# PRETERM BIRTH AND INSULIN RESISTANCE IN ADULTHOOD

# EXAMINING DETERMINANTS OF INSULIN RESISTANCE IN ADULTS BORN AT NORMAL AND EXTREMELY LOW BIRTH WEIGHT

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## ABSTRACT

The intrauterine environment and early postnatal period are important determinants of metabolic adversity in later life. The association between low birth weight at *term* birth and an increased risk for insulin resistance (IR) in adulthood is well established. However, less is known about this association in those born markedly *preterm*. Since lower birth weights are more prevalent among individuals born preterm, we compared and examined determinants of IR in adults born preterm with extremely low birth weight (ELBW; birth weight <1000g) and adults born at full term with normal birth weight (NBW; birth weight ≥2500g). Determinants of IR included central fat, total adiponectin concentrations, total lean mass adjusted for height (lean mass index, LMI) and birth weight. This project was part of a longitudinal study in a subset of 110 (60 NBW and 50 ELBW; 40 males and 70 females) adults aged 28-34 years who have been followed since birth. IR was measured by homeostasis model assessment of insulin resistance (HOMA-IR). Central fat was measured by both waist circumference (WC) and dual X-ray absorptiometry (DXA). Total lean mass was measured by DXA, total adiponectin was quantified from serum samples by enzyme-linked immunosorbant assay (ELISA), and birth weight was obtained from medical records. Compared to their NBW counterparts, we have identified for the first time at this age, that individuals born ELBW have higher central adiposity, reduced lean mass for height (LMI) but no difference in circulating adiponectin concentrations and IR. Within the cohort, central adiposity, measured by either WC or DXA, is the most important determinant of IR in adults born ELBW or NBW. Furthermore, in a subgroup analysis of ELBW adults, those born small

for gestational age (SGA) at birth have poorer health outcomes compared to those born appropriate for gestational age (AGA), having higher total body fat, central adiposity, 2-hour glucose levels, 2-hour insulin levels and lower total adiponectin concentrations. Interestingly, adults born ELBW and AGA at birth were similar to NBW controls in all clinical characteristics and health outcomes assessed. Perhaps a more unfavourable body composition characterized by higher central adiposity and lower lean mass in adults born ELBW may link small size at birth with an increased risk for type 2 diabetes and cardiovascular disease later in adulthood.

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## DEDICATION

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AGA | Appropriate for gestational age |
| AMPK | Activated protein kinase |
| BMI | Body mass index |
| CT | Computed tomography |
| CV | Coefficient of variation |
| CVD | Cardiovascular disease |
| DOHaD | Developmental origins of health and disease |
| DXA | Dual X-ray absorptiometry |
| ELBW | Extremely low birth weight |
| ELISA | Enzyme-linked immunosorbant assay |
| HMW | High molecular weight |
| HOMA | Homeostasis model assessment |
| IFG | Impaired fasting glucose |
| IGT | Impaired glucose tolerance |
| IκK-β | IkappaB kinase |
| IL | Interleukin |
| IR | Insulin resistance |
| IUGR | Intrauterine growth restriction |
| JNK | c-Jun N-terminal kinase |
| LBW | Low birth weight |
| LMW | Low molecular weight |

| | |
|---|---|
| MMW | Medium molecular weight |
| MCP-1 | Monocyte chemotactic protein-1 |
| MRI | Magnetic resonance imaging |
| NBW | Normal birth weight |
| NFκβ | Nuclear factor kappa B |
| NICU | Neonatal intensive care unit |
| NGT | Normal glucose tolerance |
| PAI-1 | Plasminogen activator-1 |
| SGA | Small for gestational age |
| T2D | Type 2 diabetes |
| TNF-α | Tumour necrosis factor alpha |
| VLBW | Very low birth weight |
| WC | Waist circumference |
| WHO | World Health Organizatio |

## CHAPTER 1: BACKGROUND

Over the last ten years, the prevalence of type 2 diabetes (T2D) has been increasing in both developed and developing countries. T2D, also known as non-insulin dependent diabetes or adult-onset diabetes, results from the body's ineffective response to insulin (i.e. insulin resistance) and reduced β-cell function. Although T2D prevalence increases with age, it is becoming more apparent in younger populations; earlier in adulthood, adolescence and even presenting in childhood (Dabela et al., 1998; Rosembloom et al., 1999), highlighting the importance of disease prevention and management. In 2008/09, ~ 2.4 million Canadians aged one year and older were living with diabetes (either type 1 or type 2), in which 1.6% of this total represented adults between 30 and 34 years of age (WHO, 2011). T2D represents approximately 90% of individuals living with diabetes, with the increasing rates largely attributable to overweight and obesity (WHO, 2011).

The link between obesity and T2D is well established. In Canada, the median body mass index (BMI) for adults with diabetes is 29 $kg/m^2$; this is four units higher than the median BMI of 25 $kg/m^2$ for adults without diabetes, highlighting that approximately three quarters (75.6%) of Canadians with diabetes are classified as overweight or obese (WHO, 2011). Furthermore, at every age the prevalence of diabetes is higher among overweight and obese individuals (WHO, 2011).

One of the major links between obesity and T2D is insulin resistance (IR): a physiological state in which the body does not respond properly to the effects of insulin on glucose homeostasis. Although metabolic complications like obesity, IR and T2D arise

due to a combination of genetic and lifestyle factors, the intrauterine environment and early postnatal period are gaining increased attention as important periods contributing to these complications in later life (Hofman et al., 2004). Epidemiological studies have highlighted the association between low birth weight and an increased risk of cardio-metabolic complications in adulthood. These associations have been described in populations of different age, sex, and ethnic origin, occurring independently of current weight and level of exercise (Fowden, Giussani, & Forhead, 2005). Attempts to uncover the mechanisms behind this association have led to the recognition that individuals with lower birth weights have impaired glucose tolerance related to both reduced insulin secretion and increased insulin resistance (Hofman et al., 1997, Gray et al., 2002).

This thesis project focuses on examining determinants of insulin resistance in adults born preterm with extremely low birth weight compared to adults born at full term with normal birth weight. The project concentrates on the importance of body composition in connecting small size at birth with an increased risk of metabolic adversity (i.e. insulin resistance, T2D and cardiovascular disease). The following sections of the literature review will be divided into three components. The first section will provide a general overview of insulin resistance and factors associated with its development. The second will explore the theory of fetal origins and fetal programming, as well as describe the growth and development of preterm, low birth weight individuals, in particular those born at ELBW. The third section will highlight what is currently known in the literature about prematurity with low birth weight, insulin resistance and the determinants discussed in section one of the literature review.

### 1.1 Insulin

Insulin is a potent anabolic peptide hormone secreted by the pancreatic β-cells in response to elevated glucose and amino acid levels. Insulin has diverse functional roles within the body; however it is best known for its regulation of normal blood glucose (euglycemia). Insulin maintains euglycemia by promoting glucose uptake in adipose tissue, skeletal muscle and the liver (Saltiel & Kahn, 2001). When blood glucose levels are high (i.e. after a meal), insulin levels rise, prompting glucose uptake in adipose tissue and skeletal muscle by stimulating translocation of the glucose transporter GLUT4 from intracellular sites to the plasma membrane (Pessin, Thurmond, Elmendorf, Coker, & Okada, 1999). In the liver, insulin inhibits glucose production (glycogenolysis and gluconeogenesis) whilst stimulating glycogen synthesis to regulate blood glucose homeostasis (Saltiel & Kahn, 2001). Insulin also plays an important role in lipid metabolism; increasing lipid synthesis (lipogenesis) in the liver and adipose tissue, whilst decreasing fatty acid release from adipose tissue into the circulation (Saltiel & Kahn, 2001).

### 1.2 Insulin resistance: a general overview

Insulin resistance (IR) is present when there is reduced responsiveness of the body's target tissues to the effects of insulin on glucose uptake, utilization and storage. It is a state that requires more insulin to maintain eugylcemia usually achieved by a lower amount of insulin in the normal state. The terms reduced insulin sensitivity and insulin resistance are often used interchangeably in the literature (i.e. lower insulin sensitivity = greater insulin resistance). As previously mentioned IR is an important feature of T2D,

preceding its development (Reaven, 2005, Tilg & Moschen, 2008). IR is also frequently associated with obesity, particularly central obesity (WHO, 2011). IR manifests when the body does not respond appropriately to rising insulin concentrations when glucose levels are elevated. Therefore, hepatic glucose output is not suppressed and there is decreased insulin-stimulated glucose uptake in adipose tissue and skeletal muscle, contributing to sustained hyperglycemia (high blood glucose) and hyperinsulinemia (high blood insulin) (Saltiel & Kahn, 2001). Eventually, insulin levels drop with accompanying β-cell dysfunction in overt T2D.

IR also precedes the development of dysglycemia (also termed pre-diabetes); a general term referring to an intermediate stage between normal glucose tolerance (NGT) and overt T2D (Defonzo & Abdul-Ghani, 2011). Dysglycemia refers to two groups of individuals, those with impaired fasting glucose (IFG) and those with impaired glucose tolerance (IGT). According to the World Health Organization criteria, IFG is defined as a fasting glucose concentration of 6.1-6.9 mmol/L, whereas IGT is defined as a 2-hour glucose concentration of 7.8-11 mmol/L after a standardized 75g oral glucose load (WHO, 2011). Furthermore, T2D is defined as a fasting glucose of ≥7.0 mmol/L *or* a 2-hour plasma glucose concentration of ≥11.1 mmol/L (WHO, 2011). IR develops simultaneously in multiple organs, however the severity of IR may differ among tissue types (Abdul-Ghani, Matsuda, Balas, & DeFonzo, 2007). Individuals with isolated IFG typically have moderate IR in the liver and normal to near-normal insulin sensitivity in muscle, while those with isolated IGT have moderate to severe IR in muscle (Defonzo & Abdul-Ghani et al, 2011). Furthermore, subjects with IFG and IGT are at high risk for

developing T2D (Gerstein et al., 2007). IFG, IGT and T2D indicate that there is insufficient insulin regardless of the degree of insulin resistance; dysglycemia develops when IR reaches the extent that the pancreatic β-cells are incapable of producing sufficient insulin to maintain normoglycemia. Therefore, identification of IFG and IGT may be indicative of IR and/or pancreatic β-cell dysfunction.

The hyperinsulinemic-euglycemic clamp technique is the gold standard for measuring whole-body insulin sensitivity (DeFonzo, Tobin, & Andres, 1979) (Refer to Appendix A). When combined with radiolabeled glucose, the individual contribution of hepatic and muscle IR to the defect in whole-body glucose disposal can be determined (DeFonzo, Simonson, & Ferrannini, 1982). The clamp method is very laborious, time-consuming and difficult to implement in clinical settings and large epidemiological studies (Abdul-Ghani et al., 2007). Therefore, in recent years, surrogate measures of whole-body insulin sensitivity have been developed from glucose and insulin concentrations (reviewed recently in Muniyappa et al., 2008 and Singh & Saxena, 2010). These measures correlate well with those derived from the clamp technique and provide a simple measure for whole-body insulin sensitivity (Abdul-Ghani et al., 2007). However, they do not provide information regarding the relative contributions of liver versus skeletal muscle to observed reductions in whole-body insulin sensitivity (Abdul-Ghani et al., 2007).

### 1.3 Insulin resistance: pathology

The pathology of insulin resistance is quite complex, and there are many determinants associated with its development (Figure 1). At the physiological level, body

composition (i.e. obesity and lower lean mass), inactivity, poor dietary habits, ageing, early life factors and certain life stages (i.e. puberty and pregnancy) are some of the determinants associated with IR development. At the molecular level, the pathology of IR is multifaceted, resulting from the complex interplay between nutrient and systemic fatty acid excess, adipose tissue inflammation, hypoxia, and endoplasmic reticulum (ER) and oxidative stresses (Hotamisligil, 2006, Regazetti et al., 2009). Inflammatory cytokines, fatty acid derivatives (ceramides and diacylglyerol), and reactive oxygen species (ROS) target key components of the insulin signalling pathway, leading to altered insulin signal transduction, reduced insulin action and increased insulin resistance in target tissues (Tanti & Jager, 2009). Partial failure of or disruptions to critical components of the insulin signalling pathway such as serine phosphorylation of insulin receptor substrate (IRS) proteins are associated with IR (Figure 2) and other metabolic disorders including: glucose intolerance, hypertension and dyslipidemia (Tanti & Jager, 2009; White, 2002). Within the scope of this thesis project, we will focus on the role of body composition (adipose tissue and lean mass), body fat distribution (central vs. peripheral adiposity) and adiponectin concentrations in relation to insulin resistance.

### 1.4 Determinants of insulin resistance: the role of body composition

The roles of both adipose tissue (amount and distribution) and skeletal muscle are key to understanding the effects of body composition on the development of IR.

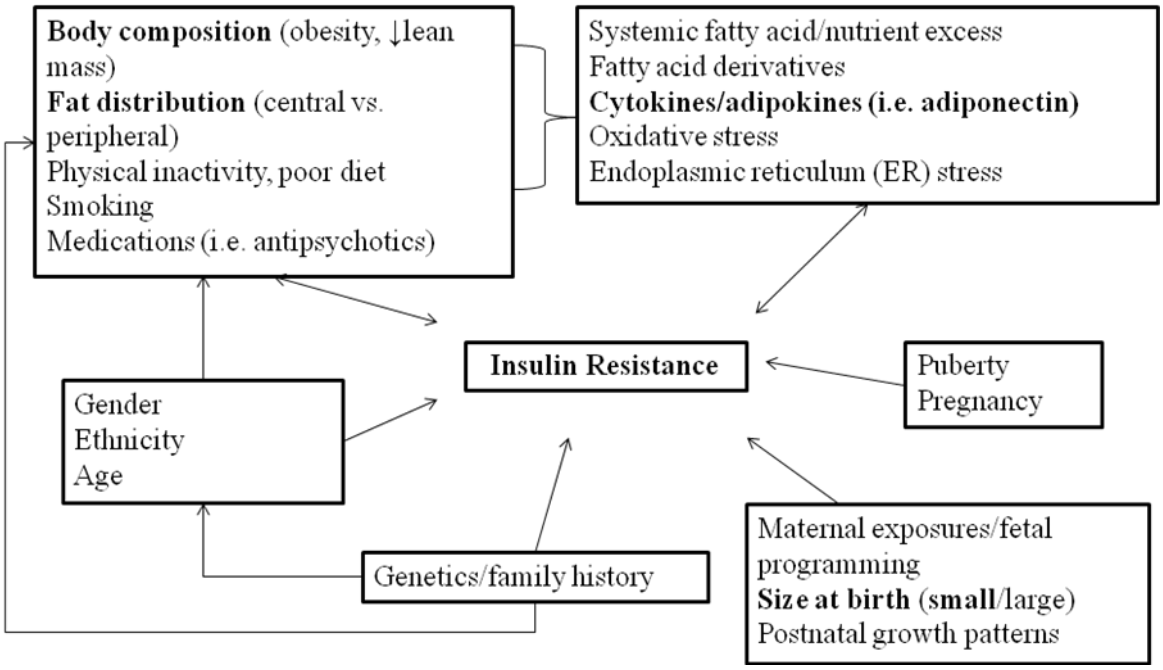


**Figure 1. Determinants of insulin resistance: physiological and molecular factors.** The determinants in bold indicate those determinants focused upon in this report in relation to insulin resistance (body composition, fat distribution, adiponectin concentrations and small size at birth). The bracket connecting the two top boxes indicates some of the molecular mechanisms of IR (i.e. systemic fatty acid/nutrient excess, fatty acid derivatives, cytokines/adipokines, oxidative stress and endoplasmic reticulum stress) associated with the physiological factors (i.e. body composition, fat distribution, physical inactivity, smoking, medications) on the right.

### 1.4.1 Adipose tissue

White adipose tissue (referred to as adipose tissue) is an endocrine organ. Adipose tissue plays a prominent role in the development of adverse metabolic conditions like obesity, dyslipidemia, CVD, insulin resistance and T2D (Andersson et al., 2008).

It is well established that obesity and insulin resistance are closely related (Kahn & Flier, 2000). Overweight and obesity are the most prominent risk factors for insulin resistance and subsequent type 2 diabetes (WHO, 2011).

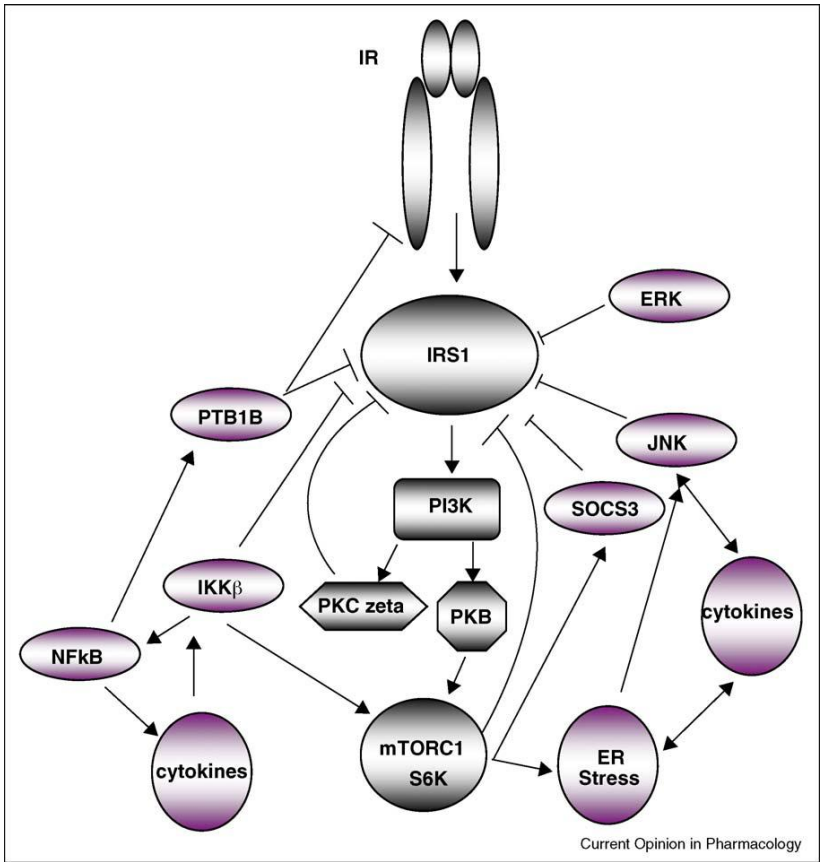


**Figure 2. The insulin signaling pathway and insulin resistance.** Insulin binds to its receptor (IR) inducing complex signaling cascades. Insulin receptor-mediated tyrosine phosphorylation of insulin receptor substrate (IRS) proteins results in the transduction of the insulin signal. Serine kinases are activated to target IRS proteins to downregulate insulin signaling. Some of these kinases such as PKC zeta and mTORC1/S6K are involved in both the propagation of the insulin signal and its termination through a negative feedback loop. Other serine kinases (purple), in particular JNK, IKKβ/NFκB, are activated by inflammatory cytokines, fatty acids, amino acids and reactive oxygen species (ROS). These kinases inhibit IRS proteins via serine phosphorylation resulting in attenuation of the insulin signal and subsequent insulin resistance. Abbreviations: IR, insulin receptor; IRS, insulin receptor substrate; PI3K phosphatidylinositol 3-kinase; mTORC, mammalian target of rapamycin complex; ERK, extracellular signal regulated kinase; JNK, c-Jun N-terminal kinase; SOCS, suppressor of cytokines signaling; ER stress, endoplasmic reticulum stress; IKKb, inhibitory-kB kinase b; NF-kB, nuclear factor-kB; PTP1B, protein tyrosine phosphatase 1B.

Excess adiposity, particularly in the central compartment of the body (central adiposity), is believed to be more detrimental to metabolic health as central fat correlates strongly with the amount of visceral adipose tissue; fat surrounding the organs within the abdominal cavity (Hayashi et al., 2008). The terms central and visceral fat are often used interchangeably in the literature, however they are not necessarily the same. Central fat will be referred to when waist circumference (WC) or dual X-ray absorptiometry (DXA) methodologies are used to measure adipose tissue in the central segment of the body. These methodologies do not distinguish between visceral and subcutaneous fat depots in the abdominal region. Visceral fat on the other hand will be referred to when visceral adipose tissue is measured in the central segment of the body using computed tomography (CT) or magnetic resonance imaging (MRI). These methodologies are able to discriminate between visceral and subcutaneous fat depots.

Excess central adiposity correlates strongly with increased IR in both animals and humans (Hayashi et al., 2008; McLaughlin, Lamendola, & Abbasi, 2011). The specific mechanisms underlying this association remain unclear; however plausible hypotheses have been suggested. They include: 1) that central adipocytes are more lipolitically active and less insulin sensitive compared to peripheral subcutaneous adipocytes (fat cells beneath the skin), 2) that products of central adipose tissue have direct access to the portal circulation compared to those of subcutaneous adipose tissue, and 3) that central obesity is associated with the dysregulation of cytokine and adipokine production and secretion, highlighting the role of inflammation in insulin resistance pathogenesis (Alberti & Zimmet, 1998; Kahn & Flier, 2000; Maury & Brichard, 2010).

#### 1.4.1.1 Adipose tissue and inflammation

A cytokine is a protein produced and released by cells that has a specific effect on the interactions/communications between cells or on the behaviour of cells. An adipokine is a cytokine produced and secreted exclusively by adipocytes (fat cells). Adipose tissue produces and secretes various cytokines and adipokines. These include the *adipokines* adiponectin and leptin, as well as a vast number of *cytokines* such as tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor (PAI-1) to name a few (Maury & Brichard, 2010). Adiponectin and leptin are exclusively produced and secreted by adipocytes, whereas *cytokines* are produced and secreted by both adipocytes and infiltrating AT immune cells (i.e. macrophages) (Maury & Brichard, 2010). Cytokines and adipokines can be pro-inflammatory (TNF-α), anti-inflammatory (adiponectin) or possess characteristics of both (IL-6) (Tilg & Moschen, 2008).

Obesity (particularly central adiposity), IR and T2D are associated with a state of systemic low grade inflammation also known as metabolic inflammation; characterized by an increase in pro-inflammatory cytokines/adipokines (i.e. TNF-α, IL-6) and a decrease in anti-inflammatory cytokines/adipokines (i.e. adiponectin) (Hotamisligil 2005; Wellen & Hotamisligil, 2005). Mechanistically, pro-inflammatory cytokines have been shown to target key components of the insulin signalling pathway leading to the subsequent activation of inflammatory pathways (i.e. JNK and IKKβ/NFκβ). Activation of these pathways heightens the inflammatory response resulting in altered insulin signal

transduction, reduced insulin action and increased insulin resistance (Maury & Brichard, 2010; Tanti & Jager, 2009).

#### 1.4.1.2 Adiponectin: an anti-inflammatory & insulin sensitizing agent

Adiponectin is an anti-inflammatory, anti-atherogenic protein with profound insulin-sensitizing properties (Maury & Brichard, 2010), produced and secreted exclusively by adipose tissue. Regarding structure, adiponectin has a carboxyl-terminal globular domain and an amino-terminal collagen domain and combines via its collagen domain to create three major multimeric forms in the human circulation: a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and a high-molecular weight (HMW) 12- to 18-mer (Waki et al., 2003). The HMW isoform is considered to be the active form of the protein (Aso et al., 2006; Ebinuma et al., 2006). Measurement of total adiponectin refers to quantification of all three multimeric forms in serum or plasma. Adiponectin can exist as full-length or a smaller, globular fragment; however, almost all adiponectin appears to exist as full-length adiponectin in circulation (Kadowaki and Yamauchi, 2005). The beneficial effects of adiponectin are mediated by two cell-membrane receptors: adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) (Kadowaki and Yamauchi, 2005). In both humans and rodents, both adiponectin receptors are expressed primarily in adipose tissue, skeletal muscle and the liver (Kadowaki and Yamauchi, 2005).

Adiponectin is inversely correlated with parameters of total adiposity; body mass index (BMI), fat mass and body fat percentage (Gavrilla et al., 2003; Lindsay et al., 2002; Stefan et al., 2002). It is also inversely associated with IR independent of fat mass (Cnop

et al., 2003; Matsurba, Makuoka, & Katayose, 2002; Yamamoto et al., 2002). Lower levels of adiponectin have been observed in patients with obesity and T2D (Arita et al., 1999, Hotta et al., 2000, Weyer et al., 2001), and lower concentrations predict future insulin resistance (Hayashi et al., 2008). Furthermore, studies have shown that central fat is a strong negative predictor of serum adiponectin and may provide a link between central obesity and insulin resistance (Cnop et al., 2003). Adiponectin also improves glucose transport and fatty acid oxidation (Maury & Brichard, 2010; Wajchenberg et al., 2002). Mechanistically, adiponectin enhances insulin sensitivity via activation of AMP-activated protein kinase (AMPK) resulting in the reduction of hepatic gluconeogenesis and an increase in skeletal muscle glucose transport (Yamuchi et al., 2002). Additionally, adiponectin decreases triglyceride content in the liver and skeletal muscle, as well as enhances fatty acid oxidation and energy consumption (Kadowaki et al., 2006). It is also believed to attenuate TNF-α expression and adipose tissue inflammation to further improve insulin sensitivity (Maury & Brichard et al., 2010).

### 1.4.2 Skeletal muscle

Skeletal muscle composes 40-50% of total body mass and is the predominant site for whole-body insulin mediated glucose disposal (accounting for approximately 80%) in the fed state (Du et al., 2010; Srikanthan and Karlamangla, 2011). Skeletal muscle is a key site for glucose and fatty acid utilization, and hence plays an important role in the pathogenesis of insulin resistance and subsequent T2D (Du et al., 2010, Phelix & Mensink, 2011). Reductions in skeletal muscle mass are associated with increased insulin resistance in both animals and humans (Du et al., 2010). A recent cross-sectional study

with over 13, 000 participants showed that across indices of skeletal muscle mass, higher muscle mass (relative to body size) was associated with improved insulin sensitivity and a lower risk of pre and overt diabetes (Phelix & Mensink, 2011). Skeletal muscle insulin resistance is apparent long before overt hyperglycemia in T2D (Defonzo & Tripathy, 2009) and may result from: 1) disruptions in fatty acid metabolism; increased fatty acid transport into the skeletal muscle (i.e. obesity induced) and/or reduced fat oxidative capacity of the skeletal muscle itself, resulting in accumulation of lipids within the tissue, and 2) metabolic inflexibility; a reduced ability to switch from fat oxidation to carbohydrate oxidation under insulin stimulated conditions (Phelix & Mensink, 2011). Both reductions and functional defects in skeletal muscle are likely to contribute to the risk of IR development and subsequent T2D.

As discussed above, both body composition (the amount of fat and lean mass) and fat distribution play important roles in the development of IR. Therefore, understanding the early origins of insulin resistance and its determinants are essential to prevention and management in both high-risk groups and the general population.

## 1.5 Early origins of metabolic disease

### 1.5.1 The developmental origins of health and disease (DOHaD) hypothesis

In the early nineties, Sir David Barker proposed that the conditions an individual experiences in the womb, shapes their susceptibility to chronic disease later in life. This is known as the developmental origins of health and disease (DOHaD) hypothesis. Also known as the fetal origins hypothesis, the theory was put forth by Barker and colleagues after the observation that lower than normal birth weights (below 5lbs) were associated

with increased cardiovascular (CV) mortality (Barker et al., 1989, Osmond et al., 1993). Around the world size at birth in relation to gestational age is a marker of fetal nutritional status (Harding, 2001), in which lower than normal birth weights are suggestive of fetal under nutrition and reduced fetal growth. Since Barker's original observation, many epidemiological studies have supported the DOHaD hypothesis confirming that impaired intrauterine growth and low birth weight (LBW) are related to the development of cardio-metabolic complications in later life. These include hypertension, hyperglycemia, obesity, T2D, and more specifically for the context of this project, insulin resistance (Doyle et al., 2003; Vestbo et al., 1996; Whincup et al., 2008).

### 1.5.2 Fetal programming and the thrifty phenotype hypothesis

The basis of the DOHaD theory states that disruptions to normal growth and development during fetal and early postnatal life result in permanent changes to multiple body systems, contributing to disease in later years (Miles, Hofman, & Cutfield, 2005). The exact mechanisms connecting low birth weight and future cardio-metabolic disease are poorly understood. However, the most common theory of fetal programming suggests that maternal conditions such as undernutrition, obesity, gestational diabetes, infection, hypertension/eclampsia, smoking, alcohol use and exogenous glucocorticoid administration, contribute to a suboptimal intrauterine environment for the developing fetus. This in turn provokes adaptive changes in the development of key endocrine and metabolic processes for continued fetal growth and survival (reviewed in Fowden et al., 2005). Proposed targets of an adverse in-utero environment include altered development and gene expression in the liver, pancreas, kidney (Luyckx & Brennanl, 2010), muscle,

adipose tissue and hypothalamic-pituitary-adrenal (HPA) axis (Gluckman, Hanson, Cooper, & Thornburg, 2008).

The physiological adjustments to an adverse in-utero environment may increase fetal survival throughout gestation, but may become permanently programmed and impact later health (Fowden et al., 2005) when the infant is exposed to a different postnatal environment. This is known as the thrifty phenotype hypothesis which suggests that poor fetal nutrition and intrauterine growth restriction contribute to metabolic programming of a phenotype that is adapted to poor but not plentiful nutrition (Miles et al., 2005). Therefore, when the baby is exposed to a completely different postnatal environment, in particular one of overnutrition (McMillen & Robinson, 2005), metabolic dysfunction results.

### 1.5.3 Preterm birth

Lower birth weights are more prevalent among infants born preterm: birth prior to 37 weeks gestation. In Canada, preterm birth affects 26,000 (8.2%) children each year and is associated with various health risks for both the mother and infant (Public Health Canada, 2008). Recent advances in neonatal intensive care technology have improved the care and survival of preterm infants; however greater risks of health problems extending beyond the neonatal period have been recognized. These include cerebral palsy, neurodevelopmental and behavioural impairments, chronic lung disease, visual and hearing impairments (Saigal et al., 2007) and more specifically for the scope of this project, an increased risk of insulin resistance and its determinants in later life (Hofman et al., 2004; Hovi et al., 2007).

According to the World Health Organization, preterm birth can be classified into three categories using gestational age as a proxy of maturity. The classifications are: preterm (born <37 weeks' gestation), very preterm (born <32 weeks' gestation) and extremely preterm (born <28 weeks' gestation) (Public Health Canada, 2008). Amongst several other countries including Canada, further classification of preterm infants according to birth weight is also used. Low birth weight (LBW) is defined as those infants weighing ≤2,500 g at birth, very low birth weight (VLBW) infants weighing ≤1,500 g at birth and extremely low birth weight (ELBW) infants weighing ≤1,000g at birth (Euser et al., 2008).

#### 1.5.3.1 Small for gestational age and intrauterine growth restriction

The two terms small for gestational age (SGA) and intrauterine growth restriction (IUGR) are both used to describe small babies. These terms are often used interchangeably throughout the literature; however it is important to note their differences. Like term infants, preterm babies can either be small or appropriate for gestational age at the time of birth. The term SGA describes an infant whose birth weight is below the 10th percentile for a given gestational age (Kramer et al., 2001). SGA at birth may be due to the fact that the infant is genetically smaller, having reached its maximum growth potential, or pathologically smaller as a consequence of prenatal growth restriction (Figueras & Gardosi, 2011). IUGR on the other hand refers to pathologically growth restricted infants that have failed to reach their full growth potential likely due to a suboptimal intrauterine environment (Clayton et al., 2007). Therefore, an infant that is SGA at birth may have also experienced IUGR.

#### 1.5.3.2 Extremely low birth weight

Birth weight is determined by both the duration of gestation and rate of fetal growth in-utero. Infants born ELBW are those born at the lowest end of the birth weight spectrum (weighing less than 1 kg at birth) and are usually born less than 28 weeks gestation. Thus, they are all premature, representing the smallest, most immature and at-risk infants surviving at birth. ELBW may result from their extreme prematurity, IUGR or a combination of both, in which some are both premature and SGA at the time of birth.

The survival of these high-risk infants has greatly improved over the last twenty years (Lorenz, 2001). In line with the DOHaD hypothesis and fetal programming theory, individuals born with extremely low birth weight provide a model to examine the effects of extreme perinatal adversity on metabolic health outcomes later in adulthood. Examination of the ELBW group will aid in understanding the observation that those at the lowest end of the birth weight spectrum have the highest risk of disease in later life (Leon et al., 2000; Stene et al., 2001).

## 1.6 Growth and development

### 1.6.1 Fetal development: adipose tissue and skeletal muscle

Extremely low birth weight infants appear to be at double jeopardy. Not only are they <1000g at birth, but they also experience the majority if not all of the third trimester of pregnancy ex-utero since they are usually born <28 weeks gestation. From the time of birth to their expected birth date (term equivalent age), infants born ELBW experience early postnatal growth failure. Therefore by term equivalent age, all ELBW infants, even

those that were AGA at birth become SGA (Ehrenkranz et al., 1999; Hack et al., 2003) (Figure 2).

The third trimester of pregnancy (28-42 weeks) is a crucial period for adipose tissue and skeletal muscle development. In humans, adipose tissue development (adipogenesis) begins in mid to late gestation of pregnancy which includes the latter half of the second trimester and the entire third trimester. Adipogenesis involves the formation of preadipocytes; precursor cells devoid of lipid but committed to the adipocyte lineage. These cells may become quiescent, proliferate to increase the number of committed preadipocytes, or differentiate into mature, lipid-containing adipocytes (Poulos, Hausman, & Hausman, 2010). The third trimester is a period of accelerated fetal weight gain and rapid adipose tissue deposition (Uthaya et al., 2005); therefore infants with younger gestational ages and/or abnormal growth in the third trimester may have premature development and altered function of adipose tissue (Enzi et al., 1981).

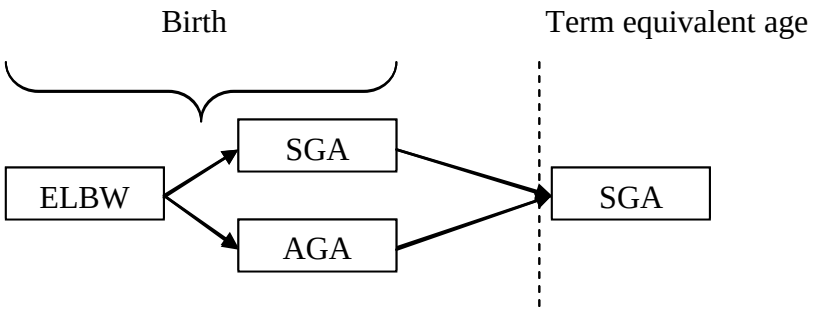


**Figure 3. Growth and gestational age classification at birth and term equivalent age in ELBW infants.**

Skeletal muscle development also begins in mid-gestation in humans and is roughly separated into three stages: embryonic, fetal and postnatal. These stages correspond to primary, secondary and postnatal myogenesis respectively (Stickland,

1978). Primary myogenesis involves the commitment of multipotent stem cells to the muscle cell lineage and the proliferation and fusing of myoblasts to form primary muscle fibres. Secondary myogenesis during the fetal stage forms most of the muscle fibres (Stickland, 1978) and is a critical stage for skeletal muscle development since there is no net increase in the number of muscle fibres after birth (Du et al., 2010). Postnatal skeletal muscle growth is mainly due to an increase in muscle fibre size without the formation of new muscle fibres (Du et al., 2010).

Perinatal events such as IUGR and extremely preterm birth may contribute to disruptions in both adipose tissue and skeletal muscle development and function. Furthermore, altered postnatal growth patterns and environmental/lifestyle factors (i.e. physical activity, diet) throughout the life course may compound previous in-utero disturbances in adipose tissue and skeletal muscle development, contributing to increased metabolic disease risk in later life.

### 1.6.2 Growth patterns, altered body composition and low birth weight

The growth trajectory from birth is a key modifier of the association between birth weight and subsequent disease risk, with lower birth weight and overweight and obesity in later life resulting in the highest risk for metabolic and cardiovascular disease (CVD) outcomes (Meriq, 2006; Ong et al., 2000). ELBW individuals experience a growth trajectory similar to those born at term and SGA. This is characterized by an initial period of postnatal growth failure to term equivalent age, followed by catch-up growth particularly in weight but not in height. Catch-up growth in weight and a subsequent increase in BMI have been observed to continue beyond infancy into childhood and

adolescence in those born ELBW (Saigal et al., 2006). This growth trajectory, particularly an accelerated increase in weight gain throughout the life course, may contribute to an unfavourable body composition in adulthood characterized by: increases in fat mass and central adiposity, as well as reductions in lean mass.

In other studies of *normal* birth weight individuals, rapid weight gain during early infancy has been associated with increases in BMI, adiposity and central fat in childhood (Ong et al., 2000). Young adults born at term and SGA have increased fat and reduced lean mass compared to their AGA counterparts (Leunissen, Stijen, and Hokken-Koelega, 2009). Additionally, SGA individuals with the most rapid catch-up growth have an increased risk of insulin resistance and T2D in adulthood (Gluckman et al., 2008).

### 1.7 What do we know about individuals born preterm with low birth weight?

#### 1.7.1 Preterm birth with low birth weight and later insulin resistance

Although lower birth weights (VLBW and ELBW) are more prevalent among preterm infants, few studies have assessed the effect of markedly *preterm* birth on insulin resistance in later life. Because only one third of all preterm children are born SGA, it has been difficult to interpret whether the effect of low birth weight on metabolic and cardiovascular risk factors are due to prematurity, SGA or both. Table 1 in Appendix D depicts a summary of the studies examining the association between individuals born preterm with LBW and later insulin resistance. The majority of these studies have focused on those born very preterm (<32 weeks gestation) with very low birth weight (birth weight <1500g), investigating insulin resistance in childhood and young adulthood. Table

2 in Appendix D highlights covariates used in analyses for each study when examining IR between preterm and term individuals.

Conflicting results have been reported in which some but not all studies have reported increased IR in individuals born preterm with LBW compared to those born at term with normal birth weight (NBW) in later life. In a study of 72 children, those born prematurely had a 40% reduction in insulin sensitivity measured during an intravenous glucose tolerance test (IVGTT) and a 50% increase in compensatory acute insulin release compared to their term-born counterparts (Hofman et al., 2004). Similar findings were reported by Regan et al. (2006) using the IVGTT and Darendelier et al. (2008) by HOMA-IR. In young adulthood (18-24 years), Hovi et al. examined IR measured during an oral glucose tolerance test in VLBW and NBW individuals. They identified that the VLBW group had significantly increased fasting insulin levels, 2-hour fasting insulin levels (weak association) and insulin resistance (HOMA-IR) compared to their NBW counterparts (Hovi et al., 2007). Compared to adults born at term, a recent study in reported a 29% reduction in insulin sensitivity after adjustment for confounders in preterm (33-36 weeks' gestation) adults aged 34-38 years using the hyperinsulinemic-euglycemic clamp technique (Mathai et al., 2012). The studies by Hovi et al. (2007) and Mathai et al. (2012) are the first studies in adulthood to show higher IR in preterm born individuals.

Additionally, differences in insulin resistance between preterm+SGA and preterm+AGA individuals have been reported by some (Bazaes et al., 2004; Fewtrell et al., 2000) but not all studies (Bo et al., 2006; Darendelier et al., 2008; Finken et al., 2006).

Willemsen and colleagues identified no influence of prematurity as an additional factor for altered insulin metabolism in short SGA children (Willemsen et al., 2008).

### 1.7.2 Preterm birth with low birth weight and body composition

Data on changes in body composition in those born preterm with low birth weight are inconsistent. Furthermore, the majority of these studies have looked at preterm, VLBW populations, with few studies in adulthood. Table 3 in Appendix D summarizes studies examining fat mass and/or percent body fat in preterm individuals with low birth weight. For the purpose of this review we will focus on the distribution of body fat.

#### 1.7.2.1 Central fat

Few of the studies examining body composition have focused on fat distribution, particularly the amount of central adiposity in preterm LBW individuals. The studies that have examined differences in central adiposity between preterm and term individuals report varying results throughout the life course. Compared to term-born counterparts, increased intra-abdominal (visceral) fat was reported in preterm infants at term equivalent age (Uthaya et al., 2005), both reductions and no difference in trunk fat in preterm children have been observed (Willemsen et al., 2008; Darendelier et al., 2008; Gianni et al., 2008), and no difference in trunk fat to leg fat ratio between NBW and VLBW young adults have been reported (Hovi et al., 2007). Central adiposity in adulthood (beyond 24 years of age) have not been examined to date, therefore more studies are needed to clarify if differences in central adiposity exist in adults born preterm compared to term.

#### 1.7.2.2 Skeletal muscle/lean mass

Individuals born preterm with low birth weight have exhibited differences in fat-free mass/lean body mass in three (Cooke et al., 2009; Hovi et al., 2007; Kajantie et al., 2010) out of nine studies (See Table 4, Appendix D). Lean body mass (LBM) was similar in preterm and term infants in 2 studies (Cooke et al., 1999; Gianni et al., 2009). Cooke and colleagues reported a lower fat-free mass compared to the reference group (Cooke et al., 2009). No differences in lean body mass adjusted for current body size have been observed in childhood and adolescence (Fewtrell et al., 2004; Gianni et al., 2009; Peralta-Carecelen et al., 2000). However, it has been reported that preterm, VLBW individuals have reduced lean mass compared to their term born counterparts later on in life in young adulthood (Hovi et al., 2007; Kajantie et al., 2010). Alterations in lean body mass have also been hypothesized to connect preterm birth with low birth weight and subsequent metabolic dysfunction in later life.

### 1.7.3 Preterm birth with low birth weight and adiponectin

Studies examining adiponectin concentrations in individuals born preterm with low birth weight have focused predominantly on infants. In childhood and adulthood, serum adiponectin is inversely associated with adiposity, body weight and BMI. However this does not seem to be the case in neonates, with adiponectin appearing to play more of a regulatory role in fetal growth and demonstrating a positive relationship with neonatal weight gain. Lower serum adiponectin concentrations at birth in preterm compared to term infants have been reported in some (Martos-Moreno et al., 2009; Yoshida et al., 2009) but not all studies (Yoshida et al., 2011; Saito et al., 2011). Furthermore, at term

corrected age, lower adiponectin levels have also been observed in most (Siahanidou et al., 2007; Siahanidou et al., 2009), but not all studies (Yoshida et al., 2009) in preterm vs. term babies. Furthermore, in infancy, adiponectin levels have also demonstrated a positive relationship with advanced gestational age at birth (Kajantie, Hytinantti, Hovi, & Andersson et al, 2004), birth weight (Kajantie et al., 2004; Siahanidou et al., 2007; Siahanidou et al., 2009; Yoshida et al., 2011) and fetal growth/weight gain (Saito et al., 2011; Siahanidou et al., 2007).

Studies examining adiponectin concentrations and its relation with metabolic health beyond infancy are lacking in individuals born preterm with low birth weight. However, we can turn to the literature comparing adiponectin concentrations in term SGA and AGA individuals to hypothesize why we would expect to observe lower adiponectin concentrations in those born preterm. Adiponectin levels in term, SGA children compared to AGA counterparts are lower in some (Cianfarani et al., 2004; Jaquet et al., 2006) but not all studies (Challa et al., 2009; Miras et al., 2010; Sancakli et al., 2008,). Furthermore, catch up growth and increased body mass index in term, SGA children are associated with lower adiponectin levels (Sancakli et al., 2008). Further examination of the adiponectin profile is needed in individuals born preterm to see if differences exist in adulthood.

### 1.8 Summary

Inconsistent results have been reported regarding differences in IR between preterm and term individuals. Furthermore, no studies to date have examined IR in extremely low birth weight populations and only one recent study in adulthood (beyond age 30) has been conducted. In assessing body composition in preterm and term individuals, the majority of studies have focused on absolute fat mass and/or percent body fat rather than fat distribution (central vs. peripheral adiposity) as their primary outcome. Therefore, this project contributes to understanding central adiposity in adults born ELBW compared to NBW peers. Additionally, the findings of lower lean mass in preterm young adults compared to term counterparts warrants examination of lean body mass in later years to determine if this difference persists with advancing age. Furthermore, differences in adipokine profile, particularly adiponectin concentrations, beyond infancy remain to be evaluated in individuals born preterm and full term. This thesis project aims to understand potential determinants of insulin resistance in adults born with the most extreme prematurity and at the lowest end of the birth weight spectrum. As prospectively assembled preterm birth cohorts are only now beginning to enter their adult years, this study provides a novel opportunity to understand the lifelong effects of extremely preterm birth on health outcomes beyond young adulthood.

# CHAPTER 2: HYPOTHESIS & OBJECTIVES

We hypothesize that disruptions to normal fetal development such as intrauterine growth restriction and preterm birth resulting in ELBW and followed by altered postnatal growth patterns may contribute to altered adipose tissue and lean mass development. These changes compounded by genetic factors, lifestyle factors (i.e. diet, physical activity) and growth patterns throughout the life may result in higher central adiposity and lower lean mass in adulthood. This may contribute to alterations in insulin sensitivity, adipokine profile (i.e. adiponectin) and glucose metabolism in adulthood (Figure 3).

## 2.1 Primary objectives

1. To compare potential determinants of IR between adults born ELBW and NBW. Determinants include: central fat, adiponectin and lean mass index.
2. To examine the relationship of central fat, adiponectin, lean mass index and birth weight with insulin resistance in a cohort of adults born ELBW and NBW.
3. To examine if interactions exist between birth weight group and significant determinants of insulin resistance (central fat, adiponectin and lean mass index) in a cohort of adults born ELBW and NBW.

## 2.2 Exploratory objectives

1. To examine if adults born ELBW have higher insulin resistance compared to their NBW counterparts.
2. To examine if adults born ELBW+SGA have higher insulin resistance compared to adults born ELBW+AGA.

3. To examine the prevalence of dysglycemia (IFG, IGT and T2D) in a cohort of adults born ELBW and NBW.

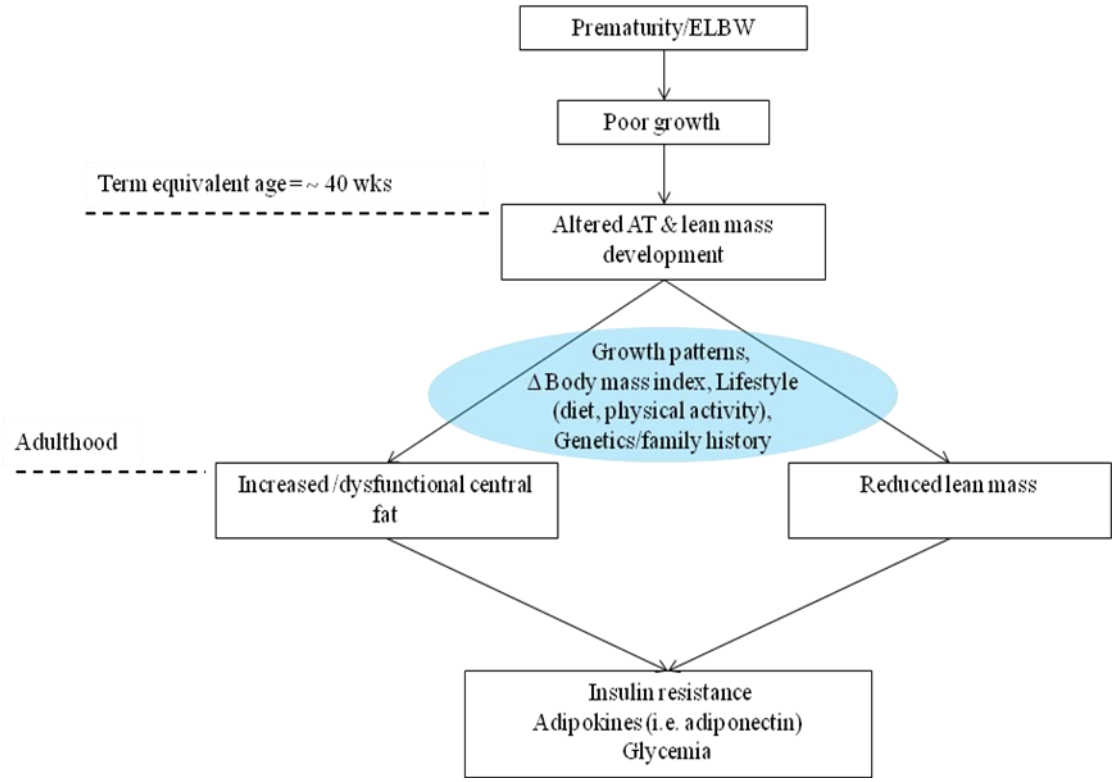


**Figure 4. Proposed model for thesis project examining the determinants of insulin resistance in adults born at normal and extremely low birth weight.** Disruptions to normal fetal development (preterm birth, ELBW, altered postnatal growth patterns) may contribute to altered adipose tissue and lean mass development at term equivalent age. These changes compounded by genetic factors, lifestyle factors and growth patterns throughout the life course may contribute to alterations in insulin sensitivity, adipokine profile (i.e. adiponectin) and glucose metabolism in adulthood.

# CHAPTER 3: METHODS

## 3.1 The McMaster ELBW cohort

The McMaster ELBW cohort is a population cohort of extreme prematurity and one of the most closely studied prospective cohorts in the world, followed since birth (1977-1982) to the present day. The cohort, assembled by Dr. Saroj Saigal, includes 150 individuals born preterm with ELBW (weighing <1000g at birth), and 150 individuals born at full term with normal birth weight (NBW). The ELBW group has been followed since birth to the present day (~30 years), whereas the NBW group was assembled and followed from 8 years of age to the present day. The NBW group was matched to the ELBW group according to sex, date of birth, birth hospital and socioeconomic status. Follow-up visits of the ELBW group have occurred at 1, 2 and 3 years corrected age and at 8 years of age, adolescence and young adulthood. Follow-up visits for the contemporaneous cohort have occurred at 8 years of age, adolescence and young adulthood. To date, retention rates of the entire cohort (ELBW and NBW) are greater than 90%.

Currently, both ELBW and NBW groups are returning for their 30-year (adult) follow-up visit where the outcomes of this thesis project will be assessed. This thesis project is a small part of a larger study, the FINCAN study, which examines adult health outcomes in both the ELBW and NBW groups with respect to metabolic, cardiovascular and mental health outcomes. FINCAN is also part of an international collaboration with a research team in Finland. The Finnish team has a similar cohort of 166 VLBW and 172

NBW controls. The Finnish VLBW cohort was born between 1978 and 1985 and was last assessed at 23 years of age.

### 3.2 Sample

The study population for this thesis project included all, non-pregnant adults born ELBW or NBW within the McMaster birth cohort that completed their adult follow-up visit for the FINCAN study prior to March 13th, 2012. The FINCAN study is currently in progress, with one year remaining for completion of the adult follow-up visits. Therefore, participants eligible for inclusion in this project represent a subset of the full cohort. Furthermore, the ELBW participants with neurological impairments are not included in this subset as they will be recruited for completion of their adult follow-up visit during summer 2012. Inclusion/exclusion criteria are listed in Table 1.

**Table 1.** Inclusion and exclusion criteria

| Inclusion criteria | Exclusion criteria |
|---|---|
| Member of McMaster ELBW and NBW cohort | Currently pregnant |
| Completion of FINCAN adult follow-up visit prior to March 13th, 2012 | |

### 3.3 The FINCAN adult follow-up study visit

All ELBW and NBW individuals have been invited back to complete their 30-year adult assessment which we shall call the FINCAN adult visit. This visit consists of two components: the metabolic and psychological components. For the scope of this thesis project, all clinical characteristics and health outcomes were assessed during the metabolic component of the FINCAN adult visit, which took place at the McMaster Children's Hospital (2G54 clinic). Participants were recruited and attended the visit on their scheduled date. Participants were instructed to fast overnight and drink lots of water

the night before and day of the visit. After consent was obtained all metabolic testing was conducted. This included: an oral glucose tolerance test, anthropometric measurements, DXA body scan, completion of questionnaires (to assess family history of disease, diet, physical activity patterns, sleep patterns, smoking/alcohol frequency, and pregnancy/fertility), carotid ultrasound, autonomic nervous system testing and accelerometry (an objective measure of physical activity). The clinical variables, health outcomes and utilized methods within the scope of this thesis project are described in detail below.

### 3.4 Health outcomes and measures

Insulin resistance is the primary outcome of interest. Other health measures included potential determinants of insulin resistance: central fat mass, lean mass index (LMI), total serum adiponectin and birth weight (Table 2). These variables were measured for all ELBW and NBW adults included in the sample.

**Table 2.** Primary health outcomes and determinants of IR measured in ELBW and NBW adults

| **Primary health outcome** | **Method** |
|---|---|
| Insulin resistance (IR) | HOMA-IR = [fasting glucose (mmol/L) x fasting insulin (pmol/L)/22.5] |
| **Determinants of primary health outcome (IR)** | |
| Central fat* | 1) Android region of DXA scan<br>2) Waist circumference |
| Adiponectin | Venous blood sample, ELISA |
| Lean mass index (LMI) | DXA, LMI = total lean mass(kg)/height(m)$^2$ |
| Birth weight | Recorded at birth by electronic scales to nearest gram. Birth weight for each participant stored in electronic database. |

Methods are describes in full detail in the following section
* Central fat was measured by two methods

### 3.4.1 Insulin resistance

**Overview:** Insulin resistance was determined by HOMA-IR (homeostasis model assessment of insulin resistance). HOMA-IR is a surrogate marker of steady-state insulin resistance and is a simple mathematical index calculated using fasting glucose and fasting insulin levels from a venous blood sample. HOMA-IR = $G_0$ x $I_0$/22.5, where $G_0$ is the fasting plasma glucose value (mmol/L), $I_0$ is the fasting plasma insulin value (mU/L) and 22.5 is a constant. The constant of 22.5 is a normalizing factor; the product of a "normal" healthy individual with respect to fasting plasma insulin (5μU/mL) and fasting plasma glucose (4.5 mmol/L) (Wallace, Levy, & Matthews, 2004). Higher values of HOMA-IR are indicative of increasing insulin resistance (lower insulin sensitivity) (Wallace et al., 2004). The online HOMA calculator from The Oxford Centre for Diabetes, Endocrinology and Metabolism was used to determine HOMA-IR for all participants using fasting glucose and fasting insulin concentrations.

**Rationale for use:** HOMA-IR has been utilized across diverse ethnicities for assessment of insulin sensitivity/resistance. It is practical for large scale epidemiological and clinical research studies, it is relatively non-invasive (requires one blood sample to determine fasting glucose and fasting insulin), is inexpensive and non labour intensive. The HOMA-IR method correlates strongly with the gold standard hyperinsulinemic-euglycemic clamp technique in both normal (r = 0.83) and diabetic subjects (r = 0.92) (Wallace et al., 2004). See Appendix A for a brief overview of the gold standard technique used for measuring insulin sensitivity/resistance, as well as additional surrogate measures of insulin resistance.

**Disadvantages:** The HOMA-IR method is a measure of basal (steady state) insulin resistance and does not measure insulin resistance under stimulated conditions (in response to a glucose load). It assumes a functioning feedback loop between the liver and pancreatic β-cells in which glucose concentrations are regulated by insulin-dependent hepatic glucose production (HGP), and insulin levels depend on the pancreatic β-cell response to glucose concentrations (Levy, Matthews, & Hermans, 1998; Mathews et al., 1985; Wallace et al., 2004). Thus, insulin resistance is reflected by the reduced suppressive effect of insulin on hepatic glucose production (Singh & Saxena, 2010). Additionally, HOMA-IR does not provide information regarding the contributions of liver versus skeletal muscle to observed reductions in whole-body insulin sensitivity (Abdul-Ghani et al., 2007).

#### 3.4.1.1 Fasting and 2-hour insulin concentrations

On the day of the FINCAN study visit, a fasting venous blood sample was taken at baseline (0 min) and 120 minutes after a 75-g oral glucose load. Serum samples were centrifuged for 10 minutes at 3000 rpm, aliquoted and stored in a -80°C for later analysis. Fasting and 2-hour insulin concentrations were quantified using a commercially available non-competitive ELISA (DIAsource ImmunoAssays S.A., Louvain-la-Neuve, Belgium) according to the manufacturer's instructions. Six ELISA kits were used. The primary antibody used in the ELISA was specific for insulin. A horseradish peroxidase-conjugated secondary antibody was used and the absorption read at a wavelength of 450 nm. Samples were run in duplicate, with baseline and 120 minutes serum samples from each participant run on the same plate. A calibration curve was plotted from known concentrations of

insulin standards using a computer software program MiraiBio MasterPlex ReaderFit: Curve Fitting Software for ELISA analysis. A four-parameter logistic function curve was fitted and the insulin concentration in unknown samples was determined by interpolation from the calibration curve. All standards, controls and unknown fell on the curve (See Appendix B, Figure 1). Controls and unknown samples fell in the middle portion of the curve. The minimum detectable concentration used was 3.94 µIU/mL or 27.36 pmol/L. Fasting and 2-hour insulin concentrations are reported in pmol/L (1 µIU/mL = 6.945 pmol/L). The *inter-assay* coefficient of variation (CV) of control 1 was 14.08% and 14.15% for control 2. The *intra-assay* CV for each duplicate was <15%. The mean intra-assay CV for all baseline samples was 5.33% and 5.63% for 120 minute samples.

#### 3.4.1.2 Fasting and 2-hour glucose concentrations

On the day of the study visit, a venous blood sample was taken at baseline (0 min, fasted state) and 120 minutes after 75-g oral glucose load. Samples were sent to the McMaster University CORE Laboratory. Plasma glucose concentrations were measured by means of a spectrophotometric hexokinase and glucose-6-phosphate dehydrogenase assay on a Roche INTEGRA analyzer.

### 3.4.2 Potential determinants of IR

#### 3.4.2.1 Central fat

Central fat was measured by two methods: waist circumference (WC) and using dual-emission X-ray absorptiometry (DXA) technology on a GE Lunar Prodigy Advance scanner (Model #8743). WC is a surrogate marker of central obesity, correlates with visceral fat mass and is related to increased cardio-metabolic risk (Despres et al., 1990;

Han et al., 1995). WC was measured to the nearest 0.1 cm using a non-stretchable standard tape measure attached to a spring balance. The measurement was taken over the unclothed abdomen at the smallest diameter between the costal margin and the iliac crest, ensuring that the tape measure was kept horizontal. Participants were instructed to relax with arms held loosely at their sides. Three measurements were taken and averaged. Central fat was also measured using a DXA scanner. DXA is a whole body scan generally used to measure bone mineral density, however it is also used to assess total body fat, fat-free mass (i.e. lean mass), and regional body fat distribution (i.e. central fat). Central fat was determined by defining specific regions of interest for the *android* region (L2-L4) using the DXA software program for analysis. The android region of interest is defined as the lower boundary at pelvis cut, upper boundary above pelvis cut by 20% of the distance between pelvis and neck cuts and lateral boundaries are the arm cuts. See Appendix C, Figure 1 for an illustration of a whole body DXA scan with the outline of the android region of interest to determine central fat. On the day of the study visit, the participant was instructed to wear loose clothing and to remove all metal items from his/her clothing and/or body. The scan was completed with the participant lying on his/her back with arms at sides and palms facing down, legs straight with feet pointed up and head positioned to look at ceiling.

**Rationale for use:** The gold standard for measuring central (visceral) fat in vivo is the computed tomography (CT) scan (Ness-Abramof & Apovian, 2008). However, this method is very costly and time consuming for use in clinical studies. WC and DXA both provide advantages over this method for the determination of central adiposity. These

include simplicity, quickness and reproducibility of results, relatively low cost and routine usage in clinical research studies. Specifically for DXA the minimal radiation exposure (< 1μSv or 1/100th of the radiation exposure of a chest X-ray) compared to a CT scan (Park, Heymsfield, & Gallagher, 2002) and the ability to assess multiple body composition variables (total body fat mass, total body fat percentage, and total lean mass) are additional advantages.

One of the main disadvantages of both WC and central fat measured by DXA is that regional fat estimates do not differentiate between visceral and subcutaneous central fat depots unlike the CT scan. However, in a study of middle-aged adult men and women with a healthy BMI, WC and central fat measured by DXA correlated highly (r = 0.85 and 0.72) with visceral adipose tissue determined by CT after adjustments for age and sex (Gradmark et al., 2010). Furthermore, in a study by Park et al in 90 non-obese healthy males between the ages of 18 and 44 years showed that correlations between central fat measured by DXA (region of interest L2-L4) correlated highly with total visceral adipose tissue measured by CT scan (r = 0.85) (Park et al., 2002).

**3.4.2.2 Lean mass index**

DXA was also used to determine total body lean mass, computed automatically by the analyzing software after the scan is complete. Total lean mass (kg) for each participant was adjusted for current adult height by calculating lean mass index (LMI = total lean mass (kg)/height ($m^2$)). Current height is a strong predictor of total lean mass (Hume, 1966) and evidence suggests that ELBW individuals are shorter than their NBW counterparts throughout the life course (Saigal et al., 2006). Therefore, LMI was used to

ensure that potential observed differences in lean mass between ELBW and NBW adults were not confounded by differences in adult height.

#### 3.4.2.3 Total adiponectin

Total serum adiponectin concentration was quantified from stored fasting serum samples obtained at the FINCAN adult visit using a commercially available non-competitive ELISA (Millipore, St. Charles Missouri, USA) according to the manufacturer's instructions. Three ELISA kits were used. The primary antibody used in the ELISA was specific for human adiponectin. A streptavidin-horseradish peroxidise conjugated secondary antibody was used and the absorption read at a wavelength of 450 nm. Serum samples were diluted 1:500 for this assay and samples were run in duplicate. A calibration curve was plotted from known concentrations of adiponectin standards using a computer software program MiraiBio MasterPlex ReaderFit: Curve Fitting Software for ELISA analysis. A 5-parameter logistic function curve was fitted and the total adiponectin concentration in unknown samples was determined by interpolation from the calibration curve. Controls and unknown samples fell in the middle portion of the curve. The lowest level of adiponectin detected by this assay is 0.00078 µg/mL (0.78 ng/mL) when using a 20 µL sample size. Final results were multiplied by a 500 dilution factor. Total adiponectin concentrations were reported in µg/mL (1 µg/mL = 1000 ng/mL). The *inter-assay* CV for quality control 1 was 22.94% and 24.32% for quality control 2. The *intra-assay* CV for each duplicate was <15%; the mean *intra-assay* CV was 6.16%.

**Rationale for use:** Adiponectin exists in the human circulation as multimers with distinct molecular sizes: trimeric low molecular weight (LMW)-adiponectin, hexameric medium molecular weight (MMW)-adiponectin, and high molecular weight (HMW)-adiponectin (Waki et al., 2003). Although the HMW isoform is considered to be the most active form of the protein (Aso et al., 2006; Ebinuma et al., 2006) and has been used extensively in animal studies, total adiponectin measures all forms of adiponectin in the circulation and has been used frequently in clinical and epidemiological studies (Inadera, 2008). A study by Almeda-Valdes et al. (2010) suggested that total adiponectin had similar utility for the identification of insulin resistance (determined by HOMA-IR) compared to both HMW adiponectin and HMW/total adiponectin ratio. Furthermore, total adiponectin was the most feasible option instead of the HMW isoform for this study. HMW adiponectin is more expensive and more difficult to measure compared to total adiponectin. It would require both total and HMW assays to be performed; HMW would be determined by subtracting total adiponectin values from HMW values.

### 3.4.3 Clinical characteristics at birth

The clinical characteristics at birth: gestational age in weeks, birth weight for gestational age (small or appropriate for gestational age; SGA or AGA), obstetrical history and maternal smoking during pregnancy were available for the *ELBW participants only.* These variables were obtained for this project to: answer exploratory objective 2 (if IR differed in ELBW adults born SGA or AGA at birth), and descriptively report pregnancy complications and maternal smoking patterns in the ELBW group. *Note:* All NBW participants were born full-term and AGA.

#### 3.4.3.1 Gestational age

Gestational age (GA) in weeks was previously determined by best estimate obtained through a combination of the mother's last menstrual period, physical examination and early ultrasound if available (Saigal et al., 2001).

#### 3.4.3.2 Birth weight for gestational age

Birth weight for gestational age (SGA/AGA) was determined at the time of birth for those born ELBW. SGA was defined as birth weight $<10^{th}$ percentile based on Canadian standards (Kramer et al., 2001). ELBW infants that were SGA at birth were considered to have intrauterine growth restriction.

#### 3.4.3.3 Maternal obstetrical history

Obstetrical history was collected from maternal medical records and stored in a database. Maternal pregnancy complications noted at the time of birth include: urinary tract infection, chronic hypertension, pregnancy induced hypertension, eclampsia, abruptio placentae, placenta previa, insulin dependent diabetes mellitus, no complications or unknown.

#### 3.4.3.4 Maternal smoking history

Maternal smoking history was collected from maternal medical records and stored in a database. Mothers had reported if they smoked during the third trimester of pregnancy (yes or no). If yes, then the number of cigarettes per day was reported. For entry into the database, maternal smoking data was entered as: non-smoker, smoker + 1-10 cigarettes/day, smoker + 11-20 cigarettes/day and smoker + >20 cigarettes/day.

### 3.4.4 Clinical characteristics in adulthood

The following clinical characteristics were measured for all ELBW and NBW participating in the FINCAN adult follow-up visit.

#### 3.4.4.1 Anthropometric variables

Height, weight and body mass index (BMI = mass(kg)/height(m)$^2$) were assessed as it has been reported previously that ELBW individuals are shorter but are comparable in weight and BMI compared to their NBW peers (Saigal et al., 2006). We wanted to see if these patterns were also observed in adulthood. Table 3 shows the methods used for anthropometric variables.

#### 3.4.4.2 Blood pressure

Systolic and diastolic blood pressure were assessed as it has been reported that individuals born preterm have higher blood pressure compared to their normal birth weight counterparts (Hovi et al., 2007). We wanted to see if these patterns were also observed in adulthood. Blood pressure was measured using the *BPTru* oscillometric device. Participants were quietly resting for ~5 minutes and were not allowed to smoke for at least 30 minutes prior to the measurement. The appropriate arm cuff size was selected and placed on the right arm of the participant. The bladder of the cuff was placed over the brachial artery. Participants were instructed to remain quiet and still for the duration of the measurement. The *BPTru* completed 6 measurements with 2 minute intervals between each measurement. Each of the 6 measurements and an average for systolic and diastolic blood pressure was recorded.

#### 3.4.4.3 Body composition

Total body fat (kg), total body fat percentage and total lean mass were measured by DXA as described previously. The three measures were calculated by the DXA analyzing software.

**Table 3.** Anthropometric variables assessed in ELBW and NBW adults and associated methods

| Anthropometric variables | Methods used |
|---|---|
| **Height** | Standing height was measured in centimeters using a wall stadiometer. The subject was bare foot and had their back square against the wall, eyes looking straight ahead. A set square resting on the scalp and a tape measurement from the wall were used to measure height to the first decimal place. Three measurements were recorded and averaged. |
| **Weight** | Weight was measured in kilograms using a platform scale to the first decimal place. The scale was standardized to 0 before each use. Three measurements were recorded and averaged. |
| **Body mass index (BMI)** | BMI was calculated using weight in kilograms divided by height in meters squared. [BMI = weight(kg)/height(m)$^2$] |

#### 3.4.4.4 Oral glucose tolerance test

Alterations in both insulin sensitivity (i.e. increased IR) and insulin secretion predict the development of dysglycemia and type 2 diabetes in individuals with normal glucose levels (Abdul-Ghani et al., 2006). Studies have indicated that preterm individuals have impaired glucose tolerance compared to their term born peers (Hovi et al., 2007). Glucose control was assessed during a 75-g oral glucose tolerance test (OGTT) with glucose and insulin levels measured at baseline and 2-hours post glucose load. We determined the prevalence of dysglycemia (IFG and IGT) as well as T2D in ELBW and NBW adults using the criteria defined by the World Health Organization: IFG (fasting plasma glucose of 6.1-6.9 mmol/L), IGT (2-hour plasma glucose of 7.8 mmol/L or

higher) and T2D (a fasting plasma glucose of ≥7.0 mmol/L or a 2-hour plasma glucose concentration ≥11 mmol/L) were used (WHO, 2011).

#### 3.4.4.5 Pancreatic β-cell function

Both insulin resistance and reduced β-cell function underlie the development of T2D (WHO, 2011). We used the homeostasis model assessment as a surrogate marker of β-cell function (HOMA%B) using the online HOMA calculator from The Oxford Centre for Diabetes, Endocrinology and Metabolism: http://www.dtu.ox.ac.uk/homacalculator/download.php. The input variables for the calculation include both fasting glucose (mmol/L) and fasting insulin (pmol/L) for each participant.

### 3.5 Statistical analysis

Statistical analysis was performed using SPSS and GraphPad Prism (version 4.0) (GraphPad Software Inc, La Jolla, CA).

#### 3.5.1 Objective 1

*Objective 1: to determine if central fat, lean mass index and adiponectin are different in ELBW and NBW adults. Other clinical characteristics and health measures were also compared between ELBW and NBW adults. These included: age, height, weight, BMI, waist circumference, blood pressure, total body fat, total body fat percentage, total lean mass, fasting and 2-hour glucose and insulin concentrations and HOMA%B.*

Data were tested for normality and equal variance. Continuous variables between ELBW and NBW adults were analyzed by independent two-tailed Student's t-test ($\alpha = 0.05$). BMI, waist circumference, central fat, fasting glucose, 2-hour glucose, fasting insulin, 2-hour insulin, HOMA%B, HOMA-IR and adiponectin were logarithmically transformed to normalize the distribution before statistical comparison between groups. A two-way analysis of variance (ANOVA) was used to analyze differences in continuous variables with birth weight group and sex as the independent variables. Least squared differences post-hoc testing was used if an interaction effect of these variables was identified.

##### 3.5.1.1 Sample size calculations for objective 1

The following section outlines the calculated power and standardized effect sizes to evaluate if total lean mass/LMI and adiponectin are different with fixed sample sizes of NBW and ELBW adults.

***Total lean mass/LMI***

Total lean mass/LMI has not been measured in ELBW and NBW adults. However, a study by Hovi et al. (2007) examined total body lean mass in VLBW (birth weight <1500 g) and NBW adults. They identified a lower total body lean mass assessed by DXA of 1.3 kg in VLBW females vs. controls and a lower total body lean mass of 3.1 kg in VLBW males vs. controls (Hovi et al., 2007). We used information from the study by Hovi and colleagues to determine the required sample size to examine differences in LMI between ELBW and NBW adults. With group sample sizes fixed at a minimum of 45 NBW and 45 ELBW and $\alpha = 0.05$, we have adequate power (>80%) to identify standardized effects of 0.60 and larger (smallest detectable difference = 2.32 kg (females) and 3.79 kg (males).

***Adiponectin***

Adiponectin concentrations have not been measured/published in VLBW/ELBW individuals beyond infancy. A nested case-control study by Spranger et al. (2003) in healthy adults aged 35-65 years reported that a 1.5 µg/mL decrease in total adiponectin at baseline was associated with incident T2D after 3 years of follow-up. Furthermore, Tabak et al. (2009) conducted a nested-case control study in 140 (cases = 55, controls = 85) healthy Caucasian adults aged 35-55 years. Study participants had no prevalent T2D or coronary heart disease at baseline. They reported that a 1.2 µg/mL decrease in total adiponectin concentrations at baseline was associated with incident T2D after 11 year follow-up. With group sample sizes fixed at a minimum of 45 NBW and 45 ELBW and $\alpha$

= 0.05, we have >80% to identify standardized effects of 0.62 and larger (smallest detectable difference = 0.575 µg/mL) in total adiponectin.

### 3.5.2 Objectives 2 & 3

*Objective 2: to examine the determinants of insulin resistance in a cohort of ELBW and NBW adults. Determinants evaluated based on the literature review include: central fat, adiponectin, lean mass index and birth weight.*

First, univariate regression analysis was used to analyze the contribution of individual predictors on insulin resistance in NBW and ELBW adults. Individual predictors examined included: age, sex, birth weight group, log BMI, log waist circumference, total fat percentage, log central fat, log adiponectin, LMI and log birth weight. Log HOMA-IR was the dependent (outcome) variable.

Second, determinants of insulin resistance were then analyzed using forward stepwise multivariate regression analysis. In this type of multivariate regression, the order of entry of predictor variables is determined *a priori*. The predictor variable that best predicts the outcome variable (the predictor having the highest simple correlation with the outcome variable) is added first to the model (Field, 2009). This same criterion is then used to add subsequent predictors into the model. The first multivariate regression analysis included the predictors: log central fat, log adiponectin, LMI and log birth weight and the dependent variable was log HOMA-IR. Predictors were entered into the model in four separate blocks (Table 4). A second multivariate regression analysis was performed with log waist circumference instead of log central fat (Table 5) as a measure of central adiposity. The dependent variable was log HOMA-IR.

**Table 4.** Order of entry of predictors in multivariate regression analysis 1

| Model 1 | Model 2 | Model 3 | Model 4 |
|---|---|---|---|
| Log central fat | Log central fat | Log central fat | Log central fat |
| - | Log adiponectin | Log adiponectin | Log adiponectin |
| - | - | LMI | LMI |
| - | - | - | Log birth weight |

**Table 5.** Order of entry of predictors in multivariate regression analysis 2

| Model 1 | Model 2 | Model 3 | Model 4 |
|---|---|---|---|
| Log waist circumference | Log waist circumference | Log waist circumference | Log waist circumference |
| - | Log adiponectin | Log adiponectin | Log adiponectin |
| - | - | LMI | LMI |
| - | - | - | Log birth weight |

*Objective 3: to examine if interactions exist between birth weight and significant determinants of insulin resistance in a cohort of ELBW and NBW adults*

Lastly, interactions terms between birth weight group (coded NBW = 0, ELBW = 1) and significant predictors determined from univariate analyses were assessed in multivariate regression analyses. The dependent variable was log HOMA-IR.

**3.5.2.1 Sample size for multivariate regression analyses**

A common guideline for sample size determination for multivariate regression is 10 cases of data per predictor in the model. There are 4 predictor variables in each multivariate regression model (Tables 5, 6), therefore a minimum sample size of 40 participants in *each birth weight group* are required ($N_{total}$ = minimum 80 participants). Another suggested rule of thumb for sample size determination using multiple linear regression proposed by Green et al. (1991) recommends a minimum sample size of 50 +8k, where k is the number of predictors in the model. This rule is suggested for testing the overall fit of the proposed regression model (i.e. testing the $R^2$; the variability in the outcome accounted for by the predictors). Therefore, with 4 predictors a sample size of at

least 82 participants is sufficient (~41 ELBW and 41 NBW). The current sample size of 90 participants (48 NBW and 42 ELBW) is sufficient to carry out this type of analysis at 80% power and $\alpha = 0.05$.

### 3.5.3 Exploratory objectives

#### 3.5.3.1 Exploratory objective 1

*Exploratory objective 1: to examine if adults born ELBW have increased insulin resistance compared to their NBW counterparts.*

Differences in insulin resistance (HOMA-IR) between ELBW and NBW adults were assessed using student's two tailed t-test ($\alpha = 0.05$). Data were tested for normality and equal variance (all variables passed these statistical tests).

This objective was exploratory as we had insufficient numbers to detect a difference in insulin resistance between ELBW and NBW groups with adequate power (≥80%). Sample size calculations indicated that 145-165 participants per group were needed (290-330 participants total) to assess this objective ($\alpha = 0.05$, power = 80%). Sample size calculations were determined from the study by Hovi et al. (2007). However, we believed this was worth pursuing as an exploratory objective as we were studying a cohort that experienced greater adversity during the neonatal period and are nearly a decade older than the cohort studies by Hovi et al. (2007). Furthermore, age is known to increase the risk of dysglycemia and insulin resistance (Geer & Shen, 2009).

#### 3.5.3.2 Exploratory objective 2

*Exploratory objective 2: to examine if adults born ELBW+SGA have increased insulin resistance compared to adults born ELBW+SGA.*

Differences in clinical characteristics and health outcomes (continuous variables) between ELBW+SGA and ELBW+AGA were assessed using Mann-Whitney (rank sum) tests due to small numbers. Fisher's exact test was used to compare categorical variables. Comparisons were also made between ELBW+AGA and NBW groups as well as ELBW+SGA and NBW groups. This objective is exploratory as we do not have sufficient numbers to detect a significant difference in insulin resistance between these 2 groups. Sample size calculations showed that ~58 participants per group (116 participants total) are required to assess this objective ($\alpha = 0.05$, power = 80%).

#### 3.5.3.3 Exploratory objective 3

*Exploratory objective 3: to examine the prevalence of dysglycemia (IFG, IGT and T2D) in ELBW and NBW adults.*

Fisher's exact test was used to compare categorical variables between ELBW and NBW adults.

# CHAPTER 4: RESULTS

## 4.1 Sample characteristics

As of March 13th, 2012 114 (63 NBW and 51 ELBW) participants completed their adult follow-up visit for the FINCAN study, and 110 of these participants (60 NBW and 50 ELBW) met the inclusion criteria for this thesis project (Figure 4). This sample represents a subset of the original cohort as the FINCAN study is currently ongoing.

More NBW than ELBW adults participated in the adult visit prior to March 13th, 2012 (54.5% vs. 45.5%) and the proportion of females participating was also higher than males (63.6% vs. 33.7%) in both NBW and ELBW groups (Table 6). The sex difference at the adult follow-up visit is greater than that of the young adult FINCAN (~23 years) follow-up visit (females = 55.4%, males = 44.6%) (data not shown).

## 4.2 Birth characteristics

Birth characteristics, obstetrical history and maternal smoking data are shown in Table 7 for the ELBW group. Of ELBW adults, 30% (15) were SGA at birth. Obstetrical history was unknown for 18% of ELBW births. From the known obstetrical history data, 44% of ELBW births were from pregnancies with no obstetrical complications. Of the ELBW births with known obstetrical complications during pregnancy, hypertension/eclampsia and abruptio placentae were most common. Of those ELBW births with a known maternal smoking history (72%), 16% were born to mothers who smoked during pregnancy.

Individuals born NBW came from healthy pregnancies with no obstetrical complications and were AGA at birth. The mean birth weight of the NBW group was 3362.4 ± 502 g (males = 3462.4 ± 487.4 g, females = 3304.5 ± 507.5 g).

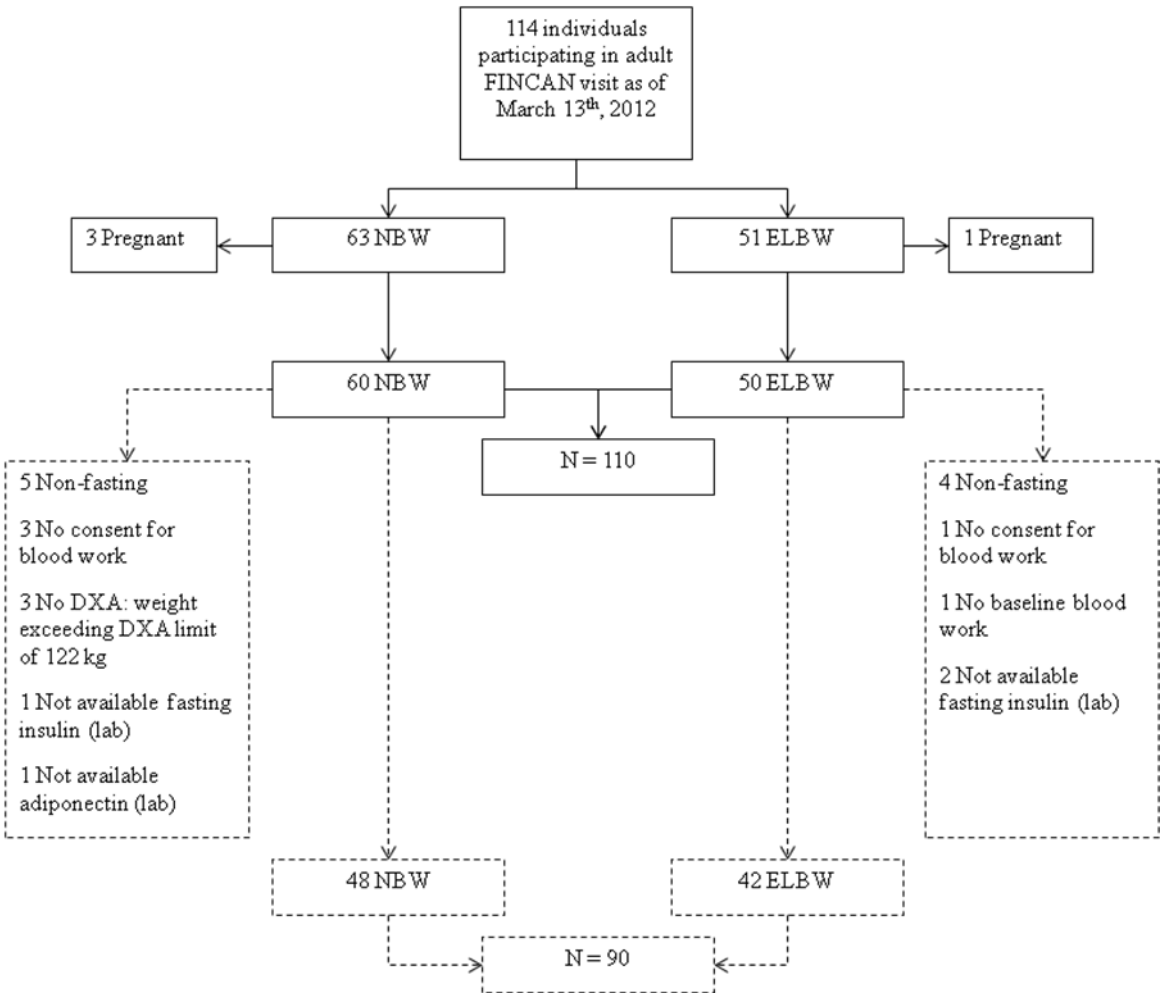


**Figure 5. Sample sizes and excluded participants for thesis project.** N = 110 (NBW = 60, ELBW = 50) for comparing clinical characteristics and health outcomes between NBW and ELBW adults. Dashed lines indicate sample size (N = 90) and excluded participants for *multivariate analyses* examining determinants of insulin resistance (primary objective #2).

**Table 6.** Current sample size by birth weight group and sex

| | **Male, n (%)** | **Female, n (%)** | **All, n (%)** |
|---|---|---|---|
| **NBW, n (%)** | 22 (36.7) | 38 (63.3) | 60 (54.5) |
| **ELBW, n (%)** | 18 (36) | 32 (64) | 50 (45.5) |
| **All, n (%)** | 40 (36.4) | 70 (63.6) | 110 |

**Table 7.** Birth characteristics, obstetrical history and maternal smoking data of ELBW group only

| | **Male (n=18)** | **Female (n=32)** | **All (n=50)** |
|---|---|---|---|
| **Birth weight, g, mean (SD)** | 826.4 (120.9) | 847 (141.5) | 839.6 (133.6) |
| **Gestation, wk, mean (SD)** | 26.2 (1.8) | 28.1 (2.0) | 27.4 (2.1) |
| **SGA, <10th percentile, n (%)** | 4 (22.2) | 11 (34.4) | 15 (30) |
| **Obstetrical history** | | | |
| **Infection** | 0 | 2 (6.25) | 2 (4) |
| **Diabetes** | 0 | 2 (6.25) | 2 (4) |
| **Hypertension/eclampsia** | 1 (5.6) | 7 (21.9) | 8 (16) |
| **Abruptio placentae** | 4 (22.2) | 2 (6.25) | 6 (12) |
| **Placental previa** | 1 (5.6) | 0 | 1 (2) |
| **Unknown** | 4 (22.2) | 5 (15.6) | 9 (18) |
| **No complications** | 8 (44.4) | 14 (43.8) | 22 (44) |
| **Maternal smoking** | | | |
| **Non-smokers, n (%)** | 10 (55.6) | 18 (56.3) | 28 (56) |
| **1-10 cigarettes/day, n (%)** | 1 (5.6) | 5 (15.6) | 6 (12) |
| **11-20 cigarettes/day, n (%)** | 0 | 1 (3.1) | 1 (2) |
| **>20 cigarettes/day, n (%)** | 1 (5.6) | 0 | 1 (2) |
| **All smoking, n (%)** | 2 (11.1) | 6 (18.8) | 8 (16) |
| **Unknown, n (%)** | 6 (33.3) | 8 (25) | 14 (28) |

### 4.3 Comparing health outcomes between adults born ELBW and NBW

Table 8 shows the unadjusted clinical characteristics and health outcomes of NBW and ELBW participants in adulthood. The proportion of males was similar between the two groups. Mean age of all participants was 33.12 ± 1.6 years. Compared to their NBW counterparts, ELBW adults were shorter ($p = 0.023$), had higher systolic blood pressure ($p = 0.023$), total percent body fat (0.029), central fat (0.018), fasting glucose ($p = 0.05$), 2-hour insulin ($p = 0.01$) and had lower total lean mass ($p = 0.011$) and lean mass index ($p = 0.018$). No differences in weight, BMI, waist circumference, diastolic blood pressure, total body fat, 2-hour glucose, fasting insulin, HOMA%B, HOMA-IR and adiponectin were identified between groups.

No interactions were identified between sex and birth weight group for any of the clinical characteristics and health outcomes in adulthood (Table 9).

**Table 8.** Unadjusted clinical characteristics and health outcomes of adults born NBW and ELBW

| | ALL | | | NBW | | | ELBW | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Valid N** | **Mean** | **SD** | **Valid N** | **Mean** | **SD** | **Valid N** | **Mean** | **SD** | **p-value** |
| **Male, n (%)** | | 40 (36.4) | | | 22 (36.7) | | | 18 (36) | | 1 |
| **Age (yr)** | 110 | 33.12 | 1.55 | 60 | 33.17 | 1.47 | 50 | 33.06 | 1.65 | 0.721 |
| **Height (cm)** | 110 | 167.22 | 11.35 | 60 | 169.46 | 10.99 | 50 | 164.53 | 11.30 | **0.023*** |
| **Weight (kg)** | 110 | 73.44 | 17.15 | 60 | 75.67 | 18.16 | 50 | 70.77 | 15.62 | 0.133 |
| **BMI (kg/m$^2$)** | 110 | 26.19 | 5.45 | 60 | 26.18 | 4.97 | 50 | 26.20 | 6.03 | 0.891 |
| **Waist circumference (cm)** | 110 | 83.18 | 13.21 | 60 | 82.95 | 12.85 | 50 | 83.45 | 13.75 | 0.904 |
| **Systolic blood pressure (mmHg)** | 110 | 111.06 | 11.75 | 60 | 108.73 | 10.99 | 50 | 113.86 | 12.12 | **0.022*** |
| **Diastolic blood pressure (mmHg)** | 110 | 72.25 | 9.44 | 60 | 70.70 | 8.19 | 50 | 74.12 | 10.55 | 0.058 |
| **Total body fat (kg)** | 107 | 23.07 | 10.19 | 57 | 22.32 | 10.03 | 50 | 23.92 | 10.42 | 0.422 |
| **Total body fat (%)** | 107 | 33.13 | 9.95 | 57 | 31.17 | 10.35 | 50 | 35.37 | 9.07 | **0.029*** |
| **Total lean mass (kg)** | 107 | 44.41 | 12.40 | 57 | 47.25 | 11.82 | 50 | 41.17 | 12.36 | **0.011*** |
| **LMI (kg/m$^2$)** | 107 | 26.39 | 6.17 | 57 | 27.70 | 5.47 | 50 | 24.91 | 6.62 | **0.019*** |
| **Central fat (kg)** | 107 | 2.16 | 1.15 | 57 | 1.92 | 1.01 | 50 | 2.43 | 1.24 | **0.018*** |
| **Fasting glucose (mmol/L)** | 98 | 5.02 | 0.68 | 53 | 4.90 | 0.69 | 45 | 5.17 | 0.65 | **0.050*** |
| **2-hour glucose (mmol/L)** | 94 | 5.55 | 1.83 | 52 | 5.32 | 1.64 | 42 | 5.84 | 2.03 | 0.190 |
| **Fasting insulin (pmol/L)** | 94 | 78.80 | 63.35 | 52 | 77.04 | 68.50 | 42 | 80.98 | 57.11 | 0.804 |
| **2-hour insulin (pmol/L)** | 92 | 294.42 | 187.02 | 50 | 259.39 | 169.70 | 42 | 336.12 | 199.83 | **0.010*** |
| **HOMA%B** | 94 | 120.97 | 64.02 | 52 | 121.92 | 62.42 | 42 | 119.80 | 66.69 | 0.450 |
| **HOMA-IR** | 94 | 1.45 | 1.14 | 52 | 1.41 | 1.23 | 42 | 1.49 | 1.03 | 0.911 |
| **Adiponectin (µg/mL)** | 96 | 11.04 | 5.78 | 52 | 11.14 | 5.99 | 44 | 10.91 | 5.58 | 0.879 |

**Table 9.** Interactions between sex and birth weight group for each clinical characteristic/health outcome

| | N | p-value |
|---|---|---|
| **Height (cm)** | 110 | 0.230 |
| **Weight (kg)** | 110 | 0.806 |
| **BMI (kg/m$^2$)** | 110 | 0.696 |
| **Waist circumference (cm)** | 110 | 0.529 |
| **Systolic blood pressure (mmHg)** | 110 | 0.483 |
| **Diastolic blood pressure (mmHg)** | 110 | 0.829 |
| **Total body fat (kg)** | 107 | 0.349 |
| **Total body fat (%)** | 107 | 0.326 |
| **Total lean mass (kg)** | 107 | 0.147 |
| **LMI (kg/m$^2$)** | 107 | 0.116 |
| **Central fat (kg)** | 107 | 0.291 |
| **Fasting glucose (mmol/L)** | 98 | 0.397 |
| **2-hour glucose (mmol/L)** | 94 | 0.982 |
| **Fasting insulin (pmol/L)** | 94 | 0.074 |
| **2-hour insulin (pmol/L)** | 92 | 0.145 |
| **HOMA%B** | 94 | 0.504 |
| **HOMA-IR** | 94 | 0.765 |
| **Adiponectin (µg/mL)** | 96 | 0.069 |

### 4.4 Determinants of IR in adults born NBW and ELBW

#### 4.4.1 Univariate regression analyses

Table 10 shows the unadjusted univariate regression analyses for each predictor variable on insulin resistance (log HOMA-IR) in adults born NBW and ELBW. Log waist circumference was the most significant predictor of insulin resistance in NBW and ELBW adults accounting for 20% of the variability in IR ($p < 0.001$). Log BMI, log central fat, log adiponectin and total body fat percentage were also significant predictors of IR, individually accounting for 17.3%, 10.3%, 7.5% and 5.1% of the variability in IR respectively. Higher waist circumference, BMI, central fat, and total body fat percentage, as well as lower adiponectin concentrations are associated with increased insulin resistance as measured by HOMA-IR. Age, sex, birth weight group (NBW/ELBW), LMI and log birth weight were not significant predictors of insulin resistance.

#### 4.4.2 Multivariate regression analyses and interactions

In a multivariate regression analysis assessing predictors of IR (Table 11), log central fat remained the only significant predictor of insulin resistance even after adjustment for log adiponectin, LMI and log birth weight (model 4, $p = 0.011$). The addition of log adiponectin in model 2 resulted in model 2 accounting for 12.5% of the variability in IR compared to model 1 which accounted for 9.8% of the variability ($p = 0.109$).

In a second multivariate regression analysis (Table 12) assessing log waist circumference, log adiponectin, LMI and log birth weight as predictors of IR, log waist circumference was the only significant predictor of insulin resistance even after

adjustment for the other predictors (model 4, p = 0.001). Using log waist circumference in the multivariate analysis instead of log central fat accounted for 17.7% of the variability in IR compared to 12.6% in the previous multivariate analysis.

Lastly, no interactions were identified between birth weight group and log waist circumference, log BMI, body fat percentage, log central fat and log adiponectin with insulin resistance in NBW and ELBW adults (Table 13).

**Table 10.** Unadjusted univariate regression analysis for predictor variables on insulin resistance (log-HOMA-IR) in adults born NBW and ELBW

| | N | Unstandardized β | Std. error | Standardized β | $R^2$ | %Variability | p-value |
|---|---|---|---|---|---|---|---|
| **Age (yr)** | 94 | -0.033 | 0.023 | -0.15 | 0.022 | 2.2% | 0.15 |
| **Sex** | 94 | -0.14 | 0.076 | -0.19 | <0.001 | <0.1% | 0.859 |
| **Birth weight group** | 94 | 0.008 | 0.072 | 0.012 | <0.001 | <0.1% | 0.911 |
| **log BMI (kg/m$^2$)** | 94 | 1.688 | 0.385 | 0.416 | 0.173 | 17.3% | **<0.001*** |
| **log WC (cm)** | 94 | 2.243 | 0.467 | 0.448 | 0.2 | 20.0% | **<0.001*** |
| **total fat (%)** | 91 | 0.008 | 0.004 | 0.227 | 0.051 | 5.1% | **0.031*** |
| **log central fat (g)** | 91 | 0.411 | 0.129 | 0.321 | 0.103 | 10.3% | **0.002*** |
| **log adiponectin (µg/mL)** | 93 | -0.395 | 0.145 | -0.274 | 0.075 | 7.5% | **0.008*** |
| **LMI (kg/m$^2$)** | 91 | 0.009 | 0.006 | 0.144 | 0.021 | 2.1% | 0.173 |
| **log birth weight (g)** | 93 | -0.03 | 0.118 | -0.026 | 0.001 | 0.1% | 0.803 |

**Table 11.** Multivariate regression analysis 1 examining predictors of insulin resistance in adults born NBW and ELBW

| | N | Unstd β | Std. Error | Std β | 95% CI Lower bound | 95% CI Upper Bound | $R^2$ | $R^2$ change | p-value |
|---|---|---|---|---|---|---|---|---|---|
| **Model 1** | 90 | | | | | | | | |
| Constant | | -1.292 | 0.428 | | -2.142 | -0.442 | 0.098 | | 0.003 |
| Log central fat (g) | | 0.402 | 0.130 | 0.313 | 0.142 | 0.661 | | | **0.003*** |
| **Model 2** | 90 | | | | | | | | |
| Constant | | -0.916 | 0.483 | | -1.877 | 0.045 | 0.125 | 0.027 | 0.061 |
| Log central fat (g) | | 0.364 | 0.131 | 0.284 | 0.103 | 0.625 | | | **0.007*** |
| Log adiponectin (µg/mL) | | -0.255 | 0.157 | -0.166 | -0.567 | 0.058 | | | 0.109 |
| **Model 3** | 90 | | | | | | | | |
| Constant | | -0.970 | 0.517 | | -1.999 | 0.059 | 0.126 | 0.001 | 0.064 |
| Log central fat (g) | | 0.360 | 0.133 | 0.281 | 0.096 | 0.624 | | | **0.008*** |
| Log adiponectin (µg/mL) | | -0.239 | 0.166 | -0.156 | -0.569 | 0.091 | | | 0.153 |
| LMI ($kg/m^2$) | | 0.002 | 0.007 | 0.033 | -0.011 | 0.015 | | | 0.762 |
| **Model 4** | 90 | | | | | | | | |
| Constant | | -0.992 | 0.700 | | -2.385 | 0.401 | 0.126 | 0.000 | 0.160 |
| Log central fat (g) | | 0.362 | 0.139 | 0.282 | 0.085 | 0.639 | | | **0.011*** |
| Log adiponectin (µg/mL) | | -0.240 | 0.167 | -0.156 | -0.573 | 0.093 | | | 0.156 |
| LMI ($kg/m^2$) | | 0.002 | 0.007 | 0.032 | -0.012 | 0.015 | | | 0.778 |
| Log birth weight (g) | | 0.006 | 0.121 | 0.005 | -0.234 | 0.246 | | | 0.962 |

**Table 12.** Multivariate regression analysis 2 examining predictors of insulin resistance in adults born NBW and ELBW

| | | | | | 95% CI | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **N** | **Unstd β** | **Std. Error** | **Std β** | **Lower bound** | **Upper Bound** | **R2** | **R2 change** | **p-value** |
| **Model 1** | 90 | | | | | | | | |
| Constant | | -4.090 | 1.027 | | -6.131 | -2.048 | 0.156 | | <0.001 |
| Log waist circumference (cm) | | 2.154 | 0.538 | 0.394 | 1.085 | 3.224 | | | **<0.001** |
| **Model 2** | 90 | | | | | | | | |
| Constant | | -3.705 | 1.206 | | -6.102 | -1.309 | 0.159 | 0.004 | 0.003 |
| Log waist circumference (cm) | | 2.006 | 0.591 | 0.367 | 0.830 | 3.182 | | | **0.001*** |
| Log adiponectin (µg/mL) | | -0.102 | 0.166 | -0.067 | -0.432 | 0.228 | | | 0.540 |
| **Model 3** | 90 | | | | | | | | |
| Constant | | -4.339 | 1.287 | | -6.898 | -1.779 | 0.177 | 0.018 | 0.001 |
| Log waist circumference (cm) | | 2.490 | 0.688 | 0.456 | 1.122 | 3.858 | | | **0.001*** |
| Log adiponectin (µg/mL) | | -0.129 | 0.166 | -0.084 | -0.460 | 0.202 | | | 0.440 |
| LMI ($kg/m^2$) | | -0.010 | 0.007 | -0.165 | -0.025 | 0.005 | | | 0.178 |
| **Model 4** | 90 | | | | | | | | |
| Constant | | -4.449 | 1.451 | | -7.334 | -1.564 | 0.177 | 0.000 | 0.003 |
| Log waist circumference (cm) | | 2.521 | 0.715 | 0.462 | 1.098 | 3.943 | | | **0.001*** |
| Log adiponectin (µg/mL) | | -0.130 | 0.168 | -0.085 | -0.463 | 0.203 | | | 0.439 |
| LMI ($kg/m^2$) | | -0.010 | 0.008 | -0.172 | -0.026 | 0.005 | | | 0.184 |
| Log birth weight (g) | | 0.020 | 0.116 | 0.018 | -0.211 | 0.250 | | | 0.867 |

**Table 13.** Interaction terms included in multivariate regression analysis

| **Interaction term** | **p-value** |
|---|---|
| Birth weight group x log waist circumference | 0.952 |
| Birth weight group x log BMI | 0.863 |
| Birth weight group x body fat percentage | 0.109 |
| Birth weight group x log central fat | 0.513 |
| Birth weight group x log adiponectin | 0.926 |

Birth weight group coded: 0 = NBW, 1 = ELBW

### 4.5 Exploratory objectives

#### 4.5.1 Prevalence of dysglycemia

The prevalence of IGT and T2D in the entire cohort was 8.5% and 3.2% respectively (Table 14). The prevalence of both IGT and T2D was higher in the ELBW compared to NBW group but this did not reach statistical significance (16.7% vs. 7.7%, p = 0.21) (Table 14).

**Table 14.** Prevalence of dysglycemia and T2D in adults born NBW and ELBW

| | **NBW (n = 52)** | **ELBW (n = 42)** | **All (n = 94)** | **p-value** |
|---|---|---|---|---|
| **IFG, n (%)** | 0 | 0 | 0 | |
| **IGT, n (%)** | 3 (5.9) | 5 (11.9) | 8 (8.5) | |
| **T2D, n (%)** | 1 (1.9) | 2 (4.8) | 3 (3.2) | |
| **All, n (%)** | 4 (7.7) | 7 (16.7) | 11 (11.7) | 0.21 |

Differences between groups for "All" determined by Fisher's exact test

#### 4.5.2 Subgroup analysis: adults born ELBW+SGA vs. ELBW+AGA

Table 15 shows the birth characteristics, maternal obstetrical history and maternal smoking data for the ELBW group by birth weight for gestational age. 15 of 50 ELBWs (30%) were born SGA. Mean gestational age was higher in ELBW+ SGA than ELBW+AGA (p = 0.0005). From ELBW pregnancies with a known maternal obstetrical history, 12 of 14 (86%) ELBW+SGA adults compared to 7 of 27 (27%) ELBW+AGA adults came from pregnancies with obstetrical complications. Among the obstetrical complications noted, hypertension/eclampsia during pregnancy was associated with a higher proportion of ELBW+SGA (p = 0.0004). From known data, maternal smoking was not different between ELBW+SGA and ELBW+AGA infants.

Table 16 shows the unadjusted clinical characteristics and health outcomes of ELBW adults born SGA and AGA at birth. Compared to their AGA counterparts, adults born ELBW+SGA are significantly shorter ($p = 0.030$), have significantly higher waist circumference ($p = 0.026$), total body fat ($p = 0.010$), total body fat percentage ($p = 0.001$), central fat ($p = 0.006$), 2-hour glucose ($p = 0.03$), HOMA%B ($p = 0.047$), and significantly lower adiponectin ($p = 0.001$). Furthermore, the prevalence of dysglycemia (IFG, IGT and T2D) is significantly higher in ELBW+SGA compared to NBW adults ($p = 0.026$).

When clinical characteristics and health outcomes were compared between ELBW+AGA and NBW adults, no significant differences were observed in any of the variables measured (Table 16). However, it is worth noting that total lean mass and LMI approached significance ($p = 0.071$ and 0.080). Therefore, adults born ELBW+AGA are similar to their NBW counterparts in all aspects except perhaps lean mass.

Table 17 shows that log waist circumference, log BMI, total fat % and log central fat were significant individual predictors of insulin resistance in ELBW adults. Waist circumference accounted for the most (18.8%) variability in insulin resistance ($r^2 = 0.188$, $p = 0.004$). BMI, total fat percentage and log central fat individually accounted for 17.6%, 12.3% and 10.4% of the variability in IR respectively. Age, sex, weight status at birth (SGA/AGA), gestational age in weeks, log adiponectin, LMI and log birth weight were not significant predictors of IR in ELBW adults.

**Table 15.** Birth characteristics, obstetrical history and maternal smoking in ELBW group by birth weight for gestational age

| | **SGA (n=15)** | **AGA (n=35)** | **All (n=50)** | **SGA vs. AGA p-value** |
|---|---|---|---|---|
| **Birth weight, g, mean (SD)** | 789 (139.6) | 861.3 (126.8) | 839.6 (133.6) | 0.079 |
| **Gestation, wk, mean (SD)** | 29 (2.1) | 26.8 (1.8) | 27.4 (2.1) | **0.0005*** |
| **Obstetrical history, n (%)** | | | | |
| **Infection** | 1 (6.7) | 1 (2.9) | 2 (4) | 0.514 |
| **Diabetes** | 2 (13.3) | 0 | 2 (4) | 0.086 |
| **Hypertension/eclampsia** | 7 (46.7) | 1 (2.9) | 8 (16) | **0.0004*** |
| **Abruptio placentae** | 1 (6.7) | 5 (14.3) | 6 (12) | 0.654 |
| **Placental previa** | 1 (6.7) | 0 | 1 (2) | 0.300 |
| **Unknown** | 1 (6.7) | 8 (22.9) | 9 (18) | 0.247 |
| **No complications** | 2 (13.3) | 20 (57.1) | 22 (44) | **0.005*** |
| **Maternal smoking** | | | | |
| **Non-smokers, n (%)** | 9 (60) | 19 (54.3) | 28 (56) | 0.765 |
| **Smokers, n (%)** | 4 (26.7) | 4 (11.4) | 8 (16) | 0.220 |
| **Unknown, n (%)** | 2 (13.3) | 12 (34.3) | 14 (28) | 0.179 |

**Table 16.** Unadjusted clinical characteristics and health outcomes of ELBW adults born SGA and AGA at birth

| | SGA | | | AGA | | | NBW | | | SGA vs. AGA | AGA vs. NBW | SGA vs. NBW |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Valid N** | **Mean** | **SD** | **Valid N** | **Mean** | **SD** | **Valid N** | **p-value** | **p-value** | **p-value** |
| **Age (yr)** | 32.53 | 1.60 | 15 | 33.29 | 1.64 | 35 | 33.17 | 1.47 | 60 | 0.169 | 0.731 | 0.159 |
| **Height (cm)** | 161.43 | 12.88 | 15 | 165.86 | 10.48 | 35 | 169.46 | 10.99 | 60 | **0.030*** | 0.203 | **0.003*** |
| **Weight (kg)** | 75.73 | 17.33 | 15 | 68.65 | 14.57 | 35 | 75.67 | 18.16 | 60 | 0.276 | 0.170 | 0.937 |
| **BMI (kg/m2)** | 29.54 | 8.72 | 15 | 24.77 | 3.75 | 35 | 26.18 | 4.97 | 60 | **0.026*** | 0.285 | 0.106 |
| **Waist circumference (cm)** | 90.35 | 14.35 | 15 | 80.49 | 12.55 | 35 | 82.95 | 12.85 | 60 | 0.051 | 0.597 | 0.072 |
| **Systolic blood pressure (mmHg)** | 116.53 | 11.49 | 15 | 112.71 | 12.36 | 35 | 108.73 | 10.99 | 60 | 0.743 | 0.090 | 0.098 |
| **Diastolic blood pressure (mmHg)** | 74.67 | 8.06 | 15 | 73.89 | 11.55 | 35 | 70.70 | 8.19 | 60 | 0.799 | 0.061 | 0.222 |
| **Total body fat (kg)** | 29.38 | 13.86 | 15 | 21.57 | 7.65 | 35 | 22.32 | 10.03 | 57 | **0.010*** | 0.785 | **0.013*** |
| **Total body fat (%)** | 39.86 | 11.27 | 15 | 33.45 | 7.33 | 35 | 31.17 | 10.35 | 57 | **0.001*** | 0.576 | **0.001*** |
| **Total lean mass (kg)** | 42.50 | 9.61 | 15 | 40.60 | 13.45 | 35 | 47.25 | 11.82 | 57 | 0.589 | 0.071 | **0.043*** |
| **LMI (kg/m2)** | 26.20 | 4.89 | 15 | 24.36 | 7.23 | 35 | 27.70 | 5.47 | 57 | 0.958 | 0.080 | 0.081 |
| **Central (android) fat (kg)** | 3.11 | 1.61 | 15 | 2.13 | 0.92 | 35 | 1.93 | 1.01 | 57 | **0.006*** | 0.392 | **0.001*** |
| **Fasting glucose (mmol/L)** | 5.02 | 0.63 | 12 | 5.23 | 0.66 | 33 | 4.90 | 0.69 | 53 | 0.360 | 0.052 | 0.728 |
| **2-hour glucose (mmol/L)** | 7.04 | 2.26 | 12 | 5.36 | 1.75 | 30 | 5.32 | 1.64 | 52 | **0.030*** | 0.957 | **0.019*** |
| **Fasting insulin (pmol/L)** | 110.62 | 65.29 | 12 | 72.88 | 50.43 | 32 | 77.04 | 68.50 | 52 | 0.091 | 0.795 | 0.066 |
| **2-hour insulin (pmol/L)** | 472.14 | 289.70 | 10 | 293.61 | 143.59 | 32 | 259.39 | 169.70 | 50 | 0.057 | 0.252 | **0.016*** |
| **HOMA%B** | 157.63 | 82.47 | 11 | 106.37 | 55.68 | 31 | 121.92 | 62.42 | 52 | **0.047*** | 0.366 | 0.119 |
| **HOMA-IR** | 1.97 | 1.22 | 11 | 1.32 | 0.92 | 31 | 1.41 | 1.23 | 52 | 0.112 | 0.862 | 0.117 |
| **Adiponectin (µg/mL)** | 6.99 | 2.26 | 12 | 12.38 | 5.77 | 32 | 11.14 | 5.99 | 52 | **0.001*** | 0.298 | **0.018*** |
| **IFG, n (%)** | 0 | | 12 | 0 | | 33 | 0 | | 53 | - | - | - |
| **IGT, n (%)** | 4 (33.3) | | 12 | 1 (3.0) | | 33 | 3 (5.7) | | 53 | - | - | - |
| **T2D, n (%)** | 0 | | 12 | 2 (6.1) | | 33 | 1 (1.9) | | 53 | - | - | - |
| **All*, n (%)** | 4 (33.3) | | 12 | 3 (9.1) | | 33 | 4 (7.5) | | 53 | 0.069 | 0.704 | **0.026*** |

*All = IFG + IGT + T2

**Table 17.** Univariate regression analyses for determinants of insulin resistance in adults born ELBW only

| | N | Unstandardized β | Std. error | Standardized β | $R^2$ | %Variability | p-value |
|---|---|---|---|---|---|---|---|
| **Age (yr)** | 42 | -0.042 | 0.035 | -0.187 | 0.035 | 3.5% | 0.236 |
| **Sex (M/F)** | 42 | 0.012 | 0.126 | 0.015 | <0.001 | <0.1% | 0.927 |
| **GA status (SGA/AGA)** | 42 | -0.152 | 0.135 | -0.175 | 0.031 | 3.1% | 0.268 |
| **GA (wks)** | 39 | -0.009 | 0.032 | -0.048 | 0.002 | 0.2% | 0.772 |
| **log BMI ($kg/m^2$)** | 42 | 1.752 | 0.598 | 0.42 | 0.176 | 17.6% | **0.006*** |
| **log WC (cm)** | 42 | 2.272 | 0.747 | 0.433 | 0.188 | 18.8% | **0.004*** |
| **total fat (%)** | 42 | 0.15 | 0.006 | 0.351 | 0.123 | 12.3% | **0.023*** |
| **log central fat (g)** | 42 | 0.534 | 0.248 | 0.322 | 0.104 | 10.4% | **0.038*** |
| **log adiponectin (µg/mL)** | 42 | -0.415 | 0.279 | -0.229 | 0.052 | 5.2% | 0.145 |
| **LMI ($kg/m^2$)** | 42 | 0.013 | 0.01 | 0.197 | 0.039 | 3.9% | 0.211 |
| **log birth weight (g)** | 42 | 0.839 | 0.824 | 0.161 | 0.026 | 2.6% | 0.315 |

## CHAPTER 5: DISCUSSION

In addition to genetic and lifestyle factors, the intrauterine and early postnatal environments are now highlighted as important determinants of cardio-metabolic disease risk in adulthood (Hofman et al., 2004). Individuals born at full term with low birth weight appear to be more susceptible to impaired glucose regulation, insulin resistance, T2D and cardiovascular disease in later life (Barker et al., 2002; Eriksson et al., 2006; Robinson et al., 1992). Although lower birth weights are more prevalent among preterm infants, fewer studies have examined the effects of markedly *preterm* birth on risk factors for these conditions in adulthood (Hovi et al., 2007). With improvements in neonatal technology over the past two decades, survival rates of preterm infants have substantially increased (Lorenz, 2001). Prospectively assembled preterm birth cohorts are only now beginning to enter adulthood where metabolic health outcomes can be evaluated. We have for the first time, compared and identified potential determinants of IR in adults born ELBW and NBW.

Compared to their NBW counterparts, we have identified that adults who were born ELBW have higher central adiposity, lower lean mass for height (LMI) but with no difference in circulating adiponectin concentrations, insulin resistance or dysglycemia. Within our cohort, central adiposity measured by either waist circumference or regional fat measured by DXA, is a significant correlate of IR in adults born ELBW or NBW. We identified no difference between the birth weight groups in the determinants of IR. We did observe however, that adults born ELBW and SGA at birth have higher total body fat, central adiposity, 2-hour glucose and insulin levels, and lower adiponectin concentrations

compared to adults born ELBW and AGA at birth. Interestingly, adults born ELBW and AGA at birth were similar to adults born NBW in the majority of health outcomes assessed, except for perhaps lean mass and systolic blood pressure. The following sections will discuss our findings according to the previously defined objectives. Finally, limitations, future directions and conclusions will be addressed.

**5.1 An "unfavourable" body composition**

Evidence suggests that individuals born at full term with lower birth weights have a greater abdominal distribution of adiposity and reduced muscle mass in adolescent and adult life (Kensara et al., 2005; Law et al., 1992; Malina et al., 1996; Singhal et al., 2003). We wanted to see if these findings were present in adults born preterm, as lower birth weights are more prevalent among preterm infants and because all ELBW adults in our cohort had a birth weight below normal at term equivalent age. Studies examining body composition outcomes in those born preterm versus term have largely focused on the quantity of both fat and lean mass, with less focus on fat distribution. We have identified for the first time in adulthood that preterm, ELBW individuals have a more "unfavourable" body composition characterized by higher central adiposity and lower lean mass for height (lean mass index, LMI) compared to their term, NBW counterparts.

Adjustments for age and sex were not made for these comparisons between groups as mean age and the proportion of males in each birth weight group were similar (Table 8). Additionally, interactions between sex and birth weight group on both central fat and LMI were not observed (Table 9). This means that the effect of age on central fat or LMI did not depend on the effect of sex. It is important to note that ageing is associated with

changes in body composition such as increases in fat mass (Beaufrere & Morio, 2000) and loss of lean body mass (Dilorio et al., 2006). Furthermore, for a given BMI, there are well known differences in body composition between men and women, including higher lean mass in men and higher total adiposity in women (Garaulet et al., 2000). Men tend to store fat centrally and thus have more visceral than subcutaneous adipose tissue compared to women. On the other hand, women tend to have a peripheral distribution of adipose tissue with greater subcutaneous than visceral adipose tissue than men (Bray et al., 2008; Kvist et al., 1988). Perhaps with a larger sample size, sex specific differences in central adiposity and LMI can be explored in adults born ELBW and NBW.

### 5.1.1 Increased central fat

The few studies that have examined central fat distribution in preterm and term individuals have reported inconsistent findings from birth to young adulthood. Higher central adiposity in individuals born preterm is only reported in one study at birth (Uthaya et al., 2005), with no differences observed in childhood compared to term counterparts. Given the consistent pattern of increases in weight with advancing age in adults, perhaps time is required to demonstrate differences between individuals born preterm and term. Alternatively, it is possible that differences in lifestyle behaviours (i.e. diet, physical activity and sleep patterns) between ELBW and NBW individuals may be influencing the gradual onset of differences in body composition.

In addition, inconsistencies in results throughout the life course may be due to methodological issues such as: examination of individuals with varying degrees of prematurity and/or birth weight, variations in sample size as well as discrepancies in

defining and measuring central adiposity. The study by Uthaya et al. (2005) used magnetic resonance imaging to measure intra-abdominal (visceral) adipose tissue in 38 very preterm (<32 weeks gestation at birth) and 29 term neonates at term equivalent age. The use of this gold standard methodology enables direct quantification of adipose tissue compartments (visceral vs. subcutaneous) in the central region. Darendelier et al. (2008), Willemsen et al. (2008) and Hovi et al. (2007) all defined the trunk region of the DXA scan as their measure of central adiposity. Darendelier et al. (2008) examined fat distribution in 96 preterm (≤37 weeks gestation at birth) and 83 term born children, whereas the study by Willemsen et al. (2008) included 479 preterm (≤36 weeks gestation at birth) and term short SGA children. The study by Hovi et al. (2007) consisted of 150 preterm, VLBW (<36 weeks gestation at birth) and 136 term, NBW young adults. It may be possible that DXA is insufficiently sensitive to detect differences in central adiposity at these ages. Furthermore, defining the trunk region as the estimate of central adiposity may include both android and gynoid fat distribution. Therefore, this may not fully represent estimates of central (visceral) adiposity.

### 5.1.2 Reduced lean mass

Similar to the study by Hovi et al. (2007), we have observed that ELBW individuals have lower total lean mass for height than their NBW peers. Current height is a strong predictor of total lean mass. Adjusting total lean mass for height (LMI) was appropriate since the ELBW group was shorter than their NBW counterparts (Table 8). Therefore, we can confirm that differences in lean mass are not attributable to the shorter stature of the ELBW group. The majority of studies, except for Cooke et al. (2009)

identified no difference in lean mass between preterm and term individuals in infancy, childhood and adolescence (Appendix B, Table 3). However, we and others (Hovi et al., 2007) have suggested otherwise in adulthood. In young adulthood (18-24 years), Hovi and colleagues (2007) found a 3.3% and 5.3% lower lean mass index in VLBW women and men respectively compared to their NBW counterparts. Lean mass was measured by DXA in all of the studies noted (Appendix B, Table 3).

Differences in lean body mass for height may not be apparent until later life (beyond childhood and adolescence), as lean body mass peaks in the late teens and twenties and begins to gradually declines after age 30 (Janssen et al., 2000; Lexell, Downham, & Sjostrom, 1986). Ageing is associated with weight gain (Beaufrere and Morio, 2000) and lean mass loss (Di lorio et al, 2006). Sarcopenia, the age-related decline in lean body mass that affects functional capacity (i.e. strength), usually presents in the elderly years. However, lower lean mass in ELBW adults at this early adult age may pose an increased risk for functional impairment, physical disability and metabolic adversity in subsequent adult years (Janssen, Heymsfield, & Ross, 2002). Therefore, it is important to consider whether individuals born preterm with low birth weight have had less accrual of lean mass or a more rapid decline in lean mass with advancing age.

### 5.1.3 Unfavourable body composition: why?

An unfavourable body composition characterized by higher central adiposity and lower lean mass for height in preterm individuals may not present until early adulthood as we and others (Hovi et al., 2007; Kajantie et al., 2010) have observed. Prematurity and low birth weight have a small genetic component, largely reflecting the quality of the

intrauterine environment (Gluckman & Hanson, 2004). In our cohort, those born ELBW were small at birth because of: extreme prematurity, IUGR (as some were smaller than they should have been given their gestational age at birth) or a combination of both. The reasons underlying preterm birth and IUGR are difficult to pin point, but can be related to a suboptimal intrauterine environment. This environment may be due to a variety of maternal, placental and fetal factors including: maternal under/overnutrition, maternal smoking/alcohol/environmental toxin exposure, infection, hypoxia, inflammation, abnormal placental development/function, steroid exposure and fetal abnormalities (Cetin & Antonazzo, 2009; Fowden et al., 2005). We propose that in addition to genetic and lifestyle factors, disruptions to the growth and development of adipose tissue and lean mass *in-utero* and during early postnatal life contribute to an increased susceptibility for an "unfavourable" body composition. We can turn to other models of low birth weight, in particular fetal under-nutrition and growth restriction, to understand early life programming effects contributing to a more unfavourable body composition.

ELBW infants are usually born <28 weeks gestation, thus the majority spend the third trimester outside of the maternal intrauterine environment. The third trimester of pregnancy (28-42 weeks gestation) is an important period for adipose tissue and lean mass development. These processes are highly sensitive to the nutritional environment and metabolic milieu; in particular concentrations of insulin-like growth factors, glucose, insulin and glucocorticoids (Aihaud, Grimaldi, & Negrel, 1992; Martin, Hausman, & Hausman 1998). It has been suggested that in infants experiencing intrauterine growth restriction (IUGR) due to a suboptimal in-utero environment, nutrients are reallocated to

optimize the growth of key body organs (i.e. the brain). This is at the expense of other organs/tissues including both adipose tissue and muscle (Desai et al., 1996; Larciprete et al., 2005; Padoan et al., 2004; Zhu et al., 2006). Not all ELBW infants experience IUGR, however we do know that all ELBW infants in our cohort experienced poor growth from birth to term equivalent age (Saigal et al., 2006). Nutrient reallocation may also occur ex-utero in ELBW infants during the time of poor postnatal growth to term equivalent age, contributing to altered adipose tissue and lean mass development. Furthermore, the number of adipocytes and muscle cells change very little after fetal and early postnatal life (Muhlhausler & Smith, 2005). Little is known about the capacity of pre-adipocytes to differentiate into mature adipocytes in human adult life. However, what we do understand highlights that most, if not all of adipose tissue development is completed in early life (Ailhaud et al., 1992; Martin, 1998). Hirsch and colleagues reported in rats that that the number of adipocytes was relatively fixed after young adulthood (Klyde & Hirsch, 1979) and more recent studies have shown a very low adipocyte turnover rate in adult humans (Spalding et al., 2008; Strawford et al, 2004). The majority of skeletal muscle development occurs during the fetal stage (secondary myogenesis) where most of the muscle fibres are formed. New muscle fibers are generated only in adulthood to replace injured tissue (Du et al., 2010). Therefore, suboptimal intrauterine conditions and disruptions to normal fetal growth (preterm birth) may confer an increased susceptibility to an unfavourable body composition from an early age.

It has been suggested that adaptations for fetal survival like nutrient reallocation only become detrimental when the postnatal nutrient environment is more abundant than

it had been prenatally (Hales & Barker, 2001). This is known as the thrifty phenotype hypothesis. Studies of prematurity and/or low birth weight have highlighted catch up growth in the early postnatal period as a potential link between low birth weight and a more unfavourable body composition (Ong et al., 2000) and metabolic adversity (Cianfarani et al., 1999; Ibanez et al., 2006) later in life. The deleterious effects of catch-up growth are not fully understood. However it is believed that the rapid adipose tissue deposition and expansion, as well as lower lean tissue gain accompanying catch up growth play key roles (Dullo et al., 2002; Ibanez et al., 2006). A study in mice showed that LBW offspring exposed to maternal under nutrition and exhibiting catch up growth from birth to 3 weeks showed: greater adipocyte size in diameter and increased adipose tissue lipogenic gene expression compared to offspring that did not exhibit catch up growth (Insagnaitis et al., 2009). Furthermore, a rat model of weight recovery reported that during catch up growth, in vivo glucose utilization under insulin stimulation was reduced in skeletal muscle but enhanced in white adipose tissue (Cettour-Rose et al., 2005). In a subsequent study, this same group identified that increased fat deposition in catch up growth was associated with: adipose tissue hyperplasia and hypertrophy, an early and sustained lipogenic capacity and an increased adipose tissue capacity for triglyceride storage (Summermatter et al., 2009). Additionally, in rats, reduced in vivo glucose utilization and lower oxidative capacity of skeletal muscle after catch up growth was identified (Cettour-Rose et al., 2005; Crescenzo et al., 2006; Summermatter et al., 2008). Thus, early postnatal growth patterns like catch up growth may contribute to altered adipose tissue and lean mass development and/or function.

Differences in body composition between individuals born ELBW and NBW cannot be fully explained by early intrauterine and early postnatal disturbances as current lifestyle behaviours are known to affect overall health. Lifestyle factors such as poor dietary habits, psychosocial stress and short sleep duration are all known to contribute to weight gain and poor metabolic health (Vgontzas et al., 2008; Wadden et al., 2008), and may even differ between individuals born ELBW and NBW. In particular, it is well established that engagement in regular physical activity is beneficial for overall health as well as protecting against obesity and other cardio-metabolic complications. Perhaps differences in lifestyle factors particularly physical activity may help to explain our finding of higher percentage body fat, greater central adiposity and lower lean mass for height in ELBW adults (Table 8). Kajantie and colleagues found that preterm, VLBW young adults (~22 years) were less physically active during leisure time compared to their NBW peers. These differences were characterized by lower frequency, intensity and average session duration of leisure time physical activity even after adjustment for confounders (Kajantie et al., 2010). In another study, ELBW adolescents aged 17 years reported less participation in sports compared to NBW controls (Rogers et al., 2005). Therefore lower physical activity in this population may contribute to a greater susceptibility of overweight and obesity. Physical activity data for ELBW and NBW adults are currently being collected as part of the FINCAN adult follow-up visit. This data however was not available for analysis in this thesis project, but provides an area of future focus to compliment results from this project. A number of factors may potentially explain why physical activity levels are lower in ELBW/VLBW populations such as:

poorer motor coordination (Burns et al., 2009; Evensen et al., 2009; Rogers et al., 2005), lower physical self confidence as well as lower perceived physical ability (Saigal et al., 2007), lower lean mass (Hovi et al., 2007; Kajantie et al., 2010), less muscle strength and lower aerobic capacity (Keller et al., 2000; Pianosi and Fisk, 2000) compared to NBW peers.

**5.2 Adiponectin: a link between body fat distribution and metabolic adversity?**

Adiponectin is believed to be a potential mediator between central adiposity and insulin resistance (Cnop et al., 2009) due to its key role in enhancing insulin sensitivity (Havel et al., 2001; Saltiel, 2001;). We compared total adiponectin concentrations between ELBW and NBW adults and identified no difference between groups (Table 8). Adiponectin concentrations are affected by several physiological factors, with increasing age, puberty status and male sex associated with lower adiponectin levels. In our analysis we did not make adjustments for age or male sex as both were similar in ELBW and NBW groups. Furthermore, interaction between sex and birth weight group with adiponectin was not found (Table 9), meaning that the effect of sex on adiponectin concentrations did not depend on the effect of birth weight group on adiponectin concentrations. Puberty status would not come into effect here as ELBW and NBW individuals are well beyond this life stage. Studies examining differences in adiponectin concentrations between preterm and term individuals have largely focused on the infancy period: at birth and term corrected age. This may not provide useful information about differences in adulthood, as adiponectin behaves differently in infancy; acting as a regulator of fetal growth and demonstrates a positive association with infant weight gain.

Although there are no studies previous to ours that examine adiponectin levels in preterm and term born individuals beyond birth, we can turn to the literature comparing adiponectin concentrations in *term* SGA and AGA individuals to hypothesize why we would expect to observe lower adiponectin concentrations in those born preterm. Adiponectin levels in *term* SGA children compared to AGA counterparts are reduced in some (Cianfarani et al., 2004; Jaquet et al., 2006), but not all studies (Challa et al., 2009; Miras et al., 2010; Sancakli et al., 2008).

Obese subjects with an excess of visceral adipose tissue have lower adiponectin concentrations and elevations in inflammatory cytokines (i.e. plasma C-reactive protein (CRP), IL-6 and TNF-α) (Cote et al., 2005; Lemieux et al., 2001). We expected to see lower adiponectin concentrations in ELBW adults as they had higher central adiposity and total percent body fat compared to their NBW counterparts (Table 8). Lower adiponectin levels are associated with higher central adiposity as well as precede the development of T2D in both humans and animals in parallel with increased IR (Hotta et al., 2001; Spranger et al., 2003). We did not find differences between the two groups in adiponectin concentrations or insulin resistance. No difference in adiponectin levels may help to explain our findings of no observed differences in IR at this age of assessment. If we had observed lower adiponectin levels in adults born ELBW with no difference in IR between the two groups, this may have provided stronger evidence that adults born ELBW may be at a greater risk of increased IR later on in adulthood.

We had also hypothesized that lower adiponectin concentrations, may have provided preliminary evidence to suggest altered adipocyte function in adults born ELBW

compared to NBW. Although differences were not observed in our population, adipocyte dysfunction in ELBW/LBW individuals should not be ruled out. Indeed reduced expression of IGF-1 and insulin signalling molecules (GLUT4, PI3K, IRS-1) in the adipose tissue of low birth weight adults compared to controls has been reported (Ozanne et al., 2006). Perhaps systemic markers are not sensitive enough to identify altered adipose tissue function at this time point. It may be worthwhile for subsequent studies to examine the HMW isoform of adiponectin in addition to total adiponectin in order to report any observed differences in total adiponectin, HMW adiponectin and the ratio of HMW to total.

**5.3 Determinants of IR**

Increased insulin resistance in individuals born preterm has been reported in some but not all studies (Appendix B, Figure 1). Since we did not have sufficient power to examine this type of difference between ELBW and NBW adults, we examined the relationship of central adiposity, adiponectin concentration, LMI and birth weight with insulin resistance in a cohort of ELBW and NBW adults. Although age and sex are important biological predictors of insulin resistance (Geer & Shen, 2009), we did not include these variables in the multivariate regression models (Tables 11 and 12) because: in the univariate analyses both age and sex were not significant independent predictors of IR in our cohort (Table 10) and with our sample size of 90 participants, including more than 4 predictors would contribute to less power and stability in our model.

### 5.3.1 Central adiposity

We identified that central adiposity was the most important correlate of insulin resistance in adults born ELBW and NBW even after adjustment for other determinants. Our observation provides support for other studies in preterm individuals that highlight the importance of current body fatness rather than size at birth in determining insulin resistance. Using an intravenous glucose tolerance test, Willemsen et al. (2009) could demonstrate no influence of prematurity on insulin sensitivity in 169 preterm and 136 term young adults aged 18-24 years. However, trunk fat and oral contraceptive use were the most important determinants of insulin sensitivity in young adulthood, independently of gestational age and size at birth. Additionally, a recent study by Mathai et al. (2012) reported a 29% lower insulin sensitivity in adults born preterm compared to their term born counterparts, stating that their result was predominantly reflected by higher BMI in the preterm group.

We did not identify interactions between birth weight group and central fat measured by DXA and between birth weight group and waist circumference on IR in our cohort (Table 13). Thus, we can conclude that higher central adiposity is associated with greater insulin resistance in adults born ELBW *and* NBW. This is in opposition to the study by Finken et al. (2006). They identified a significant interaction between birth weight standard deviation score (SDS) and adult wait circumference SDS on IR in 346 on IR preterm young adults (~19 years). They also reported that the effect of adult absolute fat mass on IR was dependent on its interaction with birth weight SDS, in which a higher absolute fat mass after lower birth weight SDS was associated with greater insulin

resistance measured by HOMA-IR. However, Finken and colleagues did not have a term, NBW comparator group. Perhaps a larger sample size may highlight interactions between birth weight and central adiposity with insulin resistance in our cohort, as we observed ELBW adults have higher central fat mass compared to their NBW peers despite no differences in IR. Our finding also suggests that while ELBW adults have higher central adiposity, it may be this difference in body composition that may potentially help to explain observed differences in IR reported by others (Figure 1, Appendix B).

Table 2 in Appendix D illustrates covariates corrected for in other studies analyzing insulin sensitivity/resistance between preterm and term individuals or preterm+SGA and preterm+AGA (Appendix C, Table 1). Only Willemsen et al. (2009) made adjustments for current adiposity (fat mass and trunk fat). Based on our findings, measures of central adiposity either by waist circumference or DXA should be adjusted for when examining differences in insulin sensitivity/resistance between preterm/low birth weight and term/NBW individuals.

Lastly, central adiposity measured by both waist circumference and DXA were significant determinants of IR in the cohort. The multivariate model including waist circumference as the measure of adiposity explained more of the variation in IR than the model with central adiposity measured by DXA (17.7% and 12.6%- model 4). Therefore waist circumference may be the preferred method as it is less time consuming, less costly and can easily be obtained in clinical settings.

### 5.3.2 Adiponectin

Although adiponectin was significantly associated with IR in the univariate analysis (Table 10), in the multivariate analyses it did not contribute significantly to explaining variability in IR. Adding adiponectin into the regression model after central fat measured by DXA (Table 11- model 2) only resulted in an additional 2.7% variation explained in IR. This may be partly due to the fact that central adiposity is a strong determinant of circulating adiponectin concentrations. In an epidemiological study of 3355 healthy participants aged 23-45 years Steffes and colleagues reported that waist circumference adjusted for BMI exhibited a relatively strong inverse correlation with total adiponectin concentrations (r = -0.5) (Steffes et al., 2004). Cnop et al. (2003) also reported relatively strong inverse correlations between waist-to-hip ratio and intra-abdominal fat measured by computed tomography in middle-aged men and women (r = -0.35 and -0.36). Perhaps in our cohort, central adiposity accounts for the variability in IR explained by both itself and adiponectin. Furthermore, this may be a consequence of our sample size as Cnop et al. (2003) showed in182 healthy participants although intra-abdominal fat accounted for the majority of the variation (41%), adiponectin accounted for an additional 3% of the variation in insulin sensitivity (p = 0.0003).

## 5.4 Exploratory objectives

### 5.4.1 Insulin resistance and dysglycemia

The study was underpowered to examine if adults born ELBW had increased IR compared to their NBW counterparts using HOMA-IR based on previous literature (Hovi

et al., 2007). Thus our negative findings cannot allow conclusion that ELBW adults have no difference in IR compared to their NBW counterparts.

Both insulin resistance and pancreatic β-cell dysfunction precede the development of T2D (WHO, 2011).We identified that ELBW adults have higher fasting glucose concentrations ($p = 0.05$) and 2-hour insulin levels ($p = 0.01$), but found no difference in the prevalence of dysglycemia (IFG, IGT or T2D), compared to NBW adults. Hyperglycemia in the basal state is associated with reduced sensitivity to insulin in the liver, in which hepatic glucose output is not suppressed (Abdul-Ghani et al., 2006). Higher 2-hour insulin concentrations after a glucose load may reflect a compensatory mechanism for peripheral insulin resistance: the β-cells of the pancreas produce more insulin as higher insulin concentrations are needed to help glucose enter the critical tissues (adipose tissue, skeletal muscle and liver) (Shanik et al., 2008). It is interesting to note that higher 2-hour insulin in the ELBW group was sufficient to lower glucose levels at the 2-hour time point (no difference in 2-hour glucose between ELBW and NBW groups). Higher fasting glucose adults born ELBW prompted us to explore differences in β-cell function since β-cell dysfunction may even be present before overt hyperglycemia (Abdul-Ghani et al., 2006). However, no difference in β-cell function was identified between the two groups. Perhaps higher fasting glucose and 2-hour insulin concentrations may reflect premature alterations in glucose and insulin homeostasis.

### 5.4.2 Subgroup analysis: ELBW+SGA vs. ELBW+AGA

Although all ELBW infants are SGA by term corrected age, birth weight for gestational age provides insight to the intrauterine conditions and prenatal growth patterns

of the fetus prior to delivery. We had hypothesized that within the ELBW group, those born SGA at birth would have higher IR in adulthood compared to those born AGA. We were underpowered to examine if adults born ELBW+SGA had increased insulin resistance compared to adults born ELBW+AGA using HOMA-IR based on previous literature (Hovi et al., 2007). Therefore, our negative findings cannot allow conclusion that no difference in IR exists between these two groups.

In our study, ELBW+SGA were slightly more mature at birth than the ELBW+AGA, and therefore spent a longer part of their third trimester in utero. However, while both groups would have experienced a similar environment in the immediate post-natal period, the ELBW+SGA experienced intrauterine growth restriction resulting in their small size for gestational age at birth. Interestingly, it is the ELBW+SGA, in our preliminary comparison of the groups that appears to demonstrate greater disturbances in body composition and glucose-insulin metabolism compared to the NBW group (Table 16). The ELBW+SGA group had higher BMI, absolute total fat mass, body fat percentage, central adiposity, 2-hour glucose and insulin levels as well as lower adiponectin concentrations compared to the ELBW+AGA group. Furthermore, these differences were not observed when comparing ELBW+AGA adults and NBW controls.

It has been proposed that the timing of in-utero insult arising from maternal, placental and fetal conditions during critical windows of development is central to the severity of future disease (Fowden et al., 2005). In 19-year old men who had been exposed to famine in-utero, the prevalence of obesity was greatest amongst those that

were exposed during the first two trimesters of pregnancy compared to those exposed during the last trimester/immediate postnatal period (Ravelli, Stein, & Susser, 1976). Furthermore, the third trimester of pregnancy is believed to be a critical window for the programming of glucose intolerance. Ravelli et al. (1998) identified reduced glucose tolerance in middle aged adults who were in-utero at the time of strict food restriction rationing during the Dutch Winter Famine at the end of World War II. Those exposed to the famine during late gestation (third trimester) had higher 2-hour glucose and insulin levels after a standard glucose load compared to unexposed individuals (Ravelli et al., 1998). This has also been confirmed in animal studies (Holeman & Van Assche, 2003; Seckl, 2004). Taken together, we suggest that the mechanisms leading to SGA at birth may have contributed to subsequent differences in body composition and metabolism later in life. Determining a certain period or trimester during pregnancy by which poor intrauterine growth has the greatest effect on future metabolic adversity requires further investigation.

The ELBW+SGA group had lower adiponectin concentrations compared to the ELBW+AGA group. Unlike our previous findings comparing ELBW and NBW adults, lower adiponectin levels may reflect downstream effects of higher total and central adiposity observed in ELBW+SGA adults (Table 16). Our results are consistent with studies in term SGA pre-pubertal children, stating that lower adiponectin concentrations may be a predisposing factor mediating small size at birth and later development of IR (Cianfarani et al., 2004; Jaquet et al., 2006). However this has not been reported in all studies (See recent review by Briana & Malamitsi-Puchner, 2009).

Lastly, we noticed that comparisons of ELBW+SGA vs. NBW and ELBW+AGA vs. NBW indicated similar values for systolic blood pressure and LMI in adults born ELBW+SGA and ELBW+AGA (Table 16). Perhaps these findings suggest potential programming effects prior to the third trimester, as most ELBW infants are born < 28 weeks gestation.

### 5.5 Limitations

The sample of adults born ELBW and NBW for this thesis project was not entirely representative of the original cohort as recruitment for the FINCAN study is currently ongoing; our entire sample represented a subset of ELBW and NBW individuals from the original cohort assembled thirty years ago. This may have contributed to sampling bias as we included those participants that completed their adult follow-up visit within the first half of the FINCAN study; from March 2011-March 2012. Sampling bias is a systematic error which results in some members of the population to be less likely included in the sample than others. Within our sample, a higher proportion of females compared to males in both birth weight groups as well as exclusion of neurologically impaired adults born ELBW may have also contributed to sampling bias. It is likely that the higher proportion of females was due to the anecdotal observation that women were more likely to complete their FINCAN visit on their scheduled date at the clinic, whereas men were more likely to re-schedule their FINCAN visit to a later date. Furthermore, a larger proportion of males at the time of the clinic visit were non-fasting (they did not remember to come fasting in the morning, $p = 0.058$). Therefore it may be possible that baseline and 2-hour glucose and insulin concentrations as well as IR values may be more reflective of female associated values. The exclusion of adults born ELBW with neurological impairments was not intentional but a matter of timing; they will be recruited for completion of their adult FINCAN visit during summer 2012. Confirmation of our reported results at the end of the FINCAN study is warranted to ensure that our findings are representative of the cohort in its entirety. Furthermore, a larger sample size will enable us to: explore our

exploratory objectives with greater power, potentially identify sex specific differences in adult health outcomes associated with prematurity and ELBW and observe whether adiponectin concentration explains a significant amount of the variability in IR in the multivariate regression analyses.

Other limitations of the study are associated with the choice of certain methodologies. A limitation of using the HOMA-IR method as a surrogate marker of insulin resistance is that it does not provide information regarding the relative contributions of liver versus skeletal muscle to observed reductions in whole-body insulin sensitivity (Abdul-Ghani et al., 2007). Furthermore, HOMA-IR is a surrogate measure of steady-state insulin sensitivity. Perhaps future studies may examine differences in insulin sensitivity between preterm and term individuals under dynamic (glucose-stimulated) conditions. Additionally, the variability in fasting insulin concentrations for the ELBW and NBW groups was quite high (Table 8). This may have contributed to a source of error in the results of the HOMA-IR calculation.

Although we were able to quantify the amount of fat mass in the central compartment of the body (central adiposity) in adults born ELBW and NBW using DXA, DXA does not differentiate between visceral and subcutaneous central fat depots unlike a magnetic resonance or computed tomography scan. Since it known that higher visceral adiposity in the abdominal region is strongly associated with poorer cardio-metabolic health, it would be very beneficial to understand differences in these central fat depots between the two groups.

### 5.6 Future directions

The results from this project highlight the importance of the intrauterine environment and early postnatal life on metabolic health parameters in adulthood. There are many opportunities to elaborate on the results presented in this project in both clinical and basic science areas.

As previously mentioned in the discussion, the underlying causes of prematurity and/or low birth weight are difficult to pinpoint. Examination of effects of specific maternal obstetrical complications (i.e. hypertension/eclampsia during pregnancy) and adult metabolic health outcomes may provide insight into common intrauterine conditions associated with future risk of cardio-metabolic complications (i.e obesity, T2D, cardiovascular disease) in preterm individuals. In our sample, the numbers of maternal obstetrical complications were relatively low and may have been incomplete, although this was consistent with knowledge at the time of initial recruitments. However, international collaboration involving data sharing and joint analyses between multiple preterm birth cohorts may provide adequate numbers to pursue these kinds of research questions.

The identification of differences in body composition in ELBW and NBW adults may prompt investigation of lifestyle factors such as dietary habits, participation in physical activity and sleeping patterns. Poor diet, physical inactivity and reduced amounts of sleep are associated with obesity and poor metabolic health (Vgontzas et al., 2008; Wadden et al., 2008). Perhaps differences in these factors may help to explain our findings of differences in body composition and the importance of central adiposity as a

predictor of IR in addition to early life disturbances. Furthermore, a more in-depth understanding of lifestyle differences between ELBW and NBW individuals can assist in the development and prescription of interventional strategies that may help to reduce the risk of cardio-metabolic complications in individuals born ELBW.

Another area of research is to identify functional differences in adipose tissue and lean mass. A study in *term* SGA and AGA children revealed that adipose tissue of those born SGA at birth had lower insulin signalling proteins (IGF-IR, IR, AKT and ERK) compared to their AGA counterparts (Iniguez et al., 2009). Furthermore, study by Jensen et al. (2007) identified an increased proportion of type II muscle fibers in low birth weight 19 year old males compared to their term born peers. In obese and type 2 diabetic individuals, the distribution of muscle fiber type is shifted toward type II fibers which are associated with lower oxidative capacity (He, Watkins, & Kelly, 2001; Oberbach et al., 2006) and reduced GLUT4 content (Daugaard et al., 2000; Gaster et al, 2001;). We have identified higher central adiposity and lower lean mass for height in ELBW adults. Examining functional tissue differences in *preterm* compared to *term* individuals could be a next step in identifying specific mechanisms behind altered tissue development from intrauterine and early postnatal insults and later metabolic adversity.

It is important to note that the McMaster ELBW cohort was assembled when improvements to neonatal care such as mechanical ventilation and intravenous feeding of nutritional formulas with increased protein and vitamin content were introduced. Breast milk was not administered to preterm infants in the 1970s/early eighties. The ELBW infants in our cohort were given intravenous fluids with proteins and fat, and an orally

higher calorie formula of 24 to 30 cal/oz depending on their toleration. It would be very interesting to examine health outcomes over the life course, in particular body composition and fat distribution, of individuals born ELBW with respect to previous (i.e. late 20$^{th}$ century) and current feeding practices after birth and in infancy.

### 5.7 Conclusions & Implications

This study demonstrates that adults born ELBW have higher central adiposity, lower lean mass for height, and no differences in adiponectin concentrations or IR compared to their NBW counterparts. Furthermore, central adiposity measured by both DXA and waist circumference is the most significant correlate of IR in both adults born ELBW and NBW, even after consideration of other correlates of interest. This finding is very exciting as it suggests alterations in body composition, specifically higher central adiposity, likely explain differences in IR reported by others, rather than some innate difference in insulin sensitivity central to prematurity or poor perinatal growth. Understanding the etiology of this difference in body composition should be the target of subsequent studies in preterm versus term populations. Our exploratory analysis also raises the hypothesis that the adequacy of the intrauterine environment, rather than birth weight, is the key determinant of adult body composition, adiponectin levels and glucose metabolism.

We have observed that poorer body composition and metabolic health outcomes are present in adulthood in those born preterm with ELBW. This may suggest an increased risk of poorer cardio-metabolic health (i.e. type 2 diabetes, cardiovascular disease) in subsequent years. Furthermore, knowledge that these kinds of health outcomes exist in adulthood may contribute to improved care and monitoring of ELBW and LBW individuals over the life course. Perhaps weight gain should be tracked throughout the life course with emphasis and prescription of healthy diet and physical activity regimens to reduce the risk of metabolic adversity in later years.

## APPENDIX A: GOLD STANDARD FOR INSULIN SENSITIVITY/RESISTANCE

### *Gold standard technique for insulin sensitivity/resistance*

The hyperinsulinemic-euglycemic glucose clamp technique developed by DeFonzo et al. (1979) is the gold standard method for directly determining insulin sensitivity in humans. See recent review by Muniyappa et al. (2008) for a description of the procedure. The clamp directly measures the effects of insulin in promoting glucose utilization under steady-state conditions in vivo (DeFonzo, Tobin, & Andres, 1979). When combined with radiolabeled glucose, it can allow for the determination of the individual contributions of hepatic and muscle IR to the defect in whole-body mediated glucose disposal (DeFonzo, Simonson, & Ferrannini, 1982). Although considered the gold standard, the clamp has many limitations; it is very costly, labour intensive, invasive, and involves intravenous infusion of insulin and frequent blood sampling. Additionally, the clamp utilizes steady-state insulin levels that may be supra-physiological resulting in an inaccurate reflection of insulin action and glucose dynamics under physiological conditions (Muniyappa et al., 2008). Use of the hyperinsulinemic-euglycemic glucose clamp technique is not recommended for epidemiological studies, large clinical investigations, or routine clinical applications (Muniyappa et al., 2008).

**Table 1.** *Additional surrogate measures of insulin sensitivity/resistance under fasting conditions*

| Methodology | Measurement | Advantages | Disadvantages |
|---|---|---|---|
| Fasting insulin, 1/fasting insulin | Reciprocal of fasting plasma insulin concentration, U/ml | Practical (cheap, not time consuming), detects IR before clinical disease occurs | Lack of standardization of the insulin assay procedure (high proportion of false positives) |
| Glucose/insulin ratio | Ratio of fasting plasma glucose (mg/dl) and insulin (U/ml) concentration | Used in patients with polycystic ovary syndrome (PCOS) | Does not appropriately reflect the physiology of insulin sensitivity |
| Quantitative insulin sensitivity check index (QUICKI) | 1/Log (fasting insulin, U/ml) + Log (fasting glucose, mg/dl | Minimally invasive, reliable and precise index of insulin sensitivity, most evaluated and validated surrogate index for IS | Normal ranges should be determined for each laboratory due to lack of standardization of the insulin assay procedure (high proportion of false positives) |

Adapted from Muniyappa et al, 2008 and Singh & Saxena, 2010

## APPENDIX B: CALIBRATION CURVES FOR ELISAS

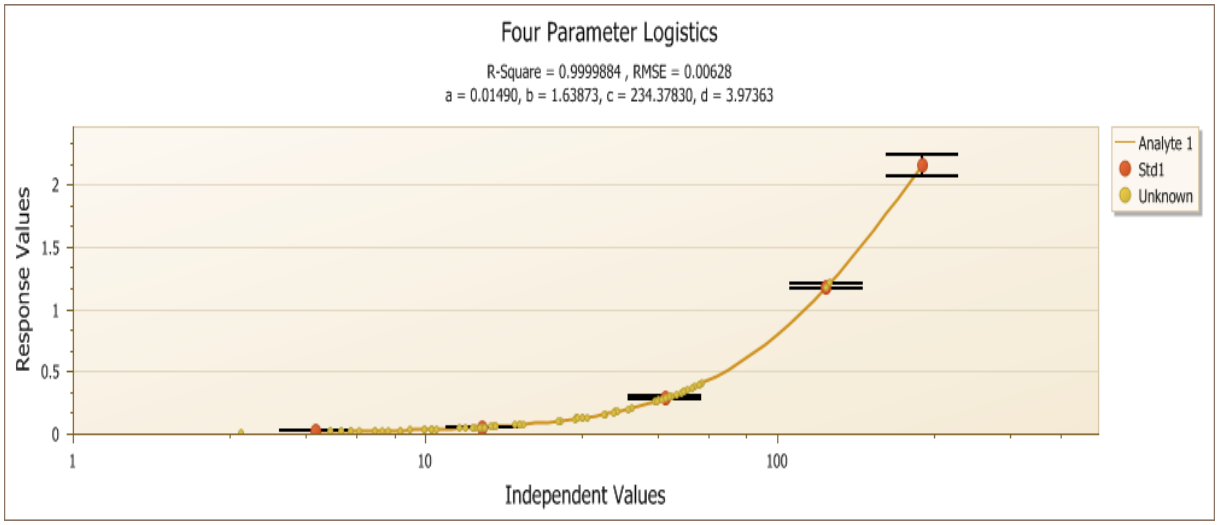


**Figure 1.** Four-parameter logistic function curve used to determine unknown fasting and 2-hour serum insulin concentrations.

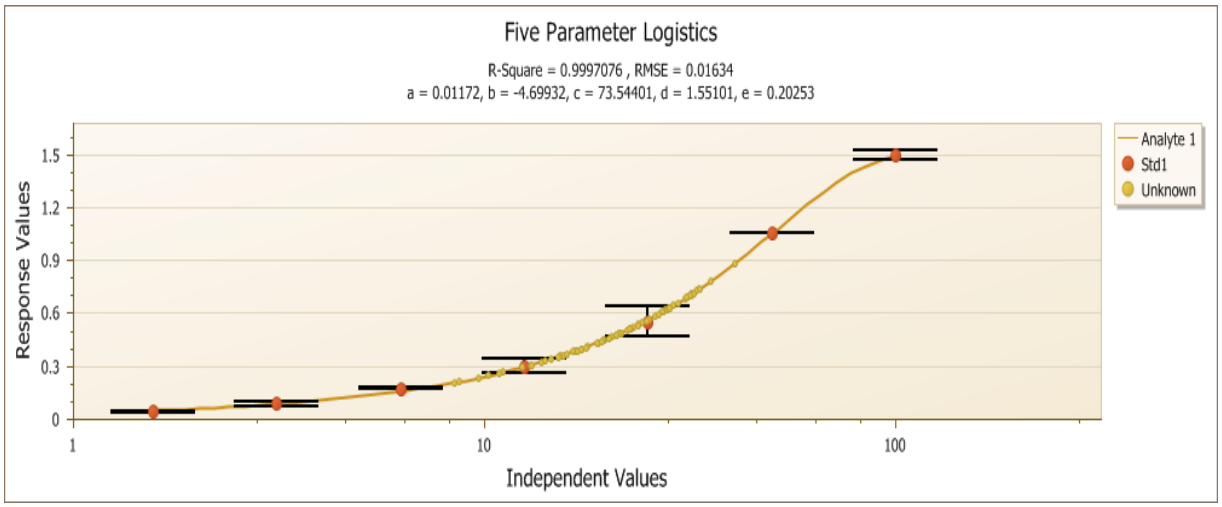


**Figure 2.** Five-parameter logistic function curve used to determine unknown fasting serum total adiponectin concentrations

## APPENDIX C: ANDROID REGION OF DXA SCAN

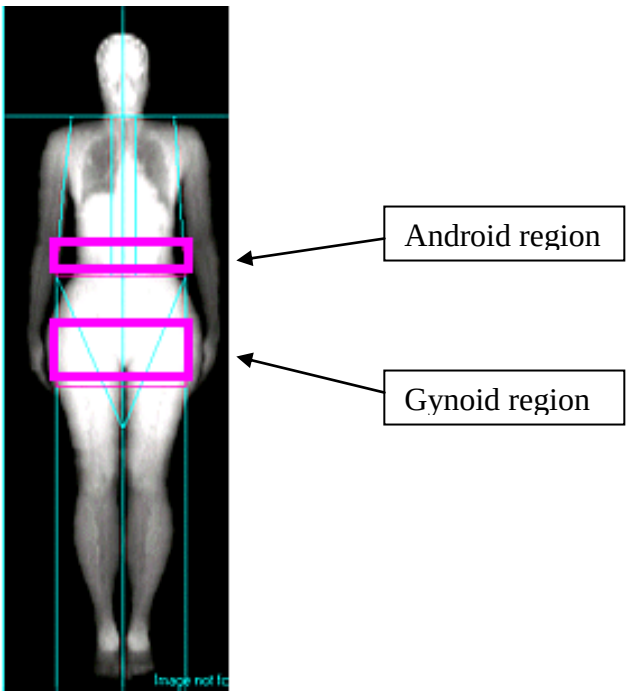


**Figure 1. Android and gynoid region of DXA scan.** The android region is defined to quantify the amount of fat in the central compartment of the body. Region of interest L2-L4 is selected using the DXA software program for analysis. The android region of interest is defined as the lower boundary at pelvis cut, upper boundary above pelvis cut by 20% of the distance between pelvis and neck cuts and lateral boundaries are the arm cuts.

## APPENDIX D: SUMMARY OF STUDIES AND HEALTH OUTCOMES

**Table 1.** Studies examining insulin resistance in those born preterm with low birth weight
(+) Indicates increased insulin resistance in preterm compared to term controls
--- Indicates no difference in insulin resistance between preterm and controls
† Study reported an inverse association between birth weight and 30-min insulin levels; lower birth weights associated with higher 30-min insulin, no control group included
†† Study reported an inverse association between birth weight and HOMA-IR; lower birth weights associated with greater HOMA-IR, no control group included

| | Birth | Childhood (4-9 yrs) | Adolescence | Young Adulthood (~19 yrs) | Adulthood (33-38 yrs) |
|---|---|---|---|---|---|
| Preterm+ELBW vs. control | ? | ? | ? | ? | ? |
| Preterm+VLBW/LBW vs. control | (+) Martos-Moreno et al., 2009 | --- Willemsen et al., 2008<br>(+) Darendelier et al., 2008<br>(+) Regan et al., 2006<br>(+) Hofman et al., 2004 | - | --- Willemsen et al., 2009<br>(+) Hovi et al. 2007 | (+) Mathai et al., 2012 |
| All preterm (SGA vs. AGA/no control) | - | --- Bo et al., 2006<br>(+) Bazaes et al., 2004<br>Fewtrell et al., 2000† | - | (+) Rotteveel et al., 2011<br>--- Finken et al., 2006†† | - |

**Appendix D (cont.)**

**Table 2.** Covariates used to when analyzing insulin sensitivity/resistance

| Study | Life stage | Covariates |
|---|---|---|
| Willemsen et al., 2008 | Childhood | Age, sex |
| Darendelier et al., 2008 | Childhood | Gestational age, birth weight |
| Regan et al., 2006 | Childhood | Age, sex, gender, weight SDS, height SDS, birth weight, nutrition parameters at birth |
| Hofman et al., 2004 | Childhood | Age, sex, height SDS, weight for length index, birth weight SDS, mid parental height SDS |
| Bo et al., 2006 | Childhood | Age, sex, gestational age, neonatal respiratory failure, gestational age status at birth (SGA/AGA), skinfold SDS, BMI SDS |
| Willemsen et al., 2009 | Young adulthood | Age, sex, birth weight, birth length, gestational age, oral contraceptive use, adult height, adult weight, adult fat mass, adult trunk fat mass |
| Rotteveel et al., 2011 | Young adulthood | Sex, height, weight, BMI, fat-free mass |
| Hovi et al., 2007 | Young adulthood | Sex, family history of diabetes, leisure time physical activity, parental education |
| Mathai et al., 2012 | Adulthood | Sex, age, BMI, antenatal steroid use |

**Appendix D (cont.)**

***Growth patterns and later insulin resistance in preterm***

Similar to individuals born full term and SGA, the importance of postnatal growth patterns, both poor postnatal growth to term equivalent age and catch up growth in weight, have been highlighted as important determinants connecting preterm birth with later insulin resistance. A study by Bo and colleagues in 34 pre-school VLBW children identified that those in the highest quartile of HOMA-IR showed weight centile crossing at 2 years, concluding that insulin resistance is better predicted by rapid postnatal weight gain independently of gestational age (Bo et al., 2006). In a prospective follow up study of 385 preterm children aged 9-12 years, 30-min insulin levels were associated with patterns of increased weight gain; those with the greatest increase in weight SDS between 18 months and current follow up had higher insulin concentrations before and after an oral glucose load (Fewtrell et al., 2000). Finken et al. showed a weak association between rapid infancy weight gain to 3 months and 1 year with higher basal insulin levels and HOMA-IR in 346 preterm, VLBW young adults. However, these associations lost statistical significance after adjustment for confounders (sex, race, gestational age, multiple pregnancy, SES, parity and hypertension during pregnancy). Rotteveel and colleagues examined whether growth until *term* age influenced insulin resistance in young adulthood in 57 individuals born very preterm with VLBW. They showed that those experiencing postnatal growth restriction (PGR) until TEA have a greater insulin resistance compared to those born AGA or without PGR. However, this difference was non-significant after adjustment for current BMI (Rotteveel et al., 2011).

**Appendix D (cont.)**

***Fat mass and percent body fat***

Table 3 summarizes studies examining fat mass and/or percent body fat in preterm individuals with low birth weight. Differences in fat mass and/or percent body fat have been observed in some (Cooke et al., 2009; Fewtrell et al., 2004; Gianni et al., 2008; Gianni et al., 2009; Uthaya et al., 2005; Willemsen et al., 2009) but not all studies (Cooke et al., 1999; Darendelier et al., 2008; Hovi et al., 2007; Peralta-Carcelen et al., 2000).In infancy at term equivalent age, Uthaya et al. (2005) reported a decrease in total fat mass in ELBW infants compared to their term born counterparts using magnetic resonance imaging. Conversely, Rawlings et al. (1999) and Gianni et al. (2009) reported greater fat mass and body fat percentage in preterm babies vs. term controls at hospital discharge assessed by DXA and PEAPOD respectively. Similar results were observed by Cooke et al. (2009). In preterm, LBW children, reduced adiposity measured by DXA was found by Fewtrell et al. (2004), Gianni et al. (2008) and Willemsen et al. (2009). However, Peralta-Carcelen et al. (2000) reported similarities in body composition assessed by DXA between NBW and ELBW adolescents despite lower height and weight in the ELBW group, which was also suggested by Darendelier et al. (2008) in preterm, low birth weight children. In early adulthood, Finken and colleagues showed that adult fat mass (assessed by skinfolds) was dependent on birth weight SDS; lower birth weight was associated with an increase in adult fat mass in VLBW young adults. However, in a fairly recent study by Hovi et al. (2007), no difference in adult fat mass or body fat percentage determined by DXA was noted in preterm, VLBW young adults compared to their normal birth weight counterparts. Evensen et al. (2009) reported similar skinfold measurements between preterm, VLBW, term, SGA and term, NBW young adults.

**Table 3.** Studies examining fat mass and/or body fat percentage in preterm, LBW

(-) Indicates reduced fat mass (g)/body fat percentage (%) in preterm compared to controls
(+) Indicates increased fat mass (g)/body fat percentage (%) in preterm compared to controls
--- Indicates no difference in fat mass (g)/body fat percentage (%) between preterm and controls
* Studies examining preterm, ELBW individuals
† In a subgroup analysis VLBW+SGA young adults had increased body fat compared to VLBW+AGA individuals
†† Showed interaction between birth weight and adult fat mass

| | Birth | Childhood (4-9 yrs) | Adolescence (~15 yrs) | Young Adulthood (18-24 yrs) | Adulthood |
|---|---|---|---|---|---|
| Preterm vs. control | (+) Gianni et al., 2009<br>(+) Cooke et al., 2009<br>(-) Uthaya et al., 2005*<br>(+) Cooke et al., 1999 | (-) Willemsen et al., 2009<br>(-) Gianni et al., 2008<br>--- Darendelier et al., 2008<br>(-) Fewtrell et al., 2004 | --- Peralta-Carcelen et al., 2000* | --- Evensen et al., 2009†<br>--- Hovi et al. 2007 | - |
| All preterm (no control) | - | - | - | Finken et al., 2006†† | - |

**Table 4.** Studies examining fat-free mass and/or lean mass in preterm, LBW
(-) Indicates reduced fat-free mass/lean mass in preterm compared to normal birth weight controls
--- Indicates no difference in fat-free mass/lean mass between preterm and controls
* Studies examining preterm, ELBW individuals

| | Birth | Childhood (4-9 yrs) | Adolescence (~15 yrs) | Young Adulthood (18-24 yrs) |
|---|---|---|---|---|
| Preterm (VLBW/LBW) vs. control | --- Gianni et al., 2009<br>(-) Cooke et al., 2009<br>--- Cooke et al., 1999 | --- Willemsen et al., 2009<br>--- Gianni et al., 2008<br>--- Fewtrell et al., 2004 | --- Peralta-Carcelen et al., 2000* | (-) Hovi et al., 2007<br>(-) Kajantie et al., 2010 |

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