

# **Intrauterine Influences on Obesity and Insulin Resistance in Pre-pubertal Children**

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*"Truth lies all around us, but is only revealed to those who search for it."*

Nicolae Iorga (1871-1940)

This thesis is dedicated to my mother for her immense love, trust, support and encouragement not only through the PhD candidature years, but throughout my entire life. I have learnt from her wisdom that giving anything less than all I could give meant sacrificing a gift.

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## Abstract

Within the paradigm of developmental origins of health and disease, an intrauterine environment that stimulates fetal overnutrition has been found to contribute to the risk of subsequent obesity in the offspring. There is compelling epidemiological evidence for a positive association between maternal obesity prior to pregnancy, gestational diabetes (GD) or excessive gestational weight gain, and the development of childhood obesity (as measured by body mass index, BMI). However, the evidence is limited and inconsistent with respect to more specific measures of adiposity (body composition or fat pattern) and insulin resistance in children. Furthermore, the long-term effects of maternal borderline gestational glucose intolerance (BGGI) on the offspring have not been considered.

Therefore, I sought to examine whether maternal obesity prior to pregnancy, gestational glucose intolerance across the entire spectrum, and gestational weight gain have deleterious effects on the development of obesity (both global and specific measures of adiposity) and insulin resistance in pre-pubertal children. These associations are particularly important from a public health perspective as, once identified, they may point towards potential windows for prevention of childhood obesity and related metabolic disorders.

This project entailed a follow-up of an existing representative, prospective birth cohort study (Generation 1 Study, n=557) in Adelaide, South Australia, recruited during 1998-2000. At the 9-10 year follow-up, rigorous anthropometric measurements were conducted in 443 children (80% of the original cohort), of whom 163 consented to provide a fasting blood sample for the estimation of insulin resistance based on homeostasis model assessment (HOMA-IR). Information on intrauterine exposures and confounders was collected from the antenatal interviews and hospital records. Maternal age, parity, smoking, pregnancy-induced hypertension, and education at the time of pregnancy were considered as potential confounders for all the associations of interest, and child current BMI z-score as a potential mediator on the pathway between the intrauterine exposures and child insulin resistance. Data were analysed using multiple linear regression and generalized linear models.

Maternal pre-pregnancy BMI was positively associated with all three obesity-related measures considered in the 9-10 year-old children (BMI z-score, percentage body fat estimated by bioelectrical impedance analysis, and waist-to-height ratio); these relationships were robust to adjustment for potential confounders (adjusted coefficients for each one kg/m<sup>2</sup> increase in maternal pre-pregnancy BMI were 0.08 (95% confidence interval 0.06, 0.10) for child BMI z-score, 0.44 (95% CI 0.31, 0.58) for percentage body fat and 0.002 (95% CI 0.002, 0.003) for waist-to-height ratio). There was no association between maternal pre-pregnancy BMI and HOMA-IR in children (with or without adjustment); however, when child current BMI z-score was included as a mediating variable, the relationship between maternal pre-pregnancy BMI and child HOMA-IR was inverse and significant (adjusted change in child HOMA-IR for each one kg/m<sup>2</sup> increase in maternal pre-pregnancy BMI was -0.83% (95% CI -1.63, -0.02)).

Intrauterine exposure to glucose intolerance during pregnancy (either BGGI or GD) was not associated with any of the three obesity-related measures in children at 9-10 years. Children of mothers who developed GD during the index pregnancy had a higher HOMA-IR; this relationship was robust to adjustment for potential confounders (adjusted change in child HOMA-IR if exposed to maternal GD was 42.9% (95% CI 20.9, 68.9)) and partly mediated by child current BMI z-score. No association was found between exposure to maternal BGGI and child HOMA-IR (with or without confounder adjustment); however, when child current BMI z-score was added as a potential mediator, exposure to BGGI was associated with a reduction in child HOMA-IR (adjusted change in child HOMA-IR if exposed to maternal BGGI was -17.9% (95% CI -29.9, -3.96)).

There were no significant associations between maternal gestational weight gain and any of the outcome measures of interest in unadjusted models. However, adjustment for pre-pregnancy BMI led to a positive association between gestational weight gain and child BMI z-score (adjusted changes in child BMI z-score for each one kg increase in maternal gestational weight gain was 0.032 (95% CI 0.007, 0.057)). Gestational weight gain was not associated with child insulin resistance, and this did not change when child current BMI z-score was included as a potential mediator on the pathway between gestational weight gain and child insulin resistance.

Potential two-way interactions between the main exposures were investigated in relation to all outcomes of interest. Two significant interactions were identified: maternal pre-pregnancy BMI and glucose tolerance status, and maternal pre-pregnancy BMI and gestational weight gain, with a synergistic effect on child waist-to-height ratio.

These results suggest that childhood obesity and insulin resistance have origins, at least in part, in intrauterine life, particularly in relation to maternal obesity at the time of pregnancy and GD. Further research to differentiate between genetic, environmental and intrauterine programming is recommended. That said, maternal pre-pregnancy BMI was the strongest predictor of child BMI z-score, while GD appeared to have an independent effect on child insulin resistance, and both clinical and public health actions to address these maternal factors are warranted for a range of reasons.



## Declaration

I, Oana Maftei certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Oana Maftei

Date: 21 December 2011

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## Abbreviations and acronyms

%BF	percentage body fat
ACHOIS	Australasian Carbohydrate Intolerance Study in Pregnancy
ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
ADIPS	Australasian Diabetes in Pregnancy Society
ALSPAC	Avon Longitudinal Study of Parents and Children
BGGI	borderline gestational glucose intolerance
BIA	bioelectrical impedance analysis
BMI	body mass index
BW	birth weight
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CT	computed tomography
DXA	dual energy X-ray absorptiometry
EPOCH	Exploring Perinatal Outcomes among Children
FFM	fat-free mass
FGIR	fasting glucose-to-insulin ratio
FM	fat mass
FSIVGTT	frequently sampled intravenous glucose tolerance test
FTO	fat mass and obesity associated gene
GD	gestational diabetes
GLM	generalised linear model
GWG	gestational weight gain
HAPO	Hyperglycemia and Adverse Pregnancy Outcomes
HbA1c	haemoglobin A1c
HDL	high-density lipoprotein
HOMA-IR	homeostasis model assessment of insulin resistance
IGF	insulin-like growth factor
IGT	impaired glucose intolerance
IL	interleukin

IOM	Institute of Medicine
IOTF	International Obesity Task Force
IQR	interquartile range
IR	insulin resistance
LDL	low-density lipoprotein
LMS	lambda-mu-sigma
MRI	magnetic resonance imaging
NDDG	National Diabetes Data Group
NGT	normal glucose tolerance
NZSSD	New Zealand Society for the Study of Diabetes
OGCT	oral glucose challenge test
OGTT	oral glucose tolerance test
OR	odds ratio
PAR	population attributable risk
PCOS	polycystic ovarian syndrome
QUICKI	quantitative insulin sensitivity check index
r	Pearson's correlation coefficient
RCT	randomised clinical trial
RR	relative risk
SD	standard deviation
SES	socio-economic status
SFT	skinfold thickness
TBW	total body water
TNF	tumour necrosis factor
TAFE	Training and Further Education
WHO	World Health Organization
WHtR	waist-to-height ratio

## Conference presentations

1. **Maftei O**, Whitrow MJ, Moore VM, Davies MJ. *Intrauterine influences on offspring obesity in prepubertal children* - oral presentation, 7<sup>th</sup> World Congress on Developmental Origins of Health and Disease, Portland, Oregon, USA, 18-21 September 2011
2. **Maftei O**, Whitrow MJ, Moore VM, Davies MJ. *Intrauterine influences on insulin resistance in prepubertal children* - oral presentation (ranked in top 10%), 7<sup>th</sup> World Congress on Developmental Origins of Health and Disease, Portland, Oregon, USA, 18-21 September 2011
3. **Maftei O**, Whitrow MJ, Moore VM, Davies MJ. *Insulin resistance in prepubertal children in relation to intrauterine exposure to overnutrition* - oral e-poster presentation, 52<sup>nd</sup> Annual Meeting of the European Society for Paediatric Research, Newcastle, UK, 14-17 October 2011

# Chapter 1 Introduction

Childhood obesity and its related metabolic disorders, including insulin resistance, are an emerging public health issue in developed countries. Given that weight reduction is notoriously difficult to achieve and sustain, identifying effective preventative strategies is imperative. Relatively recent epidemiological research has suggested that intrauterine life might provide a window for obesity prevention. This suggestion has been based on the observation that maintaining healthy body weight is more likely to be achieved by individuals with an optimal fetal environment, leaving aside genetic considerations.

## 1.1 Developmental origins of health and disease

A growing body of evidence has shown that adverse conditions occurring during intrauterine life may result in perturbations of fetal growth and 'programming' of physiological systems, including metabolism, thereby predisposing the individual to chronic disease later in life. This constitutes the 'fetal origins hypothesis', also known as the 'developmental origins of health and disease' hypothesis, originally proposed by Barker in 1980s (Barker and Osmond 1986), and subsequently supported by many other studies, both in humans and animal models, with various exposures and outcomes of interest (Barker 1994; Barker 1995; Barker 2003; Gillman 2005; McMillen and Robinson 2005; Oken and Gillman 2003).

Several studies have indicated that changes in maternal metabolic milieu during pregnancy could play a major role in fetal development, with subsequent effects on the size at birth. Both low and high birth weight for a given gestational age have been associated with a greater risk of obesity and related metabolic outcomes later in life (U-shaped curve) (Gillman 2005; Jovanovic 2000), but, as low birth weight has become less common in developed countries, the research focus has shifted towards fetal overnutrition.

A number of intrauterine conditions have been identified as independent risk factors for greater size at birth, including maternal characteristics brought to the pregnancy (such as body weight status) and occurring during pregnancy (such as gestational glucose intolerance and weight gain). Data is scarce regarding the long-term influence of these potentially modifiable risk factors on offspring weight status, body composition, fat distribution and insulin sensitivity, independently, cumulatively, or in complex interactions.

The current project sits within the paradigm of developmental origins of health and disease, aiming to investigate the influence that maternal obesity prior to pregnancy, gestational glucose intolerance, and/or gestational weight gain have on the development of obesity, increased percentage of body fat, central adiposity and insulin resistance in the child.

## **1.2 Evidence of programming of childhood obesity and insulin resistance by maternal body size and glucose intolerance during pregnancy**

There is consistent epidemiological evidence of a positive association between maternal weight status at the time of pregnancy and child global obesity, as measured by body mass index (BMI) (Boerschmann *et al.* 2010; Lawlor *et al.* 2007b; Li *et al.* 2005; Pirkola *et al.* 2010; Reilly *et al.* 2005; Whitaker 2004), although there are marked variations in the estimates reported on this association. In contrast, the evidence is scarce with respect to the influence of maternal pre-pregnancy BMI on more specific measures of adiposity in children, such as percentage body fat and fat pattern, and on insulin sensitivity in childhood.

Gestational glucose intolerance may occur during the second half of pregnancy in those women whose insulin secretion is insufficient to compensate for the increased insulin resistance that characterises any pregnancy. Various degrees of glucose intolerance may develop, ranging from severe (falling under the category of gestational diabetes) to milder (which does not meet the diagnostic criteria for gestational diabetes and which in this project is defined as borderline gestational glucose intolerance). There is compelling evidence regarding the detrimental effect of gestational diabetes on fetal development and some recent studies have shown that even lower levels of glucose intolerance may adversely affect intrauterine growth (HAPO Study Cooperative Research Group 2008). However, the long-term effects of prenatal exposure to various degrees of



maternal gestational glucose intolerance on health outcomes in the offspring subsequent to birth have been far less documented.

There is relatively abundant evidence to suggest an association between maternal gestational diabetes and the long-term risk of global obesity in the child, but it is inconsistent, with some studies identifying an increased risk of obesity (Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Egeland and Meltzer 2010; Krishnaveni *et al.* 2010; Silverman *et al.* 1991; Silverman *et al.* 1998), while others not (Gillman *et al.* 2003; Jeffery *et al.* 2006; Lawlor *et al.* 2010; Pirkola *et al.* 2010; Whitaker *et al.* 1998). Data regarding the association between maternal glucose intolerance during pregnancy and body composition or fat distribution of the child, while more limited, are also conflicting. After confounder adjustment, exposure to gestational diabetes was positively associated with offspring measures of adiposity in some studies (Catalano *et al.* 2003b; Egeland and Meltzer 2010; Krishnaveni *et al.* 2010; Wright *et al.* 2009), but not in other studies (Catalano *et al.* 2009a; Pirkola *et al.* 2010). Furthermore, there is little and inconclusive research on the association between exposure to a diabetic intrauterine environment and insulin resistance during childhood (Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Egeland and Meltzer 2010; Jeffery *et al.* 2006; Krishnaveni *et al.* 2010).

It has been recently suggested that persistent increases in maternal plasma glucose levels during pregnancy that do not fulfil the diagnostic criteria for gestational diabetes may also result in the programming of child obesity and insulin resistance. These associations have been addressed in very few studies in non-high risk populations, with mixed findings (Chandler-Laney *et al.* 2011; Egeland and Meltzer 2010; Hillier *et al.* 2007; Lawlor *et al.* 2010). There is no study investigating the long-term influence of maternal glucose intolerance during pregnancy across the entire spectrum of severity on child obesity, body composition, fat pattern and insulin resistance before puberty, with thorough adjustment for confounders.

Recent studies have suggested that, in addition to maternal obesity and glucose intolerance during pregnancy, gestational weight gain may also affect the intrauterine environment, with increases to the risk of obesity later in life and potentially obesity-related metabolic disorders. With one exception (Whitaker 2004), all published studies have found a positive association between maternal weight gain during pregnancy and obesity in children. The evidence is very limited with regard to the influence of maternal weight gain during pregnancy on more specific measures of adiposity in the child, with inconsistent results (Fraser *et al.* 2010; Oken *et al.* 2007). No studies have been published addressing the potential association between maternal excessive weight gain in

pregnancy and child insulin resistance. Moreover, no studies have examined how maternal gestational weight gain across the entire spectrum influences child body composition, fat distribution and insulin resistance before puberty.

In summary, the existing literature on these relatively new questions about how predisposition to childhood obesity and insulin resistance arises has a number of limitations including lack of data for some associations, poor confounder adjustment, and non-representative samples. In addition, most studies used categorical variables, thus limiting opportunities to determine the effect of small shifts in a particular variable on the outcome of interest. A full summary and critique of this literature is provided in Section 2.4.

### **1.3 Research questions and hypotheses**

Numerous factors are known or suspected to affect the risk of developing obesity and related metabolic disorders in children, including genetic, epigenetic and postnatal environmental factors, but, as a whole, they are beyond the scope of this project. The current study aims to shed light on the long-term influence of early life factors, in particular maternal obesity prior to pregnancy, gestational glucose intolerance (both gestational diabetes and borderline gestational glucose intolerance), and gestational weight gain on childhood obesity, body composition, fat distribution, and insulin resistance, within a contemporary Australian cohort of 9-10 year old children.

Consistent with the developmental origins of health and disease paradigm, I hypothesise that:

1. Maternal pre-pregnancy obesity is positively associated with child global obesity (as measured by BMI), percentage body fat, central adiposity and insulin resistance in pre-pubertal children; the relationship with insulin resistance is independent of child current body size.
2. Maternal glucose intolerance during pregnancy across its entire spectrum of severity (from mild glucose intolerance to gestational diabetes) is positively associated with child global obesity (as measured by BMI), percentage body fat, central adiposity and insulin resistance in pre-pubertal children; the relationship with insulin resistance is independent of child current body size.

3. Maternal weight gain during pregnancy is positively associated with offspring global obesity (as measured by BMI), percentage body fat, central adiposity and insulin resistance in pre-pubertal children; the relationship with insulin resistance is independent of child current body size.
4. Maternal pre-pregnancy obesity, glucose intolerance during pregnancy, and gestational weight gain interact and synergistically increase offspring global obesity (as measured by BMI), percentage body fat, central adiposity and insulin resistance in pre-pubertal children.

For each association considered, several potential confounders (maternal age, parity, smoking, pregnancy-induced hypertension, and socioeconomic status) will be adjusted for.

## 1.4 Thesis objectives

This thesis aims to investigate the effects of intrauterine exposure to overnutrition due to maternal obesity, gestational glucose intolerance and gestational weight gain, on global obesity, body composition, fat pattern and insulin resistance among pre-pubertal children in a prospective birth cohort study in Adelaide, South Australia. The specific objectives of this project are:

1. To access and assess historical measurements of
  - a. maternal weight status prior to pregnancy;
  - b. maternal glucose tolerance status during pregnancy;
  - c. maternal gestational weight gainin a representative cohort of women in Adelaide, South Australia;
2. To assess
  - a. global obesity (measured by BMI);
  - b. body composition (percentage body fat);
  - c. fat pattern (waist-to-height ratio);
  - d. insulin resistance (homeostasis model assessment)in pre-pubertal (9-10 year-old) children in a representative prospective birth cohort in Adelaide, South Australia;
3. To investigate the associations between maternal pre-pregnancy BMI and child global obesity, percentage body fat, waist-to-height ratio and insulin resistance at the age of 9-10 years;

4. To investigate the associations between maternal glucose intolerance during pregnancy across the spectrum and child global obesity, percentage body fat, waist-to-height ratio and insulin resistance at the age of 9-10 years;
5. To investigate the associations between maternal weight gain during pregnancy and child global obesity, percentage body fat, waist-to-height ratio and insulin resistance at the age of 9-10 years;
6. To assess interactions between maternal pregnancy characteristics in relation to offspring global obesity, percentage body fat, waist-to-height ratio and insulin resistance at the age of 9-10 years.

## 1.5 Overview of the thesis

Following the introduction and statement of hypotheses and objectives in Chapter 1, Chapter 2 describes a theoretical framework to which this project seeks to contribute. It starts by outlining the broader conceptual framework of early life origins of health and disease, and then discusses each exposure and outcome of interest with an emphasis on their measurement and public health significance. Finally, the chapter presents a summary and critique of the published literature concerning the influences of maternal obesity, glucose intolerance and weight gain during pregnancy upon childhood obesity and related metabolic disorders, delineating limitations of each study and identifying the gaps in this area of epidemiological knowledge.

Chapter 3 describes the study methods, including the study design, relevant aspects of data collection, the derivation of exposure and outcome variables examined in this thesis, and the statistical methods applied. The Generation 1 cohort, upon which this project is based, is also briefly outlined.

Chapter 4 presents the findings arising from the analyses, including descriptive statistics pertaining to the study participants (both pregnancy-related data and data on anthropometry and glucose-insulin homeostasis in children) and the results for the investigations concerning the intrauterine influences of maternal obesity, gestational glucose intolerance and gestational weight gain upon childhood obesity, body composition, fat pattern and insulin resistance.

Chapter 5 discusses the results of each association of interest in context with previous studies, outlines study strengths and limitations, describes potential underlying mechanisms, and presents implications arising from this work and recommendations for future research in the field of early origins of childhood obesity and insulin resistance.

## **1.6 Statement of contribution**

My doctoral research was partially based on data collected from a cohort of pregnant women from 1998 through to 2000. The activities for which I was responsible under my supervisors' guidance included:

1. Conceptualisation of the specific research questions;
2. Participation in fieldwork at the 9-10 year follow-up, from June 2008 through to May 2010, interviewing and performing anthropometric measurements in 144 children, representing one third of participants;
3. Data management (except for imputation of some missing data) and data analysis;
4. Presentation and interpretation of results;
5. Discussion and implications of findings.

## Chapter 2 Theoretical framework

This chapter describes the broad concept of life course which contextualises the current project and its public health significance. Section 2.1 outlines the principles underlying early life origins of health and disease, with an emphasis on the long-term influence of conditions operating *in utero* on the development of obesity and related metabolic disorders in the offspring before puberty. The modifiable intrauterine factors that this project focuses on, namely maternal obesity prior to pregnancy, gestational glucose intolerance across the entire spectrum of severity, and excessive weight gain during pregnancy are presented in Section 2.2. Each of them is described in terms of pathophysiology, demographic trends, adverse outcomes for the mother and the child, and the clinical response thus far. Section 2.3 describes the outcomes of interest for this project, specifically global obesity, body composition, fat pattern and insulin resistance in children. Details on demographic trends, adverse health outcomes and methods of assessment are considered for each of these outcomes. Epidemiological evidence from previous international studies addressing intrauterine programming of childhood obesity and insulin resistance is summarised and critiqued in Section 2.4, flagging the need for an investigation based on a contemporary cohort of pre-pubertal children.

### 2.1 Early life exposures and later development

In recent years, potential associations between factors acting at various stages of life and development of certain chronic diseases have started to be explored within the conceptual framework of the life course (Kuh and Ben-Shlomo 2004). The life course approach to chronic disease emerged in late 1980s from the necessity to fill the gap in explaining the increasing occurrence of cardiovascular disease, by challenging the aetiological model that emphasized the role of adult lifestyle (smoking, diet, and lack of exercise) (Barker and Osmond 1986). This approach refers to the study of long term effects of exposures occurring in different stages of life, from

conception through to adulthood, on later health status or development of disease (Kuh and Ben-Shlomo 2004). Exposures may include biological and psychosocial events acting before conception, during intrauterine life, infancy, childhood, adolescence, or in early adult life, which “independently, cumulatively and interactively” influence disease risk later in life (Kuh and Ben-Shlomo 2004). The life course approach has been extended to cover intergenerational effects, focussing on those factors that are transmitted from one generation to another (Kuh and Ben-Shlomo 2004).

Within this broad framework sits the paradigm of developmental origins of health and disease, which focuses on early stages of life characterised by increased levels of plasticity and vulnerability, such as the prenatal and early postnatal periods, as potential determinants of the health trajectory later in life (Gillman 2005). Developmental plasticity is the phenomenon by which a given genotype may result in different structural and functional states in response to various environmental exposures occurring at certain stages of development (Gluckman and Hanson 2007). These permanent changes in function and/or structure of organs are thought to be associated with permanent alterations in gene expression regulated by epigenetic factors (such as DNA methylation and histone modification).

From a public health point of view, the life course approach to chronic disease and particularly the developmental origins to health and disease paradigm are important for implementing health promotion and disease prevention interventions in early life.

Three specific periods in early life have been hypothesised as critically important for the development of obesity before puberty and may indicate windows for prevention: the prenatal period, infancy and the adiposity rebound (Gillman 2004), which occurs around the age of 5-6 years (Reilly *et al.* 2005). Given the positive association between body size and insulin resistance (Lawlor *et al.* 2005), it seems reasonable to consider the same periods as being critical for the development of insulin resistance in pre-pubertal children. According to the *critical period model*, which may underlie programming (Lucas 1998), exposures acting in certain periods of vulnerability during early life, characterised by rapid cell replication, marked plasticity of the systems and high sensitivity to environmental factors (Barker 1994), which have lifelong, irreversible effects on the structure and/or function of organs and systems (Ben-Shlomo and Kuh 2002). In contrast to the critical period framework for obesity development and related metabolic disorders, it is also plausible that risk factors accumulate progressively over the life span, as advocated by the *accumulation of risk model* (Ben-Shlomo and Kuh 2002). These risk factors may be independent from each other or clustered, with synergistic or antagonistic effects (Ben-Shlomo and Kuh 2002). An example of a synergistic

effect is the relationship between insulin resistance and low birth weight, which is particularly strong for individuals who become obese later in life (Lawlor *et al.* 2005).

As intrauterine life is characterised by the most rapid growth and great vulnerability to various insults, it is the focus of the current project to examine potential influences of prenatal environment on global obesity (BMI), percentage body fat, fat distribution, and insulin sensitivity in the child, thus fitting in the original conceptual framework of early origins of health and disease ('fetal origins hypothesis') proposed by Barker (1995). Numerous observational studies have investigated the development of obesity and associated metabolic disorders in relation to size at birth as a measure of convenience (Wells *et al.* 2007), widely available as part of routine medical records and with established reference norms, but which in fact is only a crude marker of intrauterine growth for a given gestational age. Most of these studies showed a positive association with attained BMI later in life (Parsons *et al.* 1999) and a negative one with central adiposity (Okosun *et al.* 2000) and insulin resistance (Law *et al.* 1995; Phillips *et al.* 1994). However, it has been suggested that certain factors acting during pregnancy may have an impact on the later risk of disease even in the absence of any effect on size at birth (Pettitt *et al.* 1987). Therefore, directly examining these intrauterine factors (instead of birth size) in relation to later development and metabolism could be of more relevance.

Birth weight has often been used as a proxy for the 'quality' of intrauterine growth. More recently, some studies have investigated pregnancy-related conditions that affect fetal development as potential early life determinants for later obesity, greater adiposity, central fat distribution and insulin resistance. **Fetal overnutrition** and subsequent large size at birth (macrosomia or large-for-gestational age) have been linked to maternal pre-pregnancy obesity, to alterations in glucose-insulin metabolism during pregnancy (from a mild degree of glucose intolerance to gestational diabetes), to increased gestational weight gain, as well as to an unbalanced diet with high intake of carbohydrates (Clapp 2002). Macrosomia has been recognised as a potential link between intrauterine conditions associated with fetal overnutrition and the later development of obesity (Vohr *et al.* 1999).

At the other extreme of birth weight distribution, **restricted fetal growth** has been associated with low maternal pre-pregnancy BMI (Godfrey *et al.* 1997), low gestational weight gain (as a proxy for insufficient nutrient supply to the fetus) (Rogers 2003), poor quality maternal diet in early pregnancy (Ravelli *et al.* 1976), low maternal intake of proteins during pregnancy (Godfrey *et al.* 1997), pregnancy-induced hypertension (Villar *et al.* 2006), preeclampsia (Sibai 2008), advanced maternal age (Odibo *et al.* 2006), low parity (Wanzhen *et al.* 2006), multiplicity (Shinwell and Blickstein 2007;



Taylor *et al.* 1998), stress (Nkansah-Amankra *et al.* 2010) and smoking during pregnancy (Power and Jefferis 2002; Rogers 2003).

Given the reshaping of the birth weight distribution observed in Western societies in recent years, with intrauterine growth restriction occurring less frequently (Australian Institute of Health and Welfare 2000; 2010), increasing attention has been directed towards larger size at birth and its intrauterine determinants as potential risk factors for obesity and related outcomes. Therefore, this project focuses on three modifiable factors known to alter intrauterine milieu, maternal pre-pregnancy obesity, glucose intolerance during pregnancy across the entire spectrum of severity, and gestational weight gain, and their relationship to the programming of obesity and insulin resistance in pre-pubertal children.

## 2.2 Conceptualising pre-birth exposures

Intrauterine life is considered the most vulnerable period for future development, with programming effects being observed as early as the pre-implantation embryo stage (Duranthon *et al.* 2008). Plasticity *in utero* is much greater compared to any other stage of life, with the fertilised ovum undergoing 42 cycles of cell division before birth, as opposed to only 5 after birth (Barker 1994). This plasticity underpins the lifelong consequences that exposure to various factors during the fetal period might have, including coronary heart disease, hypertension, cerebrovascular disease (stroke), diabetes, obesity, altered body composition (including reduced bone mineral density), lung disease, allergic disease, cancer, neuropsychiatric disorders, and infectious diseases (Kuh and Ben-Shlomo 2004).

Pregnancy is a physiologic state characterised by profound changes in multiple organ systems: cardiovascular changes (expansion of plasma volume and red blood cells mass), renal changes (greater glomerular filtration rate and increased tubular sodium reabsorption), endocrine processes involving most hormonal axes (including the adipose-derived hormones that influence energy metabolism, adiponectin (Catalano *et al.* 2006) and leptin (Kirwan *et al.* 2002)) and metabolic changes (related to glucose, lipid and protein metabolism), which are all directed towards maintaining the pregnancy and sustaining optimal fetal development (Hyttén 1991). Among the numerous factors operating during pregnancy that may affect this physiologic state, maternal obesity, gestational glucose intolerance and excessive weight gain during pregnancy are particularly

important from a public health perspective, as they are increasingly common and, in principle, modifiable.

This section presents separately the three intrauterine exposures of interest for the current project: maternal pre-pregnancy obesity (Section 2.2.1), gestational glucose intolerance across the entire spectrum (Section 2.2.2), and excessive gestational weight gain (Section 2.2.3). First, for each of them, proposed underlying mechanisms for the metabolic perturbations they induce and their main risk factors are briefly described. Next, the public health significance of each condition is highlighted, with a focus on prevalence and incidence data worldwide and the detrimental consequences both on the mother and her child. Last, clinical responses in relation to each of these maternal intrauterine factors to limit the adverse health outcomes are described. Section 2.2.4 discusses the interrelations identified by previous studies between maternal body size prior to pregnancy, gestational glucose tolerance status and gestational weight gain.

## **2.2.1 Maternal pre-pregnancy obesity**

Weight status is most commonly characterised based on body mass index (BMI), calculated as the ratio of weight (in kilograms) to squared height (in metres). In adults BMI between 25 and 29.9 kg/m<sup>2</sup> corresponds to overweight and BMI greater than 30 kg/m<sup>2</sup> corresponds to obesity (World Health Organization 1995).

### **2.2.1.1 Pathophysiology**

It has been suggested that maternal obesity at the time of pregnancy, in combination with genetic influences, profoundly affects intrauterine development both during embryonic (first 14 weeks of gestation) and fetal periods, thus explaining the perturbations in both organ formation and fetal growth often seen in pregnancies of obese women (King 2006). There is evidence that a fetus responds to the nutrient transfer and oxygen supply from the mother through the placenta in two ways: by immediate survival choices and by long-term integrated adjustments to maximise its advantages in postnatal life (Gluckman *et al.* 2007).

The underlying mechanisms of the alterations in fetal growth induced by maternal obesity appear to revolve primarily around increasing maternal insulin resistance from early to late pregnancy, which is exacerbated in obese compared to lean women (Catalano *et al.* 1999). This increased insulin resistance has three important metabolic consequences for the pregnant woman: a reduction in the

insulin-mediated glucose uptake into the cells, leading to hyperglycaemia; a decreased ability of insulin to suppress whole-body lipolysis with secondary elevated levels of plasma free fatty acids; and a decline in the suppression of amino acid turnover with an increase in plasma levels of amino acids (King 2006). Therefore, fetuses of obese mothers are exposed to relatively high amounts of all major fuel sources (glucose, lipids and amino acids), which in turn may independently contribute to fetal overgrowth.

The distribution of adipose tissue stored during pregnancy was found to be more central (between the costal and the upper thigh areas) in obese women compared to lean women (Ehrenberg *et al.* 2003), with implications for glucose-insulin metabolism, such as relative hyperinsulinemia and an earlier (second trimester) maximal glucose response to an oral glucose load relative to women with lower-body obesity (who display these changes only in the third trimester) (Landon *et al.* 1994).

Other pathologic processes linking maternal obesity with insulin resistance and extending to fetal growth include adipocyte dysfunction, with subsequent lower circulating levels of adiponectin (a fat-derived hormone with insulin-sensitising effects) (Retnakaran *et al.* 2004) and a subclinical chronic inflammation with elevated levels of circulating cytokines, such as C-reactive protein (Retnakaran *et al.* 2003).

In addition to the marked hyperinsulinemia that characterises the pregnancy of an obese woman, gestational dyslipidemia is also exaggerated in obese women, who tend to display much higher levels of triglycerides and free fatty acids, lower levels of high-density lipoprotein cholesterol, and a significant increase in fat oxidation (Okereke *et al.* 2004). These changes in maternal lipid metabolism have been shown to play a key role in the vascular complications of obesity during pregnancy, such as preeclampsia (Ramsay *et al.* 2002), and to correlate with neonatal fat mass (Schaefer-Graf *et al.* 2008).

Obesity effects on amino acid metabolism during pregnancy have been less documented. In non-pregnant obese women, insulin-stimulated protein synthesis was reduced compared to average weight women (Chevalier *et al.* 2005), due to the insulin resistance associated with obesity. Based on these findings it was suggested that the anabolic response to pregnancy might be altered in obese women, which could explain why some obese mothers have small-for-gestational age babies (Nelson *et al.* 2010).

These obesity-related perturbations in the maternal nutritional environment have been associated not only with immediate effects on fetal growth, but also with long-term outcomes in the developing

child, such as an increased risk of obesity. The long-term metabolic consequences in pre-pubertal children will be detailed in Section 2.4.1.

#### **2.2.1.2 Public health significance – Prevalence data**

Maternal obesity prior to conception is of great public health importance as it is now highly prevalent, often associated with serious adverse health outcomes both for the mother and her child, and involves significant costs to the health care system. International data on the prevalence of overweight and obesity among women show an overall increasing trend both in developed and developing countries (mainly in urban areas of developing countries) around the world (World Health Organization 2009a). Table 1 exemplifies these rates for selected high income countries, relative to WHO recommended BMI cut-off points, contrasting the figures from the late 1990s (when the cohort on which this project is based was established) and the most recent data in the 2000s (World Health Organization 2009a). Given the different sampling frames employed in different settings and age groups considered, caution is needed for direct comparisons.

**Table 1. National prevalence of overweight and obesity in women of all ages from selected developed countries** (World Health Organization 2009a)

NOTE:  
This table is included on page 14  
of the print copy of the thesis held in  
the University of Adelaide Library.

Based on these data, approximately one third of Australian women can be classified as overweight, and about a quarter as obese (World Health Organization 2009a). As risk factors for obesity accumulate over the lifespan, the prevalence of obesity tends to increase with age; hence, rates would be lower among women of childbearing age compared to those among older women. Australian national prevalence of overweight and obesity (based on BMI calculated from measured weight and height) among women of reproductive age over two time periods is presented in Table 2.

Overall, it appears that more contemporary women of childbearing age have a higher BMI compared to the previous decade.

**Table 2. National prevalence of overweight and obesity among women of reproductive age in Australia** (Australian Bureau of Statistics 2009b)

Age group (years)	Prevalence (%) of overweight (BMI = 25-29.9 kg/m <sup>2</sup> )		Prevalence (%) of obesity (BMI ≥30 kg/m <sup>2</sup> )	
	1995	2007-2008	1995	2007-2008
18-24	17.7	20.7	8.6	14.2
25-34	23.1	26.4	15.0	18.0
35-44	29.4	32.4	16.1	22.7

BMI - body mass index

Obesity prevalence in pregnant women is less documented at a national level and is mainly generated by observational studies in specific settings, but overall it seems to have risen in parallel with the rates described in the general population of women of reproductive age. A large study conducted in nine states in the USA (Pregnancy Risk Assessment Monitoring System) showed a sharp increase in the rates of pre-pregnancy obesity (defined as BMI ≥ 29 kg/m<sup>2</sup>, calculated from self-reported data) from 13% in 1993 to 22% ten years later (Kim *et al.* 2007). A more recent British national report based on first trimester BMI data from over 600,000 pregnant women indicates that the prevalence of maternal overweight increased slightly (from 22.3% in 1989 to 25.9% in 2007), while the prevalence of obesity doubled (from 7.55% in 1989 to 15.6% in 2007) (Heslehurst *et al.* 2010). In Australia, no national data on overweight and obesity rates are available specifically in an obstetric population. Current estimates of 20.2% for overweight and 13.5% for obesity were reported based on pre-pregnancy BMI data from over 11,000 women with singleton pregnancies who delivered in 1998-2002 at Mater Mother's Hospital, Brisbane (covering 8% of all pregnancies in Queensland, Australia), after excluding women with BMI < 20 kg/m<sup>2</sup> (Callaway *et al.* 2006).

### 2.2.1.3 Adverse health outcomes of maternal pre-pregnancy obesity

Maternal pre-gravid obesity carries significant risks for the health of the woman and her child, which extend beyond the pregnancy.

#### Adverse health outcomes for the mother

For the mother, obesity complications start prior to conception and can interfere with a woman's ability to conceive (Gesink Law *et al.* 2007). Following conception, overweight and obese women

have an increased risk of miscarriage (pregnancy loss before 20 weeks gestation), whether conception was spontaneous or assisted (odds ratio OR=1.67, 95% CI 1.25, 2.25) (Metwally *et al.* 2008). One of the most commonly described pregnancy complications in overweight and obese women is gestational diabetes (Guelinckx *et al.* 2008; Torloni *et al.* 2009), with OR ranging from 1.68 (95% CI 1.53, 1.84) (Sebire *et al.* 2001) to 2.71 (95% CI 1.32, 5.55) (Doherty *et al.* 2006) in overweight women and from 2.95 (95% CI 2.05, 4.25) (Callaway *et al.* 2006) to 6.5 (95% CI 3.32, 12.74) (Doherty *et al.* 2006) in obese women, after confounder adjustments. Pregnancy of an obese woman may also be complicated by hypertensive disorders (adjusted OR = 2.60 (95% CI 1.49, 4.55) in overweight and 7.93 (95% CI 4.74, 13.27) in obese women (Doherty *et al.* 2006)), including preeclampsia (Guelinckx *et al.* 2008). Maternal obesity is also known to increase the risk of venous thromboembolic events both during pregnancy and postpartum (adjusted OR = 5.3 (95% CI 2.1, 13.5)) (Larsen *et al.* 2007). Other pregnancy complications of maternal obesity may include increased risk of induced labour (adjusted OR = 2.44 (95% CI 1.72, 3.45) (Doherty *et al.* 2006)), caesarean delivery (adjusted OR = 2.2 (95% CI 1.58, 3.12) (Doherty *et al.* 2006)) with anaesthetic and operative complications (difficult intubation and placement of epidural, excessive bleeding and postpartum infections) (Sebire *et al.* 2001). After delivery, obese women are more likely to experience difficult initiation of breastfeeding (Oddy *et al.* 2006), haemorrhage, genital and urinary tract infections, or wound infections (Sebire *et al.* 2001).

In the long term, obese mothers have an increased risk of developing hypertension, type 2 diabetes, cancer (in particular endometrial cancer), osteoarthritis, non-alcoholic fatty liver disease, gallbladder disease, pancreatitis, sleep apnoea, or depression (Pi-Sunyer 2009).

### **Adverse health outcomes for the child**

For the fetus, maternal obesity increases the risk of congenital malformations (such as neural tube defects, heart defects, cleft lip or palate, hydrocephaly, limb reduction), which are often missed during the ultrasound examination and are major causes of stillbirth and infant mortality (Stothard *et al.* 2009). Macrosomia and large-for-gestational age have been consistently reported as consequences of maternal overweight (with adjusted OR ranging from 1.2 (95% CI 1.1, 1.4) (Ehrenberg *et al.* 2004) to 1.57 (95% CI 1.50, 1.64) (Sebire *et al.* 2001)) or obesity (with adjusted OR ranging from 1.6 (95% CI 1.4, 1.9) (Ehrenberg *et al.* 2004) to 2.36 (95% CI 2.23, 2.50) (Sebire *et al.* 2001)), independently of maternal gestational glucose tolerance (Jensen *et al.* 2003). Not only fetal weight is affected by maternal obesity, but also body composition (greater percentage body fat) and insulin sensitivity (higher insulin resistance) (Catalano *et al.* 2009b). Although findings from previous

studies are not always consistent with regard to the risk of preterm delivery associated with maternal obesity (Callaway *et al.* 2006; Oddy *et al.* 2006), a systematic review and meta-analysis indicated an increased risk of preterm birth in overweight and obese women (RR=1.24, 95% CI 1.13, 1.37) (McDonald *et al.* 2010).

The far-reaching implications of maternal obesity on offspring consist of an increased risk to become obese themselves (Blair *et al.* 2007; Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Li *et al.* 2005; Reilly *et al.* 2005; Whitaker 2004) or develop metabolic syndrome (Boney *et al.* 2005). A critical appraisal of the published literature addressing the link between maternal pre-pregnancy overweight or obesity and childhood obesity and insulin resistance is presented in Section 2.4.1.

All these health consequences of maternal obesity on pregnancy outcome also incur significant economic burden to health care systems, due to more intensive hospital obstetric care required by obese women and higher rates of admission to neonatal intensive care units among infants of obese mothers (Chu *et al.* 2008; Galtier-Dereure *et al.* 2000).

#### **2.2.1.4 Clinical response to the epidemic of maternal obesity**

Extended discussion of possible responses to obesity in women of reproductive age will occur in Chapter 5. For the moment it is worth noting that in order to prevent the above mentioned adverse consequences and increase the chance of achieving healthy outcomes for mothers and their children, it has been suggested that, ideally, obese women should receive counselling prior to conception regarding the perinatal and long-term risks associated with excessive weight, as well as appropriate surveillance and support during pregnancy. In this view, the American Dietetic Association and the American Society for Nutrition recommend counselling on the roles of diet and physical activity in reproductive health in all overweight and obese women of childbearing age (American Dietetic Association *et al.* 2009). It is believed that knowledge of these risks would motivate women to adopt healthy eating and exercise regimen (Stotland and Caughey 2010), thus preventing further excessive weight gain during pregnancy, which, in turn, has been associated with adverse outcomes.

Information regarding effective intervention strategies during pregnancy in obese women to improve maternal and child health outcomes is limited. It is well established though that pharmacological treatment of obesity is unsafe during pregnancy and thus should not be recommended (Yogev and Catalano 2009). Lifestyle intervention strategies for weight loss during pregnancy in obese women



are controversial, with some authors arguing for the benefits of a reduction in the risk of delivering large-for-gestational age infants, while others emphasising the impact weight loss might have on intrauterine growth restriction (Dodd and Robinson 2011). The current Institute of Medicine recommendations for obese women are to avoid weight loss during pregnancy and to gain between 5 and 9.1 kg throughout pregnancy (Rasmussen and Yaktine 2009), much less than the recommendations for women of average weight.

Physical exercise during pregnancy has been recognised as a beneficial factor that improves maternal cardiovascular function (Clapp 2000) and insulin sensitivity (Clapp 2006b), with subsequent reduction in plasma glucose levels, fetal exposure to lower levels of glucose and subsequent decreased fetal insulin secretion, with potential influence on size at birth. The exercise regimen plays an important role; while recreational exercise does not affect birth weight (Kramer and McDonald 2006) vigorous endurance physical activity has been associated with a reduction in birth weight (Clapp 2006a). However, putative risks to the fetus have been described during maternal strenuous physical activity, such as decreased oxygen supply due to lower utero-placental blood flow secondary to increased blood flow to maternal skeletal muscles (Kennelly *et al.* 2002) and decreased glucose supply due to increased glucose use by maternal skeletal muscles (Bonen *et al.* 1992).

The effect of limiting the intake of carbohydrate and saturated fat during pregnancy in overweight or obese women remains uncertain. Clapp (2002) found a reduction in gestational weight gain associated with a low glycaemic index diet in obese pregnant women, but there is limited wider evidence on the importance or otherwise of this property of maternal diet. In one other study, in addition to the reduction in gestational weight gain, lower rates of prematurity and improved maternal plasma lipids levels were found in overweight and obese pregnant women receiving a low-glycaemic load diet during pregnancy, compared to those receiving a low-fat diet (Rhodes *et al.* 2010).

Several randomised controlled trials of lifestyle interventions (advice on physical activity and/or diet) in overweight or obese pregnant women have been completed (Asbee *et al.* 2009; Thornton *et al.* 2009) and others are underway (e.g., LIMIT trial conducted in Adelaide, South Australia, “for limiting weight gain in overweight and obese women during pregnancy to improve health outcomes” (Dodd *et al.* 2011)). In the ongoing trials, in addition to the effect on limiting gestational weight gain, the interventions will be examined with respect to the reduction in maternal insulin resistance and to the rates of large-for-gestational age infants in obese women (Nelson *et al.* 2010).



## 2.2.2 Glucose tolerance status during pregnancy

Glucose metabolism undergoes major variations during pregnancy, as described in Section 2.2.2.1. When these changes fail to be controlled by maternal compensatory mechanisms, gestational glucose intolerance arises, with various degrees of severity, culminating in gestational diabetes. **Gestational diabetes** (GD) has been defined as a clinical entity characterised by “carbohydrate intolerance with variable severity and first recognition during pregnancy” (Metzger *et al.* 1998). An amendment has been recently made to this definition, in that overt diabetes detected at the first antenatal visit (before 24-28 weeks gestation) should not be considered gestational diabetes, but rather pre-existing diabetes (International Association of Diabetes and Pregnancy Study Groups Consensus Panel *et al.* 2010). Milder degrees of impaired glucose tolerance have also been described during pregnancy, but their clinical significance is still controversial, as their adverse health implications are widely unknown. Among them, **borderline gestational glucose intolerance** (BGGI) has been defined as the mildest degree of glucose intolerance during pregnancy not meeting the criteria for diagnosing gestational diabetes (Bonomo *et al.* 2005). Details on the screening for GD are presented in Section 2.2.2.2. The following section presents the definitions of gestational diabetes and borderline gestational glucose intolerance that are employed throughout this thesis. Section 2.2.2.4 gives an overview of the factors considered to increase the risk of glucose intolerance during pregnancy and constitutes the basis for identifying potential confounders that will be included in the statistical analyses of this project. Section 2.2.2.5 describes the public health significance of gestational glucose intolerance, Section 2.2.2.6 outlines the adverse health outcomes of this pregnancy-related metabolic perturbation, while Section 2.2.2.7 presents, briefly, current approaches in its management.

### 2.2.2.1 Glucose regulation during pregnancy

#### Glucose regulation in normal pregnancies

Any pregnancy is characterised by some degree of insulin resistance, commencing towards the end of the second trimester and increasing progressively with advancing gestation to levels close to those seen in patients with type 2 diabetes (Buchanan and Xiang 2005). This is, up to a certain level, physiologic, representing an adaptive response to satisfy fetal nutritional demands for optimal intrauterine growth. Insulin sensitivity is mainly impaired peripherally, in skeletal muscle and adipose tissue (Catalano *et al.* 1993b). As a consequence of the impairment in insulin sensitivity, glucose disposal in skeletal muscle and adipose tissue is reduced by about 40% in normal pregnancies,

which in turn stimulates maternal insulin secretion and suppresses hepatic glucose production in order to maintain glucose levels within a relatively normal range (Buchanan and Xiang 2005; Catalano *et al.* 1993b).

### **Glucose regulation in pregnancies complicated by glucose intolerance**

Gestational diabetes occurs predominantly during the third trimester of the pregnancy, as a consequence of increased insulin resistance and subsequent insufficient rise in insulin secretion to overcome the insulin resistance (Catalano *et al.* 2003a).

In pregnancies complicated by glucose intolerance, in particular gestational diabetes, in addition to the reduced insulin-stimulated glucose uptake by about 54% (Friedman *et al.* 1999), the first-phase insulin response is insufficient to compensate for the peripheral insulin resistance and endogenous glucose production is less suppressed in late gestation (Catalano *et al.* 1993b). In short, as a result of the enhanced insulin resistance, maternal plasma glucose levels increase and glucose tolerance fails to be maintained, with excessive transfer of maternal energy substrates, in particular glucose and amino-acids through the placenta, which influence fetal development within the so-called 'fuel mediated teratogenesis' (Freinkel 1980). Unlike glucose and other nutrients, maternal insulin does not cross the placenta and, subsequently fetal plasma levels of insulin are dependent on secretion from the fetal pancreas only, which starts from 8-10 weeks gestation (Beardsall *et al.* 2008). Given that placental glucose transport depends on diffusion down a concentration gradient only, maternal hyperglycaemia is followed by fetal hyperglycaemia. This, in turn, stimulates the fetal pancreas to secrete more insulin compared to fetuses of normoglycaemic mothers. Insulin acts as a growth-promoting factor (Beardsall *et al.* 2008). Thus, fetal hyperinsulinemia secondary to maternal hyperglycaemia is followed by an accelerated rate of growth *in utero* and subsequently greater size at birth (Vohr *et al.* 1995; Vohr and McGarvey 1997). The accelerated fetal growth, as a consequence of fetal hyperinsulinism, mainly accounts for an increase in adipose tissue and percentage body fat at birth, and not lean mass (Catalano *et al.* 1995), with potential metabolic consequences later in life. Greater adiposity, likely due to fetal hyperinsulinism, has also been observed in appropriate-for-gestational age infants of mothers with GD compared to those of women with normal glucose tolerance during pregnancy (Catalano *et al.* 2003b).

### **Mechanisms of insulin resistance during pregnancy**

A number of underlying mechanisms have been explored to explain the enhanced insulin resistance during pregnancies complicated by GD, albeit that they are still largely unknown. It has been

suggested that insulin resistance developed during pregnancy would be directly associated with the amount of maternal adiposity gained in early pregnancy, the insulin-desensitising effects of placental derived hormones (such as human placental lactogen and human placental growth hormone) (Barbour *et al.* 2007; Buchanan and Xiang 2005), and a series of adipokines (adiponectin, tumour necrosis factor (TNF)-alpha) (Barbour *et al.* 2007). More specifically, it has been found that this insulin resistance is linked to defects at post-receptor level (in the intracellular insulin signalling pathway (Catalano 2010)) in insulin-sensitive tissues, such as adipose tissue, skeletal muscle, and placenta (Colomiere *et al.* 2010).

It has been hypothesised that a certain degree of insulin resistance exists even prior to conception in women who develop GD (Buchanan 2001). Supporting this hypothesis is the finding of higher levels of C-peptide (a marker of glucose-triggered insulin release) in women with GD, compared to women who maintain normal glucose tolerance during pregnancy (in the latter category of women, C-peptide level increases between 20 and 32 weeks of gestation) (Weijers *et al.* 2002). In contrast, pregnancies complicated by milder degrees of glucose intolerance display C-peptide levels similar to normal pregnancies (Weijers *et al.* 2002), suggesting the absence of antedating insulin resistance.

#### **2.2.2.2 Screening and diagnostic tests for glucose intolerance during pregnancy**

As GD is generally asymptomatic (McIntyre *et al.* 2005), it can be detected only based on clinical tests. Screening tests have been sought given the potential for adverse health outcomes of GD, initially considering women's risk of later developing type 2 diabetes, and more recently, incorporating the harm to the offspring, in particular macrosomia (as detailed in Section 2.2.2.6). The utility of universal screening for GD, which would maximise the diagnosis and give opportunity for intervention to improve pregnancy outcomes, is still under debate (Metzger *et al.* 2007). The National Institute for Health and Clinical Excellence in the UK and the American Diabetes Association (ADA) recommend **selective screening**, on the basis of risk factor assessment, such as pre-gravid obesity, advanced age, high-risk ethnicity and family history of diabetes (American Diabetes Association 2002; 2006; National Institute for Health and Clinical Excellence 2008). In contrast, the Australasian Diabetes in Pregnancy Society (ADIPS) has recommended **universal screening** since 1998 (Government of South Australia 2007). Screening is usually recommended to be performed at 24-28 weeks of pregnancy (American Diabetes Association 2006; Hoffman *et al.* 1998). However, pregnant women at high risk of GD are recommended to undergo the screening test earlier in pregnancy (Hoffman *et al.* 1998).

### **Oral glucose challenge test**

The most validated screening method for GD consists of a non-fasting oral glucose challenge test (OGCT), with assessment of plasma glucose levels 1 hour after a 50 g oral glucose load (American Diabetes Association 2002). The sensitivity of OGCT has been estimated as 79% and specificity as 86% (O'Sullivan and Mahan 1964). The optimum cut-offs for a positive challenge test are still uncertain. Most commonly, women with a positive challenge test result (plasma glucose level exceeding the threshold of 7.8 mmol/l) are either diagnosed with GD if the glucose level is higher than 11.1 mmol/l, or tested further by an oral glucose tolerance test if the plasma glucose level is between 7.8 and 11.1 mmol/l (World Health Organization 1985). Plasma glucose levels lower than 7.8 mmol/l are considered normal (World Health Organization 1985).

### **Oral glucose tolerance test**

The oral glucose tolerance test (OGTT) is the diagnostic test for GD. It needs to be performed after an 8-14 hour fasting period and minimum three days of unrestricted diet and unlimited physical activity (American Diabetes Association 2002). These requirements are one of the reasons a screening test is usually applied first, to rule out the majority of women who do not need to be burdened in this manner. There are two protocols for the OGTT: one based on 100 g glucose load and measurement of plasma glucose level every hour for 3 hours after oral loading (initially proposed by O'Sullivan and Mahan (1964) and modified by Carpenter and Coustan (1982)), and the second, based on 75 g glucose load and plasma glucose level measurements at baseline and after 2 hours (World Health Organization 1985).

A number of sets of criteria for the diagnosis of GD have been proposed, with some variation in glucose threshold values (Table 3). There is no universal consensus in the use of a particular set of diagnostic criteria and hence the choice of particular glucose thresholds for the diagnosis of gestational diabetes is according to the local institution's policy. Moreover, it is important to note that categories of glucose tolerance during pregnancy have been proposed to facilitate interpretation, but in both OGCT and OGTT, plasma glucose level is a continuous variable. This aspect is particularly important given the linear relationship found between hyperglycaemia during pregnancy, as a continuum, rather than a threshold effect, and the risk of adverse outcomes (HAPO Study Cooperative Research Group 2008). As an alternative to the sequential screening/diagnosis strategy described above, performing the diagnostic OGTT directly without preceding it by an OGCT has been recommended as a cost-effective approach in high risk populations (American Diabetes

Association 2002). However, in the general population, the two-step approach (OGCT followed by OGTT) was more cost-effective than OGTT alone or no-screening at all (Nicholson *et al.* 2005).

**Table 3. Cut-off values for plasma glucose levels (mmol/l) recommended for the diagnosis of gestational diabetes in oral glucose tolerance test (OGTT)**

	NDDG 100 g glucose load	ADA Carpenter and Coustan 100 g glucose load	ACOG 100 g glucose load	WHO 75 g glucose load	ADIPS 75 g glucose load	NZSSD 75 g glucose load
Fasting glucose	≥ 5.8	≥ 5.3	≥ 5.8	≥ 7.8	≥ 5.5	≥ 5.5
1-h post-load	≥10.5	≥10.0	≥10.6			
2-h post-load	≥ 9.2	≥ 8.6	≥ 9.2	≥11.1	≥ 8.0	≥ 9.0
3-h post-load	≥ 8.0	≥ 7.8	≥ 8.1			

NDDG - National Diabetes Data Group; ADA - American Diabetes Association; ACOG - American College of Obstetricians and Gynecologists; WHO - World Health Organization; ADIPS - Australasian Diabetes in Pregnancy Society; NZSSD - New Zealand Society for the Study of Diabetes

### Other screening methods for gestational diabetes

Other screening methods for GD have been proposed, but they are controversial and hence less often used in practice. They include measurement of fasting plasma glucose level (HAPO Study Cooperative Research Group 2008), measurement of random plasma glucose level (Government of South Australia 2007), or measurement of glycosylated haemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>). The latter method provides an estimate of the mean plasma glucose levels in the three months preceding the test (Gabbe 2003; Lapolla *et al.* 2007) and, if measured early in pregnancy, is particularly useful to detect pre-gestational type 2 diabetes.

#### 2.2.2.3 Defining categories of gestational glucose intolerance based on oral glucose challenge test and oral glucose tolerance test

Stratification of pregnant women into glucose tolerance categories differs according to the diagnostic criteria used. Based on the American Diabetes Association criteria, the diagnosis of GD requires at least two out of four elevated plasma glucose levels above the thresholds for the OGTT over the 3 hours (American Diabetes Association 2006). If only one threshold value at OGTT is exceeded, the woman is considered as having impaired glucose intolerance (IGT), while a positive OGCT followed by a normal OGTT (no increased plasma glucose levels during OGTT) is regarded as borderline gestational glucose intolerance (Bonomo *et al.* 2005).

In Australia, based on ADIPS criteria, women are diagnosed with GD if at least one of the OGTT threshold values is exceeded (i.e., either fasting plasma glucose level is  $\geq 5.5$  mmol/l and/or 2-h post-glucose load is  $\geq 8.0$  mmol/l) (Hoffman *et al.* 1998). In this context, borderline gestational glucose intolerance (BGGI), can be defined based on a positive OGCT and a normal OGTT (Ju *et al.* 2008) (i.e., both fasting plasma glucose level is  $< 5.5$  mmol/l and 2-h post-glucose load is  $< 8.0$  mmol/l). For this project, ADIPS criteria are employed and this definition of BGGI is used throughout the thesis.

#### 2.2.2.4 Risk factors for glucose intolerance during pregnancy

##### Risk factors for gestational diabetes

Gestational diabetes is considered a multifactorial disease. The risk factors for GD, often interrelated, are outlined below, grouped from a life course perspective.

##### **Maternal and familial factors:**

- Family history of type 2 diabetes in first-degree relatives (Kim *et al.* 2009; McLean *et al.* 2006; Solomon *et al.* 1997);
- Being a member of an ethnic group with a high prevalence of diabetes, such as Australian Aboriginal, Pacific Islander, Hispanic, Native American, African American, South and East Asian (Hoffman *et al.* 1998; Solomon *et al.* 1997);
- Maternal own birth weight has an unclear relationship to the risk of developing GD. Some studies indicate an inverse relationship between own birth weight and the risk of glucose intolerance during pregnancy (Bo *et al.* 2003; Seghieri *et al.* 2002), while other studies support a U-shaped or J-shaped relationship, with both high ( $> 4000$  g) and low birth weight ( $< 2000$  g) increasing the subsequent risk of GD (Innes *et al.* 2002);
- Maternal short stature (Bo *et al.* 2001; Branchtein *et al.* 2000; Dode and dos Santos 2009; Jang *et al.* 1998; Ogonowski and Miazgowski 2010);
- Polycystic ovary syndrome (Hanna and Peters 2002).

##### **Risk factors related to previous pregnancy history:**

- Gestational diabetes in a previous pregnancy (Bottalico 2007; Dudhbhai *et al.* 2006; Radesky *et al.* 2008; Tieu *et al.* 2008), most likely due to the persistence of other risk factors;

- Previous history of macrosomia (Bottalico 2007; Corrado *et al.* 2006);
- Previous unexplained stillbirth (Robson *et al.* 2001);
- Previous history of complicated pregnancy: preeclampsia, hypertension, polyhydramnios (Bottalico 2007; Corrado *et al.* 2006).

**Risk factors related to current pregnancy:**

- Advanced maternal age (Bottalico 2007; Corrado *et al.* 2006; Dudhbai *et al.* 2006; Montan 2007; Solomon *et al.* 1997) as a continuum rather than a threshold effect;
- Parity (Dudhbai *et al.* 2006; Seghieri *et al.* 2005);
- Pre-pregnancy obesity (Torloni *et al.* 2009); in addition to pre-pregnancy obesity, a greater weight gain in the 5 years prior to pregnancy further increases the risk of GD (Hedderson *et al.* 2008);
- Gestational weight gain greater than 1.2 kg in the first trimester, or greater than 0.4 kg/week in the second and third trimesters (Bonomo *et al.* 2005);
- Recurrent glycosuria (i.e., the presence of glucose in the urine) during pregnancy (American Diabetes Association 2006; Bottalico 2007).

**Other risk factors:**

- Diet: high glycaemic index diets (Clapp 2002), increased intake of saturated fat (Bo *et al.* 2001); higher consumption of red and processed meat (Zhang *et al.* 2006a);
- Physical inactivity: regular exercise before pregnancy lowered the risk of GD (Zhang *et al.* 2006b), or even of mild gestational glucose intolerance, especially if they continued with light-moderate physical activity in early pregnancy (Oken *et al.* 2006);
- Cigarette smoking (Wendland *et al.* 2008);
- Socioeconomic status (lower SES) (Anna *et al.* 2008; Australian Bureau of Statistics 2001; Bo *et al.* 2002; Clausen *et al.* 2006).

According to ADIPS, the highest risk of developing GD is associated with maternal age over 30 years, obesity, family history of diabetes, GD in a previous pregnancy, certain ethnicity, and past history of complicated pregnancy (Hoffman *et al.* 1998).



From a public health perspective, it is important to consider whether the risk factors for GD are modifiable or not. Arguably, some of the risk factors may be modifiable only at population level and not at individual level (e.g., women's BMI at the time of pregnancy cannot be modified once they present at the prenatal visits, although BMI is potentially modifiable at population level).

### **Risk factors for borderline gestational glucose intolerance**

There are fewer studies investigating risk factors for milder degrees of glucose intolerance during pregnancy, such as BGGI, but the risk factors seem to be similar to those for GD. These risk factors include: pre-pregnancy obesity (Weijers *et al.* 2002), advanced maternal age (Ju *et al.* 2008), parity (Dudhbhai *et al.* 2006), maternal low birth weight (Bo *et al.* 2003), increased intake of fat (Saldana *et al.* 2004), and sedentary behaviours prior to and during pregnancy (Oken *et al.* 2006). Higher gestational weight gain up to the time of the screening for GD did not increase the risk of GD or BGGI (defined by a positive screening test and a negative 3 h OGTT), but it doubled the risk of impaired glucose tolerance (defined as a positive screening test and one high plasma glucose level at 3-h OGTT) (Herring *et al.* 2009). Smoking did not seem to be associated with the risk for mild gestational glucose intolerance (Ju *et al.* 2008).

#### **2.2.2.5 Public health significance – Prevalence data**

##### **Prevalence of gestational diabetes**

Gestational diabetes is affecting increasing numbers of pregnant women worldwide (Ferrara 2007; Hunt and Schuller 2007). Some reports express these rates in terms of *prevalence* (defined as the total number of all individuals affected by the condition in a given population at a designated time), while others as *incidence* (defined as the number of new individuals affected by the disease in a specified population within a defined period of time). Given that GD is a transient condition, with a relatively short duration (3-4 months), new cases do not add much to the pool of condition in the population, as other cases resolve at the same time. Therefore, the incidence of GD does not differ much from its prevalence in a population in a specified period of time.

The prevalence of GD depends mainly on the population racial and ethnic characteristics, the clinical practice for detection of diabetes before and during pregnancy, and the diagnostic criteria used (basically, using lower plasma glucose thresholds leads to a higher prevalence by including a larger number of women into the diabetic group and vice versa). At the same time, the distribution of various risk factors in the population, such as family history of diabetes, influences this prevalence.



Thus, the prevalence of GD varies in different studies according to different criteria of diagnosis and country, ranging from 2.2% in Korea (Cheung and Byth 2003), to 4% in the USA (American Diabetes Association 2006), and to 8.8% in Australia (Beischer *et al.* 1996). In Western countries, the most likely reasons for this elevated prevalence are the higher rate of pre-pregnancy obesity, the increasing maternal age at conception (Australian Bureau of Statistics 2006) and the more widespread implementation of screening strategies for GD.

Studies conducted in Australia at different points in time, some hospital-based others population-based, both retrospective and prospective, most of them using ADIPS criteria, showed different rates of GD (Hunt and Schuller 2007). Their main findings are summarised in Table 4.

**Table 4. Prevalence and incidence rates of gestational diabetes reported in Australia**

Reference	Study design, sample frame, time frame	Setting	Ethnicity (n)	Prevalence (P) or incidence (I) of gestational diabetes
Beischer <i>et al.</i> 1996	Hospital-based, retrospective, 1971-1994	Melbourne	Not specified (27,111 in 1971-1980) (16,820 in 1991-1994)	I: 2.9% in 1971-1980 I: 8.8% in 1991-1994
Davey and Hamblin 2001	Hospital-based, retrospective, 1996-1998	Melbourne	Not specified (6,032)	I: 5.2%
Ishak and Petocz 2003	Population-based, retrospective, 1988-1999	South Australia	Non-Aboriginal (225,000) Aboriginal (4,800)	P: 2.4% non-Aboriginal 4.3% Aboriginal
Kim and Humphrey 1999	Hospital-based, 1992-1996	Cairns	Aboriginal, Torres Islanders, Australian-born Caucasian, others (7,576)	I: 14.4% in 1992 13.4% in 1993 11.1% in 1994 7.3% in 1995 5.3% in 1996
Moses <i>et al.</i> 1994	Population-based, retrospective, 1993	New South Wales, Illawarra area	Australasian, Aboriginal and Pacific Islander, Asian, Northern and Southern European, others (2,152)	I: 7.2% overall 7.1% Australasian 11.9% Asian
Stone <i>et al.</i> 2002	Population-based, retrospective, data linkage, 1996	Victoria	Non-Aboriginal (59,962) Aboriginal (438)	I: 3.6% overall 3.6% non-Aboriginal 4.3% Aboriginal
Yue <i>et al.</i> 1996	Hospital-based, prospective, 1996	Sydney	Anglo-Celtic, Aboriginal, Asian (5,250)	P: 6.7% overall 3.0% Anglo-Celtic 7.3% Arab 9% Vietnamese 10% Aboriginal 15% Chinese 17% Indian

A retrospective population analysis of all deliveries in South Australia between 1988 and 1999 showed 2.5 fold higher rates of age-standardised GD in Aboriginal compared to non-Aboriginal women (Ishak and Petocz 2003). However, the trends in GD prevalence over the 12 year period studied were different, with a 72% increase among non-Aboriginal women and only 12% increase among Aboriginal women, potentially attributable to a lower accessibility of health care services in the latter group of women.

According to the most recent national data based on counts of diagnosed cases, the incidence of GD among Australian women aged 15-49 years has increased by 20% in the last 5 years, reaching 4.6% in 2005-2006 (Australian Institute of Health and Welfare 2008). The reported rate of GD in different states of Australia ranged between 2.8% in Tasmania to 5.2% in Queensland, while in South Australia it was 4.9% (Australian Institute of Health and Welfare 2009).

Advanced age places pregnant women at a higher risk of GD. Given the current trend in delayed childbearing, this adds to the burden of this condition. The latest Australian report on the incidence of GD indicated that 13% of women aged 44-49 years were affected, as opposed to only 1% among pregnant women of age under 20 years (Australian Institute of Health and Welfare 2008). Based on the same report, of all Australian women whose pregnancies were complicated by GD in 2005-2006, the highest proportion was in the age group of 30-34 years (Australian Institute of Health and Welfare 2008).

Ethnicity is another factor that influences the incidence of GD. Aboriginal and Torres Strait Islander women have a 1.5 fold increased risk of developing GD compared to non-Indigenous Australian women (Australian Institute of Health and Welfare 2008). Another interesting finding of this national report refers to the double incidence rate of GD among women born overseas, especially in Southern Asia, compared to Australian born women (Australian Institute of Health and Welfare 2008).

### **Prevalence of borderline gestational glucose intolerance**

Although milder degrees of gestational glucose intolerance, defined by an abnormal OGCT and a normal OGTT, are likely to be more prevalent than GD, fewer reports have addressed their public health impact. Similar to GD, the use of different criteria to define mild impairment of glucose tolerance during pregnancy provides different prevalence rates of this condition. In Australia, based on ADIPS criteria, borderline gestational glucose intolerance can be defined as a positive OGCT (plasma glucose level 1 hour post-50 g glucose load  $\geq 7.8$  mmol/l) but a negative OGTT (fasting plasma glucose level  $< 5.5$  mmol/l and 2-h plasma glucose level  $< 8.0$  mmol/l post-75 g glucose

load). A recent analysis of OGCT and OGTT results available from 1,804 pregnant women who participated in the Australian Collaborative Trial of Supplements (for which data collection took place in 2002-2004) indicates a prevalence of BGGI of 8%, double the prevalence of GD (Ju *et al.* 2008).

### **2.2.2.6 Adverse health outcomes of gestational glucose intolerance**

#### **Adverse health outcomes of GD**

A wide range of perinatal and long-term postpartum adverse health outcomes have been described in pregnancies complicated by gestational diabetes, affecting both the mother and the child.

#### **Adverse health outcomes for the mother**

Women with GD have an increased risk of hypertensive disorders (RR=1.54, 95% CI 1.28, 2.11) (Joffe *et al.* 1998), including preeclampsia (adjusted OR=1.6, 95% CI 1.4, 1.9) (Stone *et al.* 2002), a higher need for induction of labour (adjusted OR=3.0, 95% CI 2.7, 3.4) (Stone *et al.* 2002) and caesarean section (adjusted OR=1.7, 95% CI 1.6, 1.9) (Stone *et al.* 2002) compared to women with normal glucose tolerance during pregnancy. These adverse outcomes for the mother also have flow on effects for the infant.

Although GD is generally a transient condition and reverts to normal glucose tolerance after delivery, in 30-70% of the women it is followed by type 2 diabetes in the 5 years following the pregnancy (Kim *et al.* 2002; McLean *et al.* 2006), possibly due to a residual dysfunction of the pancreatic beta-cells (Buchanan 2001). The risk of developing overt type 2 diabetes is exacerbated if the woman is obese during pregnancy (Guelinckx *et al.* 2008). It has been suggested that GD represents early unmasked type 2 diabetes (Yue *et al.* 1996). Population attributable risk (PAR) of gestational diabetes for type 2 diabetes (i.e., the proportion of cases of type 2 diabetes in women that could be related to the previous development of GD) varies in different populations and with different diagnostic criteria. Based on four longitudinal studies conducted in Australia (Beischer *et al.* 1996; Davey and Hamblin 2001; Martin *et al.* 1995; Moses *et al.* 1994), PAR was estimated to 0.21-0.31 (Cheung and Byth 2003). As a consequence, GD along with overweight and obesity have been included in the key areas for monitoring diabetes in Australia, with a potential for primary prevention of type 2 diabetes (Australian Institute of Health and Welfare 2006).

### **Adverse health outcomes for the child**

As GD develops later during pregnancy, it does not affect the formation of fetal organs and does not cause birth defects as seen in children of mothers with diabetes antedating pregnancy. However, it occurs at a time when the fetus is growing intensively and therefore it can affect body size, body composition and glucose-insulin metabolism. Perinatal risks for the newborn of a mother with GD are well recognized and include macrosomia (Van Assche *et al.* 2001) (adjusted OR=2.0, 95% CI 1.8, 2.3) (Stone *et al.* 2002), secondary birth trauma (such as shoulder dystocia, bone fractures, and nerve palsies), respiratory distress syndrome (adjusted OR=1.6, 95% CI 1.2, 2.2) (Stone *et al.* 2002), hypoglycaemia (Langer *et al.* 2005) and neonatal jaundice (adjusted OR=1.4, 95% CI 1.2, 1.7) (Stone *et al.* 2002). Not only size at birth, but body composition is influenced by the exposure to an intrauterine diabetic environment. Increased body adiposity was described by skinfold measures and total body electrical conductivity in newborns of mothers with GD, even if they had normal birth weight (Catalano *et al.* 2003b).

The long-term detrimental consequences of exposure to GD for the child include an increased risk of obesity (Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Chandler-Laney *et al.* 2011; Egeland and Meltzer 2010; Krishnaveni *et al.* 2010; Pettitt *et al.* 1983; Pettitt *et al.* 1985; Silverman *et al.* 1991), insulin resistance (Boerschmann *et al.* 2010; Dabelea *et al.* 2000; Egeland and Meltzer 2010; Keely *et al.* 2008; Krishnaveni *et al.* 2010), impaired glucose tolerance (Malcolm *et al.* 2006; Silverman *et al.* 1995; Silverman *et al.* 1998), higher systolic blood pressure (Cho *et al.* 2000; Wright *et al.* 2009), and potentially some degree of impaired intellectual development (Rizzo *et al.* 1997; Silverman *et al.* 1998). A critical appraisal of studies addressing the long-term metabolic implications of GD in the offspring is presented in Section 2.4.2.

### **Adverse health outcomes of BGGI**

Evidence that various degrees of glucose intolerance during pregnancy, less severe than gestational diabetes, may be associated with adverse consequences is mounting. To date, the most compelling evidence on the perinatal outcomes of elevated plasma glucose levels has been provided by the multinational study on Hyperglycemia and Adverse Pregnancy Outcome (HAPO), conducted in approximately 25,000 pregnant women from the USA, Canada, Europe, Asia and Australia, enrolled during 2000-2006, all receiving a 75-g OGTT between 24-32 weeks gestation (women with overt GD, i.e., fasting plasma glucose > 5.8 mmol/l or 2-hour plasma glucose level > 11.1 mmol/l, were excluded) (HAPO Study Cooperative Research Group 2002). This large study found a positive,

continuous association between maternal plasma glucose (each of the fasting, 1-hour and 2-hour levels during OGTT) in pregnancy across the spectrum and the risk of perinatal outcomes, such as macrosomia, higher levels of umbilical cord C-peptide (fragment of insulin), neonatal hypoglycaemia and caesarean section, with no evident cut-off value for plasma glucose levels at which these risks increased (HAPO Study Cooperative Research Group 2008).

### **Adverse health outcomes for the mother**

There is some evidence that mild glucose intolerance during pregnancy contributes to the risk of preeclampsia (Dodd *et al.* 2007; Yogev *et al.* 2004), preterm birth (Yang *et al.* 2002) and need for caesarean section (HAPO Study Cooperative Research Group 2008). In the longer term, it has been shown that not only the risk of developing type 2 diabetes may be increased in women with BGGI, but also the risk of cardiovascular disease, such as acute myocardial infarction or stroke; however, this risk is lower than in women with pregnancies complicated by GD (Retnakaran and Shah 2009). Moreover, it has been shown that among women with mild gestational glucose intolerance, those with isolated hyperglycaemia at 1 hour during OGTT have the highest degree of postpartum metabolic dysregulation, similar to those with GD (Retnakaran *et al.* 2008).

### **Adverse health outcomes for the child**

Perinatal deleterious effects of intrauterine exposure to milder degrees of gestational glucose intolerance include macrosomia (Dodd *et al.* 2007; HAPO Study Cooperative Research Group 2008; Vambergue *et al.* 2000; Yang *et al.* 2002), birth injuries and neonatal hypoglycaemia (Dodd *et al.* 2007; HAPO Study Cooperative Research Group 2008). While acknowledging the potential role of mild hyperglycaemia in pregnancy in excessive fetal growth, some reports suggest that the effect might be attributed, at least partly, to maternal obesity at the time of pregnancy (Ricart *et al.* 2005). Furthermore, it has been shown that maternal elevated level of triglycerides in the second trimester of gestation independently predicted macrosomia for women with impaired glucose tolerance during pregnancy (Bo *et al.* 2004).

Increased maternal plasma glucose levels in pregnancy, even if they are not high enough for a diagnosis of GD, have been positively associated with offspring size during infancy (at 6-8, 24-36 and 96-120 weeks), and negatively associated with child's growth trajectory by the age of 2 years, known as a 'catch-down' or decelerated growth (Stenhouse *et al.* 2006). It has been suggested that the adverse outcomes of hyperglycemia during pregnancy on the offspring extend beyond infancy as a continuum across the range of maternal glucose tolerance (Beardsall *et al.* 2008), but the evidence

is limited. The few studies conducted in non-high risk populations have suggested that maternal hyperglycaemia in pregnancy, across the spectrum, is related to the risk of obesity in the child (Chandler-Laney *et al.* 2011; Hillier *et al.* 2007). A critical appraisal of studies addressing the long-term metabolic implications of milder degrees of gestational glucose intolerance in the offspring is presented in Section 2.4.2.

### 2.2.2.7 Management of gestational glucose intolerance

#### **Management of GD**

In order to assess the efficacy of preventing the adverse health implications posed by GD, several randomised trials have focussed on the effects of tight glycaemic control compared to routine antenatal care. The Australasian Carbohydrate Intolerance Study in Pregnancy (ACHOIS), a large-scale randomized-treatment trial conducted in Adelaide, Australia, demonstrated that GD management consisting of dietary advice, blood glucose monitoring, and insulin when required, improved perinatal outcomes in both mother and child (Crowther *et al.* 2005). Briefly, infants in the intervention group had reduced rates of serious perinatal outcomes (death, shoulder dystocia, fracture and/or nerve palsy), lower mean birth weight, and lower rates of macrosomia, while their mothers reported improved health-related quality of life, were more likely to need induction of labour, with no differences in the rates of caesarean section, had lower weight gain during pregnancy (between first booking and last antenatal visit) and lower rates of preeclampsia (Crowther *et al.* 2005). Based on ACHOIS findings, the current management of GD in Australia consists of dietary advice (Tieu *et al.* 2008), blood glucose monitoring (with an aim for fasting plasma glucose of 3.5-5.5 mmol/l and 2 hour postprandial plasma glucose of 4.0-7.0 mmol/l), and insulin when required (i.e., if fasting plasma glucose is  $\geq 5.5$  mmol/l at least once a week or postprandial plasma glucose is  $\geq 7.5$  mmol/l at least twice a week) (Government of South Australia 2007).

A more recent randomised clinical trial of similar scale conducted in the US, in which women with mild GD (defined as two or three plasma glucose levels above the thresholds, but fasting glucose  $< 5.3$  mmol/l at OGTT) were randomly assigned to treatment or routine care, showed no difference in the rates of a composite outcome including stillbirth, perinatal death, neonatal hyperbilirubinemia, hyperinsulinemia, and birth trauma, but found clear benefits of treatment in reducing mean birth weight, neonatal fat mass, rates of large-for-gestational-age or macrosomia, shoulder dystocia, caesarean delivery and preeclampsia (Landon *et al.* 2009).

In recent years, oral glucose lowering drugs (such as Glibenclamide or Metformin) have been proposed as an alternative to insulin during pregnancies complicated by GD. Several randomised controlled trials and observational studies are currently underway to evaluate the perinatal effects of such treatments on maternal and neonatal outcomes (Alwan *et al.* 2009). It is, at this stage, uncertain what beneficial or adverse effects they may have in the long-term for both mothers and children.

Further research is being undertaken to examine whether management of GD has long-term benefits for the mother and the child. Based on a US data-linkage study, Hillier *et al.* (2007) showed that among 5-7 year old children exposed to GD, the risk of overweight (OR=1.89, 95% CI 1.30, 2.76) and obesity (OR=1.82, 95% CI 1.15, 2.88) was attenuated if the mother received dietary advice and insulin if required (OR<sub>weight>85th</sub>=1.29, 95% CI 0.85, 1.97; OR<sub>weight>95th</sub>=1.38, 95% CI 0.84, 2.27). In contrast, data from the 4-5 year follow-up of the children whose mothers took part in the ACHOIS trial did not support a benefit of treatment for GD on weight status in children (Gillman *et al.* 2010).

### **Management of BGGI**

Intervention strategies designed to decrease the occurrence of adverse perinatal complications in women with BGGI have also started to be evaluated, but so far evidence has been insufficient to justify routine treatment. A randomised clinical trial in Italy assessed the effect of treating BGGI by offering dietary advice and blood glucose monitoring (Bonomo *et al.* 2005). Bonomo *et al.* (2005) reported a lower incidence of large-for-gestational age infants and lower neonatal ponderal index (weight divided by length<sup>3</sup> x 100) in the treatment group compared to non-treatment group or controls. Long-term benefits of treatment for BGGI are completely unknown.

### **2.2.3 Gestational weight gain**

Gestational weight gain (GWG) has been regarded as an indicator of maternal and fetal wellbeing (Institute of Medicine 1990). It has been suggested that excessive GWG, similar to maternal obesity prior to pregnancy, may induce metabolic processes that influence the intrauterine environment, with subsequent effects on fetal development.

Initial Institute of Medicine (IOM) recommendations for GWG (Institute of Medicine 1990) were directed to correct insufficient GWG and thus reduce prevalence of low birth weight infants.

Subsequently, given the increasing trend of obesity prevalence, these recommendations have been



revised (Rasmussen and Yaktine 2009) and the focus has been extended to also include excessive GWG. Current IOM recommendations for GWG are stratified by maternal weight status at the time of pregnancy (Rasmussen and Yaktine 2009), as outlined in Table 5.

**Table 5. 2009 Institute of Medicine (IOM) recommendations for gestational weight gain (GWG) for singleton pregnancies** (Rasmussen and Yaktine 2009)

NOTE:  
This table is included on page 34  
of the print copy of the thesis held in  
the University of Adelaide Library.

### 2.2.3.1 Pathophysiology

Weight gain represents a physiological process during pregnancy, aimed to facilitate transfer of nutrients across the placenta and thus maintain normal fetal development (King 2006). It comprises *products of conception* (including the fetus, placenta and the amniotic fluid), *changes in maternal tissues* (uterus, breasts, blood, and, to a smaller extent, liver and intestinal mucosa), and *changes in body composition* (with a significant increase in total body water, subcutaneous fat and protein reserves) (Hyttén 1991).

Compared to the water or protein components, the amount of fat stored during pregnancy is more strongly correlated with total GWG and is thought to contribute the most to the development of offspring obesity later in life (Committee on the Impact of Pregnancy Weight on Maternal and Child Health 2007). The majority of fat deposited during pregnancy is subcutaneous; this fat store is built progressively up to about 30 weeks of gestation, mainly in the abdominal region, back and upper thighs, representing about one third of GWG (Hyttén 1991).

The rate of weight gain is not constant throughout pregnancy and therefore the global pattern over time has a slight sigmoid shape, rather than linear (Hyttén 1991). The weekly rates are lower before 16-18 weeks of gestation (0.36 kg/wk), higher between 16-32 weeks (0.45 kg/wk), followed by slower rates towards the term (0.36 kg/wk) (Hyttén 1991). The maximum weekly weight gain was found at



20-24 weeks (Dawes and Grudzinskas 1991). GWG is influenced by maternal pre-pregnancy weight status, age, parity (Hyttén 1991), and less so by smoking, blood pressure (Dawes and Grudzinskas 1991; Widschut 2006) or diet (Widschut 2006).

There are wide variations among studies with respect to the predictors of GWG, but pre-pregnancy weight status seems to play a major role, being inversely associated with GWG. Four categories of determinants of GWG have been identified, with complex interactions among them, which are still incompletely understood and require further research. They include *biological factors* (such as maternal pre-pregnancy BMI, age, parity, stature (Hyttén 1991)), *metabolic factors* (variations in insulin (Scholl and Chen 2002) and leptin (Stein *et al.* 1998) levels), *social factors* (e.g., maternal education, socioeconomic status, smoking, substance abuse, unintended pregnancy, and health provider advice regarding physical activity and diet) (Committee on the Impact of Pregnancy Weight on Maternal and Child Health 2007) and, more recently, *genetic factors* (Dishy *et al.* 2003; Tok *et al.* 2006).

### **2.2.3.2 Public health significance - Prevalence data**

In parallel with the increasing prevalence of obesity amongst women of childbearing age, there has been an increase in the proportion of women with GWG exceeding IOM recommendations, particularly in overweight and obese women, across all population groups (Committee on the Impact of Pregnancy Weight on Maternal and Child Health 2007; Rasmussen and Yaktine 2009). US national reports based on self-reported GWG recorded in birth certificates indicate a marked increase in the proportion of women with singleton term pregnancy gaining more than 18.2 kg during pregnancy from 16% in 1990 (National Center for Health Statistics 2007) to 21% in 2006 (National Center for Health Statistics 2009), reaching a plateau in 2008 (National Center for Health Statistics 2010). At the same time, a small decline over time in the proportion of women gaining below the recommended weight was also noted (National Center for Health Statistics 2010). More recent figures based on data obtained from the Pediatric and Pregnancy Nutrition Surveillance System and the current (2009) IOM recommendations indicate that less than one third of the white, non-Hispanic US women gain within the recommended ranges, while 18.4% gain below and 52.6% above these recommendations (Centers for Disease Control and Prevention 2010). There is no national Australian data regarding weight gain in pregnancy.

### 2.2.3.3 Adverse health outcomes of excessive gestational weight gain

Independently of pre-pregnancy BMI, or in combination with it, excessive GWG has been associated with a range of adverse health outcomes for the mother and the child, both short- and long-term.

#### Adverse health outcomes for the mother

Perinatal complications of excessive GWG for the mother include an increased risk of preeclampsia (adjusted OR=2.31, 95% CI 2.15, 2.49 in normal weight women; adjusted OR=1.88, 95% CI 1.72, 2.06 in overweight women) (Cedergren 2006), caesarean section (adjusted OR=1.24, 95% CI 1.19, 1.29 in normal weight women; adjusted OR=1.23, 95% CI 1.17, 1.30 in overweight women) (Cedergren 2006), and postpartum weight retention (adjusted OR=1.68, 95% CI 1.40, 2.01 in normal weight women; adjusted OR=1.97, 95% CI 1.55, 2.50 in overweight women) (Oken *et al.* 2009; Olson *et al.* 2003). In the longer-term, women with excessive weight gain during pregnancy have a greater risk of developing obesity, in particular if associated with failure to lose weight postpartum (Rooney and Schauburger 2002). Current evidence regarding interactions between GWG and pre-pregnancy BMI in predicting these adverse maternal outcomes is inconsistent (Committee on the Impact of Pregnancy Weight on Maternal and Child Health 2007).

#### Adverse health outcomes for the child

Children whose mothers gain weight excessively during pregnancy are more likely to be large for gestational age at birth (adjusted OR=2.73, 95% CI 2.60, 2.83 in normal weight women; adjusted OR=2.14, 95% CI 2.01, 2.28 in overweight women) (Cedergren 2006; Oken *et al.* 2007; Oken *et al.* 2009) and have a greater amount of body fat at birth (Catalano *et al.* 2003b). However, maternal pre-pregnancy BMI seems to be a stronger predictor for these outcomes than GWG itself (Committee on the Impact of Pregnancy Weight on Maternal and Child Health 2007). Birth weight appeared to be best predicted by early pregnancy weight gain, while weight gain in the third trimester was not associated with birth weight (Sermer *et al.* 1995).

The long-term adverse outcomes of excessive GWG described in the offspring include a greater risk of obesity from childhood (Moreira *et al.* 2007; Oken *et al.* 2007; Schack-Nielsen *et al.* 2010; Wrotniak *et al.* 2008) through to adulthood (Schack-Nielsen *et al.* 2010; Stuebe *et al.* 2009) and a higher systolic blood pressure in childhood (Oken *et al.* 2007) and early adulthood (Mamun *et al.* 2009), the latter effect being potentially mediated by body size. Further details on the long-term influence of maternal GWG on child obesity are presented in Section 2.4.3.

#### 2.2.3.4 Clinical response - Recommended gestational weight gain

As mentioned above, given the accumulated data on adverse outcomes of excessive weight gain during pregnancy, the IOM has recently updated their recommendations to provide appropriate guidance both during pregnancy and before conception, with the ultimate goal of achieving healthy outcomes for the women and their offspring.

A small randomised controlled trial of dietary advice to limit GWG to 6-7 kg in obese women showed favourable effects on maternal insulin and leptin levels, reducing the risk of GD without lowering maternal glucose levels, which are important to fetal growth (Wolff *et al.* 2008). Accordingly, it was suggested that this kind of dietary counselling could in turn reduce the risk of obesity in the next generation; however, more research is needed in this area.

#### 2.2.4 Interrelations between maternal pre-pregnancy BMI, glucose tolerance status during pregnancy and gestational weight gain

The intrauterine milieu undergoes major metabolic changes during pregnancy, which are largely influenced by maternal body size, glucose tolerance status and gestational weight gain. These three factors are not independent of each other, but rather interrelated, centred on the amount of energy substrate passed on to the growing fetus, mainly glucose, and compensatory fetal insulin secretion.

##### **Maternal pre-pregnancy BMI and glucose tolerance status during pregnancy**

Obesity is a strong determinant for the development of glucose intolerance, not only in non-pregnant states, but also during gestation (Catalano 2010). Maternal obesity at conception is often accompanied by a (subclinical) reduction in pre-gravid insulin sensitivity (Catalano *et al.* 1999), which is further enhanced with advancing gestation; as a result, a higher degree of insulin resistance is observed in obese pregnant women compared to those with normal weight status. It has been shown that obese women who develop GD have a greater insulin response, higher insulin resistance and lower suppression of endogenous glucose production compared to obese women who maintain normal glucose tolerance during pregnancy (Catalano *et al.* 1999).

The increased insulin resistance encountered in obese women leads to a greater availability of nutrients (glucose, free fatty acids, and amino acids) to the fetus and some degree of placental dysfunction (Yogev and Catalano 2009). Subsequently, hyperglycaemia stimulates fetal insulin production, with secondary hyperinsulinemia, which contributes to fetal overgrowth.

Women who are overweight or obese when they enter pregnancy, even if they maintain a normal glucose tolerance, are more likely to have infants with a greater amount of adipose tissue compared to infants of women who are lean or average weight before conception (Sewell *et al.* 2006). This excessive intrauterine fat deposition in the fetus has been attributed to the increased availability of free fatty acids to the fetus as a consequence of maternal lipolysis secondary to the lower sensitivity to insulin that characterises obese women (Catalano *et al.* 1995; Catalano *et al.* 2009b).

Moreover, infants of obese mothers, although not always heavier at birth, are more insulin resistant (mainly peripherally, at skeletal muscle and adipose tissue level) compared to offspring of normal weight mothers (Catalano *et al.* 2009b). This correlation between maternal pre-pregnancy BMI and infant insulin resistance at birth was robust to adjustments for fat mass and percentage body fat, suggesting potential genetic or epigenetic underlying mechanisms for fetal insulin resistance (Catalano *et al.* 2009b).

It has been posited that in obese pregnant women, the subclinical chronic inflammatory state may interfere with placental growth and function (Challier *et al.* 2008), with potential negative effects on maternal insulin sensitivity and limited fat deposition, but excessive placental and fetal growth. Accordingly, it was hypothesised that fetal obesity could be attributed to the transfer of proinflammatory cytokines across the placenta (Catalano *et al.* 2009a). However, it has been shown that most cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) do not cross the placenta (Aaltonen *et al.* 2005) and thus maternal inflammation cannot be linked directly to obesity-related fetal inflammation (Catalano *et al.* 2009a).

### **Maternal pre-pregnancy BMI and gestational weight gain**

Among the factors affecting GWG, maternal weight status at entry to pregnancy appears to be important. Obese women have an overall tendency towards a lower weight gain throughout pregnancy (Chu *et al.* 2009; Lederman *et al.* 1997). The amount of fat stored during pregnancy has been shown to be negatively associated with maternal pre-pregnancy BMI (Lederman *et al.* 1997) or percentage body fat (Ehrenberg *et al.* 2003). Not only the amount, but also the distribution of fat accrued during pregnancy differs according to pre-gravid body composition; thus women with less than 25% body fat tend to accumulate fat more peripherally, while women with more than 25% body fat tend to accumulate fat more centrally, irrespective of their gestational glucose tolerance status (Ehrenberg *et al.* 2003).

## Maternal glucose-insulin homeostasis during pregnancy and gestational weight gain

Studies on the association between indicators of maternal glucose homeostasis in early pregnancy and GWG emerged after the finding of a significantly lower weight gain in insulin resistant, non-pregnant young adult Pima Indians followed-up prospectively over 3 years (Swinburn *et al.* 1991). However, the studies conducted in pregnant women on the association between insulin sensitivity and weight gain have yielded discrepant findings. Scholl and Chen (2002) found that women with higher fasting insulin levels in the first trimester of pregnancy (women regarded as having a 'thrifty' metabolism) had greater rates of GWG, with a suggestion of a stronger relationship in women with normal weight. In contrast, Stuebe *et al.* (2010) did not detect an overall association between fasting insulin levels or insulin resistance (as quantified by homeostasis model assessment) in early pregnancy and total GWG; however, when analysed separately according to maternal weight status in early pregnancy, hyperinsulinemia was associated with lower GWG in obese women and with greater GWG in lean women (BMI < 20 kg/m<sup>2</sup>). In a small cohort of lean women (with < 25% body fat) followed up at various times throughout pregnancy, it has been shown that women with reduced insulin sensitivity prior to pregnancy gained significantly less fat mass in the first 12-14 weeks of gestation compared to women without pre-gravid alterations of glucose-insulin metabolism, but the amount of maternal fat accrued between early and late pregnancy was not associated with insulin sensitivity (probably because of the greater contribution of fetal and placental tissues rather than maternal fat to weight gain after the first trimester of pregnancy) (Catalano *et al.* 1998).

Gestational weight gain has also been specifically evaluated in relation to maternal glucose tolerance status during pregnancy, with marked inconsistencies in findings. A systematic review concluded there was weak evidence for an association between GWG and impaired glucose tolerance during pregnancy (Viswanathan *et al.* 2008). Some of the discrepancies may be due to the different methodologies employed by various researchers, mainly different criteria used for screening and diagnosis of GD or milder degrees of glucose intolerance. Other difficulties in interpreting this relationship arise from the fact that treatment recommended to women with GD, primarily dietary advice, may also influence weight gain after the diagnosis. In order to overcome this limitation, some studies focussed on weight gain prior to diagnosis of GD instead of total GWG. One of these studies (Saldana *et al.* 2006) showed that weight gain ratio (observed : expected) was significantly associated with an increased risk of developing impaired glucose tolerance among overweight women, but only marginally associated with an increased risk of developing GD. Similarly, weight gain in the first two trimesters of pregnancy (but not total GWG) was positively associated with the

likelihood of developing GD (Kieffer *et al.* 2001). In contrast, no difference in weight gain in the first 28 weeks of gestation was found between women with abnormal versus normal OGCT (Hackmon *et al.* 2007).

Other authors reported lower total GWG in women with GD compared to women who maintained normal glucose tolerance (Catalano *et al.* 1993a; Kieffer *et al.* 2006), but after adjustment for pre-pregnancy weight for height this association remained significant only for underweight women (Catalano *et al.* 1993a). On the other hand, other authors found no difference in total GWG or percentage body fat between women with GD and controls (Ehrenberg *et al.* 2003).

## 2.3 Specific metabolic consequences in children

A range of metabolic perturbations have been identified in children exposed to intrauterine conditions such as maternal pre-pregnancy obesity, gestational diabetes or borderline gestational glucose intolerance, and excessive gestational weight gain, as described in Sections 2.2.1.3, 2.2.2.6 and 2.2.3.3, respectively. Although the reports regarding these changes are not entirely consistent, they include child obesity, insulin resistance, impaired glucose tolerance, and higher systolic blood pressure. The focus of this project is child obesity, body composition, fat pattern (Section 2.3.1), and insulin resistance (Section 2.3.2). These outcomes are described in terms of public health significance, namely prevalence, adverse health outcomes and methods of assessment. Both sections conclude with a listing of the outcome measures in children to be used in the current study.

### 2.3.1 Obesity, body composition and fat pattern in children

“Obesity is neither a behavior nor a disease, but rather a general phenotype of numerous pathologic biochemical processes impinging on a complex negative feedback pathway for the control of energy balance” (Lustig 2003). However, the concept that the obesity epidemic is a simple consequence of disturbances in energy balance has started to be challenged by recent research showing that the determinants of obesity are a complex mix of genetic, environmental, psycho-social, and cultural factors.

Obesity is a chronic, multifactorial condition resulting from the interaction between genetic inheritance and environmental factors, which may act both during intrauterine life (e.g., GD) and after birth (e.g., high calorie diet, reduced physical activity, behavioural, social and cultural processes).

From a genetic perspective, only a few monogenic obesity syndromes have been described, caused by mutations in genes for hormones involved in the neuroendocrine regulation of energy balance, or their receptors (such as melanocortin 4 receptor, proopiomelanocortin, leptin, leptin receptor) (Chagnon *et al.* 2003; Farooqi 2006). These mutations, by influencing eating behaviour and/or energy expenditure, lead to a positive energy balance starting in infancy (Mietus-Snyder and Lustig 2008).

Body weight status of each individual is influenced by a multitude of factors starting prenatally and spanning the life course, including genetic, biological, environmental, behavioural, and socio-economic factors, as well as the complex interactions between them (World Health Organization 2000). Risk factors for overweight and obesity in children suggested in the literature include parental obesity (Beyerlein *et al.* 2010; Parsons *et al.* 1999), birth weight (partly affected by gestational age), maternal smoking in pregnancy, rapid growth in the first two years of life, earlier adiposity rebound, dietary factors (including exclusive formula feeding in infancy), sedentary lifestyle, and social factors (such as low level of parental education) (Beyerlein *et al.* 2010; Parsons *et al.* 1999; Reilly *et al.* 2005).

Two obesity phenotypes were described over 50 years ago (Vague 1956) and they are still used in contemporary studies (Landsberg 2008): upper-body obesity (central, abdominal, android, or 'apple shape') with important metabolic complications, and lower-body obesity (peripheral, gluteal-femoral, gynoid, or 'pear shape').

Total body adipose tissue can be broadly divided into two main components: subcutaneous (superficial or deep in relation to the fascial plane) and internal, which in turn, has two sub-divisions: visceral (intrathoracic, intra-abdominal and intrapelvic) and non-visceral (intramuscular, intermuscular and paraosseal) (Shen *et al.* 2003). These compartments have different roles. Of them, intra-abdominal adipose tissue is more strongly linked to adverse metabolic complications, including insulin resistance, type 2 diabetes, dyslipidemia, and cardiovascular disease (Goran and Gower 1999).

Intra-abdominal adipose tissue is involved in modulating cardiovascular disease risk not only in obese, but in lean individuals as well (Gower *et al.* 1999). The underlying mechanisms involve secretion by the visceral fat of metabolically active factors (e.g., IL-6, TNF- $\alpha$ , adiponectin or resistin), and release of free fatty acids into the hepatic portal vein, which increase hepatic glucose production and interfere with hepatic insulin extraction (Frayn 2000).

Weight management in obese children and adolescents is multidisciplinary. According to the severity of obesity, management may include behavioural lifestyle changes regarding dietary and physical activity patterns, pharmacological approaches (e.g., Orlistat, a medication which reduces



the absorption of fat from food; Sibutramine, a medication which acts centrally to reduce food intake; Metformin, an insulin sensitizer, recommended particularly in adolescents with insulin resistance) and surgical interventions (e.g., laparoscopic banding) (Hearnshaw and Matyka 2010). Pharmacotherapy and surgery are reserved for adolescents with severe obesity (Whitlock *et al.* 2010). Although medium- to high-intensity lifestyle interventions, sometimes combined with pharmacotherapy, have short-term (i.e., 12 months) benefits in obese children and adolescents (Whitlock *et al.* 2010), sustained benefits of obesity treatment are difficult to achieve (Oude *et al.* 2009), with individuals having a tendency to return to the previously stabilised 'set point' for body weight status (Keesey and Hirvonen 1997; Oude *et al.* 2009). This has resulted in a major focus on identifying preventative strategies, particularly upstream of current behaviours, which may be responsible for the lack of response to current obesity management. In this context, investigating whether and how intrauterine exposures may contribute to the development of obesity during childhood is of great relevance.

### **2.3.1.1 Public health significance - Prevalence data**

Childhood obesity has become a major public health issue in both developed and developing countries throughout the world, due to the increasing prevalence, associated complications, and cost to the health care system.

Obesity prevalence differs across countries and comparisons are limited by the use of different reference populations, as well as by the inconsistent definitions of obesity (some based on weight for height, or weight-for-height z-scores, others on BMI centiles or BMI z-scores) (Lobstein *et al.* 2004). The use of BMI centile and BMI z-score is described in Section 2.3.1.3. A relatively recent review on the prevalence of childhood overweight and obesity in over 60 countries around the world reported an increase in all the countries (except for Russia and Poland in the 1990s) (Wang and Lobstein 2006). The average annual increase in childhood obesity prevalence was particularly high in economically developed countries, and in societies that underwent rapid socio-economic transitions (Wang and Lobstein 2006).

In the US, obesity (defined as BMI > 95<sup>th</sup> centile of 1999-2000 reference population) prevalence in children aged 6-11 years grew from 4% in 1971-1974 to 11.3% in 1988-1994 and further to 15.3% in 1999-2000 (Ogden *et al.* 2002). In the UK, the prevalence of obesity, as defined by the International Obesity Task Force (IOTF) trebled between 1984 and 1998 (from 1.7% to 5.4% in boys and from 2.6% to 7.8% in girls), while the prevalence of overweight and obesity combined doubled over the



same period of time (from 9% to 20.7% in boys and from 13.5% to 27.4% in girls) (Lobstein *et al.* 2003; Wang and Lobstein 2006).

In Australia, based on national data (Australian Health and Fitness Survey, 1985; National Nutrition Survey, 1995), the prevalence of childhood obesity (as defined by IOTF) tripled between the mid-1980s and mid-1990s (from 1.4% to 4.7% in boys and from 1.2% to 5.5% in girls) and the prevalence of overweight and obesity combined doubled over the same time period (from 10.7% to 20% in boys and from 11.8% to 21.5% in girls) (Magarey *et al.* 2001). This equates to a rise in the prevalence of overweight and obesity in school-age children by almost 1% per year (Wang and Lobstein 2006). According to the latest report in 2007-2008, a quarter of Australian school-age children were overweight or obese (Australian Bureau of Statistics 2009a). Based on data from the National Health Survey, the prevalence of childhood obesity was still increasing in 2007-2008, but at a slower rate, reaching 8% in school-age boys and 6% in girls, while the prevalence of overweight plateaued at around 17% for both sexes at this age (Australian Bureau of Statistics 2009a). The prevalence of overweight and obesity showed a socio-economic gradient, with higher rates in children living in areas of greatest relative disadvantage (20% and 12%, respectively) (Australian Bureau of Statistics 2009a).

A flattening in the prevalence of childhood overweight and obesity over the last decade has been described in several countries, such as Australia (Olds *et al.* 2010), USA (Ogden *et al.* 2008) and France (Peneau *et al.* 2009). Olds *et al.* (2010), while acknowledging paediatric overweight as a public health issue, have indicated a stabilisation in the rates of overweight and obesity (as measured by BMI) throughout childhood and adolescence in Australia after 1996, based on a meta-analysis of 41 studies conducted between 1985-2008, with raw data available for over 70,000 children. As the authors suggest, this plateau may be a result of the multiple initiatives regarding optimal physical activity and nutrition for children; a saturation of the obesogenic environment (so that predisposed children have already become overweight, while the others are resilient to high-caloric diets or sedentary lifestyle); or a potential underestimation of the issue by using a surrogate measure of overweight such as BMI, as opposed to more sensitive measures of adiposity, such as skinfold thickness (Olds *et al.* 2010). The latter explanation is supported by a meta-analysis including 154 studies on skinfold thickness measurements in children and adolescents from developed countries around the world (1951-2004), with raw data available for over 50,000 subjects (Olds 2009). The average estimated increase in percentage body fat based on Slaughter equations (Slaughter *et al.* 1988) was of 0.9% per decade, with the highest rates being identified in peri-

pubertal children (Olds 2009). Similarly, a secular increase in waist circumference in children has been reported in Australia (Dollman and Olds 2006) and the UK (McCarthy *et al.* 2003).

### 2.3.1.2 Adverse health outcomes of childhood obesity

Childhood obesity has been associated with a wide range of adverse health outcomes, of variable severity, both immediate (as described below) and in the long-term. Overweight and obesity themselves, as well as related morbidities, tend to persist from childhood into adult years, probably due to factors promoting obesity that also track over the lifespan, such as lifestyle habits (Freedman *et al.* 2005a; Parsons *et al.* 1999; Whitaker *et al.* 1997). Reviewed data from multiple studies show a greater likelihood of overweight tracking from childhood to adulthood with increasing excessive weight and increasing age (Singh *et al.* 2008). Overweight children have at least double the risk of becoming an overweight adult compared with healthy-weight children (Singh *et al.* 2008). Measures of adiposity also track from childhood into adulthood. For instance, relatively strong correlations were described between the age of 13 years and young adulthood for BMI ( $r=0.67$ ,  $p<0.001$ ), waist circumference ( $r=0.56$ ,  $p<0.001$ ), triceps ( $r=0.51$ ,  $p=0.003$ ) and subscapular ( $r=0.52$ ,  $p=0.002$ ) skinfold thickness (Steinberger *et al.* 2001). Moreover, increased cardiovascular mortality has been documented in adults who were obese during childhood (Gunnell *et al.* 1998).

Obesity-related complications accumulate with time and are more often described in adults, but they have started to be identified in children and adolescents as well. Like in adults, these complications may affect almost every organ system, particularly in morbidly obese children and adolescents (Daniels 2009; Han *et al.* 2010), as described below:

- **cardiovascular:** hypertension (Sorof and Daniels 2002), left ventricular hypertrophy (Yoshinaga *et al.* 1995), early development of atherosclerotic lesions (Berenson *et al.* 1992), endothelial dysfunction (Aggoun *et al.* 2008; Peña *et al.* 2006), and impaired arterial distensibility (Banach *et al.* 2010), chronic inflammation (Sacheck 2008), unfavourable levels of haemostatic factors (Alessi *et al.* 2000; Ferguson *et al.* 1998);
- **metabolic and endocrine:** dyslipidemia (i.e., high triglyceride levels and low high-density lipoprotein cholesterol levels) (Daniels 2009), insulin resistance (Goran *et al.* 2003; Steinberger *et al.* 2001), impaired glucose tolerance (Sinha *et al.* 2002), early onset of type 2 diabetes (American Diabetes Association 2000; Goran *et al.* 2003; Pinhas-Hamiel *et al.* 1996), metabolic syndrome (Weiss *et al.* 2004), unmasking symptoms of polycystic ovary syndrome

(Franks 2008), accelerated sexual maturation in girls (Rosenfield *et al.* 2009) and delayed transition to puberty in boys (Biro *et al.* 2006);

- **gastrointestinal:** non-alcoholic fatty liver disease (Nanda 2004), cholelithiasis (Kaechele *et al.* 2006), iron deficiency (due to reduced absorption from the gut) (Nead *et al.* 2004), vitamin D deficiency (due to increased storage in the adipose tissue) (Alemzadeh *et al.* 2008), gastro-oesophageal reflux (Koebnick *et al.* 2010)
- **pulmonary:** obstructive sleep apnoea (Mallory *et al.* 1989), asthma (Rodriguez *et al.* 2002), limited exercise tolerance (Norman *et al.* 2005);
- **renal:** glomerular hyperperfusion and hyperfiltration (Srivastava 2006);
- **orthopaedic:** lower limb malalignment (e.g., tibia vara, slipped capital femoral epiphysis) (Daniels 2009; Dietz 1998), osteoarthritis (Han *et al.* 2010);
- **central nervous system:** pseudotumour cerebri (disorder characterised by increased intracranial pressure, presenting with headaches and possibly visual impairment in severe cases) (Dietz 1998);
- **psychosocial:** depression (Britz *et al.* 2000), stigmatisation, social isolation, negative self-esteem (Griffiths *et al.* 2010), and overall impaired quality of life (Schwimmer *et al.* 2003).

The consequences of childhood obesity on the healthcare system have also started to be documented, but data is still scarce and largely limited to the USA (Trasande 2011). There is ambiguity in reports on obesity-related expenditures, as they employ different methodologies and consider different cost components (direct versus indirect). Based on American data from the 2001-2003 Medical Expenditure Panel Survey, it is currently estimated that the annual total healthcare expenditures (including drug prescription, outpatient visits and emergency room costs) for overweight and obese children (aged 6-17 years) are US\$180 and US\$220 per person, respectively, higher than for normal weight children (Finkelstein and Trogon 2008). In contrast, the only European quantification of the economic impact of childhood obesity based on the cross-sectional German Interview and Examination Survey for Children and Adolescents, did not show a significant increase in the annual total costs associated with childhood overweight or obesity (Wenig 2010). However, the German data indicated higher utilisation of healthcare services and a significant increment in annual physician costs of €27 and €62 per person among overweight and obese children, respectively, relative to their normal weight peers (Wenig 2010). Additionally, costs

associated with adult complications of obesity need consideration, due to the high rate of persistence of childhood obesity into adult life.

### 2.3.1.3 Measures of obesity

There is no universally accepted classification of overweight and obesity in children. A number of ways to measure obesity have been proposed and used, from broad indicators of body size to measures of adipose tissue at specific sites. This section describes and critiques some of these measurement tools.

#### **BMI and BMI z-score**

The most pragmatic definition of overweight, and thus most extensively used for screening and in epidemiological studies, is centred on BMI, which is calculated as the ratio of weight (in kilograms) divided by the square of height (in metres). In the international classification of adult overweight and obesity, BMI cut-off points are 25 kg/m<sup>2</sup> and 30 kg/m<sup>2</sup>, respectively, in both females and males (World Health Organization 1995). However, these preset cut-offs cannot be used for children or adolescents, as during these stages of life, growth patterns vary markedly with age and sex, with BMI being greater in boys at all ages (Berkey *et al.* 2000). BMI increases steeply during infancy peaking between 6 and 12 months, then falls during toddlerhood and the preschool years (Silverwood *et al.* 2009), and starts to increase again around the age of 5-6 years, which is known as 'adiposity rebound' (Reilly *et al.* 2005). The maximum annual increase in BMI occurs in early puberty (0.7 kg/m<sup>2</sup>/year in 11 year-old girls and 0.8 kg/m<sup>2</sup>/year in 12 year-old boys (Berkey *et al.* 2000)). BMI variation during childhood could be attributed to changes in fat-free mass among lean children (Freedman *et al.* 2005b), particularly in boys (Neovius *et al.* 2004), while among obese children this BMI variation is mainly due to changes in fat mass (Freedman *et al.* 2005b).

To account for this inconsistent change in weight relative to height across childhood, age- and sex-specific centile curves have been created using large samples from different populations, which allow comparisons between children over time, as well as between children of different ages and sex. These centiles can be internal (which are less valid if derived from a small sample) or external. Two of the external centiles widely used are the ones adopted by the Centers for Disease Control and Prevention (CDC) and by the International Obesity Task Force (IOTF). In the USA, BMI values are compared to BMI age- and sex-specific centiles curves derived from five National Health and Nutrition Examination Surveys (1963-1994) and five supplementary data sources; the 85<sup>th</sup> and 95<sup>th</sup>

percentiles are considered the lower cut-offs for 'at risk for overweight' and 'overweight', respectively, and have been adopted by CDC (Kuczmarski *et al.* 2000). In most other settings, the current definition of childhood overweight and obesity is the one adopted by IOTF (Cole *et al.* 2000), which is gaining increasing worldwide acceptance. The method used to construct these less arbitrary age- and sex-specific centile curves for BMI was developed based on pooled international data (large heterogeneous sample) collected from surveys conducted between 1963 and 1993, in six countries (Brazil, Hong Kong, Singapore, the Netherlands, UK and USA) (Cole *et al.* 2000). According to IOTF/Cole classification, the lower thresholds for 'overweight' and 'obesity' in children are defined by the age- and sex-specific centile curves corresponding to a BMI at 18 years equal to the adult cut-off points of 25 kg/m<sup>2</sup> and 30 kg/m<sup>2</sup>, respectively (Cole *et al.* 2000). In addition to these external centiles, national systems of classifying childhood obesity based on local (internal) BMI reference curves are available in some countries (Guillaume 1999), useful mainly for national purposes and not for international comparisons.

Another BMI-related measure commonly used for comparisons of obesity rates among children over time or from different populations is the *age- and sex-specific z-score*. Essentially, this is a measure of how far a child's BMI departs from the mean. In more detail, BMI z-score is computed as the deviation of one individual's BMI from the mean BMI for the reference population, divided by the standard deviation for the reference population. This calculation can be based on the widely used LMS method (lambda-mu-sigma method (Cole 1988)), which summarises the changing distribution by three curves representing the median (M), coefficient of variation (S) and skewness (L), the latter expressed as a Box-Cox power (Cole and Green 1992). It was suggested that among the metrics of relative weight for height in children, BMI z-scores would explain the variation in percentage body fat better than BMI centiles (Field *et al.* 2003).

Although simple, cheap, reproducible and extremely useful for screening of overweight status in both clinical settings and epidemiological studies, BMI and related measures have some limitations as a proxy indicator for adiposity because they do not depict the relative contributions of lean and fat mass to total body mass, nor the fat distribution. It has been shown that BMI has high specificity (98%), but a low to moderate sensitivity (36% in boys, 60% in girls) for identifying excess adiposity in children (Reilly *et al.* 1999).

A significant variation in body composition can be found for a given BMI at any age. Individuals with increased muscle mass may have a BMI as high as those with excessive deposition of fat, hence measuring only an index of relative weight to height such as BMI may be a source of

misclassification of overweight or obese. To minimise this misclassification and better identify individuals at risk for adverse health outcomes, various methods of body composition assessment have been developed to gauge the amount of fat more directly.

### **Percentage body fat**

As opposed to BMI which is a marker of relative weight for height, percentage body fat (%BF) is a more specific measure of adiposity, indicating which overweight individuals are truly overfat rather than having increased muscle mass. Variations in %BF reflect changes both in fat mass (FM) and fat-free mass (FFM).

Body composition varies considerably with age, sex, race, and is influenced by physical activity level, diet, underlying medical conditions (e.g., FM is reduced in malnutrition), and hydration status (Forbes 1978). Physiologically, after birth, the amount of body fat declines slowly until the end of infancy, when it starts to progressively accumulate throughout childhood, at similar rates in both girls and boys (Forbes 1978). With puberty, a sexual dimorphism of body fat deposition becomes apparent, with females gaining proportionately more fat, while males gain more muscle (Forbes 1978).

Due to these variations in body composition, age- and sex-specific body fat reference curves have been developed, similar to the BMI centile curves. For example, %BF estimated by dual energy X-ray absorptiometry (method described below) were linked to the IOTF cut-offs for overweight and obesity in a study involving 661 white children aged 3-18 years, from New Zealand (Taylor *et al.* 2002). In this study, %BF ranged from 18-23% in overweight boys, 20-34% in overweight girls, 24-36% obese boys, and 26-46% in obese girls (Taylor *et al.* 2002), which supported the need for age- and sex-specific definitions to classify children as overfat or obese based on %BF, instead of using one single %BF value as a cut-off. McCarthy *et al.* (2006) did this by constructing age- and sex-specific %BF reference charts and classified children with a %BF less than the 2<sup>nd</sup> centile as underfat, while the 85<sup>th</sup> and 95<sup>th</sup> centiles defined the lower limits for overfat and obese, respectively (corresponding to the overweight and obese categories of the IOTF reference curves of BMI). Their reference population was UK Caucasian children aged 5-18 years who had bioelectrical impedance analysis (method described below) in 1985.

A multitude of methods for estimating body fat are now in use, with different levels of accuracy, complexity, feasibility, and cost. They range from equations based on simple anthropometric measurements (e.g., skinfold thickness) or bioelectrical impedance analysis, to more complex

imaging techniques, such as dual energy X-ray absorptiometry, computed tomography, or magnetic resonance imaging, as well as volume displacement methods (underwater weighing, air displacement plethysmography), or dilution techniques (Lee and Gallagher 2008). All these *in vivo* methods of body composition assessment rely on numerous assumptions which may not always be met, and therefore cannot yield estimates as accurate as the direct chemical analysis (Goran 1998), but the latter method is not feasible in human studies. Having different levels of accuracy, these techniques do not provide identical estimates for body composition in the same individual, which makes interpretation and comparisons challenging. The most accurate and feasible technique, which is thus considered as the reference, is the 4-compartment model (Radley *et al.* 2009). Within this method, fat or fat-free mass are estimated based on the measurement of the other three components: total body water, body density and body mineral content (Radley *et al.* 2009). This technique is difficult to perform, often uncomfortable (e.g., when body density measurement involves underwater weighing) and time consuming, which are major issues especially in child studies.

**Skinfold thickness** (SFT) measurements in different body sites (e.g., biceps, triceps, subscapular, abdominal, suprailiac, medial calf) offer a good indicator of subcutaneous fat amount and distribution (upper versus lower body) (Mei *et al.* 2002). Unlike BMI, SFT measurement can identify non-overweight children who have excess subcutaneous adiposity (Freedman *et al.* 2007b). However, SFT measurements give no indication on the amount of visceral fat, which is the metabolically active component. Body fat estimates from SFT are prone to examiner's error and intensive training is imperative to maximise reliability of measurements.

Numerous equations have been proposed for estimating %BF from SFT in children (Deurenberg *et al.* 1990; Slaughter *et al.* 1988), with no gold standard decided upon so far. Among them, the Slaughter equations (sex-specific equations for pre-pubertal, pubertal and post-pubertal stage) (Slaughter *et al.* 1988) provide %BF estimates most highly correlated with dual energy X-ray absorptiometry estimates, and without fat-dependent bias (Rodriguez *et al.* 2005). Simpler equations such as the sum of triceps and subscapular SFT, which correlates better with DXA estimates than BMI, have proved useful for comparisons between subjects, but they are meaningless as standalone figures (Freedman *et al.* 2007b).

**Computed tomography** (CT) and **magnetic resonance imaging** (MRI) have the major advantage of providing measures of regional fat distribution (subcutaneous, visceral, intermuscular) (Lee and Gallagher 2008). However, due to their technical complexity, high cost, and, with CT, radiation



exposure, their use is limited mainly to clinical practice and few epidemiological studies for validation of 'field' methods (e.g., bioelectrical impedance analysis) (Mattsson and Thomas 2006).

**Dual energy X-ray absorptiometry (DXA)** is based on a 3-compartment model which estimates the differential absorption of X-ray energy by fat, bone and other lean tissue. Although precise with respect to body composition assessment in paediatric populations and strongly correlated with the four-compartment model (Sopher *et al.* 2004), DXA is not very accurate, particularly in obese children and adolescents, with a tendency to overestimate FM and underestimate FFM in comparison to the four-compartment model (Wells *et al.* 2010). Other limitations of this method include the high cost, the lengthy time required, and that it measures all fatty elements in the body, not only the adipose tissue (a limitation of all two- and three-compartment models).

**Bioelectrical impedance analysis (BIA)** determines the resistance of the body tissues to a small alternating electrical current (Kyle *et al.* 2004a). The adipose tissue impedes the electric current more than muscle or bone. The method then calculates the resistance index (as the ratio of height squared and resistance) to predict total body water (TBW) and, implicitly, FFM (as adipose tissue is almost anhydrous) (Wright *et al.* 2007). Fat mass is subsequently calculated by subtraction of FFM from the total body weight. Fat and lean mass vary with height and thus cannot be interpreted as standalone figures; instead, FM can be expressed as a percentage of total weight (%BF), which is an indicator of lean and fat mass adjusted for body size.

BIA can be performed using the arm-to-leg method (with electrodes on the wrist and ankle, such as Bodystat), the foot-to-foot method (using a stand-on machine, such as Tanita), and tetrapolar, with comparable results (Wright *et al.* 2007).

The accuracy and precision of this method in children have been extensively debated, with some authors describing the method as acceptable (Schaefer *et al.* 1994), while others as biased and imprecise (Reilly *et al.* 1996). It has been suggested that the lack of correlation with estimates from more accurate methods "reflects limitations in the regression equations used which, by necessity, have been developed with small numbers of children over wide age ranges" (Wright *et al.* 2007). Data from a cross-sectional study of 30 overweight or obese Australian children revealed that among the embedded equations for BIA, the Schaefer equation offered the %BF estimate closest to that estimated by DXA (Cleary *et al.* 2008).

When comparing BIA estimates to anthropometric measurements, some studies support BIA as a more precise tool for estimating adiposity than BMI (Rush *et al.* 2003; Tyrrell *et al.* 2001) or SFT



(Kettaneh *et al.* 2005; Pecoraro *et al.* 2003). In contrast, data from a cross-sectional study in non-obese pre-menarcheal girls found BIA no better than triceps SFT in predicting %BF (Bandini and Vu 1997). Similarly, weaker correlations between BIA and anthropometric measurements have been reported in pubertal boys (Kettaneh *et al.* 2005).

Relative to DXA, which is often regarded as a reference for body composition assessment particularly in children, it has been noted that BIA tends to underestimate %BF in overweight subjects and overestimate it in lean subjects (Eisenmann *et al.* 2004). However, in a small (n=17) cross-sectional study in overweight and obese preadolescent children, despite the significant differences between BIA and DXA estimates, BIA measures of body composition were strongly correlated to the ones provided by DXA (correlation coefficients for %BF, FM, and FFM were 0.85, 0.97, and 0.94, respectively) (Goldfield *et al.* 2006).

Relative to the 4-compartment model, BIA (using Tanita in a sample of overweight and obese children) provided accurate estimates of body composition at a group level, but not at individual level, with a tendency to overestimate FFM (Radley *et al.* 2009). Foot-to-foot BIA overestimates FFM and underestimates FM in both obese and non-obese children, compared with air-displacement plethysmography (Azcona *et al.* 2006). The magnitude of this problem is hard to gauge from the available data, but over- and underestimation appeared to be in the order of 5%.

Despite the debatable accuracy for estimating %BF, BIA is highly suitable for epidemiological studies in the field, particularly in children, as it is portable, easy to perform, non-invasive, quick and increasingly inexpensive. Other advantages of this method are the minimal intra- and inter-observer variability (Diaz *et al.* 1989) and the good reproducibility, with less than 1% error on repeated measurements in the same conditions (Segal *et al.* 1991), which permits ranking of individuals within a study.

Given these advantages, BIA was the method of choice for this research project. However, although BIA is a reasonable method for estimating body composition, it does not allow for the description of fat distribution, or the separate quantification of intra-abdominal (visceral) fat, which is the most metabolically active.

## Fat pattern

Fat distribution within specific body regions, or even within non-adipose tissue, is of great interest in terms of metabolism. Two major phenotypes of relative distribution of the excess fat, with unique characteristics, have been described several decades ago in relation to cardiovascular and metabolic disease risk: central and peripheral adiposity (Ashwell *et al.* 1985; Vague 1956).

The centralised distribution of fat has been linked to a cluster of metabolic disturbances including fasting hyperinsulinemia, insulin resistance, blood lipid disorders, as well as high blood pressure (Browning *et al.* 2010), both in adults (Despres and Lemieux 2006; Harris *et al.* 2000; Li and McDermott 2010) and children (Botton *et al.* 2007; Garnett *et al.* 2008; Hara *et al.* 2002; Kahn *et al.* 2005; Savva *et al.* 2000). Abdominal adiposity may be regarded as an endocrine organ secreting various hormones (e.g., leptin, adiponectin) and cytokines (e.g., TNF- $\alpha$ , IL-6), which lead to hepatic lipogenesis and insulin resistance, release of free fatty acids from adipocytes, macrophage infiltration into the adipose tissue, or ectopic lipid storage (Kershaw and Flier 2004).

Similar to %BF, fat pattern can be estimated based on simple anthropometric measures, such as waist circumference (paediatric centile curves are available (McCarthy *et al.* 2001)), waist-to-hip ratio, waist-to-height ratio, sum of SFT (e.g., triceps, biceps, subscapular, supriliac, abdominal), or ratio of central to peripheral SFT (e.g., triceps to subscapular skinfold thicknesses). More accurately, the central pattern of adiposity may be determined by the same imaging techniques (CT, MRI or DXA) used for body composition assessment, which, due to the high cost, are usually not feasible in epidemiological studies.

Waist circumference is a simple measure of central adiposity, with a relatively good sensitivity in predicting cardiovascular risk. It measures both subcutaneous and visceral adipose tissue at abdominal level. Waist circumference is more informative when used as a ratio to other body measures. The ratio of waist-to-hip has been used as an indication of 'apple' or 'pear' shape. More recent research has suggested the ratio of waist-to-height is a better predictor of cardiovascular risk (Browning *et al.* 2010).

**Waist-to-height ratio (WHtR)** was first described in the Framingham Study in 1988 in an attempt to account for some of the limitations of BMI in predicting cardiovascular disease risk in children and adolescents (Higgins *et al.* 1988) and was later adopted by other investigators as an indicator of fat distribution. This ratio has been validated as a good anthropometric measure of abdominal obesity both in adult (Ashwell *et al.* 1996; Cox *et al.* 1997; Hsieh and Muto 2005) and paediatric populations

(McCarthy and Ashwell 2006; Nambiar *et al.* 2009). However, it cannot differentiate between intra-abdominal (visceral), the metabolically active depot (Carr *et al.* 2004), and subcutaneous abdominal adipose tissue, a differentiation which requires more expensive imaging techniques.

Several studies indicated an improved ability of WHtR to predict adverse cardiovascular risk factors, such as high blood pressure and lipid disorders in children and adolescents, compared to BMI (Kahn *et al.* 2005; Savva *et al.* 2000), whereas other studies did not support WHtR superiority, probably due to the high correlation with BMI (Freedman *et al.* 2007a).

In contrast to other anthropometric measurements in children, WHtR does not significantly vary with age and therefore expression as z-scores is thought to be not warranted (Freedman *et al.* 2007a). Similar to adults, a cut-off of 0.5 for WHtR has been proposed to define an exacerbated cardiovascular risk in children (McCarthy and Ashwell 2006). It has been argued that using a single cut-off for all ages provides a simplified public health message with no loss in accuracy: “Keep your waist circumference to less than half your height” (Ashwell and Hsieh 2005; McCarthy and Ashwell 2006). However, despite the practicality of such a simple message, relationships involving WHtR are likely to be continuous.

Data from a large community-based cross-sectional study, the Bogalusa Heart Study, support the utility of 0.5 as a cut-off for WHtR in detecting children at cardio-metabolic risk, even among those with normal weight (Mokha *et al.* 2010). Normal weight children with central adiposity (defined by  $WHtR > 0.5$ ), also known as ‘metabolically obese normal weight’ (Stefan *et al.* 2008), have more unfavourable cardio-metabolic risk profiles (higher systolic and diastolic blood pressure, abnormal lipid profile, increased levels of fasting glucose and insulin, higher insulin resistance) than normal weight children without central adiposity (Mokha *et al.* 2010). In contrast, overweight and obese children without central adiposity have lower levels of these cardio-metabolic variables (‘metabolically benign obesity’ (Stefan *et al.* 2008)) compared to those with centralised distribution of the fat (Mokha *et al.* 2010).

In summary, the following obesity-related outcomes in children are considered in the current study as continuous variables: (1) BMI z-score using the IOTF age- and sex- specific centiles curves, as a measure of global obesity; (2) %BF estimated by BIA; and (3) fat pattern defined by WHtR.

### 2.3.2 Insulin resistance in children

Insulin resistance (IR) is a state characterised by an insufficient biological response of the tissues to normal or higher levels of insulin (Matthaei *et al.* 2000). The diminished insulin action involves a reduction in the glucose uptake by skeletal muscles and fat tissue, as well as a decreased suppression of hepatic glucose production; consequently, hyperglycaemia arises, followed by compensatory hyperinsulinemia (Matthaei *et al.* 2000; Savage *et al.* 2005). The critical role of IR in human disease was recognised over 20 years ago (Reaven 1988).

There is evidence that the development of IR is influenced by several genes (Matthaei *et al.* 2000), as well as obesity (Lee 2006), in particular visceral adiposity (Cruz *et al.* 2002), and also by sedentary lifestyle, diet (e.g., rich in saturated and n-6 polyunsaturated fatty acids, with a high carbohydrate to fat ratio, or low-fiber diet (Cañete *et al.* 2007)), puberty, sex, race/ethnicity, and programming by some perinatal factors (such as small size at birth, prematurity) (Lee 2006). In addition, more recent reports have considered intrauterine exposure to maternal obesity or GD as a potential determinant of IR in the offspring, independent of genetic effect (see Sections 2.4.1 and 2.4.2).

Adipose tissue plays a central role in the development of IR, possibly through the production of free fatty acids ('portal theory') and several adipocytokines ('endocrine' paradigm), such as adiponectin (an insulin-sensitizing hormone), leptin, TNF- $\alpha$ , IL-6, and resistin. All these factors have been implicated in the alteration of insulin signalling at various levels (Chiarelli and Marcovecchio 2008). The underlying mechanisms responsible for IR are not entirely understood, but they include a number of defects in the insulin signalling cascade, which affect phosphorylation of the insulin receptor and inhibitory proteins (Matthaei *et al.* 2000; Savage *et al.* 2005).

Certain sites of fat deposition have been associated with increased risk of IR. First, visceral fat, with its greater lipolytic activity relative to subcutaneous fat, generates larger amounts of free fatty acids and glycerol to be carried to the liver, with subsequent effects on hepatic insulin sensitivity (Matthaei *et al.* 2000). Second, lipid accumulation in muscular cells may alter insulin signalling at peripheral levels (Weiss *et al.* 2005). Finally, it has been recently suggested that intermuscular thigh adipose tissue, as opposed to the subcutaneous fat (Boettcher *et al.* 2009), as well as fat deposited around blood vessels (Yudkin *et al.* 2005) may also contribute to the development of IR.

Adiposity is the most important risk factor for IR, which explains almost one third of the variance in insulin sensitivity (Lee *et al.* 2006). Insulin resistance is strongly associated with obesity (Dwyer *et al.* 2002; Lee *et al.* 2006), but it can occur in non-obese individuals as well, particularly if they are

hyperlipidemic (Al-Mahmood *et al.* 2007). Moreover, it has been suggested that IR is both a result and a cause of obesity (Lustig 2003). Insulin resistance leads to hyperinsulinemia, which may interfere with leptin signal transduction in the hypothalamus promoting leptin resistance, which decreases resting energy expenditure and increases appetite, with subsequent weight gain (Lustig 2003).

A number of cross-sectional studies in children support the role of adiposity in the development of IR, based on positive correlations between FM (measured by DXA) and IR in pre-pubertal children (Gower *et al.* 1999), between central adiposity (estimated by SFT) and fasting insulin levels (Freedman *et al.* 1987), or between visceral adiposity (measured by MRI) and fasting insulin and IR (determined by the frequently sampled intravenous tolerance test) in obese Hispanic children (Cruz *et al.* 2002).

In addition to the increased risk of IR associated with a family history of type 2 diabetes or a certain ethnic background, a number of maternal factors have been recently identified as possibly contributing to IR in children. They include GD (Boerschmann *et al.* 2010; Catalano *et al.* 2009b; Egeland and Meltzer 2010), maternal diet during pregnancy (Shiell *et al.* 2000; Yajnik *et al.* 2008), older maternal age (Loos *et al.* 2002), pregnancy-induced hypertension (Himmelman *et al.* 1997), low maternal BMI (Ravelli *et al.* 1998; Shiell *et al.* 2000), and prenatal psychosocial stress (Entringer *et al.* 2008). The great majority of observational studies focussing on the early life origins of IR have described a link with intrauterine growth restriction (Newsome *et al.* 2003) and also supported a role for rapid catch-up growth after birth (Newsome *et al.* 2003; Ong and Dunger 2004; Veening *et al.* 2002). The explanations proposed for the negative association between IR and birth weight include *in utero* 'programming' by poor intrauterine nutrition and direct effect of genes influencing IR, which reduce insulin-related growth ('fetal insulin hypothesis') (Frayling and Hattersley 2001; Hattersley and Tooke 1999).

Once clinically manifested (commonly associated with *acanthosis nigricans*, which appears as hyperpigmented, velvety plaques at the base of the neck and in the flexures) IR deteriorates over time if not treated (Quinn *et al.* 2009). Metformin, usually in association with lifestyle interventions, has shown benefits in improving insulin sensitivity (along with a decrease in BMI) in children above the age of 10 years, but due to the side effects (nausea, diarrhoea and abdominal discomfort) it is not commonly recommended (Quinn *et al.* 2009).

Insulin resistance is recognised as a key feature of metabolic syndrome, which constitutes a cluster of metabolic disorders including central obesity, impaired glucose tolerance, hyperglycaemia, dyslipidemia (high level of triglycerides, low level of HDL-cholesterol) and high blood pressure (Zimmet *et al.* 2005). Several definitions for metabolic syndrome are in use for adults, the most commonly used being those proposed by the National Cholesterol Education Program (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults 2001), World Health Organization (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults 2001), and International Diabetes Federation (Alberti *et al.* 2005). Only recently has a consensus definition of metabolic syndrome in children and adolescents been established and endorsed by the International Diabetes Federation (Zimmet *et al.* 2007), which thus replaces the use of disparate definitions based on modified criteria for adults (Cook *et al.* 2003; Cruz *et al.* 2004; Ford *et al.* 2005; Weiss *et al.* 2004). The use of various definitions for metabolic syndrome has contributed to reporting of different prevalence rates in the population.

### **2.3.2.1 Public health significance – Prevalence data**

Insulin resistance in children and adolescents, largely confined to those who are overweight or obese, has become a topic of interest following the rising prevalence of obesity in the paediatric populations and the detection of type 2 diabetes at younger ages (Kaufman 2002).

Most published reports refer to the prevalence of metabolic syndrome, and not specifically to the prevalence of IR in children and adolescents. For example, US estimates based on the National Health and Nutrition Examination Survey 1999-2000 data indicate that 6.4% of adolescents (12-19 years) have a metabolic syndrome phenotype (32.1% of obese, 7.1% of overweight and under 1% of normal weight adolescents), an increase from the 4.2% reported from the 1988-1992 data collection (Duncan *et al.* 2004). Lower rates of metabolic syndrome (about 5%) were reported in younger (7-9 year-old) US children (DuBose *et al.* 2006) compared to adolescents.

A recent review of 36 studies published between August 2007 and January 2009, including general population samples and samples of overweight or obese children and adolescents (only one of these studies being conducted in Australia, in Aboriginal children (Sellers *et al.* 2008)) explicitly describing the definition employed for the diagnosis of metabolic syndrome (with clear cut-off values for all the risk factors), concluded that the mean prevalence of metabolic syndrome in children and adolescents worldwide is 10%, ranging between 2% in normal weight individuals and 32% among obese (Tailor *et al.* 2010). Based on these data, it appears that although the prevalence of metabolic syndrome is

not particularly high in children, it is 15 times higher amongst obese children, supporting screening for metabolic syndrome in this group (Tailor *et al.* 2010). This review also identified a higher prevalence of metabolic syndrome in males than females, probably due to higher rates of central obesity (Tailor *et al.* 2010).

As mentioned, very few studies have reported prevalence of IR alone in children. Data from a study of American adolescents showed that more than half of the obese adolescents had increased IR, as determined by homeostasis model assessment (HOMA-IR >4.39) (Lee *et al.* 2006). Despite using less strict criteria for defining IR, two European studies reported somewhat lower rates in 10 year-old obese children from Crete (31%, based on HOMA-IR>2.1) (Manios *et al.* 2008) and Southern Italy (40.8%, using a cut-off for HOMA-IR of 2.5) (Valerio *et al.* 2006). The difference between the US and latter two studies is likely due to the increase in IR during puberty.

Of concern, type 2 diabetes has also emerged as a health issue in obese children and adolescents, especially in individuals with family history, or in high risk ethnic groups, such as African-American, Hispanic-American, Pima Indians, Americans with an Asian descent (American Diabetes Association 2000) or the Indigenous Australian population (Maple-Brown *et al.* 2010). An increase in the prevalence of type 2 diabetes in the past decade has been documented in the US and the UK. For example, while in 1998 six per 1000 British children were prescribed an oral antidiabetic drug, in 2005 significantly more (50 per 1000) children received this type of medication (Hsia *et al.* 2009).

### **2.3.2.2 Adverse health outcomes of insulin resistance**

Insulin resistance is regarded as a pathophysiologic link between adiposity and associated metabolic disorders and chronic cardiovascular disease (Lee 2006; Weiss and Kaufman 2008). Complications associated with IR are well documented in adults and recent studies have shown that they may also occur in children and adolescents, as described below:

- impaired glucose tolerance and type 2 diabetes (Alberti *et al.* 2004), when pancreatic  $\beta$ -cell function fails to compensate for IR;
- high blood pressure (Maffeis *et al.* 2010), presumably due to an insulin-mediated effect on the sympathetic nervous system (Landsberg 1999) and on renal sodium reabsorption (Hall 1997);
- abnormal lipid profile (high triglyceride and low-density lipoprotein cholesterol levels, and low high-density lipoprotein cholesterol level), premature development of atherosclerosis (Le *et al.* 2010);



- enhanced blood coagulability due to higher levels of plasminogen activator inhibitor-1 and fibrinogen (Matsuzawa 2005);
- hepatic steatosis characterised by the accumulation of triglycerides in hepatic cells as a result of the increased flux of free fatty acids to the liver and the enhanced hepatic lipogenesis (Browning and Horton 2004);
- systemic inflammation, potentially mediating the relationship between IR and asthma (Al-Shawwa *et al.* 2007).

It is known that, similar to overweight and obesity, IR and associated morbidities track from childhood into adult life, independently of (Sinaiko *et al.* 2006) and synergistically with BMI (Morrison *et al.* 2009). This suggests that metabolic syndrome in adults could be, at least partly, prevented by early detection of IR during childhood followed by appropriate management. Given all these adverse health outcomes of IR, research directed towards the identification of early life factors which contribute to alterations in insulin sensitivity in childhood deserves priority.

### 2.3.2.3 Assessment of insulin resistance in children

A range of tools have been developed for assessing insulin sensitivity in humans and most of them have been validated in children (Lee 2006). They vary in complexity and the conditions in which they are performed (steady- or dynamic-state), which dictate their application depending on the purpose of the study. Some of these methods provide a direct measure of insulin sensitivity by quantifying insulin-mediated glucose uptake under steady-state conditions (such as hyperinsulinemic-euglycaemic glucose clamp or the insulin suppression test), while others (such as the minimal model analysis of frequently sampled intravenous glucose tolerance test and the oral glucose tolerance test) use dynamic data to generate an indirect estimate of insulin sensitivity (Muniyappa *et al.* 2008). However, all these methods are impractical in studies with large numbers of participants, especially children. As an alternative, a number of equations based on fasting insulin and glucose have been proposed and validated for clinical research and epidemiological studies of children (Gungor *et al.* 2004). They include fasting insulin level, fasting glucose to fasting insulin ratio (FGIR), the homeostasis model assessment, the quantitative insulin sensitivity check index (QUICKI). The Belfiore glycaemia and McAuley index (McAuley *et al.* 2007) are used less commonly. The most widely used methods for estimating IR are briefly described and critiqued below.



The gold standard method for assessing IR in humans is the **hyperinsulinemic-euglycaemic clamp**, valid both in adult (Morey 2003) and child populations (Arslanian 2005). It is the only reliable method to identify IR in individuals with frank hyperglycaemia or diabetes (Morey 2003). This technique involves intravenous administration of insulin to raise plasma insulin to a certain level, followed by an infusion of 20% dextrose at variable rates, thereby 'clamping' the plasma glucose concentration to a basal level. Insulin resistance is inversely proportional to the amount of glucose required to maintain basal glucose levels. The technique is extremely complex and invasive, requiring double vascular access, one for insulin and glucose infusions, and one for frequent blood sampling to measure plasma glucose level (every 2-5 minutes) and insulin level (every 10-15 minutes) over 3 hours (Arslanian 2005).

Another method of insulin sensitivity assessment is the **insulin suppression test**. This technique relies on the intravenous administration of somatostatin, which suppresses endogenous insulin production, followed by a simultaneous infusion of insulin and glucose (to ensure steady-state conditions) for 3 hours, during which repeated blood samples are collected (Muniyappa *et al.* 2008). Insulin sensitivity is inversely proportional to the plasma glucose concentration measured in the steady-state period (Muniyappa *et al.* 2008).

Among the indirect methods of estimating IR is the **frequently sampled intravenous glucose tolerance test** (FSIVGTT), which involves intravenous infusion of a certain amount of glucose, followed by a bolus of insulin, and multiple blood sampling at exact timing usually over a period of 3 hours; plasma glucose and insulin levels at specific time points are then used to derive an index of insulin sensitivity (Muniyappa *et al.* 2008).

Alternative methods have been proposed based on a 2-hour **oral glucose tolerance test** (OGTT), which requires multiple blood sampling (at baseline, 30, 60, and 120 minutes following the oral administration of 1.75 g/kg glucose). Plasma glucose and insulin concentrations measured during this test are used in specific equations to derive whole body insulin sensitivity indices (Matsuda and DeFronzo 1999; Soonthornpun *et al.* 2003). OGTT, although less cumbersome compared to the hyperinsulinemic-euglycaemic clamp, is still impractical in paediatric epidemiological studies as it involves multiple blood sampling and requires co-operation over a prolonged period of time (Muniyappa *et al.* 2008).

Although the four methods outlined above provide valuable information for physiological studies and in clinical settings, they are expensive, time-consuming and laborious (they require intravenous

catheters and multiple blood sampling), hence not suitable for application in large epidemiological studies, especially of children (Morey 2003). In contrast to these invasive methods, simple proxy indices for insulin sensitivity have been derived based on fasting plasma insulin and glucose concentrations. These methods, whilst still requiring a fasting blood sample, are less invasive and less time consuming, hence more acceptable to use in epidemiological studies of children.

Among the indices of insulin sensitivity derived from fasting measurements of insulin and glucose, **homeostasis model assessment of insulin resistance** (HOMA-IR) is now the most commonly used in epidemiological and clinical research. HOMA-IR has a physiological basis and reflects the feedback loop between tissues involved in glucose regulation, namely the liver (hepatic glucose production) and the  $\beta$ -cells (insulin secretion), in homeostatic (fasting) conditions (Matthews *et al.* 1985). The original model was derived from mathematical interpretations of the interaction between IR and  $\beta$ -cell function; these estimations are then used to predict fasting steady-state insulin and glucose concentrations (Matthews *et al.* 1985). The original model approximates IR according to the following formula (simplified from iterative equations):

$$\text{HOMA-IR} = \text{Fasting plasma insulin } (\mu\text{U/ml}) \times \text{Fasting plasma glucose (mmol/l)} / 22.5$$

(Matthews *et al.* 1985)

The denominator (22.5) is an adjustment to normal population levels; it is the product of the fasting plasma insulin (5  $\mu\text{U/ml}$ ) and fasting plasma glucose (4.5 mmol/l) levels considered normal in a healthy individual (Matthews *et al.* 1985).

Estimates of IR derived from HOMA-IR have been validated against more complex methods within individuals with normal or impaired glucose tolerance, and mild forms of diabetes, provided the blood collection takes place in steady-state conditions (Wallace *et al.* 2004). High correlations have been identified in adults between insulin sensitivity estimated by HOMA-IR and measured by the hyperinsulinemic-euglycaemic clamp ( $r=0.88$ ,  $p<0.0001$  (Matthews *et al.* 1985);  $r=0.73$ ,  $p<0.0001$  (Katsuki *et al.* 2001)), and between HOMA-IR and the index derived from the minimal model FSIVGTT ( $r=0.7$ ,  $p<0.001$  (García-Estévez *et al.* 2003)). The validity of HOMA-IR has been assessed in various populations of children and adolescents, most studies reporting good correlations against the clamp-derived index ( $r=0.91$ ,  $p<0.1$  (Gungor *et al.* 2004)) or the minimal model FSIGTT in obese children ( $r=0.89$ ,  $p<0.01$  (Conwell *et al.* 2004)). In contrast, other studies have indicated a weaker correlation between HOMA-IR and the index obtained from the minimal model FSIGTT ( $r=-0.4$ ,  $p<0.001$ ) in twins and children born small-for-gestational age (Cutfield *et al.* 2003). Compared to

other indices of IR derived from fasting plasma insulin and glucose (such as QUICKI or FGIR), HOMA-IR in obese children and adolescents is more accurate (has higher sensitivity and specificity), and therefore is more appropriate when other measures of insulin sensitivity are not possible (Keskin *et al.* 2005).

Normal IR is indicated by a value of 1 for HOMA-IR (Wallace *et al.* 2004). For diabetic adults, HOMA-IR ranges from 2.61 to 2.89 (Matthews *et al.* 1985). Among children and adolescents, the cut-off value for detecting metabolic syndrome with high sensitivity and specificity has not been conclusively established. Different mean values for HOMA-IR have been reported, ranging from 2.3 in normal weight adolescents (Lee *et al.* 2006), to 2.5 in pre-pubertal overweight children (Madeira *et al.* 2008), and 3.16 (Keskin *et al.* 2005) or 4.93 (Lee *et al.* 2006) in obese adolescents.

HOMA-IR has been deemed appropriate to quantify IR as a one-off measure and to assess longitudinal changes in IR (e.g., in order to examine the natural history of diabetes) (Wallace *et al.* 2004). HOMA-IR is chosen in the current study over other indices based on fasting plasma insulin and glucose, as it has been proven to be accurate in children and adolescents (Keskin *et al.* 2005).

## 2.4 Evidence of early origins of childhood obesity and insulin resistance

The focus of interest for this project was on the influence of three modifiable factors known to alter the intrauterine milieu (maternal pre-pregnancy obesity, glucose intolerance during pregnancy across the entire spectrum, and gestational weight gain), separately and concomitantly, on the programming of obesity and insulin resistance in pre-pubertal children. The following three sections include a summary and critique of published epidemiological studies addressing the influence that each of these intrauterine factors have on obesity and insulin resistance in childhood, identifying limitations and gaps in the existing literature. A greater emphasis is given to longitudinal studies, known to provide the strongest evidence for such associations.

### 2.4.1 Intrauterine programming of obesity and insulin resistance by maternal pre-pregnancy obesity

Epidemiological studies increasingly suggest a link between maternal pre-pregnancy obesity and metabolic perturbations in the offspring. Maternal obesity at the time of pregnancy has been suggested as an independent risk factor for obesity in the next generation (Nelson *et al.* 2010). A

summary of the longitudinal studies examining the relationship between maternal obesity status at the time of pregnancy and child obesity, adiposity, fat pattern and insulin resistance beyond infancy in low risk populations is presented in Table 6 and discussed below.

Seven longitudinal studies comprising contemporary samples of children (born between 1982 and 2000) of various ages, from around the world provide consistent evidence for a positive association between maternal overweight status at the time of pregnancy and the development of childhood obesity, as measured by BMI (Boerschmann *et al.* 2010; Lawlor *et al.* 2007b; Li *et al.* 2005; Pirkola *et al.* 2010; Reilly *et al.* 2005; Whitaker 2004). Of relevance to the age group considered in the present study, OR for maternal weight and child obesity ranged from 4 (95% CI 1.23, 13.2) in 6-11 year-old children (Catalano *et al.* 2009a) to 7 (95% CI 1.8, 27.7) in 11 year-olds (Boerschmann *et al.* 2010). Based on data from studies that included at least two age groups, it appears that the association might increase in peri-pubertal years compared with early childhood (Boerschmann *et al.* 2010; Li *et al.* 2005), but it is difficult to draw conclusions from only two studies. Variations may also be due to differences in exposure and outcome definitions (critiqued below) or to differences in populations' characteristics (e.g., in the study by Boerschmann *et al.* (2010) children were born to mothers with GD). A further increased risk of obesity was observed in children of obese mothers who also developed GD during the index pregnancy compared to those whose mothers were obese but maintained normal glucose tolerance during pregnancy (Pirkola *et al.* 2010).

The few prospective studies looking beyond weight relative to height of child in relation to maternal overweight status prior to pregnancy similarly reported a positive association for child percentage body fat in pre-pubertal years (Blair *et al.* 2007; Catalano *et al.* 2009a) and for waist-to-height ratio in adolescents (Pirkola *et al.* 2010). The latter association was found to be amplified by GD (Pirkola *et al.* 2010).

Two sets of evidence are in favour of a transmitted effect of maternal obesity through the intrauterine milieu in addition to an inherited (genetic) one on the increased risk of overweight status in the offspring. First, maternal pre-pregnancy BMI appeared as a better predictor of child body size than paternal BMI at the time of conception (Lawlor *et al.* 2007b; Reilly *et al.* 2005). Furthermore, based on data from the Avon Longitudinal Study of Parents and Children (ALSPAC), the risk of childhood obesity at 7 years increased exponentially when both parents were obese at conception (OR=10.4 (95% CI 5.11-21.3) when both parents were obese; OR=4.25 (95% CI 2.86-6.32) when mother was obese; OR=2.54 (95% CI 1.72-3.75) when father was obese) (Reilly *et al.* 2005). Second, evidence for the role of the intrauterine milieu in programming obesity risk in the child was revealed by a study

of siblings discordant for *in utero* exposure to maternal obesity, which showed a reduction in the prevalence of obesity by 52% in offspring born after bariatric surgery, reaching levels similar to the general population (Kral *et al.* 2006); in the latter study, it is possible that women also changed their diet and lifestyle, with potential positive effects on child body size.

The evidence is scarce and inconsistent with respect to the effect of maternal obesity on insulin resistance in the offspring. One family-based study conducted in young adults found a significantly higher degree of insulin resistance in offspring of mothers who were obese at the time of pregnancy compared to those of normal weight mothers (Mingrone *et al.* 2008). In a recent cohort study, Catalano *et al.* (2009b) documented for the first time that insulin resistance in the offspring exposed to maternal pre-pregnancy obesity is apparent at birth and reported a positive relationship ( $r=0.27$ ,  $p=0.002$ ) between maternal pre-pregnancy BMI and neonatal insulin resistance as measured by HOMA-IR, based on umbilical cord blood samples (mothers had fasted to prepare for caesarean section, thus ensuring steady-state conditions). These two studies are not included in Table 6 as they were not studies of children beyond infancy. Of greater relevance to the present study, a longitudinal study in 8-11 year old children of women with GD did not show an association between maternal pre-pregnancy BMI and child insulin resistance (Boerschmann *et al.* 2010).

**Table 6. Summary of longitudinal studies examining the influence of maternal pre-pregnancy overweight / obesity on child obesity and insulin resistance**

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of maternal pre-pregnancy BMI	Outcome measurement	Covariates, confounders	Effect of maternal pre-pregnancy weight status	
									Unadjusted	Adjusted
Blair <i>et al.</i> 2007	New Zealand  Auckland Birthweight Collaborative Study	Caucasian	Prospective	591	7	self-reported pre-pregnancy weight and height (after delivery)  Nwt/Owt/Ob	%BF (BIA)	maternal age, smoking, hypertension in pregnancy, SES, BW, breastfeeding, rapid weight gain in infancy, sedentary time	Overweight mothers: +3% (p<0.01) Obese mothers: +4% (p<0.01)	Overweight mothers: +3.7% (p<0.01) Obese mothers: +4% (p<0.01)
Boerschmann <i>et al.</i> 2010	Germany	Caucasian (>98%)  - all women with GD	Prospective	89 74	8 11	early pregnancy weight and height (measured by physicians)  Nwt/Owt/Ob	BMI ≥ 90 <sup>th</sup> centile (German reference)  HOMA-IR	birth size, smoking, therapy of GD	Overweight mothers: 8 y: OR=7.4 (0.8, 68.2) 11 y: OR=3.1 (0.5, 19.5)  Obese mothers: 8 y: OR=16 (1.8, 140.9) 11 y: OR=9.3 (1.8, 48.7)  No association pre-pregnancy BMI and HOMA-IR in offspring of GD mothers	Not reported for overweight mothers  Obese mothers: 8 y: OR=5.1 (1.4, 18.6) 11 y: OR=7.0 (1.8, 27.7)  No association pre-pregnancy BMI and HOMA-IR in offspring of GD mothers
Catalano <i>et al.</i> 2009a	USA	Caucasian, African American, Hispanic, Asian  - elective caesarean section	Prospective	89 63	6-11	self-reported pre-pregnancy weight (at delivery), measured height  Nwt/Owt vs. Ob	Weight centile (2000 CDC) (highest tertile)  % BF (DXA) (highest tertile)	GD, GWG, family history of diabetes, maternal age, parity, 1-h glucose screening, smoking, paternal weight and height  + %BF at birth	Obese mothers: OR=3.75 (1.39, 10.1)  Obese mothers: OR=5.45 (1.62, 18.4)	Obese mothers: OR=4.03 (1.23, 13.2)  Obese mothers: OR=7.75 (1.51, 37.4)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of maternal pre-pregnancy BMI	Outcome measurement	Covariates, confounders	Effect of maternal pre-pregnancy weight status	
									Unadjusted	Adjusted
Lawlor <i>et al.</i> 2007b	Australia  Mater University Study of Pregnancy and its Outcomes	Not specified	Prospective	3,795	14	self-reported pre-pregnancy weight and height (at first antenatal clinic)  internal age-specific SD for BMI	Internal age- and sex- specific SD for BMI	Paternal BMI at the time of pregnancy, income, parental education, maternal age, smoking  + size at birth	Not reported	+1SD in maternal pre-pregnancy BMI -> +0.36SD in child BMI (0.32, 0.40)  +1SD in maternal pre-pregnancy BMI -> +0.362SD in child BMI (0.323, 0.402)
Li <i>et al.</i> 2005	USA  National Longitudinal Survey of Youth	White, Black, Hispanic	Retrospective	2,636	2-16	self-reported pre-pregnancy weight and height (after delivery)  Uwt/Nwt/Owt/Ob	BMI $\geq$ 95 <sup>th</sup> centile (CDC)	GWG, gestational age, birth order, race/ethnicity, parity, age, smoking, alcohol use, education, family income, BW, breastfeeding	Not reported	Owt mothers: 2-6 y: OR=2.4 (1.4, 4.5) 7-10 y: OR=2.2 (1.2, 4.1) 11-16y: OR=3.3 (1.7, 6.2)  Ob mothers: 2-6 y: OR=2.8 (1.3, 6) 7-10y: OR=5.7 (2.9, 11.7) 11-16y: OR=4.3 (1.7, 10.8)
Pirkola <i>et al.</i> 2010	Finland  Northern Finland Birth Cohort of 1986	Caucasian	Prospective	4,186	16	Nwt vs. Owt/Ob	BMI $\geq$ 85 <sup>th</sup> centile (IOTF)  Waist-to-height ratio > 0.5	GD (selective screening - high risk)  Pre-pregnancy smoking, paternal BMI, size at birth	Relative to Nwt women without risk factors for GD: Owt/Ob women with normal OGTT OR=2.92 (2.03, 4.22) Owt/Ob women with GD OR=5.03 (2.54, 9.97)  Relative to Nwt women without risk factors for GD: Owt/Ob women with normal OGTT OR=2.97 (1.95, 4.51) Owt/Ob women with GD OR=4.25 (1.97, 9.16)	Relative to Nwt women without risk factors for GD: Owt/Ob women with normal OGTT OR=2.56 (1.69, 3.88) Owt/Ob women with GD OR=4.05 (1.90, 9.16)  Relative to Nwt women without risk factors for GD: Owt/Ob women with normal OGTT OR=2.60 (1.62, 4.17) Owt/Ob women with GD OR=3.82 (1.66, 8.82)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of maternal pre-pregnancy BMI	Outcome measurement	Covariates, confounders	Effect of maternal pre-pregnancy weight status	
									Unadjusted	Adjusted
Reilly <i>et al.</i> 2005	UK Avon Longitudinal Study of Parents and Children	White, non-white	Prospective	7,758	7	self-reported weight and height (in early pregnancy)  Nwt/Owt vs. Ob	BMI ≥ 95 <sup>th</sup> centile (1990 British)	SES, education, BW, gender, parity, smoking, gestational age, breastfeeding, ethnicity, maternal age	OR=4.66 (3.28, 6.64)	OR=4.25 (2.86, 6.32)
Whitaker 2004	USA  Special Supplemental Nutrition Program for Women, Infants, and Children	White, black, Hispanic, other  Low-income families	Retrospective + data linkage	8,494	2-5	weight and height measured in the first trimester of pregnancy  Uwt/Nwt/Owt/Ob/ morbidly Ob	BMI ≥ 95 <sup>th</sup> centile (CDC)	BW, gestational age, gender, race / ethnicity, parity, age, smoking, education, net GWG (self-report), marital status	Prevalence of child obesity: Nwt mothers 2 y: 6.4% 4 y: 9% Owt mothers 2 y: 9% 4 y: 14% Ob mothers 2 y: 13.9% 4 y: 22.8%	Owt mothers: 2 y: OR=1.42 (1.13, 1.79) 4 y: OR=1.75 (1.4, 2.18)  Ob mothers: 2 y: OR=2.28 (1.84, 2.83) 4 y: OR=3.07 (2.48, 3.79)  No significant interactions with covariates

%BF – percentage body fat, BIA – bioelectrical impedance analysis, BW – birth weight, CDC – Centers for Disease Control and Prevention, DXA – dual energy X-ray absorptiometry, GD – gestational diabetes, GWG – gestational weight gain, HOMA-IR – homeostasis model assessment of insulin resistance, IOTF – International Obesity Task Force, Nwt – normal weight, Ob – obese, OGTT – oral glucose tolerance test, Owt – overweight, SES – socioeconomic status, SD – standard deviation, Uwt - underweight



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## Critique of longitudinal studies investigating the influence of maternal pre-pregnancy overweight status on child obesity and insulin resistance

The summarised studies concerning the association between maternal pre-pregnancy BMI and child obesity and insulin resistance have a number of limitations, including their exposure and outcome assessment, sampling frames and representativeness. A critique of these studies and identification of their limitations follow.

### Exposure definition

All but one study defined maternal overweight status as a categorical variable, using BMI cut-offs recommended by WHO (World Health Organization 1995). Although data was available on BMI as a continuous variable in each of these studies, it was not used in analytical models. Hence the effect size of increasing pre-pregnancy BMI by one unit on child obesity risk could not be reported. The exception was Lawlor *et al.* (2007b) who reported a third of an SD increase in child BMI z-score (internally derived) for each SD increase in maternal BMI.

No studies directly measured maternal weight prior to pregnancy. Most studies based their maternal pre-pregnancy BMI calculation on pre-pregnancy weight self-reported during pregnancy (Lawlor *et al.* 2007b; Reilly *et al.* 2005) or at/after the time of delivery (Blair *et al.* 2007; Catalano *et al.* 2009a; Li *et al.* 2005). Three of these studies also used self-reported height (Blair *et al.* 2007; Li *et al.* 2005; Reilly *et al.* 2005). This has potential implications on the interpretation of the results due to information bias. Given women's tendency to under-report their weight, particularly in overweight and obese women, with subsequent potential underestimation of pre-pregnancy obesity, it is possible that the associations reported underestimate the true association. Boerschmann *et al.* (2010) and Whitaker (2004) used direct measurements of weight and height in the first trimester of gestation as proxy measures for maternal pre-pregnancy weight and height; there may be some variation from weight at conception, but for most women this is likely to be small.

### Outcome definition

Different definitions of childhood overweight and obesity were employed by these studies. All but one (Lawlor *et al.* 2007b) used externally derived BMI centiles for a specific population, two based on American reference data (Li *et al.* 2005; Whitaker 2004), one on British (Pirkola *et al.* 2010; Reilly *et al.* 2005), one on German (Boerschmann *et al.* 2010) and only one on IOTF centiles (Pirkola *et al.* 2010). Thus, the reported adjusted ORs for childhood overweight and obesity are not directly

comparable between studies, ranging from 2.28 to 7 in children aged 2-16 years. The ORs appear to increase with age; for instance, Li *et al.* (2005) reported OR of obesity in children of obese mothers ranging from 2.8 in 2-6 year olds, to 5.7 in 7-10 year olds and 4.3 in 11-16 year olds.

Only one study reported how the spectrum of maternal pre-pregnancy BMI influences child BMI (internally derived age- and sex-specific BMI z-score) (Lawlor *et al.* 2007b). This is important as it removes the influence of arbitrary cut-offs between studies and allows the investigation of smaller unit shifts in BMI.

Studies examining only child BMI as an outcome are limited to drawing conclusions on weight relative to height, and cannot comment on the amount of adiposity or fat distribution. Only three studies looked beyond BMI in examining intrauterine programming of childhood obesity by maternal pre-pregnancy overweight status. Of them, two studies focused on child %BF, estimated by BIA (Blair *et al.* 2007) or DXA (Catalano *et al.* 2009a). While both studies showed a positive association between maternal pre-pregnancy overweight status and child %BF, neither provided information on how pre-pregnancy BMI across the spectrum can influence child %BF.

The only longitudinal study assessing offspring fat pattern in relation to maternal overweight during pregnancy was conducted in the Finnish cohort of adolescents and defined abdominal obesity as waist-to-height ratio  $> 0.5$  (Pirkola *et al.* 2010). By using a dichotomous variable, small shifts in waist-to-height ratio that might occur as a result of intrauterine exposure to maternal overweight, but without shifting the ratio above 0.5, could be missed.

## Confounders

Each study adjusted for a different set of covariates, including various indicators of SES, maternal age, parity, smoking, hypertension, paternal weight, birth weight or breastfeeding (the latter two factors are more likely to be on the causal pathway than confounding factors). Glucose tolerance during pregnancy, a factor that is thought to be associated both with maternal pre-pregnancy obesity and child obesity, was taken into account only by Catalano *et al.* (2009a) and Pirkola *et al.* (2010). Similarly, adjustment for weight gain during pregnancy was done in only three studies (Catalano *et al.* 2009a; Li *et al.* 2005; Whitaker 2004). None of the studies included all the potential confounders I have identified, namely glucose tolerance during pregnancy, gestational weight gain, maternal age, parity, smoking, pregnancy-induced hypertension, and education at the time of pregnancy (see Section 3.4.3 for confounder justification). Poor confounding adjustment could result in inflated ORs if an underlying factor is driving the association.

## Sample representativeness and external validity

Although some of the samples on which these analyses were based comprised over 2,000 participants from various ethnic backgrounds (Li *et al.* 2005; Pirkola *et al.* 2010; Reilly *et al.* 2005; Whitaker 2004), others were relatively small, with under 100 participants (Boerschmann *et al.* 2010; Catalano *et al.* 2009a). However, the effect size in pre-pubertal children did not seem to be influenced by the sample size (e.g., OR=4.25 in ALSPAC (Reilly *et al.* 2005), OR=4 in the small-scale study of Catalano *et al.* (2009a)).

Studies were not always representative of the general population. For instance, the cohort of German mother-child pairs reported by Boerschmann *et al.* (2010) included only women who developed GD during the index pregnancy. Thus those findings are unlikely to be generalisable to mothers who maintain normal glucose tolerance during pregnancy, which might explain the higher OR for child obesity identified in this study compared to other studies with children of similar age. Another example of non-random sample is given by the Special Supplementation Nutrition Program for Women, Infants and Children, which included only low income families (Whitaker 2004). With regard to socioeconomic status, some of the studies did not report whether their sample comprised the full SES range (Boerschmann *et al.* 2010; Catalano *et al.* 2009a).

Another important factor that might affect representativeness in longitudinal studies with long-term follow-up like the ones described in this section is participation rate, particularly if women or children with certain characteristics (e.g., lower SES) are less likely to participate at the study outset or in follow-up. Participation at the time of outcome measurement (number followed-up divided by number of participants at baseline) ranged between 38.4% at 8 years and 31.9% at 11 years in the German study with offspring of GD mothers (Boerschmann *et al.* 2010), to 53% at 14 years in Mater University Study of Pregnancy and Its Outcomes (Lawlor *et al.* 2007b), to 55.5% at 7 years in ALSPAC (Reilly *et al.* 2005), 67.9% in Auckland Birthweight Collaborative Study (Blair *et al.* 2007) and 80% in the Northern Finland Birth Cohort (Pirkola *et al.* 2010). High non-participation can lead to response bias, which may affect the inferences drawn from the study in question. Of note, comparison of baseline characteristics between participants and non-participants were reported only in two of the studies reviewed in this section. In the study by Lawlor *et al.* (2007b) participants at follow-up had lower birth weight, were less likely to be from low-income families, their parents were more educated, their mothers were older, but parental BMI at the time of pregnancy was similar to the original cohort. In contrast, in the study by Pirkola *et al.* (2010) parental BMI at pregnancy was

lower in the subgroup taking part in the follow-up compared to the initial cohort. Without full disclosure of attrition, it is challenging to evaluate the validity of the findings.

## Conclusion

Whilst a number of longitudinal studies have found positive associations between maternal pre-pregnancy overweight status and child obesity, firm conclusions on these associations cannot be drawn due to the limitations mentioned above. Thus, this critical appraisal demonstrates a need for a systematic replication with extension of exposures, outcomes and control for potential confounders in a study comprising a representative, contemporary Caucasian population of children. With the exception of one study that examined how maternal pre-pregnancy BMI across the entire spectrum influences child BMI (using internally derived SD scores), progressive effects of discrete changes in maternal body size prior to pregnancy on child body size, body composition, fat pattern and insulin resistance have not been investigated. These are the gaps identified in the literature that this thesis seeks to fill. Removing arbitrary cut-offs (using a continuous exposure and outcome measures) is important because it can demonstrate that even incremental increases in maternal body size, regardless of meeting criteria for overweight, might increase risk in the child. The influence of maternal body size on child BMI is certainly important, but the potential effects on more specific measures of adiposity (percentage body fat or fat distribution) and insulin resistance are of even greater relevance for the future metabolic health of the child, as they are better predictors of cardiovascular risk than BMI (Goran and Gower 1999; Steinberger *et al.* 2001). Therefore, these potential associations, sparingly assessed to date, warrant examination, as they might indicate targets for obesity and insulin resistance prevention. Moreover, the incomplete adjustment for confounders in previous studies may have resulted in overestimation of the true associations.

### 2.4.2 Intrauterine programming of obesity and insulin resistance by maternal glucose intolerance during pregnancy

A growing body of epidemiological literature has been devoted to the description of long-term metabolic alterations associated with prenatal exposure to maternal glucose intolerance during pregnancy. A summary of longitudinal studies investigating intrauterine programming of obesity, body composition, fat pattern and insulin resistance in children (beyond neonatal period) by maternal glucose intolerance during pregnancy in non-high risk populations worldwide is presented in Table 7 and discussed below.

## Intrauterine programming of obesity and insulin resistance by gestational diabetes

The evidence of an association between maternal GD and long-term risk of obesity in the child, although relatively abundant, is inconsistent. Early reports came from longitudinal prospective studies in Pima Indians, the population with the highest known incidence and prevalence of type 2 diabetes in the world, which showed that *in utero* exposure to diabetes results in increased weight for height during childhood, independent of size at birth (Pettitt *et al.* 1983; 1987). It could be argued that the increased risk of obesity in this population is primarily due to genetic factors and not to the diabetic intrauterine environment. In support of the latter are the findings of a study conducted in siblings of Pima Indians (with two or more siblings per family), whose mothers developed type 2 diabetes between two pregnancies (meaning that children born before the diagnosis were not exposed to diabetes *in utero*, while subsequent siblings were) (Dabelea *et al.* 2000). Participants aged 9 years and older, who were exposed to diabetes *in utero* had higher BMI (+2.6 kg/m<sup>2</sup>,  $p=0.003$ ) compared to their unexposed siblings (who presumably had a similar risk of inheriting the same genetic predisposition), but there was no difference in BMI between siblings born before and after their father's diagnosis of type 2 diabetes. Moreover, data from the same study showed that intrauterine exposure to type 2 diabetes trebled siblings' risk of developing type 2 diabetes themselves (OR=3.7, 95% CI 1.3, 11.3) compared to those not exposed (Dabelea *et al.* 2000).

The findings from Pima Indian studies stimulated initiation of studies in populations with lower prevalence of GD, which have generated conflicting results. Studies report increased weight relative to height (Catalano *et al.* 2009a; Crume *et al.* 2011a; Egeland and Meltzer 2010; Krishnaveni *et al.* 2010; Silverman *et al.* 1991; Silverman *et al.* 1998), higher BMI growth velocity (Crume *et al.* 2011b), or greater prevalence of obesity (Boerschmann *et al.* 2010) in pre-pubertal and pubertal children exposed to maternal GD compared to those born to mothers who maintained normal glucose tolerance during pregnancy. It was also observed that BMI in adolescents of diabetic mothers (either pre-gestational diabetes or GD) correlated with fetal insulin secretion, as measured by the amniotic fluid insulin (Silverman *et al.* 1998). In contrast, other studies of populations without a high risk for diabetes did not find a robust association between GD and global obesity in children aged 5-16 years, after adjustment for potential confounders, including maternal body size (Gillman *et al.* 2003; Jeffery *et al.* 2006; Pirkola *et al.* 2010; Whitaker *et al.* 1998). One of these studies concluded that the GD influence on child obesity risk in peri-pubertal years was partially explained by the effect on birth weight (Gillman *et al.* 2003). The findings from Whitaker *et al.* (1998) and Pirkola *et al.* (2010) may have underestimated the true association between GD and child obesity as women diagnosed

with GD were given treatment (including dietary advice, glucose monitoring and insulin if needed); this treatment may have lessened the severity of fetal exposure to maternal glucose intolerance. The findings of Jeffery *et al.* (2006) need to be interpreted with caution given that maternal third trimester fasting glucose levels were documented only in a small subgroup of women (n=26) with random glucose level >6.5 mmol/l.

Similarly, there is little and inconsistent evidence on the relationship between glucose intolerance during pregnancy and child body composition. After confounder adjustment, exposure to GD was positively associated with offspring fat mass at birth (Catalano *et al.* 2003b), subscapular and triceps skinfold thickness at 3 years (Wright *et al.* 2009) and at 9.5 years (Krishnaveni *et al.* 2010), but not with percentage body fat estimated by DXA at 6-11 years (Catalano *et al.* 2009a).

Childhood central adiposity in relation to intrauterine exposure to maternal glucose intolerance was explored in four longitudinal studies (Catalano *et al.* 2009a; Crume *et al.* 2011a; Egeland and Meltzer 2010; Pirkola *et al.* 2010), again with contradictory results. The Exploring Perinatal Outcomes among Children (EPOCH) study showed increased waist circumference (by 3.2 cm, p=0.05 in fully adjusted model) and marginally greater subcutaneous adipose tissue in the abdominal region quantified by MRI (by 26 cm<sup>3</sup>, p=0.08 in fully adjusted model) in 6-13 year-old offspring exposed to diabetes *in utero* compared to those not exposed; however, the association initially observed between maternal diabetes and the ratio of subscapular to triceps skinfold thickness in the offspring was attenuated to null after adjustment for maternal pre-pregnancy BMI (Crume *et al.* 2011a). In another study, female offspring exposed to GD or impaired glucose tolerance while *in utero* had significantly greater waist circumference at age 15 years compared to those of normal glucose tolerant mothers (mean difference 5.9 cm after covariate adjustment) (Egeland and Meltzer 2010). The findings of this study are limited by the relatively small sample size (90 mothers with GD or impaired glucose tolerance, and 99 mothers with normal glucose tolerance during pregnancy), low participation rates in both groups (51.6% and 24.4%, respectively) (Egeland and Meltzer 2010) and exclusion of male children. The other two studies did not identify an association between maternal GD and offspring waist circumference at 6-11 years (Catalano *et al.* 2009a) or waist-to-height ratio in 16 year-old offspring of normal weight mothers (Pirkola *et al.* 2010). Variations may be due to differences in the age at which offspring were assessed, as well as differences in exposure and outcome definitions, to be explored later in this section.

Exposure to a diabetic intrauterine environment has also been linked to childhood glucose-insulin homeostasis, but the evidence has been inconclusive. A greater prevalence of impaired glucose

tolerance was identified in adolescents exposed to maternal diabetes *in utero* (with no differential effect between GD, type 1 or type 2 diabetes during pregnancy) compared to those not exposed and it correlated with the higher level of amniotic fluid insulin, as a marker of excessive fetal insulin secretion (Silverman *et al.* 1995). Inconsistencies also exist in relation to the risk of insulin resistance in children whose mothers developed glucose intolerance during pregnancy. Three recent studies identified increased HOMA-IR in peri-pubertal children (Boerschmann *et al.* 2010; Egeland and Meltzer 2010; Krishnaveni *et al.* 2010), while two others found no increase in younger children (Catalano *et al.* 2009a; Jeffery *et al.* 2006) of mothers with GD. Of note, adjustments for potential confounders were not performed in any of these studies with HOMA-IR as primary outcome. Moreover, only the study by Krishnaveni *et al.* (2010) considered the potential mediating effect of current BMI, which attenuated to null the association initially identified. It is possible that puberty was the driving factor for the increased HOMA-IR reported by the former three studies.

A longitudinal study linking maternal GD and offspring size at birth with later development of metabolic syndrome showed that children born large-for-gestational-age whose mothers had GD were at increased risk of developing insulin resistance by 11 years as estimated by the fasting glucose to insulin ratio  $<7$ , but not by HOMA-IR (Boney *et al.* 2005). Moreover, these children were reported to have an increased risk of metabolic syndrome based on a definition that, importantly, did not consider waist circumference, despite this conventionally being a core component of the metabolic syndrome (Zimmet *et al.* 2007).

### **Intrauterine programming of obesity and insulin resistance by milder degrees of glucose intolerance during pregnancy**

There is some epidemiological evidence suggesting that milder elevations of maternal glycaemia during pregnancy (not diagnostic for GD) may also be related to an increased risk of obesity in the offspring. Initial studies conducted in American Indians (mainly Pima Indians) showed positive associations between maternal 2-hour glucose level during OGTT and offspring age-, sex- and height-specific weight at 5-14 years (Pettitt *et al.* 1991), and between maternal third trimester glucose level and offspring BMI and waist circumference in subjects aged 10-14 years (but not other age groups), whose mothers had an abnormal OGCT, but a normal OGTT (Franks *et al.* 2006).

Few studies have addressed intrauterine programming of child obesity by maternal chronic hyperglycaemia during pregnancy in non-high risk populations. One data linkage study reported an



increased risk of overweight and obesity in children who were not macrosomic at birth and whose mothers were in the highest quartile of plasma glucose levels at OGCT (Hillier *et al.* 2007). An additional argument for a potential link between various degrees of maternal glucose intolerance during pregnancy and offspring obesity emerging from this study was the finding of lower obesity rates in children whose mothers received treatment for GD compared to those whose did not (Hillier *et al.* 2007). Importantly, no adjustment for maternal pre-pregnancy body size was performed in these analyses, which might confound the results.

Data from the ALSPAC study showed some evidence of an association between higher maternal glucose levels during pregnancy and several obesity-related measures in 9-11 year-old children (Lawlor *et al.* 2010). Maternal glycosuria (considered as a measure of a lower degree of hyperglycaemia than that characterising GD) was associated with 53% increased risk of child global overweight or obesity, and 39% increased risk of child central adiposity; these relationships were attenuated (to 35% and 31%, respectively) by adjustment for maternal pre-pregnancy BMI, but remained statistically significant (Lawlor *et al.* 2010).

Similar to some extent, in a small, retrospective study, Chandler-Laney (2011) showed that maternal plasma glucose level at OGCT as a continuous variable correlated positively with child BMI, fat mass, fat free mass and waist circumference at the age of 5-10 years.

Only one study reported on the association between various degrees of glucose intolerance during pregnancy and offspring insulin resistance during puberty (Egeland and Meltzer 2010), finding significantly greater mean HOMA-IR in girls of insulin-treated GD mothers (2.36) compared to both normal (1.58) and impaired glucose tolerance during pregnancy (1.56); no significant difference in mean HOMA-IR was found between daughters of mothers with normal and impaired glucose tolerance during pregnancy (Egeland and Meltzer 2010). The potential confounding effect of maternal pre-pregnancy BMI was not examined in this study.



**Table 7. Summary of longitudinal studies examining the influence of maternal glucose tolerance status during pregnancy on child obesity and insulin resistance**

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of glucose intolerance in pregnancy	Outcome measurement	Covariates, confounders	Effect of maternal glucose tolerance in pregnancy	
									Unadjusted	Adjusted
Boerschmann <i>et al.</i> 2010	Germany German GD offspring study & Baby-Diab study	Caucasian (>98%)	Prospective, combining two studies:	Children of GD mothers: 89 74  751 (74 OGD, 425 OT1D, 252 OND)	8 11	German Diabetes Association NGT GD T1D	BMI ≥ 90 <sup>th</sup> centile (German reference)          HOMA-IR	BMI in early pregnancy, smoking, GD therapy, birth size	Prevalence of obesity significantly higher in OGD 8 y: 20.2% 11 y: 31.1%  relative to OT1D 8 y: 11% (p=0.03) 11 y: 15.8% (p=0.01)  and OND 8 y: 10.3% (p=0.02) 11 y: 15.5% (p=0.005)  Significantly higher HOMA-IR in OGD compared to OT1D (p=0.03) and ONDM (p=0.04) (no estimates presented – graph only)	Exacerbated by BMI in early pregnancy in OGD group 8 y: 36.4% 11 y: 45.8%          Not reported
Boney <i>et al.</i> 2005	USA	White (>94%)	Prospective with a retrospective component	179  88	6, 7, 9, 11  11	National Diabetes Data Group NGT GD  4 groups: LGA / GD AGA / GD LGA / NGT AGA / NGT	BMI >85 <sup>th</sup> centile      HOMA-IR FGIR <7	Maternal pre-pregnancy BMI, GWG, SES, birth size	No difference among the 4 groups with regard to the prevalence of obesity or HOMA-IR  At 11 y, trend towards a higher incidence of FGIR <7 in LGA / GD group (p=0.08)	Interaction of LGA and GD (but no independent effect) on FGIR OR=10.4 (1.5, 74.4)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of glucose intolerance in pregnancy	Outcome measurement	Covariates, confounders	Effect of maternal glucose tolerance in pregnancy		
									Unadjusted	Adjusted	
Catalano <i>et al.</i> 2009a	USA	Caucasian, African American, Hispanic, Asian - elective caesarean section	Prospective	89	6-11	National Diabetes Data Group - NGT - GD	BMI z-score (2000 CDC)	Pre-pregnancy BMI, family history of diabetes, smoking, education, age, parity, GWG	Child BMI z-score in NGT: 0.31 GD: 0.90 (p=0.03)	Not presented, but concluded that pre-pregnancy obesity was a better predictor for child obesity	
				63			waist circumference, central to peripheral SFT ratio		No assoc with waist circumference (p=0.36) or ratio SFT (p=0.13)		
				47			%BF (DXA)		+ %BF at birth Child %BF in NGT: 27.8 GD: 31 (p=0.14)		
							HOMA-IR		HOMA-IR in NGT: 1.89 GD: 2.55 (p=0.08)		
Chandler-Laney <i>et al.</i> 2011	USA	African American (78%), European American	Retrospective	27	5-10	1-h glucose level at OGCT (from medical records)	BMI centile (CDC)	Ethnicity, resting energy expenditure, physical activity, diet	r=0.445 (p<0.05)	Not reported	
							Waist circumference		r=0.469 (p<0.05)		
							FM (DXA)		+FFM r=0.418 (p<0.05)		r=0.443 (p<0.05)
							FFM (DXA)		Height, sex r=0.122 (p>0.05)		r=0.371 (p=0.07)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of glucose intolerance in pregnancy	Outcome measurement	Covariates, confounders	Effect of maternal glucose tolerance in pregnancy	
									Unadjusted	Adjusted
Crume <i>et al.</i> 2011a	USA Exploring Perinatal Outcomes among Children	Multiethnic	Retrospective	461	6-13	National Diabetes Data Group	BMI	BW, gestational age, age, sex, race, Tanner stage, maternal age, SES, smoking, pre-pregnancy BMI	20.2 vs. 18.9 (p=0.02)	19.9 vs. 19.1 (p=0.2)
						GD NGT	waist circumfer.		69.6 vs. 65.4 (p=0.004)	68.7 vs. 65.5 (p=0.05)
							VAT (MRI) SAT (MRI)		27.8 vs. 24.2 (p=0.1) 156.4 vs. 121.7 (p=0.01)	26.4 vs. 25.3 (p=0.7) 147.1 vs. 121.0 (p=0.08)
							Subscapular to triceps SFT ratio		0.83 vs. 0.76 (p=0.01)	0.82 vs. 0.77 (p=0.2)
Egeland and Meltzer 2010	Canada	Caucasian, non-Caucasian	Prospective	189	15	National Diabetes Data Group	BMI centile (2000 CDC)	BW z-score, ethnicity, physical activity, diet, mother's 15 year weight gain, mother's current BMI, %BF, waist circumference	71.4 vs. 60.2 (p<0.01)	Attenuated (p<0.10)
						- NGT - IGT (1 abnormal value at OGTT) -GD (≥2 abnormal values at OGTT)	%BF (BIA)		31.5 vs. 28.7 (p<0.01)	Annulated (p<0.10)
							waist circumfer.		81.2 vs. 73.5 (p<0.001)	Attenuated (p<0.05)
							GD/IGT=cases NGT=controls		HOMA-IR	1.94 vs. 1.58 (p<0.05)
Gillman <i>et al.</i> 2003	USA Growing Up Today Study & Nurses' Health Study II	Nationwide, but homogeneous in terms of race / ethnicity	Linkage	14,881	9-14	Self-reported GD status	BMI centile (2000 CDC) >85 <sup>th</sup> >95 <sup>th</sup>	BW, breastfeeding, SES, diet, physical activity, age, Tanner stage, maternal current BMI	GD vs. NGT:	GD vs. NGT:
						GD NGT			OR <sub>&gt;85<sup>th</sup></sub> =1.2 (0.9, 1.5) OR <sub>&gt;95<sup>th</sup></sub> =1.4 (1.1, 2.0)	OR <sub>&gt;85<sup>th</sup></sub> =1.0 (0.7, 1.3) OR <sub>&gt;95<sup>th</sup></sub> =1.2 (0.8, 1.7)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of glucose intolerance in pregnancy	Outcome measurement	Covariates, confounders	Effect of maternal glucose tolerance in pregnancy	
									Unadjusted	Adjusted
Hillier <i>et al.</i> 2007	USA	Multiethnic	Data-linkage (electronic medical records)	9,439	5-7	Glucose levels at OGCT (universal screening)-quartiles  National Diabetes Data Group (likely treated if GD) / Carpenter and Coustan (likely untreated if GD) +OGCT, -OGTT +OGCT, +1 abnormal value at OGTT +OGCT, + ≥2 abnormal values at OGTT (GD – untreated) +OGCT, + ≥2 abnormal values at OGTT (GD – treated)	Weight (age-,sex-specific centiles) >85 <sup>th</sup> >95 <sup>th</sup>	GWG (self-reported), age, parity, ethnicity, BW, sex	Not reported	Highest vs. lowest quartile of glucose at OGCT: OR <sub>&gt;85<sup>th</sup></sub> =1.22 (1.03, 1.45) OR <sub>&gt;95<sup>th</sup></sub> =1.28 (1.02, 1.60)  Relative to -OGCT: +OGCT, -OGTT no significant change in OR <sub>&gt;85<sup>th</sup></sub> , OR <sub>&gt;95<sup>th</sup></sub>  +OGCT, +1 abnormal value at OGTT OR <sub>&gt;85<sup>th</sup></sub> =1.37 (1.01, 1.84) OR <sub>&gt;95<sup>th</sup></sub> =1.30 (0.89, 1.90)  +OGCT, + ≥2 abnormal values at OGTT (GD - untreated) OR <sub>&gt;85<sup>th</sup></sub> =1.89 (1.30, 2.76) OR <sub>&gt;95<sup>th</sup></sub> =1.82 (1.15, 2.88)  +OGCT, + ≥2 abnormal values at OGTT (GD - treated) OR <sub>&gt;85<sup>th</sup></sub> =1.29 (0.85, 1.97) OR <sub>&gt;95<sup>th</sup></sub> =1.38 (0.84, 2.27)
Jeffery <i>et al.</i> 2006	UK EarlyBird	Caucasian (mainly)	Retro- and prospective	249	8	3 <sup>rd</sup> trimester fasting glucose in women with random glucose > 6.5 mmol/l (n=26)	Weight z-score (1990 UK)	Not specified	r=-0.09 (p=0.66)	Not reported
							HOMA-IR	Current weight	Not reported	r=0.23 (p=0.29)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of glucose intolerance in pregnancy	Outcome measurement	Covariates, confounders	Effect of maternal glucose tolerance in pregnancy	
									Unadjusted	Adjusted
Krishnaveni <i>et al.</i> 2010	India	Indian	Prospective	455	9.5	OGTT Carpenter and Coustan	BMI (continuous)	Parental BMI	GD vs. NGT: Greater BMI in girls 16.4 vs. 14.3 (p<0.001)	
							SFT triceps, subscapular		Greater SFT in girls 14.9 vs. 10.5 (p<0.001) 14.1 vs. 7.6 (p<0.001)	
							HOMA-IR	Current BMI	Greater HOMA-IR Girls 1.2 vs. 0.9 (p=0.006) Boys: 1.1 vs. 0.6 (p=0.002)	Girls: not significant Boys: significant but not presented
Lawlor <i>et al.</i> 2010	UK Avon Longitudinal Study of Parents and Children	White, Non-white	Prospective	6,842	9-11	GD screened for in high risk women	BMI z-score (1990 UK)	Pre-pregnancy BMI, age, SES, parity, smoking,	Mean difference (95%CI) GD: 0.32 (0.01, 0.63) Glycosuria: 0.14 (0.01, 0.27)	Mean difference(95%CI) GD: 0.01 (-0.30, 0.63) Glycosuria: 0.09 (-0.05, 0.22)
						≥2 episodes of glycosuria	Waist circumference z- score		GD: 0.04 (0.00, 0.08) Glycosuria: 0.02 (0.00, 0.03)	GD: 0.00 (-0.04, 0.06) Glycosuria: 0.01 (0.00, 0.03)
							FM z-score (DXA)	+ height, height squared	GD: 0.07 (-0.21, 0.36) Glycosuria: 0.18 (0.06, 0.30)	GD: -0.16 (-0.43, 0.11) Glycosuria: 0.12 (0.01, 0.23)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of glucose intolerance in pregnancy	Outcome measurement	Covariates, confounders	Effect of maternal glucose tolerance in pregnancy	
									Unadjusted	Adjusted
Pirkola <i>et al.</i> 2010	Finland Northern Finland Birth Cohort of 1986	Caucasian	Prospective	4,186	16	Selective screening for GD with 75g 2-h OGTT  3 categories: - GD if one abnormal value at OGTT - normal OGTT - control (no risk factors for GD)	BMI $\geq$ 85 <sup>th</sup> centile (IOTF)  Waist-to-height ratio > 0.5	Pre-pregnancy BMI, smoking, paternal BMI, size at birth	In Nwt women, relative to women without risk factors for GD: women with GD OR=0.67 (0.24, 1.89) women with normal OGTT OR=1.18 (0.90, 1.56)	In Nwt women, relative to women without risk factors for GD: women with GD OR=0.73 (0.26, 2.08) women with normal OGTT OR=1.13 (0.83, 1.54)
Silverman <i>et al.</i> 1991	USA Diabetes in Pregnancy Study at Northwestern University in Chicago	Caucasian, Black, Hispanic, other	Prospective	124	6-8	2 <sup>nd</sup> and 3 <sup>rd</sup> trimester fasting plasma glucose	Symmetry index = relative weight / relative height	Pre-pregnancy weight to calculate PIBW	PIBW: r=0.28 (p<0.005) Amniotic fluid insulin: r=0.24 (p<0.05) GD vs. pre-gestational diabetes: not significant	Not reported

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of glucose intolerance in pregnancy	Outcome measurement	Covariates, confounders	Effect of maternal glucose tolerance in pregnancy	
									Unadjusted	Adjusted
Silverman <i>et al.</i> 1998	USA Diabetes in Pregnancy Study at Northwestern University in Chicago	Caucasian, Black, Hispanic, other	Prospective	Not clearly reported	14-17	2 <sup>nd</sup> and 3 <sup>rd</sup> trimester fasting plasma glucose	BMI (continuous)	Pre-pregnancy weight to calculate PIBW	Children exposed to diabetes (T1D, T2D, GD) <i>in utero</i> vs. control 24.6 vs. 20.9 kg/m <sup>2</sup> (p<0.001) In children exposed to diabetes (T1D, T2D, GD) PIBW: r=0.38 (p<0.001) Amniotic fluid insulin: r=0.29 (p=0.04)	Amniotic fluid insulin: r=0.29 (p<0.05)
Whitaker <i>et al.</i> 1998	USA	Non-Hispanic whites (94%)	Retrospective (medical records)	524	5-10	Control (-OGCT) GD (+OGCT, ≥2 +values OGTT) – Carpenter and Coustan (diet treated)  Non-fasting glucose level at OGCT (quartile)	BMI z-score > 1.036 (US reference)	Maternal pre-pregnancy BMI, paternal BMI at delivery  Maternal obesity BMI≥27.3 kg/m <sup>2</sup> Paternal obesity BMI≥27.8 kg/m <sup>2</sup>	Mean BMI z-score in GD vs. control offspring 0.39 vs. 0.45, p=0.40  Mothers with higher quartiles of glucose levels at OGCT had children with lower obesity rates (p=0.05)	No effect of GD on BMI z-score  No longer significant after adjustment for parental obesity

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of glucose intolerance in pregnancy	Outcome measurement	Covariates, confounders	Effect of maternal glucose tolerance in pregnancy	
									Unadjusted	Adjusted
Wright <i>et al.</i> 2009	USA Project Viva	White, Black, Hispanic, other	Prospective	1,238	3	NGT (-OGCT) IGT (+OGCT, 0/1 + value at OGTT) GD (+OGCT, ≥2 +values OGTT)	BMI z-score (2000 CDC)	Maternal age, parity, ethnicity, education, smoking, family history of diabetes, maternal pre-pregnancy BMI (self-reported weight and height), paternal BMI (self-reported weight and height), GWG (serial gestational weights), BW	No effect on BMI z-score NGT: 0.44 IGT: 0.52 GD:0.47	Effect on BMI z-score relative to NGT: IGT: 0.002 (-0.17, 0.17) GD: -0.08 (-0.37, 0.22)
							Sum of subscapular SFT and triceps SFT		No effect on sum of SFT NGT: 16.6 IGT: 17.1 GD:17.5	Effect on sum of SFT relative to NGT: IGT: 0.25 (-0.48, 0.99) GD: 1.31 (0.08, 2.55)
							Ratio of subscapular SFT and triceps SFT		+ child current BMI z-score No effect on ratio of SFT NGT: 0.64 IGT: 0.67 GD:0.65	Effect on ratio of SFT relative to NGT: IGT: 0.03 (-0.001, 0.06) GD: 0.01 (-0.04, 0.05)
							Non-fasting glucose level at OGCT (+10mg/dl)		As above As above	Not reported

%BF – percentage body fat, AGA – appropriate-for-gestational age, BIA – bioelectrical impedance analysis, BW – birth weight, CDC – Centers for Disease Control and Prevention, DXA – dual energy X-ray absorptiometry, FFM – fat-free mass, FGIR – fasting glucose to insulin ratio, FM – fat mass, GD – gestational diabetes, GWG – gestational weight gain, HOMA-IR – homeostasis model assessment of insulin resistance, IGT – impaired glucose tolerance, IOTF – International Obesity Task Force, LGA – large-for-gestational age, MRI – magnetic resonance imaging, NGT – normal glucose tolerance, OGCT – oral glucose challenge test, OGD – offspring of mothers with gestational diabetes, OGTT – oral glucose tolerance test, OND – offspring of non-diabetic mothers, OT1D – offspring of mothers with type 1 diabetes PIBW - percentage of ideal body weight relative to the Metropolitan Life Insurance Company tables, r – Pearson's correlation coefficient, SAT – subcutaneous (abdominal) adipose tissue, SES – socioeconomic status, SFT – skinfold thickness, T1D – type 1 diabetes, T2D – type 2 diabetes, VAT – visceral adipose tissue



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## Critique of longitudinal studies investigating the influence of maternal glucose intolerance during pregnancy on child obesity and insulin resistance

The summarised studies assessing the relationship between various degrees of maternal glucose intolerance during pregnancy and child obesity, percentage body fat, fat pattern and insulin resistance have been limited by the way exposure and outcome variables were defined, confounding adjustment, sampling frames and representativeness. These limitations are discussed below, highlighting the gaps that the current project aims to address.

### Exposure definition

The focus of most studies was contrasting the long-term effects of GD relative to normal glucose tolerance during pregnancy (Boerschmann *et al.* 2010; Boney *et al.* 2005; Catalano *et al.* 2009a; Crume *et al.* 2011a; Gillman *et al.* 2003; Krishnaveni *et al.* 2010; Pirkola *et al.* 2010; Whitaker *et al.* 1998), while only three studies separately considered milder degrees of glucose intolerance during pregnancy (Egeland and Meltzer 2010; Hillier *et al.* 2007; Wright *et al.* 2009). There was a wide variation in the criteria employed for diagnosing GD. For instance, Catalano *et al.* (2009a) assigned the diagnosis of GD based on the stricter NDDG criteria (National Diabetes Data Group 1979), whereas Whitaker *et al.* (1998) and Krishnaveni *et al.* (2010) on the more inclusive Carpenter and Coustan criteria (Carpenter and Coustan 1982), and Boerschmann *et al.* (2010) on local German criteria, with even lower thresholds for glucose concentration. Only one of the studies with lower thresholds for glucose levels did not find an association between maternal GD and child BMI (Whitaker *et al.* 1998). However, strong conclusions regarding associations between abnormal glucose tolerance during pregnancy and obesity-related outcomes in children are precluded. Moreover, extrapolating findings from these studies to settings where different diagnostic criteria are in use might not yield accurate estimates.

As evidence is accumulating on the increasing risk of adverse outcomes across the entire spectrum of maternal glucose tolerance during pregnancy (HAPO Study Cooperative Research Group 2008) and not only in children of mothers with overt GD, several authors have considered milder degrees of glucose intolerance during pregnancy in addition to GD, in relation to child outcomes. Inconsistent categorisation makes interpretation and comparison of results difficult. Wright *et al.* (2009), for example, defined a separate category of impaired glucose tolerance based on a positive OGCT and none or one abnormal value at OGTT, while Egeland and Meltzer (2010) combined women with impaired glucose tolerance and GD into one group ('cases'), a combination which might have

weakened the true association between maternal GD and child obesity. In addition to separate categories of glucose intolerance during pregnancy, Hillier *et al.* (2007) were able to differentiate the effect of GD treatment on child outcomes, emphasising the role of variable degrees of hyperglycaemia during pregnancy. Along the same lines of continuous risk across the range of maternal hyperglycaemia during pregnancy, Chandler-Laney *et al.* (2011) found a positive correlation between plasma glucose levels at OGCT (as a continuous variable) and metabolic consequences in the child.

Other authors used more crude measures of glucose dysregulation during pregnancy than those provided by the OGCT or OGTT. In the absence of universal screening in the UK at the time of recruiting pregnant women for ALSPAC, glycosuria was used as a proxy for impaired glucose tolerance during pregnancy (Lawlor *et al.* 2010); however, some of these women may have had undiagnosed GD as supported by the very low prevalence of GD in this cohort (0.4%). Older investigations considered second or third trimester fasting plasma glucose as an indicator of glucose homeostasis during pregnancy (Silverman *et al.* 1991; Silverman *et al.* 1998). In an even less reliable manner, the Growing Up Today Study considered maternal self-reported GD status and assessed its influence on birth weight and BMI centile in adolescence (Gillman *et al.* 2003), which might introduce information bias with subsequent implications on the interpretation of the results.

### **Outcome definition**

The definitions of childhood global obesity were heterogeneous, making comparisons between studies difficult. In some instances, analyses were based on BMI as a continuous variable (with no reference to a specific population) (Crume *et al.* 2011a; Krishnaveni *et al.* 2010; Silverman *et al.* 1998), age- and sex-specific BMI centiles (relative to CDC (Chandler-Laney *et al.* 2011; Egeland and Meltzer 2010; Gillman *et al.* 2003) or German (Boerschmann *et al.* 2010) reference data), BMI z-scores (Catalano *et al.* 2009a; Lawlor *et al.* 2010; Wright *et al.* 2009), or symmetry index, another measure of weight relative to height used in the past (Silverman *et al.* 1991). The precision of measurement for BMI could be debated in the case of the Growing Up Today Study, which relied on weight and height reported by the children themselves (Gillman *et al.* 2003). When data on children's height was not available, age- and sex-specific weight centiles (Hillier *et al.* 2007) or weight z-scores (Jeffery *et al.* 2006; Whitaker *et al.* 1998) were used to define overweight status in children despite the fact that these measures based on weight only are poor indicators of fatness (Cole *et al.* 1995). Of these studies, only the large-scale one by Hillier *et al.* (2007) found an association between maternal GD and child overweight status in younger children (5-7 year-old).

Similarly, different measures of adiposity were examined in relation to intrauterine exposure to glucose intolerance, with variable accuracy of outcome assessment, ranging from the simple measurement of subscapular and triceps SFT (Krishnaveni *et al.* 2010; Wright *et al.* 2009), to the estimation of %BF by BIA (Egeland and Meltzer 2010) or DXA (Catalano *et al.* 2009a). One study quantified visceral and subcutaneous abdominal adipose tissue by MRI (Crume *et al.* 2011a). The conflicting findings of intrauterine programming of fatness by exposure to maternal glucose intolerance could thus be attributed, at least in part, to differences in the methods of assessment. It appeared that studies with less accurate measurements (SFT or BIA), which are the only practical measures for large studies, were more likely to detect an effect (Egeland and Meltzer 2010; Krishnaveni *et al.* 2010; Wright *et al.* 2009).

In terms of fat pattern, influences of intrauterine exposure to maternal glucose intolerance were evaluated relative to offspring waist circumference (Catalano *et al.* 2009a; Chandler-Laney *et al.* 2011; Crume *et al.* 2011a; Egeland and Meltzer 2010), waist circumference z-score (Lawlor *et al.* 2010), or a ratio between central and peripheral SFT (Catalano *et al.* 2009a; Crume *et al.* 2011a; Wright *et al.* 2009), but not relative to offspring waist-to-height ratio, which is a more robust indicator of fat distribution.

Most studies in non-high risk populations focussing on child insulin resistance as an outcome of *in utero* exposure to a diabetic environment estimated it by HOMA-IR (Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Egeland and Meltzer 2010; Jeffery *et al.* 2006; Krishnaveni *et al.* 2010) and one study considered the ratio between fasting glucose and fasting insulin as a marker for insulin resistance (Boney *et al.* 2005); however, in the latter study only FGIR (and not HOMA-IR) increased the with exposure to GD in children born large-for-gestational age.

## **Confounders**

Adjustment for several covariates was considered in the majority of these studies, but none of them addressed the full set of potential confounders I have identified for the current study. The variables adjusted for in different studies included maternal age, parity, ethnicity, smoking in pregnancy, family history of diabetes, GD therapy, GWG, education (SES), size at birth, breastfeeding duration, and parental BMI (either at the time of pregnancy or at follow-up). Importantly, maternal pre-pregnancy BMI (or its proxy measure, early pregnancy BMI), a factor that is known to be associated both with glucose intolerance during pregnancy and child obesity, was only adjusted for in a relatively limited number of studies (Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Lawlor *et al.* 2010; Silverman *et*

*al.* 1991; Silverman *et al.* 1998; Whitaker *et al.* 1998; Wright *et al.* 2009). When this adjustment was undertaken, the effect was not consistent, ranging from attenuation of the GD effect on child BMI (Catalano *et al.* 2009a; Crume *et al.* 2011a; Lawlor *et al.* 2010; Whitaker *et al.* 1998), to revealing an effect not apparent without adjustment (Wright *et al.* 2009). The latter was noted in relation to the sum of subscapular and triceps SFT, in the sense that only after full adjustment, exposure to GD increased this sum by 31% relative to offspring whose mothers maintained normal glucose tolerance during pregnancy (Wright *et al.* 2009). Other authors adjusted for maternal current BMI (Gillman *et al.* 2003; Krishnaveni *et al.* 2010), which whilst not fitting the standard criteria for confounders (discussed in Section 3.4.3) may have been included as a proxy for pre-pregnancy BMI.

Some of the studies evaluating the effect of GD on child insulin resistance only addressed potential mediating role of current body size (weight or relative weight) (Jeffery *et al.* 2006; Krishnaveni *et al.* 2010), which attenuated the initial effect. However, none of these studies considered pregnancy-related factors, such as maternal pre-pregnancy BMI or GWG, as potential confounders.

### **Sample representativeness and external validity**

Sample representativeness in longitudinal studies is influenced by factors such as sample size, sampling frame, and participation rates at follow-up. Several studies comprised large numbers of participants (thousands) (Gillman 2004; Hillier *et al.* 2007; Lawlor *et al.* 2010; Pirkola *et al.* 2010), whereas others were confined to samples smaller than 100 subjects (Catalano *et al.* 2009a; Chandler-Laney *et al.* 2011). Larger scale studies were less likely to report an association between maternal glucose intolerance during pregnancy and child obesity, which suggests that the association identified in small-scale studies might be a result of chance.

Most samples included in these studies were representative of the general population, but a few of them were not recruited at random, with potential implications for external validity. For instance, studies conducted by Silverman *et al.* (1991, 1998) or Crume *et al.* (2011a) included a mixture of women with GD and pre-gestational diabetes, and although an association with child weight relative to height was found, separating the effect of hyperglycaemia induced by pregnancy from the hyperglycaemia of pre-gestational diabetes was impossible. Although the Growing Up Today Study comprised a large sample, it included only offspring of mothers who were nurses (Gillman *et al.* 2003), who, due to their health oriented profession, might have followed intensive treatment for GD with likely effects on the degree of hyperglycaemia at which the fetus was persistently exposed and subsequent effects on the post-natal outcomes. Another example of non-random sample came from

studies which included only families with private health insurance (Crume *et al.* 2011a; Hillier *et al.* 2007), thus not covering the entire socio-economic spectrum and potentially introducing selection bias. Also, the finding of a positive association reported by Egeland and Meltzer (2010) might not be generalisable to male children as their sample comprised girls only.

Participation rate, another factor that may alter sample representativeness over time, varied across studies, ranging between 36% at 5-7 years in the data linkage study reported by Hillier *et al.* (2007) and 81% in the prospective longitudinal study conducted in India (Krishnaveni *et al.* 2010). Potential sources of bias were considered by some of the authors. In ALSPAC, with a participation rate of 50% at 9-11 years, it was found that participant mothers were older, more educated and had a higher SES compared to those who did not take part at the follow-up (Lawlor *et al.* 2010). Similarly, mothers from Project Viva participating at the 3 year follow-up (58% of the original sample) were more likely to be older, white, had a higher income and lower pre-pregnancy BMI (Wright *et al.* 2009), which might explain the lack of an association between maternal GD and child global obesity (however, there was an association with child SFT). Higher participation rates at 15 years were identified among offspring of women with GD (51.6%) relative to those of mothers who maintained normal glucose tolerance (24.4%) in the prospective study conducted by Egeland and Meltzer (2010), which might contribute to the identification of an association between maternal GD and child obesity and related outcomes. The response rate in the study conducted by Boney *et al.* (2005) was not only differential (42.1% among women with GD and 10% among women with normal glucose tolerance), but also low, with further potential effects on selection bias.

Children's age also varied across studies from early childhood (Wright *et al.* 2009), pre-pubertal (Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Chandler-Laney *et al.* 2011; Hillier *et al.* 2007; Jeffery *et al.* 2006; Krishnaveni *et al.* 2010; Lawlor *et al.* 2010; Whitaker *et al.* 1998), pubertal (Egeland and Meltzer 2010; Pirkola *et al.* 2010), or combining multiple age groups (Crume *et al.* 2011a; Gillman *et al.* 2003). It was suggested that the long-term effects of GD on child obesity might become apparent only during puberty (Crume *et al.* 2011a), but no clear trend by child age was evident, as for a given age group some studies reported an association, while others did not.

## Conclusion

This critical appraisal of studies addressing intrauterine programming of child obesity and insulin resistance by maternal glucose intolerance during pregnancy shows several gaps in the literature, that I aim to address with this thesis using a contemporary population of mother-child pairs living in

Australia. To the best of my knowledge, no study has been conducted in an Australian sample, using the ADIPS criteria to define GD, looking at how maternal glucose tolerance across the entire spectrum of severity (from borderline to GD) influences child BMI, percentage body fat, waist-to-height ratio or HOMA-IR before puberty, with thorough adjustment for confounders. Although some of these relationships have been previously reported in different populations (albeit with conflicting findings), it is unlikely these estimates could be extrapolated to the Australian population, where a different set of criteria to define categories of gestational glucose intolerance has been in use. In the face of emerging evidence that any degree of maternal chronic hyperglycaemia during pregnancy has detrimental effects on the child at birth, of particular importance is assessing the longer-term influences of milder degrees of gestational glucose intolerance (which occur in a larger proportion of women compared to GD). Studying these effects in pre-pubertal children is important given that puberty may influence the outcomes of interest (Lee 2006) and interfere with the relationships identified. While there is a suggestion that maternal gestational glucose intolerance may increase the risk of obesity in the offspring, it is unclear whether it influences fat free mass or, conversely, the adiposity amount and distribution, which are more sensitive markers of adverse long-term metabolic health than BMI alone.

### **2.4.3 Intrauterine programming of obesity and insulin resistance by maternal gestational weight gain**

In recent years, it has been suggested that, in addition to maternal obesity and glucose intolerance during pregnancy, gestational weight gain may affect the intrauterine milieu and thus fetal growth, with subsequent offspring obesity risk later in life and potentially obesity-related metabolic disorders. A summary of the longitudinal studies addressing this association in mother-child pairs is presented in Table 8 and discussed below.

All but one of these studies have provided evidence of a positive, albeit relatively weak, association between maternal weight gain during pregnancy and obesity in children of different ages (Fraser *et al.* 2010; Moreira *et al.* 2007; Oken *et al.* 2007; Oken *et al.* 2008; Schack-Nielsen *et al.* 2005; Sharma *et al.* 2005; Wrotniak *et al.* 2008) and only one study in young children did not (Whitaker 2004). Of the studies supporting an association between maternal GWG and child obesity, three (Oken *et al.* 2007; Oken *et al.* 2008; Schack-Nielsen *et al.* 2010) reported a linear association, while three (Fraser *et al.* 2010; Sharma *et al.* 2005; Wrotniak *et al.* 2008) a non-linear one. Two of the latter studies (Sharma *et al.* 2005; Wrotniak *et al.* 2008) identified an interaction between GWG and

maternal pre-pregnancy BMI in relation to child obesity, with the association being strongest in the group of underweight women. It is unclear why the studies reported different types of associations, but may partly be due to heterogeneous exposure and outcome definitions.

The evidence is very limited with regard to more specific measures of adiposity. Only one study addressed the influence of maternal GWG on the sum of subscapular and triceps SFT in the offspring and found no association, despite reporting an influence on child global obesity risk (Oken *et al.* 2007). The two studies investigating the relationship between maternal weight gain during pregnancy and fat pattern yielded conflicting results, with Fraser *et al.* (2010) reporting a positive association with child waist circumference at 9 years, and Oken *et al.* (2007) finding no association with the ratio of central to peripheral SFT in 3 year old children. Besides the difference in children's age, there were differences in the measurement of weight gain in pregnancy, which might contribute to the inconsistent results.

No studies have been published to date on obesity-related metabolic disorders, such as insulin resistance, in children in relation to maternal GWG.

**Table 8. Summary of longitudinal studies examining the influence of maternal gestational weight gain on child obesity**

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of GWG	Outcome measurement	Covariates, confounders	Effect of GWG	
									Unadjusted	Adjusted
Fraser <i>et al.</i> 2010	UK Avon Longitudinal Study of Parents and Children	White, non-white	Prospective	5,154	9	GWG=last weight measured in pregnancy – first weight measured in pregnancy (obstetric records)  Inadequate / adequate (ref) / excessive (2009 IOM)	BMI continuous WC continuous FM (DXA)	Pre-pregnancy BMI (predicted pre-pregnancy weight using multilevel models, self-reported height), maternal age, parity, smoking, GWG in previous period, SES (parental occupation)	Mean difference (95% CI) relative to adequate GWG based on IOM recommendations:  Inadequate GWG: BMI: -0.29 (-0.47, -0.12) WC: -0.83 (-1.31, -0.35) FM: -217 (-497, 63)  Excessive GWG: BMI: 0.78 (0.59, 0.97) WC: 1.97 (1.46, 2.49) FM: 1162 (860, 1464)	Mean difference (95% CI) relative to adequate GWG based on IOM recommendations:  Inadequate GWG: BMI: -0.33 (-0.50, -0.15) WC: -0.90 (-1.38, -0.42) FM: -260 (-540, 21)  Excessive GWG: BMI: 0.74 (0.55, 0.94) WC: 1.93 (1.41, 2.45) FM: 1075 (773, 1378)
							BMI >85 <sup>th</sup> centile (IOTF)		Not reported	Relative to adequate GWG: Inadequate GWG: OR <sub>BMI&gt;85th</sub> = 0.80 (0.67, 0.96) OR <sub>WC≥90th</sub> = 0.79 (0.69, 0.90) Excessive GWG: OR <sub>BMI&gt;85th</sub> = 1.73 (1.45, 2.05) OR <sub>WC≥90th</sub> = 1.36 (1.19, 1.57)
							WC ≥ 90 <sup>th</sup> centile (British reference)			



Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of GWG	Outcome measurement	Covariates, confounders	Effect of GWG	
									Unadjusted	Adjusted
Oken <i>et al.</i> 2007	USA Project Viva	White, Black, Hispanic, other	Prospective	1,044	3	Total GWG = last recorded weight before delivery – self-reported pre-pregnancy weight	BMI centile <50 <sup>th</sup> (reference) / 50-84 <sup>th</sup> / 85-94 <sup>th</sup> / ≥95 <sup>th</sup> (2000 CDC)  BMI z-score (2000 CDC)  Sum of subscapular SFT and triceps SFT  Ratio of subscapular SFT and triceps SFT	Maternal pre-pregnancy BMI, age, parity, smoking, SES (income, education), ethnicity, glucose tolerance status in pregnancy, gestation length, BW z-score breastfeeding duration, sex, paternal BMI	OR <sub>≥95<sup>th</sup></sub> =1.30 (1.04, 1.62)  BMI z-score mean difference (95% CI) for each +5 kg in GWG: 0.10 (0.04, 0.16)  Sum SFT mean difference (95% CI) for each +5 kg in GWG: 0.18 (-0.06, 0.42)  Ratio SFT mean difference (95% CI) for each +5 kg in GWG: 0.003 (-0.01, 0.01)  Not reported	OR <sub>≥95<sup>th</sup></sub> =1.52 (1.19, 1.94)  BMI z-score mean difference (95% CI) for each +5 kg in GWG: 0.11 (0.05, 0.17)  Sum SFT mean difference (95% CI) for each +5 kg in GWG: 0.25 (0.00, 0.50)  Not reported  Relative to inadequate GWG: Adequate GWG: OR <sub>≥95<sup>th</sup></sub> =3.77 (1.38, 10.27) BMI z: 0.47 (0.37, 0.57)  Excessive GWG: OR <sub>≥95<sup>th</sup></sub> =4.35 (1.68, 11.24) BMI z: 0.52 (0.44, 0.61)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of GWG	Outcome measurement	Covariates, confounders	Effect of GWG	
									Unadjusted	Adjusted
Oken <i>et al.</i> 2008	USA Growing Up Today Study & Nurses' Health Study II	Nationwide, but homogenous in terms of race / ethnicity	Linkage	11,994	9-14	Total GWG (self-reported)  5-lb increments  Inadequate / adequate (ref) / excessive (1990 IOM)	BMI z-score (self-reported weight and height)  BMI centile <85 <sup>th</sup> (ref) / 85-94 <sup>th</sup> / ≥95 <sup>th</sup> (2000 CDC)	Maternal pre-pregnancy BMI (self-report), age, smoking, SES (income, paternal education), GD, ethnicity, BW, breastfeeding duration, child's age in 1996, sex, Tanner stage, diet, physical activity	Prevalence of BMI ≥95 <sup>th</sup> had a U-shaped distribution across GWG (highest among offspring of mothers with GWG <14 lb or >45 lb and lower in between)	Effect of 5-lb increase in GWG: OR <sub>85-94<sup>th</sup></sub> =1.05 (1.03, 1.08) OR <sub>≥95<sup>th</sup></sub> =1.08 (1.05, 1.13) BMI z: 0.03 (0.02, 0.04)  Relative to adequate GWG based on IOM recommendations:  Inadequate GWG: OR <sub>85-94<sup>th</sup></sub> =0.97 (0.84, 1.12) OR <sub>≥95<sup>th</sup></sub> =0.91 (0.74, 1.13) BMI z: -0.06 (-0.10, -0.01)  Excessive GWG: OR <sub>85-94<sup>th</sup></sub> =1.27 (1.12, 1.44) OR <sub>≥95<sup>th</sup></sub> =1.42 (1.19, 1.70) BMI z: 0.14 (0.09, 0.18)
Schack-Nielsen <i>et al.</i> 2005; 2010	Denmark Copenhagen Perinatal Cohort	Caucasian	Prospective	2,034 1,408 1,070 1,037 940	1 3 6 8 14	Total GWG (obstetric records)  5 categories assigned the interval middle value: <6 (5.5), 6-8 (7), 9-10 (9.5), 11-12 (11.5), 13-15 (14), >16 (16.5) kg	BMI z-score (1990 British reference)	Maternal pre-pregnancy BMI, age, parental SES, smoking, edema during pregnancy, BW	Not reported	BMI z-score mean difference (95% CI) for each 'category' of GWG: 1 y: 0.03 (0.02, 0.05) 3 y: 0.02 (0.00, 0.04) 6 y: 0.02 (0.00, 0.04) 8 y: 0.03 (0.02, 0.05) 14 y: 0.03 (0.01, 0.05)

Reference	Setting	Population	Study design	Sample size in analyses	Age at outcome (years)	Measurement of GWG	Outcome measurement	Covariates, confounders	Effect of GWG	
									Unadjusted	Adjusted
Sharma <i>et al.</i> 2005	USA	Not specified  - low income	Data linkage of Pediatric & Pregnancy Nutrition Surveillance Systems	165,013	2-4	Routinely collected GWG	BMI ≥ 95 <sup>th</sup> centile (2000 CDC)	Age, sex, ethnicity, state of birth, maternal age, height, smoking, education, BW	No estimates reported Non-linear association (p<0.0001) Effect modification by maternal pre-pregnancy BMI (p<0.0001)	Attenuated by BW adjustment, but still significant
Whitaker 2004	USA	White, Black, Hispanic, other  - low-income	Retrospective, data linkage	8,494	2-5	Net GWG=(self-reported GWG-BW) / length of gestation  Quartiles (lowest=ref)	BMI ≥ 95 <sup>th</sup> centile (2000 CDC)	Not specified for this particular exposure  For main analyses: BW, gestational age, gender, ethnicity, parity, age, smoking, education, marital status	Not reported	Not significant
Wrotniak <i>et al.</i> 2008	US National Collaborative Perinatal Project	Multiethnic	Retrospective	10,266	7	Total GWG = measured weight at delivery – self-reported pre-pregnancy weight  Inadequate / adequate (ref) / excessive (1990 IOM)	BMI ≥ 95 <sup>th</sup> centile (2000 CDC)	Maternal pre-pregnancy BMI, age, race, smoking, gestational age, child's sex, age, first born status  + BW	+ 1 kg GWG: OR=1.02 (1.00, 1.03) Inadequate GWG: OR=0.82 (0.69, 1.01) Excessive GWG: OR=1.62 (1.25, 2.12)	+ 1 kg GWG: OR=1.03 (1.02, 1.05) Inadequate GWG: OR=0.88 (0.68, 1.14) Excessive GWG: OR=1.48 (1.06, 2.06)  + 1 kg GWG: OR=1.03 (1.01, 1.05)  Inadequate GWG: OR=0.93 (0.72, 1.21)  Excessive GWG: OR=1.40 (1.00, 1.95)

BW – birth weight, CDC – Centers for Disease Control and Prevention, DXA – dual energy X-ray absorptiometry, FM – fat mass, GWG – gestational weight gain, IOM – Institute of Medicine, IOTF – International Obesity Task force, SES – socioeconomic status, SFT – skinfold thickness, WC – waist circumference

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## Critique of longitudinal studies investigating the influence of maternal gestational weight gain on child obesity

The longitudinal studies examining the association between maternal weight gain during pregnancy and child obesity, body composition and fat pattern have been limited by the definition of exposure and outcome variables, adjustment for potential confounding factors, sampling frame and representativeness. Insulin resistance in children, a common consequence of obesity, has not been studied in relation to intrauterine exposure to excessive GWG. These studies are critiqued below, with a view to clarifying gaps that the present study aims to address.

### Exposure definition

No consensus was adopted in defining GWG in the summarised studies (Table 8), with some expressing it as total (Fraser *et al.* 2010; Oken *et al.* 2007; Oken *et al.* 2008; Schack-Nielsen *et al.* 2005; Sharma *et al.* 2005; Wrotniak *et al.* 2008), while others as net GWG (separating maternal from fetal components) (Oken *et al.* 2007; Whitaker 2004). More importantly, the precision of GWG measurement varied across studies, including routinely collected data (Schack-Nielsen *et al.* 2005; Sharma *et al.* 2005) with minimal information bias, direct measurement of at least one weight during pregnancy (Fraser *et al.* 2010; Oken *et al.* 2007; Wrotniak *et al.* 2008), or self-reported GWG (Oken *et al.* 2008; Whitaker 2004) with potential information bias. In addition to continuous measures of GWG, categories ('inadequate', 'adequate', or 'excessive' weight gain based on the Institute of Medicine recommendations, 1990 or 2009) were considered as predictors for obesity risk in the offspring. Of these, the 'adequate' GWG category was chosen as the reference by most authors (Fraser *et al.* 2010; Oken *et al.* 2008; Wrotniak *et al.* 2008). In contrast, Oken *et al.* (2007) considered the 'inadequate' GWG as the reference category, which likely explains the much higher odds of obesity reported in offspring exposed to excessive GWG. Other authors used internal quartiles (Whitaker 2004) or more arbitrary categories (Schack-Nielsen *et al.* 2005) of GWG as exposure variables. These variations in measurements are likely to have influenced the associations reported by different studies and made comparisons challenging.

### Outcome definition

Various indices of childhood global obesity were used in these studies, based mainly on direct measures of weight and height, one on measurements collected from school health records (Schack-Nielsen *et al.* 2005). Oken *et al.* (2008), however, based BMI calculation on child self-reported weight and height, with potential impact on the validity of the results due to the established tendency

of adolescents to under-report body weight (Elgar *et al.* 2005). BMI was expressed as a continuous variable, not standardised for age and sex (Fraser *et al.* 2010), as a z-score (Oken *et al.* 2007; Oken *et al.* 2008), or as a centile relative to a specific population (CDC - (Oken *et al.* 2007; Oken *et al.* 2008; Sharma *et al.* 2005; Whitaker 2004; Wrotniak *et al.* 2008), or IOTF - (Fraser *et al.* 2010)). In a further point of distinction between studies, different centiles were employed as reference categories when calculating the odds of childhood obesity – e.g., 50<sup>th</sup> centile by Oken *et al.*(2007) and 85<sup>th</sup> centile by Oken *et al.*(2008). As a result of these different definitions, the reported adjusted ORs for childhood overweight/obesity in relation to maternal excessive GWG ranged from 4.35 in 3 year-old children from Project Viva (Oken *et al.* 2007), to 1.73 in 9 year-old children enrolled in ALSPAC (Fraser *et al.* 2010), and 1.42 in 9-14 year-old children from the Growing Up Today Study (Oken *et al.* 2008). In addition, as the association between maternal GWG and the risk of obesity in the offspring seems to decrease as children grow older, it could be argued that the different ages of the children included in these studies also contributed to the wide variation in odds of childhood obesity reported by different authors.

The amount of adipose tissue in children was investigated in relation to exposure to maternal GWG in two studies. In one of them, fat mass measured by DXA was increased by about one kg in 9 year-old children if the mother gained weight excessively during pregnancy, robust to confounder adjustments (Fraser *et al.* 2010). In the other study, the sum of subscapular and triceps SFT in 3 year-old children was positively associated with GWG after adjustment for a series of covariates, including parental BMI, but further adjustment for birth weight z-score attenuated the association (Oken *et al.* 2007). The same two studies evaluated the influence of maternal GWG on child fat pattern and reported opposite effects: positive association of waist circumference at 9 years with GWG (Fraser *et al.* 2010) and no relationship between GWG and the ratio of central-to-peripheral SFT in 3 year olds (Oken *et al.* 2007).

### **Confounders and effect modification**

Each of the studies on the relationship between maternal GWG and child obesity adjusted for a different set of covariates, including pre-pregnancy BMI, maternal age, parity, smoking, various indicators of SES, ethnicity, gestational length, first born status, birth weight, breastfeeding duration, or pubertal (Tanner) stage. Glucose intolerance during pregnancy, a factor considered to be on the causal pathway between maternal GWG and child obesity, was taken into account only by Oken *et al.* (2007; 2008).

Discordant findings were reported on a potential interaction between GWG and pre-pregnancy BMI in relation to child obesity. Of the studies addressing interactions, four supported an effect modification by pre-pregnancy BMI (Fraser *et al.* 2010; Oken *et al.* 2007; Oken *et al.* 2008; Schack-Nielsen *et al.* 2005) while another two did not (Sharma *et al.* 2005; Wrotniak *et al.* 2008). This difference would not seem to arise from a lack of statistical power as the studies with no interactions were large.

### **Sample representativeness and external validity**

Although published recently, two of the studies reviewed in this section comprised historical cohorts established between 1959 and 1972 (Schack-Nielsen *et al.* 2005; Wrotniak *et al.* 2008), when the prevalence of 'inadequate' GWG was higher (65%) and that of 'excessive' GWG was lower (11%) (Wrotniak *et al.* 2008) compared to contemporary Western society (National Center for Health Statistics 2010). Despite this, the associations are still likely to be relevant.

All the samples included in these analyses were large, with thousands of participants from various ethnic backgrounds. Despite the large sample sizes, two of these studies were not representative of the general population. As already mentioned, mothers of children enrolled in the Growing Up Today Study were nurses, who, as a consequence of their health oriented profession, might have had a higher rate of 'adequate' GWG (Oken *et al.* 2008). Second, the Copenhagen Perinatal Cohort comprised mainly women with current or previous pregnancy complications (such as edema, hypertension, proteinuria, pre-eclampsia) (Schack-Nielsen *et al.* 2005), which might have increased disproportionately the water component of GWG, which is less likely to have direct metabolic effects on the offspring, as opposed to the fat component.

Attrition, which may affect original sample representativeness, varied across studies and was highest in the historical cohort reported on by Schack-Nielsen *et al.* (2005) (in this study, of the 9,125 participants from the original sample, about 1,000 children were assessed at each follow-up beyond infancy). Among the contemporary cohorts, participation rates ranged between 44.8% at 9-14 years in Growing Up Today Study (Oken *et al.* 2008) and 57% at 9 years in ALSPAC (Fraser *et al.* 2010). Details regarding the likelihood of bias due to attrition were reported only by Oken *et al.* (2007) who noted that participants (49.1% of original cohort) were more likely to be white and to have lower pre-pregnancy BMI, but had similar GWG as non-participants; thus, in principle their results should not be affected by this relatively low participation.

## Conclusion

This critical appraisal of studies on intrauterine programming of child obesity by maternal GWG indicates a need for a study comprising a representative, contemporary Caucasian population of children. Australia is a site where such research should be undertaken due to the adequacy of routine data collection, relative affluence and potential generalisability to other developed Western nations. The basis for conducting such a study is that, while there seems to be some evidence of GWG effects on offspring BMI and very limited evidence on fat mass or waist circumference, no studies have examined how maternal GWG across the entire spectrum influences child percentage body fat, waist-to-height ratio and HOMA-IR before puberty. These are the gaps identified in the existing literature that the current project aims to address. Examining maternal GWG as a continuous variable has the advantage of removing arbitrary cut-offs (preferred in clinical approaches to data analysis, but less acceptable from an epidemiological perspective), thus allowing the description of the effect of incremental increases in GWG on child outcomes. It is possible that maternal GWG influences offspring overall body size, but it is still uncertain whether it affects offspring adiposity or fat distribution, measures that are more closely linked to long-term metabolic perturbations than global obesity. Hence, the current study includes these additional outcome measures. If maternal GWG appears to influence child insulin sensitivity (relationship that has not been previously investigated), this might represent a step in identifying modifiable early life factors that contribute to insulin resistance prior to puberty. In addition, in existing studies the adjustment for confounders has often been incomplete, which may have led to reporting false associations.

## 2.5 Summary

From a public health perspective, identifying specific gestational factors with a potential to influence body size, body composition, fat distribution and glucose-insulin homeostasis in the next generation appears increasingly appealing. Once these maternal factors are identified, attention could be directed to means by which they might be modified, thus contributing to the prevention of later metabolic disorders in the child, such as obesity and insulin resistance.

## Chapter 3 Methodology

This chapter outlines a brief description of the Generation 1 study in which my research project is nested (Section 3.1), relevant aspects of data collection in the 9-10 year old children, including details about approaching the families, procedures undertaken with participants, and ethical considerations (Section 3.2). Means of managing the data are presented in Section 3.3, followed by a clear definition of variables of interest (Section 3.4). The chapter concludes with a description of the analytical framework adopted (Section 3.5).

### 3.1 The Generation 1 cohort

Given that my proposed research project was based on an already established birth cohort of children, I consulted and drew upon three documents written by Associate Professor Vivienne Moore, the principal investigator in the Generation 1 Study: a paper describing in detail eligibility criteria for women and baseline data collection (Moore *et al.* 2004), an Ethics application (number H-167-2006) and a National Health and Medical Research Council Grant application (number 453655), the latter two outlining data collection for this project, which was largely designed prior to the start of my candidature.

#### 3.1.1 Study design and overall aim

The current project was an extension of the ongoing Generation 1 Study, a prospective cohort study which commenced in 1998. At its inception, the overall aim of the study was to examine early life origins of health and disease. Within the broader Generation 1 study, my project in particular aimed to provide understanding of intrauterine environment influences (namely, maternal obesity prior to pregnancy, glucose intolerance during pregnancy, and excessive gestational weight gain) on child obesity, body composition, fat pattern and insulin resistance at the age of 9-10 years.



Up to this stage, extensive data about pregnancy and growth of the children during infancy and in the first 5.5 years of life have been collected over ten waves. In this phase of follow-up, at 9-10 years of age, my focus was on describing the growth of this group of South Australian children (using anthropometric measurements, namely, BMI z-score), assessing their body composition (in particular, percentage body fat), their fat pattern (by waist-to-height ratio) and glucose-insulin homeostasis (using a fasting blood sample).

### 3.1.2 Baseline sampling process and selection criteria

The Generation 1 cohort was established prospectively between 1998 and 2000. In order to provide representative recruitment across the entire socioeconomic spectrum and cover various dietary patterns, a sampling strategy was devised in which women were enrolled in early pregnancy through the antenatal clinic at a public hospital (Lyell McEwin Hospital, Adelaide, South Australia) and the rooms of three privately practising obstetricians (centrally located in the Adelaide area, with large caseloads). At the public hospital, pregnant women were approached following a random schedule (about 5 women per week, in order to limit the number of recruited women within a manageable load for the fieldworkers). At the private practices all eligible women were approached (1-2 women per week in each practice). Approximately half of the study participants were identified through the public hospital, similar to the proportion of South Australian women who received antenatal care in a public hospital clinic at that time (Chan *et al.* 2000).

To be eligible to join the study (Moore *et al.* 2004), women had to:

- be in the first 16 weeks of gestation (so that detailed information about early pregnancy, specifically dietary intake, could be recalled accurately);
- have a singleton pregnancy (because there is an increased risk for low birth weight in multiple pregnancies (Shinwell and Blickstein 2007; Taylor *et al.* 1998));
- have spontaneously conceived (since intrauterine growth is often restricted in pregnancies occurring through assisted reproductive technology (Allen *et al.* 2006; Fisch *et al.* 1997));
- be free from certain medical conditions known to severely affect fetal growth (e.g., diabetic mothers have increased risk of delivering macrosomic babies (Koukkou *et al.* 1997; Platt *et al.* 2002));
- be at least 18 years of age (as teenage mothers are more likely to give birth to growth restricted babies (Chandra *et al.* 2002; Cooper *et al.* 1995));

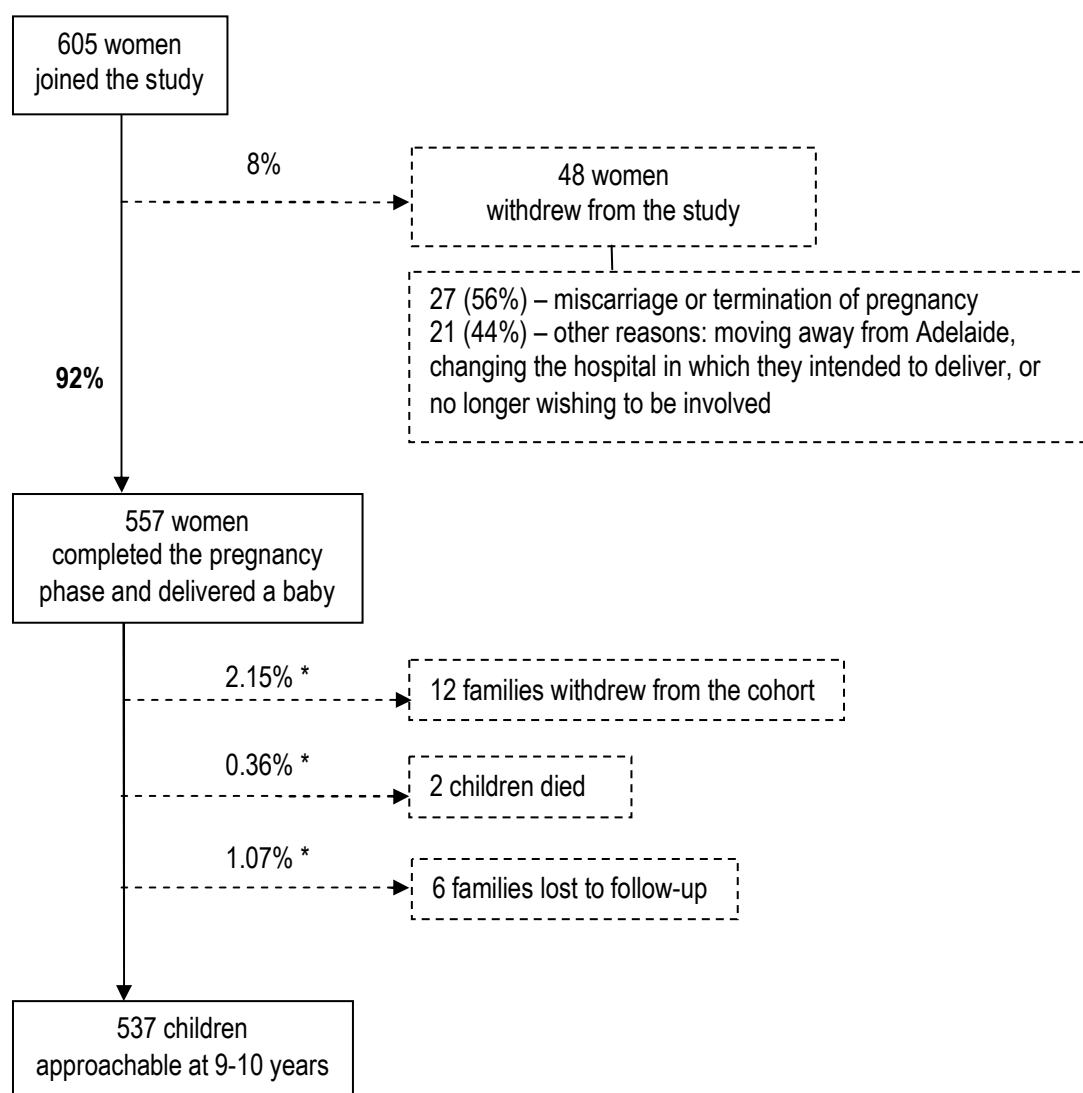
- be Caucasian (as assessment could only be done for Caucasian diets); and
- be sufficiently fluent in English to give informed consent and to complete the study questionnaires.

Among the eligible women who were approached, 65% consented to participate in the study. Of them, 557 women completed the pregnancy phase (representing 92% of women who enrolled in the study) and gave birth to a baby that survived the neonatal period (Figure 1). Women who did not complete the pregnancy phase were not invited to continue in the study. The ethics committee required exclusion of women who gave birth to babies with serious congenital abnormalities, but there were none.

### 3.1.3 Baseline data collection

Pregnant women who joined the study were interviewed by two research nurses, face-to-face, on two occasions: in early pregnancy (between 8 and 20 weeks of gestation) and in late pregnancy (between 27 and 42 weeks of gestation). A rich set of data was collected from these women both through the interviews and from antenatal records. This information included, but was not limited to, women's age, results of screening for gestational diabetes (taken from the antenatal records), pre-pregnancy weight (self-reported), height and weight measured in early and late pregnancy, parity, personal and family medical history, blood pressure, diet, physical activity, smoking, education and other social circumstances (employment status, household income, relationship status, family size), and details regarding complications of the pregnancy (such as pregnancy-induced hypertension, preeclampsia or gestational diabetes).

Children in this study were born in five hospitals in Adelaide. Special arrangements were made for measurements of the babies and placentas for study purposes by midwives. In addition, routinely recorded data and birth details were abstracted from the hospital records.

**Figure 1. Flowchart of participation**

\*% of women who completed the pregnancy phase and delivered a baby

### 3.1.4 Participation until this follow-up

After birth, detailed information was collected about family circumstances and growth of the Generation 1 children every 3 months in the first year and at 2, 3.5 and 5.5 years of age. A comprehensive description of data collected on every follow-up is not warranted here, but some aspects that have set the scene for the current phase at 9-10 years and played a critical role to the success of this project are outlined below.

Periodic tracing of participants, both manual and electronic, was an essential process for maintaining the cohort as intact as possible and thus preventing attrition, which is an important methodological issue for longitudinal studies. Irrespective of the form attrition takes (either as a premature

discontinuation of participation or declining some follow-ups but then returning to the study at future stages (Twisk and de Vente 2002)), it may compromise the internal and external validity of the study (Garcia *et al.* 2005), result in a loss of statistical power and may lead to selection bias (Kristman *et al.* 2004).

In order to minimise attrition, a series of strategies were implemented from the inception of the study and for each follow-up (Table 9). As a result of these strategies, a trusting relationship was built between the families and the research team. This has contributed towards the high participation rate achieved (over 90% at each face-to-face follow-up). Only 20 families of the original 557 (less than 4%) had withdrawn before the latest follow-up, leaving 537 families to approach at this round. The attrition that did occur in the Generation 1 study was due to the researchers' inability to relocate subjects after they had changed addresses (n=6), family's decision of no longer wishing to be involved (n=12) or subject's death (n=2) (Figure 1).

**Table 9. Main strategies to prevent loss to follow-up and enhance response rates**

Enrolment	Inform participants of requirements of the study Collect extensive contact details (address, phone number, date and place of birth, contact details of personal contacts)
Regular contact	Newsletters with study updates, holiday cards
Research team	Well trained, enthusiastic, warm, communicative, respectful, stable, flexible with scheduling
Incentives	Small tokens of appreciation, parking tickets, reimbursement for petrol

### 3.2 Data collection for the follow-up at 9-10 years of age

It is worth noting that the current thesis is one part of a much more extensive project. In short, the main outcomes of interest for this research are child obesity (defined by child BMI z-score), %BF (derived from BIA), fat pattern (indicated by WHtR), and IR (estimated by HOMA-IR). A detailed description of data collection for these parameters is presented below, along with an outline of other data collected that was not included in my analyses, but could offer a better understanding of the entire Generation 1 follow-up at 9-10 years of age.

### 3.2.1 Approaching participant families

All Generation 1 families who had indicated that they would be willing to consider future involvement in the study were contacted by letter. In some cases, retracing of the families was needed, including approaching alternate contact persons, and using electronic databases such as the electronic telephone directory and the Electoral Roll. An information sheet was sent to each family, explaining the procedures involved in this phase of the follow-up and also repeating their rights as study participants. The information sheet was followed by a phone call within a week (where possible) to answer queries and see if families wished to participate. Of the 537 mother-child pairs that were approached, 443 (82.5% of current cohort members, 80% of the original cohort) agreed to take part in this follow-up.

### 3.2.2 Procedures undertaken with participant children

The follow-up of Generation 1 participants at the 9-10 year round comprised three main parts: a visit either at home or in one of the offices assigned by the research team (for interview and anthropometric measurements), assessment of body composition, and a fasting blood sample. Each home/office visit was attended by two trained fieldworkers, a research nurse to conduct the interview with the mother, and myself or another PhD student to work with the child.

#### 3.2.2.1 Interview

After renewing the written consent for agreement to participate in the study by the mother, one fieldworker conducted an interview with the child, assessing their physical activity and diet. Consent will be described in more detail in Section 3.2.3. The child's physical activity was examined by 'Multimedia Activity Recall for Children and Adolescents' (MARCA) (Ridley *et al.* 2006). This is a validated computer-delivered, self-report proforma which collects data on a wide range of physical activities and sedentary behaviours from the previous day, providing an estimation of energy expenditure over 24 hours (Ridley *et al.* 2006). The child's diet in the past 6 months was assessed by a Food Frequency Questionnaire entitled 'Australian Children's Eating Survey' (Watson *et al.* 2006). Information on the child's physical activity and diet was not considered in this project, but is readily available for future research.

While the child was being interviewed, the other fieldworker administered a questionnaire to the mother covering aspects of her health, the child's health, health-related behaviours, family socio-economic circumstances, and parenting practices.

### 3.2.2.2 Anthropometric measurements and assessment of body composition

The study protocol included a range of anthropometric measurements in both mothers and children who agreed to take part: standing height, sitting height, skinfold thickness (triceps, biceps, subscapular, and supriliac sites), and body circumferences (head, waist, hip, mid-arm and mid-calf). These measurements were done based on a protocol (Norton and Olds 1996) endorsed by the International Society for the Advancement of Kinanthropometry (ISAK) (Marfell-Jones and Olds 2007), by one of the three research nurses involved in this study, who had been previously trained in performing them with an acceptable interpersonal variability. The measurements relevant to the current project are presented below:

- **Standing height** to the nearest 0.1 cm was measured with the participant in bare feet, having their feet together, with the heels, buttocks and the upper part of the back touching the scale, and the plane going through the lower margin of the eye socket and the notch above the tragus of the ear in a horizontal position (Norton and Olds 1996). Height measurements were performed twice for each subject using a portable stadiometer, and the mean value was used in the analyses.
- **Weight** to the nearest 0.1 kg was determined with the participant wearing minimal clothing and no shoes (Norton and Olds 1996), using Tanita body composition analyser TBF-300 (Tanita Corp., Tokyo, Japan) for a standard body build.
- **Waist circumference** to the nearest 0.1 cm was measured at the level of the narrowest point between the lowest rib margin and the iliac crest (or the mid-point between these two references if no narrowing was obvious) in a horizontal plane, at the end of a normal expiration, with the participant in standing position, balanced on both feet, with arms extended comfortably (relaxed) by their side (Norton and Olds 1996), using a flexible, non-extensible tape measure. Two to three measurements were obtained for waist circumference and the mean value was used in the analyses.

These anthropometric measurements were performed in all (n=443) participating children, except for waist circumference measurements, which had one missing value.

**Body composition** was assessed in all mothers and children who participated in this round of follow-up, using a foot-to-foot bioelectrical impedance analyser Tanita TBF-300 (Tanita Corp., Tokyo, Japan). The estimates of percent body fat, fat mass, fat free mass and total body water were derived from specific equations embedded in the machine (undisclosed by the manufacturer), based on weight, height, age and sex (all children were considered as having a 'standard' body build). This technique was chosen as it is portable, non-invasive, easy to perform, safe (Hosking *et al.* 2006) and inexpensive.

### 3.2.2.3 Fasting blood samples

Fasting blood samples were required in order to assess the child's glucose-insulin metabolism, one of the main outcomes for this project. At the end of the interview, the research nurse discussed with the family the possibility of the child having a fasting blood test (for research purposes only). If the mother's written consent and the child's verbal assent were obtained, the interviewers arranged for a domiciliary nurse from a private blood collection facility to visit the family on an agreed day, at 8 am. Fasting for 12 hours before the blood sample collection (nothing to eat or drink other than water from 8 pm the previous night) was requested. The phlebotomist checked that the child had fasted and was still willing to provide a blood sample. Families were given a local skin anaesthetic (EMLA cream) to apply about 1 hour prior to the appointment, in order to minimise pain associated with needle insertion. A fasting blood sample (12 ml) was collected from 164 children (37% of children who took part at this follow-up). Blood was initially processed by the collection laboratory, then plasma and serum aliquots were frozen and subsequently couriered in batches to the University of Adelaide Medical School for the assays and storage.

For the purpose of the current research study, the assays of interest were plasma glucose and plasma insulin. Plasma glucose was quantitatively determined with a Hitachi 912 automated sample system using the Glucose HK assay kit (Roche Diagnostics, NSW, Australia). The mean coefficient of variation was around 3.3%. The reference range for the assay used was 3.33–5.55 mmol/l. Plasma insulin was measured using a commercially available radioimmunoassay kit specific for human insulin (Linco, Millipore). The mean coefficient of variation was around 5%. The reference range for the assay used was 5–15  $\mu$ U/ml.

### 3.2.3 Ethical considerations

From its inception, the study has complied with the ethical standards for medical research involving human participants (World Medical Association Declaration of Helsinki 1997). At baseline, ethics approval was obtained from all hospitals and antenatal clinics involved in subject recruitment for the study. For this phase of the follow-up, ethics approval was obtained from the University of Adelaide Human Research Ethics Committee.

#### **Obtaining consent and co-operation from participants**

Participants were provided with written information about the study and also the opportunity to discuss any study-related queries with a research nurse. As in previous rounds, written consent for the interview and physical measurements was obtained from the mother and verbal assent from the child, at the beginning of the visit. At the end of the interview, the possibility of the child having a fasting blood test was discussed with the mother and the child. Given the fragility of any cohort, it was emphasised that both the blood test was optional and the family could continue to be part of the cohort even if they declined this component.

#### **Potential risks for participants**

Although the interviews did not contain obviously sensitive questions, participants were informed at the beginning of the interview that they were free not to answer any questions they would find uncomfortable (although they had been asked for similar information previously, mainly by the same research nurses).

The anthropometric measurements and assessment of body composition by BIA were not invasive or painful. The callipers for SFT measurement exerted some degree of pressure on a fold of skin, uncomfortable at times, but normally not painful. The protocol was to discontinue measurements if any child was distressed or found them painful and this did occur in one case.

With respect to the blood sample, overnight fasting for 12 hours before collection was not a harmful procedure. In order to prevent pain associated with needle insertion, a local skin anaesthetic (EMLA cream) was applied about one hour prior to blood collection. Also, the blood sample was collected by an experienced phlebotomist, who had been trained to reassure participants before, during and after the sample was taken, and to respond to any signs of faintness.



### **Preserving confidentiality of participants**

Confidentiality of participants has been totally preserved. They were identified on data collection forms by a study ID number and not by name. The forms have been stored in lockable filing cabinets in an area with restricted access and computerised data in password-protected files. Data analyses have concerned groups of participants, so women and their children could not be individually identifiable in any reports or papers. However, study participants were allowed to request access to their individual data and results.

### **Payment, reimbursement or other inducements**

Families were not paid for participating in this study. If they had to travel to the appointment, reimbursement of car parking and petrol was offered. There was no clinical relationship with the subjects, so they did not feel under any obligation to assist with the research in regard to their health care. The costs of this project were covered by a National Health and Medical Research Council Strategic Award for which Associate Professor Vivienne Moore, University of Adelaide, is Chief Investigator A.

## **3.3 Data management**

Data collected from the Generation 1 participants were entered into a Microsoft Office Access 2007 database by three trained data entry staff. De-identified data were stored as numerical responses and text fields. A set of codes was created to code open-ended text questions. Data files relating to this round of follow-up were linked to past data sets based on mother and child study ID numbers. A data dictionary comprising a description of each variable was added to the existing Generation 1 data dictionary.

Prior to data analysis, raw data was thoroughly examined. This preliminary process involved data cleaning and data screening through preparatory data analysis, in order to detect and correct any potential errors, observe trends (patterns) within the data, and direct towards appropriate statistical tests.

**Data cleaning**, an essential determinant of the validity of any study, was undertaken both during and after entry, following a structured cleaning framework which involved detecting, diagnosing, and editing suspected inaccuracies (Van den Broeck *et al.* 2005). During data entry, valid ranges were

checked using 'input edits', known as an error prevention process (e.g., 2007 was not accepted for an interview date at this follow-up, as all interviews took place in 2008-2010). After entry, data cleaning was conducted by a statistician who performed a random 10% check of the first-stage dataset (in which 10% of the observations were completely checked against the raw 'paper' data). This procedure has been recognised as a good alternative to the double entry method (in which two datasets with raw data are entered separately by two individuals and then compared for differences) when resources are limited (Prymachuk and Richards 2007). It was particularly useful for identifying erroneous inliers (incorrect data points falling within the expected range), which would have otherwise escaped detection. If problem variables were detected, the checking for those variables was extended to the full dataset.

Subsequently, frequency analysis was performed. Through this process values for each of the variables were checked against the expected range. This step was particularly important for data that had not been entered manually (e.g., blood test results). Discrepancies within a participant's characteristics were also sought and temporal consistency of data was checked (e.g., child's height and weight at 9-10 years was compared with their growth trajectory, using previously collected data from 2, 3.5 and 5.5 years of age). Although some children were found to be shorter/taller, or lighter/heavier than expected based on previous measurements, this was within physiological possibility and all anthropometric measurements were judged to be accurate.

**Preparatory data analysis** consisted of examination of variables distributions and identification of missing and outlying data, two common problems in epidemiological research.

**Missing data** was assessed in terms of extent (percentage of cases with missing data on a certain variable) and pattern (missing completely at random, missing at random, and missing not at random). Although there are no guidelines for what represents excessive missing data, it was suggested that up to 10% missing data on a given variable would be acceptable (Fox-Wasylyshyn and El-Masri 2005). Two aspects of missing data were encountered in this study: missing cases (i.e., cohort members who did not participate in follow-up) and missing data items (i.e., certain data not recorded for participants who had other data available). In order to determine the pattern of missing cases and assess sample representativeness, participants were compared with non-participants in terms of baseline characteristics, using independent samples t-tests (for continuous variables) and chi-squared tests (for categorical variables).

A series of methods for dealing with missing data have been proposed, such as complete case analysis (exclusion of incomplete records, with some loss of statistical power), overall mean substitution (replacement of the missing value with the average in the dataset), missing indicator method (inclusion of a newly created indicator variable on missing in multivariable analysis along with the original variable), or imputation (prediction of the missing value using other characteristics of the subject) (Donders *et al.* 2006). Among these techniques, imputation has the advantage of yielding the least biased results, for both missing at random and missing not at random data (Donders *et al.* 2006). In this study, single and multiple imputation techniques were applied only for two of the exposure variables (i.e., maternal pre-pregnancy weight, maternal weight just before delivery) and are explained in greater detail in Section 3.4.1. Imputation techniques were not used for outcome variables.

**Outliers** were defined in this study as observations that fell 1.5 times the interquartile range below the first quartile or above the third quartile (Moore and McCabe 2003). They were assessed in terms of leverage (deviation from the mean value) and influence (property of an observation whose inclusion or exclusion from the analysis would be followed by major changes in the fitted models). Although dealing with outliers is considered a matter of individual judgement (Moore and McCabe 2003), it has been suggested that extreme values on both exposure and outcome variables would be more concerning than those on solely one of these categories, and should be discarded (Prymachuk and Richards 2007). A similar approach has been recommended for true extreme values resulted from unanticipated external processes (Prymachuk and Richards 2007) (e.g., not fasting when fasting blood sample was required from children). My approach was to retain outliers wherever possible, as they generally appeared to be genuine data points.

## 3.4 Defining variables of interest

### 3.4.1 Intrauterine exposures

#### Maternal pre-pregnancy BMI

Maternal pre-pregnancy nutritional status was measured by pre-pregnancy BMI (weight in kg divided by height in m squared). This was calculated based on pre-pregnancy weight (self-reported) and height (measured by the research staff at the interview in early pregnancy, which was considered equal to the pre-pregnancy height).

Maternal pre-pregnancy weight was self-reported at the time of the first interview, in early pregnancy. It was collected as a response to the question: "How much did you weigh before pregnancy?" followed by interviewer's probe for more accuracy if a rounded weight was given as an answer. However, there was no specification regarding woman's clothing, time of the day or fasting status when the measurement had been done, all these aspects potentially contributing to the inaccuracy of self-reported pre-pregnancy weight.

Despite the wide acceptability of self-reported weight data in epidemiological studies (Spencer *et al.* 2002), it is also known that there is a great degree of discordance between subjective and objective weight measures, with an overall tendency to underestimate it by self-report, especially in overweight or obese individuals (Gorber *et al.* 2007; Rowland 1990). For this reason, the agreement between self-reported pre-pregnancy weight and directly measured early pregnancy weight required assessment in the Generation 1 women.

A majority of the women (n=502) in the Generation 1 cohort reported their pre-pregnancy weight. Of them, 21 women reported a pre-pregnancy weight that seemed unlikely when compared to the standardised estimates that corrected for weight gain during early pregnancy (these women either lost more than 0.37 kg/week or gained more than 0.80 kg/week of gestation before the first interview in early pregnancy). These 21 incongruent pre-pregnancy weight values were set to missing, and were then imputed along with the other 55 missing values in the women who did not report their pre-pregnancy weight. Both single and multiple imputations were performed. With single imputation, pre-pregnancy weight was estimated from a linear regression against maternal measured early pregnancy weight, height and age, which, based on the women without missing data, explained 90% of the variance in predicted variable; therefore, imputation was deemed appropriate. Subsequently, the multiply imputed pre-pregnancy weight was based on a regression of the singularly imputed pre-pregnancy weight, early pregnancy weight and height, and maternal age. The average of the means of pre-pregnancy weight from the five imputed data sets, the average standard deviation, minimum and maximum values were identical to the singularly imputed variable. As a result, the singularly imputed pre-pregnancy weight was used in all analyses.

### **Maternal glucose tolerance status during pregnancy**

The definition of gestational glucose tolerance status in Generation 1 women was based on the Australasian Diabetes in Pregnancy Society (ADIPS) guidelines (Hoffman *et al.* 1998). These guidelines, which were made available for routine use just before enrolment of pregnant women for

the Generation 1 study (1998-2000), recommended universal screening for GD at 26-28 weeks of gestation (Hoffman *et al.* 1998). According to these criteria, the non-fasting oral glucose challenge test (OGCT) was considered positive if 1 hour plasma glucose level was  $\geq 7.8$  mmol/l after a 50 g glucose load (Hoffman *et al.* 1998). Pregnant women with a positive challenge test were further referred for a fasting oral glucose tolerance test (OGTT). The cut-off points recommended by ADIPS for GD were fasting plasma glucose level of  $\geq 5.5$  mmol/l and/or plasma glucose level of  $\geq 8.0$  mmol/l at 2 hours post-75 g glucose load (Hoffman *et al.* 1998); this definition was imposed by the health service attended by the women and were not at the discretion of the researchers. Women with negative OGTT following a positive OGCT were included in the group of borderline gestational glucose intolerance, for the purpose of this study. There was no possibility of using a continuous form of plasma glucose levels at OGTT due to the relatively small number of women with data available from this test.

In the Generation 1 Study, OGCT and OGTT results of pregnant women were abstracted from the antenatal records. OGCT was performed at about 28 weeks of gestation (range 23-30 weeks), and OGTT at 29.5 weeks (range 20-36 weeks). Of the 557 women who completed the pregnancy phase, 535 women (96.1%) had available information regarding their glucose tolerance status; the other 22 women were excluded from the analyses involving this variable. Imputation was not used for missing data since there is no known equation for predicting glucose tolerance status with acceptable reliability on the basis of other non-metabolic data.

### **Maternal gestational weight gain**

Gestational weight gain (kg) was calculated by subtracting pre-pregnancy weight from maternal weight shortly before delivery. Measurement of maternal weight was part of the study protocol for the second interview (which was intended to occur at 30-34 weeks of gestation). For some women the second interview weight thus occurred several weeks before delivery. Of the 557 women who gave consent to be part of the cohort, 550 (98.7%) had available data on measured weight in the last trimester of pregnancy taken during the late pregnancy interview (between 27.3 and 40.6 weeks of gestation) (Table 10). The seven women without a third trimester measure of weight were excluded from the analysis of gestational weight gain as their weight shortly before delivery could not be accurately predicted.

**Table 10. Timing of last available measure of weight during pregnancy**

Time during pregnancy	Number of women (%)
3 <sup>rd</sup> trimester	550 (98.7%)
<i>Within 7 days pre-delivery</i>	41 (7.36%)
<i>8-14 days pre-delivery</i>	41 (7.36%)
<i>15-21 days pre-delivery</i>	37 (6.64%)
<i>22+ days pre-delivery</i>	431 (77.4%)
2 <sup>nd</sup> trimester	5 (0.90%)
1 <sup>st</sup> trimester	2 (0.36%)

For the 550 women with weight measured in the third trimester of pregnancy, mean (SD) gestational age at delivery was 39.4 (1.53) weeks, while mean (SD) gestational age when maternal weight was last recorded was 33.4 (2.24) weeks. Given this difference in gestational age (mean of 6 weeks), it was considered that the last recorded maternal weight was unlikely to be representative of pre-delivery weight. Therefore, maternal weight shortly before delivery was derived based on a linear regression against last recorded maternal weight (in the third trimester) and the number of weeks between last measurement and delivery, assuming a weekly gestational weight gain in the last trimester of pregnancy of 0.36 kg/week (Hytten 1991; Widschut 2006).

Total GWG was calculated by subtracting maternal self-reported pre-pregnancy weight (using imputed data as described in the previous section) from the derived maternal weight shortly before delivery. Weekly GWG was calculated as total GWG divided by gestational age at delivery. Eleven women had outlying mean weekly weight changes compared to the other Generation 1 women (they gained either less than 0.055 kg/week or more than 0.655 kg/week across the entire pregnancy). These improbable weight changes were excluded from the analysis.

### 3.4.2 Outcomes in children at the age of 9-10 years

The outcomes of interest in children were continuous measures of child global obesity, percentage body fat, fat pattern and insulin resistance. We chose to employ continuous measures as we were interested in the entire spectrum of these outcome variables in relation to maternal pre-pregnancy BMI, glucose tolerance status during pregnancy and gestational weight gain.

### **Child BMI z-score**

Child BMI was used as a global measure of obesity or overweight status. It was calculated as weight (kg) divided by squared height (m<sup>2</sup>). BMI was further converted to standard deviation scores (BMI z-scores) based on the 1990 age- and sex-specific British growth reference curves (Cole *et al.* 1995), using the 'zanthro' Stata macro (Vidmar *et al.* 2004). The age- and sex-specific reference curves were based on the lambda-mu-sigma (LMS) method (Cole 1988). In addition, for descriptive purposes, children were classified as normal weight, overweight or obese using the International Obesity Task Force (IOTF) age- and sex-specific BMI cut-offs (Cole *et al.* 2000), using the Stata function 'zmicat'. Data on BMI and thus BMI z-score was available in all 443 children who consented to participate in the 9-10 year follow-up.

### **Child percentage body fat**

Child %BF (including both subcutaneous and visceral fat) was regarded as a more specific measure of obesity and was estimated using BIA (Tanita TBF-300). Data on %BF was available for 442 children (one child had a weight of 94 kg, which was greater than the value accepted as valid for this age by the analyser and therefore could not have %BF estimated by BIA).

### **Child waist-to-height ratio**

Fat distribution in children was derived from the WHtR, as a continuous variable. Data on waist and height measurements were available for 442 children (one participant declined waist circumference measurement).

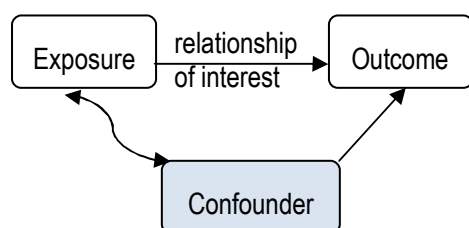
### **Child insulin resistance**

Child IR was quantified by HOMA-IR, calculated as the product of fasting plasma insulin ( $\mu\text{U/ml}$ ) and fasting plasma glucose (mmol/l) divided by 22.5 (Matthews *et al.* 1985). Data were available for 164 children (37% of participants at the 9-10 year follow-up) who gave consent for a fasting blood sample collection. One of these children had extremely high values for plasma glucose and insulin, possibly due to non-fasting, and these values were excluded from the analyses. Therefore, the analyses were based on 163 participants with complete data on fasting plasma insulin and glucose levels. Missing data for children who did not have a blood test are excluded from the relevant analyses.

### 3.4.3 Potential confounders

It is well known that simple associations between exposures and outcomes can be in fact falsely overestimated or, less frequently, obscured (MacKinnon *et al.* 2000) by the effect of a third variable. This third variable can be either a *confounder* (if it is associated with both the exposure and the outcome, without being a consequence of the exposure (Hennekens and Buring 1987)) (Figure 2), or a mediator (if the variable is on the causal pathway). In order to prevent distortion of the true association of interest and maintain validity of inferences, potential third variable effects need to be accounted for in the analysis of any causal process. Confounding and mediating factors are treated identically in statistical analysis (MacKinnon *et al.* 2000) and the distinction between them is based on accumulated knowledge about the relationship of interest.

**Figure 2. Confounders in relation to exposures and outcomes**



There is limited scientific literature on potential confounding effects in the relationships of interest for this project. Several factors have been suggested as being associated on one hand with an increased risk of pre-pregnancy obesity, gestational glucose intolerance (detailed in Section 2.2.2.4), and/or excessive GWG (detailed in Section 2.2.3.1), and on the other hand with an increased risk of childhood obesity and insulin resistance. Of these factors, potential confounders considered in this thesis were maternal age at the time of pregnancy, parity, smoking during pregnancy, presence of pregnancy-induced hypertension, and maternal highest level of education completed at the time of pregnancy (described below). In addition, each main exposure was included in turn as a potential confounder for the relationships between the other two predictor variables and the outcomes of interest. Child current BMI z-score was considered as a potential pathway variable and adjusted for in the models with child HOMA-IR as an outcome. In this thesis, multivariate analysis was performed to investigate and adjust for potential confounding and mediating effects.

**Maternal age at the time of pregnancy** was obtained by subtracting the woman's date of birth from the child's date of birth (both abstracted from antenatal records) and dividing this difference by 365.25; it was treated as a continuous variable. **Parity** at the time of study pregnancy was defined



as a binary variable (primiparity or multiparity), derived from the answer to a question regarding the number of children previously delivered. **Maternal smoking** was considered as a binary variable indicating if the mother had reported (at the early or late pregnancy interview) any smoking during the study pregnancy (yes or no). Data on **pregnancy-induced hypertension** were abstracted from antenatal casenotes and referred to any degree of high blood pressure with onset during pregnancy, including preeclampsia (treated as a binary variable). **Maternal education** was chosen as a surrogate marker for socio-economic status and was defined by the highest level of education completed by the mother at the time of pregnancy: partial high-school, high-school, Training and Further Education (TAFE) or college, and university degree.

Of note, factors such as child's size at birth, breastfeeding, diet or physical activity have also been associated both with maternal pre-pregnancy obesity, GD or GWG on one hand, and child obesity on the other, but these variables are likely to be on the causal pathway between maternal exposures and child outcomes of interest. Given that they are mediators (not confounders) and their potential influence was not within the scope of this thesis, these variables were not included in analyses.

### 3.5 Analysis plan

Statistical analyses included in this thesis covered both descriptive and inferential aspects.

**Descriptive statistics** focused on summary measures for all variables considered in this project (exposures, outcomes and potential confounders), as well as on the non-participation assessment in relation to the original Generation 1 cohort. For descriptive purposes, summary measures are reported as means, standard deviations (SD), medians, ranges, and interquartile ranges (IQR) for continuous variables (i.e., maternal pre-pregnancy BMI, maternal plasma glucose levels at OGCT and OGTT, maternal GWG, maternal age at the time of pregnancy, child BMI z-score, child %BF, child WHtR, and child HOMA-IR), and as numbers and proportions for categorical variables (i.e., maternal glucose tolerance status during pregnancy, categories of maternal pre-pregnancy BMI, categories of maternal GWG, parity, smoking, pregnancy-induced hypertension, and level of educational attainment at the time of pregnancy).

In practice, different summary measures of central tendency and variability are relevant according to the distribution of the variable of interest: mean and SD for normally distributed variables and median and IQR for variables with a skewed distribution. For the latter type of data, the most appropriate

graphical representation of the summary measures is the box and whisker plot (an example is further presented in Chapter 4, Figure 6). The box contains 50% of the data, the middle line is the median, the bottom and the top lines of the box are the 1<sup>st</sup> and 3<sup>rd</sup> quartiles (IQR), the whiskers extend to 1.5 of IQR and the dots represent the outliers. Normality was inspected for each outcome variable and a transformation was sought for those with a skewed distribution (e.g., HOMA-IR). Non-participation was examined using independent samples t-tests (for continuous variables) and chi-squared tests (for categorical variables) and is presented in Section 4.1.4.

**Inferential statistics** followed a component-based approach, with statistical models being constructed to investigate associations between each main intrauterine exposure (i.e., maternal pre-pregnancy BMI, categories of glucose tolerance during pregnancy or GWG), and the development of obesity in terms of BMI z-score, percentage body fat and central adiposity, and IR in children.

Briefly, the main components of the inferential analyses were:

1. maternal pre-pregnancy BMI and child BMI z-score, %BF, WHtR and HOMA-IR;
2. maternal glucose tolerance during pregnancy and child BMI z-score, %BF, WHtR and HOMA-IR;
3. maternal GWG and child BMI z-score, %BF, WHtR and HOMA-IR;
4. interactions between each pair of main exposures in relation to child BMI z-score, %BF, WHtR and HOMA-IR.

After examining each bivariate relationship (using Pearson's correlation), several covariates identified in previous studies as independently predicting the exposures and the outcomes were tested and adjusted for in each model, in a stepwise manner. The potential confounders controlled for in this project included maternal age, parity, smoking during pregnancy, pregnancy-induced hypertension and highest level of education completed by the time of pregnancy. Confounders were retained in the models if  $p < 0.1$ . Given the well known positive association between obesity and insulin resistance, child BMI z-score was further considered as a potential mediating variable on the pathway between each of the early origin factors addressed in this project and child HOMA-IR.

Potential interactions between each two main exposures (i.e., maternal pre-pregnancy BMI and glucose tolerance status during pregnancy, maternal pre-pregnancy BMI and GWG, and glucose tolerance status during pregnancy and GWG) were identified *a priori* and tested in relation to all outcomes of interest, with the significant potential confounders included in the models. Two exposures are considered to interact in relation to a certain outcome when their observed joint effect

is greater (synergy) or smaller (antagonism) than the expected joint effect based on their independent effects alone (Greenland 1983).

Models with continuous and (relatively) normally distributed outcomes (i.e., child BMI z-score, %BF and WHtR) were run using simple and forward stepwise multiple linear regression.

Traditionally, the logarithmic transformation is applied to positively skewed dependent variables, such as HOMA-IR, in order to obtain normal distributions. However interpreting the results of analyses based on log transformations raises a number of issues. First, this type of transformation often leads to *symmetry* of the dependent variable distribution and does not equate to *normality* (Moran *et al.* 2007). Second, appropriate 'back transformation' to the original scale of the dependent variable involves more than a simple exponentiation. When the dependent variable is log transformed in an ordinary least squares regression model, the regression coefficients estimate the expected *geometric mean* of the original variable and not the *arithmetic mean*, which would be estimated using the untransformed dependent variable (and which is of primary interest) (UCLA: Academic Technology Services and Statistical Consulting Group). In fact, the effect on the original dependent variable is in terms of percentage change, quantified as  $100 * [\exp(\beta) - 1]$ , where  $\beta$  is the regression coefficient of the independent variable (regardless of whether it is continuous or categorical) (Cole 2000). The above mentioned limitations of log transformation of a dependent variable may be overcome by applying generalised linear models (GLM), which synthesize the general techniques used for continuous and discrete data into a unified conceptual framework with a more flexible approach (Breslow 1996). In this thesis analyses with child HOMA-IR as the outcome of interest were assessed using GLM with log link function and Gaussian family.

Assumptions underpinning each statistical analysis were tested and appeared to be met. Given that the power was fixed by the sample size (established cohort), no power calculation was performed. Throughout this thesis, the cut-off for statistical significance was set at 0.05, except for the criterion of retaining confounders, in which case the cut-off was 0.1. Simultaneously testing multiple hypotheses on a single dataset may inflate the overall type I error (the probability of the observed associations being attributable to chance), thus increasing the likelihood of a false-positive conclusion (Schulz and Grimes 2005). To minimise this type I error, adjustments for multiple comparisons have been suggested, which usually involve multiplying the p-value of the observed association by the number of hypotheses tested (Schulz and Grimes 2005), thereby reducing the probability of mistakenly rejecting a true null hypothesis. However, randomly observed associations are still possible (Rothman 1990), albeit less likely. Moreover, these random associations in nature,

as opposed to simple random number sets, may be significant and are worthy of further investigation (Rothman 1990). Therefore, adjustment for multiple comparisons was considered unnecessary and not performed in the current study. All data analyses were performed using Stata IC version 10 statistical software (StataCorp, Inc., Texas USA).

### **3.6 Summary**

This chapter outlined a brief history of the Generation 1 cohort, a detailed description of the methods of data collection for the 9-10 year wave, along with the methods of analysis for each component. The next chapter presents the results of statistical analyses.

# Chapter 4 Results

This chapter presents the main findings of statistical analyses conducted in order to investigate potential associations between three maternal pregnancy-related factors likely to contribute to fetal overnutrition (maternal obesity prior to pregnancy, gestational glucose intolerance and excessive gestational weight gain), on one hand, and child obesity and insulin resistance, on the other.

## 4.1 Descriptive statistics

This first section of results focuses on the description of variables of interest for this project, including the intrauterine exposures (Section 4.1.1), the outcomes in children (Section 4.1.2), and the potential confounders identified from previous research (Section 4.1.3). Sample representativeness at this follow-up in relation to the original study group (which was considered representative of the wider population of South Australian women who gave birth in 1998-2000) is presented in Section 4.1.4.

### 4.1.1 Intrauterine exposures

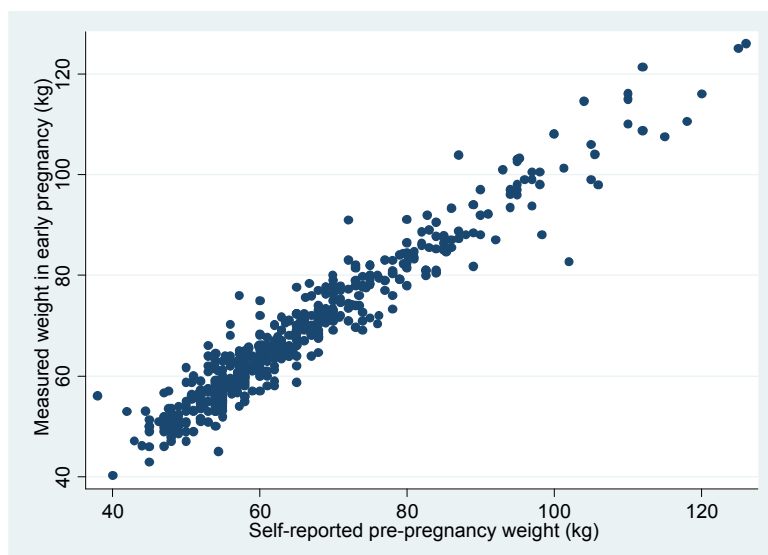
The three main exposures for this study were maternal pre-pregnancy BMI, glucose tolerance status during pregnancy, and gestational weight gain; their summary measures are presented below.

#### 4.1.1.1 Maternal pre-pregnancy BMI

As described in Section 3.4.1, maternal nutritional status prior to pregnancy was measured by BMI, using self-reported pre-pregnancy weight and height measured in early pregnancy. Pre-pregnancy weight was reported by 502 women (90.1%). Given the well known tendency of women to under-report their weight (Gorber *et al.* 2007), self-reported pre-pregnancy weight was compared to the directly measured weight in early pregnancy. Early pregnancy weight was measured between 7.7 and 20.4 weeks of gestation (mean (SD) for gestational age 14.4 (2.0) weeks) and was available for

all 557 women. Overall, there was a very strong correlation between self-reported pre-pregnancy weight and weight measured in early pregnancy ( $r=0.96$ ,  $p<0.001$ ) (Figure 3).

**Figure 3. Correlation between maternal self-reported pre-pregnancy weight and measured weight in early pregnancy**



However, for 21 women, the difference between measured weight in early pregnancy and self-reported pre-pregnancy weight for gestational age fulfilled the criteria for outliers within Generation 1 cohort (these women either lost more than 0.37 kg/week or gained more than 0.80 kg/week of gestation, assuming a constant rate of weight gain in early pregnancy). In order to maximize the dataset, the 21 improbable values of pre-pregnancy weight (likely due to poor recall or reporting) were set to missing and then imputed along with the other 55 missing values (as detailed in Section 3.4.1). Anthropometric measurements of Generation 1 women before pregnancy are presented in Table 11.

**Table 11. Maternal body size before pregnancy (n=557)**

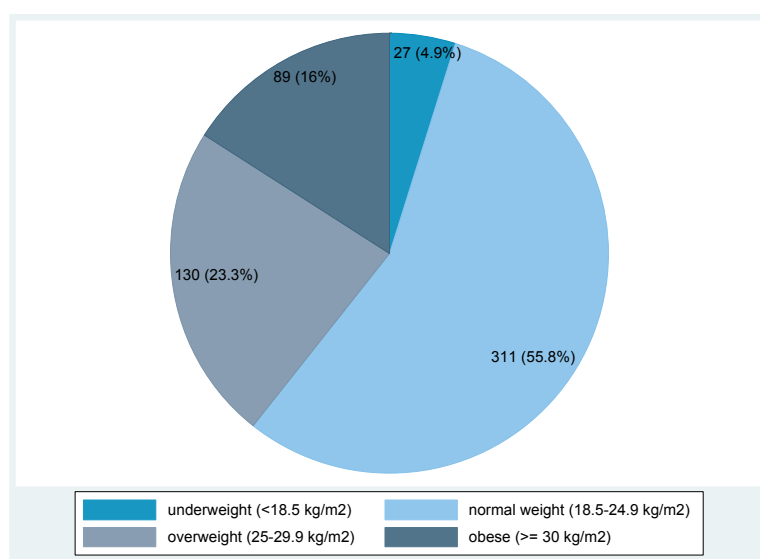
Pre-pregnancy measure	Mean	SD	Range	Median	IQR
Weight (kg)	66.9	15.0	40.0 – 126.0	63.0	56.0 - 74.6
Height (cm)	163.6	6.16	146.5 – 185.0	163.5	159.5 - 167.6
BMI (kg/m <sup>2</sup> )	25.0	5.59	14.7 – 46.6	23.6	21.2 - 27.5

IQR – interquartile range, SD – standard deviation

Based on WHO classification of BMI (World Health Organization 1995), 39.3% of Generation 1 women were overweight or obese prior to pregnancy (Table 12 and Figure 4).

**Table 12. Maternal pre-pregnancy BMI status**

Categories of pre-pregnancy BMI	Number (%) of women
Underweight (<18.5 kg/m <sup>2</sup> )	27 (4.85)
Normal weight (18.5-24.9 kg/m <sup>2</sup> )	311 (55.8)
Overweight (25-29.9 kg/m <sup>2</sup> )	130 (23.3)
Obese (≥30 kg/m <sup>2</sup> )	89 (16.0)
Total	557 (100)

**Figure 4. Maternal pre-pregnancy BMI status**

#### 4.1.1.2 Maternal glucose tolerance status during pregnancy

As described in Section 3.4.1, the guidelines proposed by the Australasian Diabetes in Pregnancy Society were used to classify maternal glucose tolerance status during pregnancy. Briefly, the screening for GD was a two stage process, comprising a non-fasting OGCT which, if positive, was followed by a fasting OGTT, which is a diagnostic test. Women with normal OGTT following an abnormal OGCT were considered to have BGGI. The plasma glucose levels from the OGCT and OGTT are summarised in Table 13.

**Table 13. Summary of plasma glucose levels at OGCT and OGTT during pregnancy for Generation 1 women**

Test (oral glucose load)	Number (%) of cohort members who underwent the test		Plasma glucose level (mmol/l)				
			Mean	SD	Range	Median	IQR
OGCT (50 g)	538 (96.6%)	1 h post- load	6.3	1.8	2.8-21.6	6.2	5.2-7.2
OGTT (75 g)	90 (16.2%)	Fasting	4.7	0.6	3.5-6.9	4.7	4.4-5
		2 h post-load	7.0	1.3	4.5-10.8	6.9	6.1-8

IQR – interquartile range, OGCT – oral glucose challenge test, OGTT – oral glucose tolerance test, SD – standard deviation

OGCT was performed in 538 women (96.6% of pregnant women enrolled in the Generation 1 cohort) as part of their standard care (universal screening for GD was introduced in 1998, when recruitment for the cohort started). Of the 538 women who underwent OGCT, 452 women (81.2%) had normal plasma glucose level (<7.8mmol/l) and 86 women (15.4%) had a positive screening test ( $\geq$ 7.8 mmol/l).

Of the 86 women with an abnormal OGCT, 80 (93.0%) had OGTT done. This test was positive in 27 women (fasting plasma glucose level >5.5 mmol/l and/or 2h-post 75 oral glucose load >8.0 mmol/l), who were thus diagnosed with gestational diabetes; OGTT was negative in 53 women who, for the purpose of this project, were included into the borderline gestational glucose intolerance category. For the six women with abnormal OGCT and without OGTT available results, a degree of glucose intolerance could be suspected (either borderline gestational glucose intolerance, or gestational diabetes), but it was not possible to clearly define their glucose tolerance status. These women were not included in further analyses regarding this exposure.

In addition to the 80 women undergoing an OGTT following an abnormal OGCT, six women had OGTT done without being preceded by the screening test; OGTT was positive in three of them, hence they were classified as GD, while the remaining three were not classifiable and were excluded from further analyses regarding this exposure. An additional four women underwent OGTT despite having normal OGCT, which was not common practice; OGTT was positive in one of them and this woman was classified as GD, while the remaining three were classified as having normal glucose tolerance.

In summary (Table 14 and Figure 5), 31 women (5.57%) of the Generation 1 cohort were diagnosed with GD, 53 women (9.52%) were included into the BGGI category, 451 women (81%) were considered as having normal glucose tolerance, and for 22 women (3.95%) glucose tolerance status could not be determined.

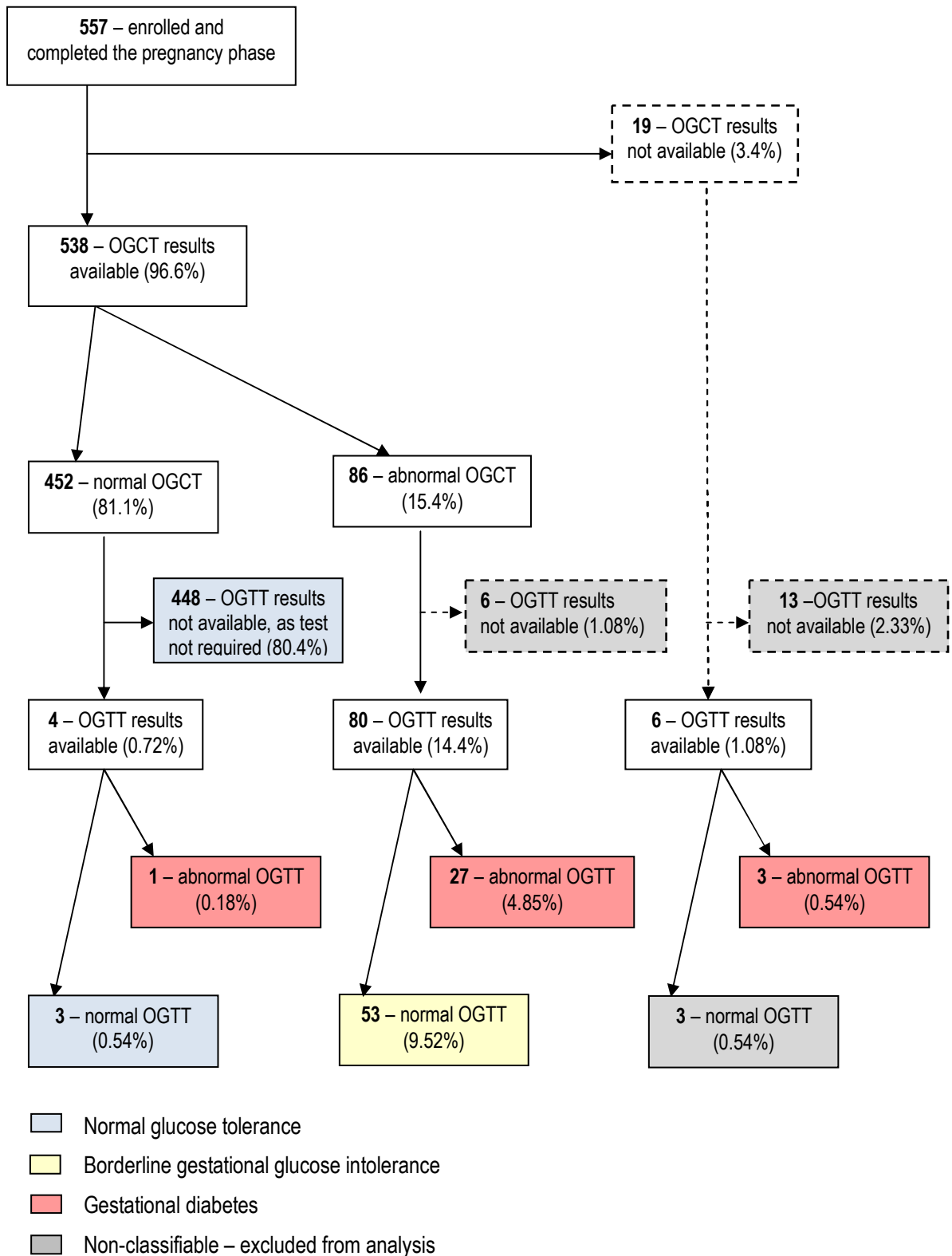


**Table 14. Glucose tolerance profiles during pregnancy in Generation 1 women**

OGCT	OGTT	Number of women	Glucose tolerance category	Criteria or assumptions
<b>Normal</b>		<b>452</b>		
	Normal	3	NGT	OGTT is a diagnostic test
	Abnormal	1	GD	OGTT is a diagnostic test
	Missing	448	NGT	Assume OGTT not recommended
<b>Abnormal</b>		<b>86</b>		
	Normal	53	BGGI	
	Abnormal	27	GD	ADIPS
	Missing	6	Non-classifiable (BGGI or GD)	
<b>Missing</b>		<b>19</b>		
	Normal	3	Non-classifiable (NGT or BGGI)	
	Abnormal	3	GD	OGTT is a diagnostic test
	Missing	13	Non-classifiable	
<b>Total</b>		<b>557</b>		

ADIPS - Australasian Diabetes in Pregnancy Society, BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance, OGCT- oral glucose challenge test, OGTT - oral glucose tolerance test

Figure 5. Results of prenatal screening for gestational diabetes in Generation 1 women



#### 4.1.1.3 Maternal gestational weight gain

As described in Methods Section 3.4.1, gestational weight gain was calculated by subtracting maternal self-reported pre-pregnancy weight (using reliable and imputed data) from maternal weight shortly before delivery. After excluding 22 (3.23%) outliers, mean (SD) total GWG in Generation 1 women (of those with weight available during the third trimester of pregnancy) was 14.0 (4.54) kg (range 2.29 to 26.4 kg); the mean (SD) weekly gestational weight gain was 0.35 (0.11) kg/week (range 0.06 to 0.65 kg/week).

Across all categories of pre-pregnancy BMI, about a third of the women gained weight adequately throughout pregnancy and almost a half gained weight excessively compared to the 2009 recommendations of Institute of Medicine (Rasmussen and Yaktine 2009) (Table 15). The highest proportion (over 60%) of excessive GWG was observed in overweight and obese women.

**Table 15. Total gestational weight gain across categories of pre-pregnancy BMI in Generation 1 women, relative to 2009 Institute of Medicine (IOM) recommendations**

Pre-pregnancy BMI category	n	IOM recommendations 2009 for GWG		
		Insufficient GWG n (%)	Adequate GWG n (%)	Excessive GWG n (%)
Underweight (<18.5 kg/m <sup>2</sup> )		<12.7 kg	12.7 – 18.2 kg	>18.2 kg
	26	10 (38.5%)	10 (38.5%)	6 (23.0%)
Normal weight (18.5-24.9 kg/m <sup>2</sup> )		<11.4 kg	11.4 – 15.9 kg	>15.9 kg
	304	57 (18.8%)	128 (42.1%)	119 (39.1%)
Overweight (25-29.9 kg/m <sup>2</sup> )		<6.8 kg	6.8 – 11.4 kg	>11.4 kg
	127	8 (6.3%)	32 (25.2%)	87 (68.5%)
Obese (≥30 kg/m <sup>2</sup> )		<5 kg	5 – 9.1 kg	>9.1 kg
	82	12 (14.6%)	18 (22.0%)	52 (63.4%)
Total	539	87 (16.1%)	188 (34.9%)	264 (49.0%)

#### 4.1.1.4 Interrelations between maternal pre-pregnancy BMI, glucose tolerance status during pregnancy and gestational weight gain

Previous research (outlined in Section 2.2.4) has shown that the three exposures of interest for this project are interrelated mainly through the amount of glucose provided to the fetus, with subsequent impact on intrauterine growth. The interrelations observed between maternal pre-pregnancy BMI, glucose tolerance status during pregnancy and GWG in Generation 1 women are reported below. They are further examined as potentially interacting with each other in relation to child obesity and insulin resistance, analyses that are presented in Section 4.2.4.

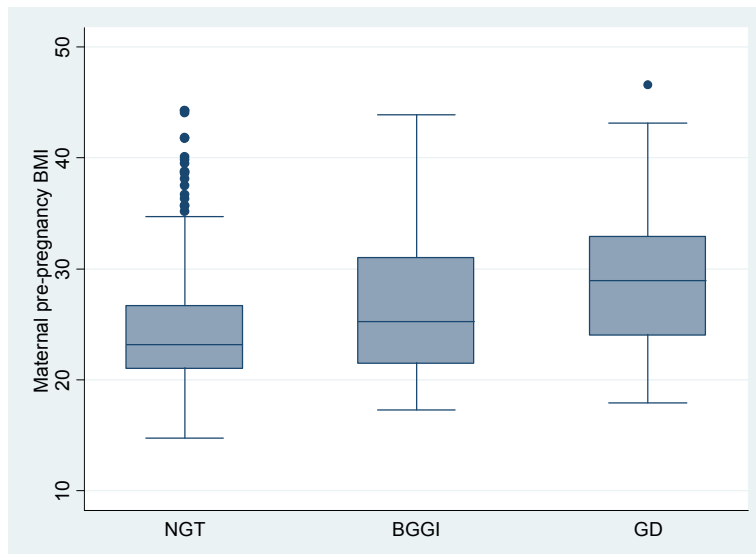
##### Maternal pre-pregnancy BMI and glucose tolerance status during pregnancy

As illustrated in Table 16 and Figure 6, pre-pregnancy BMI (mean and median, respectively) was lowest among women who maintained normal glucose tolerance during pregnancy and highest among those who developed gestational diabetes. The fact that obese women are at increased risk of glucose intolerance during pregnancy is well established; however, not only overweight or obese women develop this pregnancy-related condition. In this cohort, a quarter of the women with GD and 45.3% of those with BGGI were not overweight or obese. Even underweight women presented glucose tolerance across the entire spectrum, albeit only one developed GD.

**Table 16. Maternal pre-pregnancy BMI across the spectrum of glucose tolerance during pregnancy**

Glucose tolerance status during pregnancy	n	Mean (SD) pre-pregnancy BMI (kg/m <sup>2</sup> )	Underweight n (%)	Normal weight n (%)	Overweight n (%)	Obese n (%)
NGT	451	24.5 (5.08)	22 (4.88)	271 (60.1)	98 (21.7)	60 (13.3)
BGGI	53	27.1 (7.02)	3 (5.66)	21 (39.6)	15 (28.3)	14 (26.4)
GD	31	29.2 (6.84)	1 (3.23)	7 (22.6)	12 (38.7)	11 (35.5)
Total	535	25.0 (5.56)	26 (4.86)	299 (55.9)	125 (23.4)	85 (15.9)

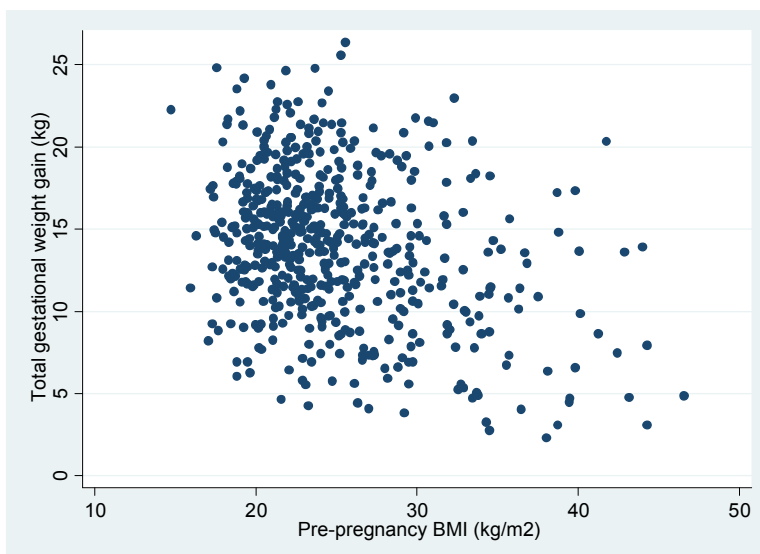
BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

**Figure 6. Maternal pre-pregnancy BMI across the spectrum of glucose tolerance during pregnancy**

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

### Maternal pre-pregnancy BMI and gestational weight gain

Similar to other studies (Cedergren 2006; Schack-Nielsen *et al.* 2010), there was a moderate negative correlation between pre-pregnancy BMI and GWG ( $r=-0.32$ ,  $p<0.001$ ). This relationship is presented in Figure 7.

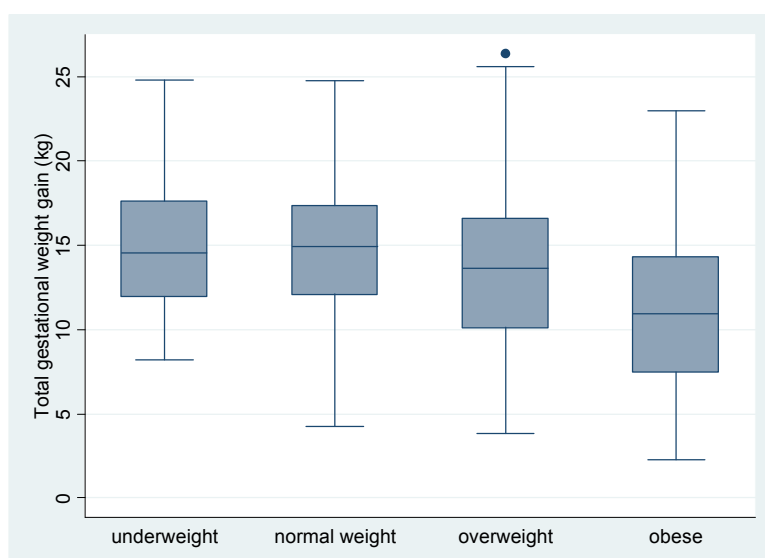
**Figure 7. Maternal gestational weight gain in relation to pre-pregnancy BMI**

As presented in Table 17 and Figure 8, underweight women gained the greatest amount of weight overall during pregnancy, while obese women gained the least, approximately 3.6 kg less compared to normal weight women.

**Table 17. Maternal gestational weight gain across categories of pre-pregnancy BMI**

Pre-pregnancy BMI category	n	Mean (SD) total GWG (kg)
Underweight (<18.5 kg/m <sup>2</sup> )	26	15.0 (4.51)
Normal weight (18.5-24.9 kg/m <sup>2</sup> )	304	14.8 (4.00)
Overweight (25-29.9 kg/m <sup>2</sup> )	127	13.5 (4.63)
Obese (≥30 kg/m <sup>2</sup> )	82	11.2 (5.10)
Total	539	14.0 (4.54)

**Figure 8. Maternal gestational weight gain across categories of pre-pregnancy BMI**



### Maternal glucose tolerance during pregnancy and gestational weight gain

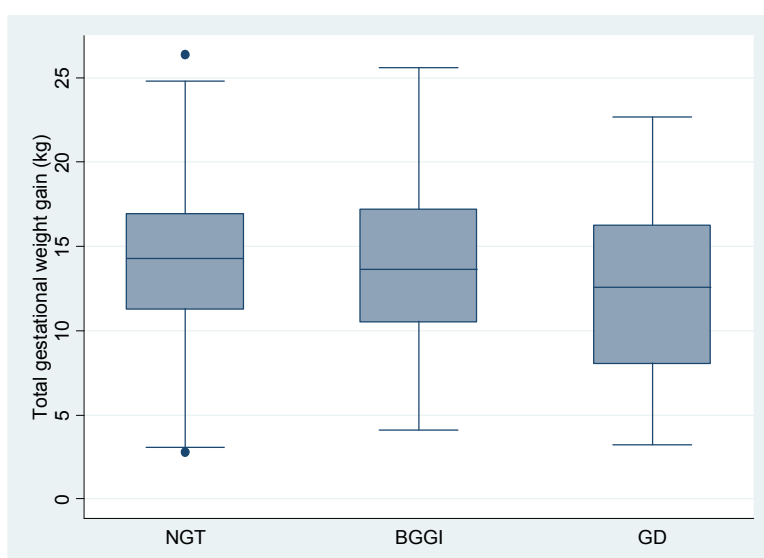
Overall, women who developed GD gained a smaller amount of weight during pregnancy (up to delivery) compared to women who maintained normal glucose tolerance during pregnancy (which aligns with the fact that they were likely to be, though not exclusively, overweight or obese).

Maternal GWG across the spectrum of glucose tolerance during pregnancy is summarised in Table 18 and Figure 9.

**Table 18. Maternal gestational weight gain and glucose tolerance status during pregnancy**

Glucose tolerance profiles during pregnancy	n	Mean (SD) total GWG (kg)
NGT	439	14.1 (4.39)
BGGI	49	13.8 (4.58)
GD	31	12.8 (5.42)
Total	519	14.0 (4.76)

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, GWG – gestational weight gain, NGT - normal glucose tolerance

**Figure 9. Maternal gestational weight gain and glucose tolerance status during pregnancy**

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

#### 4.1.2 Outcomes in children at the age of 9-10 years

The mean (SD) age of children taking part in this phase of the Generation 1 study was 9.65 (0.31) years. The outcomes considered in this project are child global obesity (measured by BMI z-score), percentage body fat (estimated by BIA), central adiposity (derived from the WHtR), and insulin resistance (defined by HOMA-IR). Their definitions were presented in Section 3.4.2.

#### 4.1.2.1 Child BMI z-score

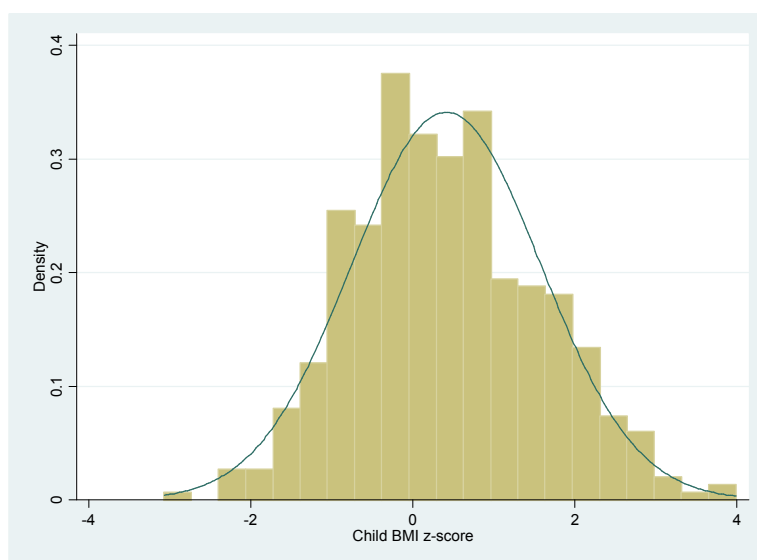
Child BMI was considered as a global indicator of obesity or overweight status. Summary measures of children's weight, height, and BMI are outlined in Table 19. Based on the age- and sex-specific growth reference curves adopted by the International Obesity Task Force (Cole *et al.* 1995), BMI can be expressed both as a continuous z-score (the distribution is presented in Figure 10) and as a three-category variable (normal weight, overweight and obese). According to the IOTF classification, 341 (77.0%) Generation 1 children were normal weight, 76 (17.1%) were overweight and 26 (5.9%) were obese (Figure 11).

**Table 19. Anthropometric measurements in Generation 1 children at 9-10 years (n=443)**

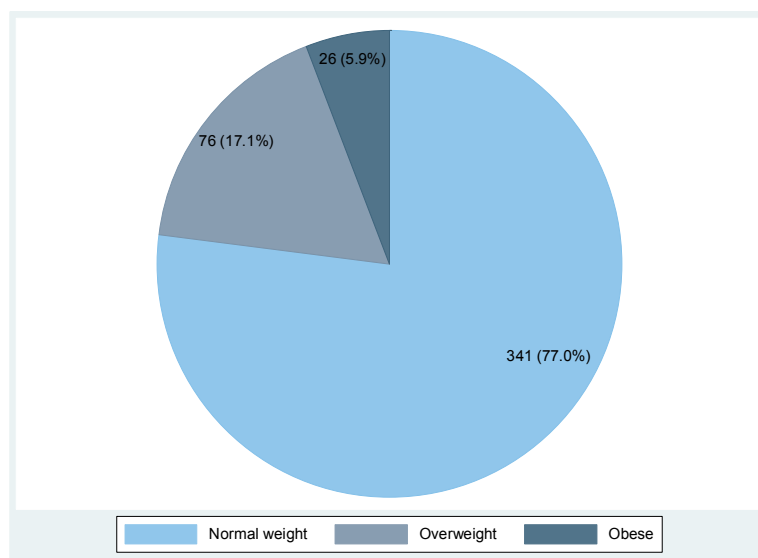
Measure	Mean	SD	Range	Median	IQR
Weight (kg)	34.4	8.27	21.9 - 94.0	32.8	28.7 - 38.1
Height (cm)	138.0	6.46	121.1 - 157.4	137.8	133.1 - 142.0
BMI (kg/m <sup>2</sup> )	17.9	3.17	12.8 - 37.9	17.2	15.8 - 19.3
BMI z-score	0.41	1.17	-3.07 - 3.99	0.34	-0.41 - 1.18

IQR – interquartile range, SD – standard deviation

**Figure 10. BMI z-score in Generation 1 children**





**Figure 11. Distribution of BMI categories in Generation 1 children**

#### 4.1.2.2 Child percentage body fat

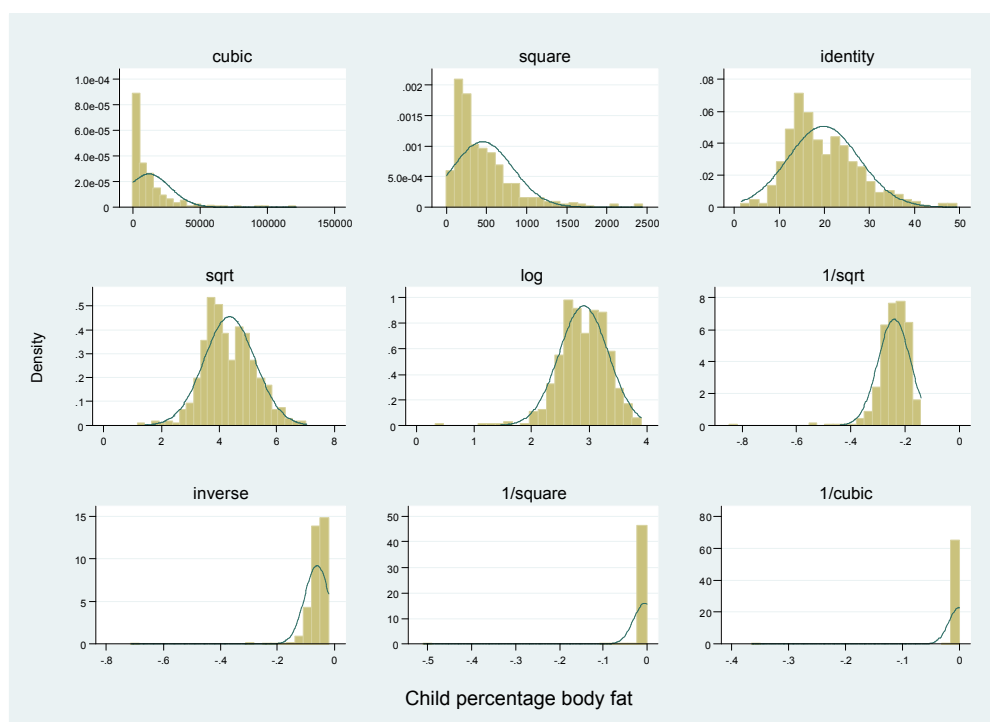
Percentage body fat was estimated by bioelectrical impedance analysis in 439 children (99.1% of those participating at the 9-10 year follow-up). Summary measures for percentage body fat in Generation 1 children are presented in Table 20.

**Table 20. Summary measures for percentage body fat in Generation 1 children (n=439)**

Measure	Mean	SD	Range	Median	IQR
Percentage body fat	19.8%	7.87%	1.4-49.5%	18.2%	14-24.7%

IQR – interquartile range, SD – standard deviation

Percentage body fat distribution was slightly skewed to the right (the graph labelled 'identity' in Figure 12). Of the transformation options generated by the 'ladder' Stata command, the square root of percentage body fat provided the best approximation to a normal distribution (Figure 12). However, after considering the options, for a simpler and more intuitive interpretation of the results, identity function was kept (no transformation was used) in the analyses involving this outcome, particularly since all assumptions of linear regression (Berry and Feldman 1985) using the untransformed variable were largely met.

**Figure 12. Histograms by transformation for child percentage body fat**

#### 4.1.2.3 Child waist-to-height ratio

Waist-to-height ratio was used as a proxy measure for central adiposity. Waist circumference was measured in 442 children (99.8% of those participating in this round of follow-up). Summary measures for waist circumference and WHtR in Generation 1 children are presented in Table 21.

**Table 21. Summary measures of central adiposity in Generation 1 children (n=442)**

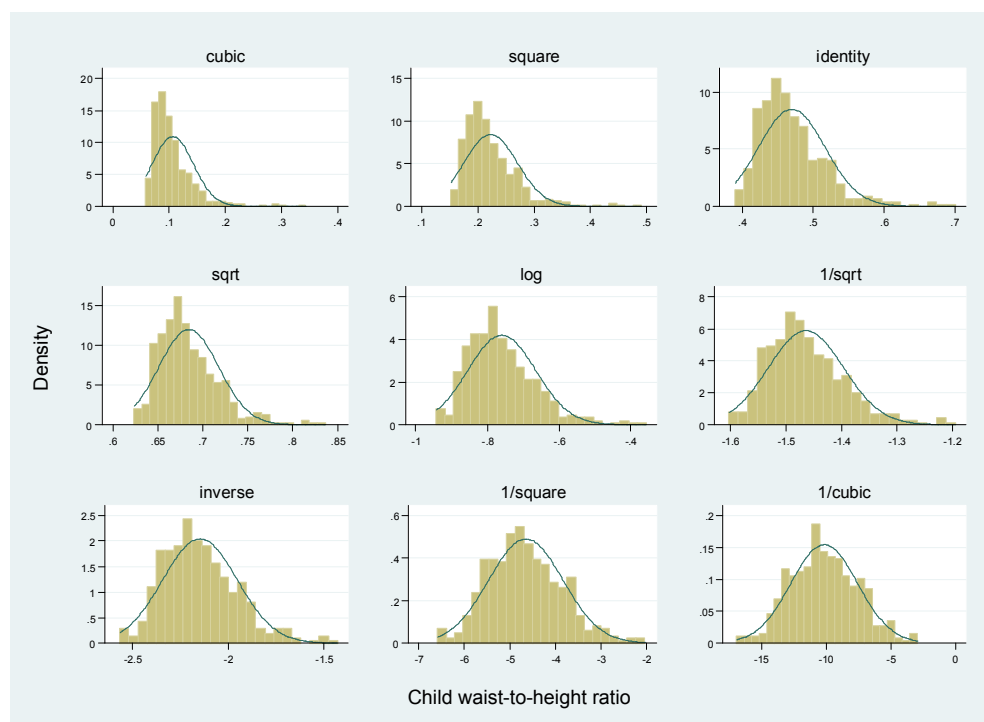
Measure	Mean	SD	Range	Median	IQR
Waist circumference (cm)	64.9	7.62	52-110.3	63.0	59.5-68.8
Waist-to-height ratio	0.470	0.047	0.390-0.700	0.460	0.436-0.492

IQR – interquartile range, SD – standard deviation

Similar to percentage body fat, WHtR was positively skewed (skewness coefficient = 1.40). In this case, the transformation consisting of the inverse of the square root technically provided the best approximation of normality, but did not represent a great improvement over identity (i.e. untransformed) (Figure 13). Again, assumptions of linear regression (Berry and Feldman 1985) using the untransformed variable were largely met, except for a minimal violation of normality of the residuals, but, given the sample size, this was unlikely to affect the estimation of the regression coefficients. In

order to facilitate the interpretation of the results, it was decided to keep identity function, particularly since linear regression modelling is known to be robust against some violations of normality (Berry and Feldman 1985).

**Figure 13. Histograms by transformation for child waist-to-height ratio**



One fifth of the Generation 1 children seen at 9-10 years of age had a WHtR greater or equal to 0.5, a cut-off that has been proposed as a marker of central adiposity (McCarthy and Ashwell 2006).

#### 4.1.2.4 Child insulin resistance

Insulin resistance was defined by HOMA-IR, which was calculated as the product of fasting insulin ( $\mu\text{U/ml}$ ) and fasting glucose ( $\text{mmol/l}$ ) divided by 22.5 (Matthews *et al.* 1985). Six observations of HOMA-IR fulfilled the criteria for outliers (falling more than 1.5 times interquartile range above the 3<sup>rd</sup> quartile or below the 1<sup>st</sup> quartile). A closer look at these extreme values showed higher fasting insulin levels in all cases, but no difference to the other Generation 1 children with respect to age, BMI, fasting glucose, triglyceride, HDL-cholesterol and LDL-cholesterol levels, except for one case with HOMA-IR of 8.82 whose LDL-cholesterol was in the 'borderline high' range (3.83  $\text{mmol/l}$ ). The mother of this child also had a pre-pregnancy BMI outside of the overall pattern of the distribution, as well as BGGI. Only this most extreme outlier for HOMA-IR was dropped from further analyses, while

the other outliers were retained. Summary measures for fasting glucose, fasting insulin, and HOMA-IR after omitting this extreme value are presented in Table 22.

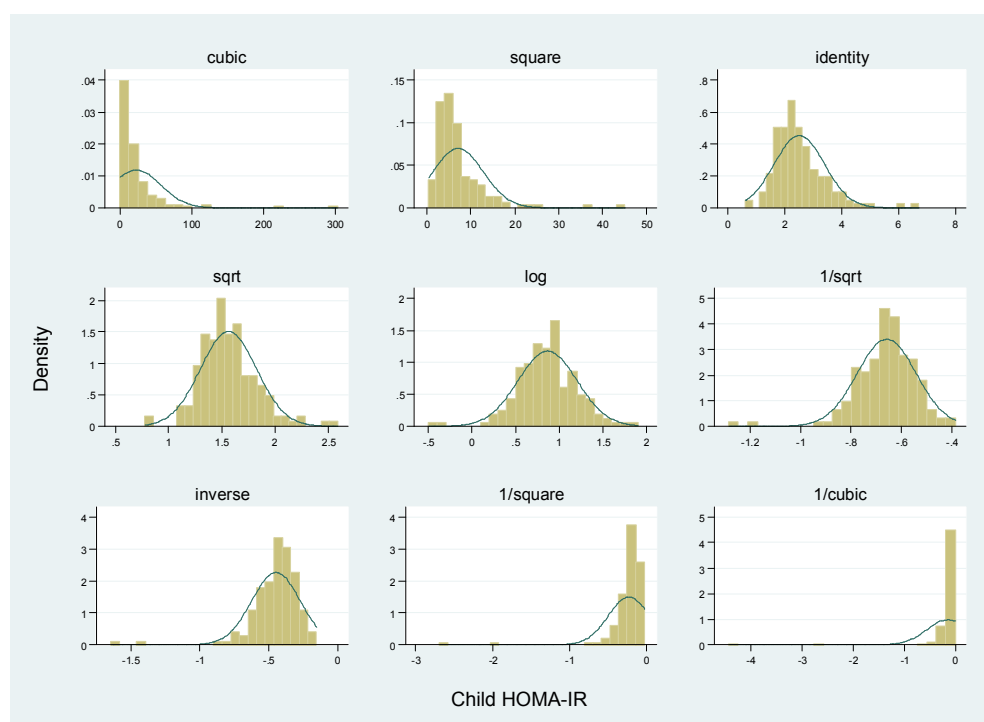
**Table 22. Summary measures for fasting glucose, fasting insulin, and HOMA-IR in Generation 1 children (n=163)**

Measure	Mean	SD	Range	Median	IQR
Insulin ( $\mu\text{U/ml}$ )	11.3	3.53	3.02-27.69	10.7	8.99-12.8
Glucose (mmol/l)	4.96	0.42	3.50-6.21	4.91	4.70-5.23
HOMA-IR	2.51	0.88	0.61-6.71	2.34	1.92-2.91

IQR – interquartile range, SD – standard deviation

The distribution of HOMA-IR showed marked kurtosis and positive skewness even after excluding the most extreme outlier (the graph labelled ‘identity’ in Figure 14). Using the ‘ladder’ Stata command, the log transformation of HOMA-IR provided the best approximation to a normal distribution (Figure 14).

**Figure 14. Histograms by transformation for child HOMA-IR**



Traditionally, the logarithmic transformation is applied to positively skewed dependent variables in order to obtain normal distributions. However interpreting the results of analyses based on log transformations raises a number of issues, which have been discussed in detail in Section 3.5. These limitations of log transformation of a dependent variable may be overcome by applying

generalised linear models (GLM), which in the present case (of child HOMA-IR) were applied with log link function and Gaussian family.

A summary of anthropometric measurements and fasting blood assays of interest is presented in Table 23.

**Table 23. Children's anthropometric measurements and fasting measures of glucose homeostasis at 9-10 years (mean, SD)**

	All children	Girls	Boys	p-value for girls vs. boys
Age (years)	9.65 (0.31)	9.64 (0.29)	9.65 (0.32)	0.688
Body size	n=443	n=221	n=222	
Weight (kg)	34.4 (8.27)	33.5 (6.91)	35.35 (9.36)	<b>0.019</b>
Height (cm)	138.0 (6.46)	137.1 (6.33)	138.9 (6.48)	<b>0.005</b>
BMI (kg/m <sup>2</sup> )	17.9 (3.17)	17.7 (2.65)	18.1 (3.61)	0.120
BMI-z score*	0.41 (1.17)	0.25 (1.04)	0.57 (1.27)	<b>0.004</b>
Percentage body fat	n=439	n=218	n=221	
Based on bioelectrical impedance analysis	19.8 (7.87)	20.6 (7.76)	19.0 (7.92)	<b>0.039</b>
Fat pattern	n=442	n=220	n=222	
Waist circumference (cm)	64.9 (7.62)	63.4 (6.34)	66.3 (8.47)	<b>0.000</b>
Waist-to-height ratio	0.47 (0.05)	0.46 (0.04)	0.48 (0.05)	<b>0.000</b>
Fasting measures of glucose homeostasis	n=163	n=89	n=74	
Insulin (µU/ml)	11.3 (3.53)	11.5 (3.46)	11.1 (3.64)	0.492
Glucose (mmol/l)	4.96 (0.42)	4.92 (0.43)	5.00 (0.40)	0.226
HOMA-IR index	2.51 (0.88)	2.54 (0.85)	2.49 (0.92)	0.731

\* Based on the 1990 British growth reference chart (age- and sex-specific)

### 4.1.3 Potential confounders

A series of factors have been identified in previous research, albeit often inconsistently, as being associated with maternal pre-pregnancy obesity, glucose intolerance during pregnancy, and/or excessive gestational weight gain, and also independently increasing the risk of obesity and related outcomes in the child. Those variables that met the criteria for potential confounders were described in Section 3.4.3. They include maternal age at the time of pregnancy, parity, smoking during

pregnancy, presence of pregnancy-induced hypertension, and maternal socioeconomic status (indicated in this study by the highest level of education attained at the time of pregnancy).

**Maternal age** at the time of study pregnancy ranged between 18.8 and 42.2 years, with a mean (SD) of 29.8 (5.02) years. Maternal pre-pregnancy BMI and maternal age were not correlated ( $r=0.005$ ,  $p=0.916$ ). Women with impaired glucose tolerance during pregnancy were older than those without (mean (SD) age in GD group 31.7 (5.49) years, BGGI group 30.9 (4.28) years, NGT group 29.6 (5.05) years;  $p=0.021$ ). An association approaching significance was found between total GWG and maternal age ( $r=-0.075$ ,  $p=0.081$ ). Maternal age at the time of pregnancy was not associated with child BMI z-score ( $r=0.026$ ,  $p=0.589$ ), %BF ( $r=0.001$ ,  $p=0.981$ ), WHtR ( $r=0.003$ ,  $p=0.949$ ), or HOMA-IR ( $r=-0.044$ ,  $p=0.575$ ).

**Parity** at the time of study pregnancy was considered as a dichotomous variable (primiparity or multiparity). One third (33.6%) of Generation 1 women had not given birth to a child prior to the study pregnancy. Women with previous births had a significantly higher mean BMI at the beginning of the current pregnancy (25.5 (5.87) kg/m<sup>2</sup>) compared to primiparous women (24.0 (4.86) kg/m<sup>2</sup>) ( $p=0.002$ ). There was no statistically significant difference in the likelihood of developing GD between primiparous (4.40%) and multiparous (6.52%) women ( $p=0.596$ ). Multiparous women gained significantly less weight throughout pregnancy (13.4 (4.64) kg) compared to women with no previous births (15.1 (4.12) kg) ( $p<0.001$ ). Parity was not associated with child BMI z-score ( $p=0.091$ ), %BF ( $p=0.149$ ), WHtR ( $p=0.843$ ), or HOMA-IR ( $p=0.316$ ).

**Maternal smoking** was reported at the early and late pregnancy interview. A binary variable was derived to indicate if the mother had reported any smoking during her pregnancy (yes or no). A total of 119 (21.4%) Generation 1 women reported smoking during pregnancy. Maternal smoking was not associated with pre-pregnancy BMI ( $p=0.730$ ), glucose tolerance status during pregnancy ( $p=0.141$ ), or GWG ( $p=0.561$ ). Similarly, maternal smoking during pregnancy was not associated with child BMI z-score ( $p=0.450$ ), %BF ( $p=0.972$ ), WHtR ( $p=0.409$ ), or HOMA-IR ( $p=0.222$ ).

**Pregnancy-induced hypertension** occurred in 63 women (11.3%) of the Generation 1 cohort. Women who developed this complication had a significantly higher mean (SD) pre-pregnancy BMI (27.6 (6.89) kg/m<sup>2</sup>) compared to those who did not (24.7 (5.33) kg/m<sup>2</sup>) ( $p<0.001$ ). Pregnancy-induced hypertension was not associated with glucose tolerance status during pregnancy ( $p=0.283$ ), or GWG ( $p=0.833$ ). Pregnancy-induced hypertension was not associated with child BMI z-score ( $p=0.828$ ), %BF ( $p=0.618$ ), WHtR ( $p=0.538$ ), or HOMA-IR ( $p=0.345$ ).

**Maternal education** was chosen as a surrogate marker for SES and was defined by the highest level of education completed by the mother at the time of pregnancy: partial high-school, high-school, TAFE or college, and university degree. One third of Generation 1 women did not complete high-school (33.8%), while half of them had undergone tertiary education, either TAFE/college (30.3%) or a university degree (18.3%) by the time of the study pregnancy. Maternal pre-pregnancy BMI was associated with the educational attainment ( $p=0.018$ ); women with a university degree had, on average, the lowest mean pre-pregnancy BMI (23.7 (4.09) kg/m<sup>2</sup>) while those with partial high-school had the highest mean BMI (25.7 (6.49) kg/m<sup>2</sup>). GD prevalence was double in women without high-school completed (8%) compared to those with a university degree (4%); however there was no significant overall association between maternal education and glucose tolerance status during pregnancy ( $p=0.607$ ). There was no significant association between GWG and maternal level of education ( $p=0.818$ ). BMI z-score and WHtR were significantly higher in children whose mothers had undergone TAFE or college by the time of pregnancy (mean (SD) BMI z-score 0.66 (1.23) and WHtR 0.48 (0.05)) compared to children of mothers who had not completed high-school (mean (SD) BMI z-score 0.25 (1.20),  $p=0.003$  and WHtR 0.47 (0.05),  $p=0.017$ ). Child %BF was overall associated with maternal education at the time of pregnancy ( $p=0.031$ ), but the relationship was not significant within each level of education. Child HOMA-IR was not associated with maternal education at the time of pregnancy ( $p=0.308$ ).

#### 4.1.4 Non-participation assessment

As with any other prospective longitudinal study, the Generation 1 cohort inevitably had a degree of non-participation. Although low (20%), non-participation could introduce attrition bias into the study and may affect the cohort representativeness. Therefore, baseline characteristics of three groups of subjects were compared: the original cohort ( $n=557$ ); those taking part in the 9-10 year follow-up ( $n=443$ ); and participants in the 9-10 year follow-up who provided a fasting blood sample ( $n=163$ ). Additionally, current anthropometric measures of children who provided a fasting blood sample ( $n=163$ ) were compared with those taking part in the 9-10 year follow-up but did not provide a fasting blood sample ( $n=280$ ). These comparisons were examined using independent samples t-tests (for continuous variables) and chi-squared tests (for categorical variables).

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**Maternal and child characteristics in the Generation 1 participants at 9-10 year relative to the original cohort**

The key baseline characteristics compared are listed in Table 24. Both the sample of participants at 9-10 years and the subgroup of participants who provided a fasting blood sample included mothers from across spectrum of glucose tolerance, weight and socioeconomic status (as defined by their education levels). Mothers and children participating in the 9-10 year follow-up were largely similar to the original Generation 1 cohort (Table 24). Mothers of children taking part in the study at 9-10 years were older ( $p=0.002$ ), were less likely to have smoked during pregnancy ( $p=0.001$ ), and overall had a higher level of education ( $p=0.017$ ) than those not participating. The subsample of participants at 9-10 years who provided a fasting blood sample was largely similar to the original cohort, except were marginally less likely to have been exposed to maternal smoking during pregnancy ( $p=0.075$ ).



**Table 24. Maternal and child characteristics in the Generation 1 participants at 9-10 year follow-up relative to the original cohort**

	Original Generation 1 cohort		Participants at 9-10 years		Participants at 9-10 years with fasting blood sample collected	
	n=557		n=443		n=163	
<b>Maternal characteristics at study pregnancy</b>	<b>Mean (SD)</b>	<b>n (%)</b>	<b>Mean (SD)</b>	<b>n (%)</b>	<b>Mean (SD)</b>	<b>n (%)</b>
Age (years)	29.8 (5.02)		30.2 (4.93)		30.3 (5.14)	
Glucose tolerance status						
Normal glucose tolerance		451 (81.0%)		354 (79.9%)		128 (78.5%)
Borderline gestational glucose intolerance		53 (9.52%)		41 (9.26%)		19 (11.7%)
Gestational diabetes		31 (5.57%)		30 (6.77%)		9 (5.52%)
Missing		22 (3.95%)		18 (4.06%)		7 (4.29%)
Pre-pregnancy weight (kg)	66.9 (15.0)		67.0 (15.4)		66.5 (16.0)	
Pre-pregnancy height (cm)	163.6 (6.16)		163.8 (6.12)		163.6 (5.43)	
Pre-pregnancy BMI (kg/m <sup>2</sup> )	25.0 (5.59)		25.0 (5.65)		24.9 (5.89)	
Gestational weight gain (kg)	14.0 (4.54)		13.7 (4.38)		13.7 (4.40)	
Parity						
Primiparous		187 (33.6%)		153 (34.5%)		47 (28.8%)
Multiparous		370 (66.4%)		290 (65.5%)		116 (70.2%)
Smoking during pregnancy						
No		438 (78.6%)		361 (81.5%)		136 (83.4%)
Yes		119 (21.4%)		82 (18.5%)		27 (16.6%)
Pregnancy-induced hypertension						
No		494 (88.7%)		395 (89.2%)		147 (90.2%)
Yes		63 (11.3%)		48 (10.8%)		16 (9.82%)
Education						
Partial high-school		188 (33.8%)		137 (30.9%)		52 (31.9%)
Complete high-school		98 (17.6%)		79 (17.8%)		29 (17.8%)
TAFE / college		169 (30.3%)		137 (30.9%)		47 (28.8%)
University degree		102 (18.3%)		90 (20.3%)		35 (21.5%)
<b>Child characteristics at birth</b>	<b>Mean (SD)</b>	<b>n (%)</b>	<b>Mean (SD)</b>	<b>n (%)</b>	<b>Mean (SD)</b>	<b>n (%)</b>
Weight (g)	3422.5 (530.9)		3421.4 (507.5)		3424.0 (518.2)	
Gestational age at birth (weeks)	39.4 (1.63)		39.4 (1.56)		39.4 (1.66)	
Sex						
Female		283 (50.8%)		221 (49.9%)		89 (54.6%)
Male		274 (49.2%)		222 (50.1%)		74 (45.4%)

### Child characteristics in the Generation 1 participants at 9-10 years who provided a fasting blood sample relative to all participating children at 9-10 years

The key child current characteristics compared are listed in Table 25. Generation 1 children taking part in the 9-10 year follow-up who provided a fasting blood sample were in general similar to those who did not, except they were slightly younger (mean (SD) age 9.58 (0.19) years versus 9.65 (0.31) years,  $p=0.003$ ). In summary, both the anthropometric measurements and the blood samples collected from the children at this follow-up could be considered reasonably representative of the South Australian population of 9-10 year old children.

**Table 25. Child characteristics in the Generation 1 participants at 9-10 years who provided a fasting blood sample collection relative to all participating children at 9-10 years**

	Participants at 9-10 years n=443	Participants at 9-10 years with fasting blood sample collected n=163	p*
Child characteristics at 9-10 years	Mean (SD)	Mean (SD)	
Age (years)	9.65 (0.31)	9.58 (0.19)	<b>0.0003</b>
BMI z-score	0.41 (1.17)	0.31 (1.10)	0.145
Percentage body fat	19.8 (7.87)	19.1 (7.22)	0.135
Waist-to-height ratio	0.47 (0.05)	0.47 (0.04)	0.127

\* p value for comparing participants with fasting blood sample collected at 9-10 years (n=163) to participants without fasting blood sample collected (with anthropometric measurements only, n=280)

## 4.2 Inferential statistics

This section presents the main findings from analyses exploring the long-term metabolic consequences for the child of three maternal factors likely to be associated with fetal overnutrition. The effects of maternal pre-pregnancy BMI on child obesity and insulin resistance in pre-pubertal years are detailed in Section 4.2.1, those of glucose intolerance during pregnancy in Section 4.2.2, and those of gestational weight gain in Section 4.2.3. Two-way interactions between these maternal factors in relation to child obesity and insulin resistance are presented in Section 4.2.4. The following potential confounders were investigated for each relationship of interest, using a step-wise approach: maternal age, parity, smoking during pregnancy, pregnancy-induced hypertension, and maternal educational attainment at the time of pregnancy (as an indicator of socio-economic status). Potential confounders with a  $p<0.1$  were considered potentially influential and retained in the model.

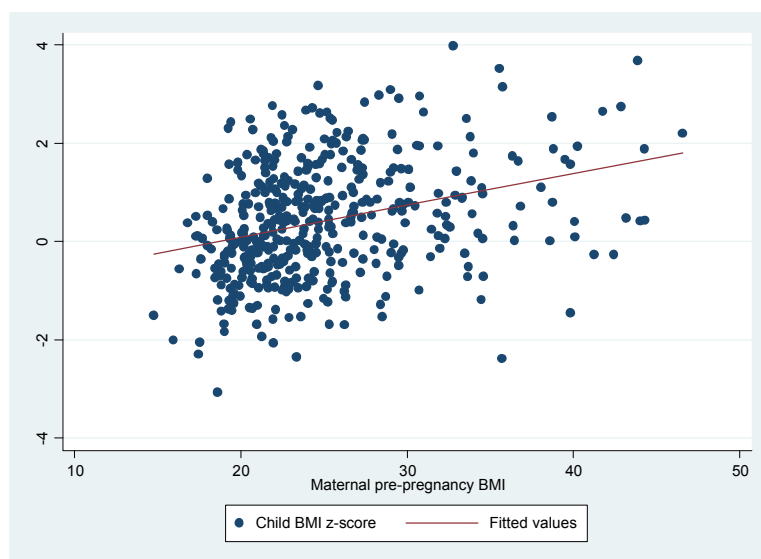
#### 4.2.1 Maternal pre-pregnancy BMI and child outcomes

A main focus of this research project was estimating the effect that maternal pre-pregnancy BMI (as a continuous variable) had on four child outcomes: child BMI z-score (calculated based on IOTF reference centiles), percentage body fat (as a continuous variable obtained from bioelectrical impedance analysis), fat pattern defined by waist-to-height ratio (as a continuous variable) and insulin resistance, defined by HOMA-IR (as a continuous variable). In addition to the above mentioned maternal factors (age, parity, smoking, pregnancy-induced hypertension, education), maternal glucose tolerance status during pregnancy and gestational weight gain were also considered as potential confounders.

##### 4.2.1.1 Maternal pre-pregnancy BMI and child BMI z-score

In bivariate analysis, a positive correlation was found between maternal pre-pregnancy BMI and child BMI z-score at 9-10 years (Pearson's correlation coefficient  $r=0.31$ ,  $p<0.001$ ); this relationship is graphically presented in Figure 15. There was no indication of a non-linear relationship.

**Figure 15. Child BMI z-score in relation to maternal pre-pregnancy BMI**



The progressive increase in child BMI and BMI z-score across categories of maternal pre-pregnancy BMI is also shown in Table 26.

**Table 26. Child BMI and BMI z-score across categories of maternal pre-pregnancy BMI**

Pre-pregnancy BMI categories	n	Mean (SD) child BMI (kg/m <sup>2</sup> )	Mean (SD) child BMI z-score	Normal weight children n (%)	Overweight children n (%)	Obese children n (%)
Underweight	18	16.0 (1.66)	-0.40 (0.96)	17 (94.4)	1 (5.56)	0
Normal weight	259	17.3 (2.63)	0.10 (0.96)	217 (83.8)	33 (12.7)	9 (3.47)
Overweight	96	18.6 (3.08)	0.56 (0.99)	62 (64.6)	27 (28.1)	7 (7.29)
Obese	70	19.6 (4.37)	0.73 (1.02)	45 (64.3)	15 (21.4)	10 (14.3)
Total	443	17.9 (3.17)	0.28 (1.02)	341 (77.0)	76 (17.2)	26 (5.87)

The relationship between maternal pre-pregnancy BMI and child BMI z-score was assessed using multiple linear regression models. Assumptions of linear ordinary least squares models were met. Child BMI z-score was positively associated with maternal pre-pregnancy BMI (Model 1, Table 27). This relationship was robust to adjustment for various potential confounders (Models 2-8, Table 27). After adjustment for GWG ( $p=0.018$ ), pregnancy-induced hypertension ( $p=0.046$ ) and maternal education ( $p=0.007$ ), for each one kg/m<sup>2</sup> increase in pre-pregnancy BMI, the child BMI z-score increased by 0.08 ( $p<0.001$ ) (Final model, Table 27).

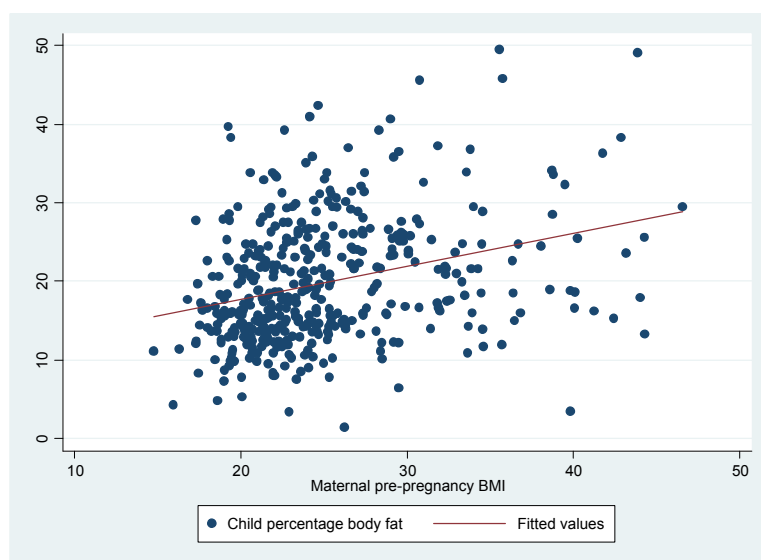
**Table 27. Estimated change in child BMI z-score in relation to maternal pre-pregnancy BMI (n=443)**

Model number	Model description	BMI z-score change for one kg/m <sup>2</sup> increase in pre-pregnancy BMI (95% CI)
Model 1	Unadjusted	<b>0.06 (0.05, 0.08)</b>
Model 2	M1 + glucose tolerance during pregnancy	<b>0.07 (0.05, 0.09)</b>
Model 3	M1 + gestational weight gain <sup>†</sup>	<b>0.07 (0.05, 0.09)</b>
Model 4	M3 + maternal age at the time of pregnancy	<b>0.07 (0.05, 0.09)</b>
Model 5	M3 + parity	<b>0.07 (0.05, 0.09)</b>
Model 6	M3 + maternal smoking during pregnancy	<b>0.07 (0.05, 0.09)</b>
Model 7	M3 + pregnancy-induced hypertension <sup>†</sup>	<b>0.07 (0.05, 0.09)</b>
Model 8	M7 + maternal education at the time of pregnancy <sup>†</sup>	<b>0.08 (0.06, 0.10)</b>
Final model	Pre-pregnancy BMI + gestational weight gain + pregnancy-induced hypertension + education (M8)	<b>0.08 (0.06, 0.10)</b>

<sup>†</sup>  $p<0.1$  for potential confounder, hence retained in the model

#### 4.2.1.2 Maternal pre-pregnancy BMI and child percentage body fat

Similar to child BMI z-score, child %BF increased with maternal pre-pregnancy BMI (Pearson's correlation coefficient  $r=0.30$ ,  $p<0.001$ ) (Figure 16). There was no indication of a non-linear relationship.

**Figure 16. Child percentage body fat in relation to maternal pre-pregnancy BMI**

As evident from the graph and further described in Table 28, children of obese mothers had the highest %BF.

**Table 28. Child percentage body fat across categories of maternal pre-pregnancy BMI**

Pre-pregnancy BMI categories	n	Mean (SD) percentage body fat
Underweight	18	15.4 (5.48)
Normal weight	257	18.3 (7.03)
Overweight	95	22.1 (7.80)
Obese	69	23.5 (9.25)
Total	439	19.8 (7.87)

The association between maternal pre-pregnancy BMI and child %BF was assessed using linear regression models. Similar to the child BMI z-score, %BF was positively associated with maternal pre-pregnancy BMI (Model 1, Table 29). This relationship remained statistically significant after controlling for potential confounders (Models 2-8, Table 29). After adjusting for GWG ( $p=0.060$ ) and maternal education ( $p=0.100$ ), for each one  $\text{kg}/\text{m}^2$  increase in pre-pregnancy BMI, child %BF increased by 0.44 ( $p<0.001$ ).

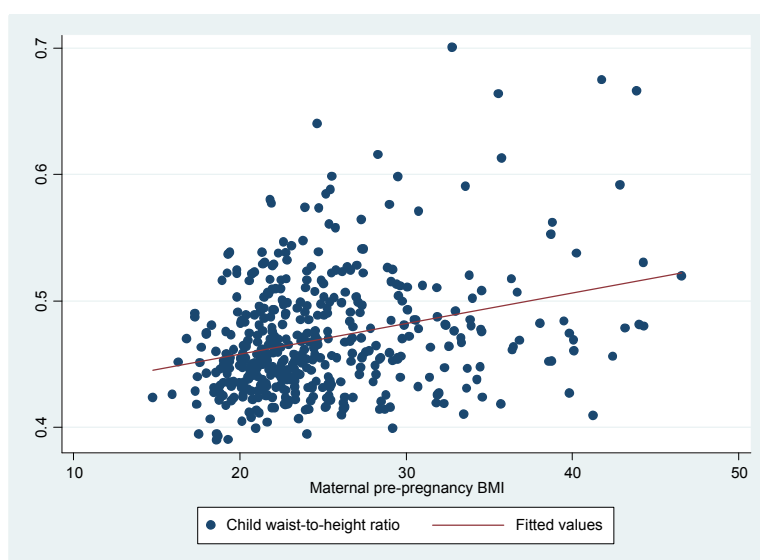
**Table 29. Estimated change in child percentage body fat in relation to maternal pre-pregnancy BMI (n=439)**

Model number	Model description	Percent body fat change for one kg/m <sup>2</sup> increase in pre-pregnancy BMI (95% CI)
Model 1	Unadjusted	0.42 (0.30, 0.55)
Model 2	M1 + glucose tolerance during pregnancy	0.45 (0.31, 0.58)
Model 3	M1 + gestational weight gain <sup>†</sup>	0.45 (0.31, 0.58)
Model 4	M3 + maternal age at the time of pregnancy	0.45 (0.31, 0.59)
Model 5	M3 + parity	0.45 (0.31, 0.58)
Model 6	M3 + maternal smoking during pregnancy	0.45 (0.31, 0.58)
Model 7	M3 + pregnancy-induced hypertension	0.46 (0.32, 0.60)
Model 8	M3 + maternal education at the time of pregnancy <sup>†</sup>	0.44 (0.31, 0.58)
Final model	Pre-pregnancy BMI + gestational weight gain + education (M8)	0.44 (0.31, 0.58)

<sup>†</sup>p<0.1 for potential confounder, hence retained in the model

#### 4.2.1.3 Maternal pre-pregnancy BMI and child waist-to-height ratio

There was an increasing trend of child WHtR with pre-pregnancy BMI (correlation coefficient r=0.29, p<0.001) (Figure 17) and across categories of maternal pre-pregnancy BMI (Table 30).

**Figure 17. Child waist-to-height ratio in relation to maternal pre-pregnancy BMI**

**Table 30. Child waist-to-height ratio across categories of maternal pre-pregnancy BMI**

Pre-pregnancy BMI categories	n	Mean (SD) waist-to-height ratio
Underweight	18	0.45 (0.03)
Normal weight	258	0.46 (0.04)
Overweight	96	0.48 (0.05)
Obese	70	0.50 (0.06)
Total	442	0.47 (0.05)

The relationship between maternal pre-pregnancy BMI and child WHtR was examined by employing linear regression analysis. There was a positive association between maternal pre-pregnancy BMI and child WHtR (Model 1, Table 31), which remained statistically significant after adjustment for potential confounders (Models 2-8, Table 31). Of the potential confounders considered, pregnancy-induced hypertension and maternal education at the time of pregnancy were considered influential and retained in the model ( $p < 0.1$ ).

**Table 31. Estimated change in child waist-to-height ratio in relation to maternal pre-pregnancy BMI (n=442)**

Model number	Model description	WHtR change for one kg/m <sup>2</sup> increase in pre-pregnancy BMI (95% CI)
Model 1	Unadjusted	<b>0.002 (0.002, 0.003)</b>
Model 2	M1 + glucose tolerance during pregnancy	<b>0.003 (0.002, 0.004)</b>
Model 3	M1 + gestational weight gain	<b>0.002 (0.002, 0.003)</b>
Model 4	M1 + maternal age at the time of pregnancy	<b>0.002 (0.002, 0.003)</b>
Model 5	M1 + parity	<b>0.002 (0.002, 0.003)</b>
Model 6	M1 + maternal smoking during pregnancy	<b>0.002 (0.002, 0.003)</b>
Model 7	M1 + pregnancy-induced hypertension <sup>†</sup>	<b>0.003 (0.002, 0.003)</b>
Model 8	M7 + maternal education at the time of pregnancy <sup>†</sup>	<b>0.002 (0.002, 0.003)</b>
Final model	Pre-pregnancy BMI + pregnancy-induced hypertension + education (M8)	<b>0.002 (0.002, 0.003)</b>

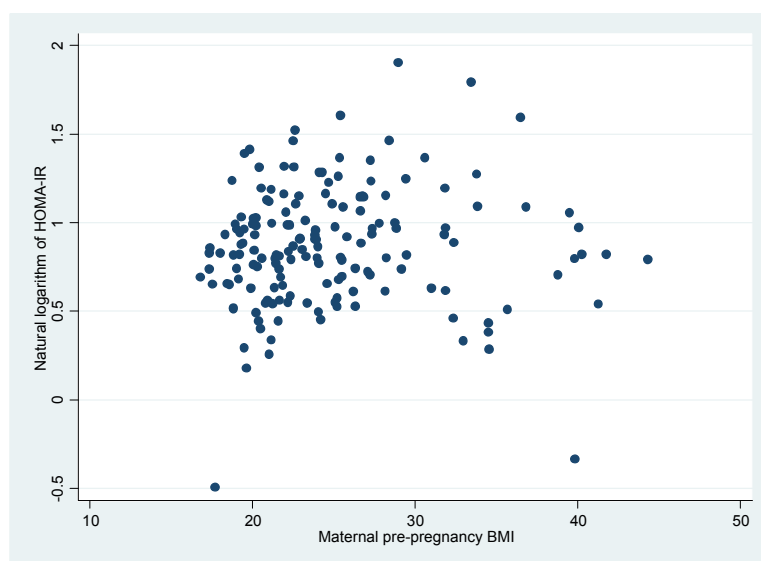
<sup>†</sup>  $p < 0.1$  for potential confounder, hence retained in the model

In summary, maternal pre-pregnancy BMI was positively associated with child BMI z-score, %BF and WHtR, and all these relationships were robust to adjustment for potential confounders.

#### 4.2.1.4 Maternal pre-pregnancy BMI and child insulin resistance

As described in Section 4.1.2.4, the distribution of HOMA-IR was positively skewed and the best approximation to a normal distribution was provided by natural logarithm of HOMA-IR. No correlation was observed between maternal pre-pregnancy BMI and natural logarithm of HOMA-IR in the child (correlation coefficient  $r=0.04$ ,  $p=0.608$ ) (Figure 18). As shown in Table 32, the mean value of HOMA-IR appeared highest among children of overweight, rather than obese, mothers.

**Figure 18. Natural logarithm of child HOMA-IR in relation to maternal pre-pregnancy BMI**



**Table 32. Child HOMA-IR across categories of maternal pre-pregnancy BMI**

Pre-pregnancy BMI categories	n	Mean (SD) HOMA-IR	Mean (SD) natural logarithm of HOMA-IR
Underweight	9	2.00 (0.56)	0.63 (0.43)
Normal weight	89	2.46 (0.72)	0.86 (0.29)
Overweight	38	2.81 (1.05)	0.97 (0.32)
Obese	27	2.49 (1.12)	0.82 (0.44)
Total	163	2.51 (0.88)	0.87 (0.34)

As child HOMA-IR was not normally distributed, in preference to applying a log-transformation, generalised linear models with log link function were applied in order to examine the relationship between pre-pregnancy BMI and child HOMA-IR. Results are presented as 'back transformed' percentage differences in HOMA-IR for one kg/m<sup>2</sup> increase in pre-pregnancy BMI. No association was found between maternal pre-pregnancy BMI and child HOMA-IR in the unadjusted model (Model 1, Table 33) or after adjustment for potential confounders (Models 2-8, Table 33). Maternal glucose



tolerance status during pregnancy and education were included in the final model, as both met the criterion for retention ( $p < 0.1$ ).

**Table 33. Estimated change in child HOMA-IR in relation to maternal pre-pregnancy BMI (n=163)**

Model number	Model description	% difference in HOMA-IR for one kg/m <sup>2</sup> increase in pre-pregnancy BMI (95% CI)
Model 1	Unadjusted	0.42 (-0.43, 1.28)
Model 2	M1 + glucose tolerance during pregnancy <sup>†</sup>	0.28 (-0.57, 1.14)
Model 3	M2 + gestational weight gain	0.28 (-0.62, 1.18)
Model 4	M2 + maternal age at the time of pregnancy	0.27 (-0.58, 1.12)
Model 5	M2 + parity	0.27 (-0.57, 1.13)
Model 6	M2 + maternal smoking during pregnancy	0.25 (-0.59, 1.11)
Model 7	M2 + pregnancy-induced hypertension	0.39 (-0.48, 1.26)
Model 8	M2 + maternal education at the time of pregnancy <sup>†</sup>	0.18 (-0.70, 1.04)
Final model	Pre-pregnancy BMI + glucose tolerance during pregnancy + education (M8)	0.18 (-0.70, 1.04)

<sup>†</sup>  $p < 0.1$  for potential confounder, hence retained in the model

Investigating whether any relationship between maternal pre-pregnancy BMI and child HOMA-IR was independent of child current body size was deemed important at the outset. Therefore, although no association was found between maternal pre-pregnancy BMI and child HOMA-IR (before and after adjustment for potential confounders), child current BMI z-score was considered as a potential mediating factor on the pathway between the early origin factor (pre-pregnancy BMI) and the later outcome (child insulin resistance). When child BMI z-score was added to the final model, there was a negative relationship between pre-pregnancy BMI and child HOMA-IR, so that for each one kg/m<sup>2</sup> increase in pre-pregnancy BMI, HOMA-IR was reduced by 0.83% (95% CI -1.63, -0.02,  $p = 0.044$ ) for a given BMI z-score.

As suggested by Lucas *et al.* (1999), the relationship between the outcome of interest and the 'early origin factor' requires further examination in context with 'later body size'. Therefore, an interaction was explored between maternal pre-pregnancy BMI and child BMI z-score in relation to child HOMA-IR. The effect modification of child current BMI z-score on maternal pre-pregnancy BMI in relation to child HOMA-IR was not statistically significant in the fully adjusted model (% difference in child HOMA-IR for the interaction term = -0.32, 95% CI -1.00, 0.37). This indicates that the negative

relationship between maternal pre-pregnancy BMI and child HOMA-IR after adjustment for child BMI z-score was consistent across child BMI z-score.

In summary, child HOMA-IR was not significantly associated with maternal pre-pregnancy BMI or exposure to other prenatal factors known to influence fetal development and metabolism. However, after controlling for child current BMI z-score, a variable likely to be a mediator on the causal pathway, maternal pre-pregnancy BMI was inversely associated with child HOMA-IR.

#### 4.2.2 Maternal glucose tolerance status during pregnancy and child outcomes

Maternal glucose intolerance during pregnancy was the second exposure of interest for this project. As described in Section 3.4.1, maternal glucose tolerance status during pregnancy was defined as a categorical, ordinal variable. The three obesity-related outcomes and insulin resistance in children were examined as potential long-term consequences of intrauterine exposure to maternal glucose intolerance. For each association examined, both unadjusted and adjusted models were run. The same set of potential confounders was examined. Potential confounders with a  $p < 0.1$  were considered potentially influential and were retained in the regression model.

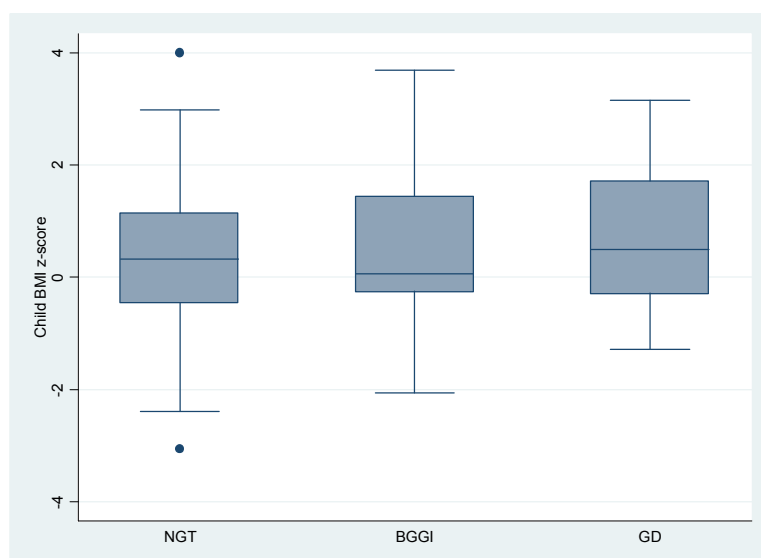
##### 4.2.2.1 Maternal glucose tolerance status during pregnancy and child BMI z-score

Inspecting the data, it appeared that mean BMI z-score in children increased with the level of glucose intolerance during pregnancy, being highest among children whose mothers had GD (Table 34 and Figure 19). However, the differences among groups were not statistically significant.

**Table 34. Child BMI and BMI z-score across the categories of maternal glucose tolerance during pregnancy**

Glucose tolerance profiles in pregnancy	n	Mean (SD) child BMI (kg/m <sup>2</sup> )	Mean (SD) child BMI z-score	Normal weight n (%)	Overweight n (%)	Obese n (%)
NGT	354	17.8 (2.94)	0.24 (1.00)	276 (78.0)	62 (17.5)	16 (4.52)
BGGI	41	18.6 (4.63)	0.32 (1.22)	31 (75.6)	3 (7.32)	7 (17.0)
GD	30	18.7 (3.58)	0.53 (0.98)	22 (73.3)	5 (16.7)	3 (10.0)
Total	425	17.9 (3.20)	0.41 (1.18)	329 (77.4)	70 (16.5)	26 (6.12)

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

**Figure 19. Child BMI z-score across categories of maternal glucose tolerance during pregnancy**

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

The association between maternal glucose tolerance status during pregnancy and BMI z-score in childhood was assessed by linear regression. Assumptions of linear ordinary least squares models were met. Simple linear regression was followed by multiple linear regression to include potential confounders.

The unadjusted mean BMI z-score of children exposed to either GD or BGGI *in utero* was not significantly different to the BMI z-score of children whose mothers had normal glucose tolerance during pregnancy (Model 1, Table 35). Of the potential confounders examined, maternal pre-pregnancy BMI, GWG, pregnancy-induced hypertension and maternal education were retained in the model ( $p < 0.001$ ,  $p = 0.052$ ,  $p = 0.046$  and  $p = 0.006$ , respectively), but their inclusion in the model did not alter the relationship between maternal glucose tolerance status during pregnancy and child BMI z-score at the age of 9-10 years (Models 2, 3, 7 and 8 Table 35).

In summary, glucose tolerance status during pregnancy was not associated with child BMI z-score at the age of 9-10 years, in unadjusted or fully adjusted models.

**Table 35. Estimated change in child BMI z-score in relation to maternal glucose tolerance status during pregnancy**

Model number	Model description	NGT (reference group) n=354	BGGI n=41	GD n=30
		Adjusted mean BMI z-score (95% CI)	BMI z-score change compared to the reference group (95% CI)	BMI z-score change compared to the reference group (95% CI)
Model 1	Unadjusted	0.37 (0.25, 0.49)	0.13 (-0.25, 0.51)	0.33 (-0.11, 0.77)
Model 2	M1 + pre-pregnancy BMI <sup>†</sup>	0.38 (0.26, 0.50)	-0.06 (-0.43, 0.30)	-0.01 (-0.44, 0.41)
Model 3	M2 + gestational weight gain <sup>†</sup>	0.40 (0.28, 0.51)	-0.11 (-0.49, 0.27)	-0.03 (-0.45, 0.40)
Model 4	M3 + maternal age at the time of pregnancy	0.40 (0.28, 0.51)	-0.12 (-0.50, 0.26)	-0.04 (-0.47, 0.39)
Model 5	M3 + parity	0.40 (0.28, 0.52)	-0.09 (-0.47, 0.29)	-0.04 (-0.46, 0.39)
Model 6	M3 + maternal smoking during pregnancy	0.39 (0.27, 0.51)	-0.10 (-0.48, 0.28)	-0.02 (-0.44, 0.41)
Model 7	M3 + pregnancy-induced hypertension <sup>†</sup>	0.39 (0.28, 0.51)	-0.08 (-0.46, 0.30)	-0.02 (-0.44, 0.41)
Model 8	M7 + maternal education at the time of pregnancy <sup>†</sup>	0.39 (0.27, 0.50)	-0.06 (-0.44, 0.32)	0.02 (-0.40, 0.44)
Final model	Gestational glucose tolerance status + pre-pregnancy BMI + gestational weight gain + pregnancy-induced hypertension + education (M8)	0.39 (0.27, 0.50)	-0.06 (-0.44, 0.32)	0.02 (-0.40, 0.44)

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance, <sup>†</sup> p<0.1 for potential confounder, hence retained in the model

#### 4.2.2.2 Maternal glucose tolerance status during pregnancy and child percentage body fat

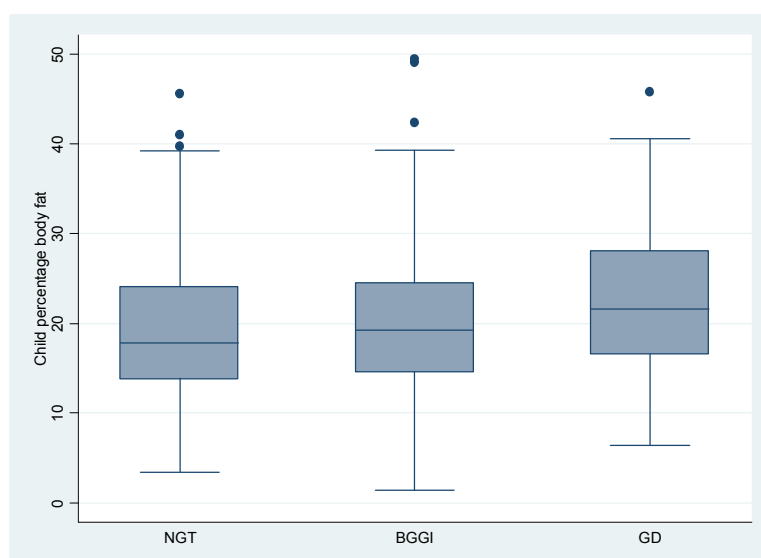
Inspection of data suggested that mean %BF in children increased with the degree of maternal glucose tolerance impairment during pregnancy (Table 36 and Figure 20) but the differences among groups were not statistically significant.

**Table 36. Child percentage body fat across the categories of maternal glucose tolerance during pregnancy**

Glucose tolerance profiles in pregnancy	n	Mean (SD) percentage body fat
NGT	350	19.3 (7.36)
BGGI	41	21.0 (10.7)
GD	30	23.1 (8.79)
Total	421	19.8 (7.90)

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

**Figure 20. Child percentage body fat across the categories of maternal glucose tolerance during pregnancy**



BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

The effect of glucose tolerance status during pregnancy on child %BF at 9-10 years was analysed based on linear ordinary least squares, both simple and multiple regression models. The unadjusted mean %BF was 3.78 percentage points greater in children whose mothers developed GD while they were *in utero* compared to children of mothers with normal glucose tolerance during pregnancy ( $p=0.012$ ) (Model 1, Table 37). The %BF in children of mothers with BGGI was not significantly different relative to the reference group (Model 1, Table 37). After adjustment for maternal pre-

pregnancy BMI the association between exposure to GD and increased %BF in children was no longer statistically significant (Model 2, Table 37). Controlling for GWG in addition to pre-pregnancy BMI did not make any further difference (Model 3, Table 37). Of the other potential confounders considered, maternal level of education met the criterion for retention ( $p=0.073$ ); however, this adjustment did not modify the effect estimates (Model 8, Table 37).

In summary, an association between exposure to maternal GD and increased %BF in children was found, but it was attenuated to the null after adjustment for maternal pre-pregnancy BMI.

**Table 37. Estimated change in child percentage body fat in relation to maternal glucose tolerance status during pregnancy**

Model number	Model description	NGT (reference group) n=350	BGGI n=41	GD n=30
		Adjusted mean % body fat (95% CI)	% body fat change compared to the reference group (95% CI)	% body fat change compared to the reference group (95% CI)
Model 1	Unadjusted	19.3 (18.5, 20.1)	1.73 (-0.82, 4.27)	<b>3.78 (0.84, 6.71)</b>
Model 2	M1+ pre-pregnancy BMI <sup>†</sup>	19.4 (18.6, 20.2)	0.48 (-1.97, 2.93)	1.64 (-1.23, 4.50)
Model 3	M2 + gestational weight gain	19.5 (18.7, 20.3)	0.05 (-2.50, 2.60)	1.62 (-1.24, 4.49)
Model 4	M2 + maternal age at the time of pregnancy	19.4 (18.6, 20.2)	0.50 (-1.96, 2.97)	1.67 (-1.21, 4.56)
Model 5	M2 + parity	19.4 (18.6, 20.2)	0.55 (-1.91, 3.01)	1.62 (-1.25, 4.49)
Model 6	M2 + maternal smoking during pregnancy	19.4 (18.6, 20.2)	0.49 (-1.97, 2.95)	1.65 (-1.23, 4.53)
Model 7	M2 + pregnancy-induced hypertension	19.4 (18.6, 20.2)	0.54 (-1.92, 3.00)	1.66 (-1.21, 4.53)
Model 8	M2+ maternal education at the time of pregnancy <sup>†</sup>	19.4 (18.6, 20.2)	0.56 (-1.89, 3.00)	1.73 (-1.13, 4.59)
Final model	Gestational glucose tolerance status + pre-pregnancy BMI + education (M8)	19.4 (18.6, 20.2)	0.56 (-1.89, 3.00)	1.73 (-1.13, 4.59)

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance, <sup>†</sup> p<0.1 for potential confounder, hence retained in the model

#### 4.2.2.3 Maternal glucose tolerance status during pregnancy and child waist-to-height ratio

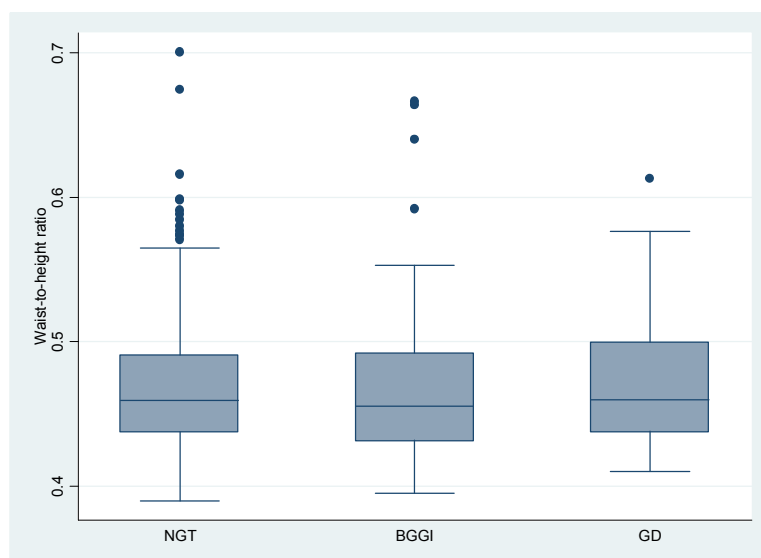
No difference in mean WHtR of the children was observed among the three categories of maternal glucose tolerance during pregnancy (Table 38 and Figure 21).

**Table 38. Child waist-to-height ratio across categories of maternal glucose tolerance during pregnancy**

Glucose tolerance profiles in pregnancy	n	Mean (SD) WHtR
NGT	353	0.469 (0.044)
BGGI	41	0.477 (0.067)
GD	30	0.471 (0.050)
Total	424	0.470 (0.047)

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

**Figure 21. Child waist-to-height ratio across categories of maternal glucose tolerance during pregnancy**



BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

The relationship between categories of maternal glucose tolerance during pregnancy and child WHtR was examined using linear regression modelling. No association was found between categories of maternal glucose tolerance during pregnancy and WHtR in the unadjusted (Model 1, Table 39) or adjusted models (Models 2-8, Table 39). Of the potential confounders considered, maternal pre-pregnancy BMI, pregnancy-induced hypertension and maternal educational level were retained in the final model ( $p < 0.1$ ).



**Table 39. Estimated change in child waist-to-height ratio in relation to maternal glucose tolerance status during pregnancy**

Model number	Model description	NGT (reference group) n=353	BGGI n=41	GD n=30
		Adjusted mean WHtR (95% CI)	WHtR change compared to the reference group (95% CI)	WHtR change compared to the reference group (95% CI)
Model 1	Unadjusted	0.47 (0.46, 0.47)	0.008 (-0.007, 0.024)	0.003 (-0.015, 0.021)
Model 2	M1+ pre-pregnancy BMI <sup>†</sup>	0.47 (0.46, 0.47)	0.001 (-0.014, 0.015)	-0.010 (-0.027, 0.007)
Model 3	M2 + gestational weight gain	0.47 (0.46, 0.47)	-0.002 (-0.018, 0.013)	-0.010 (-0.027, 0.007)
Model 4	M2 + maternal age at the time of pregnancy	0.47 (0.46, 0.47)	0.001 (-0.014, 0.015)	-0.010 (-0.027, 0.007)
Model 5	M2 + parity	0.47 (0.46, 0.47)	0.000 (-0.014, 0.015)	-0.010 (-0.027, 0.007)
Model 6	M2 + maternal smoking during pregnancy	0.47 (0.46, 0.47)	0.001 (-0.014, 0.016)	-0.010 (-0.027, 0.008)
Model 7	M2 + pregnancy-induced hypertension <sup>†</sup>	0.47 (0.46, 0.47)	0.001 (-0.013, 0.016)	-0.010 (-0.027, 0.007)
Model 8	M7+ maternal education at the time of pregnancy <sup>†</sup>	0.47 (0.46, 0.47)	0.003 (-0.012, 0.017)	-0.009 (-0.026, 0.008)
Final model	Gestational glucose tolerance status + pre-pregnancy BMI + pregnancy-induced hypertension + education (M8)	0.47 (0.46, 0.47)	0.003 (-0.012, 0.017)	-0.009 (-0.026, 0.008)

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance, <sup>†</sup> p<0.1 for potential confounder, hence retained in the model

#### 4.2.2.4 Maternal glucose tolerance status during pregnancy and child insulin resistance

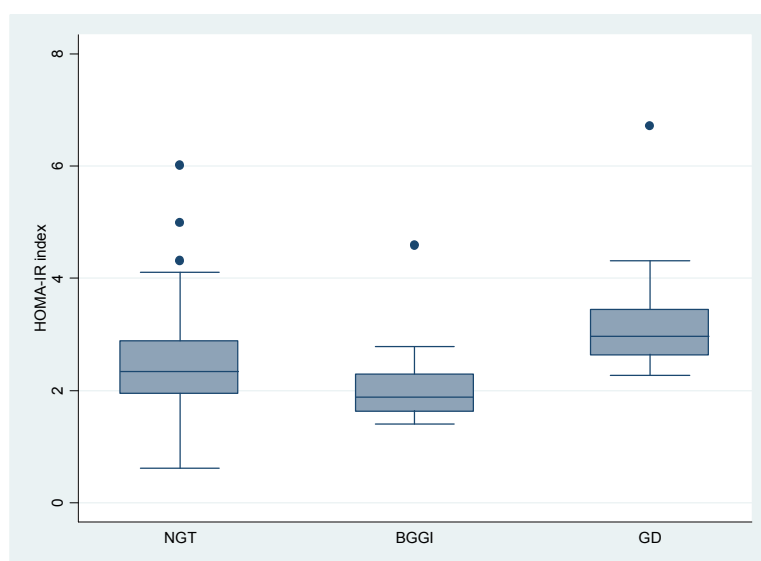
Children of GD mothers appeared to have the highest mean HOMA-IR, while the mean HOMA-IR of children whose mothers developed BGGI appeared to be lower compared to those of mothers who maintained normal glucose tolerance during pregnancy (Table 40 and Figure 22).

**Table 40. Child HOMA-IR across categories of maternal glucose tolerance during pregnancy**

Glucose tolerance profiles in pregnancy	n	Mean (SD) HOMA-IR
NGT	128	2.47 (0.80)
BGGI	19	2.11 (0.72)
GD	9	3.42 (1.38)
Total	156	2.48 (0.87)

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

**Figure 22. Child HOMA-IR across categories of maternal glucose tolerance during pregnancy**



BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance

The association between glucose tolerance during pregnancy and child HOMA-IR was assessed using a generalised linear model with log link function and Gaussian family. Data are presented as 'back transformed' means for the reference group and percentage differences for BGGI and GD.

Overall, glucose tolerance during pregnancy was associated with child HOMA-IR ( $p < 0.001$ ). In models unadjusted for confounders, mean HOMA-IR in children exposed to GD was 38.5% greater ( $p < 0.001$ ) than in children whose mothers maintained normal glucose tolerance during pregnancy

(Model 1, Table 41). The unadjusted mean HOMA-IR in offspring of women with BGGI was not significantly different to the reference group (Model 1, Table 41). Of the potential confounders investigated, maternal education met the criterion for retention ( $p=0.076$ ), but adjustment for this variable did not result in appreciable changes in the estimates of the association between glucose tolerance status during pregnancy and HOMA-IR for children (Model 8, Table 41).

In addition to the potential confounders, the child current BMI z-score was assessed as a potential mediating factor on the pathway linking maternal glucose tolerance status during pregnancy and child HOMA-IR. The addition of child BMI z-score ( $p<0.001$ ) to the final model slightly attenuated the relationship between exposure to GD and HOMA-IR, but it remained statistically significant (39.8%, 95% CI 21.9, 60.4). In the model including confounding and mediating variables, children exposed to BGGI *in utero* had significantly lower HOMA-IR compared to children of mothers with normal glucose tolerance during pregnancy (-17.9%, 95% CI -29.9, -3.96). Following the recommendations of Lucas *et al.* (1999), an assessment of whether the effect of exposure to maternal glucose intolerance during pregnancy on child HOMA-IR was different according to child BMI z-score was undertaken. A significant positive interaction was identified between GD (but not BGGI) and child current BMI z-score in relation to HOMA-IR (12.7%, 95% CI 1.55, 15.0).

In summary, *in utero* exposure to GD was associated with a significantly higher HOMA-IR in children at the age of 9-10 years compared to offspring whose mothers maintained NGT. This relationship was robust to adjustment for potential confounders and the potential mediating effect of child current BMI z-score. In addition to the independent effect of GD on child HOMA-IR, GD had a significant synergistic effect with child BMI z-score on HOMA-IR. Exposure to BGGI was not associated with child HOMA-IR in unadjusted models or after adjustment for potential confounders, but had a small negative effect on child HOMA-IR after including child current BMI z-score in the model, which was consistent across the range of child current BMI z-score.

**Table 41. Estimated change in child HOMA-IR in relation to maternal glucose tolerance status during pregnancy**

Model number	Model description	NGT (reference group) n=128	BGGI n=19	GD n=9
		Adjusted mean HOMA-IR (95% CI)	% difference in HOMA-IR compared to the reference group (95% CI)	% difference in HOMA-IR compared to the reference group (95% CI)
Model 1	Unadjusted	2.47 (2.33, 2.62)	-14.5 (-29.0, 3.00)	<b>38.5 (16.9, 64.0)</b>
Model 2	M1+ pre-pregnancy BMI	2.47 (2.33, 2.62)	-15.2 (-29.8, 2.42)	<b>37.2 (15.6, 62.9)</b>
Model 3	M1 + gestational weight gain	2.48 (2.34, 2.64)	-14.3 (-29.7, 4.13)	<b>37.8 (16.1, 63.6)</b>
Model 4	M1 + maternal age at the time of pregnancy	2.48 (2.34, 2.63)	-14.3 (-28.9, 3.27)	<b>40.4 (18.2, 66.8)</b>
Model 5	M1 + parity	2.47 (2.32, 2.62)	-13.7 (-28.5, 4.12)	<b>37.6 (16.1, 63.2)</b>
Model 6	M1 + maternal smoking during pregnancy	2.49 (2.34, 2.63)	-15.6 (-29.9, 1.68)	<b>38.5 (17.0, 63.9)</b>
Model 7	M1 + pregnancy-induced hypertension	2.47 (2.32, 2.62)	-13.1 (-28.1, 5.05)	<b>39.1 (17.5, 64.8)</b>
Model 8	M1+ maternal education at the time of pregnancy <sup>†</sup>	2.46 (2.32, 2.60)	-14.8 (-29.1, 2.46)	<b>42.9 (20.9, 68.9)</b>
Final model	Gestational glucose tolerance status + education (M8)	2.46 (2.32, 2.60)	-14.8 (-29.1, 2.46)	<b>42.9 (20.9, 68.9)</b>

BGGI - borderline gestational glucose intolerance, GD - gestational diabetes, NGT - normal glucose tolerance, <sup>†</sup> p<0.1 for potential confounder, hence retained in the model

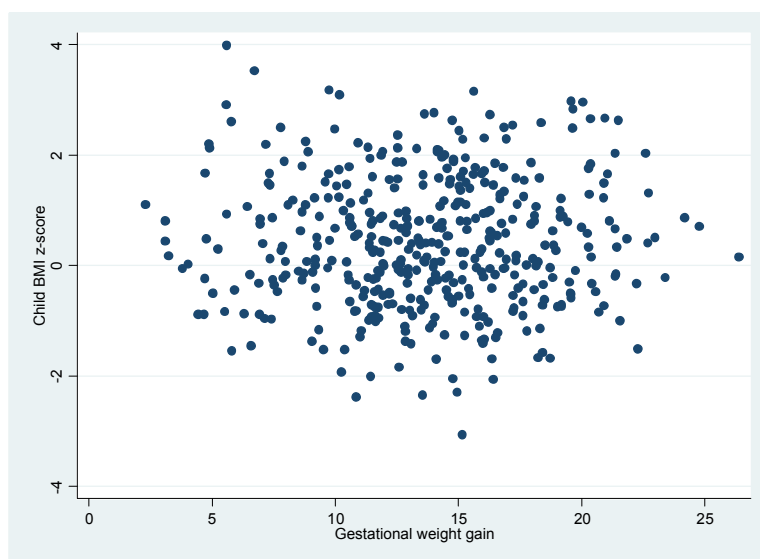
### 4.2.3 Maternal gestational weight gain and child outcomes

In this section, the focus was on investigating the relationships between maternal GWG and child BMI z-score, percentage body fat, waist-to-height ratio and HOMA-IR in pre-pubertal years. Following the unadjusted model for each relationship of interest, models were constructed with consecutive adjustments for potential confounders.

#### 4.2.3.1 Maternal gestational weight gain and child BMI z-score

Overall, no correlation was found between maternal GWG and child BMI z-score (correlation coefficient  $r=0.01$ ,  $p=0.863$ ), as presented in Figure 23.

**Figure 23. Child BMI z-score in relation to maternal gestational weight gain**



In order to further assess the relationship between maternal GWG and child BMI z-score, linear regression models were applied, including adjustments for potential confounders. There was no association between GWG and child BMI z-score in the unadjusted model (Model 1, Table 42). Adjustment for maternal pre-pregnancy BMI led to a statistically significant positive relationship between GWG and child BMI z-score (Model 3, Table 42). After additional adjustments for pregnancy-induced hypertension and maternal education (the potential confounders that met criterion for retention,  $p<0.1$ ), child BMI z-score increased by 0.032 for each one kg increase in gestational weight gain (Final model, Table 42).

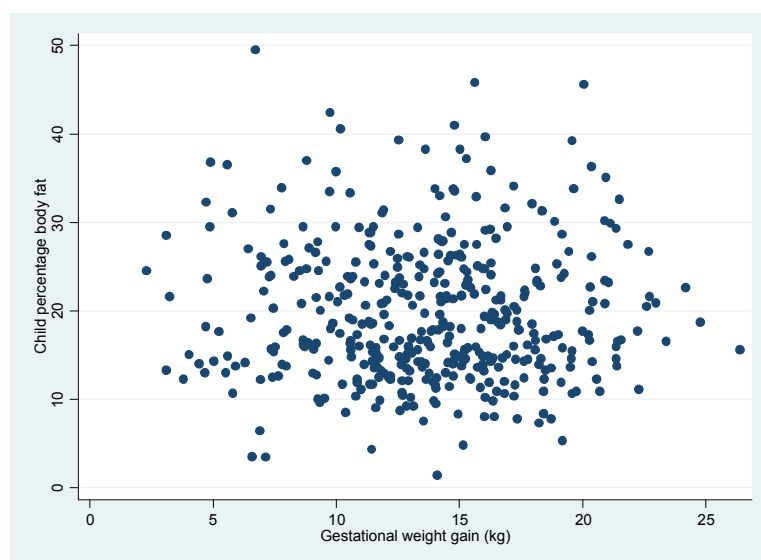
**Table 42. Estimated change in child BMI z-score in relation to maternal gestational weight gain (n=430)**

<b>Model number</b>	<b>Model description</b>	<b>BMI z-score change for one kg increase in GWG (95% CI)</b>
Model 1	Unadjusted	0.002 (-0.023, 0.028)
Model 2	M1 + glucose tolerance during pregnancy	-0.001 (-0.027, 0.026)
Model 3	M1 + pre-pregnancy BMI <sup>†</sup>	<b>0.030 (0.004, 0.055)</b>
Model 4	M3 + maternal age at the time of pregnancy	<b>0.031 (0.005, 0.056)</b>
Model 5	M3 + parity	<b>0.033 (0.007, 0.058)</b>
Model 6	M3 + maternal smoking during pregnancy	<b>0.029 (0.004, 0.055)</b>
Model 7	M3 + pregnancy-induced hypertension <sup>†</sup>	<b>0.032 (0.007, 0.057)</b>
Model 8	M7 + maternal education at the time of pregnancy <sup>†</sup>	<b>0.032 (0.007, 0.057)</b>
Final model	Gestational weight gain + pre-pregnancy BMI + pregnancy induced hypertension + education (M8)	<b>0.032 (0.007, 0.057)</b>

<sup>†</sup>p<0.1 for potential confounder, hence retained in the model

#### 4.2.3.2 Maternal gestational weight gain and child percentage body fat

Child %BF did not correlate overall with maternal weight gain during pregnancy (Pearson's correlation coefficient  $r = -0.01$ ,  $p = 0.917$ ); this is shown graphically in Figure 24. The relationship between GWG and child %BF was further examined using linear regression modelling and considering the same set of potential confounders. Maternal GWG was not associated with child %BF in the unadjusted model (Model 1, Table 43). However, after adjustment for pre-pregnancy BMI, there was a borderline positive association between maternal GWG and child %BF (Model 3, Table 43). Inclusion of other potential confounders in the models did not appreciably change the effect size and the p-value was generally borderline (the exception was Model 5, Table 43, which was statistically significant).

**Figure 24. Child percentage body fat in relation to maternal gestational weight gain****Table 43. Estimated change in child percentage body fat in relation to maternal gestational weight gain (n=426)**

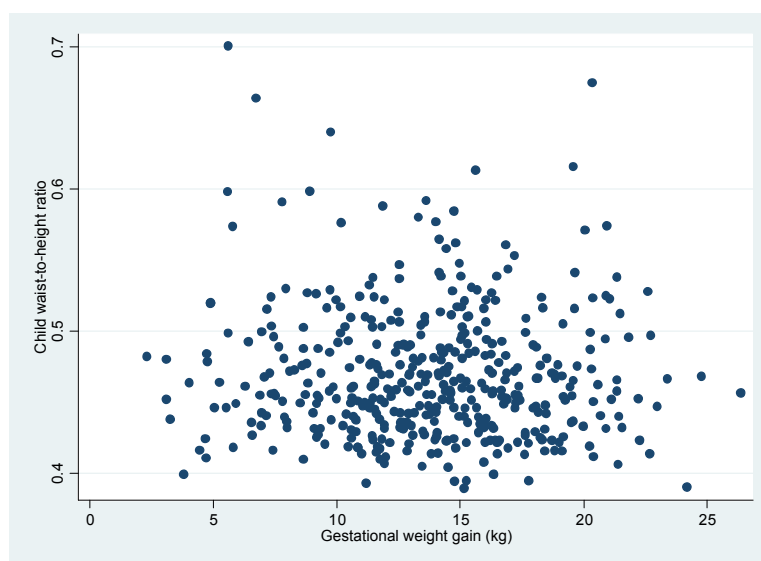
Model number	Model description	Percentage body fat change for one kg increase in GWG (95% CI)
Model 1	Unadjusted	0.009 (-0.180, 0.162)
Model 2	M1 + glucose tolerance during pregnancy	-0.034 (-0.211, 0.143)
Model 3	M1 + pre-pregnancy BMI <sup>†</sup>	0.164 (-0.007, 0.335)
Model 4	M3 + maternal age at the time of pregnancy	0.166 (-0.006, 0.338)
Model 5	M3 + parity	<b>0.181 (0.007, 0.354)</b>
Model 6	M3 + maternal smoking during pregnancy	0.165 (-0.006, 0.336)
Model 7	M3 + pregnancy-induced hypertension	0.170 (-0.002, 0.342)
Model 8	M3 + maternal education at the time of pregnancy <sup>†</sup>	0.157 (-0.014, 0.328)
Final model	Gestational weight gain + pre-pregnancy BMI + education (M8)	0.157 (-0.014, 0.328)

<sup>†</sup> p<0.1 for potential confounder, hence retained in the model

#### 4.2.3.3 Maternal gestational weight gain and child waist-to-height ratio

Bivariate analysis showed no association between maternal weight gain during pregnancy and child WHtR (correlation coefficient  $r=0.04$ ,  $p=0.385$ ); this is presented in Figure 25. In multiple linear regression, no relationship was found between maternal GWG and child WHtR, neither in unadjusted (Model 1, Table 44) nor adjusted models (Models 2-8, Table 44).

**Figure 25. Child waist-to-height ratio in relation to maternal gestational weight gain**



**Table 44. Estimated change in child waist-to-height ratio in relation to maternal gestational weight gain (n=429)**

Model number	Model description	WHtR change for one kg increase in GWG (95% CI)
Model 1	Unadjusted	0.000 (-0.001, 0.001)
Model 2	M1 + glucose tolerance during pregnancy	-0.001 (-0.002, 0.000)
Model 3	M1 + pre-pregnancy BMI <sup>†</sup>	0.000 (-0.001, 0.002)
Model 4	M3 + maternal age at the time of pregnancy	0.001 (-0.001, 0.002)
Model 5	M3 + parity	0.000 (-0.001, 0.002)
Model 6	M3 + maternal smoking during pregnancy	0.000 (-0.001, 0.002)
Model 7	M3 + pregnancy-induced hypertension <sup>†</sup>	0.001 (0.000, 0.002)
Model 8	M7 + maternal education at the time of pregnancy <sup>†</sup>	0.001 (0.000, 0.002)
Final model	Gestational weight gain + pre-pregnancy BMI + pregnancy-induced hypertension + education (M8)	0.001 (0.000, 0.002)

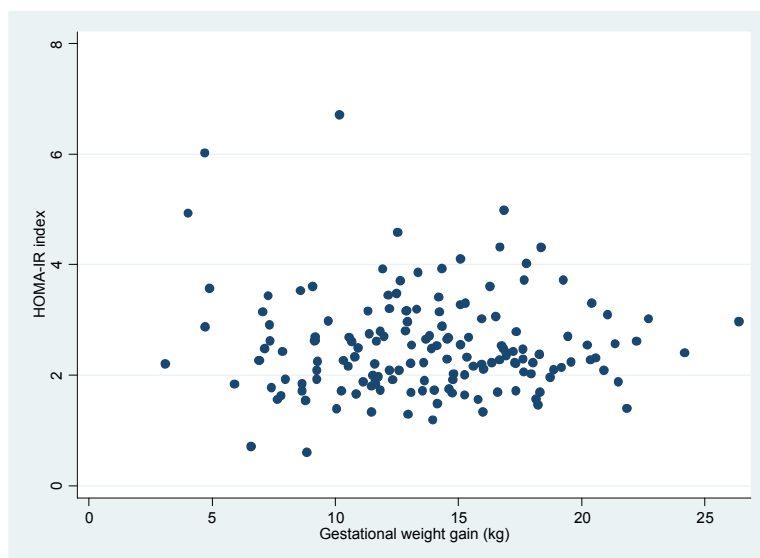
<sup>†</sup>  $p < 0.1$  for potential confounder, hence retained in the model



#### 4.2.3.4 Maternal gestational weight gain and child insulin resistance

The index of insulin resistance, HOMA-IR, in children did not correlate with maternal weight gain during pregnancy ( $r=0.04$ ), as illustrated in Figure 26.

**Figure 26. Child HOMA-IR in relation to maternal gestational weight gain**



The association between GWG and child HOMA-IR was investigated using a generalised linear model with log link and Gaussian family. No significant relationship was identified between maternal GWG and HOMA-IR in children, either in the unadjusted model (Model 1, Table 45) or after adjustment for potential confounders (Models 2-8, Table 45).

Child current BMI z-score was considered as a potential mediator on the pathway between maternal weight gain during pregnancy and child HOMA-IR, but its addition to the model did not have an effect on the association of interest ( $-0.50$ , 95% CI  $-1.51$ ,  $0.51$ ).

In summary, of the four outcomes of interest in children, only BMI z-score was associated with maternal GWG after adjustment for pre-pregnancy BMI. Neither of the specific measures of adiposity in children, nor the index of insulin resistance was associated with GWG in unadjusted or fully adjusted models.

**Table 45. Estimated change in child HOMA-IR in relation to maternal gestational weight gain (n=157)**

Model number	Model description	% difference in HOMA-IR for one kg increase in GWG (95% CI)	p
Model 1	Unadjusted	-0.32 (-1.60, 0.97)	0.626
Model 2	M1 + glucose tolerance during pregnancy <sup>†</sup>	-0.17 (-1.42, 1.10)	0.970
Model 3	M2 + pre-pregnancy BMI	-0.09 (-1.36, 1.21)	0.895
Model 4	M2 + maternal age at the time of pregnancy	-0.19 (-1.43, 1.08)	0.772
Model 5	M2 + parity	-0.11 (-1.39, 1.18)	0.863
Model 6	M2 + maternal smoking during pregnancy <sup>†</sup>	0.02 (-1.25, 1.31)	0.977
Model 7	M6 + pregnancy-induced hypertension	0.00 (-1.28, 1.29)	0.999
Model 8	M6 + maternal education at the time of pregnancy <sup>†</sup>	0.04 (-1.20, 1.29)	0.955
Final model	Gestational weight gain + glucose tolerance during pregnancy + maternal smoking in pregnancy + education (M8)	0.04 (-1.20, 1.29)	0.955

<sup>†</sup>p<0.1 for potential confounder, hence retained in the model

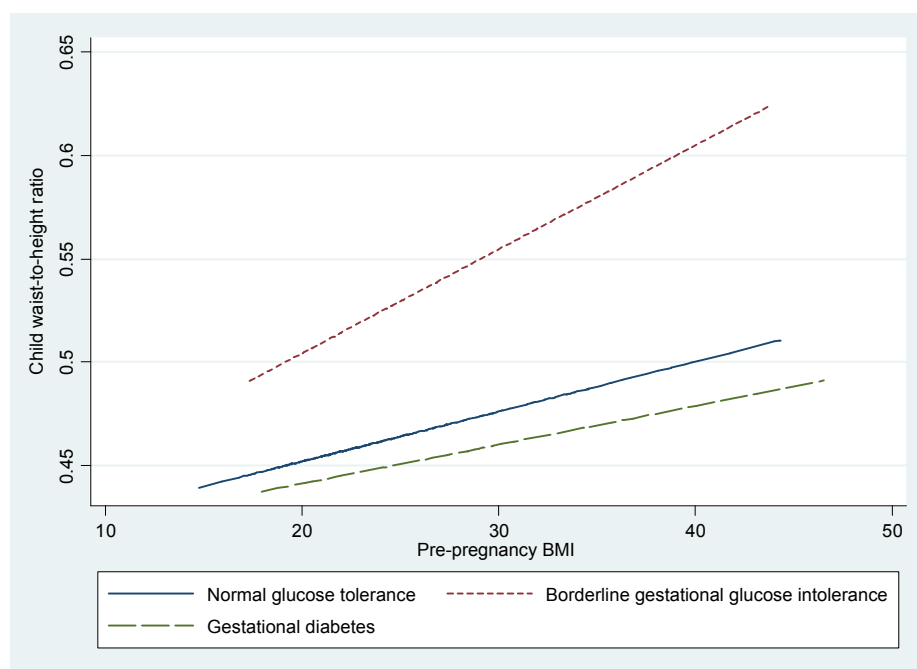
#### 4.2.4 Two-way interactions between the three intrauterine exposures of interest in relation to child outcomes

In the models described in Sections 4.2.1, 4.2.2, and 4.2.3, the relationship between each maternal intrauterine exposure and child outcomes of interest was assumed to be consistent across different levels of the other intrauterine factors. In this section, first order interactions between pairs of intrauterine exposures were explored in relation to child outcomes.

##### **Interaction between maternal pre-pregnancy BMI and glucose tolerance status during pregnancy in relation to child outcomes**

There was no significant interaction between maternal pre-pregnancy BMI and categories of glucose tolerance during pregnancy, in relation to child BMI z-score, %BF, or HOMA-IR as outcomes. A significant interaction was identified between maternal pre-pregnancy BMI and glucose tolerance status when WHtR was the outcome (p=0.033). The association between pre-pregnancy BMI and WHtR was greater in children of BGGI mothers than in those whose mothers had normal glucose tolerance during pregnancy. For every one kg/m<sup>2</sup> increase in maternal pre-pregnancy BMI, WHtR at 9-10 years increased by 0.003 more in children of mothers with BGGI than children of mothers with normal glucose tolerance (p=0.013) with adjustment for pregnancy-induced hypertension and maternal education. This interaction is presented in Figure 27.

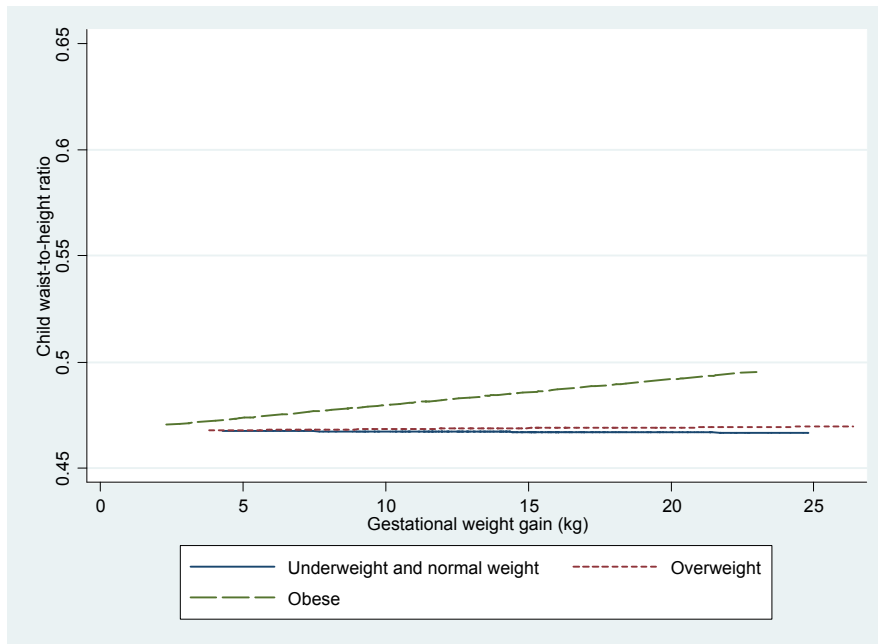
**Figure 27. Interaction between maternal glucose tolerance status during pregnancy and pre-pregnancy BMI in relation to child waist-to-height ratio**



### Interaction between maternal pre-pregnancy BMI and gestational weight gain in relation to child outcomes

No significant interaction was identified between maternal pre-pregnancy BMI and GWG in relation to child BMI z-score, %BF or HOMA-IR. There was a significant interaction between maternal pre-pregnancy BMI and GWG when child WHtR was the outcome. The association between GWG and WHtR increased with increasing pre-pregnancy BMI. For every one kg/m<sup>2</sup> increase in maternal pre-pregnancy BMI, child WHtR increased by 0.0002 more per one kg increase in GWG (p=0.010). In order to facilitate interpretation of results from this interaction, the relationship between child waist-to-height ratio and maternal GWG is presented in Figure 28, separately according to maternal pre-pregnancy BMI status. Due to the small number (n=18), underweight women were included in the same category with normal weight women (n=259).

**Figure 28. Interaction between maternal pre-pregnancy BMI and gestational weight gain in relation to child waist-to-height ratio**



**Interaction between glucose tolerance status during pregnancy and gestational weight gain in relation to child outcomes**

There was no significant interaction between maternal glucose tolerance status during pregnancy and GWG in relation to child BMI z-score, %BF, WHtR or HOMA-IR.

## Chapter 5 Discussion and conclusion

The aim of the current project was to shed light on the impact of an intrauterine environment characterised by overnutrition on obesity and insulin resistance in pre-pubertal offspring. This final chapter provides a synopsis of the main findings (Section 5.1), an outline of the strengths and limitations of this study (Section 5.2), an interpretation of the findings in the context of previous research (Section 5.3), a discussion of potential implications and recommendations for public health (Section 5.4), suggestions for future research (Section 5.5) and a concluding statement (Section 5.6).

### 5.1 Overview of major findings

This is the first prospective longitudinal study to concomitantly show in an analysis with thorough adjustment for confounders a positive, continuous association between maternal body size at the time of pregnancy, and a range of obesity measures in pre-pubertal children (from the global BMI z-score, to the more specific percentage body fat and waist-to-height ratio). These results confirm previous studies showing a positive association between maternal pre-pregnancy obesity and a child's risk of developing obesity. However, only one earlier study (Lawlor *et al.* 2007b) considered maternal pre-pregnancy BMI and child BMI as continuous variables. Thus, the current study contributes significantly to the description of progressive effects of maternal body size prior to pregnancy on child overall weight and adiposity. Maternal pre-pregnancy weight for height was not associated with child IR before or after adjustment for potential confounders.

This is the first study to report interactions between maternal body size prior to pregnancy and two other aspects of intrauterine environment in relation to child central adiposity. The positive association between maternal weight for height prior to pregnancy and child central adiposity was amplified in children whose mothers had developed mild glucose intolerance during pregnancy (compared to those whose mothers had maintained normal glucose tolerance) and in children whose mothers gained more weight during pregnancy.

Although maternal GWG has been repeatedly documented as a risk factor for obesity in the offspring, this is the first study to investigate it as a potential contributor to child insulin resistance. Maternal GWG was not associated with any of the child outcomes of interest (BMI z-score, %BF, WHtR or HOMA-IR) in unadjusted models, but a positive association was found between maternal GWG and child body size or adiposity after adjustment for pre-pregnancy weight for height.

This study advances the work in the area of intrauterine programming of child obesity and IR by maternal gestational glucose intolerance, as it addresses not only GD, but also milder degrees of gestational glucose intolerance as a potential influence. Exposure to maternal GD, but not to milder degrees of glucose intolerance, was associated with higher IR in children, with this association being robust to adjustment for potential confounders. In contrast, no association was found between maternal mild or clinically defined gestational glucose intolerance and child body size or central adiposity at 9-10 years. The positive association identified between maternal GD and child overall adiposity was attenuated to null after adjustment for maternal pre-pregnancy body size.

As IR is strongly correlated with body size and given the debate regarding the appropriateness of including offspring's current body size when analysing intrauterine effects on later outcomes, possible mediation by current body size was considered for all relationships between maternal factors and child IR. Maternal pre-pregnancy body size was inversely associated with child IR after considering child current body size as a potential mediator. The relationship between maternal GD and child IR was only partly mediated by child current body size. Moreover, maternal GD and child current body size synergistically increased child IR (there was a significant interaction). Exposure to milder degrees of glucose intolerance during pregnancy had the opposite effect, being associated with a reduction of child IR after including child current body size as a potential mediating variable. Child current body size did not mediate the effects of maternal GWG on child IR.

Of the three maternal factors associated with fetal overnutrition examined in this study, maternal body size prior to pregnancy appears to play the dominant role in programming child obesity, interacting with at least two other intrauterine exposures (GWG and BGGI) to further increase the risk of central adiposity. Maternal GD, on the other hand, appears to be an important, independent intrauterine contributor to child insulin resistance prior to puberty.

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## 5.2 Study strengths and limitations

A number of strengths pertaining to the design of this study, the assessments performed in mothers during pregnancy and children at the current follow-up, and the analyses presented in this thesis warrant mention. At the same time, several limitations in the study design, methods and analyses need to be acknowledged when interpreting the findings of this thesis. The strengths and limitations relevant to this project are addressed below.

### 5.2.1 Study design

The three major methodological strengths of this study are its prospective longitudinal design, the sample representativeness and the high participation rates at follow-up. The Generation 1 study offered a sound dataset for the purpose of this project. The richness of the available pregnancy data meant that it was possible to examine the role of specific intrauterine exposures on childhood obesity and related metabolic outcomes, while taking into account the potential influences of other covariates.

The **prospective** collection of the data minimised selection bias as all three exposures of interest (maternal BMI prior to pregnancy, gestational glucose tolerance status and weight gain) were ascertained at the time of pregnancy, well before the investigation of obesity and related outcomes in children. Given that follow-up of children in relation to obesity, fat pattern, or insulin resistance was not foreseen at the time of pregnancy, it is unlikely that the outcomes of interest would have influenced the measurement and classification of exposures.

**Sample representativeness** across a range of demographic variables is important to ensure minimal selection bias and increases the reliability of the results and statistical power (by not attenuating exposure variables). For the Generation 1 cohort, sample representativeness at baseline across SES and other demographic variables, a highly recommended but rarely achieved feature in observational studies, was obtained by balanced recruitment from public and private antenatal clinics, with high initial response rate (as described in Section 3.1.2). This is a strong feature of the Generation 1 study given the well known poor representation of low SES participants in observational studies (e.g., Project Viva cohort in the US was more likely to include well educated women (Oken *et al.* 2005); 29.3% women had a graduate degree and only 3% had not completed high school (Rich-Edwards *et al.* 2010) as opposed to 18.3% and 33.8%, respectively, in Generation 1 women). As previously reported (Moore *et al.* 2004), women who accepted the invitation to enrol

in the current study were slightly older (29 years versus 28 years,  $p < 0.05$ ) and their mean area disadvantage score based on postcode of residence was slightly higher (mean difference was 56,  $p < 0.05$ ) than those who declined to participate. These differences are small and unlikely to alter associations of interest unless the relationship between the exposure and outcome is different for non-participants, which seems improbable.

The Generation 1 cohort included only Caucasian women, thus restricting the ability to generalise the study findings to other ethnic groups in which susceptibility to obesity, glucose intolerance during pregnancy, excessive GWG, and associations with childhood obesity and insulin resistance might be different. Other ethnic groups were excluded since the initial focus of the Generation 1 study was maternal dietary intake during pregnancy, requiring use of a food frequency questionnaire that was appropriate and relevant only for Caucasian women (Moore *et al.* 2004).

**High participation rates** at each follow-up in the Generation 1 study can be largely attributed to the good rapport built with the families by the stable research team (the chief investigators and study coordinator remained the same from the inception of the cohort). At the most recent follow-up, the participation rate for anthropometric measurements was 80% of the original cohort, which was higher compared to any other prospective cohorts over similar length of time. For instance in ALSPAC, among the 10,725 original participants with complete data on GD and maternal glycosuria during pregnancy, 6,842 (64%) had available data on anthropometric measurements at the age of 9-11 years (Lawlor *et al.* 2010). Similarly 57% of the children had complete data on maternal GWG and adiposity at the age of 9 years (Fraser *et al.* 2010). As described in Section 2.4, similar longitudinal studies had even lower participation rates. Although impossible to directly assess selection bias, it appears that baseline characteristics of participants differed to those of non-participants at the most recent follow-up only with respect to mothers' age (slightly older), education (higher level of education) and smoking status during pregnancy (less likely to have smoked), as detailed in Section 4.1.4. Similar maternal characteristics were reported to diverge between participants and non-participants in the 9 year follow-up of ALSPAC study (Fraser *et al.* 2010). Such differences do not necessarily bias associations under investigation, as the biological relationships are likely to apply to mothers of all ages, levels of education, and smoking status.

When Generation 1 children were 9-10 years old, despite the high participation rate for anthropometric measurements, only 36.8% of participating children (29.3% of the original cohort) gave consent for the collection of a fasting blood sample. Four factors might explain this lower participation rate for blood collection: (1) children's aversion to needles or needle phobia; (2) the



burden of participation for children and their families (i.e., having to fast overnight and receive a separate visit by a domiciliary nurse for blood collection in addition to participation in interviews and anthropometric measurements was a considerable imposition, to which some of the families were reluctant to consent); (3) some families having relocated interstate or in rural areas not covered by the blood collection service that the Generation 1 team employed for the purpose of this study; and (4) fieldworkers' fatigue, which may have had an impact on their enthusiasm in asking for consent. However, other than being slightly older (9.65 years versus 9.58 years,  $p=0.003$ ), participating children who did not consent for blood sample collection were similar to those who did (as shown in Section 4.1.4). While no major differences were identified in the baseline characteristics of children who took part in this follow-up and those who did not, or between those who gave consent for blood sample collection and those who did not, a possibility still exists that other unmeasured or unknown factors related to the outcomes might be different between the two subgroups. However, for this to bias the reported associations the effects would need to be different in those assessed and those not assessed, which seems implausible.

One potential limitation of this study design is the **sample size**. The sample size was dictated by the size of the original cohort ( $n=557$ ) and by the participation rates at follow-up, thereby preventing changes. The relatively small sample size ( $n=443$  mother-child pairs participating at the most recent follow-up;  $n=163$  fasting blood-samples collected from the children) could have constrained statistical power and thus somewhat limited the ability to detect true effects. However, other studies with similar or smaller sample sizes have been able to show significant associations between maternal obesity prior to pregnancy and child obesity (Blair *et al.* 2007; Boerschmann *et al.* 2010; Catalano *et al.* 2009a), or between maternal glucose levels during pregnancy and child obesity or insulin resistance (Chandler-Laney *et al.* 2011; Crume *et al.* 2011a; Egeland and Meltzer 2010).

Sample size when conducting interaction analyses warrants a special mention. In order for the study to have sufficient statistical power to detect an interaction effect, sample size needs to be larger than the sample size required for estimating the main effects (Shieh 2009), hence it is plausible that this study was underpowered to detect interactions.

## 5.2.2 Measurements

### Maternal self-reported pre-pregnancy weight

As described in the Methods chapter (Section 3.4.1), pre-pregnancy weight was reported by the women in the first 16 weeks of gestation. It is well known that self-reported weight tends to underestimate real weight, particularly among obese women (Gorber *et al.* 2007). It may also be affected by recall bias, in turn influenced by age, education, or employment (Stommel and Schoenborn 2010; Yu and Nagey 1992), by non-response bias, often due to social desirability (Gorber *et al.* 2007), and sometimes by interviewer bias (Hennekens and Buring 1987), as a result of inconsistencies in interview techniques. However, given that women were recruited during their pregnancy, it was the only available indicator of pre-pregnancy weight in this study for this purpose.

A number of factors arguably minimise bias around self-reported pre-pregnancy weight in this study. Firstly, the interviewers were not aware of the study hypotheses at the time of data collection and the interviews were rigorously structured. Secondly, the time between the exposure (i.e., pre-pregnancy weight) assessment and data collection was minimised by interviewing women early in the index pregnancy. Thirdly, after data collection, checks were undertaken to identify those self-reported weights that were not plausible when compared to the weights measured in early pregnancy. In order to include data from women reporting unlikely pre-pregnancy weights (n=21) or missing data on pre-pregnancy weight (n=55), single imputation was used, the limitations and strengths of which will be discussed in Section 5.2.3. Moreover, while recognising limitations, self-reported weight is widely used and accepted in epidemiological research and has been validated against direct measures of weight (Oken *et al.* 2007).

An alternative approach to self-reported data may be given by estimating pre-pregnancy weight, either by retrospective extrapolation or using standardised estimates that correct for weight gain during early pregnancy. *Retrospective extrapolation*, while useful when at least two consecutive weight measurements in early pregnancy are available (which is not often, and not in this study), is limited by the fact that it does not account by short term fluctuations in body weight and, even more importantly, erroneously assumes constant rate of weight gain throughout pregnancy (Harris and Ellison 1998). Although mean weight gain in early pregnancy is considered minimal overall, wide variations have been reported ranging between -3 kg at 9 weeks (Van Loan *et al.* 1995) to 4.5 kg at 15 weeks (Clapp and Little 1995). In contrast to the retrospective extrapolation approach, *standardised estimates that correct for weight gain during early pregnancy* have the only limitation

that they do not take into account inter-individual variability or different weight gains for each woman in consecutive pregnancies (Harris and Ellison 1998).

### **Screening for maternal gestational diabetes**

Results of GD screening for Generation 1 women (i.e., plasma glucose levels at OGCT and/or OGTT) were abstracted from antenatal records (details were presented in Section 3.4.1). Thus, they can be considered objective and with minimal, if any, bias, particularly since the initial focus of the study was not on GD but on maternal diet during pregnancy (Moore *et al.* 2004). Over 90% of Generation 1 women underwent routine OGCT and, where required, OGTT. Given that the screening for GD based on the two-step approach became universal in Australia in 1998, when recruitment for this study had just commenced, some of the missing data on OGCT and/or OGTT could be attributed to an initially less effective implementation of the screening. Another explanation for the small amount of missing data on OGCT in women with available data on OGTT could be that some women (n=6) were referred directly for the diagnostic test OGTT, skipping the OGCT, perhaps due to a perceived high risk for GD (e.g., obesity, family history of diabetes).

By defining categories of glucose tolerance during pregnancy instead of using continuous plasma glucose levels in analyses, there is some potential for misclassification and for some reduction in statistical power (Ragland 1992; Selvin 1987), but this is believed to be unavoidable and minimal.

There is no standard international definition of BGGI. The BGGI category used in this study is directly comparable only to the category defined on the basis of the same ADIPS criteria (Ju *et al.* 2008); it does not correspond with the definitions used in studies from other settings, such as the study of Bonomo *et al.* (2005), which used NDDG criteria to define maternal glucose tolerance status. Nonetheless, based on ADIPS criteria in use in Australia, this was the only possible way to define an intermediate category of gestational glucose intolerance given that one plasma glucose level above the thresholds at OGTT (i.e., either fasting plasma glucose  $\geq 5.5$  mmol/l or 2-hour plasma glucose  $\geq 8$  mmol/l) is sufficient for the diagnosis of GD, as opposed to at least two plasma glucose levels above the thresholds at OGTT required by other criteria (e.g., NDDG) used in other settings. Thus comparing the findings of this thesis with other studies addressing health consequences of milder degrees of gestational glucose intolerance requires prudence.

Although not common practice in Australia at the time when Generation 1 women were pregnant, it is possible that some women with GD may have received dietary advice or even insulin therapy. If this treatment succeeded in controlling maternal glucose levels, hence reducing fetal exposure to the

nutrient-rich environment, the current study findings on the influence of maternal glucose intolerance during pregnancy on child outcomes might underestimate the true association in a population where treatment had not occurred. It is also possible that the GD group included women with unrecognized pre-existing glucose intolerance, as they were not screened prior to pregnancy (not common practice). However, this is unlikely to be driving the results presented in this thesis since the number of women with this condition is likely to have been low.

### **Maternal gestational weight gain**

In the current project, GWG was computed across the entire pregnancy (total GWG) from weight measured shortly prior to delivery (a strength of this study) and self-reported pre-pregnancy weight (a potential weakness, but the strategies undertaken to overcome the tendency to under-report pre-pregnancy weight have been discussed). To maximise the dataset, single imputation was used for women whose last recorded weight was measured early in the third trimester of pregnancy (details were presented in Section 3.4.1). As described in Chapter 2, most previous studies used self-reported GWG (Oken *et al.* 2008), which may also be problematic.

### **Child BMI**

Body mass index, in particular age- and sex-specific BMI centiles or z-score, is widely used in epidemiological studies as an indicator of global obesity as it is inexpensive, easy to compute, and reproducible. In this study, child BMI was calculated based on direct measures of weight and height, thus avoiding the potential bias introduced by self-reported measures. Ideally, the most stable anthropometric measurements are done in the morning after overnight fasting and after voiding (Norton and Olds 1996), to account for diurnal variation of body weight and height, both contributing to a small increase of BMI during the day (Rodríguez *et al.* 2000). However, these strict conditions were not feasible in a large-scale epidemiological study of a community sample like this, which involved home visits at a time prioritised by the families' availability.

Despite its advantages, BMI has some recognised limitations as a proxy measure of adiposity or of fat distribution, as described in Section 2.3.1.3. Hence, in this thesis, obesity measures in children were extended to include percentage body fat and fat distribution.

### **Child percentage body fat**

For the purpose of this project, %BF, a measure of fat mass adjusted for body size, was estimated by foot-to-foot bioelectrical impedance analysis (built into weight scales), a technique with multiple

advantages for research in the field, and few limitations. Bioelectrical impedance analysis has the advantage of being portable, simple, non-invasive and inexpensive. It is advantageous to skinfold thickness measurements, a common alternative, as it is not prone to examiners' error (Segal *et al.* 1991) and provides estimates for the whole body fat mass, including visceral fat (unlike the equations based on skinfold thicknesses that take into account only subcutaneous fat). Ideally, the assessment should have been undertaken in the morning after overnight fasting, given the small diurnal decrease in fat mass estimate (Rodríguez *et al.* 2000) and that food and liquid intake are known to influence total body water measurement (Kyle *et al.* 2004b). However, this strict condition was impractical for the purpose of this study, as most children were assessed after school, to minimise intrusion into their family life.

### **Child waist-to-height ratio**

For the purpose of this study, fat pattern was indicated by the WHtR, a measure that has been shown to correlate well with cardio-metabolic risk (Kahn *et al.* 2005; Savva *et al.* 2000). Using WHtR rather than waist circumference alone or waist-to-hip ratio is a strength, as this index takes into account an individual's height. Another strength is that in this study only three research nurses were involved in performing all anthropometric measurements in triplicate, thus minimising the inter-examiner variability in measurements. This is important given that body girth measurements, including waist circumference, are prone to examiner errors. As mentioned in Section 2.3.1.3, although WHtR has been shown to reliably predict cardiovascular disease risk, it cannot differentiate between the visceral and subcutaneous abdominal adipose tissue.

Imaging techniques (CT, MRI or DXA) would have provided more accurate estimates of fat distribution, particularly the differentiation between intra-abdominal and subcutaneous adipose depots, but their use was not feasible in this study. It is unclear how this extra precision could have changed the results.

### **Child HOMA-IR**

Homeostasis model assessment is a convenient index of insulin resistance that has been validated in children against more invasive and accurate methods (Gungor *et al.* 2004). Although advantageous for epidemiological studies, HOMA-IR is not without some limitations, including its inability to differentiate between hepatic and peripheral insulin resistance, and its potential to underestimate insulin resistance because the equation was originally calibrated to insulin assays used in 1970s (Wallace *et al.* 2004). Care also needs to be taken in making comparisons of insulin

resistance estimates based on data from different laboratories given the large insulin inter-assay variation and the lack of standardisation of insulin assays between laboratories (Robbins *et al.* 1996).

It has been suggested that it would be best to use the mean of three fasting concentrations for insulin collected at 5 minute-intervals in order to overcome the pulsatile nature of insulin secretion and improve intra-subject variability (Wallace *et al.* 2004). However, this was not feasible in the current study and, in fact, for practical reasons, most studies use one fasting insulin concentration to calculate HOMA-IR (Monzillo and Hamdy 2003).

### 5.2.3 Analyses

In addition to the issue of sample size (mentioned previously as a design issue), the robustness of findings presented in this thesis is directly influenced by the approach to the analyses, in particular definition of variables, handling of missing data, choice of statistical models, and adjustment for potential confounders and mediators.

A strength of this study is the way most exposure and outcome variables were included in analyses as **continuous variables**. By using continuous measures for both exposures and outcomes as opposed to categories or arbitrary cut-points, their relationships could be assessed in a more refined manner across the spectrum, reducing the misclassification associated with categorical variables and maximising statistical power (Ragland 1992; Selvin 1987).

Methods for **dealing with missing data** were discussed in Section 3.4.1. In this study, in order to maximise the dataset, single imputation was applied for exposure variables only (i.e., maternal pre-pregnancy weight, maternal weight just before delivery), because this could be done with high reliability. Subsequently, complete case analyses were undertaken, which may have had two limitations: potentially lower precision of estimates due to the reduced sample size and potentially biased estimates. However, the drop in numbers was small (n=18) and results did not change appreciably.

The index of insulin resistance, HOMA-IR in children, had a positively skewed distribution, for which logarithmic transformation is often applied to obtain a normal distribution. In this study though, given the limitations of log transforming a dependent variable (which were detailed in Section 4.1.2.4), **generalised linear models** (GLM) with log link function and Gaussian family were applied in analyses where HOMA-IR was the outcome. One main advantage of using GLM rather than log transformation was that the cumbersome appropriate 'back transformation' to the original scale

(which involves more than simple exponentiation) could be avoided (UCLA: Academic Technology Services and Statistical Consulting Group). The second advantage was that the regression coefficients directly estimated the expected arithmetic mean of the original variable (and not the less informative geometric mean, as is the case with log transformed dependent variables) (UCLA: Academic Technology Services and Statistical Consulting Group).

Thorough **adjustment for potential confounding variables** was a major strength of this study. Covariates were decided *a priori* based on theoretical and empirical evidence for an association with both the exposure and the outcome of interest, and were included in analyses using a stepwise approach. The richness of the data collected during pregnancy allowed adjustment for all the predefined potential confounders: maternal age, parity, smoking during pregnancy, pregnancy-induced hypertension, and maternal education. However, as is the case for any observational study, there could be residual confounders (unmeasured variables or unadjusted for in this project) that might inflate or reduce the observed associations.

A potential limitation is that no adjustment for multiple testing was performed in this study, which may have increased the likelihood that some of the significant findings, particularly those unexpected and not reported previously, arose by chance. However, the appropriate approach to multiple comparisons remains controversial. Some authors recommend adjustment to minimise the likelihood of mistakenly rejecting the null hypothesis using, for instance, Bonferroni correction. This approach is recommended mainly when random numbers are analysed, but with natural observations (like in this study), adjustment would increase the likelihood of a false-negative conclusion (Rothman 1990). Others argue that by reducing type I error for a null association, type II error for associations that are not null is increased, thereby reducing statistical power (Perneger 1998).

Summing up Section 5.2, this study has benefited from a number of major strengths, in particular the prospective nature of data collection, sample representativeness, high participation rates at follow-up, and, in general, high quality data. Limitations detailed above are inevitable in a large-scale longitudinal study like this. The most important one is likely to be the sample size, while the others are considered unlikely to have affected the reliability of the results presented in this thesis.

## 5.3 Relationship to studies on early origins of child obesity and insulin resistance

This section contextualises the associations observed in this study with findings from previous longitudinal studies on intrauterine influences of childhood obesity and insulin resistance. It starts (Section 5.3.1) by comparing characteristics of the Generation 1 sample with other cohorts, thus placing the observed findings in the context of other published data. It then presents a comparison of the associations of interest with previous similar studies (Sections 5.3.2 to 5.3.6), as well as an outline of potential mechanisms that might underlie the observed associations (Section 5.3.7).

### 5.3.1 Comparison of the Generation 1 sample with previous relevant studies

Mean pre-pregnancy BMI in Generation 1 women was comparable to that described in other contemporary cohorts (Table 46). The prevalence of overweight and obesity prior to pregnancy was in line with larger Australian surveys from 10 years ago, when this cohort was established (Cameron *et al.* 2003), but higher relative to some studies from other settings (Table 46). The variation in the prevalence estimates could be attributed to different rates of overweight and obesity in the background population, sample representativeness, or measurement accuracy.

As there is no international consensus to define GD and BGGI, comparisons of the prevalence estimates for GD and BGGI in this study with other studies are difficult. Prevalence in the current study was slightly higher compared to those reported in the only other Australian study using the same categories of glucose intolerance during pregnancy (Table 46). This is possibly due to the fact that only primiparous women were included in the other study (Ju *et al.* 2008), who are more likely to maintain normal glucose tolerance during pregnancy; these women were also younger compared to Generation 1 women.

Overall, only about one third (34.9%) of Generation 1 women gained weight within the 2009 recommendations of the Institute of Medicine, while almost half (49%) gained excessive weight during pregnancy. The amount of weight gained throughout pregnancy by the Generation 1 women was comparable to that reported by other contemporary international studies (Table 46).



**Table 46. Comparison of the Generation 1 sample with previous studies**

Study (Reference)	Country Year of pregnancy data collection	Mean maternal BMI prior to pregnancy (kg/m <sup>2</sup> ) (SD)	Prevalence of maternal overweight and obesity prior to pregnancy	Prevalence of GD and BGGI	Mean GWG (kg) (SD)	Mean child BMI z-score (SD)	Prevalence of child overweight and obesity
Generation 1	Australia 1998-200	25.0 (5.59)	Overweight: 23.3% Obesity: 16%	GD: 5.57% BGGI: 9.52%	14.0 (4.54)	0.41 (1.17) in 9 year-olds	Overweight: 17.7% Obesity: 5.9%
Mater University Study of Pregnancy and Its Outcomes (Lawlor <i>et al.</i> 2007b)	Australia 1981-1984	22.0 (4.1)					
Project Viva (Oken <i>et al.</i> 2007)	USA 1999-2002	24.6 (5.0)			15.6 (5.4)		
Growing Up Today Study (Oken <i>et al.</i> 2008)	USA 1996		Overweight: 11.3% Obesity: 3.6%		14.3 (5.1)		
ALSPAC (Brion <i>et al.</i> 2011; Fraser <i>et al.</i> 2010; Hughes <i>et al.</i> 2011)	UK 1991-1992		Overweight or obese: 21.3%		12.1 (5.1)	0.36 (1.19) in 11 year-olds	Overweight: 13.4% Obesity: 15.8%
Auckland Birthweight Collaborative Study (Blair <i>et al.</i> 2007)	New Zealand 1995-1997		Overweight or obese: 23.4%				
Australian Collaborative Trial of Supplements with antioxidants to pregnant women to prevent preeclampsia (Ju <i>et al.</i> 2008)	Australia 2001-2005			GD: 4% BGGI: 8%			

The prevalence of obesity in the Generation 1 children cohort was lower than the national rates (Australian Bureau of Statistics 2009a) and those from ALSPAC (Hughes *et al.* 2011). In contrast, the prevalence of overweight in Generation 1 children was similar to the rate identified by the Australian National Health Survey (Australian Bureau of Statistics 2009a), but higher than the rates reported in children from the ALSPAC cohort (Hughes *et al.* 2011) (Table 46).

Children participating in the current study appear largely similar in terms of %BF with other groups of Caucasian children. There is limited published data regarding the reference values for %BF in children and it is difficult to compare the mean found in children of the Generation 1 cohort ( $19.8\% \pm 7.87\%$ ) with those few other studies due to the different methods employed to estimate %BF. However, a simple comparison of sex-specific means of %BF in the Generation 1 cohort to the reference curves based on BIA data showed that Generation 1 girls' %BF ( $20.6\% \pm 7.76\%$ ) was between the 25<sup>th</sup> and 50<sup>th</sup> centiles, while boys' %BF ( $19\% \pm 7.92\%$ ) was between the 50<sup>th</sup> and 75<sup>th</sup> centiles (McCarthy *et al.* 2006).

Likewise, limited data exists with respect to reference values for WHtR in children. Relative to children of similar age from a British cohort (McCarthy and Ashwell 2006), Generation 1 children had higher mean WHtR. Given that the Generation 1 children were assessed more than 10 years after the children from the above mentioned British cohort, this difference may reflect the increasing prevalence of childhood obesity over time.

While current glucose assays provide similar plasma glucose concentrations irrespective of the method used, no standardised method of measuring plasma insulin has been validated to date (Staten *et al.* 2010). Thus, plasma insulin levels may vary widely according to the assay used, mainly due to variations in assay specificity, calibration procedures or conversion factors (Manley *et al.* 2007). Of the commercially available human insulin assays, radioimmunoassays are known to give the highest insulin levels (Manley *et al.* 2007). Fasting plasma insulin levels measured in Generation 1 children by a specific radioimmunoassay (mean  $11.3 \mu\text{U/ml}$ ) were similar to those reported in Bogalusa study (Freedman *et al.* 2007a), but double those reported in other studies of pre-pubertal non-obese children, which measured them by ELISA (Ong *et al.* 2004). Without a reference method for insulin measurement, indices derived on the basis of the absolute value of insulin (such as HOMA-IR) are affected by this variation. Therefore, using cut-offs to define insulin resistance is not appropriate across different studies. This was another reason for considering HOMA-IR as a continuous variable in this study.

### 5.3.2 Maternal pre-pregnancy BMI and child outcomes

This study builds on previous research concerning the association between maternal obesity prior to pregnancy and child obesity and related metabolic outcomes, by showing that the relationship is continuous. The finding of a positive association between maternal pre-pregnancy BMI and child global obesity, adiposity and fat pattern in pre-pubertal years is consistent with previous studies, as critiqued in Section 2.4.1 and summarised in Table 6. Had Generation 1 data been analysed using categorical variables for maternal pre-pregnancy BMI and child BMI, as per the approach taken in the great majority of previous studies, the magnitude of the effects would have been comparable (OR for child overweight or obesity was 1.96 (95% CI 1.19, 3.20) if the mother was overweight, and 2.19 (95% CI 1.26, 3.80) if the mother was obese prior to pregnancy). When pre-pregnancy BMI was converted to an internal standard deviation in the Generation 1 study to facilitate comparison with the only one other study which used both exposure and outcome variables in continuous format (Lawlor *et al.* 2007b), the effect of a one standard deviation increase in maternal pre-pregnancy BMI on offspring BMI z-score was of 0.43 (95% CI 0.31, 0.54) in fully adjusted model, similar to the estimates reported by the previous study (Lawlor *et al.* 2007b).

Robust associations were identified between maternal pre-pregnancy BMI and child body size and adiposity. While causality cannot be concluded, the consistency of this finding supports a relationship. It is not clear if this is a direct contribution of the intrauterine environment or if it is due to genetic factors or shared postnatal environment (i.e., similar diet and physical activity patterns). (This is discussed further in Section 5.5.) Data obtained in this project do not allow for the explicit untangling of these possibilities; however, they support the public health recommendations of avoiding obesity at the beginning of pregnancy in order to reduce the obesity risk in the subsequent generation.

No association was detected between maternal pre-pregnancy BMI and child HOMA-IR before or after adjustment for potential confounders. This null result is in agreement with the findings of the only previous prospective study of children of similar age to the Generation 1 children, but who had all been exposed to GD while *in utero* (Boerschmann *et al.* 2010). In contrast, a retrospective study in young adults reported a significantly higher degree of insulin resistance (calculated from the 2-h OGTT) in offspring of mothers who were obese at the time of pregnancy compared to those of normal weight mothers (Mingrone *et al.* 2008). Whilst the study of young adults was not without limitation (pre-pregnancy weight and height were self-reported about 20 years after delivery), overall these results suggest an effect of maternal pre-pregnancy BMI on offspring insulin resistance may

not be evident until after childhood, when HOMA-IR takes on a different distribution with a greater range of values (Li *et al.* 2003).

### 5.3.3 Maternal glucose tolerance status during pregnancy and child outcomes

The null result between GD or BGGI and child BMI z-score in both unadjusted and adjusted models is in agreement with some (Boney *et al.* 2005; Gillman *et al.* 2003; Jeffery *et al.* 2006; Lawlor *et al.* 2010; Pirkola *et al.* 2010; Whitaker *et al.* 1998; Wright *et al.* 2009), but not all (Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Chandler-Laney *et al.* 2011; Crume *et al.* 2011a; Egeland and Meltzer 2010; Krishnaveni *et al.* 2010) of the previous longitudinal studies summarised in Table 7. As discussed in Section 2.4.2, discrepancy in findings could be due to differences in the consideration of potential confounders, in particular maternal body size, which is known to be a strong predictor of offspring weight status. Of the studies that did identify a crude association between exposure to chronic hyperglycaemia *in utero* and child obesity, all reported an attenuation (sometimes to null) after adjustment for maternal BMI prior to pregnancy (Crume *et al.* 2011a; Lawlor *et al.* 2010) or at the time of follow-up (Egeland and Meltzer 2010; Gillman *et al.* 2003; Krishnaveni *et al.* 2010). This indicates that the initial relationship may have been driven by maternal body size rather than maternal GD. Similar to the current project, previous large-scale studies found no independent association between GD and child obesity before and after adjustment for various confounders, including maternal pre-pregnancy BMI (Pirkola *et al.* 2010; Whitaker *et al.* 1998; Wright *et al.* 2009).

The lack of an association between maternal GD and child BMI could reflect, at least in part, a limited statistical power to detect true effects. In addition, as mentioned earlier, management of GD may also have attenuated the associations between GD and child obesity, although this seems unlikely for the Generation 1 study, as the intensive management of GD was not routinely recommended at the time when the women were pregnant. Of note, in all the previous studies which did not detect an association between maternal GD and child obesity, women with GD received dietary advice and insulin if required (Lawlor *et al.* 2010; Pirkola *et al.* 2010; Whitaker *et al.* 1998; Wright *et al.* 2009) or, due to their profession (nurses), were likely to have had a better glycaemic profile during pregnancy (Gillman *et al.* 2003).

In contrast, data from the ALSPAC study (which has a very large sample size) showed an association between milder degrees of hyperglycaemia during pregnancy (women presenting with glycosuria and not receiving treatment) and an increased risk of obesity among offspring (Lawlor *et al.* 2010). Similarly, an increasing risk of child overweight and obesity was shown to parallel

increasing maternal glucose level at OGCT (Hillier *et al.* 2007). However, adjustment for maternal body size at the time of pregnancy was not undertaken in that study, which might confound the associations observed.

No association was identified in the current study between GD or BGGI and child body composition or fat pattern. This finding is consistent with two previous studies which reported no associations between maternal GD and percentage body fat (Catalano *et al.* 2009a) or fat pattern (Catalano *et al.* 2009a; Pirkola *et al.* 2010) in either children or adolescents. In contrast, exposure to GD was found to be positively associated with child adiposity measured by the sum of subscapular and tricipital skinfold thicknesses in 3 year old participants in Project Viva (Wright *et al.* 2009) or by subcutaneous abdominal adipose tissue (from MRI measurements), as well as with fat distribution estimated from waist circumference in 6-13 year-old children from the EPOCH study (Crume *et al.* 2011a). Two other studies with smaller sample sizes to the present research, in which maternal BMI prior to pregnancy was not considered as a potential confounder, also reported a positive association between maternal 1-hour glucose level at OGCT and offspring fat mass at the age of 5-10 years (Chandler-Laney *et al.* 2011), as well as greater waist circumference in female adolescent offspring of mothers with GD or impaired glucose tolerance (Egeland and Meltzer 2010). In this context, although no association was found in the current study between maternal glucose intolerance during pregnancy and offspring body composition or fat pattern in pre-pubertal years, it is possible that such an effect might be detectable during puberty or later in life (Singhal *et al.* 2003).

In contrast to child body size, maternal GD was a strong predictor of child insulin resistance, independent of confounders. Whilst some of the previous studies have reported higher HOMA-IR in children exposed to maternal GD compared to those whose mothers had normal glucose tolerance during pregnancy (Boerschmann *et al.* 2010; Catalano *et al.* 2009a; Krishnaveni *et al.* 2010), this is the first to present an effect after thorough adjustment for confounders.

Intrauterine exposure to maternal BGGI did not appear to be associated with child insulin resistance in Generation 1 children. This is similar to the findings of the only other study that examined the association between milder degrees of glucose intolerance during pregnancy, which indicated that exposure to maternal impaired glucose tolerance during pregnancy had no significant effect on child HOMA-IR (Egeland and Meltzer 2010).

In summary, while no significant association was identified between maternal glucose intolerance during pregnancy (across the spectrum) and child obesity or adiposity, the findings of this thesis in

conjunction with existing literature suggest that intrauterine exposure to maternal GD increases insulin resistance in the child, independent of other maternal factors.

#### 5.3.4 Maternal gestational weight gain and child outcomes

Previous research in the area of intrauterine programming by maternal GWG (critiqued in Section 2.4.3) has been dominated by studies addressing the effects on childhood obesity and their findings have been inconsistent. Data on the effects of GWG on body composition, fat pattern and insulin resistance in the child have been scarce. On the basis of Generation 1 data, no association was found between maternal GWG and child BMI z-score at the age of 9-10 years until adjusting for maternal pre-pregnancy BMI, when the association was positive, which highlights again the importance of maternal body size prior to pregnancy for child weight status.

No association was found in the current study between maternal GWG and more specific features of child adiposity. This is at odds with the one other study of similarly aged children from ALSPAC (Fraser *et al.* 2010), which found an increase in both fat mass and waist circumference with excessive weight gain during pregnancy. A potential explanation for the difference in findings might be the different statistical power of the two studies.

This is the first study to investigate the association between maternal GWG and child insulin resistance and report no significant independent effect of GWG on offspring HOMA-IR. While this might again reflect a lack of statistical power, the fact that other associations between maternal factors and child insulin resistance were observed suggests that GWG is relatively unimportant for this outcome.

#### 5.3.5 Interpreting mediation by current body size

Given the established strong correlation between an individual's obesity and insulin resistance, it was deemed important and appropriate in the current study to investigate whether any relationship between maternal intrauterine conditions and child HOMA-IR was mediated by child current body size. Examination of this mediating variable for all associations of interest was also dictated by the existing controversy regarding the appropriate analysis and interpretation of associations between pre-birth factors and later outcomes (Cole 2005; Lawlor *et al.* 2007a; Tu *et al.* 2005). In this section, mediation results are interpreted based on recommendations in the literature.

The association between maternal pre-pregnancy BMI and child IR changed from null to negative with the addition of child current BMI z-score to the model. This suggests that amongst children who grow to the same body size at age 9-10 years, those born to mothers with lower BMI prior to pregnancy have, on average, higher IR or, conversely, those born to mothers with higher pre-pregnancy BMI have, on average, lower IR. Similarly, there was no significant difference in the IR of children of mothers with normal glucose tolerance and borderline gestational glucose intolerance until child current BMI z-score was added to the model, when children of BGGI mothers had lower IR. This can be interpreted as amongst children who grow to the same size at age 9-10 years, those whose mothers had BGGI have, on average, lower IR than those whose mothers had normal glucose tolerance during pregnancy. The positive association of maternal GD and the null association of GWG with child IR did not change with adjustment for the child's current body size.

These phenomena (where a positive or null association between an early life factor and later outcome becomes negative after controlling for current size) have been described in statistical terms as the 'reversal paradox' or the Simpson's paradox, or within the generalised linear modelling framework, as the 'suppression effect' (Tu *et al.* 2005). The interpretation of this result is contentious (Cole 2005; Lawlor *et al.* 2007a; Tu *et al.* 2005). The reversal paradox has been investigated principally in relation to effects of birth size and current body size on adult blood pressure (Lucas *et al.* 1999). Rather than considering it as a 'statistical artefact' (Tu *et al.* 2005), it was suggested that the inverse relationship between birth size and the later outcome with adjustment for current body size should be interpreted as being driven by the change in body size (i.e., BMI centile crossing) (Cole 2005; Lawlor *et al.* 2007a; Tu *et al.* 2005). I propose that this approach can be extrapolated 'upstream' from birth size to intrauterine exposures that influence birth size.

An alternate interpretation is that children born to mothers with low BMI had slower intrauterine growth, followed by a period of accelerated postnatal growth (upward BMI centile crossing). Evidence elsewhere suggests that periods of rapid weight gain in any stage of life are characterised by a disproportionate gain in fat compared to lean body tissue (Dulloo *et al.* 2006), which could, in turn, increase susceptibility to IR. As per recommendations by Lucas *et al.* (1999), an interaction between maternal BMI and child current BMI was also tested for, which was not significant. It is also possible that this result reflects a non-pathologic intergenerational tracking of BMI (whereby children of high BMI mothers have high BMI themselves, but this higher BMI is not accompanied by increased IR). In future, it would be interesting to examine whether the relationship between maternal pre-pregnancy BMI and child HOMA-IR is further affected by including intermediate

measures of child body size (e.g., BMI at 2 years or BMI at the age of adiposity rebound) in the models or more specific measures of adiposity (e.g., %BF or WHtR).

The only comparable study to this has been in adult twins (aged 18-34 years), where low maternal pre-pregnancy BMI was also associated with hyperinsulinemia and IR in the adult offspring after adjusting for their current body size (Loos *et al.* 2002). However, the findings of the present study are intriguing when compared to a study of infants. Catalano *et al.* (2009b) reported higher HOMA-IR at birth in infants of obese mothers compared to those of lean mothers, which correlated strongly with neonatal adiposity. These conflicting results could suggest, again, that postnatal factors, including weight gain during childhood are on the pathway between maternal BMI and child IR.

The negative association between maternal BGGI and child IR after controlling for current body size is more complex to interpret due to the use of categorical variables and the need to consider it in context with the positive association between maternal GD and child HOMA-IR, which was only partly mediated by current body size. Potential mediation of the association between maternal GD and child IR by child current body size has been previously examined in a study, which showed that adjusting for child current BMI reduced the association in boys and attenuated it to null in girls (Krishnaveni *et al.* 2010), indicating perhaps a more important role of body size in determining IR, compared to that of exposure to GD. This is supported by my finding that the effect of GD on child HOMA-IR was amplified by child current BMI z-score (there was a positive interaction between maternal GD and child current BMI z-score in relation to HOMA-IR).

One possible interpretation of the negative association between maternal BGGI and child IR is that children of mothers with BGGI may have different allocations of fat and lean tissue and, while BMI z-score did not vary significantly across the groups, there may be enough variation such that adjusting for it reveals lower HOMA-IR for a given BMI z-score. It could be hypothesised that within this cohort, fetuses of BGGI mothers were able to convert the surplus glucose into muscle growth, hence being leaner for a given BMI. It is also prudent when interpreting this result to consider that BGGI women formed a relatively small group in this cohort. Nonetheless, this is the first study to report this interesting result and it warrants further investigation.

### 5.3.6 Two-way interactions between main exposures in relation to child outcomes

Generation 1 data revealed effect modification of maternal pre-pregnancy BMI on child central adiposity by BGGI, but not GD. The fact that no interaction was detected in this study between



maternal pre-pregnancy BMI and GD is in contrast to a large prospective cohort study in adolescents, which has reported higher risk of obesity and central adiposity (based on waist circumference) in offspring exposed concomitantly to maternal overweight at the time of pregnancy and GD compared to those exposed only to maternal overweight (Pirkola *et al.* 2010). This discrepancy in findings could reflect a lack of power in the current study.

The second interaction identified was between maternal pre-pregnancy BMI and GWG in relation to child central adiposity. Similar interactions have been reported previously, but not universally, in relation to child global obesity (Fraser *et al.* 2010; Oken *et al.* 2007; Oken *et al.* 2008; Schack-Nielsen *et al.* 2005), but not specifically with central fat deposition.

These interactions suggest there is complexity in the way the intrauterine environment affects growth and particularly the development of central adiposity. They once again highlight the important role of maternal pre-pregnancy BMI for child obesity and suggest that by addressing only gestational glucose intolerance or GWG in isolation, without optimising maternal BMI prior to conception, the reduction of central obesity in the child would be limited.

### 5.3.7 Potential underlying mechanisms

The positive associations between **maternal body size prior to pregnancy** and child body size, body composition and fat pattern observed in this study could be explained by genetic inheritance, shared poor nutritional and physical activity behaviours, and/or intrauterine programming through epigenetic changes. The current study does not allow for disentanglement of these three categories of effects.

At least a part of the direct association between maternal and child body size is genetically determined. Genetic predisposition to obesity rarely has a monogenic basis (i.e., a mutation in a single gene that is sufficient to cause obesity, such as the genes on the leptinergic-melanocortinergic pathway) and is mainly due to the combined effect of polygenic variants (the individual effect of each polygenic variant on the phenotype is small) (Hinney *et al.* 2010). Genome wide association studies have led to the detection of at least 17 confirmed polygenic variants involved in body weight regulation, including the fat mass and obesity associated gene (FTO), but many more polygenic variants await identification (Hinney *et al.* 2010). Based on longitudinal twin studies, it has been shown that the genetic influence on BMI variance strengthens during childhood (Haworth *et al.* 2008). Heritability has been described to influence a number of factors involved in obesity

development, including appetite, food preferences (Garcia-Bailo *et al.* 2008) and physical activity levels (Perusse *et al.* 1989). However, the rapid rise in childhood obesity is unlikely to be accounted for by genetic factors, and rather by changes in the environmental factors (Department of Health Public Health Research Consortium *et al.* 2007).

Another potential explanation for the association between maternal weight status prior to pregnancy and child body size could be the shared postnatal environment, including shared patterns of diet and physical activity. Mothers play an important role in shaping their children's eating behaviour through the foods they make available (diet composition), child feeding practices, their own eating behaviour and parental control (restriction to junk foods and pressure to eat healthy foods) (Scaglioni *et al.* 2008), with subsequent effects on body weight. It appears that patterns of eating and food preferences established during childhood are likely to track into adulthood (Fisk *et al.* 2011), with further potential impact on body weight. There is accumulating evidence of a direct association between maternal and child quality of diet, which appears to follow a socioeconomic gradient (McLeod *et al.* 2011). Women whose diets include more healthy foods are more likely to have children with similar dietary patterns, and, conversely, those with high intakes of junk foods or large portion sizes are more likely to have children with comparable diets (Robinson *et al.* 2007). In the same way, mothers may influence their children's physical activity through shared activities, modelling, and support (Gustafson and Rhodes 2006). That said, it should be acknowledged that there is inconsistent evidence regarding parent-child correlations of physical activity (Gustafson and Rhodes 2006), with some studies indicating similar patterns of physical activity in children and their parents especially for sedentary activity (Fogelholm *et al.* 1999; Simonen *et al.* 2002), while others not (Anderssen *et al.* 2006).

While accepting the above alternatives may play a part, biological plausibility of intrauterine programming of adiposity has been supported by experimental work in various animal models. It has been postulated that programming of obesity by fetal overnutrition involves persistent abnormalities in adipocytes (number and size) as well as dysfunction in the hypothalamic regulation of energy balance (appetite control). Both mechanisms are likely to be mediated by fetal hyperinsulinism secondary to maternal increased levels of plasma glucose. As suggested by animal studies (Mühlhäusler and Smith 2009; Mühlhäusler 2007), it could be hypothesised that human fetal adipocytes exposed to higher levels of glucose during early development may be programmed to maintain an increased capacity to form new adipocytes and to accumulate lipids in the existing fat cells.

Another potential mechanism of intrauterine programming of child obesity may involve lifelong altered programming of hypothalamic regulation of energy balance, with subsequent increased food intake and reduced energy expenditure. Fetal insulin appears to play a key role for development of neuronal networks in the hypothalamus that regulate energy balance (Bouret 2009). It is possible that hypothalamic insulin resistance secondary to an abnormal hypothalamic development leads to the release of high levels of orexigenic neurotransmitters (e.g. neuropeptide Y, the strongest promoter of appetite identified so far) and reduced sensitivity to satiety signals, as indicated by previous studies in rats (Plagemann *et al.* 1999). As a consequence, these children may have exaggerated appetite, with subsequent predisposition to become overweight and accumulate adipose tissue.

The negative association observed between maternal body size prior to pregnancy and child IR after considering child's current body size as a potential mediator might be in fact driven by rapid postnatal growth in some of these children. The role of rapid postnatal growth in the first few years of life in predicting IR during childhood or adolescence has been previously shown (Fewtrell *et al.* 2000; Singhal *et al.* 2003), with the highest level of IR observed in children born in the lowest birth weight tertile who became obese (Ong *et al.* 2004). The mechanisms underlying rapid transition from small size at birth to overweight or obesity during childhood (rapid catch-up growth) have not been fully characterised, but they appear to involve increased appetite secondary to high levels of leptin (Ong *et al.* 1999), increased number of receptors for insulin in the first years of life (Hales and Barker 2001) and a suite of factors that promote growth, including insulin-like growth factors (IGF) and their binding proteins, which regulate not only growth during childhood (Hill and Hogg 1989), but also insulin sensitivity (Jones and Clemmons 1995, Singhal, 2003 #1612).

The fact that intrauterine exposure to **maternal GD** was associated with IR in the child, independent of other maternal factors and only partially mediated by child current body size could be explained by genetic inheritance of IR or intrauterine programming. Overall heritability for insulin sensitivity has been estimated at 0.53-0.55 (Shaaf and Groop 2007). A number of genes have been identified as playing a role in cellular mechanisms of IR in GD (e.g., insulin receptor substrate 1, insulin receptor, ectonucleotide pyrophosphatase/phosphodiesterase 1) (Shaaf and Groop 2007). These genes could be inherited by the offspring, who would be thus predisposed to develop IR.

Potential underlying mechanisms of the intrauterine programming of offspring obesity and IR by maternal GD also appear to involve fetal hyperinsulinism secondary to the excessive glucose passage from the mother, which has programming effects on adipocytes development (Mühlhäusler

and Smith 2009) and on appetite-regulating neuronal network by stimulating the expression of neuropeptide Y neurons (Plagemann *et al.* 1992), with subsequent weight gain and, potentially, IR. (In contrast to previous animal studies, maternal GD in the current study was only associated with child IR and not with child body size or body composition). The exact mechanisms through which maternal GD affects child IR and not child body size or composition are not clear, but it could be posited that defects in the insulin receptor or insulin signalling proteins induced by maternal GD might be more prominent or longer-lasting compared to the imprinting effects on adipocytes and hypothalamic regulation of energy balance.

The positive association between **maternal GWG** and child body size in pre-pubertal years (after adjustment for maternal body size prior to pregnancy) might reflect genetic inheritance of the potential to gain weight or programming by fetal overnutrition. Intrauterine programming is supported by animal models of energy-rich diet during pregnancy, mimicking excessive GWG in humans. For instance, lambs of sheep that were overfed in late pregnancy had an increased appetite, hyperglycaemia, hyperinsulinemia, and greater deposition of subcutaneous fat in the postnatal period, potentially related to reduced expression of the leptin receptor in the hypothalamus and of appetite-inhibiting neuropeptides (such as cocaine- and amphetamine- regulated transcript) in response to increases in fat mass (Mühlhäusler *et al.* 2006).

While acknowledging the interrelationships between the three maternal factors considered in this study, maternal body size at the time of pregnancy appears to have a greater contribution to the development of obesity in the next generation compared to gestational glucose intolerance during pregnancy or GWG, as it profoundly shapes the intrauterine environment.

## 5.4 Implications and recommendations for public health

From a life course perspective, the intrauterine period is emerging as an important stage during which maternal factors associated with fetal overnutrition, in particular maternal obesity and gestational diabetes, appear to contribute to the programming of offspring obesity and insulin resistance. In this view, the current research suggests that one potential means of preventing childhood obesity is to optimise maternal body weight status before a woman conceives. Not only might the child's risk of global obesity be reduced if born to a mother who was not obese prior to pregnancy, but other aspects of the child's adiposity, including the central distribution of fat, might

also be improved, with potential further benefits for the child's metabolic health. Moreover, based on this study, the risk of central adiposity appears even greater in children whose mothers were obese prior to pregnancy and either developed borderline gestational glucose intolerance or gained more weight during pregnancy. This latter finding has broader implications, as these maternal conditions rarely occur in isolation and are rather interrelated. However, as responsibility for implementing appropriate interventions rests with different sectors, preventative strategies need to be comprehensive and address them in a co-ordinated multidisciplinary approach.

To address this issue, obesity-related health promotion needs to occur at both individual and population levels, targeting three specific time windows in a woman's life: pre-conception, during pregnancy and postpartum (between pregnancies).

One recommendation flowing from the research presented in this thesis is that overweight and obese women of reproductive age who are planning pregnancy should receive **preconception counselling** with respect to the risks associated with excessive weight (both for the women themselves and their future offspring). Achieving an optimal nutritional status should be encouraged through healthy diet and regular physical activity (American Dietetic Association *et al.* 2009).

However, this focussed approach is limited by the fact that pregnancy is not always planned. In Australia for instance, although there is no national data on the rates of unintended pregnancy (Australian Government and Department of Health and Ageing 2009), it has been estimated that about half (51%) of all women had experienced an unplanned pregnancy at some time during their reproductive carrier (Marie Stopes International 2006). Similar rates of unintended pregnancy have been reported in the USA (Finer and Henshaw 2006). Therefore, in order to also capture unintended pregnancies (and reduce the rates of poor pregnancy outcomes) preconception care could be made available to all women of reproductive age, irrespective of their intention to conceive. However, an inadequate uptake of such a broad and inclusive approach could be a limiting factor for its success.

Nonetheless, current recommendations from the US Centers for Disease Control and Prevention and its Select Panel on Preconception Care stipulate that preconception care should be offered to all women with a potential to become pregnant (15-44 years), both before a first and a subsequent pregnancy, aiming to identify and manage women's medical, behavioural and social risks to their own health and any pregnancy they may have (Centers for Disease Control and Prevention (CDC) and Agency for Toxic Substances and Disease Registry (ATSDR) 2006). This primary prevention approach needs to include, but not be limited to, developing a reproductive life plan, providing risk

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assessment through screening, promoting a healthy weight status, smoking and alcohol cessation, periconceptional folic acid supplementation, appropriate vaccinations, and management of chronic medical conditions (Centers for Disease Control and Prevention (CDC) and Agency for Toxic Substances and Disease Registry (ATSDR) 2006, Royal Australian College of General Practitioners, 2009 #1544).

Only recently has preconception care (including that related to women's weight status) been included among the preventive activities in general practice in Australia, broadening the former focus on the three months preceding a pregnancy (Royal Australian College of General Practitioners 2009). The effectiveness of its implementation has not been comprehensively evaluated, but data from a qualitative study have suggested that there is room for improvement in the delivery and uptake of preconception care (in general) in Australia (Mazza and Chapman 2010). The main enabling factors identified were women's willingness and motivation to engage in healthier behaviours in order to ensure the best possible intrauterine environment for the future child, while the major barriers included women's receptivity to preconception care (depending on their life stage), the view that pregnancy is a normal event (which should not require intervention prior to it occurring), the limited availability of preconception counselling and the perceived role of their doctors to be one of acute care (Mazza and Chapman 2010).

More specifically, the outcomes of counselling obese women of childbearing age before becoming pregnant are still largely unknown, but it seems that about half (46%) of such women report some weight loss (Callaway *et al.* 2009), while fewer women manage to achieve normal weight before conception (American Dietetic Association *et al.* 2009). This is not surprising given that long-term weight loss and maintenance is difficult to achieve at any stage of life (Loveman *et al.* 2011).

A number of barriers in addressing overweight and obesity prior to pregnancy, which might impede the effectiveness of preconception care, have been described in Australian women from an urban setting. These include the insufficient uptake of routine pre-pregnancy health checks (about half of women), the relatively high proportion of unplanned pregnancies (35%) and the inaccurate self-categorisation of weight, with a tendency to underestimate weight status (and potentially an inaccurate perception of the associated risks) (Callaway *et al.* 2009). An intriguing finding of this study was that only one third of the overweight and obese women having preconception health checks reported being advised to lose weight (Callaway *et al.* 2009). While the possibility exists that some women might have forgotten their doctors' advice, this finding may also suggest that some of the doctors did not actually provide lifestyle advice to overweight and obese women prior to

conception, indicating a need for healthcare providers to become more proactive in providing preconception counselling. On the other hand, other overweight and obese women participating in this study reported receiving advice to lose weight not only from their doctors, but also their partners, family members and friends (Callaway *et al.* 2009). This highlights the importance of improving knowledge regarding the benefits of healthy weight during pregnancy in the wider community.

It has been suggested that awareness of the relevance and uptake of preconception care could be improved by healthcare professionals offering it as part of consultations for other matters, sending invitation letters to all women of childbearing age, running preconception classes, or making educational materials with relevant information available in waiting rooms (Mazza and Chapman 2010). Further benefits could arise from promoting those factors that have been identified by community members themselves as enabling the uptake of preventive health care in general practice (such as continuity of care, trust and rapport), and addressing barriers (such as the lack of knowledge regarding the relevance of preventive care, time constraints and cost of consultations) (Mazza *et al.* 2011).

However, structured as a clinical initiative only, preconception care cannot be expected to achieve significant improvements in the women's health (Moos 2010). Preconception health promotion rather needs to encompass education and counselling to encourage individual behavioural change by health professionals from various settings (e.g., general practice physicians, obstetricians, gynaecologists, nurses, midwives), with further support from community-based public health programs, effective policies at local and national levels, as well as involvement of financial systems (Centers for Disease Control and Prevention (CDC) and Agency for Toxic Substances and Disease Registry (ATSDR) 2006).

A response to the concern that "preconception health is pronatalist and has the potential to frame women as nothing more than vessels for growing healthy offspring" is the recommendation to healthcare providers to simultaneously offer non-pregnancy related reasons for adopting healthy behaviours and emphasise their positive impact on the general health of women themselves, as part of primary care (Moos 2010). All women deserve this care in their own right, irrespective of their childbearing desire. Therefore any routine healthcare encounter (for instance, every time women are screened for cervical cancer) should be regarded as an opportunity to address women's health needs and promote women's own wellness as a whole, including the provision of lifestyle advice to encourage healthy weight. This strategy would have a beneficial impact on women's future health by



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reducing obesity-related complications, which in itself is an important outcome; for those women who do become pregnant, the benefits are likely to extend to the next generation.

Some obese women may enter **pregnancy** without receiving preconception counselling or without achieving significant changes in their weight. For these women, pregnancy may offer the opportunity to intervene, so that their risk of developing complications related to excessive weight, both for themselves and their children, is minimised. Obese pregnant women are recommended to restrain the amount of weight they gain (American College of Obstetricians and Gynecologists 2005), but not to lose weight, which could negatively impact on fetal growth (Dodd and Robinson 2011).

Despite the guidelines proposed by the Institute of Medicine (detailed in Section 2.2.3), there are still large inconsistencies in the advice regarding optimal GWG according to pre-pregnancy BMI given by health practitioners (Phelan 2010). Although obese women gain, on average, less weight during pregnancy compared to normal weight women, they are more likely to exceed the GWG recommended by IOM, as shown in previous studies (Althuisen *et al.* 2009; Olson *et al.* 2003) and supported by data from the Generation 1 study (Section 4.1.1.4). Difficulty adhering to the recommended GWG may partly stem from time and financial constraints, and these barriers need to be taken into account in future health promotion strategies (Phelan 2010).

A recent systematic review of randomised controlled trials of dietary and exercise interventions in pregnancy for overweight or obese women indicated no statistically significant differences in mean GWG, large-for-gestational-age infant and other outcomes, between women who did and those who did not receive the interventions (Dodd *et al.* 2010). Cognitive behavioural therapy for obesity, which is known to be effective mainly in the short term (Van Dorsten and Lindley 2008), has not been evaluated in pregnant women (Denison and Chiswick 2011), but could be a promising way forward given that it is tailored on individual's needs, providing practical strategies to address psychological barriers to the long-term adherence to healthy weight behaviour (Cooper and Fairburn 2001). Weight loss drugs (e.g., Sibutramine, Orlistat) have not been licensed for use in pregnancy (Denison and Chiswick 2011) and are not likely to be an acceptable solution. The findings from the ongoing Australian multicentre RCT evaluating the effectiveness of a combination of periodic dietary, exercise and behavioural advice in pregnancy to limit gestational weight gain in overweight and obese women (Dodd *et al.* 2011), are much awaited.

During pregnancy, many women tend to be motivated to adopt lifestyle changes for the benefit of their future offspring, and these behaviours are likely to perpetuate postpartum (Phelan 2010).



Although vigorous physical activity cannot be recommended to women during pregnancy due to the potential adverse effects on fetal growth (Kennelly *et al.* 2002), some level of exercise is beneficial for woman's cardiovascular function and insulin sensitivity, as well as for fetal growth (Clapp 2006a; Clapp 2000). However, a number of socio-demographic factors, medical (in particular obstetrical) history, and pregnancy symptoms have been identified as predictors of lower physical activity level among pregnant women, which could affect effectiveness of exercise interventions. They include older age, lower educational attainment social disadvantage in general, obesity, and early pregnancy symptoms (nausea, vomiting and lower back pain) (Foxcroft *et al.* 2011).

Therefore, exercise interventions during pregnancy tailored to take into account these factors (and potentially others as they are identified) and offer greater support to women from lower SES groups might prove more effective in controlling gestational weight gain.

Based on the current study, maternal GWG does not play an independent role in either child obesity or insulin resistance. Gestational weight gain rather interacts with maternal pre-pregnancy BMI to synergistically increase child central adiposity. This latter finding implies that optimising only GWG would be insufficient to reduce central adiposity in the child and that in addition, achieving an optimal pre-pregnancy BMI is required. That said, intervening in pregnancy to prevent excessive weight gain has immediate benefits (e.g., reducing the risk of preeclampsia or caesarean section) and gives the opportunity to reduce maternal obesity risk prior to subsequent pregnancies, given that GWG (and not pre-pregnancy weight status) is the strongest determinant of postpartum weight retention (Linné *et al.* 2004; Rooney and Schauburger 2002).

Although maternal glucose tolerance status during pregnancy does not appear to influence child body size and adiposity in this cohort, there is an indication that exposure to maternal GD increases child insulin resistance. Moreover, if children exposed to maternal GD also become obese, their risk of developing insulin resistance is even greater than for their counterparts. These findings have further implications for female offspring, who could perpetuate insulin resistance and GD cycle over subsequent generations. Management of GD with diet, exercise and insulin when required has been shown beneficial for preventing perinatal complications (except for labour induction) in both mothers and children (Alwan *et al.* 2009), so there is enough evidence to intervene in women with GD. However, there is no data on long-term outcomes of such interventions, hence it would be reasonable to recommend organising long-term follow-up of existing trials on GD management to evaluate the effects on child outcomes beyond birth, in particular on child glucose-insulin homeostasis. Based on Generation 1 data, one could not suggest the need for intervention in

women with milder degrees of gestational glucose intolerance, but more research (using data from larger cohorts) should be done to explore in more detail the influence that maternal BGGI may have during pregnancy and after birth, birth for the women and their children.

The **inter-conception period**, that is the period following childbirth and before the next pregnancy, could also provide an opportune time window for engaging with women in targeted programs to enhance postpartum weight loss, minimise weight retention and avoid obesity development, which would further impact on future pregnancies. Any program designed to improve women's weight status after childbirth needs to recognise the numerous challenges that women face during this period, including time constraints, motivation issues (sometimes related to postpartum depression) and the need for sustained support (Montgomery *et al.* 2010).

There is no standard definition of what constitutes excessive postpartum weight retention or what an optimal rate of weight loss would be (Amorim Adegboye *et al.* 2008). It has been estimated that by one year postpartum, women retain on average 0.5-4 kg (Linné and Rössner 2003; Olson *et al.* 2003). This postpartum weight retention appears to be mostly influenced by excessive GWG, low SES and parity (Linné and Rössner 2003). It has been indicated that women who do not lose the weight they had gained during pregnancy by 6 months postpartum are more likely to become obese 10 years later (Rooney and Schauburger 2002), suggesting that encouraging postpartum weight loss would be beneficial.

The best strategy to achieve postpartum weight loss has not been clearly established, mainly due to inconsistencies related to the potential effects on breastfeeding (quantity and quality of breast milk) (Amorim Adegboye *et al.* 2008). A recent systematic review concluded that attention to diet alone or associated with exercise enhance postpartum weight loss, while exercise alone does not have a significant effect on weight loss, but improves woman's cardio-vascular fitness; both approaches are safe in breastfeeding women (Amorim Adegboye *et al.* 2008).

Supporting exclusive breastfeeding for at least 6 months (World Health Organization 2001) has been shown to have multiple health benefits, including favourable effects on women's body size and their children's future metabolic health (Toschke *et al.* 2007). While a great majority of mothers initiate breastfeeding early postpartum, with rates exceeding 80% in countries such as Australia and the USA (Haas *et al.* 2006; Scott *et al.* 2006), continuation of breastfeeding during the following few months remains below the recommended targets, with less than half of the infants still receiving breast milk by the age of 6 months (Donath and Amir 2000). In other words, identifying effective

strategies to increase breastfeeding duration has been and continues to be a challenge.

Breastfeeding duration has been observed to be much shorter in obese compared to normal weight mothers (Oddy *et al.* 2006), which suggests that more effective support is needed for obese women. Breastfeeding support may be provided by a range of people with variable involvement in mothers' lives, including the fathers (particularly when living together), other family members (with frequent contact with the mother), doctors, midwives, nurses (which should extend beyond the postpartum period), lactation consultants, breastfeeding support groups, employers (for when breastfeeding mothers return to work) and last, but not least important, the community, protecting breastfeeding in public (legislation being in place in some countries, including Australia, UK, and the USA) (Clifford and McIntyre 2008).

In addition to health promotion in women at an individual level (at all stages of their reproductive life, during pre-conception period, pregnancy, or postpartum), more public health attention needs to be directed towards strategies to optimise weight status in women of childbearing age at the **population level**, by favourably influencing the obesogenic environment.

Although obesity has been recognised as a global health priority (World Health Organization 2000), population strategies developed to date to control the risk factors have been largely ineffective (Gortmaker *et al.* 2011; Swinburn *et al.* 2011), as reflected by the persistently high obesity prevalence in both developed and developing countries (Finucane *et al.* 2011), with the well known socio-economic gradient (Adler and Newman 2002).

There is growing evidence that the current obesity epidemic is due to a chronic energy imbalance gap, secondary mainly to major shifts in the global food system that have occurred over the last couple of decades (Gortmaker *et al.* 2011), with the fastest increase in high income countries (Finucane *et al.* 2011). These changes are related to the rising consumption of mass prepared food (as opposed to individual preparation of food in the past) and the greater availability of highly processed, energy-dense, nutrient-poor foods, which have become cheap, easily accessible and aggressively promoted by marketing campaigns (Gortmaker *et al.* 2011).

Based on the evaluation of cost-effectiveness of obesity interventions in adults in Australia (Vos *et al.* 2010), the two most cost effective interventions at population level are arguably the introduction of 10% tax on unhealthy foods and beverages, and making nutrition information visible at-a-glance on food packaging to help consumers select healthier foods ('front-of-pack traffic light nutrition labelling') (Sacks *et al.* 2011). However, it is not clear that such measures could be implemented soon.

It is increasingly recognised that weight is not only a matter of individual responsibility for food choices and exercise habits, but also a matter of the society that shapes the living environment, influencing personal choice. “It takes more than just willpower to maintain a healthy weight – a strong support system is necessary” (Strategies to Overcome and Prevent (STOP) Obesity Alliance 2010). The STOP Obesity Alliance launched in 2007 also highlights the importance of emphasising through consistent messages to the public health-related benefits of not being obese rather than those related to body image (appearance) (Strategies to Overcome and Prevent (STOP) Obesity Alliance 2010).

A comprehensive approach to obesity prevention needs to encompass strategies at multiple levels of the society, combining direct initiatives, which rely on the beneficial influence of certain interventions on energy balance, with documented cost-effectiveness, and indirect, cross-cutting actions, with less documented cost-effectiveness, which support the implementation of direct interventions (Institute of Medicine 2010).

As there is no consensus regarding the most effective strategies to prevent and control the complex issue of obesity worldwide, the need for sustained, integrated efforts to create successful programmes to lower obesity rates has to be a priority (Australian Government and National Preventative Health Taskforce 2009b; Gortmaker *et al.* 2011). This would be possible within a co-ordinated ‘systems approach’, involving multiple parties: governments, international agencies, private sector, civil society, health professionals and individuals (Australian Government and National Preventative Health Taskforce 2009b; Gortmaker *et al.* 2011).

**Governments** have been recognised as “the most important actors in reversing the obesity epidemic” (Gortmaker *et al.* 2011), as their role is to protect public interest. As recommended by WHO (World Health Organization 2009b) and outlined in the recent paper by Gortmaker *et al.* (2011), core actions for governments to reduce obesity prevalence are related to leadership, healthy public policies (e.g., enforcement of subsidies for healthy foods and taxes for junk foods; creation of safe walking, cycling and recreation environments), funding, supporting research in obesity prevention, workforce development, networks for co-ordination, and communications (e.g., establishing targets for the food industry on nutrient composition of foods, and ensuring nutrition claims comply with a nutrient profiling system). Therefore, commitment of the health system for obesity prevention needs to be accompanied by actions across a range of other sectors such as education, agriculture, transportation, urban planning, or finance (Australian Government and National Preventative Health Taskforce 2009b; Gortmaker *et al.* 2011).

Actions regarding obesity prevention and control have also been proposed for **international agencies** (e.g., WHO, Food and Agriculture Organisation, World Food Programme) including the need for global leadership (Gortmaker *et al.* 2011; World Health Organization 2009b). The **private sector** (encompassing food and built environment industries, and media) play a key role in influencing individuals' choice regarding their lifestyle, mainly through lobbying activities which often undermine obesity prevention policies (Gortmaker *et al.* 2011). The recommended actions include the development of less processed food products with healthier nutrient composition, voluntary restrictions on marketing promotions of unhealthy foods, appropriate nutrition labelling of food packaging (Gortmaker *et al.* 2011). **Civil society** (including consumer associations, charities, foundations, professional associations, sporting clubs) plays an important advocacy role in relation to promotion of healthier environments and lowering obesity rates (particularly in democratic countries) (Gortmaker *et al.* 2011). **Health professionals** need to monitor their patients' weight status and support them to maintain or achieve healthy weight (Gortmaker *et al.* 2011). Finally, **individuals** are encouraged to opt for a healthy lifestyle, by choosing healthy foods and activities (Gortmaker *et al.* 2011; Swinburn *et al.* 2011), but they need support from all the other sectors.

A number of issues have been identified and need consideration in relation to the implementation of policies on obesity prevention and control, such as feasibility (based on the availability of trained staff, leadership involved and existing programmes), sustainability (influenced by the level of policy support and funding), effects on equity (with regard to SES, ethnicity, gender), potential side effects (on other health conditions, household costs) and acceptability to stakeholders (Swinburn *et al.* 2005). Although high intensity interventions are usually more effective, they reach a limited segment of the population due to the higher cost, hence low intensity interventions, often with mixed effects, are considered more feasible at population level, as a greater number of people may benefit from them at lower costs (Gortmaker *et al.* 2011).

In Australia, a National Preventative Health Strategy to halt the rise in obesity prevalence has been recently put forward as part of the initiative of turning Australia into the healthiest country by 2020 (Australian Government and National Preventative Health Taskforce 2009b). In addition to the multilevel directions for action outlined above, this strategy recommends a staged implementation of actions, so that subsequent actions can be informed by evidence of effects from the previous phases, in a cyclical approach ("do, measure, report – do, measure, report") (Australian Government and National Preventative Health Taskforce 2009b). For the time being, it appears that Australian Government has had limited response to the recommendations advanced by the National

Preventative Health Taskforce in 2009 (Australian Government and National Preventative Health Taskforce 2009b) in adopting laws that would promote healthy eating, with the food industry being pressured to self-regulate its actions and practices (MacKay 2011 - in press).

While no specific strategies have been developed for women to prevent weight gain or promote weight loss, the need for such actions has been flagged in the Technical report on obesity, particularly in women with children (Australian Government and National Preventative Health Taskforce 2009a). To be effective in combating obesity among women in community settings, these strategies would have to be tailored to address personal, social and environmental constraints that women in particular may be confronted with when attempting to adopt healthy behaviours. A number of perceived barriers to healthy eating and exercise habits have been reported by young women, including lack of motivation, time limitations (mainly due to work responsibilities), lack of social support (particularly in women with children), and cost constraints (Andajani-Sutjahjo *et al.* 2004). Getting married, motherhood and commencement of paid work have been associated with lower physical activity levels in women (Brown and Trost 2003). Among overweight women, other reasons for not engaging in physical activity were feeling too fat or too embarrassed to exercise (Ball *et al.* 2000) and feeling less confident about being able to engage in physical activity (Jewson *et al.* 2008). Taken together, these findings suggest that community-based interventions to prevent obesity in women should tackle these perceived barriers, by enhancing women's motivation, being family-friendly, and providing social support for adopting healthy behaviours. For overweight women, it has been recommended that interventions should also consider developing women's skills and confidence to improve self-efficacy with regard to their physical activity behaviour (rather than targeting attitudes through health messages) (Jewson *et al.* 2008).

An example of a feasible low-intensity community-based strategy to prevent weight gain is the Healthy Lifestyle Program. A cluster-randomised controlled intervention was conducted in a population of middle SES women with children, apparently healthy, who were recruited through their children's schools in Victoria, Australia (Lombard *et al.* 2009). The intervention was based on social cognitive theory (namely goal setting, self monitoring, social support and relapse prevention training) and consisted of four face-to-face group sessions on behaviour change, followed by ongoing support for one year (Lombard *et al.* 2009). With regard to self efficacy, women appeared to be more confident in making a change towards a healthier diet than towards increasing levels of physical activity (Lombard *et al.* 2009). In this study, several factors were identified by women as facilitating behaviour change, such as intervention delivery by a health professional in a community setting

(school), various types of reminders (phone, text, mail), and handouts on physical activity and diet (Lombard *et al.* 2009).

The US Task Force to prevent obesity in women, comprising almost 20 health advocacy organisations, is currently aiming to advance understanding among policy makers with regard to the specific impact obesity has on women's health and to launch practical initiatives, that are "culturally, gender and age-appropriate", with focus on multiple levels (family, community, wider society) (Strategies to Overcome and Prevent (STOP) Obesity Alliance Task Force on Women 2010).

In summary, this section of the Discussion has outlined arguments for an intergenerational value of preconception care to all women of reproductive age, with an emphasis on behavioural change related to optimal weight status. Reasons for implementing strategies for limiting weight gain and improving glycaemic control during pregnancy were reiterated, with acknowledgement that despite the interventions trialled in these areas, they remain a largely unsolved challenge. The last body of this section described the multifaceted approach to obesity prevention and control at a population level, highlighting the need for co-ordinated action by wider community, civil society, private sector, local and national authorities.

## 5.5 Future directions for research

The work presented in this thesis invites further research principally in two key areas: to disentangle intrauterine programming from genetic or social postnatal influences, and to identify effective interventions with the potential to optimise maternal weight status prior to conception, and glycaemic control during pregnancy, with long-term beneficial effects for the offspring.

First, it is worth mentioning that, given the comprehensive data collected so far and the follow-ups planned for this cohort of children, it would be possible to undertake further work related to the current project, but beyond the scope of the present study. This may include extending the analyses carried out in this project, considering programming of other (related) health outcomes by fetal overnutrition, and investigating the changes in body size, body composition, fat pattern or insulin resistance longitudinally, in a life course approach. The relationship between maternal factors associated with fetal overnutrition and child global obesity or insulin resistance could be further investigated by considering additional potential mediating influence of other factors (that are on the pathway between the intrauterine environment and child obesity). These factors may include child's



birth weight (previously considered in numerous studies, including those by Blair *et al.* (2007), Gillman *et al.* (2003), Lawlor *et al.* (2007b), Oken *et al.* (2007)), postnatal growth trajectory (i.e., child BMI at younger ages) (Crume *et al.* 2011b), breastfeeding (Blair *et al.* 2007; Gillman *et al.* 2003; Oken *et al.* 2007; Oken *et al.* 2008; Reilly *et al.* 2005), diet, and physical activity (Reilly *et al.* 2005).

In addition, sex-specific analyses could be performed in order to identify potential differences in the associations of interest between girls and boys. This approach would be justified by the emerging evidence of sexual dimorphism in the programming of body composition (Huang *et al.* 2011; Labayen *et al.* 2006) or insulin action (Sugden and Holness 2002). The underlying mechanisms of these differences are still largely unknown, but they could be linked to the sex-specific pathways underlying the development of most organs, to different epigenetic marks leading to different gene expression, and to the influence of sex hormones (Gabory *et al.* 2009). However, by undertaking sex-specific analyses, statistical power would be reduced. (In addition to the power-related issue, sex did not meet criteria for a potential confounder and was therefore not included in the analyses presented in the current study.)

Although a research question that was beyond the scope of the current project, investigating whether fetal overnutrition contributes to the programming of child insulin secretion in addition to that of insulin resistance could help better understand the area of intrauterine programming of impaired glucose tolerance and type 2 diabetes prior to puberty. There is very little information in the current literature regarding this aspect (Bush *et al.* 2011), so more research in this area would be welcome.

Investigating longitudinal changes in child BMI z-score, %BF, WHtR and HOMA-IR in relation to intrauterine exposures could contribute to better understanding of the life course epidemiology of obesity, body composition, fat pattern and insulin resistance. This could be achieved by examining cohort members at later points in time, including at least one follow-up during adolescence, when some further intrauterine influences on metabolic outcomes might become apparent.

**Unravelling intrauterine from genetic or postnatal environmental influences** on childhood obesity and insulin resistance involves clearly demonstrating that maternal exposures of interest are a direct *cause* of these outcomes in children. The relatively limited ability to infer causation is a drawback that applies to any observational study. Although regarded as the highest quality observational study design, providing level II evidence for aetiology (surpassed only by a systematic review of level II studies) (National Health and Medical Research Council 2009), a prospective cohort study can only provide evidence of an association between exposures and outcomes, which may or



may not be causal. However, as indicated by Bradford Hill (1965), causality may be inferred if the association of interest is characterised by a combination of strength, consistency upon repetition across different studies (with different populations, settings and time points), specificity (but acknowledging that one condition may have multiple aetiologies and that one exposure could contribute to several outcomes), time sequence, biological gradient (dose-response), biological plausibility and coherence of explanation, experimental evidence (which makes epidemiological evidence more convincing), and analogy with other relationships.

In order to consolidate the evidence, as recommended by Bradford Hill (1965), findings presented in this thesis would benefit from replication in other cohort studies, which should address the main limitations identified in Section 5.2. Broadly, these approaches could include undertaking similar work within larger prospective birth cohorts, with enhanced participation at follow-up (compared to other international cohorts), to permit detection of potential associations between exposures with relatively low-prevalence (such as GD or BGGI) and obesity or insulin resistance in the offspring. In addition, glucose levels at OGCT and OGTT could be considered as continuous rather than categorical variables to predict metabolic risk in the child, given the documented linear relationship between glucose levels at OGCT and the risk of adverse perinatal outcomes (HAPO Study Cooperative Research Group 2008).

Theoretically, a randomised controlled trial (RCT) of optimising weight status in women of childbearing age, with long-term follow-up of the children would provide a more conclusive answer regarding the possibility of preventing obesity in the offspring than the current cohort study. Practically, however, such a trial could face several issues, in addition to the challenges related to costs, time and the constrained external validity. From an ethical point of view, it would seem inequitable to deprive women randomised to the controlled group of the well-known benefits for the women themselves of achieving normal BMI prior to becoming pregnant. There could be logistical problems related to timing of weight loss and timing of the pregnancy (i.e., some women might become pregnant a long time after the beginning of the trial, or in some cases, never, which would impact on the timing of the follow-up and, potentially, on sample size). Similarly, expanding on the beneficial effects of GD treatment for perinatal outcomes (Alwan *et al.* 2009), a long-term follow-up of existing RCTs to evaluate the effects of these interventions on child body size, adiposity and, more importantly, insulin resistance beyond infancy would seem appealing. Also, it appears relevant and important to conduct more research (larger cohort studies) to explore in more depth the influence of

milder degrees of gestational glucose intolerance on child body size, adiposity and IR and identify suitable management for these women.

For studies assessing early life origins of health and disease, characteristically involving a long time lag between exposures and outcomes assessment, several approaches have been recently proposed to improve the ability to infer causation and prove fetal programming (Davey Smith 2008). They include:

- comparing maternal-offspring and paternal-offspring associations: if a stronger maternal-offspring association is identified, this indicates a specific effect of intrauterine milieu (e.g., fetal overnutrition in case of maternal obesity or glucose intolerance in pregnancy) on the offspring;
- examining how a certain exposure (e.g. maternal obesity or diabetes) influences child outcomes when it occurs prior or after pregnancy;
- conducting discordant sibling exposure studies: when one sibling is born prior to the development of a certain condition in the mother (e.g., diabetes), thus being unexposed, while the younger sibling is exposed to that particular condition;
- Mendelian randomisation studies, in which maternal genotype is considered an indicator of environmentally modifiable influences on the intrauterine environment, have the advantage of minimising residual confounding in examining the association between a given exposure and the outcomes of interest (Davey Smith 2008). With respect to this project, in order to test the hypothesis that maternal obesity, glucose intolerance during pregnancy and excessive GWG, through the modified intrauterine environment, have a causal effect on child obesity and insulin resistance, it would have been helpful to assess the influence of maternal genetic variants related to obesity (i.e., FTO gene (Frayling *et al.* 2007)), blood glucose levels (i.e., glucokinase gene (Weedon *et al.* 2006)) or excessive gestational weight gain conditioned on offspring genotype (Davey Smith 2008).

The above mentioned specialised approaches could not be applied in this study, as necessary data were not available. (Indeed, such data are currently scarce worldwide.)

The above issues notwithstanding, the current findings provide further support for the well recognised need to **identify effective interventions** to prevent and control obesity and GD in women of childbearing age. Interventions that address optimising weight status prior to conception in women and improving glucose tolerance status during pregnancy, particularly in obese women,

could prove beneficial not only for the women themselves, but also for reducing children's propensity to gain weight or to develop metabolic perturbations, such as insulin resistance.

There is debate with respect to what constitutes sufficient evidence to translate research findings into practice in public health. It has been proposed that the concept of evidence should move from the 'best possible' evidence that could be provided by RCTs to the 'best available' evidence (Glasgow and Emmons 2007). In principle, an association that appears to be well documented, strong and consistent in independent studies (Bradford Hill 1965), even if not entirely specific and without a clear biological underlying mechanism, may be regarded as good evidence for action in the absence of an RCT (Baum 2008). An example of such an approach is the "Back to sleep" campaign (American Academy of Pediatrics and Task Force on Infant Sleep Position and Sudden Infant Death Syndrome 2000), which, based on previous observational (not experimental) studies, raised public awareness regarding the risk of sudden infant death syndrome associated with prone sleeping and recommended back sleep position in infants. Likewise, the well established link between smoking and lung cancer has led to the development of strategies to promote smoking cessation without the need of confirming the association in a trial (US Department of Health and Human Services 1990). Translating research findings into practice is a complex process influenced by the relevance of the health issue, sustainability of behaviour change (Colditz and Taylor 2010), associated costs (both of action and inaction) (Glasgow and Emmons 2007), and involves advocacy and lobbying (Baum 2008). Existing epidemiological evidence on the adverse effects of obesity and glucose intolerance during pregnancy (in the short- and the long-term, both for the women and their children) arguably deserves to be translated into practical approaches to optimise women's weight and improve their glucose control during pregnancy.

## 5.6 Conclusion

Located within the paradigm of developmental origins of health and disease and building on data collected from a contemporary prospective birth cohort study in Australia, this thesis has presented findings for early (*in utero*) origins of obesity and insulin resistance in pre-pubertal children, particularly in relation to maternal obesity and glucose intolerance during pregnancy. The current study has extended the earlier data on the deleterious effects of maternal pre-pregnancy obesity on child global obesity before puberty by establishing that even more specific measures of adiposity, such as percentage body fat and central adiposity, are influenced by maternal obesity prior to

pregnancy, taking into account potential confounders. Another novel contribution of this study is the finding of an exacerbated risk of accumulating abdominal fat in children whose mothers had greater body size prior to pregnancy and either gained more weight during pregnancy or developed borderline gestational glucose intolerance. This research has investigated for the first time the long-term influences of maternal gestational glucose intolerance across the spectrum, from borderline to GD, on child obesity and insulin resistance. Although global obesity and adiposity in pre-pubertal children do not appear to be associated with maternal glucose tolerance status during pregnancy, insulin resistance seems to originate, at least in part, from intrauterine exposure to maternal GD and these perturbations in glucose-insulin homeostasis are exacerbated if the child becomes obese.

The findings generated from this thesis contribute to the body of evidence in relation to major public health implications for the prevention of childhood obesity and related metabolic disorders, which are increasingly observed in Western societies. While addressing factors that occur during pregnancy, such as glucose intolerance and weight gain, is, without doubt, important, more public health strategies need to be developed to promote the benefits of women conceiving at an optimal weight status, both for the mother and the child in the future.

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