THE IMPACT OF IN UTERO EXPOSURE TO GESTATIONAL DIABETES ON INSULIN RESISTANCE IN THE OFFSPRING

by

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ABSTRACT

Background: The evolution of increased cardio-metabolic risk in offspring exposed to maternal gestational diabetes (GDM) is not well understood.

Purpose: (i) To evaluate the impact of GDM exposure on insulin resistance in early childhood, and (ii) To evaluate waist-to-height ratio as a surrogate measure of cardio-metabolic risk (CMR) in children under 5 years of age.

Methods: A prospective cohort of infants born to mothers with and without GDM underwent metabolic characterization between birth and 5 years of age.

Results: Among non-GDM children, male gender predicted a 35.1% lower HOMA-IR (p=0.03). In GDM offspring, a 1 unit increase in maternal insulin sensitivity predicted a 17.7 % decrease in HOMA-IR (p=0.002). Waist-to-height ratio was not superior in predicting CMR.

Conclusions: There are no differences in IR of children with or without GDM exposure however the determinants of IR are different. BMI-z score is the preferred measure of adiposity in this age group.
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LIST OF ABBREVIATIONS

ADP - Air-displacement plethysmography

BMI - Body Mass Index (kg/m²)

DXA – Dual-energy X-ray absorptiometry

GDM - Gestational Diabetes Mellitus

HbA1c – Hemoglobin A1c

HOMA-IR - Homeostatic Model Assessment – Insulin Resistance

IR - Insulin Resistance

IS - Insulin Sensitivity

OGTT – Oral glucose tolerance test

WHO - World Health Organization
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1. INTRODUCTION

Pregnancy and early childhood are recognized as pivotal periods for offspring development and programming of future metabolic trajectories. This crucial developmental window may also represent a time of potential plasticity allowing for modification of one’s metabolic risk. Accordingly, significant interest has been generated in initiatives which capture at-risk children and allow implementation of interventions which may mitigate future risk of disease in adolescence and adulthood. Identification of children at increased metabolic risk may be facilitated by two strategies: early recognition of their inherent environmental and genetic risk factors as well as longitudinal monitoring of their growth and adiposity.

Exposure to maternal gestational diabetes (GDM) has been recognized as an important risk factor associated with increased risk of adiposity and insulin resistance in later life. (Monasta et al. 2010) While this relationship has been clearly studied in adolescence and adulthood, there is very little known about the evolution of this risk in early childhood. Enhancing our understanding of the impact of GDM on insulin sensitivity in the offspring will be helpful in better monitoring their cardio-metabolic health.

One of the most powerful tools for monitoring cardio-metabolic health includes routine assessment of a child’s adiposity during early childhood. Traditionally, body mass index and weight for length have been used in clinical practice. Emerging measures such as waist to height ratio have not yet been examined in very young children and may represent a powerful tool through which to identify vulnerable children.
This thesis will therefore attempt to address the gaps in the literature, examining the relationship between maternal gestational diabetes and early metabolic health in the offspring, while also evaluating the utility of waist to height ratio as a measure of cardio-metabolic health in young children.

2. BACKGROUND AND LITERATURE REVIEW

2.1 Childhood Obesity

2.1.1 Impact of Obesity in Early Childhood

Childhood obesity continues to pose a pervasive public health problem, with overweight and obese children now representing nearly one-third of the Canadian population. Obesity is also being diagnosed earlier in childhood. In 2014, global estimates from the WHO estimated that 41 million children under the age of five were overweight or obese. While the prevalence has stabilized in some parts of the world, developing countries continue to experience an increase in the rates of obesity in preschool children. (Onis, Blo, and Borghi 2010) Its impact also disproportionately targets children of lower socio-economic status and ethnic minorities. (Isong et al. 2018) Obesity diagnosed in childhood can have lifelong consequences and is often accompanied by significant medical, psychological and social sequelae which can translate into tremendous healthcare utilization and costs. (Borner et al. 2016; Sonntag, Ali, and De Bock 2016). Furthermore, obesity which originates in childhood typically persists into adulthood and predicts increased mortality from cardiovascular disease in the future. (D S Freedman et al. 2004)

2.1.2 Determinants of Childhood Obesity and Metabolic Syndrome

A clear understanding of the underlying pathophysiology of childhood obesity is essential for the development of effective and well-timed interventions. However, causes of obesity are complex and
multifactorial. Cardio-metabolic risk is determined by a combination of genetic, epigenetic and lifestyle factors which drive patterns of behavior, adiposity and metabolism (see Figure 1).

### 2.1.3 Genetic Determinants of Weight and Insulin Resistance

Genetic variance plays an important role in the development of obesity, insulin resistance and type 2 diabetes, explaining differences in individual susceptibility to a shared obesogenic environment. Genetics govern the intricate behavioural and physiological systems which influence metabolism, eating behaviours and body weight. Family studies indicate that more than half of BMI heritability can be accounted for by cumulative genetic causes. (Haworth et al. 2008) Genome-wide association studies (GWAS) and candidate gene approaches are beginning to identify genetic loci which are important in determining fat mass and diabetes risk. (Frayling et al. 2007). Studying genetic forms of diabetes such as...
maturity-onset diabetes of the young (MODY) has led to the recognition of the importance of transcription factors, while single nucleotide polymorphisms identified through GWAS studies have highlighted the role of genes in involved in pancreatic beta cell function. (Bernstein, Golson, and Kaestner 2017) To date, nearly 100 genes have been associated with type 2 diabetes, yet their collective contribution to the determination of diabetes risk has been quite small. Indeed, it is estimated that only 15% of the heritability of type 2 diabetes is currently accounted for by known genetic variant loci. (Mahajan et al. 2014).

2.1.4 Environmental Determinants of Insulin Resistance

The environment plays a pivotal in programming cardio-metabolic health. Both the in utero environment and postnatal environment are important drivers of metabolic risk. The fundamental role of the postnatal environment is supported by observations of increasing population obesity despite stability of the genetic pool. Consumption of energy-dense foods, poor sleep duration and a sedentary lifestyle are all behavioural factors which have been linked with obesity. (Fatima, Doi, and Mamun 2016; Janssen and LeBlanc 2010; Harrington 2008) A comprehensive framework published by the Institute of Medicine recognizes other important drivers of obesity and highlights the contribution of diverse macro-environmental factors including systems governing food, transportation, community infrastructure, schools and health care provision. (Accelerating Progress in Obesity Prevention 2012)

2.1.5 Importance of Early Diagnosis and Intervention

Identification of youth at-risk for future obesity is a fundamental part of health prevention initiatives aimed at combatting this public health problem. Preventative interventions in vulnerable children have demonstrated a modest, but efficacious impact on weight-related outcomes and quality of life. (Peirson et
In particular, school-based interventions for children which focus on nutrition and other behavioural strategies have demonstrated significant reductions in BMI, BMI z score and prevalence of overweight and obesity. Although reductions in rates of obesity are not consistently achieved, meaningful differences in fasting insulin levels and other measures of adiposity have been accomplished through these interventions (Group 2010). Accordingly, strategic efforts are focused on modification of high-risk behaviours which are present in early childhood and have been closely linked with obesity in later life. These risk factors include parental factors such as exposure to gestational diabetes and smoking as well as infant factors such as consumption of sugar sweetened beverages, reduced physical activity, poor sleep, lack of breastfeeding and most importantly, rapid growth and obesity (Monasta et al. 2010). A systematic review of interventions targeting these identified risk factors in the first 1,000 days of life have shown a modest impact on reducing childhood BMI. (Blake-Lamb et al. 2016) Effective behavioural interventions often focused on individual or family based counseling delivered in a diverse settings including within the home, community or a hospital based environment. Intervention strategies which focus on childcare programs and curriculum have also been successful at modifying driving behaviours such as screen time, physical activity and diet (Sisson et al. 2016; Zhou et al. 2015). Modification of risk factors in early infancy has also been shown to longitudinally improve social and racial disparities in the prevalence of childhood obesity (Taveras et al. 2013). These encouraging findings have generated significant interest in interventions which target this important period of development and underscore the preventative opportunities inherent in a diagnosis of early cardio-metabolic disease in childhood. (Ekelund et al. 2007; McCormick et al. 2010).

2.1.6 The Evolution of Insulin Resistance and Progression to Type 2 Diabetes

Obesity is as a multi-systemic disorder which has a pervasive impact on physical and emotional health. Obesity-related comorbidities that emerge early in childhood include dysregulation of glucose
homeostasis, lipid abnormalities, fatty infiltration of the liver and hypertension. (Weiss and Caprio 2005)

Hard outcomes of cardiovascular disease, such as myocardial infarction or stroke, are uncommon in children, therefore other intermediate outcome measures of cardio-metabolic risk must be used. Obesity is an integral part of metabolic syndrome which is a constellation of clinical features that involve the presence of central obesity, insulin resistance, and lipid dysfunction and hypertension which herald the future development of type 2 diabetes and cardiovascular disease. (Weiss and Caprio 2005) Central adiposity refers to the accumulation of adipose tissue in and around the abdominal viscera and is estimated by measurement of waist circumference. It is highly prevalent among children and adolescents with obesity, and in particular those with central adiposity, however the prevalence of metabolic syndrome in early childhood remains unknown. (Weiss and Caprio 2005). This in part stems from the fact that there are a number of proposed definitions for metabolic syndrome in children, with no accepted consensus on the optimal diagnostic criteria. (Zimmet et al. 2007)

Hyperinsulinemia is one of the hallmarks of metabolic syndrome and insulin resistance is recognized as the most common biochemical derangement. (S. Li et al. 2003) Insulin resistance is defined as an inappropriately elevated serum insulin levels in response to either normal or high serum glucose levels. (Moller, Flier, and Flier 1991) Under physiologic conditions, insulin is responsible for mediating glucose utilization and metabolism by regulating the uptake of glucose into skeletal muscle and adipose tissue and inhibiting hepatic glucose production. Exposure to a glucose load should induce pancreatic insulin secretion to promote uptake of circulating glucose by insertion of glucose transporters into cellular membranes. Impairment in the process of insulin secretion or insulin action at the level of the receptor results in inability to restore euglycemia. Insulin resistance also results in the dysregulation of liver glycogenesis and very low-density lipoprotein production (Patel and Abate 2013).
Animal studies have demonstrated that insulin resistance is one of the earliest signs of metabolic dysfunction present in states of obesity. (Barnard et al. 1998) Abnormalities in patterns of insulin resistance have been identified in children in early infancy. (Ong et al. 2004; D A Lawlor et al. 2005) Insulin resistance is highly positively associated with BMI and the presence of obesity is one of the strongest risk factors for type 2 diabetes. (Awa et al. 2012) Insulin resistance is an early precursor of more overt glucose abnormalities and has been shown to predict progression to type 2 diabetes in adolescence. (Morrison et al. 2008) Reduced insulin sensitivity requires compensatory pancreatic beta cell insulin secretion to maintain normoglycemia. In vulnerable individuals, beta cell function may fail to generate an adequate response, giving rise to progressive hyperglycemia and the clinical symptoms of type 2 diabetes. (Elder et al. 2015)

Estimation of insulin resistance and insulin secretion can be achieved through direct techniques such as the glucose clamp technique or a minimal model approach or indirect measures derived from fasting insulin and glucose values. The hyper-insulinemic euglycemic clamp is considered to be the gold standard technique for determination of insulin sensitivity. It is calculated by measuring the rate of glucose infusion required to achieve a constant plasma glucose level for a given rate of insulin infusion. (Borai, Livingstone, and Ferns 2007) This method is invasive and technically complex. For these reasons, it is not commonly used in clinical practice and is not suitable for use in young children. Instead, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) is a more practical technique which was developed in adults, but subsequently validated in children and adolescents without diabetes against gold standard clamp procedures. (Matthews et al. 1985; Gungor et al. 2004) One of the challenges of studying cardio-metabolic disease in young children is the long latency of disease onset. Accordingly, intermediate markers of disease must instead be used. Proposed surrogate markers include insulin resistance, which is also a key mechanism underpinning the pathophysiology in both obesity and diabetes.
2.1.7 Visceral Adiposity, Insulin Resistance and Cardio-metabolic Risk

Once considered an inert storage depot for energy reserve, adipose tissue is now recognized as a fundamental determinant of systemic glucose metabolism. Adipocytes actively release bioactive proteins called adipokines which are involved in numerous metabolic processes including appetite regulation, insulin sensitivity and systemic inflammation.(Ouchi et al. 2011)

During fetal development, adipose tissue appears and expands between 5 and 29 weeks gestation.(Tarantal and Berglund 2014) Accretion of fetal fat mass is determined by maternal energy balance and therefore influenced by disease states affecting fetal nutrition including maternal obesity and gestational diabetes. Towards the end of pregnancy, adipose tissue comprises 20% of the body mass of the fetus, the majority of which is located in subcutaneous tissue. During the second year of life, fat-free mass gradually increases over time concurrent with when childhood mobility is advancing.(Butte et al. 2000)

Fat can be stored in a number of different compartments throughout the body including muscle, liver, subcutaneous tissue and viscera. Patterns of fat deposition vary according to age, gender, pubertal status, ethnicity and underlying genetics.(Tchernof and Després 2013) Intra-abdominal fat, in particular, appears to vary substantially across different ethnicities.(Modi et al. 2009) Truncal, central and abdominal obesity are all terms which attempt to describe excess adiposity confined to the thorax and abdomen. This is distinct from subcutaneous adipose tissue and includes intra-abdominal and intra-thoracic visceral adiposity.(Patel and Abate 2013) Visceral adipocytes are unique for several reasons. They demonstrate increased sensitivity to lipolysis as well as close proximity to the portal circulation. Greater lipolytic
activity results in increased mobilization and release of non-esterified fatty acids into the circulation and increased tissue insulin resistance. (Ouchi 2016) Visceral adipocytes may also produce more pro-inflammatory adipokines such as TNF-α, IL-6 and leptin. (Tchernof and Després 2013) Cytokine production which favours an inflammatory milieu is another recognized driver of hyperinsulinemia and thus subsequent cardio-metabolic disease. During periods of positive energy balance, insulin resistance has been linked to alterations in the ‘partitioning of lipids’, which involves a preferential deposition of fat into the visceral compartment. This observation may explain why certain individuals for a given degree of obesity are more prone to a metabolically unhealthy phenotype associated with increased health risks. (Weiss and Caprio 2005)

In adults, the presence of visceral fat has been clearly linked to metabolic dysregulation. (Fox et al. 2007) The same relationship appears to hold true in children and adolescents. Studies of children with obesity children with impaired glucose tolerance have demonstrated that this group has higher stores of visceral fat compared to glycemic controls, which are inversely correlated with glucose disposal. (Weiss and Caprio 2005) Greater relative proportions of visceral adipose tissue were also associated with higher 2-h glucose, insulin and triglycerides levels supporting the notion that higher relative ratios of visceral to subcutaneous fat impart a negative impact on glucose and lipid metabolism. (Taksali et al. 2008) Central adiposity in children as measured by dual-energy X-ray absorptiometry (DXA) has been associated with abnormalities in plasma lipid and lipoprotein concentrations, blood pressure and left ventricular mass. (S. Daniels and Kelly 2014) In another study, the presence of intra-abdominal fat excess as measured by DXA and computed tomography (CT) was also associated with high fasting insulin and high triglycerides. (Gower, Nagy, and Goran 1999) Clinical measures of abdominal obesity including waist circumference, waist to height ratio and waist to hip ratio have been shown to be valid measures of visceral adipose tissue in children (Karlsson et al. 2013). A systematic review examining these measures of central fat demonstrated a clear association between abdominal fat in children and cardio-metabolic
risk factors, in particular blood pressure.(Kelishadi et al. 2015) Together, these studies demonstrate the association between visceral adiposity and risk of metabolic syndrome.

2.2 Measurement of Adiposity in Children

2.2.1 Longitudinal Monitoring of Growth and Adiposity

Monitoring of longitudinal linear height and weight gain is a foundational component of pediatric primary care which allows for identification of concerning growth trajectories prior the onset of serious health consequences.(Society and Committee 2016) There are significant changes in growth and body composition in children over time which are impacted by a number of factors including age, gender, pubertal status and ethnicity. Furthermore, certain growth patterns such as early rapid infant weight gain are clearly linked with obesity in adulthood.(Brisbois, Farmer, and Mccargar 2012) Early adiposity rebound, defined as the age at which the individual’s BMI begins to increase following the nadir from early childhood, has also been clearly associated with later obesity.(Rolland-Cachera et al. 1984) While obesity prevention is ideal, the development of reliable anthropometric measures to assist in the early diagnosis of childhood obesity creates an opportunity for interventions that can potentially shift a child’s growth trajectory and prevent future cardio-metabolic disease.

2.2.2 Measurement of Body Composition in Children

Measurement of body composition and adiposity in children can be accomplished using a variety of methods. Measurements such as weight for length and BMI z score are some of the most commonly used clinical techniques. Dual-energy X-ray absorptiometry, skin fold thickness measurements, air-displacement plethysmography (ADP)(Urlando, Dempster, and Aitkens 2003) and bioelectrical impedance analysis are studies which are often used in a research setting to estimate adiposity. While
providing a more comprehensive assessment of body composition, these measures can be technically challenging, expensive and too time consuming to implement in routine use. Clinical measurements of adiposity should be simple, non-invasive, fast and inexpensive while also offering diagnostic accuracy and precision. Ideally, the measured parameter should also be associated with later risk of comorbidities and metabolic dysfunction.

2.2.3 Currently Recommended Anthropometric Measures in Early Childhood

Currently, weight for length is the growth standard used to evaluate weight status in children from birth to 2 years of age. (Barlow 2007; Society and Committee 2016; “WHO Child Growth Standards Based on Length/Height, Weight and Age.” 2006) However, there is increasing evidence to potentially support the utility of BMI in this age group. (Sani M Roy et al. 2016; Furlong et al. 2016) For children greater than 2 years old, BMI is the recommended measurement to assess for obesity and nutritional status. (S. R. Daniels, Hassink, and COMMITTEE ON NUTRITION 2015; Styne et al. 2017) BMI is a measure of weight adjusted for height and is determined by dividing a patient’s weight in kilogram by the height in metres squared. It is a well-established measure of adiposity in the growing child which is easily calculated from growth measures that are commonly collected in primary care (height and weight). It is an indirect measure but is associated with adiposity as measured by DXA as well as serum markers of cardio-metabolic risk. (Pietrobelli et al. 1998; David S. Freedman et al. 2007). Importantly, BMI has also been linked to future cardiovascular risk factors(David S. Freedman, Ogden, and Kit 2015).

2.2.4 Limitations of Existing Anthropometric Measures

Recent longitudinal studies of children have demonstrated that patterns of change in body fat percentage throughout childhood differ from temporal changes in BMI suggesting that BMI may not be the optimal
measure of adiposity. (Telford, Cunningham, and Abhayaratna 2014) As a diagnostic tool, BMI has been shown to have high specificity, but only moderate sensitivity for predicting excess body fat in children between 4 and 18 years, incorrectly classifying almost a quarter of children with excess body weight. (Javed et al. 2015) BMI has been shown to overestimate adiposity in children with significant muscle mass, and conversely underestimate those children limited fat-free mass. (David S Freedman, Wang, et al. 2009)

The association between BMI and cardio-metabolic risk factors in early childhood is somewhat conflicting. Studies have demonstrated a correlation between BMI and lipid profiles (Williams et al. 2004) as well as fasting insulin (Shea et al. 2003; Anderson et al. 2016) and leptin (Anderson et al. 2016) in children less than 5 years of age. Other studies have failed to find similar associations. (Garemo, Palsdottir, and Strandvik 2006; Uauy et al. 2010; Cowin and Emmett 2000)

This may be explained by a number of challenges inherent to this measurement. BMI reflects the weight of adipose tissue in addition to skeletal, muscle and organ mass. BMI is unable to discriminate between fat and fat-free mass, so it is therefore a measure of excess weight, not excess fat. This can result in significant variability in fat mass for a given BMI. (Maynard et al. 2001; D S Freedman et al. 2005) Additionally, changes in BMI may not represent changes in adiposity but instead changes in lean body mass, and this is particularly marked in patients who are not obese or overweight. (Demerath et al. 2006) Finally, BMI does not capture the distribution of adiposity, and yet central and peripheral fat depots are likely have differential impact on metabolic health. (Patel and Abate 2013) Given these limitations, efforts have been made to define other alternative anthropometric indicators suitable for clinical use.
2.2.5 Waist to Height Ratio in Adults

Waist circumference is a measure of abdominal obesity which is easily derived by measurement of the distance around the abdomen immediately above the superior level of the iliac crest. Since the presence of visceral fat is associated with cardio-metabolic risk, a clinical measure which captures potential alterations in body fat distribution has additional discriminatory value. (Fox et al. 2007) A measurement of greater than 102 cm in men and 88 cm in women is associated with increased cardio-metabolic risk. (NHMRC 2013) Waist circumference may represent an alternative measure to BMI, designed to act as a proxy for the degree of visceral adiposity. (Benn 1971) However, the sole use of waist circumference to identify elevated cardio-metabolic risk has limitations. Optimal screening cut-offs for waist circumference vary significantly amongst different ethnic groups, with many of the original studies being conducted in homogenous and largely European populations. (Katzmarzyk et al. 2011) Furthermore, it does not account for the impact of height and relative body proportion on obesity-related risk.

Waist to height ratio has emerged as an alternative, potentially superior measurement. In adults, the predictive value of waist to height ratio in adults is well established. A waist to height ratio greater than 0.5 is generally accepted as signifying increased risk of cardiovascular disease and metabolic syndrome across different ethnicities. (Margaret Ashwell and Hsieh 2005) This measure has been shown to be highly predictive of percent fat mass and visceral adipose tissue mass in adult men and women as measured by DXA. (Swainson et al. 2017) A systematic review which evaluated twenty-two prospective studies in adults found that waist to height ratio was significantly associated with cardio-metabolic risk factors in 72% of studies, as compared to only 58% of studies for BMI. (Browning, Hsieh, and Ashwell 2010) Similarly, meta-analyses of prospective studies in adults have demonstrated that waist to height ratio is superior to BMI in predicting incident cardiovascular disease, cardiovascular mortality and all-
cause mortality. (Savvas C Savva, Lamnisos, and Kafatos 2013; M. Ashwell, Gunn, and Gibson 2012). These studies provide cogent rationale for uptake of this measure in clinical practice.

2.2.6 Waist to Height Ratio in Pediatrics

Accumulation of visceral adiposity in children appears to pose the same risk to metabolic health as in adults. (Goran and Gower 1999) This observation combined with the recognized utility of waist to height ratio in adults has motivated efforts to evaluate this measure in school-aged children and adolescents.

Studies which have evaluated waist to height ratio in relation to its ability to identify cardio-metabolic risk in children have generally found it to be a useful measure and a potentially superior predictor of obesity comorbidity. In a cross-sectional study of adolescents aged 10-17 years, waist to height ratio was found to be comparable to other indices of central adiposity such as waist circumference and BMI in diagnosing metabolic syndrome. (Hong and Cho 2017) In a study comparing waist to height ratio against other measures of central adiposity, subjects with a waist circumference < 90th percentile but waist to height ratio > 0.5 were found to have a higher prevalence of cardio-metabolic risk factors. The authors concluded that waist to height ratio offers additional predictive potential for cardio-metabolic risk. (Chung et al. 2016) Similarly, a cross-sectional study of 14,493 subjects from the NHANES dataset demonstrated that waist to height ratio may help to further stratify children classified as obese by BMI. Obese adolescents with a waist to height ratio of greater than 0.6 showed additional risk clinical and biochemical risk factors for metabolic syndrome. (Khoury, Manlhiot, and McCrindle 2013)

Studies that have compared waist to height ratio to BMI have reported conflicting results on the relative strength of their association with metabolic risk. Longitudinal data from the Bogulosa Heart Study
demonstrated that both BMI and waist to height ratio had a comparable ability to discern adverse cardiovascular risk in children, however waist to height ratio was more strongly associated with hypertension and adverse lipid profiles in a population of overweight children.(Study et al. 2007; David S Freedman, Dietz, et al. 2009). Similarly, other studies in adolescents have reported that waist to height is a superior anthropometric tool when compared with BMI in predicting cardio-metabolic risk.(S C Savva et al. 2000; Savvas C Savva, Lamnisos, and Kafatos 2013)

However, other studies in children have found that it is comparable, but not superior to BMI. A recent meta-analysis which evaluated the discriminatory power of different anthropometric indices by using the area under the curve found that waist to height ratio was not superior to BMI and waist circumference. (Lo et al. Obesity 2016) This is supported by another meta-analysis comparing BMI and waist to height ratio in children which found that both parameters were equally were correlated with body fat as measured by DXA (Calvo et al. 2016 Nutrients).

The use of waist to height ratio in children is unique in that a single cut-off for determination of cardio-metabolic risk may not possible in early childhood since the ratio gradually falls from 0.69 at birth to 0.48 by age five.(Roswall et al. 2009) Following age five, the ratio continues to decrease over time until it reaches a plateau at 16 years of age.(Mccarthy and Ashwell 2006) This variation reflects the significant changes in height which occur during childhood. Given these longitudinal changes, it may require the use of z scores to adjust measurements for age and gender similar to BMI. A value of 0.5 has been proposed as a potential screening threshold in children and adolescents however this has not been evaluated in young children. (Lo et al. 2016)
2.2.7 Waist to Height Ratio in Early Childhood

The studies evaluating waist to height ratio in a pediatric population are largely limited to children and adolescents, with very few studies exploring the use of waist to height ratio in pre-school age children and early childhood. Given the importance of early detection of cardio-metabolic risk, use of this ratio in early childhood may be important. Much of the existing literature has used a cross-sectional study design to evaluate the ability of waist to height ratio to predict either adiposity or metabolic risk by examining its association with traditional and emerging clinical and biochemical risk markers. There are few studies that have examined this relationship prospectively.

In one study, the measure was evaluated in a cohort of 315 Brazilian pre-school children. A waist to height ratio cut-off of 0.51 in boys and 0.49 in girls was able to identify children with 2 of more risk factors for metabolic syndrome. (Campagnolo, Hoffman, and Vitolo 2011) A second study compared waist to height ratio against other measurements and their ability to predict body composition in 61 children aged 3 to 7 years. Waist to height ratio performed equally as well as BMI and waist circumference but correlated better with serum HDL levels. (Sijtsma et al. 2014) Similarly, another study looked at 232 children at 4 years of age and found that waist to height ratio was correlated with insulin resistance and HOMA-IR and comparable to other anthropometric measures including waist circumference, BMI and triceps and subscapular skin folds sum. (Aristizabal et al. 2015b)

Not all studies support the use of this measure in predicting obesity-related health consequences. A study of 439 healthy children sought to determine the association between waist to height ratio and systolic blood pressure. They concluded that the measure was not predictive of hypertension in 3 year old children. (Whitrow, Moore, and Davies 2011) In younger children, waist to height ratio may not correlate well with other clinical and direct measurements of adiposity. Corvalan et al. found a statistically
significant correlation between waist to height ratio and other indices of body fat in 4 year olds, however it was only weakly associated with biochemical metabolic markers. (Uauy et al. 2010) Taylor et al. reported that waist to height ratio was correlated with trunk fat mass as measured by DXA in 301 children aged 3 to 5, however waist circumference was overall more strongly correlated. (Taylor et al. 2008)

Taken together, these findings suggest that waist to height ratio in preschool children may be associated with early metabolic risk factors however its use in younger children has never been studied. This is balanced with some studies that have not demonstrated superiority of waist to height ratio in young children. The conflicting nature of the findings in these studies may in part be related to the nature of the population that is being studied, with cohorts enriched with more obese subjects being more likely to reveal an association between waist to height ratio and metabolic risk. A greater understanding of how this indicator functions in children from birth to three years of age will be important in determining its utility in clinical practice.

2.3 Early Life Origins of Metabolic Syndrome

2.3.1 Impact of the Fetal Environment on the Risk of Childhood Obesity and Diabetes

Recognizing that the origins of childhood obesity are complex and multifactorial, the developmental origins of disease hypothesis proposes that the earliest foundation for metabolic dysfunction begins during pregnancy, at the time of fetal development. (D. J. P. Barker 2012) The Barker hypothesis theorized that rising rates of obesity and cardiovascular disease in adulthood were linked to suboptimal nutrition during fetal life which had long lasting consequences through the effects of perinatal programing. (D. J. Barker et al. 1993) This paradigm has grown to include in utero environmental exposures such as maternal obesity and hyperglycemia which can result in fetal over-nutrition, in turn, impacting expression of genes critical for energy metabolism including pathways involved in
adipogenesis and insulin signaling. (Nicholas et al. 2015) These changes can be programmed during crucial developmental windows which in turn result in inter-generational transmission of metabolic disease.

2.3.2 Maternal Gestational Diabetes

Gestational diabetes is defined as hyperglycemia and glucose intolerance first diagnosed during pregnancy, regardless of the requirement for insulin or persistence of diabetes postpartum. (Metzger et al. 2010) The prevalence of GDM has been steadily increasing over the last several decades and is thought to be driven by increasing maternal age and obesity. Recent epidemiological studies suggest that GDM affects 6-7% of pregnancies in North America, but 16% of pregnancies globally. (Guariguata et al. 2014) This estimate varies greatly based on the ethnicity of the given population as well as the definitions and screening methods used, with reported prevalence rates as high as 25% in some countries. (Hartling et al. 2012) Universal screening of pregnant women is recommended at 24 to 28 weeks gestation, using either a one step or two step oral glucose tolerance test in order to detect and prevent fetal exposure to gestational hyperglycemia. (Farrar et al. 2017) There has been significant controversy surrounding the optimal testing strategy and diagnostic threshold for GDM (Farrar et al. 2017), recognizing that even subtle degrees of maternal hyperglycemia may impact perinatal outcomes. (HAPO Study Cooperative Research Group 2009; HAPO Study Cooperative Research Group et al. 2008)

2.3.3 Pathophysiology of Hyperglycemia During Fetal Growth and Development

GDM has been clearly identified as an exposure which alters the fetal environment in utero. This phenomenon was first described by the Pedersen hypothesis and arose from observations of an association between hyperglycemia in pregnant women with type 1 diabetes and fetal
outcomes. (PEDERSEN and OSLER 1961) The concept of “fuel-mediated teratogenesis” was later expanded upon by Freinkel to more broadly include excessive exposure to any maternal nutrient. (Freinkel 1980)

The impact of exposure to excessive glucose is dictated by both the extent and timing of the hyperglycemia. Exposure to hyperglycemia during the first trimester, which arises from pre-existing type 1 or 2 diabetes, is associated with increased rates of congenital anomalies include neural tube defects, congenital heart disease and other renal and gastrointestinal malformations. (Farrell, Neale, and Cundy 2002) This is explained by the primitive stages of cell differentiation and active process of organogenesis which occurs during early pregnancy. Excessive glucose delivery to the embryos is thought to result in oxidative stress and hypoxia. (Z. Zhao and Reece 2005) This, in turn, alters expression of genes required for normal anatomical development.

Distinct from pre-gravid type 1 or type 2 diabetes, GDM involves exposure to hyperglycemia predominantly during the third trimester upon completion of organogenesis. Beginning in mid-pregnancy, there is a progressive loss of insulin sensitivity which is mediated predominantly by placental hormone production and increasing levels of cortisol, progesterone and prolactin. (RYAN and ENNS 1988). Failure of the pancreatic beta cell mass to compensate with increased insulin secretion or inability to overcome the impairment in insulin action results in increased maternal circulating concentrations of glucose, lipids and amino acids. Glucose is transported across the placenta in a concentration dependent manner such that maternal hyperglycemia is proportionally reflected in the fetal circulation. (Gower, Nagy, and Goran 1999) Insulin production by the fetal
pancreas begins as early as 7 weeks gestation (Pipe r et al. 2004) and secretion in response to circulating glucose occurs around 12 weeks gestation.(Reiher et al. 1983) Excessive glucose transferred across the placenta drives fetal hyperinsulinemia which promotes increased fetal growth, particularly in insulin sensitive tissues such as fat. In women with comorbid obesity, elevated glucose levels are typically accompanied by other metabolic disturbances such as more significant insulin resistance (Ramsay et al. 2002) and dyslipidemia.(Catalano and Hauguel-De Mouzon 2011) Excessive gestational weight has been shown to impact adiposity outcomes in the perinatal period and later childhood. Obese pregnant women demonstrate higher triglycerides and low HDL levels when compared with lean women and women with normal glucose tolerance.(Di Cianni et al. 2005) As a result, increased availability of lipids results in greater placental metabolism of triglycerides and transport of free fatty acids into the fetal circulation. This environment of excessive energy substrate further drives fetal adipose accretion and growth. Accelerated growth of adipose tissue during this critical developmental window may program future obesogenic patterns of fat deposition(Catalano and Hauguel-De Mouzon 2011).

This pathologic intrauterine environment results in numerous complications for the neonate, although with no clear identifiable threshold for the association between hyperglycemia and adverse perinatal outcomes.(Farrar et al. 2016) In the perinatal period, offspring are at-risk of preterm delivery, need for caesarian section, shoulder dystocia, hypoglycemia and most relevantly, fetal macrosomia. The presence of maternal hyperglycemia during pregnancy has been shown to be an important determinant of birth weight in offspring.(Metzger et al. 2008; Catalano et al. 2012) A systematic review and meta-analysis of over 24,000 GDM offspring that this relationship between intrapartum hyperglycemia and increased neonatal fat mass occurs independently of
maternal obesity and BMI. (Logan et al. 2017) Similarly, adiposity in offspring unexposed to GDM is associated with intrapartum maternal glucose levels and hyperinsulinemia in the fetus, as measured by umbilical cord serum C-peptide levels. (HAPO Study Cooperative Research Group 2009) Even among children with normal birth weights, exposure to intrauterine hyperglycemia impacts fetal adipogenesis with offspring demonstrating increased adiposity despite absence of macrosomia. (Catalano et al. 2003)

Beyond these immediate changes to patterns of fetal growth, exposure to hyperglycemia is thought to program longer-term changes in fetal metabolism. (See Figure 2) Studies of GDM offspring suggest that gestational exposure appears to deleteriously affect both measures of insulin resistance at target tissues as well as insulin secretion from the pancreatic beta cell. (Bush et al. 2011) However, the biological mechanisms implicated in the longer term translation of these changes are not well understood but changes to offspring epigenetics and adipoinsular axis have been proposed as potential targets of this programming. (Ruchat et al. 2013; Singh, Karagas, and Mueller 2017)

2.4 Mechanisms of Fetal Programming

2.4.1 Epigenetic Changes as a Mechanism for Fetal Programming

The epigenome is thought to be sensitive to environmental exposures during key windows of development. While many genes require expression of both maternal and paternal loci, certain genes require expression of one parental allele, preferentially activated through the process of imprinting. Imprinting establishes specific patterns of gene expression based on their parental origin and is mediated by diverse epigenetic changes which occur at ‘imprinting control regions’ throughout the genome (Cassidy and Charalambous 2018). Epigenetic changes are defined as modifications to patterns of gene expression that do not alter the specific nucleotide sequence. (Weksberg et al. 2006) New epigenetic patterns are
established in the offspring during pregnancy and can include methylation of gene regulatory sites, changes to chromatin structure as well as micro-RNA mediated post-translational changes. Support for this theory derives from observational studies which have demonstrated differences in the methylation patterns of offspring exposed to different metabolic environments during pregnancy. (Finer et al. 2015)

Some of the earliest work evaluating this epigenetic mechanism focused on changes in methylation associated with reduced fetal nutrition in the context of intra-uterine growth restriction and famine (Tobi et al. 2014), however this has expanded to include exposure to fetal over-nutrition. Hyperglycemia during pregnancy has been associated with altered offspring methylation profiles in placental and umbilical cord blood affecting genes with putative roles in energy metabolism including lipoprotein lipase (A. A. Houde et al. 2014), leptin (Bouchard et al. 2010), adiponectin (Bouchard et al. 2012), ABCA1 (A.-A. Houde et al. 2013) and MEST. (El Hajj et al. 2013; Finer et al. 2015) Supportive evidence for the independent impact of the intra-uterine period derives from studies comparing methylation patterns in offspring born before and after maternal weight loss from bariatric surgery. Differential expression of glucoregulatory and immune genes has been demonstrated between siblings implicating the exposure of maternal obesity on patterns of gene expression. (Berglind et al. 2016; Guénard, Deshaies, et al. 2013; Guénard, Tchernof, et al. 2013) To further strengthen this hypothesis, methylation changes in the antisense non-coding RNA in the INK4 locus (ANRIL) and retinoid X receptor alpha (RXRA) in umbilical cord blood have been associated with markers of cardiovascular disease and adiposity at 9 years of age (Murray et al. 2016; Godfrey et al. 2011). Genome-wide association studies have previously identified INK4 locus as an area involved in determination of cardiovascular disease risk which is also supported by functional studies which demonstrate its involvement in vascular smooth muscle function. (Congrains et al. 2012) Similarly, the putative role of RXRA in brown fat metabolism provides biological plausibility for its involvement in cardio-metabolic disease risk. (Alvarez et al. 2000) Taken together, these suggest that the epigenome may play a part in conferring future obesogenic vulnerability which is programmed during pregnancy.
2.4.2 Adipo-insular Axis Changes as a Mechanism for Fetal Programming

The maternal-fetal adipo-insular axis is a hormonal regulatory system which is influenced by bioactive proteins called adipokines. (Fasshauer, Blüher, and Stumvoll 2014) These include proteins such as leptin and adiponectin, which are synthesized in adipose tissue and impact key metabolic processes in the body such as appetite and insulin sensitivity. Leptin is produced by adipocytes in proportion to fat cell mass, and drives energy homeostasis and body weight by signaling to hypothalamic control centres in the arcuate nucleus and insulin producing pancreatic beta cells. (Paz-Filho, Mastronardi, and Licinio 2015) Conversely, adiponectin is an insulin sensitizing hormone which also promotes pancreatic insulin secretion and has generally been found to be reduced in states of poor metabolic health. (Bacha et al. 2004) Gestational diabetes is characterized by alterations in the circulating levels of these adipokines and dysregulation of this system. (R Retnakaran et al. 2005) Excessive energy substrates in the fetal circulation during pregnancy are hypothesized to cause disequilibrium of this regulatory axis, programming changes to the fetal pancreas and other areas of the body regulating metabolism such as the hypothalamus. (Andreas Plagemann et al. 1999)

Changes in appetite and energy homeostasis may be mediated by altered levels of orexigenic and anorexigenic hormones in offspring exposed to hyperglycemia. This is supported by the fact that the cord blood of GDM offspring demonstrates increased leptin and reduced adiponectin levels. (Ortega-Senovilla et al. 2011) In a randomized control trial evaluating treatment of hyperglycemia in pregnancy, attenuation of glucose abnormalities was associated with reversal of these alterations in the adipo-insular axis. (Pirc et al. 2007) Other key proteins involved in beta cell proliferation such as betatrophin have been shown to be present in altered concentrations in GDM offspring cord serum. (Xie et al. 2016) The longer-term significance of variations in neonatal cytokine profiles is not well understood. Hypothalamic resistance to
leptin has been demonstrated in rat offspring exposed to GDM, and has also been linked to changes to neural appetitive pathways and eating behaviours. (Steculorum and Bouret 2011) In humans, exposure to GDM has been associated with altered eating phenotypes in adolescent offspring, implicating neural pathways involved in appetite and energy. (Shapiro et al. 2017) However, these changes have yet to be casually linked to longer term metabolic changes in child and adulthood. (Mantzoros et al. 2009)

2.4.3 Insights about Mechanisms of Fetal Programming Gained from Animal Models

Studies in animal models have been helpful in understanding the pathophysiology and longer term impact of maternal hyperglycemia, above and beyond the risk incurred through genetic vulnerability. Induction of glucose abnormalities in animal studies using streptozotocin and parenteral glucose infusions has clearly demonstrated that exposure to hyperglycemia during fetal
development is associated with pancreatic islet cell hyperplasia (Bihoreau et al. 1986). This appears to be followed by gradual depletion of insulin secretion by the pancreatic beta cell in the rat offspring, later in life (Aerts et al. 1988). Studies in rat models have looked at the impact of rearing genetically identical oocytes in euglycemic and hyperglycemic mothers. Offspring exposed to a hyperglycemic environment in utero demonstrate higher rates of glucose abnormalities later in life (Gill-Randall et al. 2004). Understanding gleaned from animal models is consistent with epidemiologic observations from human studies, lending support to the concept of metabolic programming.

2.4.4 Understanding the Relative Contribution of Genetics and Environment

As is the case in most disease processes, both environment and genetics can impact beta cell function and insulin sensitivity. The shared genetic risk between the mother and her offspring make it difficult to independently implicate the role of in utero hyperglycemia. Studies in pregnant women with pre-gravid type 1 diabetes have been helpful in understanding the relative contribution of shared genetics versus environmental exposures. In one study, offspring of mothers with type 1 diabetes underwent evaluation of beta cell function in early adulthood which revealed higher rates of impaired glucose tolerance despite negative islet cell antibody status. Adults with normal glucose tolerance still demonstrated subtle abnormalities in insulin secretion (Sobngwi et al. 2003). Other study designs have compared beta cell function in offspring of mothers with type 2 diabetes versus fathers with type 2 diabetes to control for genetic risk (Krishnaveni et al. 2005). These have shown early metabolic abnormalities among infants of mothers at 5 years of age, which are not present among offspring of fathers with diabetes. Earlier studies which followed offspring of nuclear families from the Pima Indian population also support the independent role of
environment. The risk of dysglycemia was 3.7 times greater in siblings born after the diagnosis of gestational diabetes, clearly implicating the role of exposure to hyperglycemia.(Dabelea et al. 2000)

2.5 Impact of Gestational Diabetes on Offspring Outcomes

2.5.1 Early Epidemiologic Evidence for Metabolic Programming in the Pima Indian Population

Gestational diabetes has well characterized consequences for the offspring in the immediate perinatal period, however epidemiologic evidence suggests that the impact of gestational diabetes (GDM) is not limited to the perinatal period, causing increased future risk of obesity and cardiovascular disease in offspring.(Marco et al. 2012; Debbie A Lawlor, Lichtenstein, and Långström 2011; Burguet 2010; D J Pettitt et al. 1983; D J Pettitt et al. 1988; Kubo et al. 2014)

Some of the earliest evidence for this association is derived from observational studies of high-risk groups such as the Pima Indian community in Arizona. This population is unique in that they have a high prevalence of type 2 diabetes owing to an underlying genetic predisposition which has not yet been entirely elucidated.(Knowler et al. 1978) Offspring of women born with GDM in this population were followed prospectively and compared with children born to women with normal glucose tolerance and pre-gravid type 2 diabetes. The results of these studies demonstrate a graded effect of diabetes, pre-diabetes and normoglycemia during pregnancy on the risk of obesity in the offspring in later childhood. They observed that 58% of children with a maternal history of GDM were classified as obese as compared to only 17% of children with no exposure to maternal GDM.(D J Pettitt et al. 1983) Differences in patterns of growth and weight gain in this high-risk group of children are also evident early in life, with changes in weight trajectories compared to the general population apparent in the first 6 months of life.(Lindsay et al. 2002; Touger et al. 2005)
Children from this population of Pima Indians exposed to GDM also demonstrate increased risk of glucose abnormalities and higher mean 2 hr glucose values on oral glucose tolerance testing at age 5 to 19 years. (D J Pettitt et al. 1991; Petitt et al. 1985) In one study, by age 34, 70% of people exposed to gestational diabetes had developed type 2 diabetes suggesting that the risk increased with age. (Petitt et al. 1985) Importantly, the relationship between maternal glycemic control during pregnancy and rates of obesity in early childhood occurred independently of maternal weight status and infant birth weight. (D J Pettitt and Knowler 1998; D J Pettitt et al. 1987)

Collectively, observations from this population raise concern that exposure to abnormal glucose tolerance during pregnancy may be implicated in the inter-generational transmission of cardio-metabolic risk, above and beyond the risk incurred through shared genetic vulnerability. They also highlighted that subtle differences in the metabolic risk profile of children exposed to hyperglycemia are appreciable in early childhood. However, these findings must be carefully contextualized as this cohort of women demonstrated higher rates of poor glycemic control and significant genetic risk which may limit the generalizability of these results to other populations.

2.5.2 Impact of GDM on Pancreatic Beta Cell Function and Glucose Metabolism in Adolescence and Adulthood

Subsequent to the earlier studies in the Pima Indian population, there have been several other prospective, observational cohorts which have sought to evaluate measures of insulin sensitivity and beta cell function in offspring of mothers with GDM in more heterogeneous populations (Egeland and Meltzer 2010; Kelstrup et al. 2013; Vääräsmäki et al. 2009; Silverman
et al. 1995; Catalano et al. 2009a; Malcolm et al. 2006; A. Plagemann et al. 1997; Holder et al. 2014; Sauder et al. 2017; A. N. Jeffery et al. 2006; Davis et al. 2013). Observational studies of post-pubertal adolescence with a history of exposure to GDM have generally demonstrated abnormalities in glucose regulation and beta cell function compared to unexposed peers.(Egeland and Meltzer 2010; Kelstrup et al. 2013; Vääräsmäki et al. 2009; Silverman et al. 1995) An early prospective American cohort followed offspring of mothers with diabetes during their pregnancy and observed that 19.3% of children had impaired glucose tolerance by the time they reached adolescence.(Silverman et al. 1995) Impaired glucose tolerance (IGT) was also independently associated with greater rates of obesity and a history of higher ammonitic fluid insulin levels in utero.(Silverman et al. 1998) These findings are however limited by the fact that their analysis combined mothers with both pre-gestational (type 1 and type 2) and gestational diabetes, which have distinctly different patterns of exposure to hyperglycemia during pregnancy. A later, but smaller study which evaluated 90 offspring of Caucasian women with GDM at 15 years post-partum, demonstrated higher fasting insulin levels and HOMA-IR when compared with control offspring but no difference in rates of overt glucose abnormalities.(Egeland and Meltzer 2010) Similar abnormalities in both measures of insulin secretion and insulin sensitivity were demonstrated in a cohort of 167 Danish women with GDM exposure followed to early adulthood and a Finish cohort of 95 GDM offspring followed to 16 years of age.(Kelstrup et al. 2013; Vääräsmäki et al. 2009). On oral glucose tolerance testing, adolescent GDM offspring demonstrated reduced insulin sensitivity and a lower disposition index (p<0.005). Studies in younger, pre-pubertal offspring of North American adolescents exposed to GDM, also revealed elevations in HOMA-IR and fasting insulin.(Catalano et al. 2009b) However, unlike the earlier studies in the Pima Indian population, none of these subsequent cohorts replicated the significant
increase in prevalence in overt type 2 diabetes among GDM offspring compared to unexposed youth.

The suggestion that the impact of hyperglycemia may only be apparent in high-risk populations and predisposed ethnic groups has been disputed by studies which have replicated this association even amongst low risk groups. A follow-up study of GDM offspring from a cohort of 89 Canadian, largely Caucasian children underwent OGTT at school age and demonstrated overall rates of dysglycemia of 6.9%. (Malcolm et al. 2006) Prevalence rates in other low risk populations have been reported, ranging from 1.1% to as high as 20%, in a small study of 15 Caucasian children with a mean age of 6.4 years, (A. Plagemann et al. 1997; Egeland and Meltzer 2010). Importantly, many of these estimates are derived from observational studies with smaller populations which do not provide a control cohort and often combine together mothers with pre-gestational and gestational diabetes. These estimates may also vary considerably depending on the age of the cohort age and prevalence of obesity.

The risk of metabolic abnormalities is clearly accentuated by the presence of obesity. A cohort of 255 obese adolescents was evaluated for exposure to GDM and at study onset, those in the GDM group were found to have greater impairment in beta cell function when compared with unexposed youth. Follow-up oral glucose tolerance testing (OGTT) 2 years later demonstrated higher rates of impaired glucose tolerance (IGT) in the exposed group, with GDM exposure conferring a 5 times greater risk of dysglycemia. (Holder et al. 2014) This relationship has been shown to persist, after even adjustment for both maternal BMI and the presence of offspring obesity, suggesting that
GDM exposure increases the risk of impaired glucose tolerance, independently of weight status. (Sauder et al. 2017)

Very few studies have evaluated insulin sensitivity and beta cell function while controlling for post-natal environmental factors to clarify if the relationships are a function of shared obesogenic lifestyle factors after birth. Alterations in measures of insulin secretion on OGTT testing have been demonstrated in a cohort of Latino GDM offspring independent of breastfeeding status. (Davis et al. 2013) The impact of other post-natal exposures such as physical activity and nutrition have been less well studied. There is also some suggestion that the long-term programming effect of hyperglycemia may not be appreciable in the presence of more mild abnormalities in glucose regulation. This is supported by the results of a small study which followed offspring of women with abnormal random glucose values during pregnancy in which there was no discernable association between maternal glycemia at 28 weeks gestation and measures of insulin sensitivity or adiposity in UK school age children. (A. N. Jeffery et al. 2006)

2.5.3 The Effect of Gestational Diabetes on the Risk of Obesity in Early Childhood

The effects of hyperglycemia on fetal development and perinatal outcomes are well described with a majority of studies demonstrating appearance of metabolic risk in early adolescence and early adulthood. However, the evolution of metabolic risk in younger children exposed to intrauterine hyperglycemia remains not well understood. There are only a few studies which have evaluated the impact of gestational diabetes during the first few years of life (Hillier et al. 2007; Vohr, McGarvey, and Tucker 1999; A. L. Deierlein et al. 2011; Tam et al. 2017; David J Pettitt et al. 2010a; Knight et al. 2007; Whitaker et al. 1997; Gillman et al. 2010).
Several studies have demonstrated altered patterns of growth and adiposity in infants and young children exposed to GDM. A large, multiethnic cohort of 10,000 offspring of GDM mothers from the U.S. was followed prospectively and evaluated at age 5 to 7 years. (Hillier et al. 2007) A clear association between rates of obesity and maternal glucose levels on oral glucose tolerance testing was observed despite the absence of macrosomia at birth and remained present after adjustment for relevant confounding variables including maternal and offspring BMI. GDM exposure was associated with 1.82 times greater risk of obesity (defined as weight > 95th percentile). Treatment of mothers with overt gestational diabetes resulted in an attenuation of this relationship, as compared with untreated mothers however it remained present. (Hillier et al. 2007)

Similar differences in rates of early obesity have been observed as early as 12 months of age. A cohort study which followed offspring exposed to GDM demonstrated that initially macrosomic infants of GDM pregnancies are at the highest risk of obesity at 1 year of age when compared to normal birth weight GDM offspring. This study highlights that sub-optimal treatment of underlying hyperglycemia accentuates adiposity risk. (Vohr and McGarvey 1997) Factors contributing to the GDM exposed offspring’s BMI may also be unique. Maternal obesity and infant BMI were found to predict BMI in GDM exposed offspring at 7 years of age whereas maternal obesity and intrapartum weight gain were predictive of non-GDM children’s weight. (Vohr, McGarvey, and Tucker 1999) This observation has not been consistently replicated in women with mild glucose intolerance not meeting criteria for GDM. Some studies have observed a correlation between maternal glucose values during pregnancy and anthropometric outcomes (Andrea L
Deierlein et al. 2011; W. Li et al. 2017), while others have not.(David J Pettitt et al. 2010a; Knight et al. 2007; Whitaker et al. 1997; Gillman et al. 2010)

There have been efforts to systematically review cohort and case-control studies investigating the impact of intrauterine hyperglycemia on childhood adiposity. One review and meta-analysis included 9 studies that examined the association between exposure to hyperglycemia on offspring BMI z score. This included a heterogeneous group of studies which grouped together maternal type 1 diabetes, pre-gestational and gestational diabetes. This review found a relationship between maternal diabetes status during pregnancy and BMI z score, however this was no longer significant upon adjustment for maternal BMI. (Philipps et al. 2011) A second systematic review analyzed 12 case-control and cohort studies, which only included women with gestational diabetes. Similarly, they found that the relationship between maternal glycemic status during pregnancy was not related to childhood BMI measures after adjustment for maternal obesity. (Kim et al. 2011) The results of these more rigorous analyses therefore suggest that in GDM exposed offspring, there are no discernable changes in weight in the first few years of life which are independently linked to maternal hyperglycemia.

In addition to absolute measures of adiposity, specific changes to patterns of body composition have been observed in offspring of GDM mothers, with children demonstrating higher overall levels of lean and fat mass, greater sum of skin fold thickness and central adiposity. (Chandler-Laney et al. 2011; Wright et al. 2009; Crume et al. 2011; Nehring et al. 2013) Participants in a cohort study of GDM offspring at 6 years of age were observed to have higher overall fat mass as well as measures of abdominal obesity despite normal BMI z scores, suggesting more pathologic
patterns of fat deposition. Similarly, greater truncal fat mass adjusted to leg fat mass was seen in offspring of GDM in early childhood despite normal BMI lending further support to the belief that absolute measures of adiposity may be inadequate at detecting more subtle metabolic abnormalities in GDM offspring present in early childhood. (Chandler-Laney et al. 2012). After adjustment for confounding factors such as maternal obesity, exposure to GDM was associated with a greater than 1.5 times risk of abdominal obesity at age 5 and 9 years.(Nehring et al. 2013; P. Zhao et al. 2016) This suggests that although there are no appreciable differences in BMI among GDM exposed offspring, after adjustment for confounders, other surrogate measures of body composition may be more sensitive at detecting differences.

2.5.4 The Effect of Gestational Diabetes on Offspring Beta Cell Function and Glucose Metabolism in Early Childhood

While the independent impact of GDM on early changes to childhood anthropometrics remains uncertain, other metabolic outcomes such as beta cell dysfunction and insulin resistance have also been studied. A small European cohort of offspring of mothers with GDM underwent oral glucose tolerance testing at 4-9 years of age and showed reduced insulin sensitivity when compared with children exposed to mothers with normal glucose tolerance and with pre-gestational diabetes, however, potential confounders such as infant and maternal weight status were not accounted for.(Wroblewska-Seniuk, Wender-Ozegowska, and Szczapa 2009) Similarly, a cohort of children from Mysore, India observed GDM offspring had higher fasting insulin levels compared to control children but only female offspring of GDM pregnancies had an increased risk of abnormal glucose tolerance at 5 years of age.(Krishnaveni et al. 2005) No significant differences were found amongst offspring of paternal diabetes mellitus, highlighting the causative role of environmental factors.
This early association strengthened over time, with greater insulin resistance appreciable in offspring of GDM pregnancies at 9 years of age.(Krishnaveni et al. 2010)

Our research group has previously published several studies focusing on adiposity and insulin resistance during early childhood among GDM offspring. We demonstrated that increasing maternal insulin resistance during pregnancy predicts greater offspring weight gain and adiposity at 12 months of age, as measured by sum of skin fold thickness. These relationships occurred independently of GDM status.(Hamilton et al. 2010) In the same cohort, environmental exposures such as maternal and offspring lifestyle habits have also been identified as important factors. Weight gain and adiposity at 1 year of age were also related to maternal physical activity levels and offspring feeding patterns. (Chu et al. 2012) Interestingly, there were no overt differences in measures of insulin resistance at 1 year of age among GDM exposed children, however the determinants of HOMA-IR were distinct in the offspring of mothers with GDM. HOMA-IR was associated with weight gain from birth to 12 months in infants exposed to GDM but in infants exposed to normal glucose levels, HOMA-IR was instead associated with infant birth weight.(Borgono et al. 2012) A similar pattern emerged for other markers of cardiovascular risk with no absolute difference in lipid measures, adipokines and inflammation markers among GDM offspring. However, only unexposed offspring demonstrated a relationship between maternal and infant adiponectin levels, suggesting that GDM exposure may be associated with an alteration in the determinants of cardiovascular risk(R Retnakaran et al. 2013). Taken together, these studies collectively demonstrate that there are distinctive perturbations in metabolic homeostasis which are present in infancy and may evolve into more overt abnormalities over time.
2.5.5 Insights from the Hyperglycemia and Adverse Pregnancy Outcomes Study

Important insights about the impact of untreated maternal hyperglycemia during pregnancy have been gained through a unique study which sought to clarify the controversy regarding optimal threshold for diagnosis of GDM. Historically, the criteria for the diagnosis of GDM were controversial with no clear consensus on the optimal testing modality and diagnostic threshold. The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study was a prospective multinational, multi-centre blinded observational study which followed over 25,000 mothers from 9 countries and 15 centres with glucose intolerance during pregnancy. (“The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study.” 2002) The primary objective was to define the threshold above which maternal glycemia is associated with increased risk of adverse outcomes in the offspring using a 75 g OGTT. They specifically sought to evaluate the impact of glucose abnormalities below the cut-off for diagnosis of type 2 diabetes and therefore excluded all women with a fasting blood glucose above 5.8 mmol/L and 2 hour blood glucose greater than 11.1 mmol/L from the study who met existing criteria for GDM. The remaining participants and treating physicians were blinded to the results of the OGTT. The primary analysis demonstrated that maternal glycemia has a continuous linear association with maternal and perinatal adverse outcomes below the previously accepted cut-off denoting gestational diabetes (HAPO Study Cooperative Research Group et al. 2008). This result was independent of the risk conferred by maternal obesity (Catalano et al. 2012). Upon completion of the primary study, ancillary studies, including several offspring cohorts, were followed into early childhood seeking to understand the long-term impact of gestational hyperglycemia. These offspring cohorts are unique in that many women who would otherwise meet criteria for GDM by current guidelines, were blinded to the
outcome, allowing us to understand the impact of hyperglycemia on offspring in untreated mothers.

The Belfast HAPO cohort which consisted of 1165 offspring children demonstrated no relationship between maternal glucose levels at 28 weeks gestation and BMI z score at 2 years of age. (David J Pettitt et al. 2010a) The same population was re-evaluated at 5-7 years age, assessing height, weight and skin fold thickness measurements. Again, no association between intra-uterine hyperglycemia and childhood anthropometrics was found after adjustment for maternal BMI. (Thaware et al. 2015) Conversely, a follow-up HAPO cohort from Hong Kong studied 970 children longitudinally and found higher blood pressures and lipid levels in offspring of GDM pregnancies at 8 years of age, with an association between umbilical cord insulin levels and glucose intolerance. (Tam et al. 2008) The same cohort demonstrated higher rates of glucose intolerance and cardio-metabolic risk factors in children exposed to GDM, which persisted after accounting for potential confounders like maternal BMI, childhood obesity or a history of being large for gestational age (Tam et al. 2017). In adolescents, these differences persisted independently of confounders, with fetal hyperinsulinemia associated with higher rates of obesity and metabolic syndrome at 15 years of age (Tam et al. 2010). Discordant results between the Hong Kong and Belfast sites may indicate that other factors (e.g. genetic background) interact to confer risk. Overall, the Hong Kong cohort was older at the time of assessment, which could also account for differences in outcomes measured. In 2017, a 10 site, 7 country, HAPO follow-up study was completed, with assessment of over 3000 children age 8-12 years. Results should be available shortly, and will help to definitely address the association of GDM with offspring adiposity and metabolic status. (HAPO 2017)
2.5.6 Challenges in the Literature

The results of these aforementioned studies have been conflicting, with not all demonstrating a compelling association between gestational glycemia and childhood metabolic risk. This may be in part due to several key factors inherent to study design. This includes heterogeneity in the diagnostic criteria used to define gestational diabetes which may augment or attenuate the relationship depending the severity of the hyperglycemia and subjects included. Variations in the racial composition of the cohort may also impact the outcome owing to the unique genetic susceptibilities of different racial groups. Depending on the duration of cohort follow-up, studies may be more or less effective at identifying overt metabolic differences between comparison groups. Variability in adjustment for important confounders such as maternal obesity status and early lifestyle factors can also greatly influence the association. Untangling the relative contribution of obesity and hyperglycemia is difficult, as the two conditions often co-occur. Finally, successful treatment of GDM during pregnancy with diet and insulin may further obscure the relationship between hyperglycemia and offspring outcomes by modifying the effect of maternal insulin resistance.
3. RESEARCH OBJECTIVES AND HYPOTHESES

3.1 Study Rationale

The existing literature indicates unresolved complexity in factors which determine early childhood metabolic health. This highlights the need for systematic analysis of *in utero* factors which determine glucose metabolism in childhood, accounting for potential confounding factors (see Figure 3). A growing body of evidence suggests that the intra-uterine environment contributes to offspring metabolic risk independently of shared genetic and environment drivers. Its effects are likely cumulative with underlying heritable and lifestyle risks. Gestational hyperglycemia and maternal obesity are exposures which often co-occur during pregnancy and share a similar outcome of fetal over-nutrition. Both appear to have independent effects on offspring metabolic health, however, their relative contributions remain unclear. Understanding their respective roles will help care providers prioritize the most important and influential components of maternal metabolic health during pregnancy.

![Figure 3 – Proposed Exposure Model](image)

*Figure 3. Proposed exposure model highlighting the confounding variables and the outcome of interest.*
Change in the patterns of insulin resistance and adiposity have been well characterized in GDM offspring in the perinatal period and later in adolescence and adulthood when overt glucose abnormalities are recognized. However, little is understood about the determinants and evolution of insulin resistance in early childhood, defined as the period between birth and five years of age. Importantly, enriching our understanding of metabolic trajectory in GDM offspring during this period of plasticity will help inform targeted public health interventions. Emerging measures of visceral adiposity such as waist to height ratio may be well suited to identify at-risk youth and allow for implementation of beneficial preventative interventions. This will ensure our health care providers can capture and help those at greatest risk.

Objectives

1. Our primary objective is to determine the association between in utero exposure to GDM on the outcome of insulin resistance (HOMA-IR) in the offspring from one to three years of age.

2. Our secondary objective is to identify the association between anthropometric measures of adiposity and cardio-metabolic health in children from one to five years of age, including (i) waist to height ratio, (ii) BMI z score, (iii) weight for length z score and (iv) sum of skin fold thickness. We defined cardio-metabolic health using biochemical outcomes measures (lipid profile, insulin resistance and adipokines) and direct measures of adiposity (body composition studies through dual-energy x-ray absorptiometry).

Hypothesis

1. We hypothesized that offspring of mothers exposed to GDM during pregnancy will continue to have unique predictors of metabolic risk. Specifically, among GDM offspring, HOMA-IR from one to three years will be associated with maternal metabolic status during pregnancy. Whereas,
HOMA-IR from one to three years among GDM non-exposed offspring will be not be associated with maternal metabolic status.

2. We hypothesized that waist to height ratio will be highly associated with cardio-metabolic risk including serum biochemical measures and direct measures of adiposity. Waist to height ratio will be more strongly correlated with these outcomes when compared with existing measures of BMI z score, weight for length z score and sum of skin fold thickness.
4. MATERIALS AND METHODS

4.1 Study Design

The current thesis is based on a previously established prospective, longitudinal cohort study, conducted in Toronto, Ontario, Canada. The original study is entitled “Insulin resistance and beta cell dysfunction in early childhood: The role of maternal and infant metabolic risk factors.” This thesis utilizes data collected during the course of the original study.

4.2 Study Population

The study cohort consists of mother-infant dyads with and without a history of maternal gestational diabetes (See Figure 4). The study population is followed during pregnancy and the postpartum period to evaluate the relationship between maternal markers of glucose metabolism and adiposity with longitudinal metabolic changes in the offspring, including growth, adiposity, insulin resistance, beta cell function and adipokine levels.

4.3 Inclusion Criteria

Subjects were included if they presented for a glucose challenge test during their pregnancy to screen for gestational diabetes mellitus and had no pre-existing diagnoses of diabetes (type 1 or type 2 diabetes).

4.4 Exclusion Criteria

Mothers were excluded if they had had a previous diagnosis of type 1 or type 2 diabetes. Offspring were excluded if they were the product of a twin pregnancy, if they were delivered prematurely (defined as less
than 32 weeks gestation), if they were low birth weight (defined as a birth weight less than 2500 g), or had a genetic syndrome interfering with growth.

4.5 Study Recruitment

Study participants were recruited at the time of their antepartum screening for gestational diabetes at the Mount Sinai Hospital outpatient laboratory. All subjects who presented for screening were approached. According to routine practice, pregnant women are universally screened during the second trimester with 50 g glucose challenge test (GCT). Any abnormalities identified during this initial screen (defined as a 1 hour plasma glucose of ≥ 7.8 mmol/L) are subsequently referred for a formal oral glucose tolerance test (3 hour 100 g OGTT). All participants enrolled in the study were recruited before or after the GCT, and underwent a diagnostic glucose tolerance test for determination of GDM status, irrespective of the GCT outcome. GDM status was defined according to the National Diabetes Data Group criteria. To meet diagnostic criteria, two of the four plasma glucose levels had be identified as abnormal, defined as a fasting glucose > 5.8 mmol/L, 1 hour glucose >10.5 mmol/L, 2 hour glucose of > 9.1 mmol/L or 3 hour glucose > 7.8 mmol/L (“Classification and Diagnosis of Diabetes Mellitus and Other Categories of Glucose Intolerance. National Diabetes Data Group.” 1979).

The study protocol was approved by the Research Ethics Board at Mount Sinai Hospital and the Hospital for Sick Children. Written consent was obtained from all participants. Mothers also provided informed consent on behalf of their infant.

4.6 Measurements and Assays

4.6.1 Maternal and Paternal Evaluation
Baseline maternal and paternal data were collected at the time of study recruitment and included demographic data from an established questionnaire. This included information about their BMI, medical history, ethnicity, smoking status, family history of type 2 diabetes or gestational diabetes, weight gain during pregnancy and obstetrical history. Information about maternal and paternal BMI were self-reported by the subject. In addition to the OGTT, other maternal fasting metabolic measures were obtained and included lipids, adiponectin, C-reactive protein and leptin levels. Insulin sensitivity was calculated using the Matsuda index (ISOGTT) which is derived from plasma glucose and insulin concentration sampled during fasting and following a glucose load (Matsuda and DeFronzo 1999):

\[
\text{IS (MATSUDA)} = \frac{10,000}{\sqrt{G_0 \cdot I_0 \cdot G_{\text{mean}} \cdot I_{\text{mean}}}}
\]

I₀ – Fasting plasma insulin concentration (mIU/l),

G₀ – Fasting plasma glucose concentration (mg/dl),

G_{\text{mean}} – Mean plasma glucose concentration during OGTT (mg/dl),

I_{\text{mean}} – Mean plasma insulin concentration during OGTT (mU/l),

Validation studies in pregnant women have demonstrated the utility of the Matsuda index in comparison with the euglycemic-hyperinsulinemic clamp technique (Matsuda and DeFronzo 1999). Area under the curve of glucose was calculated using the trapezoidal approximation method. In the postpartum period, women were also assessed at 3 months, 1 year, and regular intervals between 2 and 5 years.

4.6.2 Infant Evaluation
At delivery, within the first 48 hours of life, obstetrical data including infant birth weight and mode of delivery was collected from the institutional database at Mount Sinai Hospital. Follow-up visits were conducted at 3 months, 12 months and then at annual intervals until the age of five. Using a questionnaire, relevant post-natal lifestyle variables related infant cardio-metabolic health were collected during this time. This included questions focused on activity level, duration of screen time, and nutritional habits (i.e. duration of exclusive breastfeeding, use of formula, timing of solid food introduction).

**Infant Evaluation: Anthropometry**

Offspring anthropometry was obtained at birth, 3 months, 12 months, and then at annual intervals until the age of five years. All measurements were obtained in triplicate when possible and by a single investigator following standardized procedures.(CDC 2003) A digital pediatric scale was used to obtain the weight, rounded to the nearest gram. Up until 2 years, recumbent length was obtained using a digital board, rounded to the nearest 0.5mm. After 2 years of age, standing length was measured using a stadiometer, rounded to the nearest 0.5 mm. Abdominal circumference was estimated by measuring the circumferential distance between the lowest rib and the top of the iliac crest. Skin fold thickness measurements were obtained using a Harpenden Caliper(Pierson et al. 1991) at the triceps, biceps, supra-iliac and subscapular regions. Three measurements were taken at each site and a mean was calculated. A sum of skin fold thickness was calculated by summing each measurement to provide an aggregate measure of adiposity. These measurements have been shown to correlate well with estimates of body fat, as determined by dual-energy X-ray absorptiometry.(Schmelzle and Fusch 2002; David S Freedman et al. 2007) Supra-iliac and subscapular regions in particular are thought to act as a proxy for central adiposity.

**Infant Evaluation: Metabolic Function**
A fasting blood sample was collected from the infants at one year, three years and five years. Bloodwork was obtained first thing in the morning, following a period of fasting overnight. Fasting duration was defined as the typical length of time the child normally passes overnight without eating. Ideally, this was a period of 10 hours, but a minimum of four was required. Subjects were offered the option of topical anesthetic cream to minimize any potential pain associated with the procedure. The following measurements were included in the bloodwork: Insulin (Electrochemiluminescence Immunoassay Range: 21-118 pmol/L); Glucose (enzymatic reference method with hexokinase); Adiponectin (ELISA; Linco, Range: 1.5-100 ng/mL); Leptin (ELISA; Linco. Range: 0.5 - 100 ng/mL); Lipids (cholesterol, triglycerides, HDL, LDL [derived, not measured directly]; Fluorometric Assay).
Insulin resistance was determined using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). HOMA-IR is a surrogate measure of insulin resistance that has been validated against gold standard clamp procedures in non-diabetic young children less than 5 years. (Gungor et al. 2004) It is calculated using the following formula: (fasting plasma glucose [mmol/L] X fasting plasma insulin [mU/l])/22.5. (Matthews et al. 1985)

**Infant Evaluation: DXA Scan**

DXA scans were used to determine fat mass, fat free mass, and % body fat at age 3 and 5 years. A Lunar Prodigy whole-body scanner (GE Medical Systems, Madison, WI) was used, in conjunction with Encore 2002 software. Subjects did not require any preparation prior to the scan and caregivers were present during the study for comfort. Each scan lasted approximately 3 minutes, during which time the subject lay supine. The results from all scans were interpreted by a trained radiologist based on a written report.

**4.7 Sample Size Calculation**

We determined statistical power based on our primary objective, focusing on HOMA-IR among offspring from 1 to 3 years of age. The calculation is shown in Appendix 1. We restricted our main analysis to offspring outcomes during the first 3 years of life, as the cohort follow-up is ongoing with subjects who have not yet completed their 5 year visit. We therefore deferred analyses beyond 3 years, until completion of remaining study visits. Statistical power was calculated to detect a time-averaged difference of 0.3 in the primary outcome of HOMA-IR, measured at 2 different time points (1 and 3 years). We based this on an established standard deviation of 0.75 for infant HOMA-IR based on a previous publication from this cohort. (Borgono et al. 2012) Therefore, assuming a power threshold of 0.8 and an alpha level of 0.05, we estimated that a sample size of 128 offspring was required, with 64 in both groups (GDM and non-GDM).
We selected a within subject correlation of 0.3. (Borgono et al. 2012) We assumed a compound symmetry covariance structure for the repeated measurements which implies equal covariances and variances in the sampled population.

**Power Analysis**

**Time-Averaged Difference (Normal Data) Power Analysis**

**Numeric Results**

Two-Sided Test. Null Hypothesis: \( D = 0 \). Alternative Hypothesis: \( D \neq 0 \).

Covariance Type = Compound Symmetry

<table>
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<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Sample Allocation</th>
<th>Time Points to be Detected</th>
<th>Standard Deviation</th>
<th>Auto-corr.</th>
<th>Power</th>
<th>Sample Size 1 (N1)</th>
<th>Sample Size 2 (N2)</th>
<th>Allocation Ratio (R)</th>
<th>Time Points (M)</th>
<th>Difference (D1)</th>
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**Summary Statements**

Group sample sizes of 64 and 64 achieve 80% power to detect a difference of 0.300 in a design with 2 repeated measurements having a Compound Symmetry covariance structure when the standard deviation is 0.750, the correlation between observations on the same subject is 0.300, and the alpha level is 0.050.

**References**

Liu, H. and Wu, T., 2005. 'Sample Size Calculation and Power Analysis of Time-Averaged Difference.'


**Report Definitions**

Power is the probability of rejecting a false null hypothesis. It should be close to one.

N1 & N2 are the number of subjects in groups 1 and 2, respectively.

R is the ratio of the number of subjects in group 2 to the number in group 1 (R = N2/N1).

M is the number of time points (repeated measurements) at which each subject is observed.

D1 is the difference between the means of groups 1 and 2 under the alternative hypothesis.

Sigma is the standard deviation of a single observation. It is the same for both groups.

Rho is the correlation between observations on the same subject.

Alpha is the probability of rejecting a true null hypothesis. It should be small.

Beta is the probability of accepting a false null hypothesis. It should be small.
5. STATISTICAL ANALYSIS

The primary objective is based on offspring outcomes from 1 to 3 years of life. This is because cohort follow-up is ongoing with many subjects who have not yet completed their 5 year visit. Furthermore complete anthropometric characterization and bloodwork were not available before 1 year of age. The secondary objective is descriptive and analyzes data from one to five years of age.

Descriptive statistics were used to characterize the study participants at birth and at the different measurement intervals. Data were presented as means and standard deviation for continuous variables and percentages for categorical variables. Continuous variables were assessed for normality of distribution. Natural log transformation was performed for skewed variables when required. Since not all subjects were the same age at each visit date, age adjustments were performed for anthropometric measures and biochemical measures. When age adjustment was performed, means and standard errors were reported. Z scores were calculated for weight for length and body mass index using the WHO calculator (“WHO | WHO Anthro v3.2.2 Macros” 2018). P values < 0.05 (two tailed) were considered to be statistically significant.

Subjects were classified into GDM exposed and non-GDM exposed groups according to the outcome of the maternal OGTT completed at study enrollment (“Classification and Diagnosis of Diabetes Mellitus and Other Categories of Glucose Intolerance. National Diabetes Data Group,” 1979) To compare between GDM exposed and unexposed groups, a two sample t test was used for normally distributed variables, and the Wilcoxon test was used for skewed distributions. Chi-squared or Fisher’s exact test was used to compare categorical variables between groups.
As part of the second objective, the relationship between the different anthropometric measures, body composition indices and fasting biochemical measures of metabolic risk was assessed. Log transformation was performed for skewed variables. Pearson correlation coefficients were performed for normally distributed variables and Spearman correlation was performed for variables which remain skewed after transformation. Coefficient values of 0.0-0.3, 0.3-0.5, 0.5-0.7, 0.7-0.9 were considered to be negligible, weak, moderately strong and strong correlations, respectively. (Mukaka 2012)

To investigate the extent to which parental and offspring variables influence childhood insulin resistance, a linear mixed model regression was constructed using HOMA-IR at the outcome variable. For exposure variables, we included both parental variables (maternal and paternal age, BMI, ethnicity, smoking and family history of diabetes) and offspring variables (age, gestational age, sex, birthweight, BMI, ethnicity, change in weight and nutritional habits). Maternal pre-pregnancy BMI was considered as a potential confounder. HOMA-IR values were log transformed prior to the analyses in the regression models due to a non-normal distribution. Bivariate linear regression was initially performed between parental and offspring characteristics on the outcome of insulin resistance. A linear mixed model regression was initially constructed for the overall cohort. The core model included offspring age, sex and ethnicity as well as significant covariates, with a p value < 0.1 required for inclusion. All estimates were then also adjusted for maternal pre-pregnancy BMI. Maternal insulin sensitivity was included as a continuous variable instead of using a categorical variable such as GDM status.

Since GDM offspring are at greater risk for potential cardio-metabolic risk in early childhood (Krishnaveni et al. 2005) and maternal insulin sensitivity was significantly associated with offspring insulin resistance in the overall model, we completed two analyses assessing GDM and non-GDM offspring separately. In all models, variance inflation factor was determined to assess for multi-
collinearity (Salmerón Gómez et al. 2016). Model estimates were exponentiated back to the original scale and then reported. All statistical analysis was performed with SAS version 9.4.
6. RESULTS

6.1 Objective 1

6.1.1 Baseline Cohort Characteristics

A flow diagram of the study participants is presented in Figure 5. Demographic data for 790 offspring were collected at delivery. We excluded 57 offspring due to either prematurity (n=6), being low birth weight (n=38) or the product of a twin pregnancy (n=19) and some subjects met multiple exclusion criteria. This left 733 offspring included at the time of delivery. Of the 733 offspring, complete maternal data were available for 686 subjects and complete paternal data was available for 622 subjects.

Offspring attrition rate was the greatest within in the first year of life (18%), with an attrition rate of approximately 6% per year thereafter. Importantly, there was no differential attrition in the proportion of women with GDM and non-GDM during pregnancy.
Maternal and Paternal Demographics

The parental cohort characteristics are shown in Table 1. The average maternal age at the time of pregnancy was 34.3 ± 4.4 years. Fathers were slightly older, with a mean age of 37.1 ± 5.8 years. Maternal pre-pregnancy BMIs and reported paternal BMIs were in the overweight range, with a mean BMI of 25.3 ± 5.6 kg/m² for mothers and 27.2 ± 5.1 kg/m² for fathers. Overall, the cohort was ethnically diverse, with 70.0% of mothers identifying as Caucasian, 17.0% identifying as Asian or South Asian and the remaining 13.4% identifying as “other”. Similarly, 67.2% of the fathers identified as Caucasian, 16.4% identified as Asian or South Asian and 16.4% identified as “other”. A significant proportion of mothers (65.6%) reported a family history of type 2 diabetes, compared with 44.7% of fathers. All women with a diagnosis of GDM received glucose lowering treatment. Among the women who were treated, 31.7% received insulin therapy and 68.3% received dietary interventions.
6.1.3 Comparison of Parental Demographics in GDM and non-GDM Groups

Comparisons between parental characteristics among GDM and non-GDM offspring are shown in Table 1. The average maternal age at the time of OGTT was 34.4± 4.4 years, with GDM women undergoing their OGTT earlier in pregnancy compared with non-GDM women (28.8±3.3 vs. 29.8 ±2.8 weeks, p=0.0002). Women with GDM were more likely to be older (35.0 ± 4.6 vs. 34.2 ± 4.3 years, p=0.02) and have a greater BMI (26.3 ± 6.7 vs. 24.9 ±5.0 kg/m², p=0.0074) than normo-glycemic women. The ethnic composition of the groups was different between exposure groups, with fewer Caucasian and more South Asian and Asian women represented in GDM group (p=0.04). A family history of diabetes was also more common in the GDM women (75.7% vs 61.5%, p=0.0006).

Women with GDM had a lower Matsuda index of insulin sensitivity (3.4 ±2.1 vs. 5.30±3.2, p<0.0001), a greater blood glucose area under the curve on OGTT (27.5 ±3.7 vs.21.3 ± 3.4 mg/dl, p<0.0001) and higher fasting blood glucose level (5.0 ±0.7 vs. 4.5±0.6 mmol/L, p<0.0001). On assessment of the adipokine profile, the mothers with GDM demonstrated lower levels of adiponectin (7.0±2.5 vs. 8.1±3.1 ug/ml, p<0.0001), but no significant difference in leptin levels (43.0±23.6 vs 39.0 ±20.1, p=0.09). Finally, the mothers with GDM had evidence of greater inflammation with higher levels of CRP (7.8±7.0 vs. 5.8±5.7, p<0.0008).

There were no statistically significant differences in the paternal age, weight, ethnicity or family history of diabetes between exposed and unexposed offspring.
Table 1. Maternal and Paternal Demographic and Metabolic Characteristics

<table>
<thead>
<tr>
<th>Maternal Characteristics</th>
<th>Overall (n=686)</th>
<th>GDM (n=202)</th>
<th>Non-GDM (n=484)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (n=686)</td>
<td>34.43 ± 4.37</td>
<td>35.01 ± 4.60</td>
<td>34.19 ± 4.25</td>
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</tr>
<tr>
<td>Weeks’ gestation at time of OGTT (weeks) (n=680)</td>
<td>29.51 ± 3.00</td>
<td>28.81 ± 3.26</td>
<td>29.80 ± 2.83</td>
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<tr>
<td>Pre-pregnancy BMI (kg/m²) (n=668)</td>
<td>25.28 ± 5.56</td>
<td>26.30 ± 6.65</td>
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<td>Weight gain in pregnancy preceding OGTT (kg) (n=671)</td>
<td>10.43 ± 7.73</td>
<td>8.89 ± 10.07</td>
<td>11.04 ± 6.47</td>
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<tr>
<td>BMI at 3 months postpartum (kg/m²) (n=636)</td>
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<td>Previous GDM/macrosomia (%) (n=636)</td>
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<td>22 (12.09%)</td>
<td>28 (6.17%)</td>
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<td>Family history of diabetes (%) (n=640)</td>
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<td>140 (75.68%)</td>
<td>280 (61.54%)</td>
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<td>Current smoking (%) (n=685)</td>
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<td>Maternal ethnicity (%) (n=685)</td>
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<td>119 (59.20%)</td>
<td>318 (65.70%)</td>
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<td>437 (63.80%)</td>
<td>119 (59.20%)</td>
<td>318 (65.70%)</td>
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<tr>
<td>- South Asian/Asian</td>
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<table>
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<th>Paternal Characteristics</th>
<th>Overall (n=603)</th>
<th>GDM (n=202)</th>
<th>Non-GDM (n=484)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (n=603)</td>
<td>37.06 ± 5.82</td>
<td>37.29 ± 6.17</td>
<td>36.96 ± 5.67</td>
<td>=0.55</td>
</tr>
<tr>
<td>Family History of Diabetes (%) (n=600)</td>
<td>268 (44.67%)</td>
<td>84 (49.12%)</td>
<td>184 (42.89%)</td>
<td>&lt;0.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maternal Biochemical Metabolic Characteristics</th>
<th>Overall (n=644)</th>
<th>GDM (n=202)</th>
<th>Non-GDM (n=484)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsuda index of insulin sensitivity (n=644)</td>
<td>4.74 ± 3.05</td>
<td>3.35 ± 2.09</td>
<td>5.29 ± 3.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adiponectin (ug/ml) (n=491)</td>
<td>7.83 ± 2.97</td>
<td>7.00 ± 2.54</td>
<td>8.12 ± 3.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leptin (ng/ml) (n=484)</td>
<td>40.00 ± 21.38</td>
<td>42.96 ± 23.56</td>
<td>38.97 ± 20.50</td>
<td>=0.09</td>
</tr>
<tr>
<td>C-reactive protein (mg/l) (n=493)</td>
<td>6.34 ± 6.09</td>
<td>7.83 ± 6.99</td>
<td>5.81 ± 5.66</td>
<td>&lt;0.0008</td>
</tr>
<tr>
<td>AUĈ_{gluc} on OGTT (mg/dl) (n=684)</td>
<td>23.11 ± 4.47</td>
<td>27.50 ± 3.66</td>
<td>21.30 ± 3.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L) (n=685)</td>
<td>4.64 ± 0.62</td>
<td>4.95 ± 0.65</td>
<td>4.52 ± 0.55</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Paternal Characteristics</th>
<th>Overall (n=603)</th>
<th>GDM (n=202)</th>
<th>Non-GDM (n=484)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (n=603)</td>
<td>37.06 ± 5.82</td>
<td>37.29 ± 6.17</td>
<td>36.96 ± 5.67</td>
<td>=0.55</td>
</tr>
</tbody>
</table>
6.1.4 Offspring Demographics

The offspring characteristics are shown in Table 2. At delivery, offspring in the cohort were born at average of 38.8 ±1.3 weeks, with a birth weight of 3416 ± 432 g. Gender distribution was equal (50.1% male) and 35.4% were delivered via caesarian section.

6.1.5 Comparison of Offspring Demographics in GDM and non-GDM Groups

GDM offspring were more likely to be born earlier (38.6± 1.3 weeks vs. 39.1 ±1.3 weeks, p=0.0002) and at a smaller birth weight (3302.5±389.2 vs. 3464±440.9 g, p<0.0001). There was also a greater percentage of male subjects in the GDM cohort (53.0% vs. 47.3%, p=0.002). There were no significant differences in the post-natal nutritional patterns of the groups, with similar rates of exclusive breastfeeding at 3 months and age of introduction of solids. There were also no differences in the percentage of children who identified as Caucasian.
## Table 2. Offspring Demographics

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=733)</th>
<th>GDM (n=219)</th>
<th>Non-GDM (n=514)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of gestation (weeks)</td>
<td>38.80 ± 1.29</td>
<td>38.43 ± 1.25</td>
<td>39.11 ± 1.27</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Mode of delivery (% caesarean section)</td>
<td>231 (35.43%)</td>
<td>71 (36.79%)</td>
<td>160 (34.86%)</td>
<td>p= 0.07</td>
</tr>
<tr>
<td>Neonate gender (% male)</td>
<td>366 (50.07%)</td>
<td>116 (52.97%)</td>
<td>210 (47.30%)</td>
<td>p=0.002</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3416 ± 432.23</td>
<td>3302.45 ± 389.21</td>
<td>3464.38 ± 440.86</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Breastfed exclusively at 3 months (%)</td>
<td>350 (52.01%)</td>
<td>105 (51.98%)</td>
<td>245 (52.02%)</td>
<td>p=0.44</td>
</tr>
<tr>
<td>Age of introduction of solids (months)</td>
<td>5.51 ± 1.22</td>
<td>5.50 ± 1.26</td>
<td>5.52 ± 1.20</td>
<td>p=0.89</td>
</tr>
<tr>
<td>Child Ethnicity (% Caucasian)</td>
<td>345 (55.56%)</td>
<td>98 (54.75)</td>
<td>247 (55.88)</td>
<td>p=0.86</td>
</tr>
<tr>
<td>Change in weight from 0-12 months (kg)</td>
<td>6.20 ± 0.06</td>
<td>6.32 ± 0.10</td>
<td>6.14 ± 0.08</td>
<td>p=0.14</td>
</tr>
<tr>
<td>Change in weight from 0-36 months (kg)</td>
<td>11.28 ± 0.19</td>
<td>11.47 ± 0.25</td>
<td>11.18 ± 0.24</td>
<td>p=0.38</td>
</tr>
<tr>
<td>Change in weight from 0-60 months (kg)</td>
<td>15.08 ± 0.40</td>
<td>15.28 ± 0.57</td>
<td>14.98 ± 0.46</td>
<td>p=0.65</td>
</tr>
</tbody>
</table>

Values presented are expressed as mean ± standard deviation or number of subjects (percentage).
p value is derived by comparing between GDM and non-GDM groups.
6.1.6 Offspring Anthropometrics

Offspring anthropometrics and bloodwork are shown in Table 3. At baseline, 28.3% were offspring of GDM pregnancies. Over time, the relative percentage of GDM offspring fluctuated between 29.1% at 1 year, to 39.2% at 3 years and 31.9% at 5 years as the sample size changed over time. A total of 406 patients had measurements at one year, 112 had measurements at three years, and 94 had measurements at five years.

The average waist to height ratio, adjusted for age, was 0.59 ± 0.00, 0.52 ± 0.00 and 0.47 ± 0.00 at one, three and five years respectively. The average BMI z scores were 0.28±1.06, 0.29 ±1.05 and 0.15±0.98 at one, three and five years respectively. The average weight for length z scores were 0.30 ± 1.01, 0.25 ± 1.08 and 0.05 ± 0.97 at one, three and five years respectively. The prevalence of obesity in the overall cohort, as defined WHO criteria, increased from 1.5% at 1 year to 5.3% at 5 years. (Society and Committee 2016) The prevalence of overweight also increased from 3.0% at 1 year to 8.5% at 5 years.

6.1.7 Comparison of Offspring Anthropometrics and Biochemical Measures in GDM and non-GDM Groups

There were no significant differences in anthropometric measures between GDM and non-GDM offspring. There was a trend towards BMI z score at 3 years being higher in the GDM group, however this did not meet threshold for significance (p=0.06). Among the different biochemical measures, there was a significantly higher fasting insulin level at 5 years in the GDM group (p=0.04). Fasting insulin and HOMA-IR were also highly correlated at 5 years (ρ=0.986, p<0.0001). However, the remaining measures were not statistically different between groups. Specifically, there was no difference in the primary outcome of HOMA-IR at 1, 3 or 5 years of age.
## Table 3a. Offspring Anthropometrics and Bloodwork

<table>
<thead>
<tr>
<th>Anthropometrics</th>
<th>Overall (n=406)</th>
<th>GDM (n=118)</th>
<th>Non-GDM (n=288)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight for length z score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year (n=406)</td>
<td>0.30 ± 1.01</td>
<td>0.34 ± 1.07</td>
<td>0.28 ± 0.98</td>
<td>p=0.60</td>
</tr>
<tr>
<td>3 years (n=112)</td>
<td>0.25 ± 1.08</td>
<td>0.41 ± 1.16</td>
<td>0.15 ± 1.02</td>
<td>p=0.20</td>
</tr>
<tr>
<td>5 years (n=94)</td>
<td>0.05 ± 0.97</td>
<td>0.18 ± 1.00</td>
<td>-0.02 ± 0.95</td>
<td>p=0.35</td>
</tr>
<tr>
<td><strong>BMI z score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year (n=406)</td>
<td>0.28 ± 1.06</td>
<td>0.34 ± 1.1</td>
<td>0.25 ± 1.03</td>
<td>p=0.41</td>
</tr>
<tr>
<td>3 years (n=112)</td>
<td>0.29 ± 1.05</td>
<td>0.52 ± 1.00</td>
<td>0.13 ± 1.04</td>
<td>p=0.06</td>
</tr>
<tr>
<td>5 years (n=94)</td>
<td>0.15 ± 0.98</td>
<td>0.26 ± 1.00</td>
<td>0.10 ± 0.97</td>
<td>p=0.44</td>
</tr>
<tr>
<td><strong>Waist to height ratio</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year (n=394)</td>
<td>0.59 ± 0.00</td>
<td>0.60 ± 0.00</td>
<td>0.59 ± 0.00</td>
<td>p=0.49</td>
</tr>
<tr>
<td>3 years (n=105)</td>
<td>0.52 ± 0.00</td>
<td>0.52 ± 0.00</td>
<td>0.51 ± 0.00</td>
<td>p=0.25</td>
</tr>
<tr>
<td>5 years (n=94)</td>
<td>0.47 ± 0.01</td>
<td>0.48 ± 0.01</td>
<td>0.47 ± 0.00</td>
<td>p=0.58</td>
</tr>
<tr>
<td><strong>Sum of skin fold thickness (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year (n=391)</td>
<td>28.96 ± 0.36</td>
<td>28.71 ± 0.57</td>
<td>29.08 ± 0.45</td>
<td>p=0.60</td>
</tr>
<tr>
<td>3 years (n=96)</td>
<td>27.28 ± 0.85</td>
<td>27.50 ± 1.04</td>
<td>27.17 ± 1.10</td>
<td>p=0.82</td>
</tr>
<tr>
<td>5 years (n=90)</td>
<td>27.31 ± 1.28</td>
<td>27.76 ± 1.73</td>
<td>27.10 ± 1.54</td>
<td>p=0.75</td>
</tr>
<tr>
<td><strong>Bloodwork</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HOMA-IR</strong>* (no units)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year (n=175)</td>
<td>0.21 ± 1.1</td>
<td>0.25 ± 1.1</td>
<td>0.19 ± 1.1</td>
<td>p=0.17</td>
</tr>
<tr>
<td>3 years (n=91)</td>
<td>0.58 ± 0.1</td>
<td>0.69 ± 0.1</td>
<td>0.52 ± 0.1</td>
<td>p=0.09</td>
</tr>
<tr>
<td>5 years (n=83)</td>
<td>0.50 ± 1.09</td>
<td>0.56 ± 1.12</td>
<td>0.47 ± 1.11</td>
<td>p=0.21</td>
</tr>
<tr>
<td><strong>Fasting Insulin</strong>* (pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 year (n=175)</td>
<td>8.07 ± 1.1</td>
<td>9.43 ± 1.1</td>
<td>7.46 ± 1.1</td>
<td>p=0.15</td>
</tr>
<tr>
<td>3 years (n=91)</td>
<td>21.39 ± 2.1</td>
<td>25.53 ± 2.6</td>
<td>19.32 ± 2.7</td>
<td>p=0.08</td>
</tr>
<tr>
<td>5 years (n=83)</td>
<td><strong>21.62 ± 2.29</strong></td>
<td><strong>26.75 ± 3.11</strong></td>
<td><strong>19.06 ± 2.76</strong></td>
<td><strong>p=0.04</strong></td>
</tr>
<tr>
<td>1 year (n=175)</td>
<td>4.33 ± 0.05</td>
<td>4.36 ± 0.07</td>
<td>4.32 ± 0.06</td>
<td>p=0.72</td>
</tr>
<tr>
<td></td>
<td>3 years (n=91)</td>
<td>5 years (n=83)</td>
<td>4 years (n=91)</td>
<td>p=0.54</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Fasting glucose</strong></td>
<td>4.23 ± 0.06</td>
<td>4.28 ± 0.07</td>
<td>4.22 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>(mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.29 ± 1.01</td>
<td>4.35 ± 1.02</td>
<td>4.26 ± 1.02</td>
<td>p=0.39</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>1 year (n=175)</td>
<td>3 years (n=91)</td>
<td>5 years (n=73)</td>
<td></td>
</tr>
<tr>
<td>(mmol/l)</td>
<td>1.11 ± 0.05</td>
<td>0.69 ± 0.04</td>
<td>0.68 ± 0.04</td>
<td>p=0.84</td>
</tr>
<tr>
<td></td>
<td>1.09 ± 0.07</td>
<td>0.70 ± 0.05</td>
<td>0.71 ± 0.06</td>
<td>p=0.80</td>
</tr>
<tr>
<td></td>
<td>1.11 ± 0.06</td>
<td>0.68 ± 0.05</td>
<td>0.68 ± 0.05</td>
<td>p=0.67</td>
</tr>
<tr>
<td><strong>Leptin</strong></td>
<td>1 year (n=108)</td>
<td>3 years (n=70)</td>
<td>5 years (n=43)</td>
<td></td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>2.19 ± 0.18</td>
<td>2.83 ± 0.35</td>
<td>3.11 ± 0.66</td>
<td>p=0.47</td>
</tr>
<tr>
<td></td>
<td>2.36 ± 0.28</td>
<td>3.41 ± 0.42</td>
<td>2.83 ± 0.83</td>
<td>p=0.13</td>
</tr>
<tr>
<td></td>
<td>2.10 ± 0.23</td>
<td>2.53 ± 0.46</td>
<td>3.25 ± 0.81</td>
<td>p=0.68</td>
</tr>
<tr>
<td><strong>Adiponectin</strong></td>
<td>1 year (n=106)</td>
<td>3 years (n=27)</td>
<td>5 years (n=23)</td>
<td></td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>19881 ± 752.9</td>
<td>17049 ± 2743.1</td>
<td>12718 ± 1496.2</td>
<td>p=0.15</td>
</tr>
<tr>
<td></td>
<td>18483 ± 1156.7</td>
<td>18034 ± 3027.5</td>
<td>12941 ± 1985.9</td>
<td>p=0.72</td>
</tr>
<tr>
<td></td>
<td>20580 ± 941.45</td>
<td>16557 ± 3467.6</td>
<td>12606 ± 1678.0</td>
<td>p=0.87</td>
</tr>
</tbody>
</table>

*Age adjusted values are expressed as estimate ± standard error.

All other values are expressed as mean ± standard deviation.

p values are derived by comparing between GDM and non-GDM groups.

HOMA-IR, Homeostatic Model Assessment – Insulin Resistance.
### Table 3b. Prevalence of Obesity Among Offspring

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Obesity</th>
<th>Overweight</th>
<th>Risk of Overweight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 year (n=406)</td>
<td>1.48% (6) weight for length &gt;99.9th percentile</td>
<td>2.96% (12) weight for length &gt;97th percentile</td>
<td>18.47% (75) weight for length &gt;85th percentile</td>
</tr>
<tr>
<td>3 year (n=112)</td>
<td>0.90% (1) BMI &gt;99.9th percentile</td>
<td>4.47% (5) BMI &gt;97th percentile</td>
<td>16.96% (19) BMI &gt;85th percentile</td>
</tr>
<tr>
<td>5 year (n=94)</td>
<td>5.32% (5) BMI &gt;97th percentile</td>
<td>8.51% (8) BMI &gt;85th percentile</td>
<td></td>
</tr>
</tbody>
</table>

### Prevalence of Obesity Among GDM and non-GDM Offspring

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Obesity</th>
<th>Overweight</th>
<th>Risk of Overweight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 year (n=406)</td>
<td>2.54% (3) GDM</td>
<td>3.39% (4) GDM</td>
<td>19.49% (23) GDM</td>
</tr>
<tr>
<td></td>
<td>1.04% (3) non-GDM</td>
<td>2.78% (8) non-GDM</td>
<td>18.06% (52) non-GDM</td>
</tr>
<tr>
<td>3 year (n=112)</td>
<td>2.27% (1) GDM</td>
<td>4.55% (2) GDM</td>
<td>18.18% (8) GDM</td>
</tr>
<tr>
<td></td>
<td>0% (0) non-GDM</td>
<td>4.41% (3) non-GDM</td>
<td>16.18% (11) non-GDM</td>
</tr>
<tr>
<td>5 year (n=94)</td>
<td>3.33% (1) GDM</td>
<td>16.67% (5) GDM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.25% (4) non-GDM</td>
<td>4.69% (3) non-GDM</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as number of subjects (percentage).

Definition for weight status (Society and Committee 2016)

*BMI, body mass index; GDM, gestational diabetes mellitus.*
6.1.8 Comparison of Offspring Body Composition in GDM and non-GDM Groups

Offspring body composition is shown in Table 4. There were no statistically significant differences in measures of truncal fat and total body fat percentage at 3 and 5 years among GDM and non-GDM groups, even after adjustment for age.

<table>
<thead>
<tr>
<th>3 years</th>
<th>Percentage of Truncal Fat</th>
<th>Overall</th>
<th>GDM</th>
<th>Non-GDM</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21.74 ± 5.64</td>
<td>19.88 ± 4.32</td>
<td>23.73 ± 6.33</td>
<td>p=0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=31)</td>
<td>(n=16)</td>
<td>(n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of Body Fat</td>
<td>28.65 ± 4.91</td>
<td>27.31 ± 3.50</td>
<td>30.07 ± 5.87</td>
<td>p=0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=31)</td>
<td>(n=16)</td>
<td>(n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 years</td>
<td>Percentage of Truncal Fat</td>
<td>20.32 ± 4.77</td>
<td>19.50 ± 4.70</td>
<td>20.94 ± 4.88</td>
<td>p=0.44</td>
</tr>
<tr>
<td></td>
<td>(n=31)</td>
<td>(n=12)</td>
<td>(n=16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of Body Fat</td>
<td>27.21 ± 3.84</td>
<td>27.08 ± 4.17</td>
<td>27.31 ± 3.72</td>
<td>p=0.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=28)</td>
<td>(n=12)</td>
<td>(n=16)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values presented are expressed as mean ± standard deviation

p values are derived by comparing between GDM and non-GDM groups.

GDM, gestational diabetes mellitus.
6.1.9 Determinants of HOMA-IR in Overall Cohort

A linear mixed model was built to look at the determinants of insulin resistance from 1 to 3 years of age. After adjustment for offspring age, gender and ethnicity and maternal weight status, offspring age, weight change from birth to 3 years of age, gender and maternal insulin sensitivity remained significantly independently associated with insulin resistance. Older age (per year) was associated with 7.5% greater HOMA-IR ($\beta=0.03154$, $p<0.001$), greater weight gain between birth and 3 years of age (by kg) was associated with a 16.8% greater HOMA-IR ($\beta=0.1559$, $p<0.02$) and higher maternal insulin sensitivity (by 1 unit on the Matsuda index) was associated with 6.8% lower HOMA-IR ($\beta=-0.07023$, $p<0.05$). Finally, male gender was associated with 36.5% lower HOMA-IR compared with female gender ($\beta=-0.4541$, $p=0.009$).
<table>
<thead>
<tr>
<th></th>
<th>Log Estimate</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n=99, 144 observations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-2.5839</td>
<td>-4.6961</td>
<td>-0.4718</td>
<td>0.017</td>
</tr>
<tr>
<td>Offspring age</td>
<td>0.03154</td>
<td>0.02160</td>
<td>0.04148</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Offspring male gender</td>
<td>-0.4541</td>
<td>-0.7913</td>
<td>-0.1168</td>
<td>0.009</td>
</tr>
<tr>
<td>Offspring ethnicity</td>
<td>-0.07732</td>
<td>-0.4354</td>
<td>0.2807</td>
<td>0.67</td>
</tr>
<tr>
<td>Offspring birthweight</td>
<td>-0.03993</td>
<td>-0.4525</td>
<td>0.3726</td>
<td>0.85</td>
</tr>
<tr>
<td>Offspring BMI z Score at 1 and 3 years</td>
<td>-0.08811</td>
<td>-0.2806</td>
<td>0.1043</td>
<td>0.37</td>
</tr>
<tr>
<td>Offspring weight change from birth to 3 years of age</td>
<td>0.1559</td>
<td>0.03087</td>
<td>0.2809</td>
<td>0.02</td>
</tr>
<tr>
<td>Maternal pre-pregnancy BMI</td>
<td>-0.01621</td>
<td>-0.05027</td>
<td>0.01786</td>
<td>0.35</td>
</tr>
<tr>
<td>Maternal insulin sensitivity</td>
<td>-0.07023</td>
<td>-0.1313</td>
<td>-0.00911</td>
<td>0.05</td>
</tr>
<tr>
<td>Previous GDM or Macrosomia</td>
<td>0.2421</td>
<td>-0.2666</td>
<td>0.7507</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*BMI, body mass index; GDM, gestational diabetes mellitus.*
6.1.10 Determinants of HOMA-IR in GDM and non-GDM Cohorts

Using separate models, we evaluated the associations between parental and infant characteristics on the outcome of HOMA-IR between 1 and 3 years, in both GDM and non-GDM offspring separately. This step of the analysis was performed as part of the a priori analysis for 3 reasons. Firstly, differences have previously between described between GDM non-GDM infants at 1 year of age in this cohort (Borgono et al. 2012). Secondly, GDM children are at greater risk for insulin resistance and cardio-metabolic disease, therefore, we hypothesized that they may have different determinants of insulin resistance. Finally, from the univariate regression, we observed a significant association between GDM status and offspring HOMA-IR ($\beta=0.2838$, SE 0.2133, $p=0.02$). This remained significant, when GDM status was included in the overall model of HOMA-IR, instead of maternal insulin sensitivity.

The GDM model is shown in Table 6 and the non-GDM model is shown in Table 7. In both GDM and non-GDM offspring, older age was associated with a higher in HOMA-IR during early childhood. In the GDM group, for each additional year, HOMA-IR was increased by 2.5% and in the non-GDM group, it was increased by 3.0% ($\beta=0.02495$, $p<0.001$, $\beta=0.02939$, $p<0.0001$). In the GDM group, greater maternal insulin sensitivity was significantly associated with a 17.7% lower in HOMA-IR between 1 and 3 years of age ($\beta=-0.1947$, $p=0.002$). This remained significant after adjustment for maternal pregnancy BMI (shown in Table 6). Conversely, in the non-GDM group, offspring gender was significantly associated with HOMA-IR. Specifically, being male was associated with a 35.1% lower HOMA-IR compared to female offspring ($\beta=-0.4317$, $p=0.03$). This remained significant after adjustment for maternal pre-pregnancy BMI (shown in Table 7). There was a trend towards increasing weight gain between birth and three years being significantly associated with HOMA-IR in non-GDM offspring however this did not meet threshold for significance ($p=0.06$). Therefore, in summary, greater HOMA-IR in GDM offspring
was associated with increasing age and reduced maternal insulin sensitivity. Conversely, greater HOMA-IR in non-GDM offspring was associated with increasing age and female gender.
<table>
<thead>
<tr>
<th>Table 6. Determinants of Insulin Resistance (HOMA-IR) from 1 to 3 years of age in GDM Offspring (linear mixed model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=39, 65 observations)</td>
</tr>
<tr>
<td>Log Estimate β</td>
</tr>
<tr>
<td>Intercept</td>
</tr>
<tr>
<td>Offspring age</td>
</tr>
<tr>
<td>Offspring male gender</td>
</tr>
<tr>
<td>Offspring ethnicity</td>
</tr>
<tr>
<td>Weight change from birth to 3 years of age</td>
</tr>
<tr>
<td>Maternal insulin sensitivity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Determinants of Insulin Resistance from 1 to 3 years of age in GDM Offspring Adjusted for Maternal Pre-pregnancy BMI (linear mixed model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=39, 65 observations)</td>
</tr>
<tr>
<td>Log Estimate β</td>
</tr>
<tr>
<td>Intercept</td>
</tr>
<tr>
<td>Offspring age</td>
</tr>
<tr>
<td>Offspring male gender</td>
</tr>
<tr>
<td>Offspring ethnicity</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
</tr>
<tr>
<td>Weight change from birth to 3 years of age</td>
</tr>
<tr>
<td>Maternal pre-pregnancy BMI</td>
</tr>
<tr>
<td>Maternal insulin sensitivity</td>
</tr>
</tbody>
</table>

*HOMA-IR, Homeostatic Model Assessment – Insulin Resistance; GDM, gestational diabetes mellitus; BMI, body mass index.*
Table 7. Determinants of Insulin Resistance from 1 to 3 years of age in non-GDM Offspring (linear mixed model)

<table>
<thead>
<tr>
<th></th>
<th>Log Estimate</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=60, 81 observations)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-2.8435</td>
<td>-4.2344</td>
<td>-1.4525</td>
<td>0.0001</td>
</tr>
<tr>
<td>Offspring age</td>
<td>0.02939</td>
<td>0.01604</td>
<td>0.04275</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Offspring male gender</td>
<td>-0.4317</td>
<td>-0.8169</td>
<td>-0.04643</td>
<td>0.03</td>
</tr>
<tr>
<td>Offspring ethnicity</td>
<td>-0.2973</td>
<td>-0.7337</td>
<td>0.1391</td>
<td>0.18</td>
</tr>
<tr>
<td>Weight change from birth to 3 years of age</td>
<td>0.1088</td>
<td>-0.00546</td>
<td>0.2230</td>
<td>0.06</td>
</tr>
<tr>
<td>Maternal insulin sensitivity</td>
<td>-0.00506</td>
<td>-0.07014</td>
<td>0.06002</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Determinants of Insulin Resistance from 1 to 3 years of age in non-GDM Offspring Adjusted for Maternal Pre-pregnancy BMI (linear mixed model)

<table>
<thead>
<tr>
<th></th>
<th>Log Estimate</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=60, 81 observations)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-2.7721</td>
<td>-4.5511</td>
<td>-0.9930</td>
<td>0.003</td>
</tr>
<tr>
<td>Offspring age</td>
<td>0.03298</td>
<td>0.01974</td>
<td>0.04622</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Offspring male gender</td>
<td>-0.4476</td>
<td>-0.8704</td>
<td>-0.02484</td>
<td>0.04</td>
</tr>
<tr>
<td>Offspring ethnicity</td>
<td>-0.3805</td>
<td>-0.8682</td>
<td>0.1073</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Weight change from birth to 3 years of age</td>
<td>0.1123</td>
<td>-0.016551</td>
<td>0.2412</td>
<td>0.09</td>
</tr>
<tr>
<td>Maternal pre-pregnancy BMI</td>
<td>-0.0056</td>
<td>-0.05375</td>
<td>0.04262</td>
<td>0.82</td>
</tr>
<tr>
<td>Maternal insulin sensitivity</td>
<td>-0.00357</td>
<td>-0.07987</td>
<td>0.07274</td>
<td>0.93</td>
</tr>
</tbody>
</table>

HOMA-IR, Homeostatic Model Assessment – Insulin Resistance; GDM, gestational diabetes mellitus; BMI, body mass index.
6.2 Objective 2

6.2.1 Correlation between Anthropometric Measures and Biochemical Metabolic Markers

Correlation between anthropometric measures and biochemical markers are shown in Table 8. Correlation between the anthropometric measures with contemporaneous fasting biochemical measures of cardio-metabolic risk demonstrated that weight for length and BMI z score were associated with the greatest number of biochemical measures and demonstrated the greatest magnitude of association. At three years of age, BMI z score and weight for length were associated with leptin and HOMA-IR; and both measures were also correlated with fasting insulin and leptin at 3 and 5 years. Only weight for length was significantly associated with fasting insulin at one year of age. Conversely, waist to height ratio was associated with only leptin, and demonstrated a comparatively low correlation coefficient but strengthened over time over time. In general, the magnitude of the association for all of the anthropometric measures and biochemistry strengthened over time, with higher correlation coefficients as the children grew older. None of the anthropometric measures were consistently associated with serum lipid or adiponectin levels.
### Table 8. Pearson Correlation between Anthropometrics and Biochemical Markers of Metabolic Risk at 1, 3 and 5 years

<table>
<thead>
<tr>
<th></th>
<th>1 year ρ (p value)</th>
<th>3 years ρ (p value)</th>
<th>5 years ρ (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waist to height ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>0.08 (p=0.30) (n=171)</td>
<td>0.10 (p=0.36) (n=87)</td>
<td>0.21 (p=0.07) (n=73)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.14 (p=0.06) (n=170)</td>
<td>-0.07 (p=0.53) (n=87)</td>
<td>0.06 (p=0.61) (n=73)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.03 (p=0.68) (n=171)</td>
<td>0.08 (p=0.48) (n=87)</td>
<td>0.17 (p=0.16) (n=73)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td><strong>0.37 (p&lt;0.0001)</strong> (n=107)</td>
<td><strong>0.36 (p=0.003)</strong> (n=66)</td>
<td><strong>0.64 (p&lt;0.0001)</strong> (n=43)</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.14 (p=0.06) (n=171)</td>
<td>0.20 (p=0.06) (n=87)</td>
<td>0.15 (p=0.22) (n=73)</td>
</tr>
<tr>
<td><strong>Sum of Skin Fold Thickness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>0.07 (p=0.38) (n=165)</td>
<td>-0.08 (p=0.46) (n=81)</td>
<td><strong>0.30 (p=0.01)</strong> (n=70)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.0009 (p=1.0) (n=165)</td>
<td>-0.08 (p=0.50) (n=81)</td>
<td>0.02 (p=0.85) (n=70)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.03 (p=0.68) (n=165)</td>
<td>-0.09 (p=0.41) (n=81)</td>
<td>0.11 (p=0.39) (n=70)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td><strong>0.39 (p&lt;0.0001)</strong> (n=108)</td>
<td><strong>0.29 (p=0.02)</strong> (n=63)</td>
<td><strong>0.88 (p&lt;0.0001)</strong> (n=42)</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td><strong>0.15 (p=0.048)</strong> (n=165)</td>
<td>0.02 (p=0.84) (n=81)</td>
<td>0.21 (p=0.08) (n=70)</td>
</tr>
<tr>
<td><strong>BMI Z Score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>-0.04 (p=0.63) (n=174)</td>
<td><strong>0.25 (p=0.01)</strong> (n=91)</td>
<td><strong>0.26 (p=0.03)</strong> (n=73)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.08 (p=0.28) (n=173)</td>
<td>0.07 (p=0.50) (n=91)</td>
<td>0.02 (p=0.88) (n=72)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.10 (p=0.18) (n=171)</td>
<td><strong>0.24 (p=0.02)</strong> (n=91)</td>
<td>0.20 (p=0.09) (n=72)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td><strong>0.43 (p&lt;0.0001)</strong> (n=108)</td>
<td><strong>0.52 (p&lt;0.0001)</strong> (n=70)</td>
<td><strong>0.67 (p&lt;0.0001)</strong> (n=43)</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.07 (p=0.33) (n=174)</td>
<td>0.15 (p=0.16) (n=91)</td>
<td>0.09 (p=0.43) (n=73)</td>
</tr>
<tr>
<td><strong>Weight for Length Z Score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td><strong>0.18 (p=0.02)</strong> (n=174)</td>
<td><strong>0.27 (p=0.01)</strong> (n=91)</td>
<td><strong>0.27 (p=0.02)</strong> (n=73)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>-0.02 (p=0.76) (n=173)</td>
<td>0.09 (p=0.41) (n=91)</td>
<td>0.009 (p=0.94) (n=72)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.14 (p=0.06) (n=171)</td>
<td><strong>0.25 (p=0.02)</strong> (n=91)</td>
<td>0.19 (p=0.10) (n=72)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td><strong>0.42 (p&lt;0.0001)</strong> (n=108)</td>
<td><strong>0.51 (p&lt;0.0001)</strong> (n=70)</td>
<td><strong>0.65 (p&lt;0.0001)</strong> (n=43)</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.07 (p=0.35) (n=174)</td>
<td>0.14 (p=0.19) (n=91)</td>
<td>0.11 (p=0.36) (n=73)</td>
</tr>
</tbody>
</table>
Correlations are adjusted for age

HOMA-IR, Homeostatic Model Assessment – Insulin Resistance; BMI, body mass index.

6.2.2 Correlation between Anthropometric Measures and Body Composition

Correlation of anthropometric measures and body composition are shown in Table 9. Correlation was determined between the anthropometric measures and fat mass at three years of age, as estimated by DXA. Waist to height ratio was weakly correlated with overall fat mass (0.41, p=0.03). The remaining anthropometric measures, sum of skin fold thickness, weight for length z score and BMI z score were weakly or moderately correlated with all three measures of adiposity including body fat percent, truncal fat percent and overall fat mass. All measures were most strongly correlated with overall fat mass.

<p>| Table 9. Pearson Correlation between Anthropometrics and Body Composition |
|--------------------------------------------------|------------------|------------------|
| <strong>Body Fat Percent (%)</strong> | <strong>Truncal Fat Percent (%)</strong> | <strong>Overall Fat Mass (g)</strong> |</p>
<table>
<thead>
<tr>
<th>ρ (p value)</th>
<th>ρ (p value)</th>
<th>ρ (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist to height ratio (n=28)</td>
<td>0.23 (p=0.24)</td>
<td>0.35 (p=0.72)</td>
</tr>
<tr>
<td>Sum of skin fold thickness (n=24)</td>
<td>0.44 (p=0.03)*</td>
<td>0.42 (p=0.04)*</td>
</tr>
<tr>
<td>Weight for length z score (n=28)</td>
<td>0.42 (p=0.03)*</td>
<td>0.52 (p=0.005)*</td>
</tr>
<tr>
<td>BMI z score (n=28)</td>
<td>0.40 (p=0.03)*</td>
<td>0.51 (p&lt;0.006)*</td>
</tr>
</tbody>
</table>

Correlation between Anthropometrics and Body Composition Adjusted for Age
<table>
<thead>
<tr>
<th></th>
<th>ρ (p value)</th>
<th>ρ (p value)</th>
<th>ρ (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Waist to height ratio</strong></td>
<td>0.23 (p=0.23)</td>
<td>0.34 (p=0.09)</td>
<td>0.41 (p=0.04)*</td>
</tr>
<tr>
<td>(n=28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sum of skin fold thickness</strong></td>
<td>0.44 (p=0.04)*</td>
<td>0.42 (p=0.04)*</td>
<td>0.53 (p=0.01)*</td>
</tr>
<tr>
<td>(n=24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weight for length z score</strong></td>
<td>0.42 (p=0.04)*</td>
<td>0.52 (p=0.006)*</td>
<td>0.65 (p=0.000)*</td>
</tr>
<tr>
<td>(n=28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI z score</strong></td>
<td>0.40 (p=0.04)*</td>
<td>0.51 (p=0.008)*</td>
<td>0.61 (p=0.01)*</td>
</tr>
<tr>
<td>(n=28)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*BMI, body mass index.*
6.2.3 Correlation between Anthropometric Measures at 1 Year and Body Mass Index at 5 Years

The relationship between measures of adiposity and BMI z score at 5 years of age were evaluated to determine the correlation between early childhood anthropometrics and future risk of obesity. These results are shown in Table 10. Weight to height ratio at one year of age was associated with BMI z score at 5 years (0.28, p<0.0067), however, BMI z score, sum of skin fold thickness and weight for length measured at 1 year were more predictive of weight status at 5 years.

| Table 10. Correlation between Anthropometrics at 1 year and Future BMI at 5 years |
|-----------------------------------------------|------------------|
| BMI z score at 5 years                        | ρ (p value)      |
| **Waist to height ratio at 1 year**           | **0.28, p<0.007**|
| (n=90)                                        |                  |
| **Sum of skin fold thickness at 1 year**      | **0.34, p<0.0001**|
| (n=89)                                        |                  |
| **Weight for length z score at 1 year**       | **0.56, p<0.0001**|
| (n=91)                                        |                  |
| **BMI z score at 1 year**                     | **0.53, p<0.0001**|
| (n=91)                                        |                  |

*Correlations are age adjusted.

BMI, body mass index.
7. DISCUSSION

7.1 Objective 1 - Insulin Resistance and Adiposity among Offspring Exposed to GDM

The primary objective of this thesis was to understand the association between \textit{in utero} exposure to GDM and insulin resistance in the offspring. Longitudinal observation of offspring in this cohort demonstrated significant clinical and anthropometric differences in GDM offspring at delivery. By 1 year of age, these differences disappear, leaving no appreciable differences in the absolute biochemical or anthropometric outcomes. However, children exposed to hyperglycemia \textit{in utero} appear to have unique determinants of insulin resistance, with HOMA-IR in offspring of GDM mothers predicted by maternal insulin sensitivity during pregnancy.

The impact of maternal hyperglycemia on insulin resistance in the first few years of life has not been well described and this study is one of the first to report on the longitudinal patterns of insulin resistance during early childhood in GDM exposed children. Other studies have reported on insulin sensitivity in older children and a similar subtle pattern of metabolic disturbance has been described. A birth cohort study in Mysore, India prospectively followed 630 children over time and also observed differences in GDM exposed offspring at birth which were no longer appreciable at 1 year of age. (Krishnaveni et al. 2005) In contrast to our study, GDM children from the Mysore cohort were heavier at birth. Increased measures of adiposity re-emerged among GDM females at 2 years, followed by higher fasting insulin concentrations and greater risk of IGT at 5 years of age, also in girls. The metabolic risk in GDM offspring augmented as children grew older, with greater differences between groups over time. By 9 years of age, GDM offspring had significantly higher fasting insulin concentrations and HOMA-IR, after adjustment for maternal BMI. (Krishnaveni et al. 2010) The timing of these changes is also consistent with a smaller study of GDM offspring from Poland who demonstrated higher measures of insulin resistance in GDM children but unadjusted for maternal BMI, at 4 to 9 years of age. (Wroblewska-Seniuk, Wender-
In our study, by 5 years of age, GDM offspring demonstrated no differences in our primary outcome of HOMA-IR, however, they did have higher fasting insulin levels, replicating what has been previously observed in the cohort from Mysore, India.

The evolution of adiposity among GDM offspring has been much more extensively characterized during early childhood. However, the findings are conflicting. Similar to what we observed in our cohort, earlier studies in the Pima Indian population described resolution of macrosomia in GDM infants by 1 year of age, and with recurrence of greater adiposity in GDM offspring later in childhood. (Silverman et al. 1998) This finding has been replicated within cohort studies that controlled for important confounders such as maternal obesity. A large American cohort of 28,000 mother infant dyads were followed prospectively and reported that GDM status confers an increased risk of adiposity at age 7, after accounting for maternal BMI and birthweight (Hillier et al. 2007). Similar findings were replicated in another large American cohort of untreated women with hyperglycemia who failed the 50g 1 hour glucose challenge, but went on to pass the 3 hour 100g OGTT. (Andrea L Deierlein et al. 2011) GDM children demonstrated higher BMI z-score compared to their unexposed peers, as early as 3 years of age. However, an increased risk of adiposity among GDM children is not a consistent finding, with other studies showing no differences between exposed and unexposed children (Whitaker et al. 1998; Gillman et al. 2010; A. Lawlor et al. 2010; Thaware et al. 2015; David J Pettitt et al. 2010b) as well as two meta-analysis that found no differences in either BMI z score (Philipps et al. 2011) or obesity (Kim et al. 2011).

The discordant findings about adiposity risk in GDM children could be explained by important differences between studies. Varying treatment practices of women with GDM and subsequent differences in glycemic control achieved during pregnancy may impact exposure to hyperglycemia. Differences in the age and ethnic composition of cohort may alter the likelihood of appreciable metabolic
abnormalities. Finally, the screening practices and diagnostic thresholds used to define GDM remain highly controversial and there is heterogeneity in the diagnostic tests and criteria used (Farrar et al. 2017). For example, using the two step, 100-gram OGTT, the Carpenter-Coustan (Carpenter and Coustan 1982) criteria are more stringent compared to the National Diabetes Data Group (“Classification and Diagnosis of Diabetes Mellitus and Other Categories of Glucose Intolerance. National Diabetes Data Group.” 1979), with a lower threshold for GDM diagnosis. IADPSG thresholds derived from the HAPO trial, using the one step, 75-g OGTT may also be more sensitive (Metzger et al. 2010) More conservative criteria result in the inclusion of women with more mild glucose intolerance, and captures offspring with less significant hyperglycemia during pregnancy. A combination of these factors may account for the reported discrepancies in the literature and speak to the importance of accurately defining gestational diabetes.

We did not find significant differences in absolute measures of insulin resistance or adiposity in GDM offspring, however we did observe that GDM children had different factors which were associated with their measures of insulin resistance. Examination of our study design highlights several factors which may explain these results. Firstly, we followed children during early childhood - a time when any degree of metabolic dysfunction is likely in very early stages. Younger children may be less likely to clinically manifest appreciable metabolic differences. Secondly, review of the parental characteristics also suggests that our cohort may have had milder maternal metabolic phenotype, potentially resulting in a less significant offspring exposure to hyperglycemia. Maternal subjects had low rates of obesity, with an average BMI of 25.3 kg/m² in the overall cohort, and 26.3 kg/m² in the GDM group. Our cohort also had very low reported prevalence of smoking during pregnancy. While our cohort was ethnically diverse, Caucasian mothers still comprised more than half of the subjects and may have less significant beta cell dysfunction (Hasson, Apovian, and Istan 2015). Taken together, the parental characteristics and young age of the cohort subjects may be associated with a less deleterious metabolic phenotype. In turn, this would translate into less exposure burden during fetal development and less metabolic disturbances in the
offspring. Finally, mothers in our cohort who met criteria for a diagnosis of GDM received glucose lowering treatments which may have further attenuated cardio-metabolic differences in their offspring. Identified women were offered standard of care treatment which consisted of either nutritional counselling and/or insulin treatment depending on the severity of hyperglycemia. In contrast, offspring follow-up of untreated mothers provides a unique opportunity to understand the impact of hyperglycemia, not biased by the effect of glucose lowering treatments. Ancillary cohort studies from HAPO have evaluated insulin resistance among offspring of women with mild untreated hyperglycemia. They describe a lower oral disposition index and higher rates of impaired glucose tolerance at 7 years of age. Reported adiposity outcomes among studies with untreated GDM mothers remain discordant. Follow-up of the European HAPO offspring, exposed to mild untreated hyperglycemia, did not have demonstrable differences at 2-3 years or again at 5 to 7 years of age with respect to BMI, after adjustment for maternal obesity. However, two multi-ethnic, large American epidemiologic studies of untreated mother-infant pairs reported higher rates of obesity at 3 years of age and 5 to 7 years of age. In this cohort, we did not observe significant fetal macrosomia in GDM offspring. In fact, GDM offspring were born with a smaller birth weight when compared with unexposed offspring. This may relate to efficacious treatment of GDM mothers during pregnancy in the form of nutritional counselling or insulin therapy. This may mitigate excessive fetal growth during the third trimester, and resultant neonatal macrosomia. This would make detection of potential metabolic differences in exposed children more subtle and difficult to detect in early life.

We sought to evaluate the determinants of insulin resistance in both exposed and unexposed offspring. In the overall cohort which included both GDM and non-GDM offspring, we demonstrate that increasing age, reduced maternal insulin sensitivity and greater weight gain between birth and 3 years are associated with increasing insulin resistance during early childhood. These relationships occur independently of
maternal weight status. However, among children exposed to \textit{in utero} hyperglycemia, insulin resistance is associated with maternal insulin sensitivity and age. In contrast, female gender and increasing age appear to be the primary drivers of greater insulin resistance in unexposed offspring. These results reinforce that the pathways to the development of insulin resistance are different in GDM vs. non-GDM offspring.

These findings are consistent with the paradigm which considers hyperglycemia as an independent and perhaps additive risk factor to maternal weight status. This result is supported by more recent studies that have robustly evaluated the impact of each exposure separately and then together.\cite{Sauder et al. 2017} The two risk factors often co-occur with each other, putting the fetus at heightened risk for fetal over-nutrition during pregnancy. Interestingly, in this cohort, maternal pre-pregnancy BMI was not significantly associated with offspring insulin resistance on its own, or within the model. Maternal weight gain during pregnancy showed a similarly non-significant relationship with offspring insulin resistance. Other studies have reported non-significance of maternal weight status, however this is after adjustment for offspring measures of adiposity\cite{Boyne et al. 2010; Perng et al. 2014}. This finding in our cohort may in part be explained by lower mean maternal BMI, and thus potentially more limited exposure to maternal obesity. Importantly, maternal insulin sensitivity remains an important determinant of offspring insulin resistance, even after adjustment for maternal weight status.

In the overall cohort, early childhood exposures, specifically weight gain, contributed significantly to insulin resistance during 1 to 3 years of age. Within the non-GDM cohort, there was also a trend towards greater weight gain being significantly associated with higher insulin resistance at age 3 years. Previously we have seen that weight gain in the first year of life was associated with HOMA-IR at 1 year of life only among GDM offspring.\cite{Borgono et al. 2012} In our cohort, other post-partum environmental exposures such as breastfeeding and age of introduction of solids were not significantly related to insulin resistance.
and as a result, they were not included in the model. However, environmental risk factors in childhood such as nutritional patterns and physical activity may ultimately be reflected in patterns of weight gain. Weight status, in particular, elevated BMI, is a strong risk factor for insulin resistance. (Cree-Green, Triolo, and Nadeau 2013) Indeed, the presence of adiposity appears to beget greater insulin resistance. (Hosking et al. 2011) Abnormal weight changes during early childhood have been in turn linked with cardiovascular disease and type 2 diabetes. (Leunissen et al. 2009) Cross-sectional studies in 696 school-aged children from Brazil have previously demonstrated that weight gain over a 2 year follow-up period is correlated with higher HOMA-IR values. (Lourenço et al. 2014) Similarly, increased fat accretion on DXA has been linked with greater insulin resistance in 238 European children. (Hosking et al. 2011) Our findings are consistent with this literature, confirming that excessive weight gain from birth to 3 years is associated with greater insulin resistance in the overall cohort.

We observed an interesting relationship between gender and insulin resistance which was unique to the non-GDM cohort. Specifically, being female in unexposed offspring was associated with greater insulin resistance from 1 to 3 years of age. This relationship was not present in GDM offspring. Biological differences in insulin sensitivity among males and females have been clearly described during puberty (Moran et al. 2008), with recent prevalence studies confirming that females are over-represented among children and adolescents with obesity and type 2 diabetes (SEARCH for Diabetes in Youth Study Group* et al. 2007; Skinner and Skelton 2014). Sex differences in insulin resistance are also described in childhood prior to the onset of puberty, with studies observing greater insulin resistance among females during infancy and young childhood. (Shields et al. 2007; Murphy et al. 2004; S. C. Jeffery et al. 2018)

Whether this association between gender and insulin resistance is the result of intrinsic vulnerability or post-natal lifestyle factors is not clear. Despite smaller birth weights, female babies do demonstrate
greater insulin resistance, suggesting an underlying intrinsic cause for this resistance. (Shields et al. 2007) At birth, females demonstrate differences in their distribution of adiposity, with more central fatness compared to males. Gender dimorphism with respect to fat accumulation appears to be related to cord insulin levels, particularly in males. However, in females, this patterning is not as strongly associated with cord insulin levels, suggesting other factors may be contributing to this observation. (Eder et al. 2016) To support this, greater insulin resistance in girls has been shown to persist after adjustment for body composition and pubertal status suggesting that it is not simply related to adiposity. (Moran et al. 2008) In fact, it has been postulated that greater insulin resistance demonstrated in females may relate to inherent differences in genetic loci impacting the determination of insulin sensitivity. (Wilkin and Murphy 2006)

A similar finding has also been observed in the Mysore cohort, with only GDM exposed females demonstrating higher fasting insulin levels at 5 years of age on OGTT. (Krishnaveni et al. 2005) The authors speculate on possible contributions from endocrine as well as shared behavioural patterns with regards to physical activity from spending more time with their GDM mothers. In our cohort, it is of interest that exposure to gestational hyperglycemia appears to disrupt this association, such that gender is no longer associated with insulin resistance in GDM offspring. We speculate that perhaps this relationship is subsumed by the impact of maternal GDM status.

With this cohort study design, we observed a relationship between GDM exposure and offspring insulin sensitivity. However, we cannot definitively conclude that the relationship between maternal insulin sensitivity and offspring insulin resistance reflects in utero programming rather than shared genetic risk. Other groups which have employed study designs to follow sibling offspring before and after a diagnosis of maternal GDM have observed higher rates in GDM exposed offspring compared to unexposed siblings, suggesting that this relationship is mediated by programming during a critical developmental
window.(Dabelea et al. 2000) Within the non-GDM cohort in this study, the absence of an association between maternal and offspring insulin sensitivity lends support to the hypothesis that in-utero metabolic programming is the mechanistic mediator of increased risk. We propose that below a certain threshold of glycemia, observed in the non-GDM cohort, maternal insulin sensitivity is no longer a significant determinant of offspring insulin resistance. Although not yet studied, we hypothesize that this may be explained by the fact that a glycemic threshold must be surpassed before epigenetic modification of genes involved in regulation of energy metabolism and determination of insulin resistance take places. If this is truly an effect of metabolic programming, we would expect that the association between maternal insulin sensitivity and offspring insulin resistance observed in the GDM group should become less significant over time, as opposed to a more persistent association which might result from shared genetic risk.
7.2 Objective 2 - Anthropometric Measures in Early Childhood

Our secondary objective was to identify the anthropometric measure of adiposity most strongly associated with cardio-metabolic risk and adiposity in early childhood from one to five years of age. We demonstrated that BMI for age and weight for length were superior anthropometric measures in terms of their association with both adiposity and cardio-metabolic markers, further supporting their use in routine clinical practice. Importantly, our study illustrates that waist to height ratio is associated with contemporaneous biochemical measures of fat mass and total body fat as measured by DXA. Waist to height ratio at one year of age is also highly associated with future obesity at five years of age as defined by BMI. However, unlike in adolescents and adults, waist to height ratio is inferior to existing anthropometric measures of adiposity in early childhood including BMI or weight for length in its association with biochemical metabolic risk or indices of adiposity.

The literature on the use of waist to height ratio in children and adolescents is mixed. Our findings are in line with the group of studies in preschool and early school age children that do not support the use of waist to height ratio. A study of 439 healthy children by Whitrow et al. sought to determine the association between waist to height ratio and systolic blood pressure; they showed that it was not predictive of hypertension in 3 year old children.(Whitrow, Moore, and Davies 2011) Similarly, Corvalan et al. found a statistically significant correlation between waist to height ratio and other indices of body fat in 4 year olds, however it was only weakly associated with biochemical metabolic markers.(Uauy et al. 2010) Finally, Taylor et al. reported that waist to height ratio was correlated with trunk fat mass as measured by DXA in 301 children aged 3 to 5, however waist circumference was overall more strongly correlated.(Taylor et al. 2008). However, there are studies which have demonstrated predictive utility of the waist to height ratio in younger school aged children, aged 3 to 7.(Aristizabal et al. 2015a; Sijtsma et al. 2014; Campagnolo, Hoffman, and Vitolo 2011) Specifically, it appears to be a good proxy for HOMA-
IR and correlates well with other measures of adiposity. The discordance in these studies may reflect several characteristic factors inherent to the different cohorts evaluated in these studies. The significant changes in body proportion which occur in early life render cohort age an important confounding factor. The prevalence of obesity within the cohort may also impact these findings, with population enriched with overweight and obese children more likely to find a significant association. Finally, ethnicity is a recognized moderator of waist to height ratio and metabolic status in adults, which presumably remains relevant in youth as well.

Our results suggest that waist to height ratio may not offer the same advantages in early childhood as it does in adolescence and adulthood. These findings are contrary to our initial hypothesis that waist to height ratio would demonstrate strong correlation with measures of adiposity and cardio-metabolic risk, similar to what has been observed in older children and adolescents. We hypothesize that there may be several reasons to account for this. Firstly, there are significant changes in body proportion which occur between birth and the first few years of life related to differential growth of different body segments. As a result, rapid growth and significant changes in height may invalidate the relationship between waist circumference and length. In contrast, in adults and adolescents, height is a more stable measure by which to standardize changes in waist circumference. Secondly, other studies in neonates have demonstrated that waist circumference is not strongly correlated with measures of truncal and overall adiposity. (Chen et al. 2017) This may be in part due to the technical difficulties which accompany this measurement including movement, variations in feeding status and accurate land marking of the ribs and iliac crest.

Finally, even in our cohort, enriched with “at-risk” offspring exposed to a GDM pregnancy and a maternal history of metabolic disease, signs of metabolic dysfunction in early childhood are subtle. Indeed, the mean BMI z scores in our cohort (0.28±1.06, 0.29 ±1.05 and 0.15±0.98 at one, three and five
years respectively) were greater than reported BMI z scores from another cohort of similar aged children in Toronto, Ontario. (Furlong et al. 2016) Despite this, overt differences in their clinical patterns of adiposity may still be difficult to appreciate. While abnormalities in patterns of insulin resistance have been identified in early infancy (Ong et al. 2004; D A Lawlor et al. 2005), the substantial accumulation of central adiposity which occurs in adolescents and adults may not have had time to develop (Modi et al. 2009). This may reduce the relevance of waist to height ratio as a predictor of cardio-metabolic risk in early childhood.

A second important finding from this research addresses the ongoing question about the optimal anthropometric measures in early childhood for predicting future obesity (S. M. Roy et al. 2016). One of the challenges of obesity prevention stems from the fact that there is no consensus regarding the optimal way to define obesity in early childhood. At present, weight for length is the recommended anthropometric measure for evaluation of growth in young children less than 2 years of age. (Stephen R Daniels, Hassink, and COMMITTEE ON NUTRITION 2015) However, more recently, the WHO Child Growth Standards were published and included BMI references for children under the age of 2 years. (WHO Multicentre Growth Reference Study Group 2006). In young children, there appears to be good agreement between weight for length and BMI (Furlong et al. 2016), however, recent evidence suggests that BMI may be more predictive of future obesity risk. (S. M. Roy et al. 2016) Sum of skin fold thickness is a useful measure, however its cumbersome technique and significant intra-observer variability limit in use in practice.

In our study, both weight for length and BMI z score demonstrated comparably strong associations between fasting biochemical measures and fat mass as measured by DXA. Both measures at 1 year of age were comparably correlated with future BMI z score at 5 years. Interestingly, at one year of age, weight
for length z score was significantly associated with fasting insulin and leptin, while BMI z score was only associated with leptin. Over time, both BMI z score and weight for length showed increasing and comparable correlations with either fasting insulin or HOMA-IR as well as leptin. This may suggest that weight for length begins to correlate with insulin resistance at an earlier age than BMI. Indeed, weight for length had the strongest relationship to fasting insulin over the 3 time points. This is further supported by studies which have compared BMI and weight for length z score and found that the greatest degree of discordance between the two measures exists in the first year of life during the peak of infant adiposity (Sani M Roy et al. 2016). Nonetheless, based on the fact that weight for length is not recommended as a measure of adiposity beyond two years of age, we suggest that BMI z-score remains the most practical measure to assess overweight/obesity in the clinical setting longitudinally.

7.3 Strengths and Limitations

This study has several strengths. To our knowledge, this study is the first to evaluate insulin resistance in GDM offspring as well as waist to height ratio in a cohort of children in this age group. Other strengths of this study include the prospective and longitudinal evaluation of offspring with criterion measures of anthropometric and metabolic outcomes. Methods involved the use of multiple measures to assess adiposity including anthropometric measurements, serum biochemical markers and body composition indices. We obtained indices of insulin resistance derived from fasting blood work samples in very young children. Data was collected using repeated measures with standardized techniques longitudinally, allowing us to evaluate the outcome over time. The selected longitudinal modeling technique incorporates all available data while making accommodations for missing data points and avoiding removal of subjects with incomplete information.
Our study also has some limitations, many of which are related to the longitudinal nature of our cohort study design. As a result of subject attrition, there was a reduction in the number of subjects over time. However, the drop-out rate remains greatest from 3 months to 1 year, with relatively stable rates of attrition after this time. Many of the mother-infant dyads in this cohort are first time parents with otherwise healthy children. The newborn period represents a difficult time for parents and families, rendering study follow-up visits more challenging to attend. Furthermore, parents of healthy children may not be as motivated to participate in follow-up visits. It is possible that children with a history of GDM may have been more motivated to participate in study follow-up, thus biasing the composition of the cohort over time. However, the proportion of women with GDM remained relatively consistent in the years with no differential attrition. This makes it less likely that subject drop-out resulted in a systematic bias resulting from the inclusion of children with significantly different cardio-metabolic status. Furthermore, our selected modeling approach allows inclusion of subjects despite missing data which is related to attrition.

As a result of the relatively small numbers of participants at 3 and 5 years, we were not able to analyze the results based on specific ethnic group. Ethnic background is an important variable which is known to moderate the strength of association between adiposity and cardio-metabolic outcomes (David S Freedman et al. 2008). To address this, we instead classified subjects into Caucasian and non-Caucasian groups which likely accounted for some of the ethnic variation in growth and cardio-metabolic status.

While our cohort included representation from a diversity of ethnic groups, 70.0% of the mothers and 67.2% of the fathers identified as Caucasian. In contrast, the city of Toronto is comprised of a greater percentage of visible minority groups, with only 47.7% identifying as Caucasian (Statistics Canada 2017). Furthermore, on average, our cohort was comprised of a higher education level which is a proxy for
socioeconomic status (Hamilton et al. 2010). This may limit the generalizability of these findings to a larger, more socially or ethnically diverse population. As we chose to focus primarily on biologic drivers of metabolic health, our analysis did not account for parental education level or socio-economic status. In general, women of higher socioeconomic status are less likely to be obese and have lower rates of cardiovascular disease (Havranek et al. 2015). They are also more likely to experience superior health outcomes and care (Ibáñez et al. 2018). This translates into less disease burden and in utero exposure for offspring and makes potential differences between offspring more subtle.

In our cohort, we collected information about pre-pregnancy maternal BMI and weight gain preceding OGTT, however we were unable to determine overall maternal weight gain, estimate glycemic control during pregnancy or account for maternal lifestyle factors. Successful treatment of gestational diabetes resulting in improved glycemic control during pregnancy has been shown to attenuate the effects of increased adiposity in the offspring (Hillier et al. 2007). Maternal glycosylated haemoglobin (HbA1c) and neonatal cord C-peptide have been proposed as potential means to quantify gestational hyperglycaemia (O’Rahilly et al. 1987). Similarly, both the extent and timing of gestational weight gain have been identified as important determinants metabolic risk in the offspring (Ravi Retnakaran et al. 2018). Finally, maternal lifestyle behaviours such as physical activity and nutrition are recognized to affect maternal insulin sensitivity and glycemic outcomes during pregnancy (Bgeginski et al. 2017). These variables were not controlled for and may account for some of the observed variation in offspring insulin resistance.

Finally, estimation of insulin resistance and insulin secretion can be achieved through direct techniques such as the glucose clamp technique or minimal model approach or indirect measures derived from fasting insulin and glucose values. We chose HOMA-IR which is a surrogate measure that is easy to use
as it is technically simple and cost-effective. It has been validated in youth with normal glucose
tolerance (Gungor et al. 2004), as well as children and adolescents with obesity, (Conwell et al. 2004)
however it may be less accurate compared to direct techniques. (Lorenzo et al. 2010) In addition to the
reduced accuracy, some additional drawbacks of these measures include the fact that they are not
commonly used in clinical practice, and therefore no clear diagnostic thresholds for abnormal insulin
resistance have been defined children.
7.4 Clinical Implications of this Work

Taken together, these findings build upon our existing understanding of metabolic programming and can hopefully inform future initiatives to shift metabolic trajectories in vulnerable offspring. The results of this study demonstrate that metabolic differences among offspring exposed to GDM are subtle with no differences in their absolute measures of insulin resistance or adiposity in early childhood. However, there is a strong body of literature which supports the concept that this group of children remain at higher metabolic risk later in life. This highlights the importance of identifying maternal GDM status as a risk factor in a young child who may otherwise be clinically indistinguishable from their peers.

Pregnancy and early childhood represent a crucial opportunity for health prevention efforts. Our findings would suggest that infants who are exposed to reduced maternal insulin sensitivity during pregnancy and who then experience greater weight gain during early childhood, are at the greatest risk of insulin resistance in early childhood. Therefore, targeting women with risk factors for impaired insulin sensitivity (i.e. history of previous gestational diabetes or fetal macrosomia) in the peri-conception period may be one strategy for improvement of inter-generational metabolic outcomes. Physical activity, in particular aerobic or resistance exercise, has been shown to improve glycemic control among pregnant women with GDM and could may represents a promising intervention. (Harrison et al. 2016) Achievement of tight glycemic control during pregnancy may also limit excessive fetal exposure to hyperglycemia during pregnancy. In the post-pregnancy period, careful monitoring of offspring adiposity and promotion of health behaviours which mitigate excessive weight gain represents another tactical approach. Interestingly, there is some evidence that GDM exposure may impact hypothalamic satiety signaling and in turn, offspring eating behaviours. (Shapiro et al. 2017) This has important implications for GDM offspring, potentially putting them at-risk for excessive weight gain. Targeted anticipatory guidance to families with GDM offspring, counseling about the risks sugar sweetened beverages and excessive screen
time may be beneficial. Indeed, these factors have been shown to be closely linked with early obesity and metabolic syndrome and may have particular impact in at-risk offspring (Chan et al. 2014) Multi-disciplinary educational interventions which target prevention of childhood obesity in kindergarten classes have been shown to effectively stabilize BMI z scores and reduce prevalence of obesity in the short term, however longer term studies are required to demonstrate the sustainability of such efforts.(Hu et al. 2017) Our findings also reinforce the value of clinical growth monitoring using BMI and weight for length in order to effectively screen for cardio-metabolic risk.

Our results demonstrate that fasting insulin levels demonstrated a trend of being higher among GDM offspring at 5 years of age, however the significance of this finding remains unclear. This may represent an early sign of dysfunction as fasting hyperinsulinemia has been shown to be highly associated with future obesity. Young children, aged 5 to 9 years, with normal glucose tolerance, were followed prospectively and found to have an association between their fasting insulin level in early childhood and risk of correlated with future weight gain.(Odeleye et al. 1997) However, future analyses, focusing on these outcomes at 5 years, will be required to better understand this relationship.

The finding of gender differences with regards to insulin resistance is of particular clinical relevance. If female gender is indeed associated with greater insulin resistance in young childhood, this raises additional concern as these young girls grow older and become the future generation of pregnant mothers. Investing in initiatives which target the girls in early childhood will potentially impact the vicious cycle of inter-generational transmission of cardio-metabolic disease. Although some evidence suggests that these metabolic differences are biologically intrinsic and present at birth(Shields et al. 2007), efforts can be made to moderate this risk. There is some evidence to suggest that female toddlers are more sedentary, engaging in less physical activity than their male counterparts(Hager et al. 2016). Finding gender-specific
ways to engage young girls in programs may be an effective avenue through which to achieve improve cardio-metabolic health. Certainly, early adoption of healthy exercise habits may have long lasting benefits for their adult life. As children’s choices around nutrition and physically activity often closely mirror that of their parents, programs which engage both mothers and daughters may represent an effective strategy to help both present and future pregnancies.(Reed et al. 2017)
8. CONCLUSIONS

In conclusion, we present evidence that absolute measures of metabolic function are not significantly different in GDM exposed children in the first three years of life, however the factors which determine their insulin resistance are unique. In both groups, increasing age is associated with increasing insulin resistance, highlighting the natural history of insulin sensitivity in childhood. Maternal insulin sensitivity is associated with insulin resistance only among GDM offspring whereas gender is an important determinant in unexposed children. In light of the current findings, we hypothesize that GDM offspring have a distinct pathway towards the future development of metabolic disturbance. Intrauterine exposure to hyperglycemia appears to alter glucose and insulin metabolism in offspring, ultimately mediating the increased transmission of cardio-metabolic disease evidenced in later life.

Ensuring the use of accurate anthropometric measures for detection of cardio-metabolic risk in early childhood is critical as this allows for appropriate identification of at-risk children. This may help to further stratify at-risk children, such as those with a history of exposure to maternal obesity or gestational diabetes. Our findings lend further support to the routine use of weight for length or BMI z score in early childhood. Unlike among adults, measures of central adiposity such as waist to height ratio are not superior to BMI and weight for length. Finally, weight for length and BMI z score are comparable in their association with contemporary measures of adiposity and biochemical cardio-metabolic risk in early childhood.
9. FUTURE DIRECTIONS

Further insight remains to be gained from continued follow-up of this cohort. Increasing age and environmental exposures are likely to progressively uncover more overt metabolic dysfunction in exposed and unexposed children. Following insulin resistance and adiposity during the peri-pubertal period may help to further elucidate the long-term trajectory of GDM exposed children. These findings have also highlighted potential areas for clinical intervention. Indeed, pathways to insulin resistance in GDM offspring are distinct from their unexposed peers and therefore unique strategies targeting children with a history of GDM could be implemented. Future studies should encompass evaluation of these proposed interventions, looking at relevant short term outcomes of adiposity and longer term measures such as impaired glucose tolerance and cardio-metabolic disease. While insulin resistance is a relevant clinical outcome, other emerging biomarkers may help to further elucidate the mechanistic pathways which underlie the metabolic differences in GDM offspring. Differences in gene methylation patterns, microbiome composition and other novel biomarkers may yield important clues about the evolution of metabolic risk.

Our findings add to the growing body of literature which supports the use of BMI in early childhood to identify children with overweight and obesity. The ability to use one single measure of adiposity across the entire pediatric and adult age range is advantageous for healthcare providers and patients. However, the ability of BMI to predict future health risk is not well understood. Further research evaluating the associations between early childhood BMI and longer term outcomes of cardio-metabolic disease is required.
While it appears that both gestational hyperglycemia and maternal pre-pregnancy obesity independently impact metabolic risk in offspring, further exploration of other moderating factors such as gestational weight gain will be important. In women with normal glucose tolerance during pregnancy, both gestational weight gain and pre-pregnancy BMI are independently associated with offspring adiposity and leptin levels. (Perng et al. 2014) For obese pregnant women with GDM, limiting excessive weight gain during the remainder of the pregnancy may represent another opportunity to mitigate fetal over-nutrition. Finally, sex differences in insulin resistance during early childhood may be an important area of further investigation. Our findings raise important questions about gender-related vulnerabilities in insulin sensitivity, however little is understood about the fundamental genetic and hormonal differences which may explain this.
Figure 1 – Early Childhood Determinants of Cardio-metabolic Risk

- Maternal Genetic Risk
- Paternal Genetic Risk
- Ethnicity
- Maternal Smoking

- Physical Activity
- Nutrition
- Sleep Patterns
- Overall Health and Disease
- Patterns of Weight Gain

- In Utero Factors
- Pre-Conception Factors
- Post Natal Factors

Offspring Cardio-metabolic Risk

- Adiposity (BMI Z Score)
- Insulin Resistance (HOMA-IR)
Figure 2 – Pathophysiology of Maternal Gestational Diabetes and Offspring Cardio-Metabolic Risk
Figure 3 – Proposed Exposure Model

Exposure: Maternal Gestational Diabetes

Confounders: Maternal Obesity

Covariates: Offspring Age, Offspring Gender, Offspring Ethnicity

Outcome: Offspring Insulin Resistance
Figure 4 – Flow Diagram showing Study Protocol

Study Recruitment

<table>
<thead>
<tr>
<th>Non-GDM</th>
<th>Birth</th>
<th>1 year</th>
<th>3 years</th>
<th>5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDM</td>
<td></td>
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Anthropometrics, Bloodwork, DXA scan
Figure 5 - Flow Diagram Showing Derivation of Study Population

- **Recruitment**: N=790
- **At Birth**: A, n=733
- **3 months**: A, n=592
- **12 months**: A, n=406
  - BW, n=175
- **36 months**: A, n=112
  - BW, n=91
- **60 months**: A, n=94
  - BW, n=83

Twin Pregnancy
Small for Gestational Age
Prematurity < 32 weeks
Genetic Syndrome
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12. APPENDIX

Statement of Contributions

As part of this thesis project, my role included direct participation in the established longitudinal study as well as analysis of study data. Participation in the study involved subject recruitment, completion of study visits, data collection and entry. The data used in the analysis included both subjects collected prior to and during my involvement in the study.

Study Concept: N.C. and J.H.

Study Design: J.H., R.R. and N.C.

Statistical Analysis: S.C. and N.C.

Result Interpretation: N.C. and J.H.

Manuscript Revision: J.H., R.R., A.H. and C.B.
J.H. – Jill Hamilton

R.R. – Ravi Retnakaran

S.C. – Shiyi Chen

A.H. – Anthony Hanley

C.B. – Catherine Birken