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The effects of post-term birth, hyperemesis gravidarum, and birth order on childhood metabolism and body composition

Ahila Ayyavoo

A thesis submitted in partial fulfilment of the requirements for the degree of PhD

The University of Auckland, 2013
Abstract

Background: There is increasing evidence that early life events have major implications for health in later life. For example, studies at Liggins Institute have shown adverse metabolic outcomes in children and adults born small-for-gestational-age or preterm. In this thesis, glucose homeostasis and body composition (amongst other assessments) were evaluated in three under-explored groups of children, i.e. those i) born post-term (≥42 weeks gestation), ii) born of mothers who suffered from severe hyperemesis gravidarum, and iii) first-borns.

Participants: Healthy pre-pubertal children, aged 4–11 years, naturally conceived, born of singleton pregnancies and of birth weight appropriate-for-gestational-age (birth weight >-2 and <2 standard deviation scores), recruited in Auckland, New Zealand.

Methods: Primary outcome was insulin sensitivity measured using intravenous glucose tolerance tests and Bergman’s minimal model. Other assessments included body composition from whole-body dual-energy X-ray absorptiometry, fasting hormonal concentrations and lipid profiles, 24-hour ambulatory blood pressure monitoring, and inflammatory markers.

Results: We found that i) post-term children had a 34% reduction in insulin sensitivity in comparison to controls born at term, and also displayed a number of early markers of the metabolic syndrome; ii) children born to mothers who experienced severe hyperemesis gravidarum had insulin sensitivity that was 20% lower than that of controls; and iii) although first-born children were taller and slimmer, these children had a 27% reduction in insulin sensitivity and higher daytime blood pressure compared to later-borns.

Conclusions: We have identified three common groups of children (those born post-term, born of mothers who suffered from hyperemesis gravidarum, and first-borns) who are likely to be at an increased risk of developing type 2 diabetes mellitus and other metabolic and cardiovascular diseases later in life. Therefore, long-term follow up of these groups into adulthood is important, so that potential long-term adverse health effects can be better evaluated.
Wisdom is the ability to discern the truth in statements made by anybody, whoever they may be.

Someone asking "Whose child is this who has done so well?" is one of the best gifts a parent can receive.

– Thiruvalluvar – Tamil poet from the 2nd century BC

Dedicated to Appa, Amma, Baskar, Ashwath, and Advaith
for their love and unstinting support
Acknowledgements

I owe my heartfelt gratitude to many for having taken me successfully through this journey in research. I will always be thankful to Eachanari Vinayagar who has been my friend right through my life's journey. He has helped me by giving the strength of mind to continue along the chosen path, while being away from my loved ones for almost three years. The time spent with our group at Liggins Institute has been one of the best times of my life. I will always thank the stars for having had the opportunity to work with 'stars'.

I owe an enormous amount of gratitude to my supervisor Prof Wayne Cutfield. He has been a great source of support, inspiration, and guidance. I could not have wished for a better mentor. I would like to express my heartfelt appreciation for the motivation and encouragement from my co-supervisor Assoc Prof Paul Hofman. I am deeply thankful to my mentor in India, Prof Raghupathy, for encouraging me all along. Raghu, Wayne, and Paul have initiated my interest in Paediatric Endocrinology, and this interest has developed into an obsession under their tutelage.

I am grateful to José Derraik, who has been a pillar of strength from the start to finish. I owe him another round of thanks for his immense contribution in every aspect of my research and thesis. I am deeply thankful to our research nurses, Janene Biggs, Kathrynn Wrightson, Gail Gillies, and Christine Brennan for the help and support during every step of the research. I would like to thank Dr Craig Jefferies for his valuable comments. I am thankful to my advisers Prof Peter Stone, Dr Lynn Sadler, and Assoc Prof Frank Bloomfield for their help, and to Dr Yannan Jiang for very valuable statistical input. Eric Thorstensen and his group have kindly assisted with laboratory work. I am indeed happy to have had the support of my friends Silmara Gusso, Martin de Bock, Mrinal Murali, Sarah Hopkins, and the Starship team. I will always remember Eleanor Surtida with gratitude for giving me such a beautiful start to my journey in New Zealand. I am thankful to Gravida: National Centre for Growth and Development, the Australasian Paediatric Endocrine Group, and Ms Maureen Trotter for funding this research.

My parents Ayyavoo and Parvathy can’t be thanked enough for the love, support, and encouragement they have provided me all through my life. The sacrifice of my loved husband Baskar has provided me the opportunity to pursue my dream. My sons Ashwath and Advaith have been my pride and joy and God’s best gift to me. Their love and belief in me along with those of my sisters Charmila, Amirtha Mekhala, and Mithila have been at the root of all my achievements. My family loved me deeply enough to manage without me for three years, and had the strength to tell me to go out and achieve my goals. This acknowledgement would not be complete without
acknowledging the support of my new family in New Zealand – the VS family and friends. Prabha Hariswamy, Pushpa, and Lakshmi with their families will be a part of my family forever.

I am deeply indebted to the beautiful families, who had spent a lot of time and effort helping us by being a part of our research. I have loved meeting them and getting to know them personally. I have been humbled by their generosity.
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List of abbreviations

11β-HSD2 – 11β-hydroxysteroid dehydrogenase type 2
AIR – acute insulin response
BMI – body mass index
BMI SDS – body mass index standard deviation score
BP – blood pressure
CV – inter-assay coefficient of variation
DI – disposition index
DXA – dual-energy X-ray absorptiometry
DHEAS – dehydroepiandrosterone sulphate
FSIGT – frequently sampled intravenous glucose tolerance test
GH – growth hormone
GLP1 – glucagon-like peptide 1
HCG – human chorionic gonadotropin
HDL-C – high-density lipoprotein cholesterol
HPA – hypothalamo–pituitary–adrenal
IGF – insulin-like growth factor
IGFBP – insulin-like growth factor binding protein
IR – insulin receptor
LDL-C – low-density lipoprotein cholesterol
LGA – large-for-gestational-age
LMP – last menstrual period
m-TOR – mammalian receptor of rapamycin
OGTT – oral glucose tolerance test
SD – standard deviation
SDS – standard deviation score
SGA – small-for-gestational-age
SEM – standard error of the mean
SHG – severe hyperemesis gravidarum
S\text{G} – glucose effectiveness
S\text{I} – insulin sensitivity index
T2DM – type 2 diabetes mellitus
List of publications from this thesis


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Pre-pubertal children born post-term have reduced insulin sensitivity and other markers of the metabolic syndrome

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First-born children have reduced insulin sensitivity and higher daytime blood pressure compared to later-born children.

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Post-term births: are prolonged pregnancies too long?

Nature of contribution by PhD candidate: Conception of the commentary and writing

Extent of contribution by PhD candidate (%): 90%

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Childhood consequences of maternal hyperemesis gravidarum: is offspring health being thrown up?

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Is being first-born another risk factor for metabolic and cardiovascular diseases?

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Severe hyperemesis gravidarum is associated with reduced insulin sensitivity in the offspring in childhood

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Chapter 1. Introduction

1.1. Fetal programming

It is well-recognized that early life events (including the environment in utero and during infancy) have far reaching consequences into adult life\textsuperscript{1-3}. The gestational period is one of rapid growth, involving replication, differentiation and maturation of different organ systems. As a result, changes in the intra-uterine environment can lead to alterations (adaptations) in the developing embryo\textsuperscript{4}. For example, the fetus may adapt itself to a reduced availability of resources by optimizing the use of the nutrients to ensure survival\textsuperscript{5}. These adaptations may be cardiovascular, metabolic, or endocrine, and could alter the structure and/or function of various tissues, by promoting the growth of certain organs at the expense of others.

While these adaptations favour survival in the short-term, they may be a liability at a time when the environment is favourable with abundant nutrition, as per the "thrifty phenotype hypothesis"\textsuperscript{6,7}. Through developmental "programming" an insult or stimulus at critical period of fetal growth and development could have long-lasting effects\textsuperscript{6,8}. Thus, the concept of "developmental origins of health and disease" proposes that early life events may result in permanent phenotypic alterations in the offspring\textsuperscript{9,10}, which may extend to subsequent generations\textsuperscript{2}.

1.1.1. Low birth weight or small-for-gestational-age

Studies on fetal programming started on children born of low birth weight or small-for-gestational-age. Small babies were initially termed "low birth weight" if they weighed less than 2500 grams at birth\textsuperscript{5}. However, research identified a range of normal weights for a particular gestational period, and the term small-for-gestational-age (SGA) was coined\textsuperscript{11,12}. SGA babies are generally defined as those born with a birth weight (and/or length) less than \textminus{}2 standard deviations (SD)\textsuperscript{13}. Alternatively, the World Health Organization defines SGA as weighing less than the 10\textsuperscript{th} percentile for gestational age\textsuperscript{12,14-16}. Both definitions have been based on immediate and late adverse effects displayed by those born SGA in large cohort studies.

The placental weight and birth weight are influenced by maternal nutrition\textsuperscript{17}. Birth weight is an indirect indicator of poor intra-uterine milieu\textsuperscript{18}, and adverse health outcomes have been demonstrated in association with decreasing birth weight, even in offspring born appropriate-for-gestational-age\textsuperscript{19}. The risk of developing type 2 diabetes mellitus (T2DM), hypertension, and hyperlipidaemia (the metabolic syndrome) is ten times higher in a cohort of men born weighing less
than 2.95 kg compared to those with a birth weight greater than 4.31 kg\textsuperscript{20}, demonstrating a continuous association between birth weight and later disease risk. This is independent of duration of gestation and other possible confounding variables, including tobacco use, alcohol consumption, and socio-economic status\textsuperscript{20}, with similar observations made by other researchers\textsuperscript{21-24}. In another study, the odds of developing insulin resistance syndrome increased 1.72 times for each tertile drop in birth weight\textsuperscript{22}.

SGA children have insulin resistance that starts in childhood\textsuperscript{25} and persists through early adulthood\textsuperscript{26}, being associated with an increased risk for various metabolic disorders later in life\textsuperscript{20}. Studies on survivors of the Dutch Famine\textsuperscript{27} and research in the UK by Barker and colleagues\textsuperscript{28} have shown that the offspring of mothers exposed to nutritional or physiological stress during pregnancy are at increased risk of metabolic and cardiovascular disease later in life. In fact, those born SGA are at a greater risk of T2DM, hypertension, cardiovascular events, cerebrovascular diseases, and cancer\textsuperscript{10,29-32}. In addition, being born SGA is associated with several other adverse outcomes in later life, including obesity\textsuperscript{33}, relative short stature\textsuperscript{34}, behavioural and cognitive issues, scholastic under-achievement\textsuperscript{35}, polycystic ovarian disease\textsuperscript{36}, hypergonadotropinemia, reduced uterine size, and ovarian volume\textsuperscript{37}. Further, rapid weight gain during infancy or childhood in SGA children increases the risk of coronary heart disease, T2DM, and hypertension later in life\textsuperscript{38,39}.

1.1.2. Preterm birth

Another cohort that has been the focus of considerable research are children born preterm (<37 weeks gestation). Hofman et al. postulated that both preterm and SGA infants face an adverse environment at similar stages in early life\textsuperscript{40}. While preterm infants face these adverse conditions ex utero, for SGA infants the insult occurs in utero\textsuperscript{40}. Therefore, it is not surprising that those born preterm are also exposed to developmental programming that leads to long-term adverse health effects.

Changes in adiposity have been observed in preterm individuals. Magnetic resonance imaging (MRI) scans at birth showed that preterm infants had more intra-abdominal fat and less subcutaneous adiposity than term infants\textsuperscript{41}. At 3 months of age, following rapid weight gain preterm infants displayed increased total and subcutaneous adiposity\textsuperscript{41}. This early difference in the distribution of adipose tissue may be a mechanism predisposing preterm individuals to an adverse adiposity profile in later years\textsuperscript{41}.

A prospective follow-up study on preterm subjects in the Netherlands revealed an increase in the prevalence of hypertension\textsuperscript{42}. A large study in Sweden on 165,136 men showed an inverse
relationship between gestational age and systolic blood pressure at 18 years of age\textsuperscript{43}, showing an increased risk for elevated blood pressures in those born preterm.

A recent study in the Liggins Institute showed that in their mid-thirties adults born preterm had greater abdominal adiposity (more truncal fat and higher android fat to gynoid fat ratio) compared to those born at term\textsuperscript{44}. Although women born preterm and at term were of similar weight and BMI, men born preterm were on average 20 kg heavier and of greater BMI than men born at term\textsuperscript{44}. Adults born preterm also displayed a less favourable lipid profile, including lower HDL-C concentrations and greater total cholesterol to HDL-C ratio\textsuperscript{44}. Interestingly, there was evidence of intergenerational effects, with children of parents born preterm having more body fat and greater abdominal adiposity than the pre-pubertal offspring of parents born at term\textsuperscript{44}.

Importantly, as observed in those born SGA\textsuperscript{25}, preterm infants demonstrate a similar reduction in insulin sensitivity in childhood\textsuperscript{40} that persists into mid-adulthood\textsuperscript{45}. However, the incidence of adverse metabolic outcomes in late adulthood is still unknown, as preterm cohorts have not been followed beyond mid-adulthood.

1.1.3. Large-for-gestational-age

At the other end of the weight spectrum, there are those infants that are considered to be 'too large', i.e. large-for-gestational-age (LGA). LGA babies are defined as having a birth weight above the 90th percentile for gestational age\textsuperscript{46}. Maternal gestational diabetes, obesity, parity, and male gender are independent risk factors for being born LGA\textsuperscript{47}. However, mothers who are overweight or obese have the greatest risk of giving birth to a LGA baby.

Although this cohort has not been studied in depth, increased size at birth has been shown to be associated with an increased risk of overweight and obesity in childhood\textsuperscript{48}. Those born LGA also appear to be at increased risk of developing the metabolic syndrome, even in their pre-pubertal years\textsuperscript{49}. LGA subjects have features suggestive of insulin resistance and oxidative stress in childhood\textsuperscript{50}, features that are likely associated with an increased risk of metabolic diseases later in life.
1.1.4. Post-term birth

This section contains an abridged version of a manuscript currently in press in the Journal of Pediatrics.

- **Authors:** Ayyavoo A, Derraik JGB, Hofman PL, Cutfield WS.
- **Title:** Post-term births: are prolonged pregnancies too long?
- **Journal:** Journal of Pediatrics
- **Impact factor:** 4.12
- **Journal’s aims and scope:** “The Journal [of Pediatrics] seeks to publish high quality original articles that are immediately applicable to practice (basic science, translational research, evidence-based medicine), brief clinical and laboratory case reports, medical progress, expert commentary, grand rounds, insightful editorials, “classic” physical examinations, and novel insights into clinical and academic pediatric medicine related to every aspect of child health. Published monthly since 1932, The Journal of Pediatrics continues to promote the latest developments in pediatric medicine, child health, policy, and advocacy.”

1.1.4.1. Post-term birth definition & incidence

Post-term pregnancy is defined by the World Health Organisation as the end of gestation at ≥42 completed weeks of gestation, measured from the first day of the last menstrual period and based on a 28-day cycle\(^{51}\). However, in reality this definition is arbitrary, and there are no clear scientific data underpinning it.

As pregnancies that last beyond 42 weeks have been associated with adverse events historically, they have been considered a separate at risk group. It is clear that post-term births remain a common event worldwide, and management of post-term pregnancies is somewhat variable and therefore best-practice guidelines are lacking.

There are limited data across countries on long-term trends in the incidence of post-term births. Pregnancies were previously dated based on the last menstrual period (LMP), but now rely on both LMP and early ultrasound scans improving accurate of gestation timing. Data from 81 singleton pregnancies after in vitro fertilization (where conceptions can be accurately timed) showed that ultrasound scans in the first 20 weeks underestimated gestation length by just 2.8 days (standard error of the mean = 0.2), so that fetal age was determined to within 7 days in more than 95% of cases\(^{52}\). Thus, in Finland for example, the incidence of post-term births dropped from 10.3% to 2.7% once ultrasound scans became the standard technique to date pregnancies\(^{53}\).
Nonetheless, post-term births are a common occurrence worldwide. The rate of post-term births varies considerably between and within countries, and in the developed world it ranges from 0.4% to 11%. In Sweden, the incidence of post-term births is 7.5% compared to 5.6% for preterm births. As a result, the Swedish birth registry recorded nearly 311,000 children born post-term in 1983–2006.

It is important to note that in many nations (particularly in poorer countries) mothers may not have access to ultrasound scans and will also be unsure of the LMP date. Therefore, it is likely that the incidence of post-term births in many countries is higher than that officially recorded.

1.1.4.2. Aetiology of post-term birth

The two most common factors associated with post-term birth are a first-degree relative born post-term and maternal obesity. There is considerable evidence that maternal factors are associated with prolonged gestation in humans. Maternal genetic factors may account for up to 30% of all post-term births, but other studies suggested a lower contribution of 14%. Maternal gestational age was found to be correlated with the gestational age of the fetus, indicating that mothers born post-term are more likely to give birth to post-term infants. Recently, from a cohort of 36 children born post-term, we observed that 90% of children had another family member born after 42 weeks of gestation, with 65% of children having a sibling and 29% having a mother also born post-term.

Similar evidence has been provided by parent-offspring data from Norway and Danish medical and twin registries. Notably, maternal obesity has been shown to be associated with a 1.4-fold increase in the risk of delivery beyond 41 weeks gestation.

Animal models have also shown that there are paternal factors associated with prolonged gestation. Cattle studies suggest that the sire has a significant effect on length of gestation, and "short gestation length semen" is currently marketed as a tool to maximize profits in the dairy industry. A possible paternal effect has not been adequately studied in humans, but gestational age is better correlated among siblings born to the same parents than among siblings born to different fathers, suggesting a possible paternal role in the length of human gestation. Although paternal body mass index (BMI) did not alter the rate of post-term deliveries, a linear relationship has been observed between paternal gestational age and that of the offspring, which was confounded by the birth weight of the father. Thus, it is possible that paternal factors may also be associated with post-term birth in humans.

There is also evidence that the fetus itself can affect gestational length, as shown by the common occurrence of prolonged gestations among anencephalic babies and infants born following in vitro
fertilisation using vitrified blastocysts. Lunde et al. suggested that fetal genetic factors could explain 11% of the variation in gestational length.

Environmental factors have also been described to be associated with prolonged gestation. Increased duration of pregnancy has been noted with omega 3 fatty acids supplementation. Further, aspirin has been previously shown to increase gestational length and the incidence of post-term births, due to the inhibitory effects on prostaglandins synthesis, which are important regulators of human gestation and labour.

It is important to stress that no human studies have elucidated the exact mechanisms (including genetic) leading to post-term birth, which is a likely result of the combination of the described factors.

1.1.4.3. Perinatal and neurological outcomes

Studies describing adverse neonatal outcomes following post-term birth do not distinguish between the effects of prolonged gestation itself from other factors (maternal and fetal) possibly responsible for both prolonged gestation and poorer outcomes.

Most of the earlier observational studies on the effects of post-term birth have focused on the immediate perinatal period. There is an increased rate of peripartum complications in association with prolonged pregnancies, including macrosomia, traumatic deliveries, post-maturity syndrome, intrapartum fetal distress, hypoxic encephalopathy, umbilical cord complications, fractures, peripheral nerve injury, pneumonia, and septicaemia. Data on 27,000 women found that the lowest risk for fetal death occurred at 38 weeks gestation, and that resuscitation was least likely to be required at 39 weeks of gestation. Another large study on singleton pregnancies in Sweden demonstrated an increase rate of fetal death beyond 40 weeks + 3 days. Beyond 41 weeks of pregnancy, there is an elevated risk for abnormal non-stress tests, respiratory distress, oligohydramnios, caesarean sections, and stillbirths.

Studies have also indicated a higher risk of perinatal and obstetric complications in prolonged pregnancies, including increased rate of perinatal death and stillbirth with signs of chronic hypoxia and malnutrition. Retrospective analysis suggested that the rate of stillbirth increases from a nadir of 0.35/1,000 pregnancies at 41 weeks gestation to 2.12/1,000 pregnancies at 43 weeks, and that perinatal morbidity increases 2-fold from 40 to 42 weeks. A recent meta-analysis reported a minimal increase in meconium aspiration syndrome in post-term infants but no increase in perinatal mortality.
However, according to a recent guideline from the World Association of Perinatal Medicine there is no conclusive evidence that induction of post-term pregnancies before 42 weeks gestation improves fetal, maternal and neonatal outcomes (as compared to expectant management)\textsuperscript{91}. A randomized controlled trial did not find any effects on perinatal mortality or morbidity when comparing labour induction against serial antenatal monitoring in post-term pregnancies\textsuperscript{92}. The 2003 meta-analysis of 16 RCTs compared labour induction to expectant management of post-term pregnancies, and found that induction at 41 weeks was associated with a slightly lower Caesarean rate (20.1% versus 22%), but no improvements in NICU admission rates, meconium aspiration, APGAR scores, or perinatal mortality\textsuperscript{93}. Further, a recent Canadian study suggested that induction of labour at 41 weeks gestation in uncomplicated low risk post-term pregnancies increased severe neonatal morbidity (especially among infants born to multiparous women), with no apparent improvement in the rate of stillbirths or perinatal mortality\textsuperscript{94}. Thus, the optimal management of post-term pregnancies is still unclear\textsuperscript{90}.

### 1.1.4.4. Neurological outcomes in childhood

Post-term birth is associated with adverse neurological and neurodevelopmental outcomes in childhood. An increased incidence of epilepsy in infancy is noted following instrumental and caesarean deliveries in post-term children\textsuperscript{95}. A population based study in Norway revealed an increase in the incidence of cerebral palsy in children born at or after 42 weeks gestation\textsuperscript{96}.

Post-term birth is also associated with behavioural and emotional problems, including attention deficit hyperactivity disorder in early childhood\textsuperscript{97}. A small study suggested that IQ in children born after 41 weeks gestation was lower than their siblings born at term\textsuperscript{98}. However, other early childhood studies on IQ and developmental milestones have yielded conflicting results\textsuperscript{99-102}.

### 1.1.4.5. Long-term metabolic and cardiovascular effects

There have been practically no studies examining the long-term effects of post-term birth. Recently however, in a Swedish cohort with detailed longitudinal height and weight data from birth to 16 years of age, nearly half of post-term boys were overweight or obese at age 16 years compared to 13% of term boys\textsuperscript{103}. This difference in BMI was evident at 3 years of age and became progressively greater as these boys aged, so that post-term adolescent boys were on average 11 kg heavier than their term counterparts\textsuperscript{103}. Notably, this difference was not due to genetically determined obesity\textsuperscript{103}. There is therefore evidence suggesting that those born post-term are also likely to be at risk of long-term adverse health outcomes, similarly to individuals born preterm or SGA.
1.1.5. Hyperemesis gravidarum

This section contains an abridged version of a manuscript already published online in the American Journal of Obstetrics and Gynecology.

- **Authors:** Ayyavoo A, Derraik JGB, Hofman PL, Cutfield WS.
- **Title:** Hyperemesis gravidarum and long-term health of the offspring
- **Journal:** American Journal of Obstetrics and Gynecology
- **Year of online publication:** 2013
- **Impact factor:** 3.88
- **Journal’s aims and scope:** "Covering the full spectrum of the specialty, American Journal of Obstetrics & Gynecology, “The Gray Journal,” presents the latest diagnostic procedures, leading-edge research, and expert commentary in maternal-fetal medicine, reproductive endocrinology and infertility, gynecologic oncology, and urogynecology as well as general obstetrics and gynecology."

1.1.5.1. Background

According to the Fairweather criteria, hyperemesis gravidarum is characterized by severe vomiting in pregnancy that requires antenatal hospital admission before 20 weeks gestation\(^{104,105}\). The International Statistical Classification of Disease and Related Health Problems ICD-9 Code 643 defines hyperemesis gravidarum as persistent and excessive vomiting starting before the end of the 22\(^{nd}\) week of gestation\(^{106}\). Others define hyperemesis gravidarum as the presence of intractable vomiting during pregnancy associated with dehydration, electrolyte and/or metabolic disturbances, as well as a weight loss \(\geq 5\%\)\(^{107,108}\). More recently, the Hyperemesis Education and Research Foundation has defined it as "a debilitating and potentially life-threatening pregnancy disease marked by rapid weight loss, malnutrition, and dehydration due to unrelenting nausea and/or vomiting with potential adverse consequences for the mom-to-be and the newborn(s)"\(^{109}\).

Nausea and vomiting is seen in 50 to 90\% of pregnancies\(^{110,111}\), and a prospective study of 363 singleton pregnancies in the UK revealed that nausea occurred in 28\%, while 52\% of mothers experienced both nausea and vomiting\(^{112}\). However, the reported incidence of hyperemesis gravidarum is much less, varying among studies from 0.3 to 3.6\%\(^{105,111,113-116}\). There is considerable variation among nations, with the incidence of hyperemesis gravidarum ranging from 0.3\% in Sweden\(^{113}\) to 1.5\% in the USA\(^{114}\) and 3.6\% in Japan\(^{116}\).

Diagnosis of hyperemesis gravidarum is mainly clinical\(^{117}\). The onset of hyperemesis gravidarum usually occurs at 4–8 weeks gestation and can last up to 14–16 weeks\(^{106}\). However, symptoms may
actually continue into mid- and late gestation\(^\text{106}\). There are mild and severe forms, with the severe form usually defined by the presence of at least one of the following features: ketonuria, increased blood urea, increased haematocrit, and abnormal electrolytes\(^\text{118}\). Severe forms may lead to starvation and dehydration, which can result in severe ketonuria and abnormal liver enzymes\(^\text{119}\).

1.1.5.2. Aetiology of hyperemesis gravidarum

There is still no conclusive evidence on the exact factors underpinning the pathogenesis of hyperemesis gravidarum. However, numerous factors have been suggested to play a role, including human chorionic gonadotropin (HCG), thyroid hormones, oestrogen, progesterone, leptin, adrenal hormones, immunological components, serotonin, and *Helicobacter pylori* infection\(^\text{120-124}\). Other suggested factors are previous molar pregnancy, psychiatric illness, pre-existing diabetes, gastrointestinal illnesses, asthma, singleton female pregnancies, and twinning\(^\text{125}\).

Although numerous factors have been proposed, many believe that hormonal factors play a major role. HCG is structurally similar to thyroid stimulating hormone (TSH)\(^\text{126}\), and may act as a thyroid stimulator in pregnancies involving hyperemesis gravidarum\(^\text{127}\), where elevated HCG concentrations have been observed\(^\text{128}\). Enhanced TSH receptor sensitivity has been suggested as a cause of elevated thyroid hormones in hyperemesis gravidarum\(^\text{129}\). However, while a number of studies have demonstrated elevated thyroid hormones in hyperemesis gravidarum\(^\text{127,130}\), these observations are not consistent. Wilson *et al.* for example, found no association between hyperemesis gravidarum and HCG or thyroid hormones\(^\text{131}\), and in reality there is no conclusive evidence for a role of thyroid hormones in its aetiology. Serotonin is another hormone hypothesized to play a role, but mothers experiencing hyperemesis gravidarum were shown to have normal serotonin concentrations\(^\text{132}\).

Studies have indicated that the incidence of nausea and vomiting was similar in pregnancies in the same mother\(^\text{112,133}\), suggesting a possible genetic background to hyperemesis gravidarum. Fejzo *et al.* also observed a greater incidence of severe hyperemesis gravidarum amongst relatives of affected individuals\(^\text{134}\). However, another study revealed no association between nausea and vomiting in earlier and later pregnancies\(^\text{135}\). Others have suggested that environmental influences may play a role, while the fetal genotype has less influence\(^\text{136}\).

1.1.5.3. Maternal consequences of hyperemesis gravidarum

It has been hypothesized that nausea and vomiting during pregnancy protects the women and her embryo from harmful substances in food\(^\text{137}\), conferring an evolutionary advantage to maximize fitness and survival. It has also been proposed that nausea and vomiting in pregnancy may assist
with nutritional partitioning during placental development\textsuperscript{135,138}. However, nausea and vomiting in the more severe form (i.e. hyperemesis gravidarum) can have devastating effects in some mothers, and the personal, social, and economic burden of hyperemesis gravidarum are considerable\textsuperscript{139}.

In the past, hyperemesis gravidarum was associated with greater maternal mortality\textsuperscript{140}, which has been considerably reduced with improvements in medical care. In the UK, recorded maternal mortality due to hyperemesis gravidarum was reduced from 159 per million pregnancies in 1931–40 to 3 per million in 1951–60 with the introduction of intravenous fluid replacement\textsuperscript{141}. The famous English novelist Charlotte Brontë (author of Jane Eyre) is widely regarded as having perished due to hyperemesis gravidarum, but her death has been more recently attributed to tuberculosis with secondary Addison disease\textsuperscript{142}. Two maternal deaths due to hyperemesis gravidarum were recorded in 1991–93 in the UK\textsuperscript{141}, and such deaths are somewhat rare nowadays.

Nonetheless, despite the major reduction in maternal mortality, extensive maternal morbidity still results\textsuperscript{117,143} and serious complications may ensue, including Wernicke’s encephalopathy\textsuperscript{143,144}, pneumo-mediastinum\textsuperscript{145}, and spontaneous oesophageal rupture\textsuperscript{146}. Some mothers experience major nutritional disturbance, requiring enteral (via a nasogastric tube) or total intravenous nutrition\textsuperscript{147} that impairs maternal weight gain\textsuperscript{148}. There are also considerable psycho-social effects on the mother, including behavioural and cognitive dysfunction, and emotional distress that may lead to post-traumatic stress disorder\textsuperscript{149-151}.

1.1.5.4. Perinatal outcomes

Data from the 1950s suggested that mothers experiencing hyperemesis gravidarum were less likely to spontaneously abort\textsuperscript{152,153}. A meta-analysis found that nausea and vomiting during pregnancy were associated with a reduction in abortion rates, but there was no consistent link with perinatal mortality\textsuperscript{154}. Another recent meta-analysis concluded that hyperemesis gravidarum had no effect on APGAR scores or on the incidence of perinatal death or congenital anomalies\textsuperscript{155}. However, a study on over 520,000 births showed an increased rate of fetal and neonatal deaths\textsuperscript{111}.

Hyperemesis gravidarum leads to a reduction in maternal weight gain throughout pregnancy\textsuperscript{148}, which may result in suboptimal fetal outcomes\textsuperscript{104,156}. Hyperemesis gravidarum is associated with increased rates of SGA infants and premature delivery\textsuperscript{104,111,155}. Other studies also showed reductions in gestational age and birth weight, as well as an increase in postnatal hospital stay\textsuperscript{115,148}. However, findings are not always consistent and other studies observed no effect on birth weight\textsuperscript{104,108,115}. Interestingly, several studies revealed an increase in the proportion of females being born in pregnancies with hyperemesis gravidarum\textsuperscript{155}. 
1.1.5.5. Long-term outcomes in the offspring

Intellectual development assessed in early and late infancy did not reveal any abnormalities in the offspring of mother who experienced hyperemesis gravidarum. However, in utero exposure to hyperemesis gravidarum has been associated with an increased risk of depression, bipolar disorder, and anxiety. Hyperemesis gravidarum has also been suggested to lead to an increased risk of testicular cancer in the offspring.

Surprisingly, there are no studies examining the long-term metabolic and cardiovascular outcomes in the offspring. As the children born of mothers who experienced hyperemesis gravidarum would possibly be exposed to an undernutrition insult similar to that observed in the Dutch famine, these children may be similarly at an increased risk of experiencing adverse health outcomes in the long-term.

1.1.6. Birth order

This section contains an abridged version of a manuscript published in the journal Future Cardiology.

- **Authors:** Ayyavoo A, Derraik JGB, Hofman PL, Cutfield WS.
- **Title:** Is being first-born another risk factor for metabolic and cardiovascular diseases?
- **Journal:** Future Cardiology
- **Year of publication:** 2013
- **Volume:** 9
- **Pages:** 447–450
- **Journal’s aims and scope:** “Future Cardiology highlights the new molecular approach to advancing cardiovascular therapy. Coverage will also reflect the major technological advances in bioengineering in cardiology in terms of advanced and robust devices, miniaturization, imaging, system modelling and information management issues. We also take a new approach to the way information is structured and delivered, so that its value is maximized to the reader. Accessible ‘at-a-glance’ formats are important in an increasingly time-constrained clinical community.”

1.1.6.1. Background

Birth rates have been steadily declining throughout the world, particularly in Europe and many Asian countries. This reduction in birth rates is a result of a number of factors, including government policies (e.g. one-child policy in mainland China), greater family planning, personal
choice, and economical constraints. As a result, there has been a large increase in the number of one-child families, and consequently a considerable increase in the proportion of first-born children within many populations.

Thus, any adverse health outcomes that are associated with being first-born (primogeniture) would likely affect an ever-increasing proportion of the world's population. Notably, only recently has evidence emerged on the consequences of primogeniture to long-term metabolic and cardiovascular health risks.

1.1.6.2. Changes in birth weight

A reduction in birth weight among first-borns has been previously noted\textsuperscript{162,163}, suggesting that primogeniture may be associated with a degree of nutrient restriction \textit{in utero}. Although the underlying causes are still unclear, there is suggestion that changes in placentation may account for the observed differences in birth weight\textsuperscript{164}. Multiparous women who had earlier uncomplicated pregnancies have better trophoblast invasion and placentation compared to women who are pregnant for the first time\textsuperscript{165,166}. Beneficial immunomodulating effects are also seen in multiparous pregnancies\textsuperscript{165}. These factors could account for improved nutrient flow to later-born fetuses, consequently improving fetal growth in later pregnancies.

Exposure to nutritional or physiological insult at different periods of gestation has been associated with various adverse outcomes in the offspring\textsuperscript{27}. However, the timing of possible stressors to first-born fetuses is not known. A stress early in pregnancy does not affect birth weight as much as insults late in the third trimester. Thus, first-borns may be subjected to an adverse \textit{in utero} environment late in pregnancy, which would explain a reduction in birth weight. Alternatively, it is possible that first-borns may be subjected to a less favourable intra-uterine environment throughout the entire pregnancy.

1.1.6.3. Phenotypic differences & cardiovascular risk

Irrespective of the timing and nature of possible stressors \textit{in utero}, it is clear that first-borns are phenotypically different to later-borns in both childhood and adulthood\textsuperscript{162,167}. Studies have shown that first-born children were taller (with a progressive reduction in height with subsequent births) and had greater circulating insulin-like growth factor I (IGF-I) concentrations than later-born children\textsuperscript{167}. There was a reduction of 1.3 cm in height in second-born children compared to first-borns, with a further 2 cm decrease from second- to third-born children\textsuperscript{167}. Other studies revealed similar difference in height between first- and later-borns\textsuperscript{168}. Taller stature in childhood is positively
associated with overweight status and obesity later in life\textsuperscript{169}, and a large study in Brazil of over 2,000 men found that first-borns were taller and had greater fat mass than later-borns\textsuperscript{170}.

There is also mounting evidence that first-borns have an increased risk of adverse health outcomes later in life. Earlier studies have shown that first-borns were more likely to develop type 1 diabetes mellitus\textsuperscript{171}, allergic disorders\textsuperscript{172}, and psychological issues\textsuperscript{173}. In a prospective cohort study, primogeniture was a significant risk factor for increased adiposity in young adulthood\textsuperscript{174}. Nearly four decades ago a study in young adults suggested that first-borns were at a greater risk of developing hypertension\textsuperscript{175}. An unpublished study presented at an American Heart Association forum 12 years ago by Ferratini and colleagues indicated that primogeniture was associated with an increased rate of heart disease in a population of 358 patients\textsuperscript{176}.

The previously mentioned Brazilian study also found that, in association with their increased fat mass, first-borns had higher metabolic risk z-score compared to later-borns\textsuperscript{170}. The metabolic z-score was calculated as the average of the z-scores of fat mass, lipid profile, and blood pressure, with a higher score indicative of a higher risk for the metabolic syndrome\textsuperscript{170}. The same research group observed a similar increase in metabolic risk among first-born women\textsuperscript{177}. As a result, there is mounting evidence suggesting that primogeniture is likely to be a contributing factor to overall metabolic and cardiovascular disease risks later in life.

1.2. Glucose metabolism and insulin resistance

An overview of normal glucose metabolism and its homeostasis help us understand the perturbations that could happen in children exposed to adverse early life events. In addition, it also sheds light on the metabolic changes that have implications for long-term health.

1.2.1. Glucose

Glucose is the most important source of energy for most cells. Glucose homeostasis is maintained by the balance between the glucose entering the circulation (glucose appearance) and the rate of glucose removal from the circulation (glucose disappearance). The major source of glucose in blood is derived from intestinal absorption following ingestion of food. The liver is the other source of glucose for metabolism, via glycogenolysis (breakdown of glycogen) and gluconeogenesis (formation of glucose from lactate and amino acids) in the fasting state. Euglycaemia is maintained through the interaction of various hormones, including insulin, glucagon, amylin, glucagon-like peptide 1 (GLP1), cortisol, catecholamines, growth hormone, glucose dependent insulinotropic peptide, leptin, adiponectin, ghrelin, and resistin\textsuperscript{178}.  


Nutrient signalling is triggered by glucose and amino acids through various pathways that control energy metabolism, growth, and tissue differentiation. This happens through three pathways: i) hexosamine signalling; ii) m-TOR (mammalian receptor of rapamycin); and iii) adenosine monophosphate activated protein kinase (AMPK). These pathways do not function in isolation, being rather interconnected and linked to insulin signalling and release of other hormones (including those from the adipose tissue).

Glucoregulatory hormones try to maintain blood glucose in a narrow range of 4 to 7 mmol/l even during periods of fasting and feeding. This tight control is maintained by a fine balance between glucose absorption from the intestine, production from the liver, and uptake and utilization by the peripheral tissues.

1.2.2. Insulin

Insulin is a polypeptide hormone that exerts a range of physiological effects on various tissues by binding to the insulin receptor (IR). Insulin has numerous actions on target tissues including stimulation of glucose and amino acid uptake, promoting production of several metabolic enzymes, as well as enhancing the synthesis of lipids, proteins, DNA, and RNA. IR is a di-sulphide linked tetradimer with two α and β chains. Human insulin receptor resembles several members of tyrosine-specific kinase family. Insulin stimulates phosphorylation of the tyrosine and serine residues of the IR, particularly in the β sub-unit. These initiate the subsequent actions of insulin in adipocytes, skeletal muscle, placental membranes, liver, and several other tissues. Reduction in tyrosine kinase activity is seen in insulin resistance and individuals with T2DM.

Importantly, insulin is the major hormone involved in glucose disappearance (c.f. glucagon that is important for the appearance of glucose). Insulin works to maintain blood glucose levels down after a post-prandial peak, and over a period of time the concentration of glucose gradually declines to fasting levels. Immediately after food intake, insulin drives the disappearance of glucose into muscle and adipose tissue. Simultaneously, insulin also suppresses the hepatic output of glucose, via a paracrine-endocrine interaction with pancreatic α-cells that suppresses glucagon. Glucose transport into muscle cell occurs through the recruitment of glucose transporter type 4 (GLUT-4) receptors by insulin, so that the number of GLUT-4 receptors on the surface of muscle cells and fat cells increases at the expense of the intracellular pool.

1.2.3. Glucose disposal

Non-insulin dependent glucose uptake is the major form of glucose disposal in β-cells of the pancreas, in the liver, intestine, brain, cornea, kidney, and red blood cells. The transporters involved
in the transcellular transport are GLUT-1 transporters. Glucose disposal is different in the fasting and post-prandial states. Most of the glucose disposal (approximately 75%) occurs in tissues with non-insulin dependent glucose uptake (brain and splanchnic organs) in a state of fasting. Only 25% of uptake would be in insulin dependent tissues, primarily skeletal muscle. While the non-insulin dependent glucose uptake would remain the same, the insulin mediated glucose uptake would be affected in an insulin resistant state\textsuperscript{193,194}.

Post-prandial glucose homeostasis is maintained by three processes: i) secretion of insulin; ii) glucose uptake by liver, gut, and muscle in response to insulin and glucose in the circulation; and iii) suppression of hepatic glucose output. Abnormalities in the handling of glucose at the level of β-cells, liver, and/or muscle can lead to insulin intolerance. Muscles are responsible for 70–80% of insulin mediated glucose uptake in the post-prandial state, and are therefore important components of insulin resistance\textsuperscript{195}.

1.2.4. Insulin resistance

Insulin resistance is a complex metabolic state that involves interaction of several mechanisms in different pathophysiologic states\textsuperscript{196}. Although insulin acts in many ways in various tissues, defective insulin action in target tissues such as muscle, liver, and adipose tissue are responsible for insulin resistance\textsuperscript{196}. The latter is characterized by the impairment of glucose signalling in the muscle, resulting in poor glucose disposal\textsuperscript{196}.

The presence of two simultaneous defects in insulin dependent glucose uptake characterizes T2DM, i.e. both β-cell secretory dysfunction and insulin resistance\textsuperscript{193}. Insulin resistance is the primary defect in T2DM\textsuperscript{193}. It is therefore a major risk factor for T2DM development\textsuperscript{197}, and may precede the onset of T2DM in normoglycaemic individuals by a decade\textsuperscript{198}. Following insulin resistance, β-cell function ultimately fails to compensate for the increased insulin requirements, leading to the development of T2DM.

The glucose-insulin interaction and metabolism in fasted and post-prandial states in both non-diabetic and diabetic individuals have been schematically represented by Aronoff et al.\textsuperscript{178} (Figure 1.1). In a normal insulin sensitive state, insulin suppresses gluconeogenesis and glycogenolysis in the liver and promotes glycogen synthesis. Consequently, hepatic glucose output increases in a state of insulin resistance\textsuperscript{199}. Thus in subjects with T2DM, increased body fat, abdominal adiposity, serum free fatty acids, and other inflammatory markers\textsuperscript{200,201} are associated with elevated hepatic glucose output.
1A. For nondiabetic individuals in the fasting state, plasma glucose is derived from glycogenolysis under the direction of glucagon (1). Basal levels of insulin control glucose disposal (2). Insulin’s role in suppressing gluconeogenesis and glycogenolysis is minimal due to low insulin secretion in the fasting state (3).

1B. For nondiabetic individuals in the fed state, plasma glucose is derived from ingestion of nutrients (1). In the bi-hormonal model, glucagon secretion is suppressed through the action of endogenous insulin secretion (2). This action is facilitated through the paracrine route (communication within the islet cells) (3). Additionally, in the fed state, insulin suppresses gluconeogenesis and glycogenolysis in the liver (4) and promotes glucose disposal in the periphery (5).

1C. For individuals with diabetes in the fasting state, plasma glucose is derived from glycogenolysis and gluconeogenesis (1) under the direction of glucagon (2). Exogenous insulin (3) influences the rate of peripheral glucose disappearance (4) and, because of its deficiency in the portal circulation, does not properly regulate the degree to which hepatic gluconeogenesis and glycogenolysis occur (5).

1D. For individuals with diabetes in the fed state, exogenous insulin (1) is ineffective in suppressing glucagon secretion through the physiological paracrine route (2), resulting in elevated hepatic glucose production (3). As a result, the appearance of glucose in the circulation exceeds the rate of glucose disappearance (4). The net effect is postprandial hyperglycemia (5).
1.2.5. Insulin resistance and later diseases

Apart from T2DM, insulin resistance is a predictor of later risk for other metabolic and cardiovascular diseases\(^ {20,21,28,198,202-206}\). A study in non-obese healthy adults followed up for 4–11 years showed that insulin resistance is a forerunner for the later onset of not only T2DM, but also hypertension, cardiovascular disease, stroke, and cancer\(^ {204}\).

Insulin plays an important biological role in cardiovascular tissue, skeletal muscle, and adipose tissue. Hypertension is induced by several changes in association with insulin resistance, including: i) decreased endothelial cell production of nitric oxide (nitric oxide production stimulates vasodilatation)\(^ {207,208}\); ii) increased myosin light chain activation with vasoconstriction\(^ {209}\); iii) reduced skeletal muscle glucose transport that leads to reduced whole body glucose uptake and lower insulin sensitivity in skeletal muscle\(^ {210}\); and iv) attenuation of insulin/IGF-I mediated blockade of angiotensin 2 vasoconstrictive effects\(^ {211,212}\).

Insulin resistance increases the risk for coronary heart disease and stroke in healthy middle-aged man on its own, and in association with lipid factors and lifestyle\(^ {204,206,213}\). The biological effects previously noted to occur in vascular tissue is also observed in the heart, increasing the risk of heart diseases. Although T2DM (as discussed a consequence of insulin resistance) is associated with a two- to four-fold increase in coronary heart disease risk, the presence of hypertension, dyslipidaemia, and/or obesity further multiplies the risk of ischaemic heart disease in T2DM patients\(^ {214-216}\). Insulin resistance can accelerate atherosclerosis and is therefore a risk factor for ischaemic stroke due to atherothrombus\(^ {217,218}\). Insulin resistance has been associated with lacunar infarcts and large artery atherosclerosis in patients with stroke\(^ {219}\).

Insulin resistance has been demonstrated to be a risk factor for several other diseases. It is a probable risk factor for neuro-degenerative diseases\(^ {220}\). Epidemiological studies have shown that obesity and insulin resistance (both characterised by hyperinsulinaemia) are associated with an increased risk of breast, colon, prostate, and kidney cancer\(^ {221-223}\). There is also a well-described association between insulin resistance and non-alcoholic steato-hepatitis, irrespective of the presence of obesity\(^ {224}\). Thus, insulin resistance has numerous long-term health implications, and some of these adverse changes may already occur in childhood in certain groups (e.g. preterm subjects).
1.3. Hypotheses and aims

1.3.1. Hypotheses

- Post-term fetuses are exposed to in utero stressors associated with prolonged gestation, so that (similarly to those born SGA or preterm) they are at an increased risk of adverse metabolic outcomes in the long-term.
- Children born to mothers with severe hyperemesis gravidarum are exposed to nutritional stress in utero during early gestation, leading to programmed metabolic changes in post-natal life.
- Birth order is associated with changes in placentation and nutritional supply to the fetus, affecting growth and metabolism in the long-term, so that birth order is a contributing factor to the increasing rates of hypertension and type 2 diabetes mellitus worldwide.

1.3.2. Aims

- To assess whether post-term birth affects insulin sensitivity, as well as other metabolic parameters and body composition in childhood.
- To assess whether severe hyperemesis gravidarum affects glucose homeostasis and body composition in the offspring in childhood.
- To assess whether birth order is associated with metabolic (and growth) alterations in childhood.
Chapter 2. Measuring insulin sensitivity and body composition

This chapter will provide of a brief rationale and explanation for the two most important assessments used in all studies in this thesis along with a short section on statistics:

- Insulin sensitivity
- Body composition

2.1. Measurement of insulin sensitivity

As previously discussed, insulin resistance is a risk factor for several metabolic diseases, including T2DM, hypertension, coronary heart disease, stroke, and cancer\textsuperscript{204,225}. In particular, insulin resistance is a precursor to alterations in blood glucose levels that lead to T2DM. As a result, various direct and indirect methods to measure insulin sensitivity have been developed from animal and human models\textsuperscript{226} (Table 2.1).

<table>
<thead>
<tr>
<th>Method</th>
<th>Measurement of Insulin Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct measurements</td>
<td></td>
</tr>
<tr>
<td>Hyperinsulinemic euglycemic glucose clamp</td>
<td>Steady-state GIR = M. SK\textsubscript{clamp} = M/(G × ΔI), where M is normalized for G (steady-state blood glucose concentration) and ΔI (difference between fasting and steady-state plasma insulin concentrations)</td>
</tr>
<tr>
<td>IST</td>
<td>SSPG concentration during constant infusions of insulin and glucose with suppressed endogenous insulin secretion</td>
</tr>
<tr>
<td>Minimal model analysis of FSIVGTT</td>
<td>Minimal model uniquely identifies model parameters that determine a best fit to glucose disappearance during the modified FSIVGTT; SI; fractional glucose disappearance per insulin concentration unit; S\textsubscript{G/I}; ability of glucose per se to promote its own disposal and inhibit HGP in the absence of an incremental insulin effect (i.e., when insulin is at basal levels)</td>
</tr>
<tr>
<td>Surrogates derived from fasting steady-state conditions</td>
<td>Surrogates derived from dynamic tests (OGTT)</td>
</tr>
<tr>
<td>GI ratio</td>
<td>Reciprocal of fasting plasma insulin concentration, JU/ml</td>
</tr>
<tr>
<td>HOMA</td>
<td>Ratio of fasting plasma glucose (mg/dl) and insulin (JU/ml) concentration</td>
</tr>
<tr>
<td>QUICKI</td>
<td>HOMA-IR = [(fasting insulin (JU/ml)) × (fasting glucose (mmol/l))]/22.5</td>
</tr>
<tr>
<td>Surrogates derived from dynamic tests (OGTT)</td>
<td>QUICKI = 1/[Log (fasting insulin, JU/ml)] + Log (fasting glucose, mg/dl)]</td>
</tr>
<tr>
<td>Matsuda index</td>
<td>Minimal model uniquely identifies model parameters that determine a best fit to glucose disappearance during the modified FSIVGTT; SI; fractional glucose disappearance per insulin concentration unit; S\textsubscript{G/I}; ability of glucose per se to promote its own disposal and inhibit HGP in the absence of an incremental insulin effect (i.e., when insulin is at basal levels)</td>
</tr>
<tr>
<td>Gutt index: IS\textsubscript{Gutt, 120}, mg\textsuperscript{1}·mmol\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>IS\textsubscript{Matsuda} = 10,000 × [G\textsubscript{fasting (mg/dl)} × I\textsubscript{fasting (mU/l)}] × (S\textsubscript{G/I} × I\textsubscript{120})</td>
</tr>
<tr>
<td>Avignon index, SiM</td>
<td>QUICKI = 1/[Log (fasting insulin, JU/ml)] + Log (fasting glucose, mg/dl)]</td>
</tr>
<tr>
<td>Stumvoll index</td>
<td>SI\textsubscript{Matsuda} = 0.156 × 0.0000459 × I\textsubscript{120 (mU/l)} - 0.000321 × VD</td>
</tr>
</tbody>
</table>

GIR, glucose infusion rate; M, glucose disposal rate; IST, insulin suppression test; SSPG, steady-state plasma glucose; FSIVGTT, frequently sampled intravenous glucose tolerance test; S\textsubscript{G/I}, insulin sensitivity index; S\textsubscript{G/I}, glucose effectiveness index; HGP, hepatic glucose production; GI ratio, glucose/insulin ratio; HOMA, homeostasis model assessment; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; OGTT, oral glucose tolerance test; G\textsubscript{mawt}, mean plasma glucose concentration during OGTT; I\textsubscript{max}, mean insulin concentration during OGTT; G\textsubscript{mawt}, plasma glucose concentration during fasting; G\textsubscript{max}, plasma glucose concentration at 120 min; BW, body weight; L\textsubscript{i}, plasma insulin concentration during fasting; I\textsubscript{120}, plasma insulin concentration at 120 min; VD, glucose distribution volume (150 ml/kg BW).
2.1.1. Direct methods

The gold-standard technique to measure insulin sensitivity is the hyperinsulinaemic-euglycaemic clamp, developed by DeFronzo and colleagues. The hyperglycaemic clamp is a variation of this technique, and it measures β-cell sensitivity to glucose. In contrast, the euglycaemic insulin clamp measures tissue sensitivity to insulin.

The hyperinsulinaemic-euglycaemic clamp is accepted as the reference standard for the measurement of insulin sensitivity, against which all other methods are assessed. However, it is expensive and labour intensive, so that it is not easily adopted in clinical practice, particularly in paediatrics.

Another direct method to measure insulin sensitivity is the insulin suppression test developed by Shen and colleagues and modified by Harano. However, its use involves similar limitations to the hyperinsulinaemic-euglycaemic clamp, and it does not assess hepatic insulin sensitivity. In addition, it involves a universal insulin infusion rate in all subjects that results in dissimilar steady state plasma insulin, leading to errors.

2.1.2. Frequently sampled intravenous glucose tolerance test, modified with insulin

There are also a number of indirect methods to measure insulin sensitivity. One of the most widely used techniques is the frequently sampled intravenous glucose tolerance test (FSIGT) using the "minimal model" developed by Bergman and colleagues. Bergman's minimal model measures the metabolic insulin sensitivity indirectly, using paired samples of glucose and insulin obtained during a FSIGT. Bergman's model was later modified by Cutfield and colleagues for paediatric use, being therefore used as a 90-minute FSIGT.

In brief, three baseline samples are drawn at -20, -10, and 0 minutes. A 25% dextrose infusion (at 0.3 g/kg) is started at 0 minute and lasts for one minute. Blood samples are drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19 minutes. Insulin (0.015 units/kg) is intravenously administered as a bolus at 20 minutes, and further samples are drawn at 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 60, 70, 80, and 90 minutes. Glucose and insulin concentrations are measured on these paired samples, and analysed using the minimal model software.

The Bergman’s minimal model software (MINMOD) is defined by two coupled differential equations (with four model parameters each), describing either glucose or insulin dynamics. There are a number of parameters derived from the minimal model (Table 2.2; Figure 2.1), most importantly:
- Insulin sensitivity index (SI) – the fractional glucose disappearance per insulin concentration unit
- Glucose effectiveness (SG) – the ability of glucose to stimulate its own disposal and suppress the hepatic glucose production in the absence of incremental insulin effect
- Acute insulin release (AIR) – measure of insulin secretory capacity
- Disposition index (DI) – ability of β-cells to compensate for insulin resistance

Note that the modification of Bergman’s model involves the previously described administration of insulin at the 20th minute. This modification was introduced as the peak insulin concentration may overlap with the maximal glucose concentration. Thus, the insulin infusion at the 20th minute improves the accuracy of measurement of SI and SG, by overcoming the interference caused by the overlap.

<table>
<thead>
<tr>
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<th>Name</th>
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<td></td>
<td>[l⁻¹.min⁻¹]</td>
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2.1.3. Simple surrogate indices

Surrogate indices can be derived from 'steady state' or 'dynamic' situations. The 'steady state' in the hyperinsulinaemic-euglycaemic clamp (reference standard) is defined as a period $>30$ min ($\geq 60$ min after initiation of insulin infusion), during which the coefficient of variation for blood glucose, plasma insulin, and glucose infusion rate is $<5\%$. Steady state indices are more rigorous, and may determine more accurate values for glucose disposal rate and $S_I$. Simple surrogate indices derived from similar fasting steady state situations include fasting insulin (with normal fasting glucose levels), fasting glucose to insulin ratio (FGIR), homeostasis model assessment (HOMA), and quantitative insulin sensitivity check index (QUICKI). While the first two tests have several flaws and are undependable, HOMA and QUICKI have been of use in numerous epidemiological and clinical studies. QUICKI for example, has been described as a
simple, inexpensive, and useful method for epidemiological studies, being minimally invasive and well-correlated with the hyperinsulinaemic-euglycaemic clamp technique in adults\textsuperscript{226}. In obese pubertal children, HOMA was found to be more reliable than QUICKI and FGIR in diagnosing insulin resistance\textsuperscript{239}. However, overall, HOMA is no better than fasting insulin in assessing insulin sensitivity in childhood\textsuperscript{240}. All surrogate indices of insulin sensitivity correlate only modestly with the clamp technique in children\textsuperscript{241}.

Surrogate indices from dynamic tests are derived from oral glucose tolerance tests (OGTT) or meal tolerance tests. The many available include the Matsuda\textsuperscript{242}, Avignon\textsuperscript{243}, McAuley\textsuperscript{244}, Stumvoll\textsuperscript{245}, Belfiore\textsuperscript{246}, and Gutt\textsuperscript{247} indices. Although OGTT are more physiological than the intravenous tests, the assessment of insulin sensitivity via oral tests is subjected to interference from variable gut absorption, incretin effect, and variable splanchnic blood flow\textsuperscript{226}.

Amongst the OGTT, the Matsuda index\textsuperscript{242} is likely to be one of the most commonly used. It is a sensitive method that is well correlated with the insulin sensitivity index obtained from hyperinsulinaemic-euglycaemic clamp studies\textsuperscript{242}. As a result, large clinical and epidemiologic studies use the Matsuda index as a surrogate measure of insulin resistance instead of the FSIGT. In addition, the Matsuda index has a relatively strong association with the disposition index from the FSIGT, being therefore also useful in measuring beta cell compensation\textsuperscript{248}.

Nonetheless, a number of the indirect surrogate indices to measure insulin sensitivity have been shown to be relatively sensitive, and are consequently useful in light of their much simpler methodology. For example, Ascaso et al.\textsuperscript{249} have compared some of these indices to the minimal model approximation of the metabolism of glucose (MMAMG) as measured by the FSIGT. Many indices were well-correlated with the MMAMG, with the McAuley index being the most accurate\textsuperscript{249}.

**2.1.4. Conclusion**

Although the various methods of measuring insulin resistance have been validated in adults, this has not been done for paediatric use. The exception is the modified FSIGT with minimal model analysis, which is therefore the best method available to assess insulin sensitivity in young children. As previously discussed, it has a number of advantages over the gold-standard hyperinsulinaemic-euglycaemic clamp technique: less labour intensive, does not require constant adjustments of intravenous infusions, and steady state conditions are not necessary. In addition, indices of insulin sensitivity, glucose effectiveness, and β-cell function are all obtained from the single dynamic test\textsuperscript{226}, and the SI coefficient of variation is comparable to that of hyperinsulinaemic-euglycaemic
clamp studies\textsuperscript{226,234,250,251}. As the 90 minute FSIGT analysed by modified minimal model is a safe, accurate, and well-validated technique to measure insulin sensitivity in children\textsuperscript{232}, it was the adopted method in all of our studies.

2.2. Body composition

2.2.1. Importance of adipose tissue distribution

The incidence of obesity is steadily increasing worldwide, and so is the public health burden of associated diseases\textsuperscript{252,253}. Adipose tissue is a key factor in modulating the whole body lipid flux and thereby influencing the lipid and glucose homeostasis\textsuperscript{254}. Therefore, obesity is a strong predictor of T2DM, the metabolic syndrome, and coronary heart disease risk\textsuperscript{20,216,255,256}. Importantly, not only fat mass per se is important, but the distribution of adipose tissue is also strongly associated with long-term health outcomes.

Abdominal adiposity is associated with an increased risk for metabolic complications such as insulin resistance, dyslipidaemia, and ischaemic heart disease\textsuperscript{257,258}. Epidemiological studies have shown that preponderance of fat in the abdominal region (abdominal adiposity) rather than obesity per se is associated with an increased risk for T2DM and cardiovascular disease\textsuperscript{257}. As a result, the International Diabetes Foundation prioritises central adiposity as an essential feature of the metabolic syndrome\textsuperscript{256}. More specifically, in contrast to abdominal subcutaneous fat, visceral fat in the abdomen has been found to be metabolically active\textsuperscript{259}, being a risk factor for an adverse lipid profile, hyperinsulinaemia, insulin resistance, hypertension, T2DM, and coronary artery disease\textsuperscript{260-262}.

Abdominal adiposity affects both free fatty acid concentrations and glucose metabolism. While hepatic fat and visceral adipose tissue influence hepatic insulin resistance\textsuperscript{263}, free fatty acids induce insulin resistance in muscle\textsuperscript{264}. Insulin resistance in muscle is induced by the free fatty acid blockade of insulin-induced activation of IRS-1 associated phosphatidylinositol 3 kinase\textsuperscript{264}. Thus, excess abdominal adipose tissue increases free fatty acid concentrations that can directly affect insulin signalling, drive triglyceride synthesis, increase hepatic gluconeogenesis, and reduce glucose uptake in the muscle\textsuperscript{265}. Consequently, accurately determining the distribution of adipose tissue in the body is of great importance for studies evaluating long-term metabolic risks.
2.2.2. Measuring body composition

Body composition in children and adults can be evaluated using a number of direct and indirect methods, which vary somewhat in complexity\textsuperscript{266}. Further, body composition may be analysed at several levels, from the atomic level using complex and expensive machinery\textsuperscript{267,268} to more crude evaluations obtained with measuring tapes.

The body mass index (BMI, calculated as weight (kg) divided by the squared height (m)) is a commonly used surrogate measure, as it is very simple and easy to use as a rough indicator of overall adiposity. However, it is highly limited, and it is inaccurate when used in persons of athletic build with a greater proportion of muscle tissue\textsuperscript{269}. Abdominal circumference, mid-arm circumference, and skin-fold thickness are other anthropometric measurements that are of some use in clinical situations\textsuperscript{266,270}.

Better indirect tools are bioelectrical impedance analysers that estimate fat mass and fat-free mass, by measuring the resistance of the body as a conductor to a very small alternating current\textsuperscript{270-272}. The resulting measurements are obtained from regression equations based on certain sample populations\textsuperscript{266}. In view of their portability and their relatively low cost, bioelectrical impedance analysers are useful in certain research scenarios. However, these machines are somewhat inaccurate in obese subjects and cannot be used inter-changeably with other methods\textsuperscript{273}. Nonetheless, other indirect techniques can be resource-intensive and highly affected by operator inter-variability, such as hydrodensitometry (underwater weighing) and air displacement plethysmography\textsuperscript{266,274}.

In clinical paediatric studies such as ours, we are interested in tissue level evaluation of body composition, especially the amount and distribution of bone, adipose, and muscle tissues\textsuperscript{266}. Computerized tomography (CT) and magnetic resonance imaging (MRI) are more accurate and provide direct measurements of body composition, gaining therefore increasing popularity. Unfortunately, both techniques are expensive. In addition, CT scans involve relatively high levels of irradiation and MRI scans are very slow, which limit their wider applicability\textsuperscript{266}. As a result, dual energy X-ray absorptiometry scans are widely used for in the analysis of body composition in research and clinical settings\textsuperscript{275}.

2.2.3. Dual energy X-ray absorptiometry (DXA)

DXA scanners are full-body scanners that are relatively compact (Figure 2.2). These scanners use an X-ray tube operating at 80kVp coupled with a K-edge filter, resulting in the emission of two different energy levels, whose attenuation are used to measure body composition\textsuperscript{276}. Following a
full-body scan, a number of parameters are directly measured while others are automatically estimated by computer software\textsuperscript{277,278}. Output parameters include body mass, fat mass, fat-free mass, and bone mineral density.

DXA scanners are participant- and operator-friendly, are relatively quick, and involve very low irradiation levels (<5 mrem)\textsuperscript{266,277}. For example, the total body effective dose of radiation given to 5- and 10-year-old children are 0.03 and 0.02 μSv, respectively, values that are way below the background daily irradiation of 7 μSv\textsuperscript{279}. DXA scanners have also been well-validated against other anthropometric measurements. Importantly, the distribution of adipose tissue can be measured with precision and accuracy, which has more clinical relevance than the total measure of fat mass\textsuperscript{280,281}.

However, as with any other technique, DXA scanners have some limitations\textsuperscript{282}. The machine itself can only be used with subjects within certain height, weight, and width limits (Figure 2.2), so that it cannot be used on morbidly obese subjects for example\textsuperscript{283}. DXA scanners may also underestimate bone mineral content and overestimate fat-free mass\textsuperscript{284}.

\textbf{Figure 2.2.} Child undergoing DXA scan on a Lunar Prodigy 2000. The machine is located at the Maurice and Agnes Paykel Clinical Research Unit (Liggins Institute, University of Auckland).
2.2.3.1. DXA scanning and output

All DXA scans carried out in our studies were performed on a Lunar Prodigy 2000 (General Electric, Madison, Wisconsin, USA) (Figure 2.2), located at the Maurice and Agnes Paykel Clinical Research Unit (Liggins Institute, University of Auckland). The machine is loaded with the paediatric software Encore 2007 v.11.40.004, which uses reference values matched for age and sex for American and European populations. Quality control checks are performed every day with a standard block provided by the manufacturer. Also, to minimise inter-personal variation among scans, these were all performed by the same trained investigator.

The participant is positioned in the DXA scanner facing upwards (Figure 2.2 & Figure 2.3), after removal of all metals objects (e.g. coins, earrings, and watches). The head end is positioned approximately 3 cm beneath the horizontal edge of the measuring table, with head, trunk, legs, and feet positioned so that the body is located at the centre of the machine (Figure 2.3). The hands are kept flat on the sides of the body, with the palms facing down.

Figure 2.3. Positioning of subject's body on the DXA scanner.
Parameters of adipose tissue distribution are determined by the manufacturer’s software, based on an automated sectioning of specific areas of the body (Figure 2.4 & Figure 2.5). Such measures include android fat, gynoid fat, regional fat, and the android fat to gynoid fat ratio. The lower boundary of the android region is upper border of the pelvic line of demarcation; its lateral boundaries are those of the arms when they are in the normal position for a whole body scan; while the upper boundary is placed at a position that is equivalent to 20% of the distance between the pelvis and femoral neck cuts. For the gynoid region, the upper boundary is a line drawn at 1.5 times the height of the android region below the pelvis cut line; its lateral boundaries are the outer leg cuts; and the lower boundary is set such that it is twice the height of android region. The pelvis cut line is manually adjusted in each scan, so that the software displays the android and gynoid regions (and their ratio) automatically.

Figure 2.4. Description of body cuts used in the analysis. Adapted from the Encore 2007 software Operators’ manual.

1 Head: The Head cut is located immediately below the chin.
2 Left and right arm: Both arm cuts pass through the arm sockets and are as close to the body as possible. Ensure the cuts separate the hands and arms from the body.
3 Left and right forearm: Both forearm cuts are as close to the body as possible and separate the elbows and forearms from the body.
4 Left and right spine: Both spine cuts are as close to the spine as possible without including the rib cage.
5 Left and right pelvis: Both pelvis cuts pass through the femoral necks and do not touch the pelvis.
6 Pelvis top: The Pelvis Top cut is immediately above the top of the pelvis.
7 Left and right leg: Both leg cuts separate the hands and forearms from the legs.
8 Center leg: The Center Leg cut separates the right and left leg.
2.2.3.2. Relevance of DXA measures

The DXA-derived android to gynoid fat ratio is well-correlated with the intra-abdominal visceral fat measured by CT or MRI scans\textsuperscript{286}. This is important as MRI-measured visceral fat is associated with cardiovascular risk factors, such as altered lipid profile and high blood pressure\textsuperscript{286}. Higher android to gynoid fat ratio is associated with an adverse lipid profile and higher blood pressure even in childhood\textsuperscript{287}. In addition, DXA-derived android fat (irrespective of overall obesity) correlates well with overall blood pressure, atherogenic indices, fasting and 2-hour OGTT blood glucose, HbA1c, total cholesterol, LDL-cholesterol, and triglycerides in adults of both sexes\textsuperscript{288}. As a result, with an increase in obesity-related metabolic disorders, the DXA analysis of body composition is an easy and rapid method to analyse fat distribution in children before the more overt onset of obesity.

2.3. Statistics

2.3.1. Power and sample size calculation

Insulin sensitivity was the primary outcome in all three studies. Thus, the sample size calculation was based on the differences in this outcome in each of the three groups in comparison to controls. Power calculation was carried out a priori on insulin sensitivity based on previous data on healthy pre-pubertal children obtained via an identical FSIGT protocol\textsuperscript{26}. This gave a sample size of 36 participants to detect an insulin sensitivity index difference of $3.6 \times 10^{-4} \cdot \text{min}^{-1} \cdot (\text{mU/l})$ between two
groups (approximately 30%) with 90% power at 5% level of significance, assuming a standard deviation of $4.4 \times 10^{-4}\text{-min}^{-1}\text{-mU/l}$.

### 2.3.2. Statistical Analyses

Potential differences between 'treatment' and control groups at baseline were tested using one-way ANOVA or non-parametric Kruskal-Wallis, while sex ratio and ethnic composition data were compared with Fisher’s exact tests (all in Minitab v.16, Pennsylvania State University, State College, PA, USA). Similar tests were conducted to compare children included in a particular study to those who were not contactable or declined to participate.

Random effects mixed models were used to compare the primary and secondary outcomes between groups. Important confounding factors were adjusted for in the analyses, including ethnicity, birth weight SDS, birth order, age, and gender. Other factors were controlled for as required, depending on the outcome response of interest: for lipids, hormones, and outcomes associated with glucose homeostasis – BMI SDS was included; for anthropometric data – the appropriate parental factor (i.e. mean parental BMI or mid-parental height); and for blood pressure parameters – height and total body fat percentage. For the primary outcome (insulin sensitivity) models were run separately for boys and girls. The interaction effect between group and gender was tested in all models, and secondary outcomes were assessed separately for boys and girls if there was indication of a differential response to the analysed early life event between genders.

All multivariate analyses were performed in SAS version 9.3 (SAS Institute Inc. Cary NC, USA). When necessary, response variables were log-transformed to approximate normality. All statistical tests were two-tailed and maintained at a 5% significance level. Age data are presented as means ± standard deviation. Outcome data are presented as model-adjusted means (estimated marginal means adjusted for the confounding factors in the models), with associated 95% confidence intervals.
Chapter 3. Pre-pubertal children born post-term have reduced insulin sensitivity and other markers of the metabolic syndrome

Preface

This chapter contains an unaltered reproduction of a manuscript currently in press in the journal PLOS ONE.

- **Authors:** Ayyavoo A, Derraik JGB, Hofman PL, Mathai S, Biggs J, Stone P, Sadler L, Cutfield WS.
- **Title:** Pre-pubertal children born post-term have reduced insulin sensitivity and other markers of the metabolic syndrome
- **Journal:** PLoS ONE
- **Year of publication:** 2013
- **Volume:** 8
- **Page:** e67966
- **Impact factor:** 3.73
- **Journal’s aims and scope:** “PLOS ONE features reports of original research from all disciplines within science and medicine. By not excluding papers on the basis of subject area, PLOS ONE facilitates the discovery of the connections between papers whether within or between disciplines.”
Abstract

**Background:** There are no data on the metabolic consequences of post-term birth (≥42 weeks gestation). We hypothesize that post-term birth adversely affects insulin sensitivity, as well as other metabolic parameters and body composition in childhood.

**Methods:** 77 healthy pre-pubertal children, born appropriate-for-gestational-age were studied in Auckland, New Zealand: 36 born post-term (18 boys) and 41 (27 boys) born at term (38–40 weeks). Primary outcome was insulin sensitivity measured using intravenous glucose tolerance tests and Bergman’s minimal model. Other assessments included hormonal and lipid profiles, body composition from whole-body dual-energy X-ray absorptiometry, 24-hour ambulatory blood pressure monitoring, and inflammatory markers.

**Results:** Insulin sensitivity was 34% lower in post-term than in term children (7.7 vs. 11.6 x10^{-4} min⁻¹·(mU/l); p<0.0001). There was a compensatory increase in acute insulin response among post-term children (418 vs 304 mU/l; p=0.037), who also displayed lower glucose effectiveness than those born at term (2.25 vs 3.11 x10^{-2}·min⁻¹; p=0.047). Post-term children not only had more body fat (p=0.014) and less fat-free mass (p=0.014), but also had increased central adiposity with more truncal fat (p=0.017) and greater android to gynoid fat ratio (p=0.007) compared to term counterparts. Further, post-term children displayed other markers of the metabolic syndrome: lower normal nocturnal systolic blood pressure dipping (p=0.027), lower adiponectin concentrations (p=0.005), as well as higher leptin (p=0.008) and uric acid (p=0.033) concentrations. Post-term boys (but not girls) also displayed a less favourable lipid profile, with higher total cholesterol (p=0.018) and LDL-C (p=0.006) concentrations, and total cholesterol to HDL-C ratio (p=0.048).

**Conclusions:** Post-term children have reduced insulin sensitivity and display a number of early markers of the metabolic syndrome. These findings could have important implications for the management of prolonged pregnancies. Future studies need to examine potential impacts later in life, as well as possible underlying mechanisms.

Introduction

Post-term birth (≥42 weeks gestation) remains a relatively common event worldwide, although its incidence varies across countries. For example, in Europe the incidence of post-term birth ranges from 0.4% in Austria to 8.1% in Denmark [1]. Post-term birth is associated with a greater incidence of peri-partum complications affecting both the mother and newborn. As a result, pregnancies at risk of post-term delivery are often induced, but recommendations on the appropriate time for induction are not universal, ranging from 41 to ≥42 weeks gestation [2,3].
Nonetheless, little is known about the short- and long-term consequences associated with post-term birth, although two studies have reported adverse neurological outcomes in childhood [4,5]. We have recently shown in a longitudinal study of a Swedish cohort that post-term males had accelerated weight gain during childhood, and an associated increased risk of obesity in adolescence [6]. As a result, at 16 years of age the combined rate of overweight and obesity among post-term males was 47% compared to 13% for participants born at term [6].

There are however, no data on the metabolic consequences of post-term birth. Post-term fetuses are likely exposed to in utero stressors associated with a prolonged gestation, which may be analogous to those experienced by small-for-gestational-age (SGA) infants prior to birth and by preterm infants in the early neonatal period. Studies have shown that those born SGA or preterm are at an increased risk of adverse metabolic outcomes in adulthood, including insulin resistance, type 2 diabetes mellitus, hypertension, hyperlipidemia, stroke, as well as cancer [7,8]. As a result, this study aimed to assess whether post-term birth would affect insulin sensitivity, as well as other metabolic parameters and body composition in childhood.

Methods

Ethics statement
Ethics approval for this study was provided by the Northern Y Regional Ethics Committee (Ministry of Health, New Zealand) and the Auckland District Health Board Research Review Committee. Written informed consent was obtained from parents or guardians, as well as verbal or written consent from each child as was appropriate to their age.

Participants
Healthy, developmentally normal pre-pubertal children born from singleton pregnancies and aged 4–11 years were recruited for this study between November 2010 and November 2011. Potential post-term participants were born at a single centre (National Women’s Health, Auckland City Hospital, Auckland, New Zealand), and identified from its obstetrics database. Control participants (term children) were friends of participants (Figure 3.1). Each recruited post-term child was asked to invite up to two friends born at term, so that participants in both groups were approximately matched for age and socio-economic status.
Gestational ages were determined by ultrasound scans performed <20 weeks gestation. Each scan was performed by a qualified radiologist, and all scans were subsequently re-examined and validated by the first author (AA). Data from 81 singleton pregnancies after in vitro fertilization (where conceptions can be accurately timed) showed that ultrasound scans in the first 20 weeks underestimated gestation length by just 2.8 days (standard error of the mean = 0.2), so that fetal age was determined to within 7 days in more than 95% of cases [9]. Nonetheless, in our study, only term children born 38–40 weeks gestation (following spontaneous labour) were recruited, to account for possible errors in gestational age estimation. This effectively created a gestational age difference of approximately two weeks separating term and post-term groups.

Exclusion criteria included signs of puberty (Tanner stage 2 breast development in girls and testicular volume >3 ml in boys or evidence of adrenarche), premature birth, being born SGA (birth weight <-2 standard deviation scores (SDS)) or as a result of in vitro fertilization, having genetic syndromes, receiving medication that could affect insulin sensitivity, as well as having a first-degree relative or grandparent with pre-diagnosed diabetes, the metabolic syndrome, or any of its features other than central adiposity. Children were also excluded if born to mothers with gestational diabetes, pre-eclampsia, gestational or pre-existing hypertension, chronic illnesses, or maternal drug use during pregnancy (including tobacco and alcohol).
Clinical assessments
All children were assessed at the Maurice & Agnes Paykel Clinical Research Unit (Liggins Institute, University of Auckland). Data on each child were collected during a single visit to the clinic. A number of neonatal parameters were recorded, including birth weight, ponderal index, gestational age, and maternal age at time of delivery. Birth weight data were transformed into SDS [10].

Primary outcome
Insulin sensitivity was assessed using a 90-minute modified frequently sampled intravenous glucose test (FSIGT), modified with insulin, and analysed using Bergman’s minimal model software [11]. Three baseline samples were drawn at -20, -10, and 0 minutes. A 25% dextrose infusion (at 0.3 g/kg) started at 0 minute and lasted for one minute. Blood samples were drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19 minutes. Insulin (0.015 units/kg) was then administered intravenously as a bolus at 20 minutes, and further samples were drawn at 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 60, 70, 80, and 90 minutes. No episodes of hypoglycaemia (blood glucose <4 mmol/l) were recorded in any of the participants throughout the study.

Secondary outcomes
Children’s heights were measured using a Harpenden stadiometer. Weight and body composition data were obtained using whole-body dual-energy X-ray absorptiometry (DXA, Lunar Prodigy 2000, General Electric, Madison, WI, USA), specifically: total body fat, fat-free mass, truncal fat, and android fat to gynoid fat ratio. The latter parameters are provided by the manufacturer’s software based on an automated sectioning of specific areas of the body [12]. Studies in children have shown that proportionally greater adiposity in the upper body (i.e. android fat) is associated with an increased risk of adverse metabolic outcomes [13].

Height SDS were derived from Tanner/Whitehouse reference data [14], and weight and body mass index (BMI) SDS according to British 1990 standards [10,15]. Maternal and paternal weight and height were recorded for all participants. Mean parental BMI was calculated as the average of maternal and paternal BMI. Mid-parental height was calculated using standard formulas [16]. Ethnicity was recorded by self-report using a prioritised system, such that if multiple ethnicities were selected, the patient was assigned to a single category, following a hierarchical system of classification [17].

24-hour ambulatory blood pressure monitoring was carried out following the clinical visit, when participants were fitted with a Spacelabs 90217 monitor (Spacelabs Medical Inc., Redmond, WA, USA) on the non-dominant arm. Measurements were performed every 20 minutes from 07:00–
22:00, and every 30 minutes from 22:00–07:00. Only profiles with more than 14 daytime and 7 nocturnal readings over a 24-hour period were included for analysis (as per British Hypertension Society recommendations). Parameters reported were mean arterial blood pressure, and nocturnal systolic and diastolic blood pressure dipping.

Following an overnight fast, baseline blood samples were drawn to measure serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, insulin-like growth factor I (IGF-I), IGF binding protein 1 (IGFBP-1), leptin, total adiponectin, androstenedione, dehydroepiandrosterone sulphate (DHEAS), glucose, insulin, highly-sensitive C-reactive protein (CRP), and uric acid concentrations. Other parameters derived from the FSIGT were the acute insulin release (insulin secretory capacity), glucose effectiveness (glucose-mediated glucose uptake), and disposition index (increase in insulin secretion to compensate for insulin resistance and maintain normoglycaemia).

Assays
Glucose and uric acid concentrations were measured on a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) by enzymatic colorimetric assay (Roche, Mannheim, Germany), with an inter-assay coefficient of variation (CV) of 2.1 and 1.8%, respectively. Insulin concentrations were measured using an Abbott AxSYM system (Abbott Laboratories, Abbott Park, IL, USA) by microparticle enzyme immunoassay, with a CV of 5.7%. Total cholesterol, HDL-C, LDL-C, and triglyceride concentrations were measured using a Hitachi 902 autoanalyser, with CV of 8.9, 11.4, 10.1, and 5.3% respectively. Commercially available ELISA kits E20, E01, E07, and E09 (Mediagnost, Reutlingen, Germany) were used for quantitative determination of serum IGF-I, IGFBP-1, leptin, and adiponectin concentrations, respectively; assay sensitivities were 0.09, 0.2, 1.0, and 0.6 ng/ml, with CV of 3.1, 9.4, 6.7, and 3.0%, respectively. CRP concentrations were also measured with an ELISA kit (USCN Life Science Inc., Wuhan, China), with CV of <10%. DHEAS and androstenedione concentrations were measured using Finnigan TSQ Quantum Ultra AM triple quadrupole mass spectrometer controlled by Finnigan Xcaliber software (Thermo Electron Corporation, San Jose, CA, USA); mean CV were 18.4% for DHEAS and 8.2% for androstenedione.

Statistical Analysis
Potential differences between post-term and term groups at baseline were tested using one-way ANOVA or non-parametric Kruskal-Wallis, while sex ratio and ethnic composition data were compared with Fisher’s exact tests (all in Minitab v.16, Pennsylvania State University, State
College, PA, USA). Similar tests were conducted to compare post-term children included in this study to those who were not contactable or declined to participate.

Random effect mixed models were used to compare the primary and secondary outcomes between post-term and term groups. Important confounding factors were adjusted for in the analyses, including ethnicity, birth weight SDS, birth order, age, and gender. Other factors were controlled for as required, depending on the outcome response of interest: for lipids, hormones, and outcomes associated with glucose homeostasis – BMI SDS was included; for anthropometric data – the appropriate parental factor (i.e. mean parental BMI or mid-parental height); and for blood pressure parameters – height and total body fat percentage. For the primary outcome (insulin sensitivity) models were run separately for boys and girls. The interaction effect between group and gender was tested in all models, and secondary outcomes were assessed separately for boys and girls if there was indication of a differential response to post-term birth between genders.

All multivariate analyses were performed in SAS version 9.3 (SAS Institute Inc. Cary NC, USA). When necessary, response variables were log-transformed to approximate normality. All statistical tests were two-tailed and maintained at a 5% significance level. Age data are presented as means ± standard deviation. Outcome data are presented as model-adjusted means (estimated marginal means adjusted for the confounding factors in the models), with associated 95% confidence intervals.

Results

Participants
There were 59 post-term children born in 2000–2001 in the National Women’s Hospital obstetrics database who met the study criteria and could be traced (Figure 3.1). As 21 declined to participate, we recruited 38 post-term participants (Figure 3.1). Two participants had to be subsequently excluded, one due to maternal ill-health and another child could not be cannulated; thus a total of 36 post-term children (18 boys and 18 girls) were studied (Figure 3.1).

Post-term children who were not contactable or declined to participate were of similar birth weight (p=0.16), current age (p=0.58), and sex ratio (p=0.99) as those who participated. Through post-term children a total of 49 term controls volunteered, but 8 did not meet inclusion criteria, so that 41 participated in the study.

Children were aged 9.4 ± 1.9 years (range 4.0–11.9 years); age, birth weight, ponderal index, sex ratio, and ethnic composition were all similar between post-term and term groups (Table 3.1). In
addition, there were no differences in parental variables between groups, namely maternal age, pre-pregnancy maternal BMI, and mean parental BMI at the time of the study (Table 3.1).

**Insulin sensitivity and other parameters of glucose homeostasis**

Insulin sensitivity was 34% lower in among post-term children compared to term children (7.7 vs. 11.6 $10^{-4}$min$^{-1}$·(mU/l); $p<0.0001$) (Figure 3.2). Glucose effectiveness was also 28% lower among post-term children in comparison to those born at term ($p=0.047$; Table 3.2). However, the lower insulin sensitivity was compensated by an acute insulin response that was 38% higher among post-term children ($p=0.037$; Table 3.2). Disposition index, fasting glucose and insulin concentrations were not different in post-term and term children (Table 3.2).

Insulin sensitivity was 33% lower among post-term boys (8.5 vs $12.7\times10^{-4}$min$^{-1}$·(mU/l); $p=0.001$) and 44% lower among post-term girls (5.6 vs $10.0\times10^{-4}$min$^{-1}$·(mU/l); $p=0.005$). However, a significant compensatory increase in acute insulin response was only observed in post-term boys compared to term counterparts (531 vs 347 mU/l; $p=0.039$), but not among post-term girls (367 vs 304 mU/l; $p=0.49$).

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<thead>
<tr>
<th></th>
<th>Post-term children</th>
<th>Term children</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Maternal age at childbirth (years)</td>
<td>32.5 ± 5.2</td>
<td>33.6 ± 4.7</td>
<td>0.39</td>
</tr>
<tr>
<td>Maternal pre-pregnancy BMI (kg/m²)</td>
<td>25.3 ± 6.0</td>
<td>24.7 ± 5.2</td>
<td>0.62</td>
</tr>
<tr>
<td>Mean parental BMI (kg/m²)</td>
<td>28.1 ± 4.4</td>
<td>27.8 ± 4.9</td>
<td>0.65</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.7 ± 1.3</td>
<td>9.1 ± 2.2</td>
<td>0.33</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>42.2 ± 0.2</td>
<td>39.5 ± 0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>0.53 ± 0.95</td>
<td>0.35 ± 0.83</td>
<td>0.39</td>
</tr>
<tr>
<td>Ponderal index</td>
<td>25.8 ± 2.8</td>
<td>27.0 ± 4.4</td>
<td>0.34</td>
</tr>
<tr>
<td>Sex ratio (boys)</td>
<td>50%</td>
<td>66%</td>
<td>0.17</td>
</tr>
<tr>
<td>Ethnicity (New Zealand Europeans)</td>
<td>72%</td>
<td>83%</td>
<td>0.28</td>
</tr>
</tbody>
</table>
Figure 3.2. Insulin sensitivity (primary outcome) among children born post-term or at term.
Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models.

Table 3.2. Secondary outcomes among children born post-term or at term.
Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models.

<table>
<thead>
<tr>
<th></th>
<th>Post-term children</th>
<th>Term children</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td><strong>Glucose homeostasis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute insulin response (mU/l)</td>
<td>418 (339–516)</td>
<td>304 (235–392)</td>
<td>0.037</td>
</tr>
<tr>
<td>Glucose effectiveness (10^{-2}/min)</td>
<td>2.25 (1.52–3.33)</td>
<td>3.11 (2.01–4.83)</td>
<td>0.047</td>
</tr>
<tr>
<td>Disposition index</td>
<td>3539 (2406–5205)</td>
<td>3576 (2271–5630)</td>
<td>0.95</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>5.72 (4.38–7.49)</td>
<td>5.20 (3.76–7.19)</td>
<td>0.43</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>86.1 (82.9–89.2)</td>
<td>87.2 (83.4–91.0)</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Hormonal profile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>6.96 (4.99–8.92)</td>
<td>4.49 (2.13–6.86)</td>
<td>0.008</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>7.82 (4.64–9.20)</td>
<td>10.75 (9.32–12.17)</td>
<td>0.005</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>204 (168–240)</td>
<td>200 (157–243)</td>
<td>0.78</td>
</tr>
<tr>
<td>IGFBP-1 (ng/ml)</td>
<td>10.6 (7.8–13.5)</td>
<td>17.6 (14.6–20.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>0.44 (0.33–0.54)</td>
<td>0.59 (0.48–0.70)</td>
<td>0.055</td>
</tr>
<tr>
<td>DHEAS (nmol/l)</td>
<td>4.59 (2.53–6.66)</td>
<td>6.12 (3.59–8.64)</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>73.4 (70.0–76.8)</td>
<td>76.2 (72.0–80.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Nocturnal systolic dipping (%)</td>
<td>9.7 (6.2–13.2)</td>
<td>13.5 (9.2–17.8)</td>
<td>0.027</td>
</tr>
<tr>
<td>Nocturnal diastolic dipping (%)</td>
<td>15.6 (11.1–20.1)</td>
<td>19.4 (13.8–25.1)</td>
<td>0.079</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>457 (63–851)</td>
<td>490 (6–974)</td>
<td>0.86</td>
</tr>
<tr>
<td>Uric acid (μmol/l)</td>
<td>229 (211–247)</td>
<td>201 (181–220)</td>
<td>0.033</td>
</tr>
</tbody>
</table>
**Anthropometry**

Although post-term and term children were of similar BMI SDS (0.30 vs 0.11; p=0.45), there were considerable differences in adiposity and fat distribution between the two groups (Figure 3.3). Post-term children had greater body fat (22.9 vs 19.9%; p=0.014) and lower fat-free mass (77.1 vs 80.1%; p=0.014), as well as more truncal fat (21.2 vs 18.0%; p=0.017) and greater android to gynoid fat ratio (0.708 vs 0.613 p=0.007) (Figure 3.3). In addition, children born post-term were approximately 0.5 SDS taller (p=0.022; Figure 3.3). However, this pattern was mostly driven by their increased adiposity, so that addition of body fat into the statistical model removed the significant difference in height between the two groups (p=0.20).

*Figure 3.3. Anthropometric parameters among children born post-term or at term.*
Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models. *p<0.05 and **p<0.01 for post-term vs term children.*
**Blood pressure**
The 24-hour ambulatory blood pressure monitoring showed that post-term children experienced lower nocturnal blood pressure dipping. This occurred for systolic blood pressure dipping, with a similar trend also observed for diastolic dipping (Table 3.2).

**Hormonal profile & inflammatory markers**
Post-term children had higher leptin (+55%) and lower adiponectin (-37%) concentrations, as well as lower serum IGFBP-1 concentrations (-40%; Table 3.2). DHEAS concentrations were not different among groups (p=0.11), but post-term children tended to have lower androstenedione concentrations (-25%; Table 3.2). Among inflammatory markers, post-term children had higher serum uric acid concentrations (+14%; Table 3.2).

**Lipid profile**
Overall, children born post-term had a less favourable fasting lipid profile compared to term children (data not shown). However, there was a differential response between sexes, so that the less favourable lipid profile was only observed among post-term boys but not girls (Table 3.3). Specifically, post-term boys had higher total cholesterol (+29%) and LDL-C (+53%) concentrations, tended to have higher triglyceride concentrations (+40%), and also had higher total cholesterol to HDL-C ratio (+30%) compared to term controls (Table 3.3).
Table 3.3. Lipid profiles among children born post-term or at term.
Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models.

<table>
<thead>
<tr>
<th></th>
<th>Post-term Children</th>
<th>Term Children</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole cohort (n)</strong></td>
<td>36</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.84 (3.27–4.40)</td>
<td>3.29 (2.62–3.96)</td>
<td>0.034</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.13 (1.72–2.55)</td>
<td>1.67 (1.17–2.17)</td>
<td>0.016</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.81 (0.64–0.98)</td>
<td>0.69 (0.49–0.89)</td>
<td>0.12</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.27 (1.04–1.50)</td>
<td>1.23 (0.95–1.50)</td>
<td>0.69</td>
</tr>
<tr>
<td>Total cholesterol : HDL-C</td>
<td>3.19 (2.73–3.66)</td>
<td>2.68 (2.11–3.25)</td>
<td>0.037</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Boys (n)</strong></th>
<th>18</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.04 (3.73–4.71)</td>
<td>3.14 (2.32–3.97)</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.23 (1.74–2.71)</td>
<td>1.46 (0.85–2.06)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.84 (0.67–1.05)</td>
<td>0.60 (0.47–0.76)</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.32 (1.03–1.60)</td>
<td>1.24 (0.89–1.59)</td>
</tr>
<tr>
<td>Total cholesterol : HDL-C</td>
<td>3.32 (2.73–3.90)</td>
<td>2.56 (1.83–3.30)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Girls (n)</strong></th>
<th>18</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.57 (2.80–4.34)</td>
<td>3.88 (3.04–4.71)</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.01 (1.52–2.49)</td>
<td>2.22 (1.70–2.75)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.78 (0.53–1.03)</td>
<td>0.71 (0.44–0.97)</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.17 (0.92–1.42)</td>
<td>1.30 (1.03–1.57)</td>
</tr>
<tr>
<td>Total cholesterol : HDL-C</td>
<td>3.21 (2.65–3.77)</td>
<td>2.85 (2.21–3.49)</td>
</tr>
</tbody>
</table>

**New Zealand Europeans**

As New Zealand Europeans made up approximately three-quarters of participants, a subgroup analyses could be carried out with the exclusion of other ethnicities. These data are provided in a supplementary table (Table 3.4). Overall trends remained, but statistical power was considerably reduced with the reduction in n (Table 3.4). Still, insulin sensitivity was 30% lower in post-term than in term groups (8.7 vs 12.5 x10^4·min⁻¹·(mU/l); p=0.003) (Table 3.4). In addition, compared to term controls, post-term children of New Zealand European ethnicity had lower systolic blood pressure dipping, higher leptin concentrations, lower IGFBP-1 concentrations, and tended to have greater abdominal adiposity and higher uric acid concentrations (Table 3.4).
Table 3.4. Study outcomes among children of New Zealand European ethnicity who were born post-term or at term. Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models.

<table>
<thead>
<tr>
<th></th>
<th>Post-term children</th>
<th>Term children</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>26</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.66 (0.34–0.98)</td>
<td>0.37 (0.07–0.66)</td>
<td>0.20</td>
</tr>
<tr>
<td>BM ISDS</td>
<td>0.33 (-0.06–0.71)</td>
<td>0.25 (-0.13–0.64)</td>
<td>0.79</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>23.2 (21.3–25.0)</td>
<td>21.1 (19.3–23.0)</td>
<td>0.14</td>
</tr>
<tr>
<td>Fat-free mass (%)</td>
<td>76.8 (75.0–78.7)</td>
<td>78.9 (77.0–80.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Truncal fat (%)</td>
<td>20.5 (18.4–22.6)</td>
<td>18.7 (16.7–20.8)</td>
<td>0.24</td>
</tr>
<tr>
<td>Android fat to gynoid fat ratio</td>
<td>0.71 (0.66–0.77)</td>
<td>0.64 (0.59–0.69)</td>
<td>0.056</td>
</tr>
<tr>
<td><strong>Glucose homeostasis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity (x10^4∙min^-1∙(mU/l))</td>
<td>8.7 (7.4–10.3)</td>
<td>12.5 (10.8–14.4)</td>
<td>0.003</td>
</tr>
<tr>
<td>Acute insulin response (mU/l)</td>
<td>269 (211–342)</td>
<td>217 (172–272)</td>
<td>0.22</td>
</tr>
<tr>
<td>Glucose effectiveness (10^-2/min)</td>
<td>1.69 (1.26–2.27)</td>
<td>2.41 (1.86–3.11)</td>
<td>0.088</td>
</tr>
<tr>
<td>Disposition index</td>
<td>2403 (1802–3206)</td>
<td>2393 (1844–3106)</td>
<td>0.98</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>4.51 (3.67–5.53)</td>
<td>4.09 (3.36–4.99)</td>
<td>0.51</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>82.8 (80.6–85.1)</td>
<td>84.7 (82.5–86.8)</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Hormone concentrations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>5.95 (4.81–7.08)</td>
<td>4.19 (3.16–5.22)</td>
<td>0.032</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>8.13 (6.83–9.67)</td>
<td>9.88 (8.38–11.6)</td>
<td>0.12</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>191 (165–217)</td>
<td>189 (164–214)</td>
<td>0.92</td>
</tr>
<tr>
<td>IGFBP-1 (ng/ml)</td>
<td>9.92 (7.79–12.6)</td>
<td>15.4 (12.3–19.4)</td>
<td>0.013</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>0.34 (0.27–0.44)</td>
<td>0.50 (0.40–0.62)</td>
<td>0.034</td>
</tr>
<tr>
<td>DHEAS (nmol/l)</td>
<td>2.26 (1.46–3.49)</td>
<td>3.96 (2.69–5.83)</td>
<td>0.074</td>
</tr>
<tr>
<td><strong>24-hour blood pressure monitoring</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>75.4 (72.9–78.0)</td>
<td>78.5 (75.9–81.0)</td>
<td>0.11</td>
</tr>
<tr>
<td>Nocturnal systolic dipping (%)</td>
<td>7.5 (5.1–10.0)</td>
<td>11.3 (8.9–13.8)</td>
<td>0.041</td>
</tr>
<tr>
<td>Nocturnal diastolic dipping (%)</td>
<td>14.4 (11.4–17.5)</td>
<td>17.1 (14.0–20.2)</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>228 (81–374)</td>
<td>240 (95–385)</td>
<td>0.91</td>
</tr>
<tr>
<td>Uric acid (μmol/l)</td>
<td>224 (202–245)</td>
<td>195 (174–216)</td>
<td>0.061</td>
</tr>
<tr>
<td><strong>Lipid profile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.26 (3.81–4.71)</td>
<td>3.95 (3.56–4.35)</td>
<td>0.33</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.52 (2.20–2.85)</td>
<td>2.27 (1.98–2.56)</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.78 (0.66–0.91)</td>
<td>0.74 (0.62–0.85)</td>
<td>0.61</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.31 (1.14–1.48)</td>
<td>1.30 (1.14–1.45)</td>
<td>0.92</td>
</tr>
<tr>
<td>Total cholesterol : HDL-C</td>
<td>3.36 (3.03–3.68)</td>
<td>3.11 (2.82–3.41)</td>
<td>0.29</td>
</tr>
</tbody>
</table>
Discussion

This study shows that pre-pubertal children born post-term have lower insulin sensitivity in comparison to term children. Further, post-term children also displayed other early markers of the metabolic syndrome such as greater body fat and central adiposity, lower nocturnal blood pressure dipping, higher serum uric acid concentrations, as well as a less favourable lipid profile among post-term boys.

The metabolic syndrome comprises a combination of insulin resistance with or without glucose intolerance with any two of the following: central obesity, high blood pressure, dyslipidemia, pro-inflammatory state, or prothrombotic state [18]. A reduction in insulin sensitivity alone can predict the metabolic syndrome in adulthood [19], which in turn is an independent risk factor for later mortality from coronary heart disease and cerebrovascular disease [20]. The observed reduction in insulin sensitivity was similar to that observed among pre-pubertal children born SGA or preterm [21,22]. SGA children have insulin resistance that starts in childhood [21] and persists through early adulthood [23], being associated with an increased risk for various metabolic disorders later in life [7]. Infants born preterm also demonstrate a similar reduction in insulin sensitivity in childhood [22], which we have recently shown to persist into mid-adulthood [8]. Importantly, the lower insulin sensitivity in post-term children was not due to adrenarche, as indicated by higher adrenal androgen levels among term children. Thus, the reduction in insulin sensitivity we observed in children born post-term may persist into adult life.

Whole body glucose uptake is dependent upon three factors: insulin secretion (acute insulin response), insulin action (insulin sensitivity), and glucose uptake independent of insulin (glucose effectiveness) [24]. Severe defects in at least of two of these factors are necessary for the development of type 2 diabetes mellitus [24]. We observed impairments in two factors (insulin sensitivity and glucose effectiveness), indicating that post-term children may be at increased risk of later type 2 diabetes mellitus.

We also observed an overall increase in insulin secretion, particularly among post-term boys. A similar trend was observed among girls, but the increase in acute insulin response was of lesser magnitude and not significant likely due to reduced statistical power (lower n for control girls). With the greater insulin secretion, there was an expected reduction in serum IGFBP-1 concentrations amongst post-term children. Insulin resistance leads to a compensatory increase in portal insulin secretion, which suppresses IGFBP-1 concentrations [25]. Hyperinsulinemia and low IGFBP-1 are also associated with increased likelihood of developing cardiovascular disease [26]. The less favourable lipid profile observed amongst post-term boys was still within the normal
range, so that the importance of these findings is unknown. However, blood lipid levels in childhood track into adulthood [27], and could precede later dyslipidemia. Furthermore, post-term children had higher concentrations of uric acid, which is also a marker of cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus [28]. A follow-up study showed that elevated uric acid levels in childhood are predictive of higher blood pressure in adult life [29].

Further, post-term children had increased body fat, lower fat-free mass, and greater central adiposity than term controls. The elevated abdominal adiposity in particular, is associated with cardiovascular disease in adulthood [30]. There was also an adverse adipokine profile in post-term children, characterized by higher leptin and lower adiponectin concentrations, consistent with a greater central fat distribution and insulin resistance [31]. These proteins are secreted by adipose tissue [32], with high leptin concentrations (leptin resistance) and low adiponectin being both associated with the metabolic syndrome [32,33]. Interestingly, we recently studied a Swedish cohort longitudinally from birth to 16 years of age, and post-term males displayed greater adiposity that markedly increased in adolescence [6]. Nearly half of post-term adolescent males were overweight or obese, and were on average 11 kg heavier than those born at term [6]. Interestingly, both sexes were adversely affected by post-term birth in this current study. In light of our earlier study in adolescents born post-term, it is conceivable that post-term males may experience more marked changes in body composition and metabolism over time. Nonetheless, our data suggest that insulin resistance in post-term children precedes the major changes in body composition observed in adolescence [6].

Post-term children also had markedly lower nocturnal systolic blood pressure dipping, with a similar trend observed for diastolic dipping. This observation is important, as the normal reduction in nocturnal blood pressure dipping is a risk factor for cardiovascular mortality in adults, irrespective of overall blood pressure [34]. There is a normal reduction in nocturnal blood pressure in children and adults that is >10% [35], but post-term children already displayed a mean systolic dipping below this threshold. Unfortunately, there are no long-term data on the effects of reduced nocturnal dipping in childhood, which would better clarify the long-term cardiovascular consequences to our post-term cohort.

The underlying factors associated with the observed metabolic changes in post-term children are unclear. We speculate that the factors underpinning the metabolic programming in children born post-term may be associated with genetic inheritance or an adverse fetal environment in late gestation, which is unlikely to be associated with nutritional compromise. The Dutch Famine study showed an increased risk of glucose intolerance in adulthood among those exposed to famine in late
gestation compared to early gestation [36]. However, an under-nutrition insult in utero is unlikely to occur in most prolonged pregnancies, as our post-term infants had comparable birth weights (when adjusted for gestational age) and ponderal indices to term children.

Whilst the cause of the adverse environment is unclear, there are changes in placental histology seen in post-term pregnancies [37]. Nonetheless, post-term birth is common in first-degree relatives, suggesting an element of genetic inheritance [38]. Therefore, a common genetic variant or epigenetic modification may possibly lead to prolonged gestation, and, at least in part, underpin the observed metabolic changes in post-term children. In addition, prolonged gestation could also be associated with an increased stress response in the fetus (e.g. higher glucocorticoid levels) [39], which may lead to the metabolic changes we observed later in post-natal life. However, animal models of excessive glucocorticoid exposure are usually associated with reduced birth weight and alterations in the hypothalamic-pituitary-adrenal axis [39], neither of which was seen in our cohort.

In conclusion, our study shows that pre-pubertal children born post-term have lower insulin sensitivity and a number of early markers of the metabolic syndrome. These include increased body fat, greater central adiposity, lower nocturnal blood pressure dipping, and higher uric acid concentrations, as well as a less favourable lipid profile in post-term boys. Interestingly, the range of adverse metabolic outcomes in post-term children greatly exceeds those observed in SGA or preterm children. Thus, our findings in post-term children could have major implications for the management of prolonged pregnancies. It is important to study post-term cohorts in adulthood to adequately identify the long-term health risks associated with prolonged gestation. Further, future studies are necessary to establish the underlying causes and mechanisms of these changes in post-term children.

Acknowledgements

Thanks to Dr Yannan Jiang (Department of Statistics, University of Auckland) for very valuable statistical input. Eric Thorstensen (Liggins Institute, University of Auckland) and his group kindly assisted us with laboratory work. We also acknowledge the Paykel Trust for long-term support of the Maurice & Agnes Paykel Clinical Research Unit at the Liggins Institute, University of Auckland.
References


Chapter 4. Severe hyperemesis gravidarum is associated with reduced insulin sensitivity in the offspring in childhood

Preface

This chapter contains the unaltered (and yet to be corrected) version of a manuscript published online in the Journal of Clinical Endocrinology and Metabolism.

- **Authors:** Ayyavoo A, Derraik JGB, Hofman PL, Biggs J, Bloomfield FH, Cormack BE, Stone P, Cutfield WS.
- **Title:** Severe hyperemesis gravidarum is associated with reduced insulin sensitivity in the offspring in childhood.
- **Journal:** Journal of Clinical Endocrinology and Metabolism
- **Year of publication:** 2013
- **Volume:** 98
- **Issue:** 8
- **Pages:** 3263-3268
- **Impact factor:** 6.43
- **Journal’s aims and scope:** “The Journal of Clinical Endocrinology & Metabolism is the world’s leading peer-reviewed journal for endocrine clinical research and cutting edge clinical practice reviews. Each issue provides the latest in-depth coverage of new developments enhancing our understanding, diagnosis and treatment of endocrine and metabolic disorders. Regular features of special interest to endocrine consultants include clinical trials, clinical reviews, clinical practice guidelines, case seminars, and controversies in clinical endocrinology, as well as original reports of the most important advances in patient-oriented endocrine and metabolic research.”
Abstract

**Background:** Hyperemesis gravidarum alters maternal (and possibly fetal) nutrition throughout pregnancy, but there are no data on long-term effects on offspring metabolism. Thus, we aimed to assess whether severe hyperemesis gravidarum affects glucose homeostasis and body composition in the offspring in childhood.

**Methods:** Healthy pre-pubertal children (aged 4–11 years) born at term were studied: offspring of mothers who were admitted to hospital with severe hyperemesis gravidarum (SHG; n=36) and offspring of mothers from control pregnancies (Control; n=42). Primary outcome was insulin sensitivity measured using intravenous glucose tolerance tests and Bergman’s minimal model. Other assessments included lipid and hormonal profiles, and body composition using whole-body dual-energy X-ray absorptiometry.

**Results:** Insulin sensitivity in SHG children was 20% lower than in controls (8.49 vs 10.60 x10^-4 ·min^-1·(mU/l); p=0.014). SHG children also had higher fasting insulin (6.88 vs 5.04 mIU/l; p=0.024) and lower IGFBP-1 (11.8 vs 19.0 ng/ml; p=0.004) concentrations than controls. Baseline cortisol concentrations were 22% higher in SHG offspring (256 vs 210 nmol/l; p=0.021). Children in both groups were anthropometrically similar.

**Conclusion:** Children born to mothers who experienced severe hyperemesis gravidarum have lower insulin sensitivity, which may increase their long-term risk of developing diabetes mellitus. Follow up of SHG offspring is essential to determine later risk of metabolic disease.

**Introduction**

Hyperemesis gravidarum is a severe form of morning sickness in pregnancy, involving at least one antenatal hospital admission before 20 weeks of gestation(1, 2). The onset of hyperemesis gravidarum usually occurs at 4–8 weeks gestation, and in most cases the condition greatly improves by 14–16 weeks(2). However, some women suffer from hyperemesis gravidarum throughout pregnancy(3). While nausea and vomiting during pregnancy is seen in 50–90% of pregnancies(4, 5), hyperemesis gravidarum occurs only in 0.3–2% of pregnancies(2,5-7). A genetic basis to hyperemesis gravidarum has been suggested due to an apparent familial link(8), and there is a higher prevalence in certain ethnic groups(9).

Severe hyperemesis gravidarum (SHG) is usually defined as the presence of at least one of the following features: ketonuria, increased blood urea, increased haematocrit, and abnormal electrolytes(10). Other authors identify SHG by the presence of intractable vomiting during
pregnancy associated with dehydration, electrolyte and/or metabolic disturbances, as well as a weight loss ≥5%(11, 12). Some women require enteral (via a nasogastric tube) or total intravenous nutrition(13). SHG is associated with considerable maternal stress and may lead to post-traumatic stress disorder and other psychological effects(14, 15). Previous studies have assessed a number of maternal factors potentially associated with SHG, but no associations were found with human chorionic gonadotrophin, estrogen, thyroid hormones, leptin, or immunological components(16, 17).

There have been conflicting reports on the short-term effects of SHG in the offspring, which include premature birth, reduced birth weight, and increased hospital stay(7,18). Exposure to SHG in utero has also been linked to an increased risk of psychological and behavioral impairments later in life(19). However, there are no data on possible long-term effects on offspring metabolism. We hypothesize that children born to mothers with SHG are exposed to nutritional stress in utero during early gestation, leading to programmed metabolic changes in post-natal life. Hence, we aimed to assess whether SHG affects glucose homeostasis and body composition in the offspring in childhood.

**Materials and methods**

**Ethics approval**

Ethics approval for this study was provided by the Northern Y Regional Ethics Committee (Ministry of Health, New Zealand). Written informed consent was obtained from parents or guardians, as well as verbal or written consent from each child as was appropriate to their age.

**Participants**

Healthy, developmentally normal pre-pubertal children aged 4–11 years were recruited for this study in June–November 2011. Potential participants were the offspring of pregnant women admitted to the National Women’s Hospital (Auckland, New Zealand) with SHG and electrolyte abnormalities (including low serum bicarbonate (with or without acidosis) and hyponatremia), identified from a database. All potential participants from the database that met our study criteria were invited to participate. Control children were born to mothers without hyperemesis gravidarum or other pregnancy-associated complications. These children were recruited via study participants, so that all participants were approximately matched for age and socio-economic status.

All recruited children were naturally conceived, born at term (37–41 weeks gestation), from singleton pregnancies, and of birth weight appropriate-for-gestational-age (birth weight -2 to 2 standard deviation scores (SDS)(20)). Exclusion criteria included signs of puberty (Tanner stage 2
breast development in girls and testicular volume >3 ml in boys or evidence of adrenarche), genetic syndromes, receiving medication that could affect insulin sensitivity, as well as having a first-degree relative or grandparent with diabetes, the metabolic syndrome, or any of its features other than central adiposity. Children were also excluded if born to mothers with gestational diabetes, pre-eclampsia, gestational or pre-existing hypertension, thyroid dysfunction, chronic illnesses, or maternal drug use during pregnancy (including tobacco and alcohol).

Clinical assessments
All children were assessed at the Maurice & Agnes Paykel Clinical Research Unit (Liggins Institute, University of Auckland). Data on each child were collected during a single visit to the clinic. A number of neonatal parameters were recorded, including birth weight, and gestational age. Birth weight data were transformed into SDS(20).

Primary outcome
Insulin sensitivity was assessed using a 90-minute modified frequently sampled intravenous glucose test (FSIGT), modified with insulin, and analysed using Bergman’s minimal model software(21). Tests were performed between 7:00 and 8:30 am, following a fasting period of at least 10 hours. Three baseline samples were drawn at -20, -10, and 0 minutes. A 25% dextrose infusion (at 0.3 g/kg) started at 0 minute and lasted for one minute. Blood samples were drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19 minutes. Insulin (0.015 units/kg) was then intravenously administered as a bolus at 20 minutes, and further samples were drawn at 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 60, 70, 80, and 90 minutes. Note that no episodes of hypoglycaemia (blood glucose concentration <4 mmol/l) were recorded in any of the participants throughout the study.

Secondary outcomes
Children’s heights were measured using a Harpenden stadiometer. Weight and body composition data were obtained using whole-body dual-energy X-ray absorptiometry (DEXA, Lunar Prodigy 2000, General Electric, Madison, WI, USA), specifically total body fat and abdominal adiposity (represented by the android fat to gynoid fat ratio). Height SDS were derived from Tanner/Whitehouse reference data(22), and weight and body mass index (BMI) SDS according to British 1990 standards(20, 23). Parental weights and heights were measured by the investigators in 95% of mothers and 80% of fathers, with the remaining measurements reported. Mean parental BMI was calculated as the average of maternal and paternal BMI. Mid-parental height was calculated using standard formulae(24). Ethnicity was recorded by self-report using a prioritised system, such that if multiple ethnicities were selected, the patient was assigned to a single category, following a hierarchical system of classification(25).
At the start of the FSIGT, baseline blood samples were drawn to measure serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), insulin-like growth factor I (IGF-I), IGF-II, IGF binding protein 1 (IGFBP-1), IGFBP-3, cortisol, leptin, adiponectin, androstenedione, dehydroepiandrosterone sulphate (DHEAS), glucose, and insulin concentrations. Secondary outcomes from the FSIGT included acute insulin release (insulin secretory capacity), glucose effectiveness (glucose-mediated glucose uptake), and disposition index (ability of β-cells to compensate for insulin resistance).

**Assays**
Glucose concentrations were measured on a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) by enzymatic colorimetric assay (Roche, Mannheim, Germany), with an inter-assay coefficient of variation (CV) of 2.1%. Insulin concentrations were measured using an Abbott AxSYM system (Abbott Laboratories, Abbott Park, IL, USA) by microparticle enzyme immunoassay, with a CV of 5.7%. HDL-C, LDL-C and total cholesterol concentrations were measured using a Hitachi 902 autoanalyser, with CV of 11.4, 10.1, and 8.9% respectively. Commercially available ELISA kits E20, E30, E01, E05, E03A, E07, and E09 (Mediagnost, Reutlingen, Germany) were used for quantitative determination of serum IGF-I, IGF-II, IGFBP-1, IGFBP-3, leptin, and adiponectin concentrations, respectively; assay sensitivities were 0.09, 0.02, 0.2, 0.1, 1.0, and 0.6 ng/ml, with CV of 3.1, 5.0, 9.4, 9.6, 6.7, and 3.0%, respectively. Cortisol, DHEA, and androstenedione concentrations were measured using Finnigan TSQ Quantum Ultra AM triple quadrupole mass spectrometer controlled by Finnigan Xcaliber software (Thermo Electron Corporation, San Jose, CA, USA), with mean CV of 5.8, 18.4, and 8.2%, respectively.

**Power and sample size calculation**
Power calculation was carried out a priori on the primary outcome (i.e. insulin sensitivity) based on previous data on healthy pre-pubertal children obtained via an identical FSIGT protocol(26). This gave a sample size of 36 participants to detect an insulin sensitivity index difference of $3.6 \times 10^{-4} \text{min}^{-1} \text{ (mU/l)}$ between two groups (approximately 30%) with 90% power at 5% level of significance, assuming a standard deviation of $4.4 \times 10^{-4} \text{min}^{-1} \text{ (mU/l)}$.

**Statistical Analysis**
Sex ratio and ethnic composition data in both groups were compared with Fisher’s exact tests in Minitab v.16 (Pennsylvania State University, State College, PA, USA). Random effects mixed models were used to compare the primary and secondary outcomes between SHG children and the controls. Important confounding factors were adjusted for in the analyses, including ethnicity, birth weight SDS, birth order, age, and gender. Other factors were controlled for as required, depending
on the outcome response of interest: for lipids, hormones, and outcomes associated with glucose homeostasis – BMI SDS were included; and for anthropometric data – the appropriate parental factor (i.e. mean parental BMI or mid-parental height). Multivariate analyses were performed using SAS v.9.3 (SAS Institute Inc. Cary NC, USA). Parameters associated with glucose homeostasis were log-transformed to approximate normality. All statistical tests were two-tailed and maintained at a 5% significance level. Age data are presented as means ± standard deviation. Outcome data are presented as model-adjusted means (estimated marginal means adjusted for the confounding factors in the models), with associated 95% confidence intervals.

Results

A total of 300 pregnancies met our study criteria and were invited to participate. Only the first 40 respondents were enrolled, as this figure equated to the required sample size (36 participants) plus an additional 10% to account for study exclusion or failure. Subsequently, four children were excluded due to chronic illness (n=1), having a sibling with type 1 diabetes mellitus (n=1), severe attention deficit-hyperactivity disorder (n=1), or withdrawal at clinical assessment due to fear of injections (n=1).

As a result, 36 SHG children (18 boys) were included in the study. SHG participants and non-participants were of similar birth weight (p=0.29), gestational age (p=0.10), and sex ratio (p=0.72), but participants were slightly younger (8.6 vs 9.2 years of age; p=0.020). Mothers of all SHG children were admitted to hospital due to SHG in the first trimester of gestation at least once, but some mothers as many as 4 times. Median duration of hospitalization was 5 days (range 1–31 days). In 32 of the 36 pregnancies, hyperemesis persisted beyond 16 weeks gestation and continued throughout pregnancy.

Among controls, 47 children volunteered to participate, but five failed to meet the inclusion criteria due to: being born small-for-gestational-age; parental diagnosis with type 2 diabetes during study screening; early signs of puberty; conception following in vitro fertilization; or maternal gestational diabetes. Thus, 42 controls (27 boys) were included in the study.

Overall, participants were aged 8.8 ± 1.9 years, and most (79%) were of New Zealand European ethnicity. SHG and Control children were of similar age (p=0.16), gestational age (p=0.70), birth weight (p=0.10), sex ratio (p=0.25), and ethnic composition (p=0.41) (Table 1).
Table 4.1. Baseline characteristics of children born from mothers who suffered from severe hyperemesis gravidarum (SHG) versus those from control pregnancies.
Data are mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>SHG</th>
<th>Control</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>8.6 ± 1.4</td>
<td>9.0 ± 2.1</td>
<td>.16</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>39.3 ± 0.8</td>
<td>39.3 ± 0.7</td>
<td>.70</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.41 ± 0.33</td>
<td>3.54 ± 0.38</td>
<td>.10</td>
</tr>
<tr>
<td>Sex ratio (boys)</td>
<td>50%</td>
<td>64%</td>
<td>.25</td>
</tr>
<tr>
<td>Ethnicity (New Zealand European)</td>
<td>74%</td>
<td>83%</td>
<td>.41</td>
</tr>
</tbody>
</table>

Table 4.2. Secondary outcomes among children born from mothers who suffered from severe hyperemesis gravidarum (SHG) versus those from control pregnancies.
Secondary outcomes among children born from mothers who suffered from severe hyperemesis gravidarum (SHG) versus those from control pregnancies.

<table>
<thead>
<tr>
<th></th>
<th>Hyperemesis</th>
<th>Control</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.61 (0.31–0.91)</td>
<td>0.36 (0.04–0.68)</td>
<td>.09</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>0.22 (−0.19 to 0.64)</td>
<td>0.40 (−0.01 to 0.82)</td>
<td>.47</td>
</tr>
<tr>
<td>Android fat to gynoid fat ratio</td>
<td>0.60 (0.53–0.68)</td>
<td>0.64 (0.56–0.72)</td>
<td>.48</td>
</tr>
<tr>
<td>Total body fat, %</td>
<td>21.9 (18.8–25.0)</td>
<td>21.5 (18.3–24.6)</td>
<td>.82</td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.90 (3.62–4.18)</td>
<td>3.89 (3.60–4.19)</td>
<td>.96</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.27 (1.15–1.40)</td>
<td>1.35 (1.21–1.48)</td>
<td>.35</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.34 (2.08–2.60)</td>
<td>2.15 (1.87–2.43)</td>
<td>.25</td>
</tr>
<tr>
<td>Total cholesterol to HDL-C ratio</td>
<td>3.24 (2.92–3.57)</td>
<td>3.04 (2.69–3.38)</td>
<td>.32</td>
</tr>
<tr>
<td>Glucose homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose effectiveness, $10^{-2}$/min</td>
<td>2.80 (2.16–3.44)</td>
<td>2.75 (2.08–3.41)</td>
<td>.90</td>
</tr>
<tr>
<td>Disposition index</td>
<td>3095 (2430–3941)</td>
<td>2898 (2251–3731)</td>
<td>.67</td>
</tr>
<tr>
<td>Fasting insulin, mIU/L</td>
<td>6.88 (5.56–8.50)</td>
<td>5.04 (4.04–6.28)</td>
<td>.024</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>4.76 (4.62–5.88)</td>
<td>4.71 (4.58–4.84)</td>
<td>.55</td>
</tr>
<tr>
<td>Hormone concentrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline cortisol, nmol/L</td>
<td>256 (224–292)</td>
<td>210 (184–241)</td>
<td>.021</td>
</tr>
<tr>
<td>IGF-1, ng/mL</td>
<td>181 (156–207)</td>
<td>183 (157–209)</td>
<td>.88</td>
</tr>
<tr>
<td>IGF-II, ng/mL</td>
<td>651 (610–693)</td>
<td>668 (624–711)</td>
<td>.54</td>
</tr>
<tr>
<td>IGFBP-1, ng/mL</td>
<td>11.8 (7.9–15.6)</td>
<td>19.0 (15.1–22.8)</td>
<td>.004</td>
</tr>
<tr>
<td>IGFBP-3, ng/mL</td>
<td>2955 (2657–3254)</td>
<td>3435 (3122–3749)</td>
<td>.014</td>
</tr>
</tbody>
</table>
Figure 4.1. Insulin sensitivity and other parameters of glucose homeostasis in children born from mothers who suffered from severe hyperemesis gravidarum (SHG; black bars) versus those from control pregnancies (gray bars). Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models.

Insulin sensitivity and other parameters of glucose homeostasis

Insulin sensitivity in SHG children was 20% lower than controls' (8.49 vs 10.60 x10^{-4}·min^{-1}·(mU/l); p=0.014) (Figure 1). Although not statistically significant, there was indication of a 30% increase in acute insulin release among SHG children (433 vs 333 mU/l; p=0.14) (Figure 1). SHG children displayed greater area under the curve for both glucose (9886 vs 9429 mg/dl; p=0.016) and insulin (1752 vs 1453 mIU/l; p=0.037), and tended to display greater peak glucose concentrations (258 vs 240 mg/dl; p=0.076) (Figure 1). SHG children also had higher fasting insulin concentrations (p=0.024), but glucose effectiveness, disposition index, and fasting glucose concentrations were similar between groups (Table 2).

Other secondary outcomes

Baseline cortisol concentrations were 22% higher in SHG children than in controls (p=0.021). Further, SHG children had lower IGFBP-1 (-38%; p=0.004) and lower IGFBP-3 (-14%; p=0.014) concentrations compared to controls (Table 2). However, SHG and Control children were anthropometrically similar, as were their lipid profiles (Table 2). There were also no differences in other hormone concentrations measured, including leptin (p=0.39), adiponectin (p=0.46), DHEAS (p=0.96), or androstenedione (p=0.63) concentrations (data not shown).
Discussion

This study provides the first evidence of long-term adverse metabolic outcomes in the offspring of mothers who suffered from severe hyperemesis gravidarum, manifested as lower insulin sensitivity. Importantly, the magnitude of this reduction (20%) is similar to that seen with medications used to treat diabetes in adults, such as metformin(27). There have been no studies examining the long-term consequences of reduced insulin sensitivity in childhood. However, longitudinal studies in adults show that a reduction in insulin sensitivity is associated with increased risk of developing type 2 diabetes mellitus, hypertension, coronary heart disease, stroke, and cancer many years later(28, 29).

Our study shows that SHG children appear to have an isolated abnormality of reduced insulin sensitivity, so that overall glucose disposal seems normal as indicated by the disposition index. The higher fasting insulin concentrations in SHG children were associated with lower serum IGFBP-1 concentrations. Insulin resistance leads to a compensatory increase in portal insulin secretion, which suppresses IGFBP-1 concentrations(30). Hyperinsulinemia and low IGFBP-1 are also associated with increased likelihood of developing cardiovascular disease(31).

It is well-established that adverse events early in life are associated with long-term changes that may lead to later metabolic and cardiovascular disease(32, 33). Dutch Famine studies have shown an increased risk of glucose intolerance in adulthood among those exposed to famine in any stage of gestation(34, 35). In addition, the Dutch Famine cohort displays an atherogenic lipid profile and higher risk of coronary artery disease and breast cancer in adult life(34). In our study, SHG led to hospitalization in early gestation in all pregnancies, but vomiting continued throughout pregnancy in most cases. Thus, we speculate that in our SHG cohort nutritional compromise would have been greater in the first trimester of pregnancy, with some amelioration in later pregnancy leading to birth weight appropriate-for-gestational-age. There are similarities between our cohort and the Dutch Famine cohort who were exposed to famine in early gestation, who were also born of normal birth weight(36). Importantly, both groups show adverse changes in glucose regulation manifest as hyperinsulinemia (during an oral glucose tolerance test) among Dutch Famine survivors(36) and reduced insulin sensitivity in SHG children. These observations highlight the importance of early gestation in the development programming of metabolism.

The mechanisms underpinning the changes observed in our SHG cohort are unknown, but possibly similar to those affecting Dutch Famine survivors exposed to undernutrition in early gestation. Interestingly, periconceptional exposure to the Dutch Famine was associated with hypomethylation of the igf2 gene in later adult life(37). Thus, Heijmans et al.’s study raises the possibility that
epigenetic modification of gene expression may be a possible mechanism programming metabolism in our SHG cohort.

We also observed that SHG children had higher baseline cortisol concentrations than controls. This has been shown to occur in child and adult cohorts that faced a suboptimal intra-uterine environment such as those born of low birth weight(38, 39). Adults who were born of low birth weight had elevated baseline serum cortisol concentrations, which were associated with insulin resistance(40). Although early morning cortisol is commonly used as a measure of cortisol secretion, it is less precise than assessment of circadian cortisol rhythm. Nonetheless, our findings suggest an alternative explanation for the observed reduction in insulin sensitivity in SHG children may be programming of the hypothalamus-pituitary-adrenal axis due to an adverse environment in utero. Importantly, this programming may occur in the absence of a reduction in birth weight. For example, an unbalanced maternal diet (high-meat and low-carbohydrate) in human pregnancy is associated with epigenetic changes in genes controlling glucocorticoid action in subjects born at term and of normal birth weight(41, 42). Further, these epigenetic changes were noted in mid-adult life and were associated with increased adiposity and higher blood pressure(41, 42).

In conclusion, our study shows that children born to mothers who suffered from SHG have lower insulin sensitivity than those from control pregnancies. These children may be at an increased risk of developing insulin resistance and associated diseases later in life, such as type 2 diabetes. Our findings need to be corroborated in larger studies, and SHG cohorts should be assessed in adulthood to adequately identify any associated long-term health risks. Further, future studies are necessary to establish the underlying causes and mechanisms of the observed reduction in insulin sensitivity in SHG children.

Acknowledgements

Thanks to Dr Yannan Jiang (Department of Statistics, University of Auckland) for valuable statistical input. Eric Thorstensen (Liggins Institute, University of Auckland) and his group kindly assisted with laboratory work. We also acknowledge the Paykel Trust for long-term support of the Maurice & Agnes Paykel Clinical Research Unit at the Liggins Institute, University of Auckland.

References

Chapter 5. First-born children have reduced insulin sensitivity and higher daytime blood pressure compared to later-born children

Preface

The following is the unaltered version of an article published in the Journal of Clinical Endocrinology and Metabolism.

- **Authors:** Ayyavoo A, Savage T, Derraik JGB, Hofman PL, Cutfield WS.
- **Title:** First-born children have reduced insulin sensitivity and higher daytime blood pressure compared to later-born children.
- **Journal:** Journal of Clinical Endocrinology and Metabolism
- **Year of publication:** 2013
- **Volume:** 98
- **Issue:** 3
- **Pages:** 1248–1253
- **Impact factor:** 6.43
- **Journal’s aims and scope:** “The Journal of Clinical Endocrinology & Metabolism is the world's leading peer-reviewed journal for endocrine clinical research and cutting edge clinical practice reviews. Each issue provides the latest in-depth coverage of new developments enhancing our understanding, diagnosis and treatment of endocrine and metabolic disorders. Regular features of special interest to endocrine consultants include clinical trials, clinical reviews, clinical practice guidelines, case seminars, and controversies in clinical endocrinology, as well as original reports of the most important advances in patient-oriented endocrine and metabolic research.”
Abstract

**Background:** Evidence suggests that first-born children and adults are phenotypically different to later-borns. Therefore, we aimed to assess whether birth order would be associated with changes in metabolism in childhood.

**Methods:** We studied 85 healthy pre-pubertal children aged 4–11 years, born 38–40 weeks gestation, and of birth weight appropriate-for-gestational-age: 32 first-born and 53 later-born children. Clinical assessments included measurement of children’s: height, weight, fasting lipid and hormonal profiles, DEXA-derived body composition. Children also underwent 24-hour ambulatory blood pressure monitoring, and frequently-sampled intravenous glucose tests with Bergman’s minimal model. Data are adjusted means and 95% confidence intervals.

**Results:** First-born children were approximately 3 cm taller (Height SDS 0.88 vs 0.39; p=0.009) and were slimmer (BMI SDS -0.05 vs 0.39; p=0.048) than later-borns. Consistent with their taller stature, first-borns also had a 27% increase in IGF-I concentrations (227 vs 173; p=0.002). Insulin sensitivity was reduced by 21% among first-borns compared to later-borns (8.4 vs 10.6 x10^-4/min/[mU/l]; p=0.019). Further, 24-hour ambulatory blood pressure monitoring showed that first-borns had higher daytime systolic (+5 mmHg; p=0.032) and diastolic (+4 mmHg; p=0.029) blood pressure. Blood lipids were unaffected by birth order.

**Conclusions:** While first-borns were taller and slimmer, these children had reduced insulin sensitivity and increased daytime blood pressure compared to later-borns. Thus, first-borns may be at a greater risk of metabolic and cardiovascular diseases in adult life. This finding may have important public health implications, in light of a worldwide trend towards smaller families.

Introduction

There has been a steady reduction in fertility worldwide (1), with a more severe decline in birth rates observed in many European and Asian countries (2). Several reasons may account for the reduction in birth rates, including government policies such as the one-child policy in mainland China (3), greater family planning, personal choice, and economical constraints. Irrespective of underlying causes, there has been a large increase in the number of single-child families (1), and a consequent increase in the proportion of first-born children.

A number of studies suggest that birth order affects the offspring in the long-term. There is evidence of phenotypic differences in childhood (4-6) and in adulthood (7-9). In addition, previous studies have shown that first-borns are at a greater risk of developing type 1 diabetes (10), as well
as of having increased blood pressure (11). As a result, the increasing proportion of first-born children could affect the incidence of cardiovascular and metabolic diseases in a given population.

Alterations in the fetal environment (including changes in fetal nutrition) are associated with an increased risk of disease in adulthood (12). There is ongoing research investigating possible causes for the global epidemic of type 2 diabetes, including large population genetic studies which have had limited success (13, 14). The prevalence of hypertension is also increasing in industrialised countries, without clear explanation (15). As the aetiology of type 2 diabetes and hypertension are multi-factorial, we hypothesised that birth order may be a contributing factor. Thus, we aimed to assess whether birth order adversely affects glucose metabolism in childhood.

Materials and methods

Ethics approval
Ethics approval for this study was provided by the Northern Y Regional Ethics Committee (Ministry of Health, New Zealand). Written informed consent was obtained from parents or guardians, as well as verbal or written consent from each child as was appropriate to their age.

Subjects and recruitment
Only healthy, developmentally normal, pre-pubertal children aged 4–10 years, born 38–40 weeks gestation were recruited for this study in 2010–2011. Potential participants were identified from the obstetrics database at the National Women’s Health, Auckland City Hospital (Auckland, New Zealand). All children were naturally conceived, born of singleton pregnancies, and of birth weight appropriate-for-gestational-age (birth weight >-2 and <2 standard deviation scores (SDS)). Exclusion criteria included signs of puberty (Tanner stage 2 breast development in girls and testicular volume >3 ml in boys or evidence of adrenarche), receiving medication that could affect insulin sensitivity, as well as having a first-degree relative or grandparent with pre-diagnosed diabetes, the metabolic syndrome or any of its features other than central adiposity. Children were also excluded if born to mothers with gestational diabetes, pre-eclampsia, gestational or pre-existing hypertension, chronic illnesses, or prolonged maternal drug use (including tobacco).

Clinical assessments
All clinical assessments were carried out at the Maurice & Agnes Paykel Clinical Research Unit (Liggins Institute, University of Auckland). Data on each child were collected during a single visit to the clinic. A number of neonatal parameters were recorded, including birth weight, gestational age, and maternal age at time of delivery. Birth weight data were transformed into SDS (16).
**Primary outcome**

The primary outcome was insulin sensitivity, which was assessed using a 90-minute modified frequently sampled intravenous glucose test (FSIGT), modified with insulin, and analysed using Bergman’s minimal model software (17). Three baseline samples were drawn at -20, -10, 0 minutes. A 25% dextrose infusion (at 0.3 g/kg) started at 0 minute and lasted for one minute. Blood samples were drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 19 minutes. Insulin (0.015 units/kg) was then intravenously administered as a bolus at 20 minutes, and further samples were drawn at 22, 23, 24, 25, 27, 30, 35, 40, 45, 50, 60, 70, 80, and 90 minutes. Values derived from the minimal model included the insulin sensitivity index and acute insulin release (measure of insulin secretory capacity).

**Secondary outcome**

Children’s heights were measured using a Harpenden’s stadiometer, while weight and body composition data were obtained using whole-body dual-energy X-ray absorptiometry (DEXA, Lunar Prodigy 2000, General Electric, Madison, WI, USA). Height SDS were derived from Tanner/Whitehouse reference data (18) and weight and body mass index (BMI) SDS according to British 1990 standards (16, 19). Maternal and parental weight and height were recorded for all participants. Mean parental BMI was calculated as the average of maternal and paternal BMI. Mid-parental height was calculated using standard formulas (20). Ethnicity was recorded by self-report using a prioritised system, such that if multiple ethnicities were selected, the patient was assigned to a single category, following a hierarchical system of classification (21).

Following an overnight fast, baseline blood samples were drawn to measure serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, insulin like growth factor I (IGF-I), IGF-II, IGF binding protein 1 (IGFBP-1), IGFBP-3, leptin, adiponectin, androstenedione, dehydroepiandrosterone sulphate (DHEAS), cortisol, glucose, and insulin.

24-hour ambulatory blood pressure was assessed following the clinical visit, when participants were fitted with a Spacelabs 90217 monitor (Spacelabs Medical Inc., Redmond, WA, USA) on the non-dominant arm. Measurements were performed every 20 minutes from 0700–2200, and every 30 minutes from 2200–0700. Only profiles with more than 14 daytime and 7 nocturnal readings over a 24-hour period were included for analysis (22).

**Assays**

Glucose concentrations were measured on a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) by enzymatic colorimetric assay (Roche, Mannheim, Germany), with
an inter-assay coefficient of variation (CV) of 2.1%. Insulin concentrations were measured using an Abbott AxSYM system (Abbott Laboratories, Abbott Park, IL, USA) by microparticle enzyme immunoassay, with a CV of 5.7%. Total cholesterol, HDL-C, and LDL-C concentrations were measured using a Hitachi 902 autoanalyser, with CV of 8.9%, 11.4, and 10.1, respectively. Commercially available ELISA kits E20, E30, E01, E05, E03A, E07, and E09 (Mediagnost, Reutlingen, Germany) were used for quantitative determination of serum IGF-I, IGF-II, IGFBP-1, IGFBP-3, leptin, and adiponectin respectively; assay sensitivities were 0.09, 0.02, 0.2, 0.1, 1.0, and 0.6 ng/ml, with CV of 3.1, 5.0, 9.4, 9.6, 6.7, and 3.0%, respectively. Cortisol, DHEAS, and androstenedione were measured using Finnigan TSQ Quantum Ultra AM triple quadrupole mass spectrometer controlled by Finnigan Xcaliber software (Thermo Electron Corporation, San Jose, CA, USA); mean CV were 5.8% for cortisol, 18.4% for DHEAS, and 8.2% for androstenedione.

**Statistical analysis**

Children were separated into two groups according to their birth order: first-borns and later-borns. Comparisons between birth order groups were carried out using linear mixed models in SAS v.9.2 (SAS Institute, Cary, NC, USA). All models accounted for important confounding factors, mainly gender, ethnicity, birth weight SDS, gestational age, and maternal age. All models included family identification number as a random factor, to account for the clustering of siblings. Other factors were controlled for as required, depending on the outcome response of interest: for lipids, hormones, and outcomes associated with glucose homeostasis – age and BMISDS were included; for anthropometric data – age and the appropriate parental factor (i.e. mean parental BMI or mid-parental height); and for blood pressure parameters – height and total body fat percentage. Where appropriate, data were log-transformed to approximate a normal distribution. Age data are presented as means ± standard deviations, while other data are means and 95% confidence intervals adjusted for the confounders in multivariate models.

**Results**

In total, 85 children aged 8.7 ± 1.9 years (range 4–11) took part in the study, including 32 first-borns (69% boys) and 53 later-borns (57% boys). Among the latter group, there were 35 second-borns, 8 third-borns, and 8 fourth-born children.

First-borns were lighter at birth than later-borns (p=0.009), despite similar gestational ages (p=0.75; Table 1). There were no differences in biological maturity between birth order groups, as all children were pre-pubertal and had similar DHEAS (p=0.18) and androstenedione (p=0.28) concentrations (data not shown).
First-borns were taller (p=0.009) and had lower BMI (p=0.048) than later-borns (Table 1), despite correction for parental variables (height and BMI) in the model. There was also indication of an effect on bone mineral content, which appeared to be 9% higher among first-born children (p=0.059; Table 1). In keeping with the differences in stature, IGF-I concentrations were also increased in first-borns compared to later-borns (p=0.002; Table 2).

Assessment of glucose homeostasis showed a 21% reduction in insulin sensitivity among first-borns compared to later-borns (8.4 vs 10.6 x 10^{-4} /min/[mU/l]; p=0.019) (Figure 1). However, there was no significant increase in the compensatory acute insulin response (350 vs 284 mU/l; p=0.16) (Figure 1).

24-hour ambulatory blood pressure monitoring also revealed that first-borns had higher daytime systolic (p=0.032) and diastolic (p=0.029) blood pressure than later-borns (Table 2). Lipid profiles were unaffected by birth order (Table 2), and both groups had similar fasting serum leptin (3.54 vs 3.15 ng/ml; p=0.46) and adiponectin concentrations (10316 vs 9616 ng/ml; p=0.50).
Figure 5.1. Insulin sensitivity (primary outcome) and acute insulin release among first-born (black bars) and later-born (grey bars) children.
Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models.

Table 5.2. Secondary outcomes on first-born and later-born children.
Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models.

<table>
<thead>
<tr>
<th></th>
<th>First-Borns</th>
<th>Later-Borns</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>32</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>24-h ambulatory BP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime systolic BP, mm Hg</td>
<td>113.6 (107.3–118.0)</td>
<td>107.7 (103.3–112.1)</td>
<td>.032</td>
</tr>
<tr>
<td>Nocturnal systolic BP, mm Hg</td>
<td>97.6 (91.6–103.6)</td>
<td>96.6 (91.6–101.6)</td>
<td>.66</td>
</tr>
<tr>
<td>Daytime diastolic BP, mm Hg</td>
<td>69.2 (65.2–73.2)</td>
<td>65.3 (62.1–68.6)</td>
<td>.029</td>
</tr>
<tr>
<td>Nocturnal diastolic BP, mm Hg</td>
<td>55.8 (51.4–60.2)</td>
<td>54.3 (50.7–58.0)</td>
<td>.38</td>
</tr>
<tr>
<td>Diastolic dip, %</td>
<td>17.2 (11.9–22.5)</td>
<td>15.8 (11.5–20.1)</td>
<td>.51</td>
</tr>
<tr>
<td>Systolic dip, %</td>
<td>12.2 (8.4–16.1)</td>
<td>9.9 (6.8–13.0)</td>
<td>.14</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.88 (3.44–4.31)</td>
<td>3.83 (3.50–4.16)</td>
<td>.80</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.34 (2.00–2.69)</td>
<td>2.22 (1.96–2.49)</td>
<td>.44</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.30 (1.15–1.45)</td>
<td>1.30 (1.19–1.42)</td>
<td>.93</td>
</tr>
<tr>
<td>Total cholesterol: HDL-C</td>
<td>3.05 (2.71–3.42)</td>
<td>2.95 (2.69–3.23)</td>
<td>.53</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>227 (199–256)</td>
<td>173 (151–194)</td>
<td>.0002</td>
</tr>
<tr>
<td>IGFBP-1, ng/mL</td>
<td>690 (637–744)</td>
<td>668 (628–709)</td>
<td>.39</td>
</tr>
<tr>
<td>IGFBP-3, ng/mL</td>
<td>9.79 (6.70–14.31)</td>
<td>13.45 (10.10–17.90)</td>
<td>.085</td>
</tr>
<tr>
<td>Glucose homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>84.3 (81.6–87.0)</td>
<td>85.3 (83.3–87.4)</td>
<td>.32</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>5.49 (4.17–7.22)</td>
<td>5.93 (4.81–7.30)</td>
<td>.53</td>
</tr>
</tbody>
</table>
**Discussion**

This study shows that first-born children have reduced insulin sensitivity and higher daytime blood pressure compared to later-borns. The observed 21% reduction in insulin sensitivity has not been described previously, and is comparable to that seen as a result of obesity in childhood (23, 24). Decreased insulin sensitivity is known to precede a number of important adult diseases years before they occur, such as type 2 diabetes mellitus, coronary artery disease, stroke, hypertension, and cancer (25, 26).

Our first-born cohort also had higher daytime systolic and diastolic ambulatory blood pressure compared to later-born children. Previous studies have described increased blood pressure in first-compared to later-borns in childhood (11, 27) and adolescence (6), but they utilized single blood pressure measurements that are less precise. Elevations in blood pressure in childhood often track into adulthood (28, 29), and our findings suggest that first-born children may be at a greater risk of later hypertension. Higher blood pressure and the observed reduction in insulin sensitivity indicate that first-borns are at a greater risk of developing the metabolic syndrome (30). The latter comprises a combination of insulin resistance with or without glucose intolerance with any two of the following: central obesity, high blood pressure, dyslipidemia, pro-inflammatory state, or prothrombotic state (31).

We also observed that first-borns were taller and slimmer than later-borns, with increased IGF-I concentrations. Higher IGF-I levels are associated with lower birth weight, and were directly related to present height and systolic blood pressures in a cohort of 444 prepubertal children studied by Fall et al (32). However, the observed differences among first-born children in our study were independent of birth weight, which was controlled for in all our analyses.

There is no obvious mechanism that could explain the combination of adverse metabolic but positive auxological changes seen in first-born children. There is some evidence that implantation and placentation during first pregnancy leads to physical changes in the uterus (33-35). These changes become more evident in second and subsequent pregnancies, and are associated with more efficient placental invasion of the uterine wall and consequent improvement in nutrient flow to the fetus (33-35). Thus, first-borns may be exposed to a degree of nutrient restriction in utero compared to later-borns (36), which may lead to alterations in glucose metabolism and blood pressure later in life.

Indeed, it is thought that this disparity in placentation between first and later pregnancies is partially responsible with an approximate 200 g increase in birth weight among later-borns (37). This
difference in birth weight is similar to the 250 g that was observed in our study. Lower birth weight is well recognized to be associated with reduced insulin sensitivity in children (38, 39) and adults (40-43). However, our multivariate models accounted for birth weight and gestational age among other important confounders, suggesting that the reduction in insulin sensitivity among first-borns with other factors.

In conclusion, first-born children were taller and slimmer than later-borns. However, first-borns also had lower insulin sensitivity and higher daytime blood pressure, which suggest that first-borns are likely to be at a greater risk of metabolic and cardiovascular diseases later in life compared to their siblings. These findings have important public health implications, in view of a growing trend towards smaller families and the consequent increase in the proportion of first-borns in the population. Thus, we speculate that the increase over the past decades in the proportion of first-borns worldwide may be a contributing factor to the global increase in prevalence of type 2 diabetes and hypertension. It should be highlighted that pre-pubertal children were studied to remove the confounding effects of puberty and adult life-style factors (e.g. smoking, alcohol, prescription drugs) on insulin sensitivity. However, large adult studies should be carried out to corroborate our findings. Further, research is needed to elucidate underpinning mechanisms of altered growth and metabolism in first-borns.

Acknowledgements

We acknowledge the Paykel Trust for long-term funding of the Maurice & Agnes Paykel Clinical Research Unit at the Liggins Institute, University of Auckland. We thank the National Research Centre for Growth and Development and the Australasian Paediatric Endocrine Group for financial support. We also thank Eric Thorstensen (Liggins Institute) and his group for assistance with laboratory work.

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Chapter 6. Discussion

6.1. Summary of study observations

Pre-pubertal children born post-term have several early features of the metabolic syndrome:

- 34% reduction in their insulin sensitivity in comparison with term children
- a compensatory increase in acute insulin response, but a reduction in glucose effectiveness
- more body fat and less fat-free mass
- central adiposity, with greater truncal fat and higher android to gynoid fat ratio
- lower nocturnal systolic blood pressure dipping, displaying a similar tendency for diastolic dipping
- higher leptin and lower adiponectin concentrations
- higher uric acid concentration
- less favourable lipid profile in post-term boys, with higher total cholesterol and LDL-C concentrations, and higher total cholesterol to HDL-C ratio

Pre-pubertal children born to mothers who experienced severe hyperemesis gravidarum also suffer long-term adverse health effects:

- a 20% reduction in insulin sensitivity compared to controls
- higher fasting insulin and lower IGFBP-1 concentrations
- baseline cortisol concentrations that were 22% higher

Birth order was shown to affect glucose homeostasis and growth in pre-pubertal children. As a result, first-born children:

- had insulin sensitivity that was 21% lower than that of later-borns
- had higher daytime systolic and diastolic blood pressure
- were approximately 3 cm taller and also slimmer than later-borns, with an associated increase in IGF-I concentrations

The above findings have been discussed in the respective manuscripts, and this discussion will focus primarily on possible underlying triggers/mechanisms.
6.2. Early life programming of metabolism

A suboptimal intra-uterine environment may programme long-term metabolism via a number of pathways\(^\text{289}\), including:

(a) Placental alterations can affect nutrient supply during critical stages of fetal development, having long-term effects on the number, proportion, and function of specific organ systems\(^\text{290-295}\).

(b) Epigenetic changes in genes responsible for growth and metabolism can affect specific enzymes, growth factors, transporters, and/or hormone receptors\(^\text{296-298}\).

(c) Changes in appetite and behavioural regulation, which may lead to long-term modification of appetite modulation, food preference, and physical activity in post-natal life\(^\text{289}\).

These pathways are part of a very complex system with numerous interactions, including the effects of glucocorticoids\(^\text{299}\) (Figure 6.1).

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**Figure 6.1. Suboptimal intra-uterine conditions and early metabolic programming.**
Adapted from Fowden et al.\(^\text{289}\) with permission from Elsevier. "Diagram illustrating the relationships between suboptimal intrauterine conditions, early life programming of endocrine systems and circulating hormone concentrations and the subsequent development of metabolic dysfunction. Endocrine systems in square boxes. Circulating hormones in ovals. Metabolic dysfunctions in ovoid boxes. (+) Positive effect; (-) negative effect. */+ Hormones that appear twice on the diagram."
6.2.1. Placenta and fetal nutrition

When the fetus faces a situation of prolonged reduction in oxygen supply or hypoglycaemia, the fetus maintains oxidation by the metabolism of glucose obtained from glycogenolysis and gluconeogenesis and by increasing the protein and subsequent amino acid breakdown\textsuperscript{300,301}. Due to the increase in protein and amino acid breakdown, net fetal protein synthesis and growth diminishes\textsuperscript{302}. So, in states of poor substrate availability, energy metabolism is maintained at the expense of growth. In animal models, restricted maternal nutrition led to preservation of brain growth at the expense of other organs such as pancreas, heart, muscles, and kidneys\textsuperscript{303}.

During a normal pregnancy, the small spiral arteries undergo a series of changes to develop the utero-placental circulation. Migration of the endovascular tropohoblast into the small spiral arteries converts them into distended vessels with low resistance and high conductance. This improves blood supply to the fetus in the last trimester, so that blood flow is 10-fold greater than in a non-pregnant uterus\textsuperscript{304}. An impaired invasion of the maternal decidua by fetal trophoblast cells is hypothesized as the reason for insufficient transformation of spiral arteries into vessels of low resistance in SGA babies\textsuperscript{304,305}. Hence, intra-uterine growth restriction is associated with varying degrees of placental insufficiency. Blood circulation is therefore uneven, with inefficient oxygen and nutrient exchange, so that there is reduced glucose and amino acid supply to the fetus\textsuperscript{306}. There are associated changes in the fetus and placenta, in order to overcome the defective nutritional supply. These changes can be observed even in uncomplicated pregnancies, and for example, non-primipara mothers have better trophoblast invasion and placentation compared to women who are pregnant for the first time\textsuperscript{166,307}.

The major determinant of growth \textit{in utero} is the maternal supply of nutrients to the fetus through the placenta. Poor maternal nutrition has consequently been linked to reduction in offspring birth weight via placental alterations\textsuperscript{308}. Therefore, nutritional insults during different stages of gestation may lead to adaptation in fetal and placental physiology. These adaptations may alter the structure and function of various tissues, potentially leading to metabolic diseases in later life\textsuperscript{31}. The link between placenta and developmental programming has been well-illustrated by Cetin \textit{et al.}\textsuperscript{309} (Figure 6.2).
Placental efficiency (evaluated on the fetal to placental weight ratio) is influenced by maternal nutrition, fetal oxygen supply, and uterine blood flow. Across the normal weight range, larger placentae support more fetal weight. Not surprisingly, SGA babies have smaller placentae and reduced placental-fetal ratio. Placental dysmaturity detected by ultrasound in growth-restricted babies can predict the occurrence of SGA births. The rate of apoptotic cells in the placenta is also higher in pregnancies complicated with intra-uterine growth restriction (IUGR) than in normal uncomplicated pregnancies. Similarly, an increase in apoptosis is observed in the placenta of post-term pregnancies, indicating placental degeneration. In addition, there are other placental changes in post-term pregnancies that are similar to those observed in those of SGA babies, including progressive degeneration of chorionic villi; diffuse calcifications; oedema; intervilli thrombosis; infarcts; perivillous deposits of fibrins; significant amount of necrosis and fibrinoid deposition; as well as...
smaller vascular diameters and increased vascular wall thickness that imply a decrease in perfusion surface\textsuperscript{319-321}.

6.2.2. Epigenetics

Epigenetics has been proposed as a mechanism underpinning programmed changes in individuals exposed to a sub-optimal environment \textit{in utero} or postnatally. Epigenetic changes are alterations in gene expression not caused by changes in DNA sequence, which may be associated with phenotypic changes in the offspring\textsuperscript{322}. As a result, chromosome without DNA alterations can result in a stably heritable phenotype\textsuperscript{323}.

Epigenetic changes may involve DNA methylation or histone modification. Histone modification has varied roles in biological processes, including gene regulation\textsuperscript{324}. DNA methylation happens in position 5 of the cytosine residue of the cytosine–guanine dinucleotide (CpG)\textsuperscript{325}. Methylation of CpG clusters results in transcriptional repression, while hypomethylation leads to transcriptional activity\textsuperscript{326}. The effects of CpG methylation are mediated through several histone modifications\textsuperscript{327}, and is illustrated in Figure 6.3.

Maternal nutrition and the availability of dietary methyl group donors during critical periods of development could influence DNA methylation and subsequent gene expression\textsuperscript{325,328}. During re-programming, the genome of the pre-implantation embryo is demethylated and cytosine methylation is re-established prior to re-implantation\textsuperscript{326}. These methylation patterns persist through fetal and post-natal life\textsuperscript{324}. The cells’ ability to change their behaviour as per the external and internal environmental cues is termed phenotypic plasticity and forms the basis of epigenetics\textsuperscript{329}.

Evidence for an effect of maternal diet on gene expression has been recently shown by the association of an unbalanced maternal diet with DNA methylation at the 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) promoters and the IGF-II differentially methylated regions (H-19 ICR)\textsuperscript{330}. Methylation at the 11β-HSD2, H-19 ICR, and glucocorticoid receptor is positively associated with adiposity and blood pressure in adulthood in the offspring\textsuperscript{330}.
Most of the mothers with hyperemesis gravidarum in our study had an unbalanced diet early in pregnancy, with many having it right through pregnancy. Thus, similar epigenetic modifications may underpin the metabolic changes in the offspring of mothers with severe hyperemesis gravidarum. In the case of post-terms, nutritional or physiological stress associated with prolonged gestation could lead to programmed changes in metabolism. Even if there are common genetic variants (polymorphism) leading to prolonged gestation and influencing metabolism, these effects could be amplified by epigenetic modification.
6.2.3. Appetite and behavioural regulation

It is recognized that intra-uterine and post-natal events can lead to programming of post-natal appetite, food preference, gut function, and physical activity\textsuperscript{331}. Such effects have been demonstrated in animal models. The offspring of undernourished Wistar rat dams were significantly less active at all ages\textsuperscript{332}. Although these effects were independent of post-natal nutrition, sedentary behaviour was exacerbated by a post-natal hypercaloric diet\textsuperscript{332}. Other rat study also showed that locomotor activity and feeding behaviour are influenced by perinatal programming\textsuperscript{333}.

Animal studies have shown that growth patterns \textit{in utero} are associated with alterations in post-natal function of a wide range of hormonal systems, including those regulating appetite and food intake such as leptin\textsuperscript{289,334,335}. As a result, the offspring of under-nourished rat dams have increased food intake in early postnatal life, which is amplified in later life by postnatal hypercaloric nutrition\textsuperscript{336}. The authors' hypothesized that hyperinsulinemia and hyperleptinemia resulting from altered fetal development would program later hyperphagia, obesity, and hypertension\textsuperscript{336}. This observation has also been made in humans, by examining the food preferences of adult women born after intra-uterine growth restriction\textsuperscript{337}. There was a continuous association between growth restriction levels and later carbohydrate-protein ratio intake in these women\textsuperscript{337}.

However, although such changes have been observed in growth-restricted individuals, there are no data on any of the groups we studied (i.e. post-terms, offspring born after hyperemesis gravidarum, and first-borns).

6.2.4. Hormones and programmed changes

6.2.4.1. Glucocorticoids

As previously mentioned, the three above-described pathways are part of a very complex system\textsuperscript{299}. For example, abnormalities in insulin, catecholamine, cortisol, growth hormone, and IGF systems have been observed in children and adults who are born too small or too thin\textsuperscript{289,338,339}. However, it is believed that glucocorticoids have an important role mediating numerous interactions in this complex system.

Endocrine adaptations in low birth weight babies could involve the placental glucocorticoid system and programming of the hypothalamo–pituitary–adrenal (HPA) axis\textsuperscript{340}. The HPA plays a vital role in stressful conditions both before and after birth, underlying the response mechanism to physiological and psychological challenges, consequently aiding survival\textsuperscript{289}. In the scenario of an
adverse early life environment, there are associated stress responses in the fetus/infant, whose effects may persist throughout life even, in non-adverse conditions\textsuperscript{341}.

Importantly, placental 11β-HSD protects the fetus from the effects of high maternal corticosteroids by inactivating them to inert products\textsuperscript{342}. Protein restriction during pregnancy produces an attenuation of activity of placental 11β-HSD, resulting in higher exposure to maternal corticosteroids\textsuperscript{342,343}. Fetal exposure to maternal corticosteroids during critical periods of prenatal development could also initiate organisational effects or imprinting responses that persist in later life\textsuperscript{340}. This may at least in part explain observations in adult men born of low birth weight, who were shown to have dysregulated HPA axis with poor pituitary-adrenal responses\textsuperscript{344}. In another study, adult men born of low birth weight have elevation of plasma cortisol and associated higher systolic blood pressure, serum triglyceride levels, and insulin resistance\textsuperscript{338}.

Thus, low birth weight may be associated with programming of the HPA axis, and result in insulin resistance syndrome in adult life\textsuperscript{338}. In addition, analysis of plasma cortisol and adrenocorticotrophin (ACTH) concentrations in fetal blood performed after cordocentesis in AGA and SGA fetuses revealed interesting results. Fetal hypoglycemia in SGA fetuses was associated with higher plasma cortisol concentrations but lower ACTH levels, suggesting that the pituitary may be inhibited in chronically hypoglycemic SGA fetuses\textsuperscript{345}. A similar mechanism may partly explain the reduced insulin sensitivity and elevated cortisol levels we observed in children born to mothers with severe hyperemesis gravidarum.

### 6.2.4.2. Other hormones

A number of hormones (other than glucocorticoids) play a major role in fetal growth, via both anabolic and catabolic actions on fetal metabolism. Such hormones (including insulin and IGF) also affect placental endocrine functions and nutrient-transfer capacity\textsuperscript{310}.

Intra-uterine growth is influenced by levels of IGF-I and IGF-II and their binding proteins IGFBP-1, IGFBP-2, and IGFBP-3. The IGF system is up- or down-regulated by hormones such as insulin and cortisol that are sensitive to nutrition levels\textsuperscript{299,346,347} in utero. As IGF-I release is mediated by the effects of glucose on insulin\textsuperscript{348}, maternal under-nutrition can lead to a decrease in IGF-I concentrations in IUGR babies, and ultimately affect various organ systems.

Therefore, not surprisingly, IUGR fetal cord serum has markedly lower concentrations of IGF-I, IGF-II, and IGFBP-3\textsuperscript{349}. Further, the placentae of IUGR fetuses have lower expression of IGF2 and IGF1 receptors (a counter regulatory mechanism to growth retardation) compared to the placentae
of AGA fetuses\textsuperscript{350}. A reduction in IGF-I concentrations can further reduce pancreatic β-cell development in IUGR fetuses\textsuperscript{351,352}.

The complex interplay between a sub-optimal intra-uterine environment and various endocrine and metabolic events in fetal and post-natal life have been summarised in the diagram by Fowden \textit{et al.} (Figure 6.1). Importantly, alterations in IGF–IGFBP system have been observed in all the three cohorts examined in this thesis (i.e. post-terms, offspring born after hyperemesis gravidarum, and first-borns). However, the mechanisms behind the changes in IGF-IGFBP system in our cohorts are yet to be elucidated.

\section*{6.3. Directions for future research}

We have analysed the effects of post-term birth, maternal hyperemesis gravidarum and birth order on the metabolic status and body composition of pre pubertal children. The possible mechanisms that could programme these alterations have been explored in section 6.2. While the first-borns have been analysed with simple and crude methods at adulthood in a few studies, the other two groups have never been the subject of such research before. While the metabolic alterations have been demonstrated in all the cohorts with sophisticated methods, the definite underlying pathophysiological process can only be analysed in further studies. Further, it is important to assess whether our findings do apply across different ethnic groups.

\subsection*{6.3.1. Post-terms}

Approximately 68,000 babies were born post-term in the year 2000 in eight countries from the PERISTAT study in Europe\textsuperscript{55}. Children being born post-term are more common than previously assumed. In Sweden for example, the incidence of preterm births is 5.6\% compared to 7.5\% for post-terms\textsuperscript{55,59,60}. A similar situation applies to many other countries where the incidence of post-term births is greater than that of preterms. However, while the latter group has attracted a considerable amount of research in the last two decades, there are very little data on the long-term outcomes for those born post-term. Therefore, the features suggesting increased risk for the metabolic syndrome in post-term children are novel findings, and our study highlights the need to better examine this under-researched group.
There are a number of aspects that require investigation:

1. Do the observed adverse outcomes in metabolism and body composition persist or magnify with advancing age? And if so, how do diet and life-style modifications affect long-term health risks?
2. Post-term children displayed indicators of adverse cardiovascular status, and more comprehensive analysis of their cardiovascular function in childhood and adulthood are of interest.
3. Though structural changes in post-term placentae mimic changes in SGA pregnancies, the endocrine functions in post-term placentae are unknown.
4. We observed adverse outcomes in post-term children in the absence of a reduction in birth weight, suggesting different underlying mechanisms to those affecting subjects born SGA.
5. 90% of the post-term participants in our cohort had another family member born post-term, suggesting a common gene variant predisposing to post-term pregnancies. It would therefore be of interest to investigate possible genetic factors that affect length of gestation.
6. A few of the fathers of post-term children were also born after prolonged pregnancies, indicating a possible paternal effect on gestational length (in addition to the likely role played by the mothers). This hypothesis is supported by the "short gestation length sperm" marketed in the cattle industry, and studies into similar paternal effects in humans would be of great interest.
7. It would be of great interest to evaluate long-term outcomes in the offspring of pregnancies randomized to labour induction versus conservative management, in order to clarify the actual effects of prolonged gestation per se.

### 6.3.2. Hyperemesis gravidarum

One of the top twelve triumphs in 2012 for “Million Moms Challenge” (coalition of 30 large organizations that aims to improve the lives of mothers and children) has been to raise awareness for hyperemesis gravidarum. Mothers who experienced severe hyperemesis gravidarum go through a rather traumatic experience, and they invariably display a great interest in understanding what that means for their children. However, long-term outcomes on this group have not been investigated, and considerably more research into the effects of hyperemesis gravidarum is needed.

1. Dutch Famine studies have demonstrated that sub-optimal nutrition early in pregnancy results in metabolic changes in late adulthood. Does hyperemesis gravidarum lead to similar offspring outcomes in adulthood?
2. Recent animal evidence revealed that serotonin blockade could affect pancreatic β-cell development\textsuperscript{355}. As serotonin antagonists are regularly used in the management of hyperemesis gravidarum, it is important to assess the possible effects of such medications on the offspring in the long-term. In addition, while many mothers experiencing hyperemesis gravidarum take metoclopramide (partial 5-hydroxytryptamine receptor agonist), a few also take an additional anti-emetic that is a serotonin antagonist (5-hydroxytryptamine receptor antagonist). Thus, the possible effects of administering both drugs together on long-term offspring outcomes are also of interest.

3. Almost all mothers in our study experienced severe hyperemesis gravidarum right through pregnancy. However, hyperemesis gravidarum often abates by mid-gestation. Future studies comparing long-term outcomes in the offspring of both groups are warranted.

4. Possible placental changes in association with hyperemesis gravidarum have not been previously assessed, and should be the focus of future investigations.

6.3.3. First-borns

With the on-going trend towards smaller families worldwide, our findings of adverse health outcomes in this group are particularly relevant. As a result, further investigations into the long-term effects of birth order are warranted.

1. Studies on first-born adults need to be carried out using gold-standard techniques to determine whether the lower insulin sensitivity observed in pre-pubertal children are magnified or attenuated with increasing age.

2. Analysis of cardiovascular function in childhood and adulthood (particularly in comparison to later-born siblings) would provide valuable information into the long-term risks among first-borns.

3. Our findings corroborated other studies showing that first-borns are taller than latter borns\textsuperscript{167,356}. As there is some evidence suggesting that taller stature in childhood is positively associated with overweight status and obesity later in life\textsuperscript{275}, studying body composition in adulthood using DXA or MRI scans would provide important information on risk factors associated with the metabolic syndrome.

4. While the placentae of first-borns have been analysed for structural changes and immunomodulation in comparison to the placentae of later-borns, there are no data on placental growth factors, hormonal concentrations, or epigenetic changes.
6.4. Strengths & limitations

6.4.1. Strengths of our studies

- The greatest strength in all of our studies has been the use of very precise and sophisticated tools to evaluate metabolic and cardiovascular function in very well-defined study groups.
- We analysed insulin sensitivity by submitting FSIGT data to the modified minimal model, which is the most sensitive method that could be performed with ease in children. As discussed in Chapter 2, the minimal model is equivalent to the ‘gold standard’ hyperinsulinaemic-euglycaemic clamp for assessing insulin sensitivity in children.232
- Analysis of body composition using DXA provided valuable and accurate measurements of body fat and adipose tissue distribution in all of our studies, while exposing the children to the least amount of radiation.277,357,358
- Various biochemical parameters were measured to obtain a comprehensive metabolic picture of each participant, including measurements of IGFs, IGFBPs, adipokines, hormonal concentrations, lipid profiles, and uric acid.
- Our controls have been recruited from friends of the participants, so that both groups were relatively matched in terms of socio-economic status and general daily activities, factors known to have an impact on anthropometry and other health outcomes.
- Anthropometric data of all children were individually adjusted for their genetic potential (i.e. mid-parental height or mean parental BMI), as parental anthropometry is strongly associated with offspring anthropometry.360-362
- Our inclusion criteria were very strict, removing subjects that could have metabolic abnormalities that might have confounded the results (e.g. children born SGA or preterm, and those with close relatives with diabetes).
- Our studies examined only pre-pubertal children, as puberty is known to affect insulin sensitivity.363
- All the assessments (including FSIGT and anthropometric measurements) were carried out by the same investigator, thereby eliminating inter-observer variation.
- All assessments were also carried out by a Paediatric Endocrinologist, who had the relevant clinical background to appropriately examine the children as well as evaluate the data obtained and the clinical information reported by parents.
- As 7% to 16% of pregnant women are unsure of their dates, using LMP for prediction of gestational age is potentially inaccurate. Positive predictive values of LMP for preterm and term deliveries are 0.949 and 0.775, respectively, but much worse for post-term births at
Hence, the use of ultrasound scans performed before the 20th week of pregnancy to determine gestational ages ensured their reliability in our post-term study. Further, we adopted a clear-cut difference of two weeks between the post-term and term groups, virtually ensuring that there were no overlaps between groups.

- For our hyperemesis gravidarum cohort, we recruited only mothers with the severe form, i.e. those who had been admitted to hospital with weight loss or electrolyte disturbance. As a result, all mothers in this group had been exposed to considerable nutritional stress, ensuring the exclusion of mothers with more mild hyperemesis gravidarum who could have compensated for their vomiting with an adequate food intake.

6.4.2. Limitations

- In light of the level of intervention required, our studies were carried out on a relatively small number of children (approximately 36 per group). The database used for the recruitment of the participants was a decade old. Thus, difficulty in tracing these participants had further limited the number of subjects. Electronic medical records of the database started at a later date. This led to difficulty in manually scrutinising case records and some missing data with a reduction in the number of eligible subjects.

- Our study participants were from a number of different ethnicities. Although ethnicity was controlled for in all our analyses, it is not possible to ascertain whether our observed findings would indeed apply across all ethnicities. A majority of the subjects in all the three studies were New Zealand Europeans. Future analyses on other ethnicities could clarify particular metabolic risks.

- We have observed numerous adverse health risks in the cohorts examined in childhood. However, we have not yet conducted studies to ascertain whether these changes persist (or magnify) into adulthood.

- We have not yet examined the possible trigger mechanisms underpinning the observed changes.

- Our studies were cross-sectional and not longitudinal studies. Although we believe our control subjects were representative of the general population, these were not identified beforehand. Future longitudinal studies would help in determining long-term health risks.
6.5. Final remarks

Our findings in first-borns, post-terms, and the offspring of mothers who experienced hyperemesis gravidarum are novel, and of relevance to more than half of the world's population. Future research must firstly confirm that these risk factors for metabolic disease persist into adulthood, and are not transient phenomena. Follow-on adult studies in SGA and preterm subjects have shown that childhood metabolic risk factors do persist into adult life.

In the case of those born post-term, the observed changes may be a result of physiological stress caused by prolonged gestation and/or of common gene variants that could programme both length of gestation and long-term metabolism. Teasing out the relative contributions of the two factors on metabolic outcomes is important to better understand the cause of long-term programming of metabolic diseases, and inform the management of post-term pregnancies.

We studied a cohort born of mothers with a very severe form of morning sickness (i.e. hyperemesis gravidarum) who had reduced insulin sensitivity and elevated cortisol levels. However, it is unclear whether less severe forms of morning sickness (which occur far more commonly) are also associated with metabolic abnormalities in the offspring. Further, as most of the mothers in our study cohort had been treated with different anti-emetics, evaluating the possible mitigating or worsening effects of these medications on metabolic outcomes in the offspring would help formulate better protocols to manage the severe vomiting in pregnant mothers.

Regarding birth order, with the on-going trend towards smaller families, more than half of the world’s population are first-borns. Any effects on long-term metabolic risks in this group would have enormous impact on health care systems worldwide.

Interestingly, insulin resistance has been an important and common feature in all three cohorts researched for this study. Until now, most studies have found insulin resistance to be associated with obesity in children. However, all of our cohorts revealed the presence of insulin resistance in the absence of obesity. Hence, we speculate that insulin resistance may actually precede obesity, and this issue also needs further investigation.

This PhD project has already served as a starting point for several new investigations. A study is already underway (within our own institute) to examine the metabolomics of post-term children in comparison with those born at term. Grant proposals have been submitted seeking funding to study adult post-term cohorts and to carry out MRI scans on the hearts of post-term children during exercise. Other studies are under consideration, including proposals to assess metabolic risks in first-born adults and to analyse metabolic gene variants in post-term children and their families.
Lastly, while diet and lifestyle factors are known to increase the risk for metabolic diseases, the importance of early life events for our long-term health is gaining ever-increasing attention. Our research into three new under-researched cohorts of children has paved the way for larger studies across all age groups. If these changes persist into adulthood, search for factors that could modify and improve the health outcomes in these groups would be invaluable.
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