _____ months
  - □ baby was never formula fed

197
**COW’S MILK**

90. Are you feeding your baby cow’s milk?

- [ ] yes □ age when cow’s milk started: _____ months
  - □ whole (homogenized)
  - □ 2%
  - □ 1%
  - □ skim
  - □ other: _______ (please specify)

- [ ] no □ have not started yet
  - □ discontinued at age: _____ months

**OTHER TYPES OF MILK**

91. Are you feeding your baby any other type of milk (i.e.: soya milk, goat’s milk)?

- [ ] yes □ age when milk was started: _____ months
  - □ type of milk: ____________________________

- [ ] no □ □ have not started
  - □ discontinued at age: _____ months

**OTHER MILK PRODUCTS**

92. Are you feeding your baby any other milk products? (such as yogurt, cottage cheese, hard cheese or processed cheese/slices)

- [ ] yes □ started at age: _____ months

- [ ] no □ □ have not started
  - □ discontinued at age: _____ months

**FRUITS AND VEGETABLES**

93. Are you feeding your baby fruits?

- [ ] yes □ age when started: _____ months

- [ ] no □ □ have not started
  - □ discontinued (all fruits): _____ months

94. Are you feeding your baby fruit/juices?

- [ ] yes □ age when started: _____ months

- [ ] no □ □ have not started yet
  - □ discontinued at age: _____ months
Are you feeding your baby vegetables?

☐ yes ☐ age when started: _____ months

☐ no ☐ have not started yet
☐ discontinued at age: _____ months

Are you feeding your baby any meats such as beef, veal, pork, lamb, organ meats, or fish?

☐ yes ☐ age when started: _____ months

☐ no ☐ have not started yet
☐ discontinued at age: _____ months

Are you feeding your baby any foods such as lentils, kidney beans, white beans, pinto beans or soya bean products such as bean curd?

☐ yes ☐ age when started: _____ months

☐ no ☐ have not started yet
☐ discontinued at age: _____ months

Are you feeding your baby eggs?

☐ yes ☐ whole egg started at: _____ months
☐ egg white only started at: _____ months
☐ egg yolk (yellow) only started at: _____ months

☐ no ☐ have not started yet
☐ discontinued whole egg at: _____ months
☐ discontinued egg white only at: _____ months
☐ discontinued egg yolk (yellow) only at: _____ months

Are you feeding your baby any vitamin/mineral supplements?

☐ yes ☐ type: __________________ usage & dose: ______________

☐ no ☐ type: __________________ usage & dose: ______________
100. Does the supplement(s) contain iron (if applicable)?

☐ yes
☐ no

§ HARLEY'S BISCUITS

101. Are you feeding your baby Harley's biscuits?

☐ yes
☐ less than 1 biscuit/week
☐ 1 biscuit/week
☐ less than 1 biscuit/day
☐ 1 biscuit/day
☐ more than 1 biscuit/day
☐ more than 2 biscuits/day

☐ no

§ CEREALS

102. Do you usually feed your baby any infant cereals?

☐ yes
☐ no

QUESTIONNAIRE FINISHED. THANK YOU.
INFANT IRON STUDY

KEEPING 3-DAY FOOD RECORDS

We are asking you to keep a 3-day food record between your baby's 8th and 9th month birthday and again on their 10th and 11th month birthday. It is very important that we know exactly how much food and drink (not including water) your baby eats during these 3 days. That is why we are giving you a measuring set. Please use this to help you figure out how much your baby is eating (not just how much you make, but how much the baby eats).

We are giving you some examples of how to keep these records. Please keep the records on a Thursday, Friday and Saturday or on a Sunday, Monday and Tuesday. We will call you when it is time to start.

If you have any questions please call George Yeung at 813-5142.
FOOD RECORD

<table>
<thead>
<tr>
<th>Date:</th>
<th>/     /</th>
<th>Family #</th>
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<tbody>
<tr>
<td>Baby's Birthdate:</td>
<td>/     /</td>
<td>Baby's Sex:</td>
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<td>day month year</td>
<td>r male</td>
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<td></td>
<td>r female</td>
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</table>

**DAY 1, 2, 3 please circle**

**TIME: _____ am / pm please circle**

**MEAL or SNACK please circle**

Foods or drinks at this meal or snack:

- 
- 
- 

How the food was made and how much:

<table>
<thead>
<tr>
<th>Food or drink:</th>
<th>Home-made (H)</th>
<th>Store-made (S)</th>
<th>How much was eaten</th>
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</table>

How the food was made and how much:

<table>
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<tr>
<th>Food or drink:</th>
<th>Home-made (H)</th>
<th>Store-made (S)</th>
<th>How much was eaten</th>
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</table>

202
<table>
<thead>
<tr>
<th>Food or drink:</th>
<th>Home-made (H)</th>
<th>How much was eaten</th>
<th>Store-made (S)</th>
</tr>
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</table>

Recipe for: ____________________________

Recipe for: ____________________________

Recipe for: ____________________________

Recipe for: ____________________________

How much this recipe makes: ____________________________

How much this recipe makes: ____________________________

How much this recipe makes: ____________________________

How much this recipe makes: ____________________________
Dear Parents,

Welcome to the Infant Iron Study! We appreciate your interest and commitment to the study and look forward to getting acquainted with you and your infant during the next six months. Our research nurse will visit you when your child is approximately 6, 8, 10 and 12 months old, for a total of four visits. The approximate time required and the procedures associated with each visit is outlined in the last section under Appointments.

This booklet has been prepared as a guide and information source for you during the study. You will find included:

(i) A copy of the initial information letter which you received.
(ii) More detailed information about the study.
(iii) A copy of the Consent to Participate form.
(iv) An example of the daily Food use and Health Summary sheet.
(v) Appointment Calendar and Routine for Revisits.
(vi) Contact Numbers - Handy phone numbers in order to contact us.

If at any time you wish to contact us, please call:

George Yeung  
Department of GI/Nutrition, Hospital for Sick Children  
555 University Ave., Toronto, Ontario, M5G 1X8  
(416) 813-5142

Thank you for your participation.
For years the Hospital for Sick Children has been interested in infant nutrition. We invented Pablum! We are now starting a new study of infant nutrition and need your help.

We want to ensure that all babies get enough iron in their diets because iron is an essential nutrient. There are small amounts of iron in many infant foods. We are not sure, however, what is the best combination of infant foods to meet an infant's iron needs. Our goal is to find out the best way to provide enough iron in a baby's diet.

What will be expected of you?

Infants will be chosen randomly (by the toss of a coin) so that half of the infants in the study will be fed infant foods according to your choice, and half will be provided with foods which we will give to you. These foods will include: infant cereals, pureed meat (Heinz products) and cow milk.

If your infant is chosen to be in the cereal, meat and milk group, we will provide you of charge, with the infant cereals, meats and coupons for milk until your infant reaches one year of age. You may also feed your infant fruits and vegetables if you wish.

If your infant is chosen to be in the other group (you choose any foods for the baby), we will not provide you with infant foods, but will supply you with something of similar value as the foods (like clothing and laundry detergent, etc.). We will do this since we wish both groups to be treated equally.

The study will start when your baby is 6 months old and last until they are 1 year of age. At the start of the study, a nurse will visit you in your home, to collect information on what your baby eats, to measure your baby's length and weight and to take a small blood test (from the baby's finger) to check your infant's iron stores. The nurse will visit your home every 2 months during the study to repeat the measurements and the blood test. Each time we take the blood, we will send a report to your doctor. At the end of the study, we will also provide you with information about the study results.

If you are interested, we would like to be able to contact you by telephone to answer your questions and talk about you joining the study. Please sign the attached Consent to Contact form, staple or tape it closed and mail it to us. If you prefer, you may call us.

For more information please contact: Office of the Infant Iron Study, Division of Gastroenterology/Nutrition, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, M5G 1X8 (416) 813-5142.

206
INFANT IRON STUDY
CONSENT TO CONTACT FORM

I would like more information about the study. Please phone me at:

Telephone Number: ________________________________

The best time is between __________ and __________

on which day(s) of the week ____________________________

*Please print your name and address:*

Name: ____________________________________________

Address: __________________________________________

__________________________________________________

__________________________________________________

Signature: __________________________

Date: __________________________
Infant Iron Study

Principal Investigator: Dr. Stanley Zlotkin
Study Co-ordinator: George Yeung, PhD Candidate
Study Nurse: Karen Cowan
Mary Beth Zavitz

Why we are doing this study:

Iron is a very important nutrient for growing infants and children. It helps to make red blood cells. Unfortunately, some infants may not receive enough iron in their diet, which may result in iron deficiency. We are trying to find out how to prevent iron deficiency in infants by looking at different combinations of infant foods to see which foods are the best suppliers of iron.

We seek your help!

This study looks at different ways to ensure that your baby is getting enough iron in their diet. There are small amounts of iron in many infant foods. We are not sure, however, what is the best combination of infant foods to meet an infant's iron needs. The goal of this project is to clearly answer this question so that your doctor can advise parents like yourself about feeding their babies.

What will be expected of you?

Normally, when an infant reaches 6 months of age, they are offered various ‘weaning’ foods like infant cereals, pureed meat, fruits and vegetables along with breast milk, formula or cow milk. In our study, infants will be chosen randomly (by the toss of a coin) so that half of the infants in the study will be fed infant foods according to your choice (the control group) and half will be provided with foods which we will give to you. These foods will include infant cereals, pureed meat (Heinz products) and cow milk. If your infant is chosen to be in the ‘cereal, meat and milk group’ we will provide you (free of charge) with the infant cereals and meats and coupons for milk until your infant reaches one year of age. You may also feed your infant fruits and vegetables if you wish.

If your infant is chosen to be in the control group (you choose any foods for the baby), we will not provide you with infant foods, but will supply you with something worth the same as the foods (like laundry detergent). We will do this since we wish both groups to be treated equally.

If you agree, your infant will be enrolled in the study from 6 months of age until 12 months of age.
Before starting the study, a nurse from our study will visit you in your home, at a good time for you, to collect information on what your baby eats, to measure your baby's length and weight and to take a small blood test (from the baby's finger) to check your infant's iron stores. The same nurse will visit your home every 2 months during the study to repeat the measurements and the blood test. Before we start we will tell your doctor that your infant is in the study and we will send a report to your doctor. At the end of the study we will also provide you with information about the study results.

Are there any risks?

- The blood samples taken for tests may cause small bruising.
- If we find out that your baby has low blood iron, we will let you know, stop the study and notify your doctor and suggest treatment.

What are the benefits to your child?

Because of your baby being in this study:

- We will be able to check on his/her iron stores and detect any problems early on.
- The information gained from this study will help other babies in the future.

Participation is voluntary.

This study is voluntary. If you choose not to enter your baby in this study, he or she will still receive the high standard of care given to all children who visit the Hospital for Sick Children.

Is the study confidential?

Your baby will be identified only by his or her initials and study number. The study results may also be presented at a medical meeting or in a medical publication. However, your baby's records will be treated confidentially and at no time will your baby's identity be revealed.

For information and advice about any part of the study, please contact George Yeung (or nurse) at:

Division of Gastroenterology/Nutrition
The Hospital for Sick Children

555 University Ave.
Toronto, Ontario
M5G 1X8
(416) 813-5142
INSTRUCTIONS (for treatment subjects only)

FEEDING

Please feed your baby the three foods that we provided:

- whole cow milk - any amount up to 1 litre per day (same as homogenized).
- cereal - 2 / 3 cup of cereal per day (before mixing)
- meat - 1-1½ jars of strained baby meat per day

Also feel free to feed your baby anything else you would normally feed.

- no formula or other milk
- no vitamin or mineral supplements with iron in them.

Milk Coupons can only be used to buy Sealtest or Lactancia whole (homogenized) milk. Stores that carry these include:

- Loeb
- IGA
- Knobhill
- Longos
- Price Club / Costco

Some other stores may sell Sealtest or Lactancia milk as well, but they may not accept these milk coupons.

DAILY RECORD

Please use the supplied calendar to record how much your baby ate each day, and how he or she felt that day (e.g. fever, cough, etc.). This is an example of a baby that started the study on Aug. 15, 1995:

AUGUST 1995

<table>
<thead>
<tr>
<th>SUN</th>
<th>MON</th>
<th>TUE</th>
<th>WED</th>
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<td>C</td>
<td>M</td>
<td>C</td>
<td>M</td>
<td>M</td>
</tr>
</tbody>
</table>

210
In the lower box, please mark how much cereal and meat your baby ate:

- C3 ate more than 2/3 of a cup
- C2 ate 2/3 of a cup
- C1 ate 1/3 of a cup
- C0 ate less than 1/3 of a cup

- M3 ate more than 2 jars of meat
- M2 ate 2 jars of meat
- M1 ate 1 jar of meat
- M0 ate less than 1 jar of meat

In the upper box, please mark how your baby felt (more than one may be used):

- W well (no significant problems)
- M fussy but not sick (e.g. teething)
- D diarrhoea (more than 4 loose stools today)
- C cold or cough
- O other type of illness
- F fever, not bad
- S very sick, high fever, needed to go to the doctor or clinic (not a normal visit)

Name: ________________________________
INSTRUCTIONS (control subjects only)

FEEDING
Please feel free to feed your baby whatever you would normally feed him or her.

DAILY RECORD
Please use the supplied calendar to record how your baby felt each day (e.g. ill, cough, etc.). Following is an example of a baby that started the study on Aug. 15, 1995:

AUGUST 1995

<table>
<thead>
<tr>
<th>SUN</th>
<th>MON</th>
<th>TUE</th>
<th>WED</th>
<th>THU</th>
<th>FRI</th>
<th>SAT</th>
</tr>
</thead>
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</table>

Please mark how your baby felt (more than one may be used):
- W well (no significant problems)
- M fussy but not sick (e.g. teething)
- D diarrhoea (more than 4 loose stools today)
- C cold or cough
- O other type of illness
- F fever, not bad
- S very sick, high fever, needed to go to the doctor or clinic (not a normal visit)

Name:__________________________

212
The nurse will come and see you again when your baby is about 8 months, 10 months, and 12 months old. We will call you about 2 weeks before each date to arrange a good day and time for each visit.

Two or three days before the actual visit, we will call to remind that we are coming.

It is important we can visit your near these dates:

<table>
<thead>
<tr>
<th>Birthdate:</th>
<th>/</th>
<th>/</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tr>
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<table>
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</tr>
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<td></td>
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<table>
<thead>
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</thead>
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<tr>
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<td>year</td>
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<td>month</td>
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<td></td>
<td></td>
<td>year</td>
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</tbody>
</table>

**REVISITS**

If your baby’s iron is found to be low, the nurse will come back for another small blood sample within a few days so that we can check it again. If the iron is still low, then we will stop the study and supply your baby with formula with iron and/or iron drops.

All the results will be sent to your baby’s doctor when we finish the study.
CONTACT NUMBERS

<table>
<thead>
<tr>
<th>Role</th>
<th>Name</th>
<th>Contact Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Coordinator</td>
<td>George Yeung</td>
<td>office (416) 813-5142</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pager (416) 442-8753</td>
</tr>
<tr>
<td>Assistant Coordinator</td>
<td>George Karasmanis</td>
<td>office (416) 813-5142</td>
</tr>
<tr>
<td>Principle Investigator</td>
<td>Dr. Stan Zlotkin</td>
<td>office (416) 813-6170</td>
</tr>
<tr>
<td>Secretary</td>
<td>Lila Shakur</td>
<td>office (416) 813-6171</td>
</tr>
<tr>
<td></td>
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<td>fax (416) 813-4972</td>
</tr>
<tr>
<td>Lab Coordinator</td>
<td>John Kjarsgaard</td>
<td>office (416) 813-5999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pager (416) 377-1460</td>
</tr>
</tbody>
</table>

- Please call George Yeung at the office, (416) 813-5142, for general questions.
- If you require more food or other supplies, please call John at (416) 813-5999.
1. Contents for this booklet.
2. General nurse flowchart.
3. Elaboration of nurse flowchart.
4. Checklist for each visit.
6. Nurse equipment list.
ELABORATION OF FLOW CHART

1. The nurse goes to the appointment. She must bring tide or food to each appointment, depending on whether that infant is in the control or treatment group. The nurse goes through that infant’s section of the infant binder. This will involve giving the welcome booklet to the parents, having the parents fill out the questionnaires, and collecting anthropometric data in triplicate and blood, as described on the following page.

2. After each appointment, the nurse returns to the HSC lab to put the blood sample in the fridge.

At the HSC lab, the nurse takes her new appointment sheet (if there is one) from the "out" infant binder and removes any new sections (if there are any) from the "out" infant binder of the HSC lab and transfers them into her own binder. She then transfers any completed infant sections from her infant binder to the "in" infant binder of the HSC lab (i.e. she picks up any new infant papers and drops off any completed infant papers).

The nurse picks up food, tide, baby clothes, and whatever else she needs for the next few appointments.

3. The nurse updates her own daily planner from the appointment sheet, if there are any changes.

4. The nurse returns home and awaits the next appointment.

NOTES

- In general, since each nurse will have some supplies of tide and baby-food at home, it will not be necessary for the nurse to come to HSC before a home visit, only after.

- Supplies (food, tide, baby clothes) are picked up from HSC after a home visit, while dropping off blood samples and completed infant questionnaires.
HOME VISIT 1

AGE: 6 MONTHS

Forms required:

Put into Nurse’s Binder:
1. Welcome Booklet, Certificate of Participation
2. Food Use and Health Summary to cover time between visit #1 and visit #2
3. Consent to Participate
4. Consent to Contact Paediatrician
5. Backgrounder Questionnaire
6. Initial Dietary History Questionnaire Retrospective 0-6 months.
7. Anthropometric Measurements form

Supplies and equipment required:
1. Depending on whether infant is control or treatment, bring
   - treat - for 1 month (1½ boxes of meat, 3 boxes of cereal, $40 milk coupons)
   - control - tide, baby clothes, etc. Exact quantities will be assigned later.
2. 1/3 c measuring cup from measuring set (mark down colour chosen, on Progress Sheet, so that mother will receive rest of set in same colour)

For anthropometric measurement:
1. Measuring tape (kept in nurse’s bag)
2. Measuring plane
3. Health-o-meter portable paediatric scale

For blood taking purposes: (Items 1-14 kept in nurse’s carrying case)
1. 2 translucent trays for equipment
2. alcohol swabs (20)
3. gauze, 2x2’s (20)
4. Softclix Lancets (20)
5. Softclix Automatic Lancing Device
6. microtainer with E.D.T.A. (20)
7. small blue cooler bag with one frozen freezer flask
8. biohazard bags for blood sample (20)
9. disposable rubber gloves (20)
10. clinical chemistry requisition forms (20)
11. blue pads (5)
12. plastic bags for disposal purposes (10)
13. small Sharps container
14. Grabbers (6 singles)
HOME VISITS 2 & 3

AGE: 8 MONTHS AND 10 MONTHS

Forms required:

Put into Nurse's Binder:
1. Criteria Check
2. Anthropometric Measurement Form
3. 3-day dietary record
4. Food Use and Health Summary to cover time between this visit and next

Supplies and equipment required:
1. Depending on whether infant is control or treatment, bring
   - treat - for 1 month (1½ boxes of meat, 3 boxes of cereal, $40 milk coupons)
   - control - tide, baby clothes, etc. Exact quantities will be given later.

For anthropometric measurements:
1. Measuring tape (kept in nurse's bag)
2. Measuring plane
3. Health-o-meter portable paediatric scale

For blood taking purposes: (Item 1 - 14 kept in nurse's carrying case)
1. 2 translucent trays for equipment
2. alcohol swabs (20)
3. gauze, 2x2's (20)
4. Softclix Lancets (20)
5. Softclix Automatic Lancing Device
6. microtainer with E.D.T.A. (20)
7. small blue cooler bag with one frozen freezer flask
8. biohazard bags for blood sample (20)
9. disposable rubber gloves (20)
10. clinical chemistry requisition forms (20)
11. blue pads (5)
12. plastic bags for disposal purposes (10)
13. small Sharps container
14. Grabbers (6 singles)
AGE: 12 MONTHS

1. Criteria Check
2. Anthropometric Measurement Form
3. Gift for infant

Forms required:
Clip into Nurse's Binder:
1. Criteria Check
2. Anthropometric Measurement Form
3. Certificate of Completion

Supplies and equipment required:
For anthropometric measurements:
1. Measuring tape (kept in nurse's bag)
2. Measuring plane
3. Health-o-meter portable paediatric scale

For blood taking purposes: (Items 1-14 kept in nurse's carrying case)
1. 2 translucent trays for equipment
2. alcohol swabs (20)
3. gauze, 2x2's (20)
4. Softclix Lancets (20)
5. Softclix Automatic Lancing Device
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9. disposable rubber gloves (20)
10. clinical chemistry requisition forms (20)
11. blue pads (5)
12. plastic bags for disposal purposes (10)
13. small Sharps container
14. Grabbers (6 singles)
BLOOD LETTING

The nurse should assess the home and decide where the blood letting process and taking of anthropometric measurement could be best accomplished and ask permission to set up there. Remember: the nurse knows what must be done and what space is required and needs to “take charge” in a non-threatening manner.

Purpose:
To obtain 200 μL of blood by finger prick.

Supplies and equipment to be used:

1. Alcohol swabs, microtainers, gauze, 2x2's
2. Softclix lancets with E.D.T.A. biohazard bags
3. Disposable rubber gloves, clinical chemical requisition forms, and blue pads
4. Forms, paper bags, sharps container, Grabbers

Procedure followed by nurse:

1. On Visit 1, show the mother the Softclix lancet and the microtainer and show her how much blood you will be taking.
2. Have the mother hold the infant and give her an activated grabber to hold on her infant's thumb and thumb region (explain that this facilitates a better blood flow). This should be kept on infant's thumb and thumb region for at least 2 minutes.
3. While mom is warming infant's thumb:
   - Label the clinical chemical requisition form with the following information:
     Date, Family #, Visit # or Revisit, sex and birthdate of baby.
   - Stick tab from the chemical requisition onto the anthropometrics sheet next to the quantity of the blood sample. Also stick a tab onto the microtainer with the blood sample.
   - Set up your equipment, wash your hands and put on gloves.
4. Position infant for blood letting process.
5. Cleanse the finder with alcohol swab and let it air dry.
6. Puncture skin, using Softclix Lancing Device and Softclix lancet.
7. With a sterile gauze square wipe away the first drop of blood.
8. Hold the infant's thumb in a dependant position, and using an intermittent gentle pressure (milking action) collect the required amount of blood into the microtainer.
9. Apply gauze to the puncture site and have the mother apply pressure to stop bleeding.
10. DO IMMEDIATELY (to prevent BLOOD CLOTTING) - While mom is holding gauze on infant's thumb, immediately cap microtainer containing blood sample and perform an invert the tube and shake it routine 10 TIMES.
11. Place microtainer with blood sample into biohazard bag.
12. Tear off top copy of requisition form and clip into binder (leave spare tabs on requisition sheet).
13. Place biohazard bag into small blue cooler bag and close bag up immediately.

NOTE: A frozen freezer flask must be placed in the cooler bag each morning before visits begin. Bloods must be kept cold, but not frozen, at all times, therefore they must be kept
in the blue cooler bag until transfer into the HSC. lab fridge.
14. Check infant’s finger, clean puncture site.
15. Dispose of used supplies and put blood letting equipment away.

NOTE: All blood letting procedures will collect approximately 200 \( \mu \text{L} \) of blood.

**REVISITS:**
- only blood letting is performed (no anthropometric data collected)
- 200 \( \mu \text{L} \) is collected as usual
- be sure to mark on the anthropometrics sheet and the chemical requisition form that it is a revisit.
ANTHROPOMETRICS

Purpose:
To obtain accurate measurements of infant height, weight and head circumference at age 6, 8, 10, and 12 months. Each anthropometric measurement is done 3 times at each visit. Record the measurements on the record form under the appropriate visit.

Head Circumference:

Equipment:
1. Tape measure
2. Anthropometric measurements record form

Procedure:
Head circumference is taken around the greatest circumference (refer to diagram). Have mother hold the infant sitting upright or lay the infant down on a table while measuring. (Reference: Alexander, M and Brown, M. Pediatric Physical Diagnosis Toronto, McGraw-Hill, 1974, p.40)

Height:

Equipment:
1. Franklin measuring board (Note: Please wipe down once per week)
2. Anthropometric measurements record form

Procedure:
1. Place the plane on a hard flat surface.
2. Lay the infant on the plane, with the head at the top. The infant should be lying flat and centred on the board.
3. Have a second person cup the infant's head and position his/her head against the top of the plane. Infant should be facing straight up with head flat against the plane's headboard.
4. Hold the plane footpiece in one hand, while placing the other hand on the infant's shins or knees. Press down on infant's legs to straighten while moving the footpiece into position against the infant's feet.

Weight:

Equipment:
1. Health-o-meter portable paediatric scale
2. Blue pad.
3. Anthropometric measurements record form

Procedure:
1. Place blue pad on scale, zero scale, calibrate against the 5 lb. weight, undress infant, except for diaper.
2. Place infant on scale, weigh, remove infant, let scale zero before next measurement.
SUPPLIES LIST

BLOOD COLLECTION/FIELD SUPPLIES FROM H.S.C. LABORATORY

Each nurse should check her supplies bi-monthly and place an order with George Yeung. Each nurse should have in her supplies the following:

<table>
<thead>
<tr>
<th>Date of Order</th>
<th>Item Description</th>
<th>Base</th>
<th>Order Received</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Softclix Sterile Lancets</td>
<td>200 (1 box)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Softclix Automatic Lancing Device</td>
<td>1 unit</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microtainer with E.D.T.A.</td>
<td>4 boxes (20 tubes /box)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.D.I. alcohol preps medium B33907W DIN0480452</td>
<td>2 boxes 200/box</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nu gauze sponges 5cmx5cm Johnson &amp; Johnson (H5125) code 240339</td>
<td>4 boxes 25 env /box</td>
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</tr>
<tr>
<td></td>
<td>Transvelopes biohazard bags (6 x 8.5)</td>
<td>50</td>
<td>50/pkg</td>
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<tr>
<td></td>
<td>Safeguard blue underpads</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>disposable gloves, stretch vinyl (small) B.D. Tru-touch # 48 2205</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>plastic bags, (approx. 11&quot;x4&quot;), # 5 bags</td>
<td>50</td>
<td></td>
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<tr>
<td></td>
<td>Clinical Chemistry Lab Requisition forms</td>
<td>1 pkg 100/pkg</td>
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APPENDIX D: PRODUCT LISTS
**Infant Iron Study**

**Infant Food Order Form**

DATE: __________

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>IN STOCK</th>
<th>TO ORDER</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>JUNIOR FOODS:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chicken rice with vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ham and egg breakfast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vegetables and turkey</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>STRAINED MEAT DINNERS:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>beef with vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chicken with vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>veal with vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>STRAINED MEATS:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>beef with broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>beef liver with broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chicken with broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ham with broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lamb with broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>turkey with broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>veal with broth</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**STRAINED MEAT WITH VEGETABLE COMBO:**

- vegetables, beef and liver

**INFANT CEREALS:**

- barley
- mixed
- oatmeal
- rice
- infantsoy
CONTROL GROUP ONLY

VISIT #1

winter jacket
gloves

VISIT #2

polar fleece top
robe

VISIT #3

sweat top
sweat bottom
raincoat
touque

Note that for VISIT #4 both groups get tide, treatment group gets a book and squeeze toy and control group gets teddy bear.
Sample Size Estimation

Calculations are based on analyses relating to the hypothesis that there will be a difference in the number of endpoints.

Assumptions: A recently completed study on the prevalence of iron depletion and iron deficiency anaemia across Canada (Zlotkin et al, in press) show the incidence of iron depletion to be 33.9% across Canada and 17.2% in Toronto. Iron depletion was found in 37% of infants in disadvantaged families in Montreal (7). Thus a conservative estimate of the rate of iron depletion in disadvantaged infants in Toronto is at least 30%.

Based on our experience from a recently completed study by Beaton et al. (NHRDP Project 6606 4104 61 ), it is reasonable to assume that a cereal alone group would achieve an end point rate of 20% based on a low ferritin value (iron depletion). There is no data available to estimate end points rates from the 'meat' group. A clinically significant improvement from the control (30%) is estimated to be an incidence of 10%. Thus the following assumptions would be reasonable for the purpose of estimation of required sample sizes:

Group Rate of End-points
Control (no intervention)30%
Meat + Cereal 10%

The end-point is + or -. That is, we are dealing with binomial distributions. The approach to estimation of sample size in such a situation is described below:

\[ p_1 = 0.30 \]
\[ p_2 = 0.10 \]
\[ p = p_2 + p_1/2 = 0.20 \]

standardised difference = \[ (p_2 - p_1)/p(1 - p)^{1/2} \]
\[ = 0.20/0.20(0.80)^{1/2} \]
\[ = 0.5 \]

The standardised difference is, in principle, based on the ratio of the differences of interest to the standard deviation of the observations. In other words, the difference between the control and treatment groups are expressed as a multiple of the standard deviation.

Based on a monogram for calculating sample size or power, with a desired power of 0.80 at a level of significance of 0.05

\[ N = 110 \]
\[ ie. \ 55 \text{ per group x 2 groups.} \]

APPENDIX F: DISPARITY OF SERUM TRANSFERRIN RECEPTOR MEASUREMENTS AMONG DIFFERENT ASSAY METHODS
Abstract

Objective: To highlight differences in the quantification of transferrin receptor (TfR) concentration (a reliable index of iron deficiency) between three different assay methods.

Design: Methods comparison of TfR measurements in ‘elevated’ and ‘normal’ human sera using the Ramco, Quantikine and ‘Lab’ assays.

Setting: The Hospital for Sick Children, Toronto, Ontario, Canada.

Subjects: Pooled TfR for elevated and normal human sera obtained from the Ramco TfR assay kit.

Main outcome measures: Differences between TfR concentrations in normal and elevated samples and repeatability for each assay method and limits of agreement in TfR quantification between assay methods.

Results: The mean TfR concentrations for the elevated reference serum samples was higher than the normal reference samples within each individual assay ($P < 0.001$); however, measurement agreement between methods was poor.

Conclusion: Recognition of the relative differences in the values obtained from each of the assays should affect the interpretation of TfR concentration as an index of iron deficiency.

Sponsorship: This research was supported by the Ministry of Agriculture, Food and Rural Affairs Ontario.

Descriptors: transferrin receptor, iron deficiency, enzyme-linked immunoassay, iron
Introduction

Iron deficiency anemia is the most prevalent nutrition-related health problem in the world (Scrimshaw, 1991). It is identified with a combination measurement of hemoglobin plus at least one of ferritin, free erythrocyte protoporphyrin, serum iron, transferrin, or iron-binding capacity (Life Science Research Office, 1984). Individuals at risk of iron deficiency anemia have low serum ferritin concentrations (corresponding to low stores of iron) and low serum transferrin concentrations (corresponding to minimal availability of iron to the erythrocyte).

Both ferritin and transferrin measurements suffer from a lack of specificity and sensitivity (Ahluwalia et al., 1993; Baynes, 1996; Borel et al., 1991; Cooper & Zlotkin, 1996; Dallman & Reeves, 1984; Lipschitz et al., 1974). A new indicator of iron status, the circulating soluble transferrin receptor (TfR), provides the only accurate assessment of functional iron deficiency, which occurs between the initial depletion of storage iron and the overt development of anemia (Ahluwalia et al., 1993; Anttila & Cook, 1997; Cook et al., 1993; Cook & Skikne, 1989; Cooper & Zlotkin, 1996; Punnonen & Irlala, 1997; Skikne et al., 1990).

Soluble TfR is released from cells into the vascular compartment in direct proportion to the number of cellular receptors expressed (Flowers et al., 1989; Kohgo et al., 1986). Serum TfR always circulates bound to ligand. The serum receptor is an 85-kDa truncated extracellular domain of the cell-surface receptor, produced through proteolytic cleavage by a serine protease, predominantly at the surface of the exosome within the multivesicular body (Baynes, 1996; Shih et al., 1990). Unlike other methods of assessing iron availability to the developing erythrocyte, increases in TfR concentration are specific to iron deficiency (Ferguson et al., 1992; Punnonen et al., 1994).

Several enzyme-linked immunoassay (EIA) systems have been developed to measure TfR, but each system reports a different normal range (Table 1). In healthy adults, the Ramco assay reports a normal TfR range of 2.9–8.3 mg/L; the Quantikine assay, 0.85–3.05 mg/L. An indirect enzyme-linked
immunosorbent assay (ELISA) developed in our laboratory ('Lab') provides a TfR range of 2.4–8.0 mg/L in healthy infants (Cooper & Zlotkin, 1996).

The differences in the reported normal ranges appear to be greater than those that would be expected from intra- and inter-assay variability and/or differences in sensitivity (limit of detection) between the three methods. Despite the differences in the reported normal ranges, absolute TfR concentrations derived from different assay methods have been directly compared in the literature, which leads to confused interpretation (Nielsen et al., 1994). Thus, the objective of our study was to examine the limitations of comparison of assay results, by measuring and reporting on the differences in TfR concentration between three assay methods on the same reference serum samples.

Materials and methods
Two commercially available TfR kits were used in the study, both EIAs: the Ramco Human TfR Assay Kit (Ramco Laboratories Inc., Houston, TX, USA) and the Quantikine Human TfR Immunoassay (R&D Systems, Minneapolis, MN, USA). Two reference sera supplied in the Ramco kit was used throughout the study, for all assays: one reported by Ramco to be in the normal range (4.3–7.7 mg/L) and the other, elevated (9.1–15.9 mg/L) for TfR.

Between 18 and 24 replicates of the 'normal' and 'elevated' reference sera were assayed with each of the Ramco, Quantikine and Lab assays. Student's t-test was used to compare normal versus elevated samples within each assay method.

Repeatability and measurement agreement within each of the assays was determined according to the method of Bland and Altman (1986):

\[
\text{Limits of agreement} = (\text{mean}_1 - \text{mean}_2) \pm 2(\text{corrected SD of differences})
\]

\[
\text{Corrected SD of differences} = \left[ \frac{(\text{SD of differences})^2 + \frac{1}{4}(\text{SD}_1)^2 + \frac{1}{4}(\text{SD}_2)^2}{2} \right]^{1/2}
\]
The standard deviation of differences for each assay was determined by one-way analysis of variance. All statistical analyses were performed using SAS software, version 6.12 (SAS Institute, Cary, NC, USA). P values < 0.05 were considered significant.

The British Standards Institution (1979) has defined repeatability within an assay as acceptable if 95% of differences between individual replicates and the mean of all replicates are within two standard deviations (±2 SD) of the mean. Bland and Altman (1986) defined limits of agreement between two methods (e.g. the results from Ramco versus Quantikine assays) as the differences between the means of the two methods plus or minus twice the corrected standard deviation of the differences. In simple terms, the limits of agreement describe the range by which one method over- or underestimates another. If this over- or underestimation is clinically significant, the methods do not agree and cannot be used interchangeably. In this study, we defined clinically significant limits of agreement as over- or underestimation wide enough to cause misdiagnosis between normal iron status and iron deficiency.

Results

TfR measurements for elevated and normal sera with each of these assay methods are shown in Table 1. Mean TfR concentrations for the elevated reference serum samples were higher than the normal reference samples within each individual assay (P < 0.001). The intra-assay coefficient of variation for each assay for both elevated and normal samples is shown in Table 2.

For both the Ramco and Lab assays, 95% of the differences between individual replicates and the mean of all replicates fell within 2 SD of the mean (i.e., they were with the limits deemed acceptable by the British Standards Institute); for the Quantikine assays, 92% fell within 2 SD (Figure).

The mean limits of agreement among the three assay methods (±1 SD) were 7.71 ± 0.65 mg/L for the Ramco kit; 3.20 ± 0.32 for the Quantikine kit; and 6.52 ± 0.23 for the Lab kit. Between the Ramco
and Lab assays, the upper and lower limits were 3.12 and −0.74, respectively (corrected SD, 0.96); between Ramco and Quantikine, 10.95 to −1.93 (corrected SD, 3.22); and between Lab and Quantikine, 8.08 and −1.43 (corrected SD, 2.39).

Discussion
Comparing serum TfR measurements between two commercially available kits (Ramco and Quantikine) and an assay developed in our lab showed a disparity in measurements of absolute TfR levels between each of the methods for both the elevated and normal serum samples. Despite this disparity, all methods were able to distinguish between elevated and normal serum samples (Table 1).

The measured intra-assay coefficients of variation for the Ramco and Quantikine assays obtained during this study were higher than those reported by the manufacturer of the kits (Table 2). This is likely due to the larger number of replicates used by the Ramco (n = 60) and Quantikine (n = 48) manufacturers. However, the repeatability coefficients of both the Ramco and Lab assays were acceptable according to the British Standards Institution (1979), whereas that of the Quantikine assay kit was not. Repeatability is important in method comparisons because high variation in repeated measurements on the same sample depresses agreement between the two methods.

Since differences between assays were all greater for the normal than for the elevated samples, it is likely that calculated limits of agreement are broader for higher TfR values and narrower for lower values. However, the overall disparity between assay methods makes comparisons of TfR values derived from different methods unacceptable. For example, we recently reported (Yeung & Zlotkin, 1997) the mean TfR value for normal healthy infants 9–15 months of age to be 4.4 mg/L, using our ‘Lab’ assay. Values above 7 mg/L were above the 95th percentile, considered to be indicative of iron deficiency. Based on the upper limit of agreement, the Ramco assay could overestimate this mean of 4.4 mg/L by as much as 3.12 mg/L. Thus, a Ramco value of 7.52 mg/L would be incorrectly interpreted as a high value
if compared directly to normative data based on our Lab assay. This would lead to misdiagnosing an infant who has a serum TfR concentration within the normal range as being clinically deficient in iron.

The two methods therefore cannot be used interchangeably. The potential for such misinterpretation is far greater when comparing values derived from the two commercial assays, because of the wider limits of agreement between the Ramco and Quantikine assays.

The considerable overlap in the normal ranges reported for these three methods of TfR quantification (Table 1) has already led to this kind of confusion in interpretation. Nielsen and associates (1994) directly compared results using the Quantikine method with literature values based on the Ramco kit. The authors noted an inverse correlation between serum ferritin and TfR concentrations, as might be expected in patients with exhausted iron stores; but they state that the elevations in TfR were only moderate (mean, 2.7 mg/L) compared to a similar study of patients with iron deficiency anemia by Ferguson and colleagues (1992). In Ferguson et al’s study, however, TfR values were based on the Ramco assay, and consequently, were markedly higher (mean, 14 mg/L). In fact, a number of Nielsen et al’s patients had dramatic elevations in TfR compared to Quantikine’s reported normal ranges and the values obtained in our current study.

The publication of normative percentile estimates for TfR has increased the likelihood that laboratory values for TfR will be misinterpreted. We recently developed percentile estimates for TfR concentrations for 9–15-month-old infants, using our Lab assay (Yeung & Zlotkin, 1997); that was the first publication of a normal range for TfR in the infant population. TfR concentrations above this range may indicate iron deficiency, but only when our Lab assay is used for the measurement. Values from other assay methods on the same sample might be higher or lower, creating a great potential for misinterpretation and misdiagnosis.

Data from the current study did not explain the data variation between assay methods. The disparities are likely caused by differences in antibody binding constants. All three methods are enzyme-
linked immunoassays with either monoclonal or polyclonal antibodies produced against purified placental TfR to establish a standard curve. The Ramco and Quantikine assays both utilize a polyclonal capture antibody bound to the solid phase; however, Ramco uses a monoclonal detector antibody, whereas Quantikine uses a polyclonal detector antibody. The Lab assay binds TfR directly to the solid phase with a monoclonal capture and a polyclonal detector antibody. Differences in the avidity of either the capture or detector antibody between purified human placenta-derived TfR and normal human serum TfR would alter the quantification of TfR samples. Studies of the differences in antibody binding affinities may explain the disparity.

Despite the disparity in quantification of TfR between methods, Ramco, Quantikine and ‘Lab’ assays were all able to distinguish between elevated and normal serum samples. We have shown that the limits of agreement between each pair of assay methods are wide enough to cause misdiagnosis; thus, the methods cannot be used interchangeably. Recognition of the relative differences in the values obtained from each of the assays will have a major effect on the interpretation of TfR concentration as an index of iron deficiency.
Acknowledgment

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References


Life Science Research Office (1984): *Assessment of the iron nutritional status of the US population*


Table 1 Comparison of transferrin receptor concentrations assayed in two reference samples, ‘normal’ and ‘elevated’

<table>
<thead>
<tr>
<th>Assay</th>
<th>Kit</th>
<th>Reported* (mg/L)</th>
<th>Observed† (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kit Type</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Ramco</td>
<td>EIA</td>
<td>2.9–8.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Quantikine</td>
<td>EIA</td>
<td>0.85–3.05</td>
<td>1.5</td>
</tr>
<tr>
<td>‘Lab’</td>
<td>ELISA</td>
<td>2.4–8.0</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* For healthy adults (commercial kits) or healthy infants (Lab kit).
† All data for ‘elevated’ sera differed significantly from those for ‘normal’ sera measured with the same kit (P<0.001).
Table 2 Sensitivity and inter- and intra-assay variation for transferrin receptor concentration

<table>
<thead>
<tr>
<th>Assay</th>
<th>Reported Sensitivity (mg/L)</th>
<th>Reported Variation (%) CV</th>
<th>Observed Intra-assay Variation (%) CV</th>
<th>Elevated</th>
<th>n</th>
<th>Normal</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramco</td>
<td>0.7</td>
<td>3.6–8.1</td>
<td>2.3–5.8</td>
<td>8.4</td>
<td>22</td>
<td>14.1</td>
<td>18</td>
</tr>
<tr>
<td>Quantikine</td>
<td>2.0</td>
<td>3.0–7.8</td>
<td>4.3–9.9</td>
<td>10.5</td>
<td>24</td>
<td>6.1</td>
<td>24</td>
</tr>
<tr>
<td>'Lab'</td>
<td></td>
<td></td>
<td></td>
<td>6.4</td>
<td>20</td>
<td>8.9</td>
<td>20</td>
</tr>
</tbody>
</table>

CV, coefficient of variation
Figure. Repeated measures of transferrin receptor (TfR) concentrations with the Ramco assay kit, the Quantikine assay kit, and the 'Lab' assay. SD, standard deviations.