DETERMINANTS OF GROWTH AND BODY COMPOSITION IN SINGAPOREAN CHILDREN

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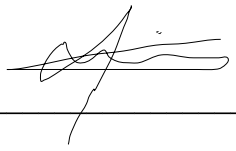
DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.



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Summary

Background

A growing body of evidence suggests that obesity and cardiometabolic disorders in adulthood may originate early in life. As adverse early-life size and growth patterns may influence pathways determining subsequent adiposity and metabolic disease, insights into the determinants of early infant size, growth and development would be crucial to understand the pathways affecting future metabolic compromise in Asian populations.

Objectives

This dissertation aims to:

i) uncover the factors which influence size and body composition of newborns in Singapore
ii) study the factors that can predict patterns of growth and adiposity in early childhood, during the first three years of life

Methods

This study is centered on the Growing Up in Singapore Towards healthy Outcomes (GUSTO) birth cohort, a comprehensively phenotyped parent-offspring cohort in the first few years of life, recruited from Singapore's two major public maternity units [National University Hospital (NUH) and KK Women's and Children Hospital (KKH)]. Extensive maternal evaluations including ultrasound scans for fetal growth, physical examinations and oral glucose tolerance testing were conducted during pregnancy. Umbilical cord specimens were obtained at delivery and analysed by genome-wide

methodologies for RNA expression, DNA methylation characterization and genotyping. Infant growth was monitored frequently at multiple timepoints, complemented by body composition assessments using multiple measurement methods including anthropometry and air displacement plethysmography.

Results

A new reference for size-at-birth of Singapore newborns at 35 to 41 weeks of gestation was established, as well as a formula for estimating body composition amongst Asian neonates using subscapular skinfolds (SSF), weight (W), gender (G), and gestational age (GA) [prediction equation for neonatal fat mass = -0.022 + (0.307 × W) - (0.077 × G) - (0.019 × GA) + (0.028 × SSF), $R^2 = 0.811$]

I. *In-utero determinants of size, body composition and growth*

Maternal glycemia at 26-28 weeks' gestation, especially during the fasting state, showed strong positive continuous associations across the range of glucose levels in relation to excessive neonatal adiposity [for each standard deviation increase in fasting glucose, OR=1.31, 95% CI: 1.10–1.55 for large-for-gestational age; OR=1.72, 95% CI: 1.31–2.27 for percent body fat above 90^{th} centile; OR=1.64, 95% CI: 1.32–2.03 for sum of skinfolds above 90^{th} centile], even at values below those defined as hyperglycemia, after correcting for potential confounders. The effect of gestational glycemia on postnatal growth was limited to growth deceleration during the first 3 weeks to 3 months of life [B(95%C.I)= -0.23(-0.42,-0.04) for weight standard deviation score (SDS)], followed by transient growth acceleration between 9-15 months [B(95%C.I)= 0.26(0.05,0.48) for weight SDS, B(95%C.I)= 0.26(0.05,0.47) for

body mass index (BMI) SDS]. The effect of maternal BMI during pregnancy on postnatal growth persisted from birth into early childhood. Maternal obesity, ethnicity, and parity may confer different susceptibility to greater adiposity in response to maternal glycemia only at two years of age.

II. *Postnatal determinants of adiposity and growth*

Varied effects of breastmilk intake on early postnatal growth amongst offspring exposed and unexposed to gestational diabetes (GDM) *in-utero* were observed. Offspring of mothers without GDM who were on breastmilk feeding predominantly exhibited growth deceleration in the first year of life [B(S.E) = -0.31(0.09) for weight SDS; B(S.E) = -0.22(0.09) for length SDS; B(S.E) = -0.22(0.09) for BMI SDS], whilst offspring of GDM mothers with greater estimated breastmilk intake however, did not exhibit the same decelerated growth during the early postnatal period [B(S.E) = 0.45(0.19) for weight SDS; B(S.E) = 0.46(0.19) for BMI SDS].

III. *Genetic determinants of size, adiposity and growth*

The transcriptomic profiles of 15 genes (*AVP, IFI6, SET, HMGB2, RAD1, AIFM1, APAF1, CALM2, NUDT1, IKBKG, CCNL1, LIG1, PITX, TNIP2, RBP*) from umbilical cords showed significant associations with fetal growth between 2nd to 3rd trimester of pregnancy, as well as with subsequent postnatal growth. Offspring with polymorphic variants of a known adiposity-associated gene [melanocortin-3-receptor (*MC3R*)] also showed greater predisposition to overweight and obesity during early childhood [OR=2.23, 95% CI: 1.08-4.61 for overweight at 2-years; OR=2.05, 95% CI: 1.17-3.58 for overweight at 3-years].

Conclusion

This study has identified the various developmental risk factors influencing adverse infant size, growth and adiposity outcomes during the first three years of life in a multi-ethnic Asian population. Future studies would involve examining whether these risk factors that operate during early growth and development would have long-term repercussions on increasing prevalence of obesity, diabetes and other cardio-metabolic disorders.

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LIST OF ABBREVIATIONS

%BF	Percent body fat
11β-HSD1	11β-hydroxysteroid dehydrogenase type 1
11β-HSD2	11β-hydroxysteroid dehydrogenase type 2
2h-PG	2-hour post-challenge glucose
AC	Abdominal circumference
ADP	Air-displacement plethysmography
AGA	Appropriate-for-gestational age\
AIFM1	Apoptosis-inducing factor, mitochondrion-associated, 1
ALSPAC	Avon Longitudinal Study of Parents and Children
ANOVA	Analysis of variance
APAF1	Apoptotic peptidase activating factor 1
AVP	Arginine vasopressin
B	Regression coefficient
BDNF	Brain-derived neurotrophic factor
BEBQ	Baby Eating Behavior Questionnaire
BF	Breastfeeding
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BPD	Biparietal diameter
CALM2	Calmodulin 2
CCNL1	Cyclin L1
CEBQ	Child Eating Behaviour Questionnaire
CI	Confidence interval
Ct	Threshold cycle
D_2O	Deuterium oxide
DARLING	Davis Area Research on Lactation, Infant Nutrition and Growth
DOHaD	Developmental Origins of Health and Disease
DXA	Dual-energy X-ray absorptiometry
EDF	Equivalent degrees of freedom

EFSOCH	Exeter Family Study of Childhood Health
EPOCH	Exploring Perinatal Outcomes among Children
ETV5	Ets variant 5
FGR	Fetal growth restriction
FL	Femur length
FPG	Fasting plasma glucose
FTO	Fat-mass and obesity-associated gene
GA	Gestational age
GAIC	Generalised Akaike Information Criterion
GDM	Gestational diabetes mellitus
GNPDA2	Glucosamine-6-phosphate deaminase 2
GUSTO	Growing Up in Singapore Towards healthy Outcomes
HAPO	Hyperglycemia and Adverse Pregnancy Outcomes
HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
HMGB2	High mobility group-box 2
IADPSG	International Association of Diabetes and Pregnancy Study Groups
ICC	Intraclass correlation
IGF-1	Insulin growth factor 1
IGF-1R	Insulin growth factor 1 receptor
IGF-2	Insulin growth factor 2
IGFBP-1	Insulin growth factor binding protein 1
IFI-6	Interferon, alpha-inducible protein 6
IKBKG	Inhibitor Of Kappa Light Polypeptide Gene Enhancer In B-Cells
IL-6	Interleukin-6
IVF	In-vitro fertilization
KCTD15	Potassium channel tetramerisation domain containing 15
KKH	KK Women’s and Children Hospital

LD	Linkage disequilibrium
LGA	Large-for-gestational age
LIG1	Ligase 1
LOA	Limits of agreement
MAD	Median absolute deviations
MC3R	Melanocortin-3-receptor
MC4R	Melanocortin-4-receptor
MRI	Magnetic resonance imaging
NCD	Non-communicable diseases
NEGR1	Neuronal growth regulator 1
NHANES	National Health and Nutrition Examination Survey
NUDT1	Nucleoside diphosphate linked moiety X-type motif 1
NUH	National University Hospital
ODM	Offspring of diabetic mothers
OGTT	Oral glucose tolerance test
OR	Odds ratio
PITX	Pituitary homeobox 2
PHLDA2	Pleckstrin homology-like domain family A member 2
PPAR	Peroxisome proliferator-activated receptor
PROGRAM	Programming Factors for Growth and Metabolism
qRT-PCR	Quantitative real-time polymerase chain reaction
RBP	Retinol binding protein
RXRA	Retinoid-X receptor alpha
SD	Standard deviation
SDS	Standard deviation score
SET	SET Nuclear Oncogene
SFT	Skinfolds thickness
SGA	Small-for-gestational age
TMEM18	Transmembrane protein 18
TNF-α	Tumor-necrosis factor alpha

TNIP2	TNFAIP3 interacting protein 2
VDR	Vitamin D receptor
WHO	World Health Organization

Chapter 1: Introduction and literature review

1.1 Introduction

Chronic non-communicable diseases (NCDs) represent a major burden and public health problem in Southeast Asia. Diseases such as stroke, cancer, obesity, diabetes and cardiovascular disease threaten this region with a rapidly growing population of almost 600 million people(1). The Global Burden of Disease projected an estimated 2.6 million people from the 10 countries in Southeast Asia died from chronic NCDs in 2005. With increasing exposure to risk factors, these numbers are estimated to increase to 4.2 million deaths by 2030(2). The proportion of age-adjusted deaths due to chronic NCDs was observed to be the greatest in countries with highest gross national incomes such as Singapore and Brunei, and 30% of all deaths due to chronic NCDs occurred in people of age 15-59(1), which represented the labour force of Southeast Asia and the most productive age group. This situation not only affects families, but entire economies, thus stressing the need for urgent action and a strong stance to combat against chronic NCDs.

1.2 Developmental Origins of Health and Disease

There is a growing body of research which suggests that adult chronic NCDs such as diabetes, obesity and cardiometabolic disorders (hypertension, heart disease etc) partly originates during intrauterine life. One of the earliest evidence echoing this hypothesis of developmental plasticity conferring later disease risk came from the Dutch famine study, which examined the offspring of women who conceived during the Dutch famine of 1944, when an embargo was placed on all food supplies to the Netherlands(3, 4). It was observed that

offspring of women exposed to the famine in early gestation showed significantly higher body mass index (BMI) and waist circumferences during adulthood compared to those who were not exposed to the famine. Later on in the 1980s, David Barker and his colleagues conducted a series of retrospective cohort studies at regions in England that had the highest rates of infant mortality, and observed that these regions also had the highest rates of mortality from coronary heart disease decades later(5). As the most commonly registered cause of infant mortality at the time was low birthweight, these observations led to the hypothesis otherwise known as the "Developmental Origins of Health and Disease (DOHaD)", which states that adverse influences early in development, and particularly during intrauterine life, can result in permanent changes in physiology and metabolism, which result in increased disease risk in adulthood. Over time, numerous epidemiological data and studies have demonstrated robust associations between small birth size and a greater risk of chronic disease including coronary heart disease(6-8), hypertension(9), stroke(7, 10), type 2 diabetes(11), and osteoporosis in later life(12, 13) (Table 1.1). These observations have led to a worldwide recognition that the DOHaD hypothesis has major public health implications. A recent World Health Organization Techinical Consultation concluded that, "The global burden of death, disability, and loss of human capital as a result of impaired fetal development is huge and affects both developed and developing countries"(14). Despite the known associations between small birth size and later disease risk, the report advocates a move away from simply low birth weight, to broader considerations of maternal well-being, and achieving the

optimal environment for the fetus to maximize its potential for a full and healthy life.

Table 1.1 Diseases linked with birthweight

Replicated and widely accepted association with small birth size
Hypertension
Coronary artery disease
Osteoporosis
Type 1 diabetes mellitus
Stroke
Dyslipidemia
Elevated clotting factors
Impaired neural development
Described but less well replicated and accepted association with small birth size
Chronic lung disease
Depression
Schizophrenia
Behavioural problems
Reduced uterine and ovarian size
Precocious pubarche
Breast cancer
Testicular cancer

Adapted from de Boo HA et al 2006 (15)

Exposures to risk factors during critical developmental periods can have long-term consequences, especially if the environment during childhood and adulthood differs from that which is predicted during fetal and infant life. This "mismatch" between the exposed environments at different stages in life may cue developmental responses leading to increased risk of disease later in life(16). Such concepts are crucial to current life-course strategies that aims to prevent and treat NCDs. In the life-course approach, risk of future disease continually increases as a result of declining plasticity and ability to mount sufficient responses to new challenges. Even though the greatest increase in

risk occurs in adult life, the trajectory towards that risk occurs much earlier before and during pregnancy, influenced by factors such as mother's diet and body composition, and also by fetal, infant and childhood development(17). Adopting a life course approach allows for identification of at-risk phenotypes and markers of risk at an early stage, with the possibility of implementing interventions at varying timepoints. Nutritional and other lifestyle interventions in early life may have a large effect on disease risk later, whilst later interventions may be impactful for vulnerable groups(17). These preventive measures would require a long-term investment, but may be more effective than population screening programs that identify the early stages of disease.

1.3 Epidemiological observations and experimental studies of DOHaD

1.3.1 Epidemiological observations

1.3.1.1 Maternal/*in-utero* factors

As described earlier, one of the most convincing evidence linking nutritional deficiency during pregnancy with development of adult metabolic disease was the Dutch famine study of 1944-1945(3, 4). During this period, daily rations to each individual were restricted to 400-800 calories per day, and this caloric restriction in pregnant mothers subsequently led to increased risks of glucose intolerance and heart disease in their offspring later in life(18). Such relationships in global nutrition during pregnancy with future disease outcomes have been reported in other epidemiological studies. A study on 626 subjects in Scotland whose mothers' food intake had been recorded during pregnancy showed that a high-meat, low-carbohydrate diet during pregnancy was associated with higher diastolic blood pressure in the offspring,

independent of maternal blood pressure, body size and smoking during pregnancy(19). In a double blind, randomised controlled trial exploring the long-term effect of calcium supplementation during pregnancy on the offspring's blood pressure during childhood, the authors found that calcium supplementation during pregnancy was associated with reduced mean systolic blood pressure, compared with the placebo group. The risk of high systolic blood pressure was also reduced amongst subjects receiving calcium supplementation, particularly among overweight children(20). These findings were echoed in another observational study by Gillman MW et al, on 936 6-month old infants from the Project Viva cohort, which showed reduced maternal mid-gestational calcium intake was associated with increased systolic blood pressure(21).

Besides maternal nutrition, exposure to an altered *in-utero* environment has been shown to have consequences later in life. Offspring of pregnancies complicated by maternal diabetes have been shown to have greater birth weight, and an increased risk of developing type 2 diabetes in later life as reported by Silverman BL et al(22) and Plagemann A et al(23). Observational studies have also documented how exposure to maternal diabetes *in-utero* may induce cardiovascular dysfunction in offspring during adulthood. Offspring of mothers with type 1 diabetes have been shown to have higher concentrations of markers of endothelial dysfunction and cholesterol-to-HDL ratio when compared with offspring of nondiabetic pregnancies(24). In another study, Bunt JC et al examined the effect of maternal diabetes status during pregnancy with cardiovascular disease in offspring during childhood in a cohort of Pima Indians. The authors reported that offspring of diabetic

pregnancies had significantly higher concentrations of HbA1c, higher systolic blood pressure and lower concentrations of HDL independent of adiposity, when compared to offspring of non-diabetic pregnancies(25). Taken together these studies highlight how exposure to diabetes *in-utero* would confer increased risk of developing metabolic diseases later in life.

Table 1.2: Summary of epidemiological studies associating maternal/*in-utero* factors with later metabolic diseases

Study	Sample size	Population source	Exposure	Outcome	Confounders addressed	Pattern of association
Ravelli AC et al 1976	702	Amsterdam	Mothers exposed to famine during gestation	Offspring glucose and insulin response	-	Prenatal exposure to famine associated with decreased glucose tolerance
Roseboom TJ 2000	736	Amsterdam	Mothers exposed to famine during gestation	Prevalence of coronary heart disease	Birth weight	Prevalence of coronary heart disease was higher in those exposed to famine in early gestation
Shiell AW et al 2001	626	Scotland	Consumption of meat and fish during pregnancy	Diastolic blood pressure in offspring	Maternal blood pressure, body size, smoking	High-meat, low-carbohydrate diet during pregnancy associated with higher diastolic blood pressure in the offspring
Gillman MW et al 2004	936	United States	Food frequency questionnaires during the second trimester of pregnancy	Systolic blood pressure in offspring	demographic, anthropometric, dietary, social, and economic variables	Reduced maternal mid-gestational calcium intake associated with increased systolic blood pressure
Belizan JM et al 1997	591	Argentina	Mothers randomly assigned calcium supplementation during pregnancy	Mean blood pressure, rate of high blood pressure in offspring	Randomized controlled trial	Calcium supplementation during pregnancy associated with reduced mean systolic blood pressure, compared with the placebo group.
Plagemann A et al 1997	198	Germany	Mothers with gestational diabetes and pregestational insulin-dependent diabetes	Offspring plasma insulin and blood glucose	Age and gender	Offspring of diabetic mothers showed impaired glucose tolerance and higher insulin levels
Silverman BL et al 1991	124	United States	Mothers with gestational diabetes and pregestational diabetes	Offspring birth weight	Age and gender	Offspring of diabetic mothers showed higher birth weights (birth weight > 90th percentile)
Manderson JG et al 2002	118	Belfast	Mothers with Type 1 diabetes	Offspring markers of endothelial dysfunction (plasma glucose, insulin, lipids etc)	Age, gender and social class	Offspring of diabetic mothers have higher concentrations of markers of endothelial dysfunction and cholesterol-to-HDL ratio
Bunt JC et al 2005	42	Pima Indian	Mothers with gestational diabetes	Offspring anthropometry, blood pressure glucose levels, HbA1c, cholesterol	Age and gender	Offspring of diabetic pregnancies had significantly higher concentrations of HbA1c, higher systolic blood pressure and lower concentrations of HDL

1.3.1.2 Size-at-birth

The observations that small size-at-birth and thinness during infancy is associated with increased risk of coronary heart disease, stroke, type 2 diabetes mellitus and the metabolic syndrome have been extensively replicated(26-28). Studies on associations between small size-at-birth and subsequent hypertension/coronary heart disease have been confirmed by many different groups of investigators in several countries, including the United Kingdom(29), United States(30), Sweden(31), Finland(32) and India(33). One of the strongest epidemiological data to date was described by Leon DA et al, who conducted record linkage between the Swedish Medical Birth Registry, the Military Conscription Register and censuses, and studied 165,136 men born in Sweden between 1973 and 1976 and conscripted into military service from 1990 to 1996. The study reported that systolic pressure was independently inversely associated with birthweight-for-gestational age, and with gestational age itself but not with birth length for gestational age, highlighting that the fetal programming of later blood pressure is a function of accretion of fetal soft-tissue mass rather than of linear bone growth(34). In another longitudinal study, Law CM et al examined the blood pressures in 346 British men and women aged 22 years whose size had been measured at birth and for the first 10 years of life. Findings were similar with that of Leon DA et al, where systolic pressure was observed to have an inverse association with birth weight [systolic pressure increased by 1.3 mm Hg (95% CI: 0.3-2.3) for every standard deviation score decrease in birth weight](35).

Various epidemiological studies have also linked size-at-birth to risk of obesity in later adulthood. The associations between high birth weight and future obesity risk have been extensively reported by investigators from China(36, 37), Brazil(38) and Argentina(39). A recent meta-analyses also revealed that high birth weight (>4000 g) was associated with approximately two-fold increased risk of later obesity [OR (95%CI): 2.07(1.91–2.24)] when compared to subjects with birth weight less than 4000g(40). In another study conducted by Loos RJ et al, each kilogram increase in birth weight amongst males predicted an increase in adult body weight by 4.2 kg(41), with similar findings observed in females(42). A different picture emerges however when looking at the associations between low birth weight and future obesity. A meta-analyses of 10 studies showed low birth weight (BW < 2500 g) was associated with decreased risk of obesity in comparison to subjects with birth weight above 2500g(40). Similarly, in the third National Health and Nutrition Examination Survey (NHANES III, 1988–1994), children born small-for-gestational age (SGA, defined as birth weight <10th percentile for gestational age) remained slightly smaller than their peers. Interestingly however, this deficiency in size was largely due to reduction in lean tissue mass, without a reduction in fat mass, hence indicating that SGA infants had higher percentage body fat(43). Gale CR et al also showed similar findings in a cohort of 143 men and women, aged 70-75 years, where low birth weight was associated with reduced lean tissue mass, but had highest total body fat(12).

Being born SGA has also been shown to be associated with various metabolic manifestations later in life, such as insulin resistance and type 2 diabetes(44). The initial hypothesis by Barker and colleagues proposed that type 2 diabetes associated with small size-at-birth was due to impaired β-cell function at a critical stage of fetal development(45). One of the earliest evidence of small size-at-birth being associated with elevated insulin levels in adults was published in 1993(46). Since then, insulin resistance has been reported in many studies amongst children and adults who were born SGA(47-49). More importantly, the observation of decreased insulin sensitivity in these individuals was independent of confounding factors, such as BMI and age. Data from a French cohort of healthy subjects assessed at 20 years old showed that insulin and proinsulin concentrations were higher in SGA compared with AGA (appropriate-for-gestational age) newborns, after correcting for BMI and gender, which may be representative of an early marker of insulin impairment(50). The relationship between size-at-birth and later insulin resistance, glucose intolerance and type 2 diabetes however, is not restricted only to those who were SGA, but for those who had high birth weights as well. In Pima Indians, the relationship between birth weight and glucose tolerance was U-shaped, with higher plasma glucose concentrations observed at both ends of the birth weight spectrum(51). Similar observations were noted in a large study of US nurses, where the relationship between birth weight and adult Type 2 diabetes was reverse-J-shaped(52).

Table 1.3: Summary of epidemiological studies associating size-at-birth with later metabolic diseases

Study	Sample size	Population source	Exposure	Outcome	Confounders addressed	Pattern of association
Frankel S et al 1996	1258	South Wales	Birthweight	Coronary heart disease deaths non-fatal cardiovascular events	-	A graded association between low birthweight and later cardiovascular disease
Curhan GC et al 1996	71100	United States	Birthweight	Blood pressure, physician diagnosed hypertension	Age, BMI, parental history of hypertension	Lower birth weight associated with increased odds of hypertension
Leon DA et al 1996	15000	Sweden	Birthweight	Mortality from ischaemic heart disease	Period of birth	Cardiovascular disease showed an inverse association with birth weight for both men and women
Eriksson JG et al 1999	3641	Finland	Birthweight	Death from coronary heart disease	Gestational age	Death from coronary heart disease was associated with low birth weight and, more strongly, with a low ponderal index at birth
Fall CHD et al 1998	506	India	Birth ponderal index	Glucose and insulin metabolism	-	High ponderal index at birth associated with lower insulin increment
Leon DA et al 2000	165136	Sweden	Birth weight for gestational age	Systolic blood pressure	Examination age, year, center of conscription	Systolic pressure was independently inversely associated with birthweight-for-gestational age
Law CM et al 2002	346	Britain	Birth weight	Systolic blood pressure	BMI	Systolic pressure was observed to have an inverse association with birth weight

Table 1.3 (continued)

Study	Sample size	Population source	Exposure	Outcome	Confounders addressed	Pattern of association
Hirschler et al 2008	1027	Argentina	Birth weight	Obesity	Gender, Age	High birth weight (> 4000g) associated with increased risk of later obesity
Montiero et al 2003	1071	Brazil	Birth weight	Obesity	Gestational age, gender	High birth weight (> 4000g) associated with increased risk of later obesity
Wang et al 2009	10897	China	Birth weight	Obesity	Gestational age	High birth weight (> 4000g) associated with increased risk of later obesity
Yang wt al 2009	11338	China	Birth weight	Obesity	Parental education, BMI	High birth weight (> 4000g) associated with increased risk of later obesity
Loos RJ et al 2001	229	Belgium	Birth weight	Body weight, height , BMI	-	Each kilogram increase in birth weight amongst males predicted an increase in adult body weight by 4.2 kg
Loos RJ et al 2002	238	Belgium	Birth weight	Body weight, height , BMI	-	Each kilogram increase in birth weight amongst females predicted an increase in adult body weight by 1.3 kg
Hediger ML et al	4431	United States	Small-, large-for-gestational age	mid-upper arm circumference mid-upper arm muscle area Skinfolds	Age	SGA infants remain smaller and LGA infants larger in size through early childhood. Upon assessment of skinfolds and arm circumference measurements, the deficiency in size was found to be largely due to reduced lean tissue mass without a reduction in fat mass
Gale CR et al	143	United Kingdom	Birth weight	Body composition	Age	Low birth weight was associated with reduced lean tissue mass, but had highest total body fat

Table 1.3 (continued)

Study	Sample size	Population source	Exposure	Outcome	Confounders addressed	Pattern of association
Phipps K et al 1993	266	United Kingdom	Birthweight	Impaired glucose tolerance	BMI	Subjects found to have impaired glucose tolerance or non-insulin-dependent diabetes mellitus had lower birthweight, a smaller head circumference and were thinner at birth
Phillips DI et al 1994	81	United Kingdom	Birth ponderal index	Insulin resistance	Gestational age, BMI, social class	Men and women who were thin at birth, as measured by a low ponderal index, were more insulin resistant
Flanagan DE et al 2000	163	Australia	Birthweight, length	Insulin sensitivity, secretion	BMI	Small size at birth is associated with increased insulin resistance and hyperinsulinemia in young adult life
Veening MA et al 2002	53	Amsterdam	Small-for-gestational age	Glucose tolerance, insulin sensitivity	BMI, % body fat	Reduced insulin sensitivity in SGA children
Leger J et al 1997	236	France	Small-for-gestational age	Insulin, proinsulin levels	Gender, BMI	Insulin and proinsulin concentrations were higher in SGA compared with AGA (appropriate-for-gestational age) newborns
Dabelea D et al 1999	3061	Pima Indians	Birthweight	Glucose, insulin concentrations	Later body size	2-h glucose concentrations showed a U-shaped relationship with birth weight
Rich-Edwards JW et al 1999	69526	United States	Birthweight	Type 2 diabetes	Age, BMI	Age-adjusted relative risks suggested a reverse J-shape association between birthweight and risk for type 2 diabetes

1.3.1.3 Infant and Childhood growth

Infants born small or low birth weight have a predisposition to gain weight more rapidly than their peers, and this is particularly true for infants who experienced a period of growth restriction *in-utero* as a result of maternal smoking, or due a primiparous pregnancy(53). An article by Melinda Yeung had highlighted the role of early postnatal weight gain as a crucial window of opportunity to prevent future obesity and related metabolic manifestations such as insulin resistance(54). A prospective cohort study conducted on 108 infants from Santiago, Chile highlighted that fasting insulin was significantly higher in SGA infants with catch-up growth in weight compared with those who did not exhibit catch-up growth(55). Similarly, another study conducted in United Kingdom on 153 children reported that diastolic blood pressure was greater in children whose weight z score increased in the first nine months of life, with similar findings obtained for mean arterial pressure and systolic blood pressure, highlighting how a period of faster weight gain in infancy was associated with higher later blood pressure(56). Findings from the Stockholm Weight Development Study also echoed similar observations, where rapid weight gain in the first six months of life predicted clustered metabolic risk at age 17 years(57). Similarly, the Programming Factors for Growth and Metabolism (PROGRAM) study reported that rapid weight gain in the first three months of life was associated with several determinants of cardiovascular disease and type 2 diabetes in early adulthood, such as insulin sensitivity, serum high-density lipoprotein cholesterol level and triglyceride levels(58). The observations of rapid weight gain and future disease risk is not restricted to infancy only, but also during periods of early childhood. A

longitudinal study on 4630 men in Helsinki, Finland reported significant associations between rapid gain in weight after 1 year of age with increased risk of coronary heart disease during adulthood(59). Similar findings were also reported in the Brompton study cohort in southern England, where subjects who had been small at birth but gained weight rapidly during early childhood (1 to 5 years) had the highest adult blood pressures(35).

Table 1.4: Summary of epidemiological studies associating infant and childhood weight gain with later metabolic diseases

Study	Sample size	Population source	Exposure	Outcome	Confounders addressed	Pattern of association
Soto N et al 2003	108	Chile	SGA with catch-up growth	Fasting insulin insulin sensitivity	Age, gender	Fasting insulin was significantly higher in SGA infants with catch-up growth in weight compared with those who did not exhibit catch-up growth.
Singhal A et al 2007	153	United Kingdom	SGA with catch-up growth	Blood pressure	Age, gender, social class	Diastolic blood pressure was greater in children whose weight z score increased in the first 9 months of life
Ekelund U et al 2007	128	Sweden	Weight gain in infancy and childhood	Metabolic risk score	Gender, birth weight, gestational age, height, maternal fat mass, and socioeconomic status	Rapid weight gain during the first 6 months of life predicted clustered metabolic risk at age 17 years
Leunissen RWJ et al 2009	217	Netherlands	Weight gain in infancy and childhood	Cardiovascular disease markers	Gestational age, gender, age, social class	Rapid weight gain in the first 3 months of life was associated with several determinants of cardiovascular disease and type 2 diabetes
Eriksson JG et al 2001	4630	Finland	Weight gain in childhood	Death from coronary heart disease	Age, gender	After age 1 year, rapid gain in weight and body mass index increased the risk of coronary heart disease
Law CM et al 2002	346	Britain	Birth weight and weight gain in childhood	Systolic blood pressure	BMI	Lower birth weight and greater weight gain between 1 and 5 years of age were associated with higher systolic blood pressure in young adult life

1.3.2 Experimental studies of DOHaD

One of the most widely accepted explanations underlying the DOHaD hypothesis is programming, whereby insults or stimulus that occurs during critical periods in the differentiation and maturation of cells and tissues may have irreversible long-term effects on development(15). Mechanisms which are well-recognized illustrating the effects of programming on future development include altered maternal and fetal nutrition, especially maternal under- and over-nutrition(60). Many studies have proven that it is remarkably easy to alter postnatal physiology by manipulation of maternal nutrition during pregnancy in experimental animals. A recent study documented that offspring of rat dams who were fed a high fat diet during pregnancy and lactation had significantly smaller pups, but had elevated adiposity compared to controls, regardless of the diet post-weaning. These rat offspring also exhibited hyperinsulinemia and hyperleptinemia(61). An earlier study by Bayor et al examining the effect of maternal "junk food" diet during pregnancy on offspring adiposity also echoed similar observations. The authors found that offspring of dams fed on "junk food" diet during pregnancy exhibited increased adiposity, which was not reversible when the offspring were switched to a normal chow diet after weaning(62). Taken together, these two studies highlighted the importance influence of maternal overnutrition during pregnancy in long-term health of offspring, which may have implications in preventing of later childhood obesity. In addition, animal studies have also illustrated how offspring of rats exposed to a high-fat diet during pregnancy demonstrate impaired glucose tolerance, glucose homeostasis and hypertension later in life(63, 64).

Evidence linking maternal undernutrition (e.g. protein and/or caloric restriction) and future disease risk have come largely from animal models. A recent study has shown how protein restriction during pregnancy consequently led to intrauterine growth restriction in the offspring, which was followed by catch-up growth and an impairment in insulin signaling pathways in adipose tissue(65). Other studies have also found how protein restriction during pregnancy in rats consequently leads to hyperinsulinemia and impaired glucose tolerance(66). Similarly, offspring of rats who were exposed to a period of caloric restriction during late gestation in pregnancy demonstrated insulin resistance and changes in vascular function in adulthood(67). Many rat model studies on caloric restriction during pregnancy have also been shown to result in lower body weight, and subsequent development of metabolic syndrome traits such as adiposity, hyperinsulinemia and hyperleptinemia in the offspring later in life(68-70). These effects were not only restricted to rats; similar observations were also noted in guinea pigs as well as sheep(71, 72).

Table 1.5: Summary of experimental studies on DOHaD

Study	Sample size	Animal model	Exposure	Outcome	Pattern of association
Howie GJ et al 2008	24	Rats	Maternal high fat diet during pregnancy	Litter weight and adiposity	Rat dams who were fed a high fat diet during pregnancy and lactation had significantly smaller pups, but had elevated adiposity compared to controls
Bayol SA et al 2008	24	Rats	Maternal "junk food" diet during pregnancy	Litter perirenal fat depots	Offspring of mothers fed with junk food diet exhibited increased perirenal fat pad mass relative to body weight and adipocyte hypertrophy compared with offspring which were never exposed to the junk food diet.
Taylor PD et al 2005	20	Rats	Maternal high fat diet during pregnancy	Whole body insulin sensitivity	Rats exposed to a high-fat diet during pregnancy demonstrate impaired glucose tolerance and homeostasis in adulthood
Fernandez-Twinn DS et al 2004	8	Rats	Maternal isocaloric low protein diet	Litter weight, glucose tolerance, insulin measurements	Protein restriction during pregnancy in rats consequently leads to hyperinsulinemia and impaired glucose tolerance
Berends LM et al 2013	8	Rats	Maternal isocaloric low protein diet	Litter weight, glucose tolerance, insulin measurements	Protein restriction during pregnancy consequently led to intrauterine growth restriction in the offspring, which was followed by catch-up growth and an impairment in insulin signaling pathways in adipose tissue
Holemans K et al 1998	18	Rats	Food-restricted during pregnancy	Litter vascular function	Food restriction during the second half of pregnancy and/or lactation may effect subtle changes in vascular function
Kind KL et al 2002	31	Guinea pigs	Food-restricted during pregnancy	Offspring blood pressure	Maternal feed restriction reduced birth weight and increased systolic blood pressure in young adult male offspring
Gardner DS et al 2005	10	Sheep	Nutrient-restriction during early & late gestation	Offspring adiposity, glucose tolerance	Prenatal undernutrition, specifically during late gestation associated with glucose intolerance

1.4 Determinants of infant size and early-life growth patterns

Given that adverse size-at-birth, body composition and early-life growth patterns might influence the pathways determining disease outcome in later life, it could be postulated that prenatal, perinatal and postnatal factors that determine subsequent infant size, body composition and growth patterns may also have potential roles in influencing future disease risk. Birth size and body composition of newborns is often determined by a complex interplay of both environmental and genetic/epigenetic factors. Recent epidemiologic observations have highlighted the influence of perinatal events on fetal size, body composition and growth(73, 74). There is also evidence of relationships amongst maternal nutrition and metabolic status during pregnancy with neonatal size, body composition and later health outcomes(60, 75), illustrating how perinatal events affecting birth outcomes and early postnatal growth may contribute to a greater risk of disease in adulthood. In addition, recent data has also suggested that postnatal events influencing size and growth of the infant may also play a key role in the programming of metabolic diseases in later life

1.4.1 Maternal/*in-utero* determinants

Nutrition during pregnancy is a key regulator of fetal growth, and hence represents an important determinant of birth size and body composition. Studies have shown that imbalances in maternal diet during pregnancy would affect both birth weight as well as placental weight, as highlighted by Godfrey et al(76). Moore VM et al also reported how percentage of energy derived from protein showed a positive association with birth and placental weight, independent of energy intake and weight gain during pregnancy, and after

correcting for confounders such as maternal age, parity, and smoking, supporting the proposition that maternal dietary composition has an influence on fetal growth(77). Similarly in the Pune Maternal Nutrition Study, it was shown that higher fat intake during pregnancy was associated with neonatal length, birth weight and triceps skinfold thickness (SFT). The study also noted interesting findings of strong associations between folate status and intakes of foods rich in micronutrients with birth weight, suggesting the role of maternal micronutrient intake as a plausibly important limiting factor for fetal growth(78).

In addition, exposure to an altered *in-utero* environment has been shown to be an important determinant of birth size and body composition. The recent Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study documented how a standard deviation (SD) increase in fasting, 1-hour and 2-hour plasma glucose levels during 24-32 weeks of gestation was associated with a 38-42% increase in odds of having a large-for-gestational age baby, 36-42% increase in odds of having a baby with sum of SFT above 90^{th} percentile and 35-44% increase in odds of having a baby with percent body fat above the 90^{th} percentile(73, 74). Findings from the Exeter Family Study of Childhood Health (EFSOCH) study also reported a positive association between maternal glycemia during pregnancy with birth weight, with the effects showing no demonstrable difference by three months of age(79). Touger et al however, reported that offspring of diabetic pregnancies had a dramatically different postnatal growth pattern from offspring of non-diabetic pregnancies. Offspring of diabetic pregnancies showed reduced change in weight z-score and attained height in the first 1.5 years of life. Subsequently, these offspring exhibited

greater weight gain and catch-up growth in height compared to offspring of non-diabetic pregnancies(80). Findings from the Exploring Perinatal Outcomes among Children (EPOCH) study also echoed similar observations; offspring of diabetic pregnancies showed higher BMI growth velocity between 10-13 years of age, increasing by 4.56 kg/m^2 compared to 3.51 kg/m^2 in offspring of non-diabetic pregnancies, and independent of demographic variables, socioeconomic factors and maternal pre-pregnancy BMI(81). Taken together, these studies highlight how maternal glucose during pregnancy may be an important determinant of birth size, neonatal adiposity as well as offspring postnatal growth.

Maternal factors during pregnancy are also known to play a significant role in influencing infant postnatal growth. In the ALSPAC birth cohort, it was shown that maternal factors such as mother's pregnancy weight gain, smoking during pregnancy and parity were closely related to postnatal catch-up and catch-down growth in the infant, and the effect was observed to be more striking amongst primiparous infants, who were more likely to be growth-restrained *in-utero*, were thinner at birth and showed greater postnatal catch-up growth(53). Multiparous infants however, were more likely to show catch-down growth. More recently, a study using data from three contemporary cohorts based in Portugal, Italy and Chile showed how prenatal maternal characteristics such as maternal smoking, pre-pregnancy overweight and underweight, parity and gestational hypertension were associated with different aspects of infant growth related to size, velocity and tempo, offering greater insights into the mechanisms and determinants of infant growth(82).

Table 1.6: Summary of maternal/*in-utero* determinants of infant size, adiposity and growth

Study	Sample size	Population source	Exposure	Outcome	Confounders addressed	Pattern of association
Godfrey K et al 1996	596	United Kingdom	Maternal average daily nutrient intake during pregnancy	Birth & placental weight	Gender, gestational age, maternal height, BMI	High carbohydrate intake in early pregnancy suppresses placental growth, especially if combined with a low dairy protein intake in late pregnancy.
Moore VM et al 2004	556	Australia	Maternal estimated daily intakes of protein, fat and carbohydrate	Birth & placental weight	Maternal age, parity, smoking	Percentage of energy derived from protein was positively associated with birth and placental weight
Rao S et al 2001	633	India	Maternal estimated daily intakes of macro- & micronutrients	Birth weight, length, skinfold thickness	Gender, parity, gestational age	Higher fat intake during pregnancy was associated with neonatal length, birth weight and triceps skinfold thickness. Birth isze showed strong associations with folate status and with intakes of foods rich in micronutrients
Metzger B et al 2008	25505	Multi-countries	Maternal glycemia during pregnancy	LGA	Gender, ethnicity, parity study center, BMI, smoking, alcohol use, family history of diabetes	Continuous association of maternal glucose level with offspring increased birthweight
Touger L et al 2005	249	Pima Indian	Mothers with diabetes before or during pregnancy	Offspring growth up to 7.7 years	Age and gender	Offspring of diabetic mothers showed significant "catch-down" in weight by 1.5 years, and subsequently exhibited greater weight gain and catch-up growth in height compared to offspring of non-diabetic pregnancies
Ong KK et al 2002	1335	United Kingdom	Maternal smoking, parity, pregnancy weight gain	Offspring growth up to 5 years	Age and gender	Pregnancy weight gain, smoking during pregnancy and parity were closely related to postnatal catch-up and catch-down growth in the infant
Pizzi C et al 2014	4622	Portugal, Italy, Chile	Maternal smoking, pre-pregnancy BMI, parity, gestational hypertension	Offspring growth size, velocity and tempo	Gender, gestational age	Maternal smoking, pre-pregnancy overweight and underweight, parity and gestational hypertension, are associated with different aspects of infant weight growth

1.4.2 Postnatal determinants

Of the numerous biological and environmental factors that are known to affect growth and adiposity during infancy and childhood, infant feeding is often recognised to be one of the most prominent determinants(83). One of the first few documented studies on infant nutrition and growth was conducted by Katherine Dewey, as part of the Davis Area Research on Lactation, Infant Nutrition and Growth (DARLING) Study(84). The authors reported that infants who were breastfed had significantly lower weights between 6-18 months of age, and had lower weight-for-length z-scores between 4-18 months of age compared to formula-fed infants. Breastfed infants also gained weight less rapidly after three months of age, highlighting the differing influences of breast- and formula-milk on early infant growth. Other studies reviewed by Dewey K et al also reported similar observations, where breastfed infants gained significantly less weight in the first year of life(85). More recently, findings from the Southampton Women's Survey showed that infants who were breast fed in the first six months of life gained weight, length and adiposity more slowly than formula-fed infants, independent of age at introduction of solids and maternal factors(86). Findings from the Millennium Cohort Study also echoed similar observations; infants who did not receive breast milk grew faster than those whose mothers initiated breastfeeding, as did those who were breastfed for less than four months versus those breastfed for four months or longer(87). Earlier data from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort also highlighted the influence of infant energy intake on postnatal weight gain and adiposity. The authors noted that infants who had higher energy intake at 4 months predicted greater weight

gain between 0-1, 0-2 or 0-3 years of age, and higher body weight and BMI at 1-5 years of age(88). More recently, findings from the Generation R cohort study showed that shorter breastfeeding duration as well as non-exclusive breastfeeding was associated with higher childhood general and abdominal fat measures(89). Taken together, these studies highlight how infant feeding is often one of the most prominent determinants of infant growth and adiposity.

As of late, there is a growing body of research looking at other infant mediators and determinants of growth and adiposity, such as infant sleep and temperament. One of the first few documented studies on infant temperament and later adiposity was conducted by Pereira GR et al, who examined the relationship between infant temperament and adiposity in healthy infants(90). The authors found that infants who exhibited higher scores for difficult temperament correlated positively with SFT at 18 months of age. Another study by Wells JCK et al also showed that infants who were much more easily soothed had significantly leaner SFTs at 2.5-3 years of age(91). In a longitudinal study by Carey WB et al, weight-for-height percentile gains between 4-5 and 8-9 years of age showed significant correlations with eight of nine difficult temperament characteristics, and also with a cumulative "index of difficulty"(92). Findings from Colorado Adoption Study also showed that male infants with less attention span, and female infants who were more easily soothed showed greater increases in weight z-scores and were more likely to be overweight/obese by 6 years of age(93). While the role of infant temperament in pediatric growth and obesity onset warrants greater research, these studies highlight the plausibility of infant temperament as a determinant for later growth and adiposity.

In parallel with the current obesity epidemic, many studies in children have shown an inverse relationship between sleep duration with adiposity measures. A systematic review of 4 cohort studies (San Francisco, Ho Chih Minh, NHANES and ALSPAC) reported that short sleep duration was associated with increased risk of overweight and obesity(94). Another systematic review of 20 longitudinal studies reported a consistent positive relationship between short sleep duration with childhood weight gain(95). Similarly, a meta-analysis of 30 published studies on sleep duration and obesity risk in children showed that a shorter sleep duration was associated with 89% increase in odds of obesity during childhood(96). While there is still a need for a greater knowledge base regarding the association between sleep duration with weight gain and obesity, these studies again highlight the plausibility of infant sleep duration as a determinant for later growth and adiposity.

Table 1.7: Summary of postnatal determinants of infant size, adiposity and growth

Study	Sample size	Population source	Exposure	Outcome	Confounders addressed	Pattern of association
Dewey K et al 1993	87	United States	Breast- or formula-feeding in first 12 months	Infant weight, length, weight-for-length z-score, rate of weight and length gain	Age and gender	Infants who were breastfed had significantly lower weights between 6-18 months of age,had lower weight-for-length z-scores between 4-18 months of age and also gained weight less rapidly after 3 months of age, compared to formula-fed infants.
Baird J et al 2008	1335	United Kingdom	Infant milk feeding and dietary assessment	Conditional growth during first year of life	Maternal education, parity, smoking, gender	Infants who were breast fed in the first 6 months of life gained weight, length and adiposity more slowly than formula-fed infants, independent of age at introduction of solids and maternal factors
Ong KK et al 2006	582	United Kingdom	Estimated energy intake of breastfed, mixed- and formula-fed infants	Weight gain and BMI	Gender	Infants who had higher energy intake at 4 months predicted greater weight gain between 0-1, 0-2 or 0-3 years of age, and higher body weight and BMI at 1-5 years of age.
Durmus B et al 2014	5063	Netherlands	Breastfeeding duration, exclusivity, age of introduction of soild foods	Total fat mass, preperitoneal abdominal fat	Sociodemographic, lifestyle,childhood factors	Shorter breastfeeding duration as well as non-exclusive breastfeeding was associated with higher childhood general and abdominal fat measures
Wells JCK et al 1997	30	United Kingdom	Infant temperament	Childhood body composition	Infant percentage fat, skinfold thicknesses	Infants who were much more easily soothed had significantly leaner skinfold thicknesses at 2.5-3 years of age
Carey WB et al 1988	138	United States	Infant temperament	Weight gain and obesity	-	Weight-for-height percentile gains between 4-5 and 8-9 years of age showed significant correlations with eight of nine difficult temperament characteristics, and also with a cumulative "index of difficulty
Marshall NS et al 2008	11335	United States, United Kingdom, Vietnam	Infant sleep duration	Childhood BMI	Race, gender, education	Negative linear relationship between sleep duration and childhood BMI
Magee L et al 2012	10959	United States, United Kingdom, New Zealand, Canada	Infant/child sleep duration	Childhood weight gain	Race, gender, education, media use, smoking, breastfeeding, physical activity	Significant negative relationship between sleep duration and weight gain
Cappuccio FP et al 2008	30002	United States, France, Germany, Brazil, Portugal, China, Taiwan	Child sleep duration	Childhood obesity	Age, gender	Short sleep duration in children was associated with increased odds of childhood obesity

1.4.3 Genetic determinants

Individuals born small, or have undergone a period of growth-restraint *in-utero*, are often at greater risk of developing chronic diseases later in life. The relationship between fetal growth and size-at-birth with future disease risk is usually explained by uteroplacental insufficiency of the maternal intra-uterine environment(97, 98), which may alter organ function and metabolic milieu of the individual, thereby increasing risk of disease in adulthood(26, 27). In addition to this, there may also be genetic or epigenetic factors that function to reduce fetal growth and size-at-birth, which may also increase susceptibility to diseases later in life(99).

Over the last decade, with advances in the area of genetic epidemiology, a great number of gene expression studies have been performed, and significant associations were found between birth weight and gene expression levels. Studies involving candidate gene approach have identified significant associations with birth weight for placental expression levels of *PHLDA2*(100), *FTO*(101), *IGF-1*, *IGFBP-1*(102), *11β-HSD1*(103) and *11β -HSD2*(104). Microarray experiments on growth-restricted placentas have also identified increased expression of certain genes such as leptin, soluble vascular endothelial growth factor receptor, human chorionic gonadotropin, follistatin-like 3, and hypoxia-inducible factor 2α(105). Experimental studies in animals have also yielded similar observations; a recent study involving a non-human primate model identified genes involved in key metabolic signalling pathways were differentially expressed according to the birth weight of the primate(106).

In addition, genes influencing fetal growth and size-at-birth may also affect postnatal growth. Some of these genes include *IGF-1, IGF-2* and *IGF-1R*. Genes which encode insulin receptors and also the post-receptor signalling cascades might also be implicated in postnatal growth. Findings from the ALSPAC birth cohort reported that 8 variants of genes known to be associated with childhood and adult BMI (*FTO, MC4R, TMEM18, GNPDA2, KCTD15, NEGR1, BDNF*, and *ETV5*) had large positive effects on early infancy weight and length gain, as well as reduced risk of early infancy failure to thrive(107). A retrospective study conducted by Keen RW et al demonstrated that variants in the Vitamin D receptor (*VDR*) gene had a significant trend association with infant weight at 1 year of age, with the homozygote recessive group exhibiting higher weights(108), indicating that early fetal or infant environment may interact with an individual's underlying genotype to program early growth outcomes.

It has also been suggested that early-life environmental exposures inducing altered phenotypes later in life may be mediated by epigenetic mechanisms, which involve changes in gene expression without alterations to the genetic material(17). Such changes would include DNA methylation, covalent modification of histones as well as non-coding RNAs. Evidence from animal models have demonstrated how epigenetic processes in non-imprinted genes may be an important link between early life exposures during pregnancy and later cardiometabolic outcomes during adulthood(109). Lilycrop K et al had earlier described how offspring of protein-restricted rats showed reduced methylation in glucocorticoid receptor (*GR*) and peroxisome proliferator-activated receptor (*PPAR*) genes(110). Sinclar KD et al also described how

restricting the supply of vitamin B12 in periconceptional diet of mature female sheep altered the methylation status of 4% of 1,400 CpG islands in the offspring, and that this modest early dietary intervention led to adult offspring that were both heavier and fatter(111). In humans, earlier studies on a variety of pathological states (such as Russell-Silver Syndrome) have provided evidence on the relationship between DNA methylation patterns with retarded fetal growth(112). Evidence has also pointed to a similar relationship between DNA methylation patterns and fetal growth amongst a set of clinically normal newborns(113). Until recently, data describing observations on how epigenetics may mediate the relationship between early environmental exposure with altered growth and body composition in humans were limited. However, a recent study by Godfrey K et al(114) on women from the Southampton Women's Survey showed associations between levels of retinoid-X receptor alpha (*RXRA*) methylation with carbohydrate intake, and that higher methylation of a single CpG within the *RXRA* promoter measured in umbilical cord was robustly associated with greater adiposity in their offspring at later childhood, thus providing novel evidence of the putative role for epigenetic changes in non-imprinted genes in relation to early development in humans.

Table 1.8: Summary of genetic determinants of fetal growth, size-at-birth and postnatal growth

Study	Sample size	Tissue specimen	Gene	Outcome	Pattern of association
Bassols J et al 2010	147	Placenta	*FTO*	Fetal weight and length gain	Placental FTO mRNA expression was associated with increased fetal-to-placental weight ratio in infants from primiparous women, and was associated with increased fetal weight and length and placental weight in infants from nonprimiparous women
Apostolidou S et al 2006	200	Placenta	*PHLDA2*	Birth weight	Maternally expressing PHLDA2 expression levels in placenta negatively correlated with size-at-birth
Koutsaki M et al 2011	47	Placenta	*IGF-I, IGFBP-1*	Fetal growth restriction	IGF-I and IGFBP-1 exhibited significantly lower expression levels in FGR group compared to controls
McTernan CL et al 2001	86	Placenta	*11β -HSD2*	Fetal growth restriction	Placental 11beta-HSD2 mRNA levels were significantly decreased in intrauterine growth restriction pregnancies when compared with gestationally matched, appropriately grown placentae
Mericq V et al 2009	74	Placenta	*11β -HSD1, 11β -HSD2*	Size-at-birth	Lower expression and activity of 11beta-HSD1 in the chorionic plate of the small-for-getational age placentas
McCarthy C et al 2007	8	Placenta	Microarray gene expression analysis	Fetal growth restriction	Microarray experiments identified increased expression of certain genes including leptin, soluble vascular endothelial growth factor receptor, human chorionic gonadotropin, follistatin-like 3, and hypoxia-inducible factor 2 in the IUGR
Emerald BS et al 2011	8	Umbilical cord, skeletal muscle, liver of Cynomolgus macaque	Microarray gene expression analysis	Birth weight	1973 genes which were differentially expressed in the three tissue types between average and low birth weight animals
Keen RW et al 1996 et al	66	Blood samples	Vitamin D receptor	Weight at 1 year	Statistically significant trend for VDR genotype against weight at the age of 1 year, with the ‘‘tt’’ homozygote group having 7% higher weight
Elks CE et al 2010	7146	Blood samples	Obesity risk alleles (*FTO,MC4R,TMEM18, GNPDA2,KCTD15, NEGR1,BNDF,ETV5*)	Early infancy and childhood weight gain	8 variants of genes known to be associated with childhood and adult BMI had large positive effects on early infancy weight and length gain, as well as reduced risk of early infancy failure to thrive

Table 1.9: Summary of studies showing how epigenetics may mediate later growth and body composition

Study	Sample size	Tissue specimen	Exposure	Epigenetic modification	Outcome	Pattern of association
Lilycrop KA et al 2007	10 rat pups	Liver	Maternal nutrition	DNA methylation	PPARα and GR1 expression	Unbalanced prenatal nutrition induces persistent, gene-specific epigenetic changes that alter mRNA expression in PPARα and GR1
Sinclair KD et al 2007	37 sheep offspring	Liver	Maternal vitamin B12	DNA methylation	Weight, Body composition	Clinically relevant reductions in specific dietary inputs to the methionine/folate cycles during the periconceptional period can lead to widespread epigenetic alterations to DNA methylation in offspring, which led to both heavier and fatter adult offspring
Godfrey K et al 2012	317 subjects	Umbilical cord tissue	Maternal pregnancy diet	DNA methylation	Offspring adiposity	Higher methylation of RXRA was associated with lower maternal carbohydrate intake in early pregnancy, previously linked with higher neonatal adiposity

1.5 Rationale of study

Currently there is a great concern regarding the predicted increase of metabolic diseases in both developed and developing Asian countries, which suggests that the pattern of development of metabolic disease merits a more detailed investigation(115). Multi-ethnic populations, such as that of Singapore (consisting of Chinese, Malays and Indians), may exhibit a physiological difference in susceptibility to metabolic risk(116). Statistics from the National Health Survey in Singapore reported that the prevalence of type 2 diabetes in Singapore has increased from 1.9% in 1975 to 11.2% in 2010, which is now one of the highest in the developed world. Findings from the survey also reported a 10.8% obesity prevalence in Singapore (117), and a recent study reported that amongst Singaporean Chinese pre-schoolers aged 6-72 months, the prevalence of overweight and obesity was 7.0% and 5.3% respectively(118). While this is lower than other developed countries such as the United States, it is still a cause for concern as childhood obesity can persist into adulthood, increasing the risk of later cardiovascular diseases.

There is also scarcity of data on early-life outcomes in Asian populations. Given that early-life growth patterns influence the pathways determining metabolic disease, new insights into early infant development would be crucial for the understanding of pathways to metabolic disease in Asian populations. Interventions that were targeted towards modifying lifestyles of adults have yielded disappointing results thus far, and hence it is apparent that other approaches focused on prevention should be explored. Identifying the prenatal, perinatal and postnatal factors that determine subsequent growth and body composition of infants may enable clinicians to

select individuals who are at risk of developing metabolic disease in later life. Clinical intervention at early stages for individuals at risk may allow for prevention of metabolic disease outcomes in the future.

To examine the potential roles of fetal and developmental factors influencing infant size, body composition and subsequent growth, data from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study was utilized. This birth cohort is one of the first in Singapore, and comprises the most comprehensively phenotyped parent-offspring cohort during the first few years of life(119). One of the primary objectives of the study is to examine developmental factors that can predict patterns of growth and body composition in infancy and childhood, and if these factors that operate during early development would affect pathways to future metabolic compromise and altered body composition. Other study objectives include identifying maternal determinants of the offspring's epigenetic state and associations with other indices of early life experience that may influence growth and body composition.

Given the scarcity of data on early-life outcomes in Asian populations, this study would help to fill this gap in knowledge with tracking of development, growth and other parameters throughout the antenatal period, birth and the first few years of life, providing new insights into development in the early part of the human life course. Infant growth is monitored frequently at multiple timepoints, complemented by body composition assessments using multiple measurement methods including anthropometry, magnetic resonance imaging (MRI), bioelectrical impedance analysis (BIA) and air displacement plethysmography (PEA POD). This presents an important opportunity to

investigate the developmental pathways underlying infant size, body composition and subsequent growth, which may in turn explain variable disease risk later in life in the three major ethnic groups.

1.6 Study aims, objectives and hypotheses

This study aims to add new information on the developmental risk factors that can influence size-at-birth, adiposity as well as patterns of growth during infancy and childhood in a multi-ethnic Asian birth cohort.

The main hypothesis in this dissertation relates to the developmental origins of growth and adiposity, which is:

- Exposure to an adverse *in-utero* and postnatal environment, as well as having certain predisposing genetic risk factors would influence an offspring's size-at-birth, adiposity as well as patterns of growth during infancy and childhood

1.6.1 Aims

1. To establish new norms for size-at-birth and body composition in a cohort of Singapore infants (Chapters 3 & 4), as tools necessary to explore later hypotheses on size-at-birth and infant adiposity
    a. To establish reference values and charts for size-at-birth from 35-41 weeks of gestation, based on the healthy GUSTO infants
    b. To establish and validate a fat-mass prediction formula that is specific for the GUSTO cohort during the early postnatal period, using PEA POD® measurements as reference, and to compare the performance of the prediction equation with that of Slaughter's, for estimating neonatal fat mass in our cohort
2. To examine the associations of maternal/*in-utero* factors with size and adiposity of Singapore infants at birth (Chapter 5)

a. To investigate the relationship between maternal glycemia during pregnancy with neonatal size and adiposity in GUSTO infants
b. To compare fasting with post-challenge glucose levels in influencing excessive neonatal adiposity outcomes in Asian mothers

3. To examine the associations of maternal/*in-utero* factors with early postnatal growth and adiposity during the first three years of life (Chapter 6)
    a. To examine the influence of maternal body mass index (BMI) during pregnancy on early postnatal growth of offspring during the first three years of life in the GUSTO cohort
    b. To examine the influence of maternal glycemia during pregnancy (fasting and post-challenge glucose levels) on early postnatal growth of offspring during the first three years of life in the GUSTO cohort
4. To examine the associations of postnatal factors with early postnatal growth and adiposity during the first three years of life (Chapter 7)
    a. To examine the effect of infant milk feeding on early postnatal growth of offspring in the first three years of life, exposed and unexposed to gestational diabetes *in-utero* in the GUSTO cohort
5. Finally, to identify potential genetic markers of fetal growth, subsequent postnatal catch-up growth and adiposity in a cohort of Singapore infants (Chapters 8 & 9)

a. To evaluate the relationship between fetal growth and subsequent postnatal growth with transcriptomic profiles of umbilical cords in the GUSTO cohort

b. To evaluate the association between polymorphic variants of known adiposity-associated genes (melanocortin-3-receptor [*MC3R*] and fat-mass and obesity associated gene [*FTO*]) with early childhood adiposity

1.6.2 Hypotheses

I. Anthropometric measures such as body weight and SFT are predictive of fat mass in newborns

II. Higher maternal glucose levels during pregnancy is associated with higher neonatal adiposity.

III. Higher maternal glucose and adiposity during pregnancy leads to increased adiposity and postnatal growth during the first 3 years of life

IV. Offspring of mothers exposed to gestational diabetes *in-utero* (ODM) who have greater estimated breastmilk intake and less formula milk exposure will exhibit reduced weight gain, compared to ODM's who have reduced breastmilk intake and more formula milk exposure.

V. Offspring with growth restriction *in-utero* and subsequent catch up growth have unique gene expression profile which is predictive of catch up growth

VI. Offspring with polymorphic variants of known adiposity-associated genes (*MC3R* and *FTO*) are predisposed to overweight and obesity during early childhood

Chapter 2: Materials and Methods

2.1 Study population

The Growing Up in Singapore Towards Healthy Outcomes (GUSTO) study is a population-based prospective cohort study, designed to test specific hypotheses related to the developmental pathways to obesity and cardio-metabolic disorders in Chinese, Malay and Indian participants in Singapore. The study has been described in detail(119). Briefly, pregnant women aged 18 years and above were recruited during their first trimester antenatal ultrasound dating scan at Singapore's two major public maternity units, namely National University Hospital (NUH) and KK Women's and Children Hospital (KKH), between June 2009 and September 2010. Subjects approached were Singapore citizens or permanent residents who were of Chinese, Malay or Indian ethnicity with homogeneous parental ethnic background, had the intention of delivering in NUH or KKH and residing in Singapore for the next five years. Informed written consent was obtained from each participant on the day of the study. This study was approved by both the National Healthcare Group Domain Specific Review Board and Sing Health Centralized Institutional Review Board.

2.2 Details of eligibility criteria

The following eligibility criteria were used for consideration of recruitment to the GUSTO study:

- Subjects receiving chemotherapy or psychotic drugs were excluded
- Subjects with type I diabetes mellitus were excluded

- Only women who agreed to donate birth tissues (umbilical cord, cord blood, placenta) at delivery were included
- Subjects less than 18 years of age were excluded
- Subjects who had no intention to deliver in NUH or KKH were excluded
- Subjects who were of more than 14 weeks of gestation at the point of recruitment were excluded
- Subjects who had non-homogeneous parental ethnic background, or were not of Chinese, Malay or Indian ethnicity were excluded
- Subjects who were not Singaporean or Singapore Permanent Residents were excluded
- Subjects who suffered miscarriage, planned to terminate their pregnancy, or had multiple *in-vitro* fertilization (IVF) pregnancies were excluded

Figure 2.1 illustrates the process of recruitment in the GUSTO study. A total of 3751 families were screened, of which 2034 met the eligibility criteria and 1247 women (response rate 61.3%) were recruited for the study. During recruitment, the eligibility criteria were designed to allow examination of differences between ethnically homogeneous groups, hence recruitment was completed with oversampling of Malays and Indians. Of the 1247 women, 1162 conceived naturally and 85 conceived through IVF. A total of 1176 babies were delivered, the first baby was born on 30 November 2009 and the last baby was born on 1 May 2011.

Figure 2.1: Flowchart of recruitment of GUSTO participants

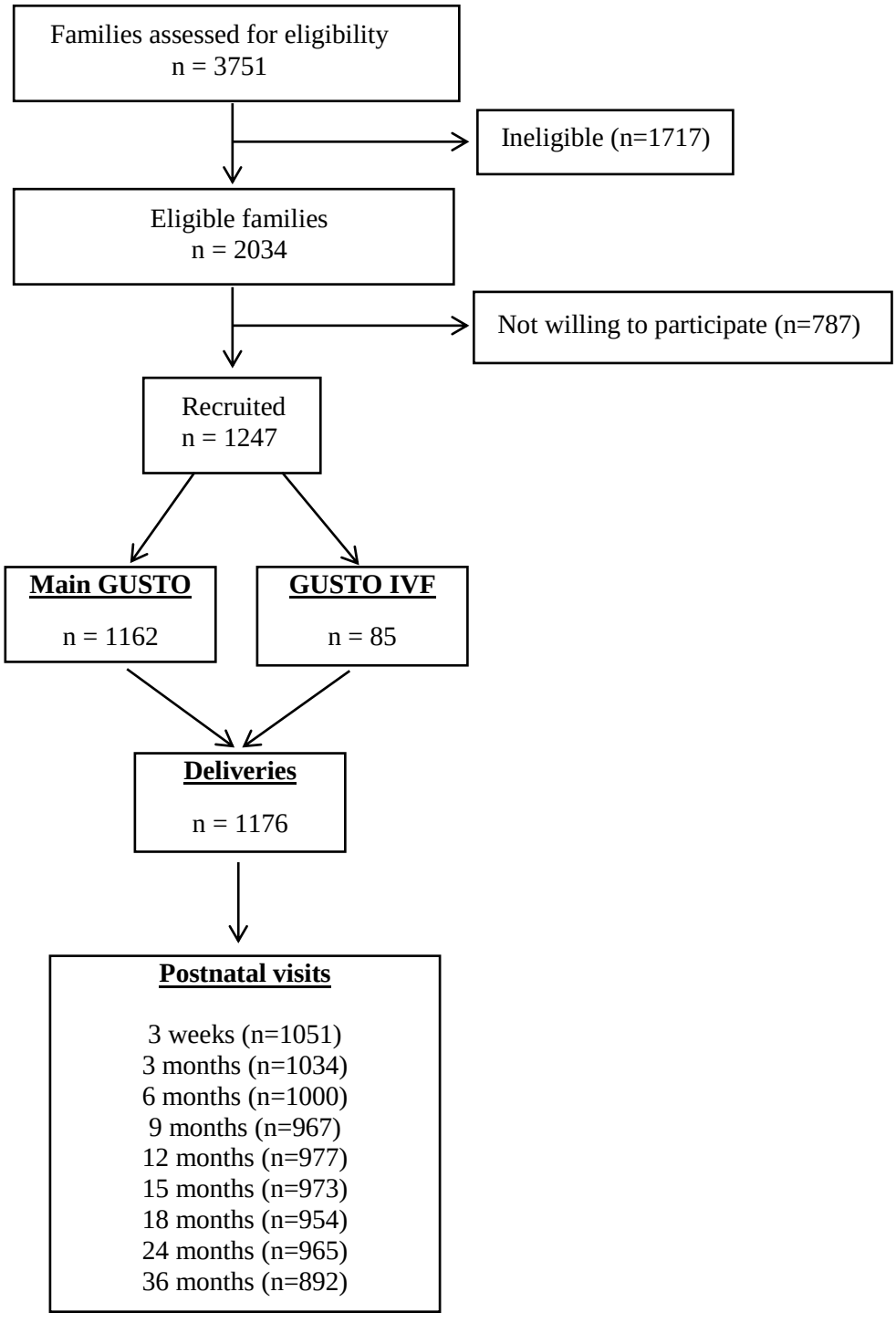


Table 2.1: Reasons for ineligibility of the 1717 families

Reasons	n	Percentage (%)
No intention to deliver in NUH or KKH	469	27.3
More than 14 weeks gestation	416	24.2
Non-homogeneous parental ethnic background	330	19.2
Not residing in Singapore for the next 5-years	178	10.3
Miscarriage	151	8.8
No intention to donate cord, cord blood and/or placenta	71	4.1
Planned to terminate pregnancy	36	2.1
In-vitro fertilization multiple pregnancies	30	1.7
Less than 18 years old	14	0.8
Non-Singaporean or Singapore Permanent Resident	14	0.8
Chronic diseases (e.g. Type 1 Diabetes Mellitus)	6	0.4
Not of Chinese, Malay or Indian ethnicity	3	0.2
Total	1717	100.0

2.3 Clinical measurements

2.3.1 Antenatal period

2.3.1.1 General questionnaire and physical examinations

During the recruitment visit (< 14 weeks gestation) and at the first clinic visit (26-28 weeks gestation), questionnaires were administered to the pregnant women to ascertain demographic, socio-economic, lifestyle (e.g. physical activity and exercise, alcohol consumption) maternal well-being, obstetric and medical history data. Physical examinations were conducted during the first clinic visit, where anthropometry such as weight (SECA 803 Weighing Scale, SECA Corp, Hamburg, Germany), height (SECA 213 Portable stadiometer, SECA Corp), skinfold thicknesses (measured on the right side of the body using Holtain skinfold calipers, Holtain Ltd, Crymych, UK) and mid-arm circumferences (SECA 212 Measuring Tape, SECA Corp) were measured. Routine antenatal clinical and laboratory data were abstracted from the hospital case notes, including measurements of blood pressure, full blood count and urine dipstick. Blood was collected for an oral glucose tolerance test at 26–28 weeks of gestation and analyses of other biochemical markers. Hair samples were collected for toxicology screening (exposure to lead, metals) and to determine steroid levels. Buccal swabs were collected for DNA to investigate the role of epigenetic processes.

2.3.1.2 Oral Glucose Tolerance Testing

All participants underwent a 75-gram oral glucose tolerance test (OGTT) after an overnight fast between 26-28 weeks of gestation, and venous glucose was measured by colorimetry (Advia 2400 Chemistry system

[Siemens Medical Solutions Diagnostics, Deerfield, IL, USA] and Beckman LX20 Pro analyser [Beckman Coulter, USA]). During the study period, glucose management was performed when mothers were diagnosed with gestational diabetes by World Health Organization criteria(120) (fasting or 2-hr plasma glucose concentrations greater than 7.0 or 7.8 mmol/L respectively). Results of the study were communicated to health practitioners, and mothers that were positively diagnosed were placed under either a diet- or insulin-treatment for management. Mothers with elevated fasting or 2-hour plasma glucose were subjected to the same glucose management protocol.

2.3.1.3 Foetal biometry and assessment of gestational age

Gestational age (GA) was assessed by ultrasonography (Aloka SSD-4000, Osaka, Japan). In all women, GA was first assessed in the first ultrasound dating scan during recruitment in the first trimester. They returned to the hospital again at 19-21, 26-28 and 32-34 weeks gestation to have follow-up ultrasound scans for assessment of fetal biometry. Intrauterine growth parameters, namely biparietal diameter, head and abdominal circumferences, femur as well as humerus lengths were measured at the above-mentioned timepoints. Scans were conducted in a standard manner at both hospitals by trained ultrasonographers.

2.3.2 Post-natal period

2.3.2.1 Anthropometry and body composition measurements

Within the first 24-hr after delivery, body composition of the neonate was assessed by anthropometry. Two SFTs (triceps and subscapular) were measured in triplicates using Holtain skinfold calipers (Holtain Ltd, Crymych,

UK) on the right side of the body, recorded to the nearest 0.2 mm. Percent body fat (%BF) and fat mass was measured using bioelectrical impedance analysis (BIA) as well as PEA POD, a non-invasive air-displacement plethysmography (Life Measurement Inc., Concord, CA, USA), which measured body volume and coupled with body weight, was used to calculate body density. %BF could then be calculated from the body density, assuming that the body consists of two components, fat mass and fat-free mass, each with a known density, from the following equation(121):

$$\%\ \text{fat} = \left[\frac{D_F D_{FFM}}{D_B(D_{FFM} - D_F)} - \frac{D_F}{D_{FFM} - D_F}\right] * 100\%$$

Where D_F = density of fat mass, D_{FFM} = density of fat-free mass and D_B = body density

Serial anthropometric measures of early growth trajectories at 3 weeks post-delivery as well as at 3, 6, 9, 12 and 15 months of age are made in the child's home by trained observers. At 18, 24 and 36 months of age, anthropometry measurements were assessed during routine clinic visits. Infant weight from birth to 18 months of age was measured to the nearest gram using a calibrated scale (SECA 334 Weighing Scale, SECA Corp). Weight at 24 and 36 months was measured to the nearest kg using calibrated scales (SECA 813 Weighing scale, SECA Corp). Standard test weights of 1, 2, 5, 10 and 20kg were used to calibrate the weighing scale. Recumbent length from birth to 24 months of age was measured from the top of the head to the soles of the feet using an infant mat (SECA 210 Mobile Measuring Mat, SECA Corp), to the nearest 0.1 cm, with the aid of two research staff. One person supported the infant's head and ensures that the head is positioned in the Frankfort horizontal plane. A second person aligned the legs by placing one hand gently but with mild pressure over the knees. The child's standing height at age 18,

24 and 36 months was also measured using a stadiometer (SECA 213 Portable Stadiometer, SECA Corp). Head circumference was also measured with the aid of two research staff. The infant is held by a health professional while the examiner uses a tape measure to measure the child's head. Maximum head circumference was measured across the frontal bones of the skull and over the occipital prominence at the back of the head, using a non-stretchable measuring band (SECA 212 Measuring Tape, SECA Corp). Mid-arm and abdominal circumferences were also measured using a non-stretchable measuring band. For reliability, all measurements were taken in duplicates.

2.3.2.2 Infant feeding assessment

Mothers were asked on the process of infant milk-feeding, based on a 24-hour recall, at routine house visits when the infants are 3 weeks, 3, 6, 9 and 12 months of age. In accordance with World Health Organization (WHO) guidelines(122), milk-feeding practices were classified into the following:

i. Exclusive breastfeeding: Infant received only breastmilk from his/her mother or a wet nurse, and no other liquids or solids, with the exception of drops or syrups consisting of vitamins, mineral supplements or medicines

ii. Predominant breastfeeding: Infant predominantly received breastmilk, and may also have received water or water-based drinks (sweetened and flavoured water, teas), fruit juice, oral rehydration salts solutions, drops and syrup forms of vitamins, minerals and medicines.

iii. Partial breastfeeding: Infant is receiving breastmilk, but is also being given other food or food-based fluids, such as formula milk or weaning foods.

iv. Formula-feeding: Infants receives no breastmilk at all

In our data collection, breastmilk intake either directly from the breast or expressed, were classified as breastfeeding.

2.4 Biospecimens

2.4.1 Collection and analysis of biospecimens

Rinsed umbilical cord, cord blood and placenta were obtained at delivery by trained personnel. Umbilical cord specimens were analysed by genome-wide methodologies for RNA expression, DNA methylation characterization and genotyping. Methods to survey gene expression analysis include the Illumina HumanHT-12 v4 Expression BeadChips (cat#BD-103-0204, Illumina) with 47,231 transcript probes, and the Illumina Infinium Methylation human 450K bead array, a bead-based technology for genome-wide methylation analysis. Genotyping was also performed on DNA extracted from frozen umbilical cords using the Illumina omniexpress + exome array platform.

2.4.2 RNA extraction

Umbilical cord tissue (300mg) was first placed in a sterile Dispomix tube and homogenized for 55s for 3 cycles in 3 ml of Trizol using the Dispomix (Medic Tools, AG, Zug, Switzerland). After spinning down the debris, the supernatants were divided equally into three 2ml tubes. 200ul of chloroform were added to each tube, vortexed vigorously and centrifuged for 15min at 4˚C. The aqueous phase was carefully transferred to a new tube containing 1ul of linear acrylamide. An equal amount of isopropanol was added and mixed by inversion. After incubating at -20˚C overnight to precipitate the RNA, the pellet was obtained by centrifuging at 13,200 rpm for

10 min at 4˚C. The RNA pellet was washed twice in 70% (v/v) ethanol, air-dried and resuspended in RNase-free water. The isolated RNA was then purified using the RNeasy Mini Kit (Qiagen, Hilden, Germany). On-column DNase digestion was carried out before the first wash step according to the manufacturer's instructions. The purified RNA was then eluted in 30 µl of RNase-free water and stored at -80°C. RNA concentration and purity were measured using a nanodrop ND-8000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), and RNA integrity was determined using the Agilent 2100 Bioanalyzer and RNA 6000 Nano Labchips (Agilent Technologies, Santa Clara, CA, USA).

Chapter 3: A new reference for gestational age-specific size-at-birth of Singaporean infants

3.1 Summary

Background: There is a lack of representative data for local gestational age-specific size-at-birth percentile charts. We aim to construct reference values and charts for size-at-birth from 35 to 41 weeks of gestation, based on a healthy local population sample.

Methods: A prospective observational birth cohort study which recruited pregnant mothers from two major public hospitals with obstetric service in Singapore at <14 weeks gestation, and data was collected for birth weight, length and head circumference of infants born from November 2009 to May 2011. Percentile curves were created separately for male and female infants using the lambda-mu-sigma (LMS) method.

Results: Smoothened curves for birth weight, length and head circumference centiles were created from 863 infants (460 males, 403 females). For a male and female Singapore infant at 38 weeks gestation, the 10-50-90th centile values for weight would be 2663-3096-3597 vs. 2571-2966-3417 grams, for length 46.4-48.6-51.1 vs. 45.6-48.0-50.4 cm, and for head circumference 32.0-33.5-35.2 vs. 31.4-32.9-34.6 cm. There were no statistically significant differences between ethnic groups.

Conclusion: The new centile charts in this study may be used as reference charts for size-at-birth for a subgroup of near-term and term infants

3.2 Introduction

Size-at-birth (birth weight, length and head circumference) is a simple clinical measure that is of importance to both neonatologists and obstetricians. It is commonly used in clinical practice to assess neonatal health. Birth weight and length have been demonstrated to be determinants of perinatal morbidity and survival(123); they are important clinical indicators that are widely used for evaluation of prenatal growth and for identifying infants who are at higher risk of mortality(124, 125). Head circumference is often used by clinicians to identify infants with malformations in the central nervous system and carry prognostic implications(126). Size-at-birth charts thus provide a basis for the assessment of growth and monitoring.

There are readily available size-at-birth-for-gestational age reference charts for newborns of several populations, such as Canada(127), Sweden(128), United States(129, 130) and United Kingdom(131). Despite evidence to assume postnatal growth trajectories are similar across countries and ethnicity(132-134), there is insufficient data on whether size-at-birth-for-gestational age is comparable across countries and ethnicity. In addition, some of these suffer from limitations related to the measurement of gestational age. Some of the charts measured gestational age to the nearest week(135), rather than truncating it to completed weeks, as recommended by World Health Organization guidelines(136). Even for charts that were measured in completed weeks, they are limited by using gestational age based on date of onset of last menstrual period(137) which has been shown to underestimate those born preterm and overestimate those with post-term gestational ages when compared with early ultrasound measurements(138). Thus in this study,

we aimed to construct gestational age-specific size-at-birth percentile charts, based on healthy infants from the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) Birth Cohort study, which would overcome some of the deficits discussed.

3.3 Materials and Methods

3.3.1 Study population, assessment of gestational age (GA) and neonatal anthropometry measurements

Details regarding the study population, assessment of gestational age and measurement of neonatal anthropometry have been described in Section 2.1, Section 2.3.1.3 and Section 2.3.2.1.

3.3.2 Exclusion criteria

In order to give size-at-birth reference values based on a "healthy" cohort, we excluded deliveries with (a) stillbirths, (b) twins, (c) complications during pregnancy with potential effects on fetal growth (gestational diabetes mellitus, anaemia, hypertension / pre-eclampsia) and (d) newborns with significant neonatal malformations. There were also a limited number of observations in the lower gestational ages (less than 35 weeks); these data were also excluded from the analysis. Outlying data points more than three times the inter-quartile range were identified using box-whisker plots, by gestational age and gender for each variable. These data points were then cross-referenced against hard-copy data; incorrect entries were then rectified into the database to ensure high-quality data was used for analysis.

3.3.3 Statistical analysis and chart development

The LMS method(139) using maximum penalized likelihood was utilized to create anthropometric centiles for birth weight, length and head circumference. This method estimates anthropometric measurement centiles in terms of three age-sex specific cubic spline curves: L curve (measure of skewness based on the Box-Cox transformation), M curve (median) and the S

curve (coefficient of variation). The transformation creates a standardized variable:

$$z(t)= \frac{[y(t)/M(t)]^{L(t)} - 1}{L(t)S(t)}$$

where t represents GA and y represents anthropometric measurement. Knowing the values of the three parameters L(t), M(t) and S(t), the 100α percentile is given by:

$$P_{100\alpha}(t) = M(t)\,[1 + L(t)S(t)Z_{\alpha}]^{1/L(t)}$$

where Z_{α} is the standard normal deviate that gives 100α% cumulative probability,

GA and sex-specific size-at-birth centile charts for 3rd, 10th, 25th, 50th, 75th, 90th and 97th percentiles were generated using LMS Chartmaker software (Medical Research Council, UK). The modeling process began with an initial set of "equivalent degrees of freedom" (EDFs) for the parameters L, M and S respectively and the transformation that gave the lowest deviance was preferred. The EDFs were then changed, first for M, then S and L, using the GAIC(3) [Generalised Akaike Information Criterion with a penalty constant of 3] as a guide. Graphical examinations are then used to fine-tune and confirm the choice of parameters to be used.

Quantile regression models for size-at-birth variables for 10th, 50th and 90th percentiles were performed to assess their association with GA and ethnicity. The interaction effect between GA and ethnicity were also explored in above models which were stratified by gender.

3.4 Results

3.4.1 Demographics and clinical characteristics

Anthropometric measurements were completed on the infants of 1163 mothers. A total of 300 infants were excluded from analysis as a result of being born with significant neonatal malformations, or born to mothers with pregnancy complications (gestational diabetes, anaemia, pre-eclampsia). Infants whose mothers had no oral glucose tolerance test (OGTT) data were also excluded in this study. After exclusion, 863 infants were included in the analysis. Table 3.1 summarizes the characteristics of the mothers and study infants.

3.4.2 Gestational age-specific size-at-birth of Singapore infants

Tables 3.1 and 3.2 shows the frequency distribution (n) of GA, LMS values and the observed 3rd, 10th, 25th, 50th, 75th, 90th, 97th percentile of size-at-birth variables for male and female infants. At each GA, male infants consistently exceeded female infants in all three variables. The EDFs for the fitted LMS spline curves for both male and female infants were respectively: 2, 3 and 1 for weight and head circumference; and 2, 2 and 1 for length. Increasing any of the LMS parameters by one EDF for birth weight, birth length and head circumference only increased the GAIC(3) score by 2.3, 2.5 and 2.9 units respectively. Figures 3.1-3.3 show the gestational age-specific smoothed percentile curves for birth weight, length and head circumference respectively, for each gender. The curves are smoothed and evenly spaced with no biologically implausible bumps preterm or flattening out post-term.

Table 3.1: Characteristics of mothers and the study infants

Measures	n	Mean (SD) or %
Maternal Age (yr)	863	30.4 ± 5.1
Marital Status (%)		
Married	812	96.1
Single	33	3.9
Highest Education attained (%)		
Below ""A" levels / diploma	356	41.8
"A" levels / diploma or higher	496	58.2
Type of housing (%)		
Government	734	86.2
Private	118	13.8
Household Income (%)		
Below $6000	572	71.0
Above $6000	234	29.0
Ethnicity (%)		
Chinese	507	58.7
Malay	227	26.3
Indian	129	14.9
Infant gender (%)		
Male	460	53.3
Female	403	46.7
Gestational age at delivery (wks)	863	38.4 ± 1.2
Infant birth weight (g)	863	3109 ± 411
Infant birth length (cm)	863	48.7 ± 2.1
Infant birth HC (cm)	863	33.4 ± 1.8

Table 3.2: Birth weight (g), length (cm) and head circumference (cm) centiles by gestational age (weeks) for boys

					Birth weight						
GA	**n**	**L**	**M**	**S**	**3rd**	**10th**	**25th**	**50th**	**75th**	**90th**	**97th**
35	6	-1.15	2566	0.12	2108	2234	2379	2566	2789	3027	3310
36	24	-0.76	2756	0.12	2248	2390	2552	2756	2990	3233	3509
37	80	-0.38	2937	0.12	2376	2537	2716	2937	3182	3428	3697
38	142	0.02	3096	0.12	2481	2663	2860	3096	3350	3597	3858
39	127	0.43	3248	0.12	2576	2780	2996	3248	3511	3757	4010
40	75	0.83	3391	0.12	2657	2888	3124	3391	3661	3907	4152
41	6	1.23	3532	0.12	2731	2992	3250	3532	3810	4055	4294
					Birth length						
GA	**n**	**L**	**M**	**S**	**3rd**	**10th**	**25th**	**50th**	**75th**	**90th**	**97th**
35	6	-4.12	46.5	0.04	43.7	44.5	45.4	46.5	47.7	49.0	50.5
36	24	-3.07	47.2	0.04	44.3	45.1	46.1	47.2	48.5	49.7	51.1
37	80	-2.01	47.9	0.04	44.8	45.8	46.7	47.9	49.2	50.4	51.7
38	142	-0.96	48.6	0.04	45.4	46.4	47.4	48.6	49.9	51.1	52.3
39	127	0.09	49.4	0.04	46.0	47.0	48.1	49.4	50.6	51.8	53.0
40	75	1.14	50.1	0.04	46.5	47.6	48.8	50.1	51.3	52.5	53.6
41	6	2.19	50.8	0.04	47.0	48.3	49.5	50.8	52.1	53.2	54.3
					Birth head circumference						
GA	**n**	**L**	**M**	**S**	**3rd**	**10th**	**25th**	**50th**	**75th**	**90th**	**97th**
35	6	-1.52	32.3	0.04	30.2	30.9	31.5	32.3	33.1	33.9	34.7
36	24	-1.59	32.7	0.04	30.7	31.3	32	32.7	33.6	34.4	35.2
37	80	-1.67	33.2	0.04	31.1	31.7	32.4	33.2	34	34.8	35.7
38	142	-1.75	33.5	0.04	31.4	32.0	32.7	33.5	34.3	35.2	36.0
39	127	-1.83	33.8	0.04	31.7	32.3	33	33.8	34.7	35.5	36.4
40	75	-1.91	34.1	0.04	32	32.6	33.3	34.1	35	35.8	36.7
41	6	-1.99	34.3	0.04	32.2	32.8	33.5	34.3	35.2	36.1	37.0

GA: Gestational age

Table 3.3: Birth weight (g), length (cm) and head circumference (cm) centiles by gestational age (weeks) for girls

					Birth weight						
GA	**n**	**L**	**M**	**S**	**3rd**	**10th**	**25th**	**50th**	**75th**	**90th**	**97th**
35	10	1.19	2424	0.11	1907	2074	2241	2424	2604	2765	2921
36	18	0.81	2620	0.11	2084	2252	2425	2620	2817	2997	3177
37	51	0.43	2800	0.11	2250	2418	2595	2800	3014	3215	3420
38	118	0.05	2966	0.11	2404	2571	2751	2966	3196	3417	3650
39	127	-0.33	3125	0.11	2554	2719	2902	3125	3371	3615	3879
40	73	-0.71	3289	0.11	2708	2873	3058	3289	3552	3821	4123
41	6	-1.09	3444	0.11	2854	3018	3205	3444	3724	4020	4365
					Birth length						
GA	**n**	**L**	**M**	**S**	**3rd**	**10th**	**25th**	**50th**	**75th**	**90th**	**97th**
35	10	2.07	45.5	0.04	42.0	43.2	44.3	45.5	46.7	47.7	48.7
36	18	1.68	46.3	0.04	42.9	44.0	45.1	46.3	47.5	48.6	49.7
37	51	1.35	47.2	0.04	43.7	44.8	45.9	47.2	48.4	49.5	50.6
38	118	1.11	48	0.04	44.5	45.6	46.7	48.0	49.2	50.4	51.5
39	127	0.96	48.8	0.04	45.2	46.4	47.5	48.8	50.1	51.2	52.4
40	73	0.83	49.6	0.04	46.0	47.1	48.3	49.6	50.9	52.1	53.3
41	6	0.71	50.4	0.04	46.8	47.9	49.1	50.4	51.8	53.0	54.2
					Birth head circumference						
GA	**n**	**L**	**M**	**S**	**3rd**	**10th**	**25th**	**50th**	**75th**	**90th**	**97th**
35	10	-2.68	32	0.04	30	30.6	31.2	32	32.8	33.7	34.6
36	18	-2.23	32.3	0.04	30.3	30.9	31.5	32.3	33.2	34	34.9
37	51	-1.78	32.6	0.04	30.5	31.2	31.8	32.6	33.5	34.3	35.2
38	118	-1.31	32.9	0.04	30.7	31.4	32.1	32.9	33.8	34.6	35.5
39	127	-0.83	33.2	0.04	31	31.7	32.4	33.2	34.1	34.9	35.8
40	73	-0.33	33.7	0.04	31.4	32.1	32.8	33.7	34.5	35.4	36.2
41	6	0.17	34.1	0.04	31.8	32.5	33.3	34.1	35	35.8	36.6

GA: Gestational age

Figure 3.1: Birth weight for gestational age centiles for boys (A) and girls (B)

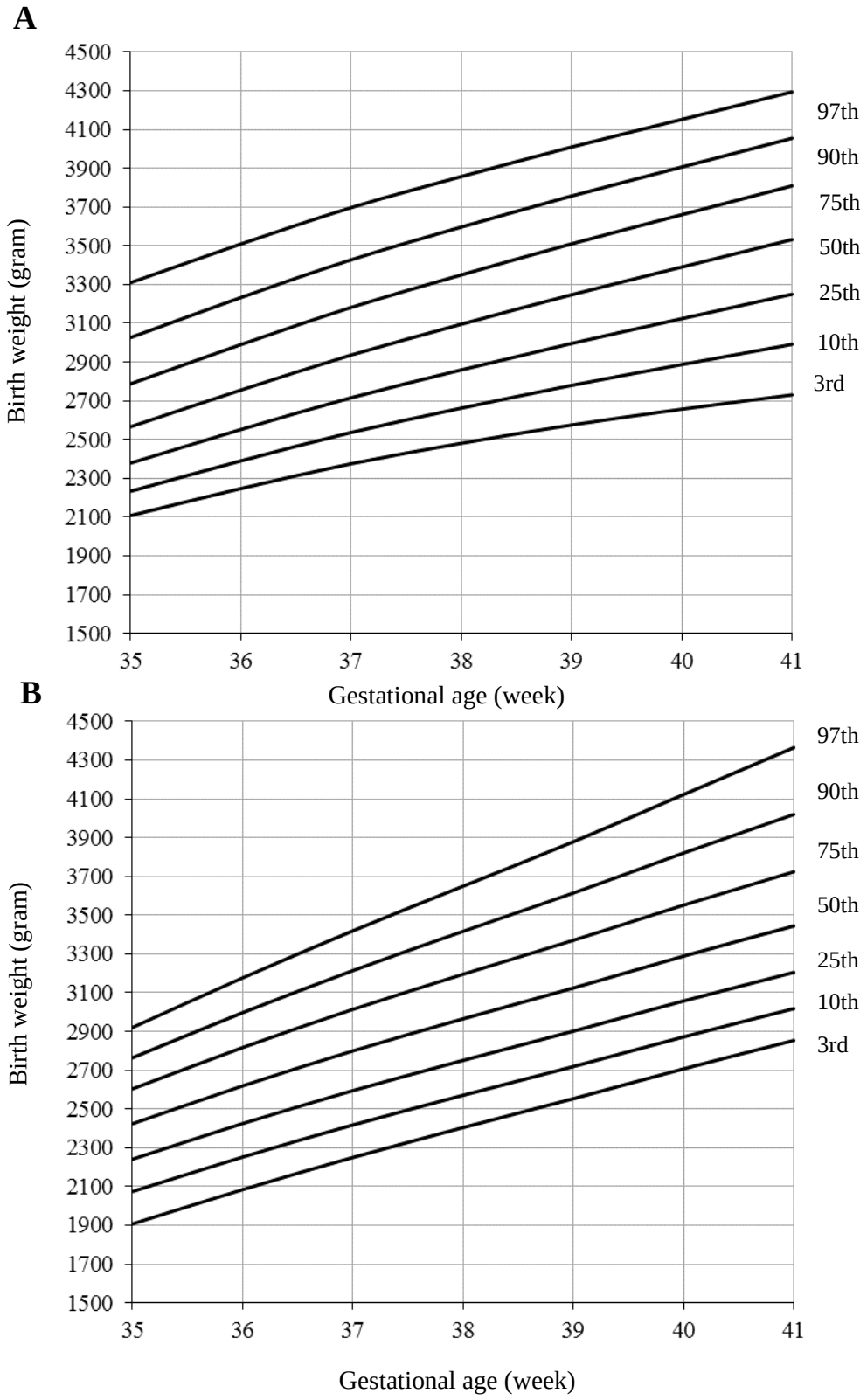


Figure 3.2: Birth length for gestational age centiles for boys (A) and girls (B)

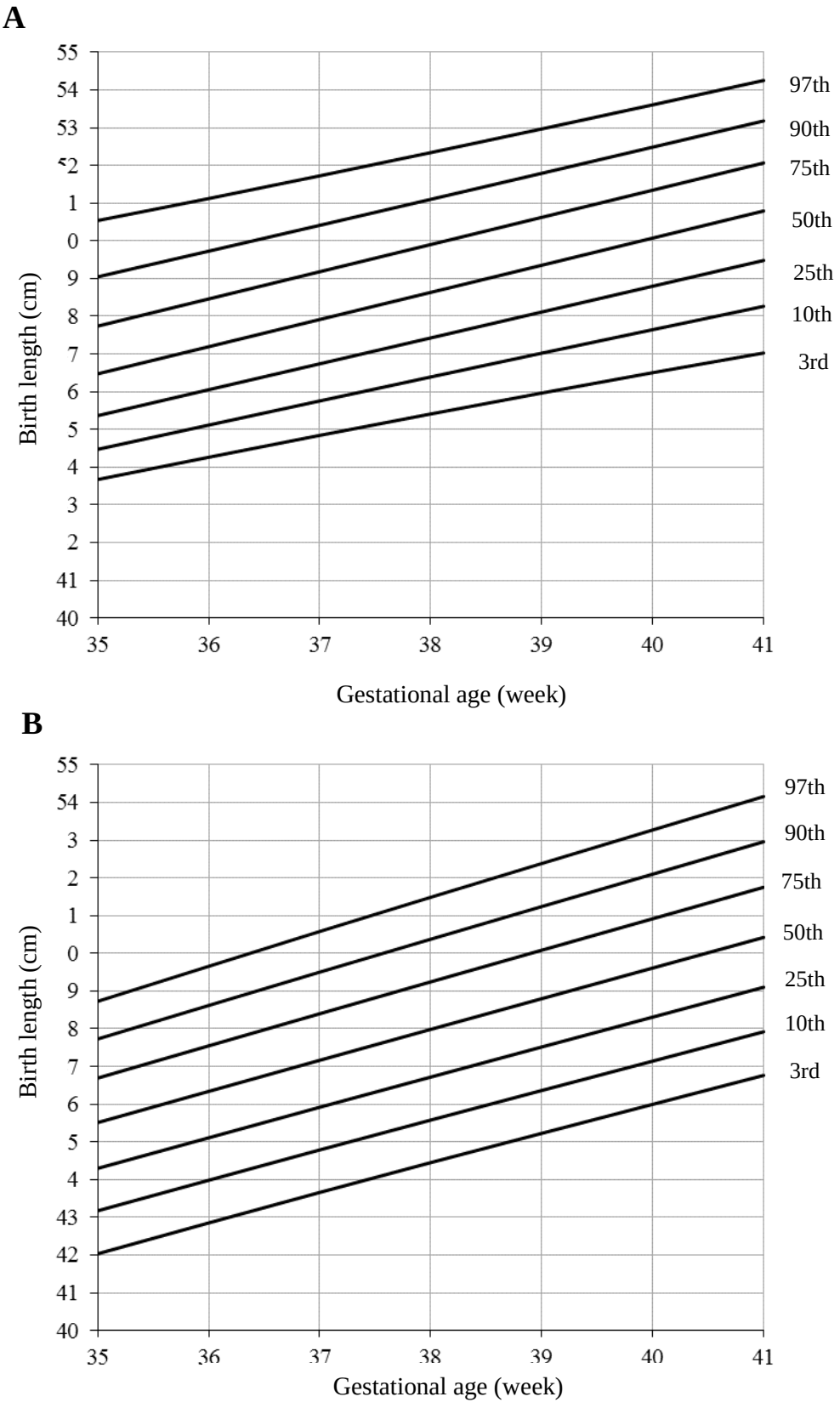
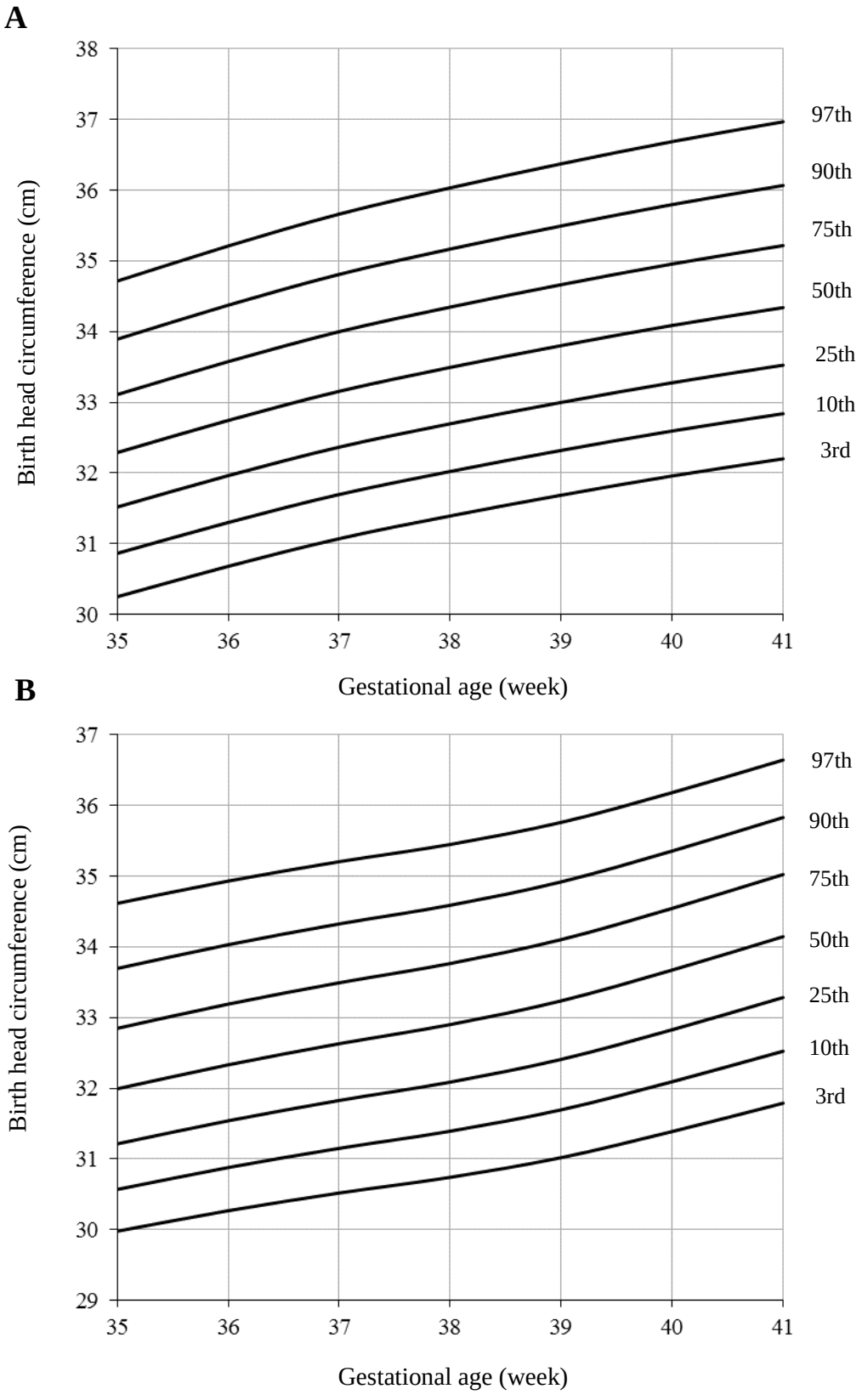


Figure 3.3: Birth head circumference for gestational age centiles for boys (A) and girls (B)



Quantile regression models for 10th, 50th and 90th percentiles of the size-at-birth variables showed no statistically significant evidence for association between the size-at-birth variables and ethnicity for both genders in models with quadratic terms for gestational age and ethnic-GA interaction terms (each P>0.05). In more parsimonious models that excluded the statistical non-significant quadratic and interaction terms (Table 3.4), out of 18 regression analyses (2 gender * 3 anthropometric variables * 3 percentiles), there was only 1 statistically significant ethnic difference: median head circumference was 0.5 cm smaller in Indian boys than Chinese boys (P<0.05) (Table 3.4).

3.4.3 Comparison of reference size-at-birth values with other cohorts

We also compared the birth weights of male and infants in our cohort with population-based references for singleton infants from Finland(140). When compared with infants from the Finland cohort, GUSTO male term and preterm infants had birth weights between 28 to 423 gram lower at the 10th, 50th and 90th percentiles (corresponding to -1.28SD, mean and +1.28SD for the Finland cohort) respectively. GUSTO female preterm and term infants had birth weights between 5 to 488 grams lower at the 10th, 50th and 90th percentiles when compared with the Finland cohort (Table 3.5).

Table 3.4: Quantile regression models for 10th, 50th and 90th percentiles of the size-at-birth variables

	10th percentile		50th percentile		90th percentile	
Weight (g)	B(95%CI)	p	B(95%CI)	p	B(95%CI)	p
Malay	35.5(-37.6,186.6)	0.571	5.0(-93.3,103.3)	0.920	71.3(-73.6,216.3)	0.334
Indian	-196.5(-401.5,8.5)	0.060	-105.0(-240.0,30.0)	0.127	-125.3(-311.9,61.2)	0.187
GA	106.5(61.2,151.8)	**<0.001**	140.0(104.0,176.0)	**<0.001**	171.7(102.2,241.2)	**<0.001**
Length (cm)						
Malay	0.3(-0.4,1.0)	0.340	-0.3(-1.0,0.3)	0.318	-0.7(-1.7,0.4)	0.203
Indian	0.3(-1.2,1.9)	0.673	-6.0e-16(-0.9,0.9)	1.000	0.3(-0.6,1.3)	0.491
GA	0.7(0.4,1.0)	**<0.001**	0.7(0.4,0.9)	**<0.001**	0.7(0.3,1.0)	**<0.001**
Head Circumference (cm)						
Malay	-1.3e-15(-0.4,0.4)	1.000	-4.4e-16(-0.4,0.4)	1.000	-4.6e-16(-0.7,0.7)	1.000
Indian	-0.5(-1.1,0.06)	0.080	-0.5(-0.9,-0.1)	**0.011**	-0.5(-1.2,0.2)	0.150
GA	0.3(0.1,0.4)	**0.001**	0.5(0.3,0.7)	**<0.001**	0.3(0.04,0.6)	**0.027**

B = regression coefficient

Table 3.5: Comparison of the 10th, 50th and 90th percentiles of gestational age (week) specific birth weights (gram) of Singapore male and female infants with from those of Finland

	Male						Female					
GA	**GUSTO**			**Finland**			**GUSTO**			**Finland**		
	10th	**50th**	**90th**	**-1.28SD**	**Mean**	**+1.28SD**	**10th**	**50th**	**90th**	**-1.28SD**	**Mean**	**+1.28SD**
35	2234	2566	3027	2175	2731	3287	2074	2424	2765	2079	2642	3205
36	2390	2756	3233	2418	2991	3564	2252	2620	2997	2323	2899	3475
37	2537	2937	3428	2663	3239	3815	2418	2800	3215	2561	3132	3703
38	2663	3096	3597	2892	3456	4020	2571	2966	3417	2774	3326	3878
39	2780	3248	3757	3075	3625	4175	2719	3125	3615	2948	3483	4018
40	2888	3391	3907	3216	3766	4316	2873	3289	3821	3094	3624	4154
41	2992	3532	4055	3325	3883	4441	3018	3444	4020	3200	3735	4270

3.5 Discussion

We report the percentiles and smoothened curves of size-at-birth variables in a cohort of Singapore neonates born at 35-41 completed weeks of gestation. Our study utilized more precise dating and measurement techniques which differed from population-based references. Also, the present sample is selected from GUSTO to form a "healthy" cohort for the purpose of providing growth standards. These charts assume optimal growth and can be used to identify near-term or term newborns at risk of adverse health outcomes associated with abnormal intrauterine growth. There has been much controversy on inclusion of only "healthy newborns" in deriving growth charts. Ideally, a study population which includes both low- and high-risk pregnancies and both normal and abnormal perinatal outcomes would be more suitable as a reference chart. Standard charts on the other hand are based on low-risk pregnancies with a normal outcome, and the growth curves would reflect optimal, healthy, linear growth of newborns who fully expressed their growth potential. When "population referenced" and "standard" charts are applied to an individual fetus or infant, interpretation of the findings would differ. Using a population referenced would yield a relative fetal size in relation to the total population; a standard chart will assess a fetal size in comparison to normally grown fetuses. Thus, a standard chart may have more clinical utility than a population referenced chart(141).

The availability of local gestational age-specific size-at-birth percentile charts is essential for obstetricians & neonatologists in their perinatal practice. Earlier charts generated by Cheng et al(135) for the Singaporean population are now several decades old; this would not be reflective of infants that were

born in more recent years, where there has been a rapid improvement in the quality of living and health indices. For example, Cheng et al's charts showed that at 40 weeks gestation, birthweight at 50^{th} percentile was approximately 3.0kg, whereas the GUSTO charts showed birthweight at 50^{th} percentile was approximately 3.3kg for the same gestational age. It has been well established that increased maternal education, income, and social status contribute to increased birth weight(142), thus our charts takes into account an improved standard of living, of which more than half of mothers in the cohort are better-educated (completed at least GCE 'A' Levels or polytechnic education) and resided in better accomodation (at least 4-5 room HDB flats). The average maternal age of the GUSTO participants was 30.4 years, slightly older than that reported in birth weight charts by Tan et al(137) at 29.2 years; this appears to be reflective of the trend of delayed childbearing amongst women here in Singapore(143).

Our charts also pooled Chinese, Malay and Indian infants together into one chart. Earlier studies on birth weights in the Singapore population had identified that Indians have the highest proportion of low birth weight(144, 145), and also tend to weigh lesser than their Chinese and Malay counterparts(146). Similarly, Indians in our study sample exhibited smaller birth weights compared to Chinese and Malays; however these differences were not statistically significant. Additionally, there was no statistical evidence to suggest that size-for-gestational age pattern was different between ethnic groups at the 10th, 50th and 90th percentiles. In a recent discussion about the cross-ethnic applicability of the WHO Multicenter Growth Reference Study child growth standards, it was suggested that a difference in

median of less than +/- 0.4 SD is a clinically non-significant difference(147). In the present study, there was a lack of clinical significance between the three ethnic groups. Comparing the statistically significant difference in median head circumference between Indian and Chinese boys and the statistically non-significant differences in medians of birth weight and length against the SD reflected by the M and S curves of the LMS models, there was no clinically significant difference across ethnic groups. These observations substantiate our pooling of the three ethnic groups together into one overall chart, rather than stratifying into ethnic-specific charts. These factors differentiate the GUSTO charts from other local published charts, hence providing a new reference for size-at-birth of Singapore newborns.

Despite evidence to assume postnatal growth trajectories is similar across countries and ethnicity(132-134), there is much less data on whether size-at-birth is comparable across countries and ethnicity. We compared the birth weights of our infants with published gender-specific references for birth weight-for-gestational age from the Finland cohort(140); both the GUSTO as well the Finland study reported gestational age based on ultrasound assessment, and only "healthy newborn" infants were included. The time period of the Finland study was from 1996-2008, relatively closer with that of GUSTO and hence allowing for comparison of the growth curves between these two studies. GUSTO term infants were observed to be lighter across the 10th, 50th and 90th percentiles when compared with the Finland cohort. This implies that the use of non-local charts could lead to misclassification of small-for-gestational-age (SGA) or large-for gestational-age (LGA) infants. Using the Finland charts with our data led to overestimation of SGA infants

and underestimation of LGA infants; the consequence of which some infants appropriate-for-gestational-age (AGA) would be wrongly identified as SGA and LGA infants would be overlooked as AGA. This supports the suggestion that population-specific growth charts should be used for classification of infants into SGA, AGA or LGA; additionally, it seems that compared with Caucasian infants, Asian infants are smaller at birth.

The use of ultrasound for more precise dating of GA, performed at the first trimester period presents as a strength of this study. Ultrasound dating measurements are usually used to confirm last menstrual period dates or if menstrual history is unreliable(148). Unlike other studies(135, 137) where GA was based on date of onset of last menstrual period, which can underestimate the number born preterm, and overestimate post-term GA, ultrasound dating helps to avoid erroneous estimation of GA(138). Some studies have used anthropometric data documented in birth records(149). While this technique allows for inclusion of a large sample number of infants, it often necessitates statistical exclusion of extreme outliers arising from errors in documentation(150, 151). Questions may also arise whether anthropometric data from past records have been reliably measured. Thus, the prospective nature of the GUSTO cohort adds strength to the study; it ensures all anthropometric variables were accurately and reliably measured, hence exclusion of extreme outliers in data was not required.

Similar to most population-based references, our growth charts have one important limitation, which is the cross-sectional nature of the data. The data analyzed in this study are based on size-at-birth anthropometrics of different newborns at different gestational ages, which is not reflective of the

intrauterine growth of the fetus. Hence, these charts would not be suitable for evaluation of fetal growth velocity. A longitudinal analysis of individual fetuses would be required for a more detailed study on intrauterine growth velocity. Another limitation of the present study is that the sample size of GUSTO was not estimated for the purpose of constructing size at birth reference charts. However, the use of modern analytic methods like LMS makes good use of data in that each data point contributes toward the estimation of the centiles of not only the gestational age interval it belongs to but also the adjacent gestational ages. Hence the relatively small sample size was partially compensated by the relatively efficient statistical methods. Future studies with even larger sample size and for a wider range of gestational weeks will be useful. Additionally, the GUSTO sample in this study does not represent the local newborn population; practical logistic issues hindered obtaining samples from other maternity units, which could have further strengthened the applicability of the findings. The GUSTO data however, was collected from two of Singapore's major maternity units (NUH and KKH) which strengthen the applicability of the findings as compared to single-center studies.

Our study also lacks sufficient sample size at preterm (less than 37 weeks), as well as at post-term GA (41 weeks). Our cohort is also limited by the small sample size for the Indian population, which necessitates the need for further research especially regarding the size-for-gestational age pattern for the Indian population. Whilst growth curves at the lower gestational ages (24-34 weeks) would add significant clinical value, our charts would still be useful for clinicians and researchers; it represents a new reference for birth weight,

length and head circumference based on a recent population of near-term and term infants, which would be crucial to evaluate birth size correctly. Birth size references representing optimal foetal growth and based on the current population are sparse especially for Singapore, where existing charts are based on populations that are decades old. As changing social trends may influence birth size, birth size references based on a more recent population would be useful for correct evaluation of newborns as small-, appropriate- or large-for-gestational age. Correct identification of SGA infants is important since these infants are at an increased risk of perinatal morbidity and mortality and long-term adverse consequences such as neurodevelopmental problems, adult-onset cardiovascular disease, and metabolic alterations(16). For researchers, our charts would allow for proper categorization of groups of infants to study etiologic determinants of birth size as well as short- or long-term prognosis.

Our study findings provide a new standard chart for size-at-birth of Singapore newborns 35-41 weeks of gestational age. For clinicians, it allows for a more suitable classification of infants as small-, appropriate- or large-for-gestational age. Given the importance of the relationship between size-at-birth and future health risks, these charts would be useful for the care of newborns, and also for future GUSTO studies involving etiologic determinants of birth size.

Chapter 4: Body fat in Singaporean infants: Development of body fat prediction equations in Asian newborns

4.1 Summary

Background: Prediction equations are commonly used to estimate body fat from anthropometric measurements, but are often population-specific. We aimed to establish and validate a body composition prediction formula for Asian newborns, and compared its performance with that of a published equation.

Methods: Two hundred and sixty-two neonates from a prospective cohort study had body composition measured using air-displacement plethysmography (PEA POD). Using fat mass measurement by PEA POD as a reference, stepwise linear regression was utilized to develop a prediction equation in a randomly selected subgroup of 62 infants measured on days 1–3, which was then validated in another subgroup of 200 infants measured on days 0–3.

Results: Subscapular skinfold thickness, weight, gender and gestational age were significant predictors of neonatal fat mass, explaining 81.1% of the variance, but not triceps skinfold or ethnicity. By Bland–Altman analyses, our prediction equation revealed a non-significant bias with limits of agreement (LOA) similar to those of a published equation for infants measured on days 1–3 (95% LOA: (-0.25, 0.26) kg vs. (-0.23, 0.21) kg) and on day 0 (95% LOA: (-0.19, 0.17) kg vs. (-0.17,0.18) kg). The published equation, however, exhibited a systematic bias in our sample.

Conclusion: Our equation requires only one skinfold site measurement, which can significantly reduce time and effort, thus aid its application to other Asian neonatal populations

4.2 Introduction

Excess adiposity is a major risk factor for adverse health outcomes and chronic diseases(152). Body fat assessment in infants is important not only as an indicator of nutritional status, but also because of the increasing evidence of its role in the developmental origins of health and disease later in life. Techniques such as dual-energy X-ray absorptiometry (DXA) and quantitative nuclear magnetic resonance have been used in research studies, which provide non-invasive, accurate and precise measurements of body composition, but both techniques are generally unsuitable for large scale pediatric use. DXA often requires infants to lie still during scanning thus making the implementation of the technique challenging. Additionally, estimates of infant body composition measured by quantitative nuclear magnetic resonance without appropriate mathematical adjustment fares poorly when compared to 4-compartment model, deuterium oxide dilution (D_2O) technique, and air-displacement plethysmography (ADP) for infants(153). The infant-sized ADP instrument, PEA POD® provides a reliable and accurate assessment of body fat in infants(154-156), and has been shown to provide better estimates than other techniques such as DXA(157). A recent study by Ellis KJ et al(158) on 49 healthy infants demonstrated no significant difference in the mean percent body fat (%BF) obtained from PEA POD® (16.9 ± 6.5%) and the reference

four-compartment model (16.3 ± 7.2%), and the regression between %BF obtained by both did not deviate significantly from the reference line of identity y=x (R^2 = 0.95; SEE = 1.4%BF), thus making the PEA POD® a reasonably accurate method for body composition assessment in children. Additionally, PEA POD® takes into account that the hydration status of neonates differs from adults and that hydration of fat-free mass decreases with age(159, 160) unlike other reference methods such as DXA, which assumes constant hydration. The machine however, is bulky and expensive, and its use is restricted only to fixed locations such as hospital settings. Therefore, it would be desirable to have a prediction equation for estimation of total body fat in infants using a combination of anthropometric variables. This would allow for quick estimations of body composition without the need for specialized laboratories or expensive equipment.

Skinfold thickness (SFT) measurements provide estimates of subcutaneous fat layer(161) which can be easily converted into values of %BF or fat mass via prediction formulas(162, 163). SFT measurements are fast and non-invasive bedside methods, which can be performed with high reproducibility so long as great care is taken with fieldworker training and quality control. SFT measurements for body composition assessment in adolescents and children have been widely used in clinical research and epidemiological settings(164, 165). Studies have shown that SFT measurements from single-site SFTs were highly correlated with total fat mass in infant subjects(166). In older children, SFT measurements have been shown to correlate with body fat measured by DXA(167). Questions have been raised however, on their validity in infancy, given the age-related variability in

hydration status as well as variability in skinfold compressibility among neonates(168, 169). Generalized skinfold prediction equations, such as those by Slaughter(170) for estimating body composition in infants have been developed, although these equations are population-specific(171) and may not be suitable for Asian infants. In the GUSTO study, %BF and fat mass data as measured by PEA POD® was collected from a subgroup of infants. Thus in this study, we aimed to establish and validate a fat-mass prediction formula that is specific for the GUSTO cohort during the neonatal period using PEA POD® measurements as reference, and hypothesized that anthropometric measures such as body weight and SFT would be predictive of fat mass in newborns. Additionally, we compared the performance of our prediction equation with that of a published equation(170), for estimating neonatal fat mass in our cohort.

4.3 Materials and Methods

4.3.1 Study population and body composition assessment

Details regarding the study population and body composition assessment were described in detail in Section 2.1 and Section 2.3.2.1. For our analysis population, only healthy, singleton, term infants ranging in gestational age between 37 to 40 weeks was considered. A subgroup of babies born in KKH whose parents consented had PEA POD measurements. In order to give reference values based on a "healthy" cohort, we excluded data from infants with birthweight below 2.5kg and also those with %BF below 5%, as we have reason to believe that PEAPOD measurements of less than 5% body fat for healthy term infants may have been erroneously taken by the observers. After exclusion, a total of 262 infants, from day 0-3 after birth, were analyzed in this study.

4.3.2 Model Derivation

For purposes of model derivation, infants whose measurements were taken at day 0 (< 24 hours after delivery) were excluded; this was based on a recent study that had suggested there was a significant weight loss during the time period of less than 24 hours after delivery(172). Hence, in deriving the prediction model for neonatal fat mass, only infants whose measurements were taken from day 1-3 were considered (n = 88). The subjects were divided into two groups using the SPSS random number generator. The "derivation group" included two-thirds of the subjects and was used to derive the prediction equation for neonatal fat mass. The "validation group" consisted of the remaining subjects. Stepwise linear regression was utilized to derive the

best model to predict neonatal fat mass from the "derivation group". The starting maximum model included all independent variables. It is well-documented that body composition differs significantly between male and female infants(173, 174); thus infant gender was included in the equation. The dependent variable was fat mass (in kg) as measured by PEA POD®. The independent variables used for development of the prediction equation were gender, ethnicity, weight, abdominal circumference, triceps and subscapular SFT, gestational age (GA) and day of measurement post-delivery.

4.3.3 Statistical analysis

The reliability of the newly-derived model and the published equation were assessed on the "validation" group and the subgroup of day 0 infants; differences between measured and predicted values were tested for significance from zero using one-sample paired t-tests. Technical errors (TEs) and intraclass correlations (ICC) were computed to evaluate reproducibility of SFT measurements(175, 176) TEs were calculated by:

$$TE = \sqrt{(\Sigma d^2 / 2N)}$$

where d = difference between smallest and largest measurements for an individual

%BF values predicted from Slaughter's equation(170) were converted to fat mass using the following equation:

$$\text{Fat mass}_{\text{Slaughter}} = (\%BF_{\text{Slaughter}} / 100) \times \text{weight}$$

Bland & Altman analysis(177) was used to compare fat mass prediction from Slaughter's equation and the newly-derived model with measurements obtained from PEA POD®, by determining the bias and limits of agreement (L.O.A). Bias was defined as (Fat Mass $_{\text{prediction}}$ - Fat Mass

$_{PEAPOD}$). L.O.A was determined by mean bias ± 1.96 SD, to indicate the possible extent of variation between predicted fat mass and PEA POD® measurement for any subject. All analysis was performed using SPSS version 19.0 (IBM SPSS Statistics, Armonk, NY).

4.4 Results

4.4.1 Demographics and clinical characteristics

Baseline characteristics of the infants at day 0 and days 1-3 are illustrated in Table 4.1. No significant differences were observed in the anthropometric measurements between derivation and validation groups. The reproducibility of SFT measurements in our study are illustrated in Table 4.2. We noted small mean differences between smallest and largest measurements for triceps (0.16mm for boys, 0.15mm for girls) and subscapular SFT (0.15mm for boys, 0.14mm for girls). ICC for triceps SFT was high in both boys (r = 0.994) and girls (r = 0.997), and the same observation was noted for subscapular SFT (r = 0.996 for boys, r = 0.997 for girls). TE for triceps (0.19mm for boys, 0.15mm for girls) and subscapular SFT (0.15mm for boys, 0.14mm for girls) were also quite small in our study, indicating high reproducibility of SFT measurements.

4.4.2 Predictors of neonatal fat-mass in a cohort of Singaporean neonates

Stepwise linear regression analysis identified GA, weight, subscapular SFT and gender to be significant predictors of neonatal fat mass (Table 4.3), which explained 81.1% of the variance in neonatal fat mass (R^2 = 0.811). Day of measurement post-delivery, abdominal circumference, triceps skinfold and

ethnicity were not significant predictors of neonatal fat mass. The final GUSTO equation, which was used in subsequent analyses, was:

Fat Mass$_{GUSTO}$ = -0.022 + (0.307*weight) - (0.077*Gender) + (0.028*subscapular SFT) - (0.019*GA),

where gender = 1 for male, 0 for female

Table 4.1: Characteristics of study subjects

	Day 1-3 (n = 88)		**Day 0 (n = 174)**
	Derivation (n = 62)	Validation (n = 26)	
Male (%)	50.0	73.1	49.7
Chinese (%)	45.2	34.6	48.0
Malay (%)	38.7	46.2	36.3
Indian (%)	16.1	19.2	15.7
Fat mass by PEAPOD (kg)*	0.36 ± 0.14	0.36 ± 0.14	0.34 ± 0.11
Gestational age (wks)*	38.5 ± 1.1	38.5 ± 0.9	38.5 ± 1.0
Weight (kg)*	3.2 ± 0.4	3.4 ± 0.4	3.2 ± 0.3
Abdominal Circumference (cm)*	29.4 ± 2.3	28.8 ± 2.4	28.8 ± 1.9
Subscapular skinfold (mm)*	5.1 ± 1.0	5.1 ± 1.0	5.3 ± 1.1
Tricep skinfold (mm)*	5.7 ± 1.2	5.5 ± 1.2	5.9 ± 1.2
day of measurement (days)			
1st day (%)	48.4	57.7	-
2nd day (%)	41.9	19.2	-
3rd day (%)	9.7	23.1	-

*Values expressed as mean ± SD

Table 4.2: Mean results and reproducibility of skinfold thickness (mm) for the study subjects

	Mean difference ± SD between minimum and maximum measurements	TE (mm)	ICC
Boys (n = 136)			
Triceps	0.16 ± 0.22	0.19	0.994
Subscapular	0.14 ± 0.16	0.15	0.997
Girls (n = 126)			
Triceps	0.15 ± 0.15	0.15	0.996
Subscapular	0.14 ± 0.16	0.14	0.997

Values are presented as mean ± SD
TE: Technical error of measurement
ICC: Intraclass correlation

Table 4.3: Regression coefficients of independent variables for prediction models of fat mass (Dependent variable: fat mass in kg measured by PEAPOD)

Predictive Variables	Standardised Coefficients				R^2	Prediction equations for neonatal fat mass
	W	G	GA	SSF		
W	0.804*				0.623	-0.638 + 0.307*W
W + G	0.853*	-0.303*			0.736	-0.655 + 0.326*W - 0.087*G
W + G + G.A	0.902*	-0.303*	-0.238*		0.774	0.437 + 0.350*W - 0.087*G - 0.030*G.A
W + G + G.A + SSF	0.805*	-0.268*	-0.147*	0.206*	0.811	-0.022 + 0.307*W - 0.077*G - 0.019*G.A + 0.028*SSF

* $p < 0.05$ for statistically significant standardized regression coefficient
W = Weight in kg, G = Gender (male = 1, female = 0), G.A = gestational age in weeks, SSF = subscapular skinfold

4.4.3 Comparison with other published fat-mass estimating equations

Fat mass predicted using the GUSTO equation exhibited a moderately strong correlation with Fat mass $_{PEAPOD}$ [r = 0.567, p = 0.003], which is similar compared with fat mass predicted using Slaughter's equations [r = 0.570, p = 0.002].

The accuracy of each prediction equation for the "validation group" was assessed using Bland-Altman plots (Figures 4.1A-B), which revealed the bias and L.O.A for body fat predicted by GUSTO and Slaughter's equation. The mean bias for GUSTO equation is 0.003kg ($p > 0.05$), similar with the mean bias for Slaughter's equation, at -0.01kg ($p > 0.05$). The L.O.A for GUSTO equation was (-0.25, 0.26) kg, which is also similar with Slaughter's equation, (-0.23, 0.21) kg. There was no significant relationship between the mean and difference of the measured and predicted values, but the relationship approached significance for Slaughter's equation. (r = 0.05, p = 0.802 for GUSTO equation; r = -0.40, p = 0.063 for Slaughter's).

We also assessed GUSTO and Slaughter's equation with infants who had their measurements taken on day 0, as illustrated in Figures 4.2A-B. The mean bias for GUSTO equation is -0.01kg ($p > 0.05$), similar with the mean bias for Slaughter's equation, at 0.002kg ($p > 0.05$). The L.O.A for GUSTO equation was (-0.19, 0.17) kg; again, this is similar with that of Slaughter's equation, (-0.17, 0.18) kg. There was no significant relationship between the mean and difference of the measured and predicted values for the GUSTO equation (r = 0.102, p = 0.126), but the relationship was significant for Slaughter's equation (r = -0.252, p = 0.001). Again, this is indicative that

Slaughter's equation has a tendency to underestimate fat mass as body fatness increased, and overestimate as body fat decreases.

Figure 4.1A: Bland-Altman plot comparing measured fat mass against predicted fat mass using equations by GUSTO for infants measured on days 1-3 post-delivery

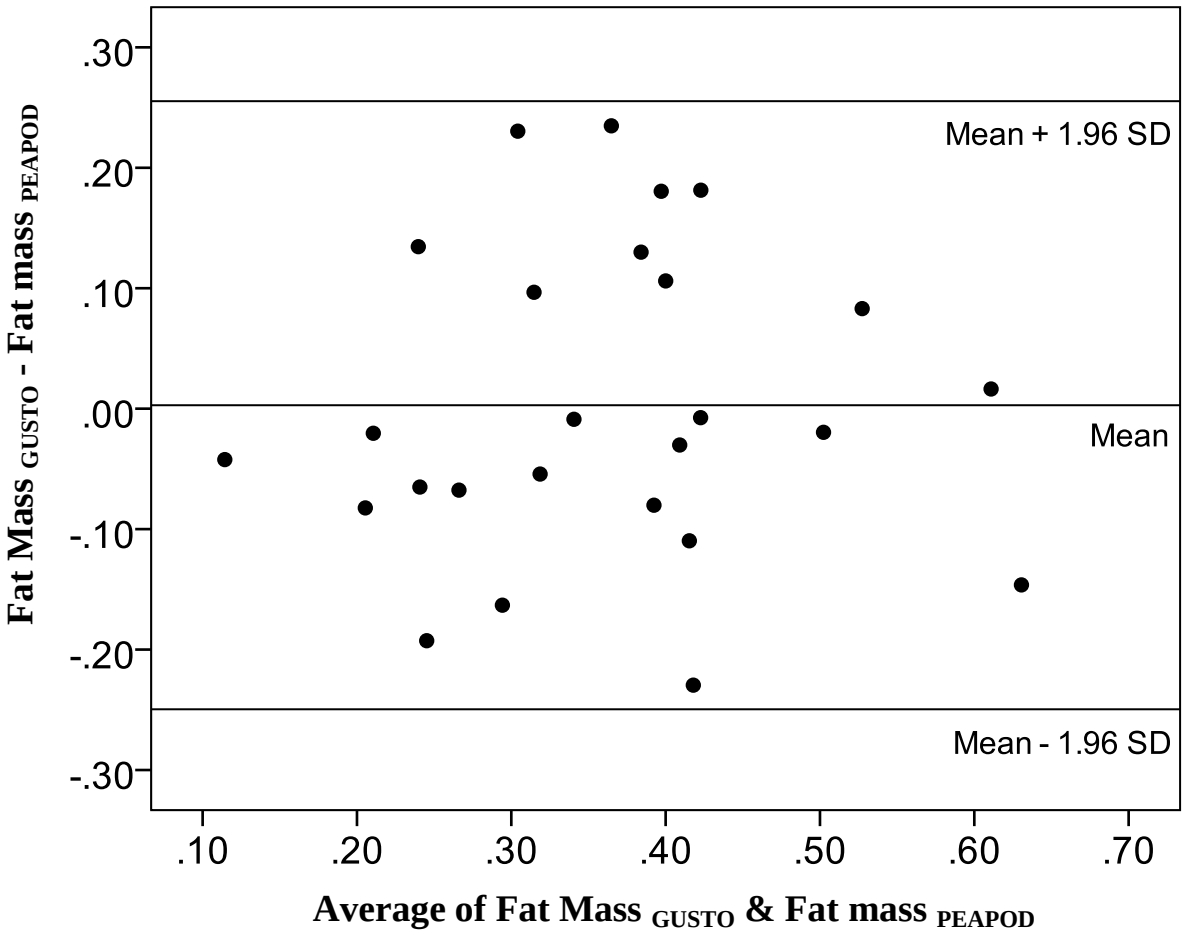


Figure 4.1B: Bland-Altman plot comparing measured fat mass against predicted fat mass using equations by Slaughter for infants measured on days 1-3 post-delivery

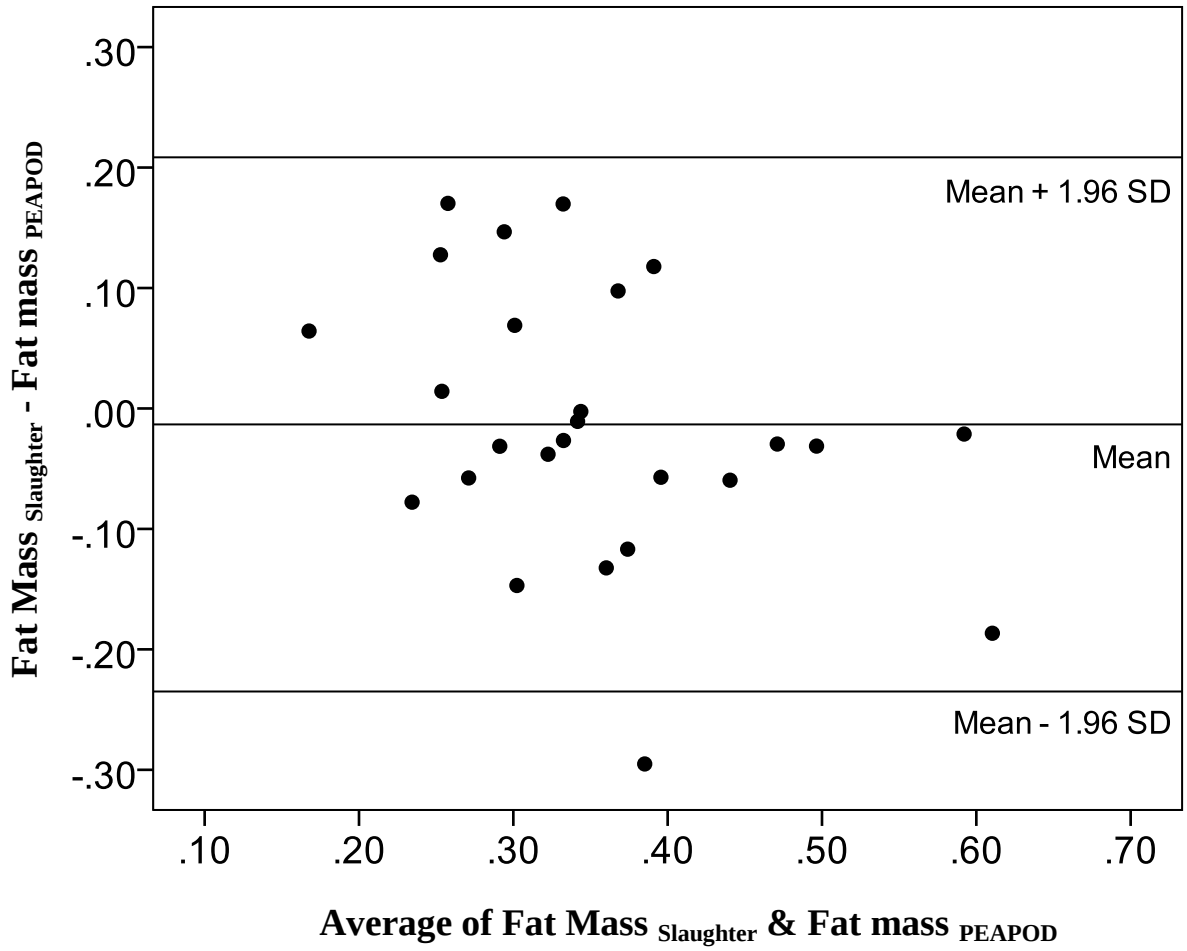


Figure 4.2A: Bland-Altman plot comparing measured fat mass against predicted fat mass using equations by GUSTO for infants measured on day 0

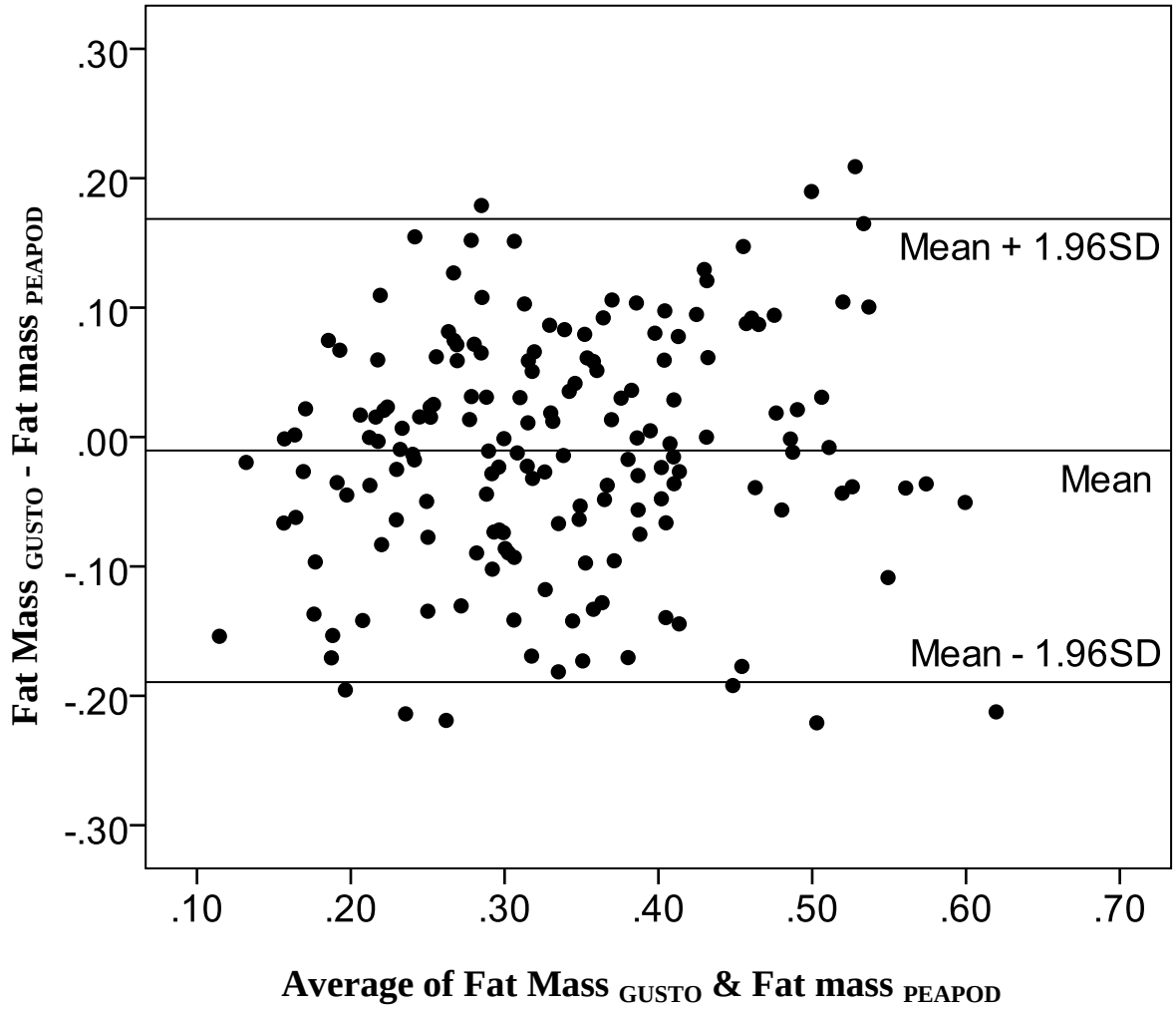
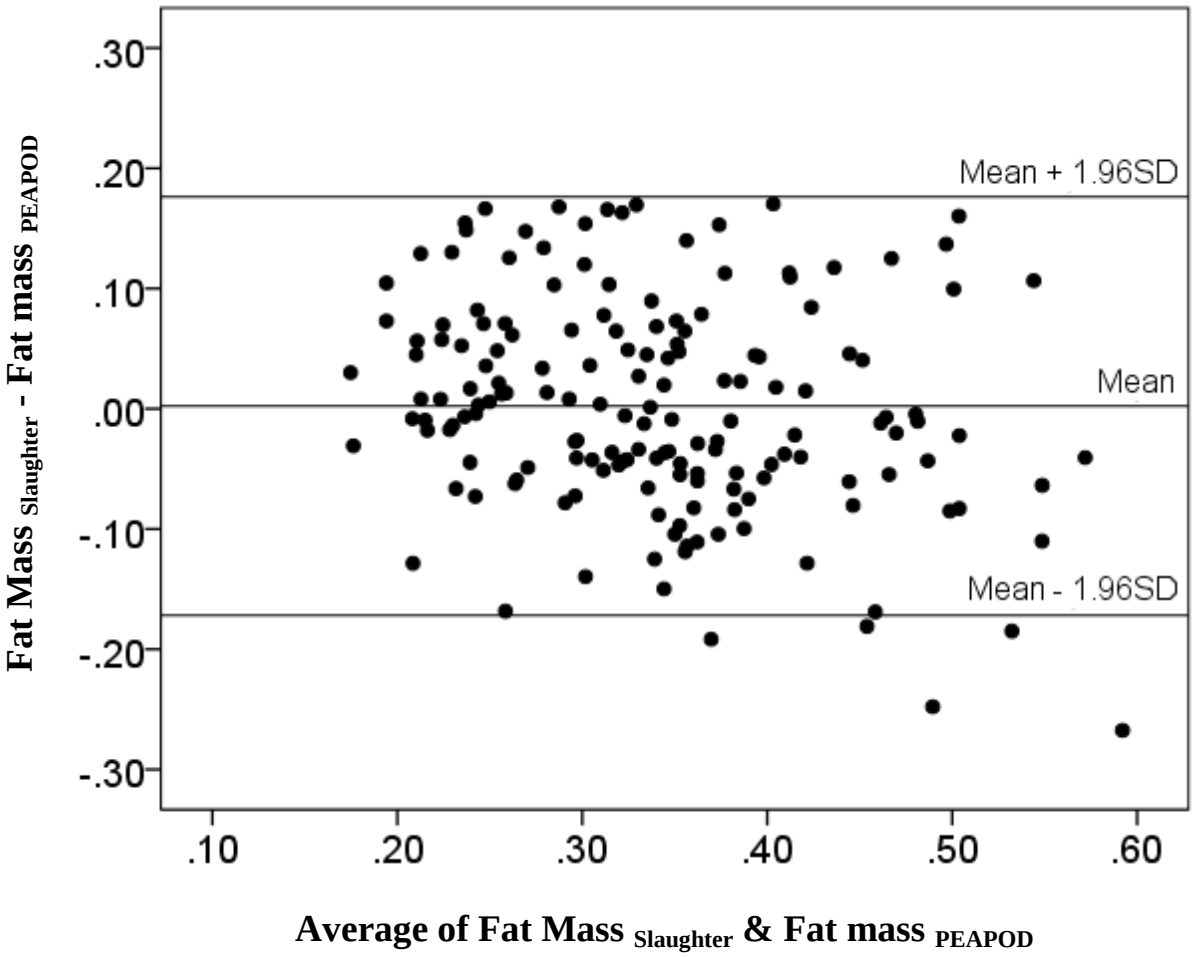


Figure 4.2B: Bland-Altman plot comparing measured fat mass against predicted fat mass using equations by Slaughter for infants measured on day 0



4.5 Discussion

We have developed a new fat mass prediction equation for neonates and compared it with the equation of Slaughter et al and with measurements obtained by PEA POD®. To our knowledge, this study is one of the few that incorporates PEA POD® as a reference method to cross-validate fat mass prediction equations. Our study utilized absolute fat mass rather than relative body fat (i.e. %BF) as the outcome variable, as early studies have identified absolute fat mass as a more desirable outcome when estimating body composition from anthropometric variables in infants and children(178). Recent studies had also identified poor predictability when attempting to correlate anthropometric measurements with relative body fat(179). SFT measurements have also been identified to be more useful in estimating fat mass rather than relative body fat(166). Consistent with other studies, we found that weight, gender and gestational age were significant contributors in estimating neonatal fat mass(179, 180). Our study found that subscapular SFT improved the prediction of fat mass. SFT has been widely accepted as a predictor of body fat(181) and can be measured directly using well-calibrated callipers. As with all quantitative biological measures, it is important to minimize error. In our study, we observed a high reproducibility of SFT measurements consistent with other studies conducted in children(182) which reflected that our observers were well-versed and trained in SFT measurement.

In our stepwise multiple regression analysis, it was somewhat surprising that triceps skinfold was not a significant predictor but subscapular skinfold is. This might be due to greater within-subject variability for triceps SFT measurements as observed in our study group. Subcutaneous fat is also

known to be unevenly distributed around the circumference of the limbs, which may explain why triceps SFT is not predictive of fat mass. Early studies have also highlighted that subscapular SFT are more predictive of fat mass than other single-skinfold sites for infants(166). Given the difficulty in performing skinfold measurements in newborns, and wide inter- and intra-individual variability in such measurements, our equation can significantly reduce time and effort in large birth studies. Ethnicity was also not a significant contributor in predicting neonatal fat mass. The lack of contribution of ethnicity in the prediction of body fat is also consistent with earlier literature on adult Singaporean Chinese, Malays and Indians, which had noted a similar observation(183, 184). This allowed us to derive a prediction model which does not require input of ethnicity for fat mass prediction, and thus may be helpful in its application to other Asian neonatal populations. We still believe there is significant difference in body composition between Caucasian and Asian babies, but our study appeared to suggest that the difference between Asian babies of different ethnicity is much less.

Our model was derived from infants whose measurements were taken on days 1-3, and excluded infants whose measurements were taken on day 0. This was based on a recent report that had identified significant initial weight loss measured at day 0(172) due to differences in hydration status. It is well-documented that neonates become lighter than they were at birth because of change in hydration status(159, 160, 185). We chose to exclude infants with anthropometric measurements taken on day 0 as the difference in hydration status may influence body fat estimates made by PEA POD®, which may in turn impact the ability of our model to estimate neonatal fat mass if day 0

infants were included. We also showed that our model, derived from infants at days 1-3 post delivery, could still estimate neonatal fat mass of infants at day 0 with a small mean bias and no significant systematic bias, unlike Slaughter's equation (Fig. 2A-B). This illustrates the applicability of our model for other newborns in the GUSTO cohort who did not have PEA POD® measurements.

Earlier studies(183, 184) highlighted that existing prediction formulas for body fat(186) were not applicable for Singaporean adults and adolescents, because of the populations in which these equations were developed in, which were mainly Caucasian. Our study revealed that Slaughter's equation may not be as applicable in Singaporean infant population, as evident by the systematic bias exhibited. This is indicative that prediction formulas for body fatness are population-specific, and existing equations may not be entirely useful for multi-ethnic Asian populations. The GUSTO equation had no significant systematic bias when estimating neonatal fat mass, indicating that the equation is in agreement with PEA POD®-derived fat mass and a general applicability of the equation to other newborns in the GUSTO cohort who did not have PEA POD® measurements. Given that the GUSTO equation was developed in a largely Asian cohort, it could also be applied to estimate body fat of Asian newborns in other studies.

Our model has its limitations; it was based on healthy, term infants and thus, not representative of small (i.e. preterm, low birth weight) infants. Our study sample had a wide distribution of fat mass, ranging from 0.13kg to 0.86kg with majority of the infants having fat mass ranging from 0.20kg to 0.50kg. Our model estimated neonatal fat mass with a coefficient of determination (R^2) of 81.1%, which is slightly lower than R^2 values reported

in other studies(180, 181). This might be explained by differences in methodologies (such as availability of SFT sites) used in other studies; our study lacked suprailiac and biceps SFT, which are also surrogate measures of central and peripheral adiposity respectively(187). The addition of these SFT sites to our model might improve the prediction of neonatal fat mass. Additionally, though we demonstrated high technical precision of SFT measurements, our model also showed somewhat broad limits of agreement. While this suggests that error in individual measurements could be large, the small mean bias observed in our equation suggests that our model is suitable for comparisons between groups of infants.

In conclusion we have developed a new fat mass prediction equation for use in Asian neonates. This equation can be used as a non-invasive method to obtain quick *in-vivo* estimate of fat mass in groups of infant subjects, but would be of almost no use in any individual infant. In order to obtain more accurate assessments of body composition of an individual infant, prediction estimates should be followed up with more sophisticated techniques of body composition measurement such as PEA POD. Given that our equations were developed in a largely Asian cohort, it can be extrapolated to estimate body fat of Asian newborns in other studies. More importantly, the GUSTO equation can be utilized to estimate fat-mass of neonates in the GUSTO cohort who did not have body composition measurements by PEA POD, which would be useful for future body composition studies.

Chapter 5: Effect of maternal glycemia on neonatal adiposity in a multi-ethnic Asian birth cohort

5.1 Summary

Background: Gestational hyperglycemia increases the risk of obesity and diabetes in offspring later in life. We examined the relationship between gestational glycemia and neonatal adiposity in a multi-ethnic Asian cohort.

Methods: A prospective mother-offspring cohort study recruited 1247 pregnant mothers and performed 75-g, 2-hour oral glucose tolerance tests at 26–28 weeks' gestation. Glucose levels were available for 1081 participants. Neonatal anthropometry (birth weight, length, triceps, and subscapular SFT) was measured, and percentage body fat (%BF) was derived using our equation. Associations of maternal glucose with excessive neonatal adiposity [large for gestational age (LGA); %BF; and sum of SFT (ΣSFT) > 90^{th} centile] were assessed using multivariable logistic regression analyses

Results: Strong positive continuous associations across the range of maternal fasting and 2-hour glucose in relation to excessive neonatal adiposity were observed; each SD increase in fasting glucose was associated with 1.31 (95%CI: 1.10– 1.55), 1.72 (95%CI: 1.31–2.27) and 1.64 (95%CI: 1.32–2.03) increases in odds ratios for LGA, %BF and ΣSFT>90th centile, respectively. Corresponding odds ratios for 2-hour glucose were 1.11 (95% CI 0.92–1.33), 1.55 (95% CI 1.10–2.20), and 1.40 (95% CI 1.10–1.79), respectively. The influence of high maternal fasting glucose on neonatal ΣSFT was less pronounced in Indians compared with Chinese (interaction $P < 0.005$).

Conclusion: A continuous relationship between maternal glycemia and excessive neonatal adiposity extends across the range of maternal glycemia. Compared with Chinese infants, Indian infants may be less susceptible to excessive adiposity from high maternal glucose levels.

5.2 Introduction

Obesity presents a massive health challenge as it rapidly becomes a worldwide epidemic(188). Increasingly, recent evidence has pointed to significant relationships between hyperglycemia during pregnancy and increased adiposity and later life glucose intolerance in the offspring(189). It is also well established that maternal hyperglycemia is associated with increased birth weight and macrosomia, and studies have identified its long-lasting effects on the offspring, resulting in higher obesity rates(190, 191) and type 2 diabetes(192). More recently, the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study showed the effect of maternal glycemia on neonatal adiposity and adverse neonatal outcomes is continuous across the range of maternal glucose concentrations, even at levels below diagnostic criteria for gestational diabetes (GDM)(73, 74).

Both fasting and 2-hour post-challenge glucose levels contribute to GDM; however the relative contributions of fasting and post-challenge hyperglycemia towards excessive neonatal adiposity remain uncertain. Past studies have shown that post-challenge glucose levels exhibit a strong linear relationship with adverse birth weight outcomes (large-for-gestational age or macrosomia)(74, 193, 194). Other studies however, reported fasting glucose levels were better than post-challenge levels in identifying risk of

macrosomia(195, 196). Recent diagnostic criteria by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) have highlighted the importance of fasting glucose in the detection and diagnosis of hyperglycemic disorders during pregnancy, which may in turn play a crucial role in identifying risk of excessive neonatal adiposity. Thus in this study, we investigated the relationship between maternal glycemia with neonatal adiposity, and hypothesized that higher maternal glucose levels during pregnancy would be associated with increased neonatal adiposity, and also compared fasting with post-challenge glucose levels in influencing excessive neonatal adiposity outcomes in Asian mothers.

5.3 Materials and Methods

5.3.1 Study population, general questionnaires and oral glucose tolerance testing

Details regarding the study population, questionnaires to ascertain demographics, lifestyle and socio-economic status and oral glucose tolerance testing have been described in Section 2.1, Section 2.3.1.1, Section 2.3.1.2.

5.3.2 Neonatal anthropometry measurements and body composition

Details regarding neonatal anthropometry measurements have been described in Section 2.3.2.1. Neonatal fat-mass was estimated using the following validated equation as described in Chapter 4 from our GUSTO cohort:

Fat Mass = -0.022 + (0.307*weight) - (0.077*Gender) + (0.028*subscapular SFT) - (0.019*Gestational Age)

where gender = 1 for male, 0 for female

Predicted fat mass values were converted to %BF using the following equation:

%BF = (Predicted fat mass / 100) x weight in kg

5.3.3 Definition of excessive neonatal adiposity outcomes

Large-for-gestational age (Birth weight > 90th centile): Centile charts for 3 newborn gender-ethnic groups (Chinese, Malay and Indian) in the GUSTO cohort were determined using the LMS method, and were described in detail in Chapter 3. Briefly, this method estimates the anthropometric measurement

centiles in terms of three age-sex specific cubic spline curves: L curve (measure of skewness based on the Box-Cox transformation), M curve (median) and the S curve (coefficient of variation). A newborn would be considered to have birth weight-for-gestational age above 90th percentile if the weight was greater than 90th centile for the infant's gender and gestational age.

Percent body fat (%BF) > 90th centile: %BF above 90th centile was defined using the same methods as for birthweight-for-gestational age > 90th centile, with gestational ages of 34-41 weeks included.

Sum of SFT (ΣSFT) > 90th centile: ∑SFT above 90th centile was defined using the same methods as for %BF > 90th centile, with gestational ages of 34-41 weeks included.

5.3.4 Statistical analyses

Descriptive statistics were reported as means and standard deviations for continuous variables and percentages for categorical variables. Differences in demographic and clinical characteristics across ethnicities were calculated using chi-square analysis and one-way analysis-of-variance (ANOVA). Fasting and 2-hour glucose measurements were divided into six categories, adapted from the HAPO Study protocol(74). For fasting glucose: Category 1 (< 4.2 mmol/l), Category 2 (4.2-4.4), Category 3 (4.5-4.7), Category 4 (4.8-4.9), Category 5 (5.0-5.2), Category 6 (≥ 5.3). For 2-hour glucose level during OGTT: Category 1 (< 5.1 mmol/l), Category 2 (5.1-6.0), Category 3 (6.1-6.9), Category 4 (7.0-7.7), Category 5 (7.8-8.7), Category 6 (≥ 8.8)

Our primary explanatory variables for analyses were maternal blood glucose levels, considered as a categorical and continuous variable. As a categorical variable, odds ratios were calculated for each measure of fasting and 2-hour glucose, higher by one category (categories 2-6), with reference to the lowest glucose category (category 1). For glucose as a continuous variable, odds ratios were calculated for each measure of fasting and 2-hour glucose higher by 1 unit (i.e. 1 mmol/L), and also by 1 standard deviation score (SDS) of glucose. The primary outcomes analysed were large-for-gestational age, %BF and ΣSFT > 90^{th} percentile. For each outcome, two logistic regression models were used for calculation of odds ratios, with Model I including adjustment for variables used to define the 90th centile (i.e. gender and gestational age at delivery) and Model II including additional adjustment for maternal age, BMI at time of OGTT, education (below or above "A" levels/diploma), parity (nulliparous or multiparous), and ethnicity (Chinese, Malay, Indian). To account for the impact of ethnic differences in socio-economic status on the outcome, an interaction term between education and ethnicity was included as a covariate in the model. All analysis was performed using SPSS version 19.0 (IBM, SPSS Statistics, Armonk, NY).

5.4 Results

5.4.1 Demographics and clinical characteristics

Data on glucose levels were available for 1081 subjects. Characteristics of these participants are shown in Table 5.1; 57.2% were Chinese, 25.5% Malay and 17.3% Indian. Mean maternal BMI at the time of OGTT was 26.2 kg/m^2, with Malay mothers exhibiting the highest BMI across the three ethnic groups ($p<0.001$). The mean glucose levels for the participants were 4.4 mmol/L and 6.6 mmol/L for fasting and 2-hour plasma glucose, respectively, with Indian mothers exhibiting the highest fasting glucose ($p=0.018$). Mean gestational age at delivery was 38.2 weeks and the mean birthweight was 3089 g. Skinfold measurements were available for 959 infants whose mothers had corresponding glucose measurements.

5.4.2 Relationship between maternal glycemia during pregnancy and frequency of excessive neonatal adiposity outcomes

Overall, we observed that the frequency of excessive neonatal adiposity outcomes rose in a linear fashion across increasing fasting and 2-hour glucose categories, even across the normal range (Figures 5.1A-C). The proportion of infants increased from 7.1% to 26.8% for LGA, 2.4% to 17.9% for %BF > 90th centile and 7.7% to 38.2% for ∑SFT > 90th centile across six fasting glucose categories. Across 2-hour glucose categories, the proportion of infants increased from 6.1% to 17.9% for LGA, 0.7% to 8.0% for %BF > 90th centile and 4.5% to 18.3% for ∑SFT > 90th centile. A chi-square test revealed a significant linear trend for all three excessive adiposity outcomes only for fasting glucose ($\lambda^2 = 30.08$ for LGA, $\lambda^2 = 25.26$ for ∑SFT > 90th percentile, λ^2

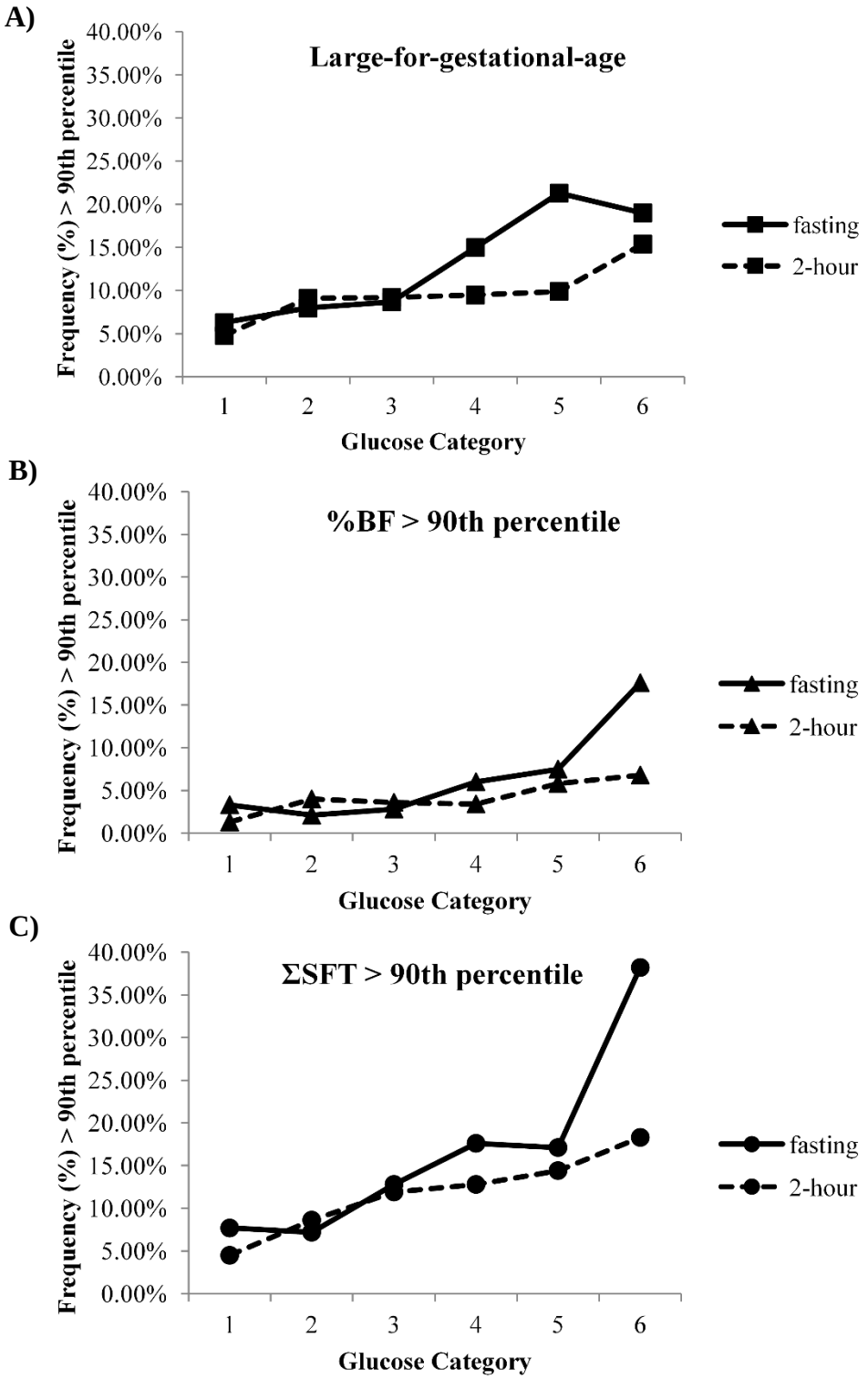
= 39.73 for ∑SFT > 90th percentile; $p < 0.001$ for all three outcomes), but not for 2-hour glucose. To test for the possible non-linear relationship between fasting glucose and birth size, squared terms for fasting glucose SDS was added to a regression model; the coefficient of the squared fasting glucose term was not statistically significant, hence highlighting that the relationship between fasting glucose and birth size is not non-linear

Table 5.1: Demographics and clinical characteristics of mothers and newborns

Mothers	**Chinese N = 618**	**Malay N = 276**	**Indian N = 187**	**Total N = 1081**	**P value#**
Age (yr)	31.7 ± 4.8	29.0 ± 5.5	29.9 ± 4.7	30.7 ± 5.1	**< 0.001**
Marital Status (%)					0.301
Married	96.5	94.8	98.3	96.4	
Single	3.3	5.2	1.7	3.5	
Divorced	0.2	0.0	0.0	0.1	
Highest Education attained (%)					**< 0.001**
Below ""A" levels / diploma	30.7	69.9	31.1	40.8	
"A" levels / diploma or higher	69.3	30.1	68.9	59.2	
Type of housing (%)					**< 0.001**
Government	81.2	93.4	87.4	85.4	
Private	18.8	6.6	12.6	14.6	
Parity (%)					**0.003**
Nulliparious	50.3	41.7	38.0	46.0	
Multiparous	49.7	58.3	62.0	54.0	
Body Mass Index (kg/m2)	25.0 ± 3.5	28.2 ± 5.4	27.1 ± 4.3	26.2 ± 4.4	**< 0.001**
Plasma Glucose (mmol/l)					
Fasting	4.3 ± 0.5	4.4 ± 0.7	4.5 ± 0.5	4.4 ± 0.5	**0.018**
2-hr	6.6 ± 1.4	6.4 ± 1.9	6.6 ± 1.5	6.6 ± 1.6	0.056
Received glucose management (%)					**0.001**
No	80.7	89.5	77.4	82.4	
Yes	19.3	10.5	22.6	17.6	
Neonates					
Gestational Age (weeks)	38.3 ± 1.5	38.1 ± 1.3	38.1 ± 1.8	38.2 ± 1.5	**0.020**
Gender (%)					
Male	52.3	55.4	51.9	53.0	0.642
Birth weight (g)	3105 ± 440	3113 ± 416	3000 ± 504	3089 ± 447	**0.010**
Predicted Body fat (%)	9.9 ± 2.3	10.0 ± 2.4	9.5 ± 2.2	9.9 ± 2.3	0.127
Sum of skinfold thickness (mm)	10.4 ± 2.3	10.4 ± 2.3	10.0 ± 2.1	10.3 ± 2.3	0.132

[#]p value across the 3 ethnic groups, by Chi-square analysis (categorical) or one-way ANOVA (continuous)

Figure 5.1: Fasting and 2-hour glucose associations with excessive adiposity outcomes; (A) Large-for-gestational-age, (B) %BF > 90th percentile and (C) ΣSFT > 90th percentile.



For fasting glucose (mmol/L): Category 1 (< 4.2), Category 2 (4.2-4.4), Category 3 (4.5-4.7), Category 4 (4.8-4.9), Category 5 (5.0-5.2), Category 6 (≥ 5.3). For 2-hour glucose level during OGTT (mmol/L): Category 1 (< 5.1), Category 2 (5.1-6.0), Category 3 (6.1-6.9), Category 4 (7.0-7.7), Category 5 (7.8-8.7), Category 6 (≥ 8.8)

5.4.3 Association between maternal glycemia during pregnancy with excessive neonatal adiposity outcomes

The associations of maternal glucose with excessive neonatal adiposity outcomes are illustrated in Table 5.2, including odds ratios (OR) and 95% confidence intervals for each category compared to the lowest category. For all three measures of excessive neonatal adiposity, we observed a continuous and graded association across increasing levels of maternal glycemia for both fasting and 2-hour glucose. For the outcome of LGA in Model I, the OR was 4.18 for highest category for fasting glucose, and 3.18 for highest 2-hour glucose category; there was modest attenuation of the odds ratios with adjustment for additional confounders in Model II for both glucose measures. For %BF and ∑SFT > 90th centile, the OR for fasting glucose were attenuated, but became larger for 2-hour glucose. OR of having LGA and ∑SFT > 90th centile were elevated within the normal fasting glucose range (category 4, 4.8-4.9 mmol/L), and also for the outcome of %BF and ∑SFT > 90th percentile for normal 2-hour glucose range of 6.1-7.7 mmol/L (categories 3 and 4). Additionally, we observed significant trends ($p < 0.0005$) of increasing likelihood of having LGA, %BF and ∑SFT > 90th centile with fasting glucose levels in six increasing categories; on the contrary, no significant trend was observed for likelihood of having excessive adiposity outcomes with increasing 2-hour glucose categories.

Table 5.2: Odds ratios for association between maternal glucose categories and excessive neonatal adiposity

	Large-for-gestational age			%BF > 90th percentile			ΣSFT > 90th percentile		
FPG[a]	**n**	***Model I OR (95% CI)**	**#Model II OR (95% CI)**	**n**	***Model I OR (95% CI)**	**#Model II OR (95% CI)**	**n**	***Model I OR (95% CI)**	**#Model II OR (95% CI)**
1	365	ref	ref	296	ref	ref	336	ref	ref
2	323	1.19 (0.68-2.09)	1.24 (0.70-2.21)	251	1.69 (0.63-4.51)	1.61 (0.59-4.37)	292	0.93 (0.51-1.69)	0.89 (0.48-1.68)
3	205	**1.79 (1.01-3.20)**	1.68 (0.92-3.06)	159	2.44 (0.89-6.71)	2.28 (0.82-6.39)	180	1.74 (0.96-3.16)	1.66 (0.88-1.60)
4	80	**2.69 (1.33-5.43)**	**2.50 (1.19-5.25)**	58	3.24 (0.91-11.49)	3.15 (0.87-11.48)	68	**2.65 (1.26-5.58)**	**2.74 (1.26-5.96)**
5	47	**3.96 (1.80-8.71)**	**3.49 (1.53-7.98)**	37	**8.56 (2.68-27.35)**	**7.14 (2.09-24.38)**	41	**2.48 (1.00-6.15)**	1.80 (0.66-4.96)
6	41	**4.18 (1.86-9.39)**	**3.19 (1.34-7.58)**	28	**11.33 (3.23-39.75)**	**7.25 (1.85-28.44)**	34	**8.44 (3.72-19.15)**	**6.30 (2.52-15.78)**
	p for trend	**< 0.0005**	**0.008**		**< 0.0005**	**0.012**		**< 0.0005**	**< 0.0005**
2-hr PG[b]	**n**	***Model I O.R (95% CI)**	**#Model II O.R (95% CI)**	**n**	***Model I O.R (95% CI)**	**#Model II O.R (95% CI)**	**n**	***Model I O.R (95% CI)**	**#Model II O.R (95% CI)**
1	164	ref	ref	134	ref	ref	154	ref	ref
2	252	1.80 (0.84-3.86)	1.71 (0.79-3.74)	206	3.91 (0.47-32.90)	4.43 (0.51-38.52)	232	1.97 (0.81-4.77)	2.01 (0.80-5.03)
3	281	2.03 (0.96-4.27)	1.90 (0.89-4.08)	227	**9.20 (1.20-70.49)**	**11.11(1.40-88.48)**	253	**2.78 (1.19-6.49)**	**3.08 (1.28-7.43)**
4	166	2.10 (0.95-4.66)	1.68 (0.74-3.82)	126	**9.04 (1.11-73.39)**	**9.51(1.13-80.30)**	148	**3.09 (1.26-7.60)**	**2.70 (1.05-6.97)**
5	120	2.03 (0.87-4.75)	1.76 (0.73-4.26)	86	**11.61 (1.40-96.26)**	**12.00(1.37-105.50)**	104	**3.57 (1.40-9.09)**	**3.54 (1.31-9.60)**
6	78	**3.18 (1.34-7.57)**	**2.54 (1.04-6.17)**	50	**12.28 (1.33-113.13)**	**14.73(1.51-143.30)**	60	**4.84 (1.77-13.19)**	**4.92 (1.70-14.24)**
	p for trend	0.207	0.484		0.102	0.106		**0.026**	0.050

FPG: Fasting plasma glucose; 2-hr PG: 2-hour plasma glucose; OR: odds ratio

*Adjusted for variables used in estimating 90th percentiles (gender and gestational age at delivery)

#Adjusted for gestational age at delivery, ethnicity, gender, maternal age, parity, maternal education, maternal education*ethnicity and maternal BMI @ 26 weeks pregnancy

[a] For fasting glucose: Category 1 (< 4.2 mmol/l), Category 2 (4.2-4.4), Category 3 (4.5-4.7), Category 4 (4.8-4.9), Category 5 (5.0-5.2), Category 6 (≥ 5.3).

[b] For 2-hour glucose level during OGTT: Category 1 (< 5.1 mmol/l), Category 2 (5.1-6.0), Category 3 (6.1-6.9), Category 4 (7.0-7.7), Category 5 (7.8-8.7), Category 6 (≥ 8.8)

We also analyzed the relationships between maternal glucose analysed as a continuous variable and excessive neonatal adiposity outcomes; each 1 mmol/L increase in fasting glucose was associated with 1.64 (95% CI 1.20-2.25), 2.73 (1.64-4.53) and 2.50 (1.68-3.71) increases in odds ratios for LGA and %BF and ΣSFT > 90th centile, respectively. Corresponding odds ratios for 2-hour glucose were 1.07 (0.95-1.19), 1.32 (1.06-1.64) and 1.24 (1.06-1.44), respectively (data not shown). For all three measures of excessive neonatal adiposity, there were significant associations with both measures of maternal glycemia, except for the relationship between 2-hour glucose and LGA. We also noted that the strength of association between fasting glucose and excessive neonatal adiposity was greater for all three outcomes as compared to 2-hour glucose. In view of the greater variability in measurement for 2-hour glucose, we also analyzed the odds ratios of having excessive adiposity outcomes for each measure of glucose higher by 1 standard deviation score (SDS). Each 1 SDS increase in fasting glucose was associated with 1.31 (95% CI 1.10-1.55), 1.72 (1.31-2.27) and 1.64 (1.32-2.03) increases in odds ratios for LGA and %BF and ΣSFT > 90th centile, respectively (Table 5.3). Corresponding odds ratios for 2-hour glucose were 1.11 (0.92-1.33), 1.55 (1.10-2.20) and 1.40 (1.10-1.79), respectively (Table 5.3). Similarly, we noted that for all three measures of excessive neonatal adiposity, there were significant associations with both measures of maternal glycemia, except for the relationship between 2-hour glucose and LGA. Additionally, there were no significant non-linear associations for both glucose measures.

Table 5.3: Relationship between maternal glucose and excessive neonatal adiposity

Outcomes	Model I OR*	95% CI	Model II OR#	95% CI
Large-for-gestational age				
Fasting glucose+	**1.40**	**1.18-1.65**	**1.31**	**1.10-1.55**
2-hour glucose+	1.18	0.99-1.39	1.11	0.92-1.33
%BF > 90th percentile				
Fasting glucose+	**1.91**	**1.46-2.49**	**1.72**	**1.31-2.27**
2-hour glucose+	**1.66**	**1.20-2.29**	**1.55**	**1.10-2.20**
ΣSFT > 90th percentile				
Fasting glucose+	**1.77**	**1.44-2.17**	**1.64**	**1.32-2.03**
2-hour glucose+	**1.45**	**1.16-1.80**	**1.40**	**1.10-1.79**

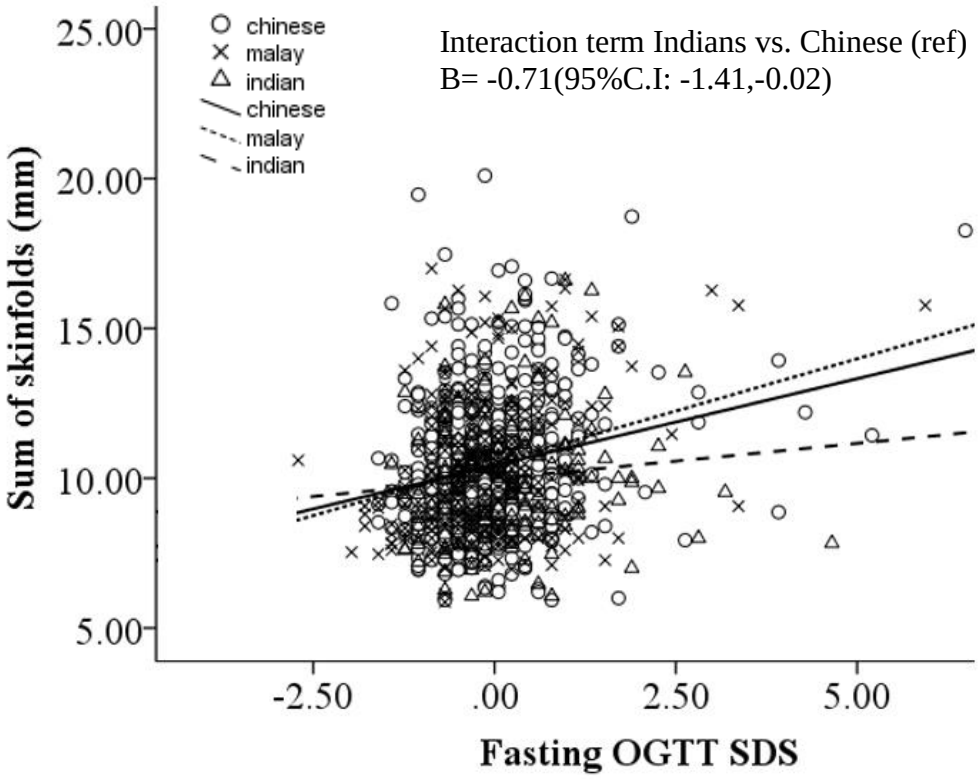
*Adjusted for gestational age at delivery and gender
#Adjusted for gestational age at delivery, ethnicity, gender, maternal age, parity, maternal BMI at OGTT, maternal education, maternal education*ethnicity
+ Odds ratios are per 1 SD increase in glucose

5.4.4 Effect of ethnicity on the association between maternal glycemia during pregnancy and excessive neonatal adiposity outcomes

When birth weight, %BF and ∑SFT were modeled as continuous variables in multiple linear regression analyses with adjustment for the same confounders in model II, mean differences between the higher (categories 2-6) and lowest categories (category 1) for both glucose measures ranged between 0.03-0.18kg for birth weight, 0.17-1.90% for %BF and 0.12-1.58mm for ∑SFT ($p < 0.05$ for both glucose measures in all outcomes). Additionally, we identified a significant negative interaction [B = -2.65 (95% C.I: -4.49,-0.81), p = 0.005] between high fasting glucose levels (category 6) with Indian ethnicity (relative to Chinese), in relation to outcome of ∑SFT. Compared to Chinese, Indians with high fasting glucose levels (category 6) is associated with lesser increase in neonatal ∑SFT. A subgroup analysis amongst women of the three ethnic groups with abnormally high glucose levels (i.e. fasting or

2-hour OGTT category ≥ 5) also identified a significant interaction between fasting glucose with Indian ethnicity [B = -0.71 (95% C.I: -1.41,-0.02), p = 0.032] for the outcome of ∑SFT. Compared to Chinese, every SD increase in fasting glucose in Indians is associated with lesser increase in neonatal ∑SFT; these are demonstrated graphically in Figure 5.2. No significant interactions were noted for 2-hour glucose with ethnicity in relation to excessive neonatal adiposity outcomes within the same subgroup.

Figure 5.2: Association between fasting glucose SDS with sum of SFT in relation to ethnicity. Open circles (o) represent Chinese, crosses (x) represent Malays and triangles (Δ) represent Indians. Dark solid line represents Chinese, dotted line represents Malays and dashed line represents Indians.



5.5 Discussion

Our findings in this study have demonstrated a continuous association between maternal glycemia and measures of excessive neonatal adiposity. The relationship was present for each maternal glucose measurement and persisted even when potential confounders such as BMI, age, ethnicity, gestational age and socio-economic status were taken into account. Firstly, consistent with that of the HAPO study, we observed that maternal glucose measured at a single point during pregnancy was effective in identifying excessive neonatal adiposity outcomes. Secondly, we noted that the relationship was graded across the range of maternal glucose levels. Hence, maternal glycemia appears to influence neonatal adiposity not just at high glucose levels, but across the normal range of glucose. In our cohort, we noted significant increases in excessive adiposity outcomes at glucose levels below those defined as hyperglycemia, similar to findings from the HAPO study.

Interestingly, in our cohort we noted that the trend of graded increases in the frequency and likelihood of excessive neonatal adiposity outcomes across the range of maternal glucose categories were significant only for fasting glucose but not for 2-hour glucose. Significant interactions for fasting glucose and Indian ethnicity in relation to ΣSFT > 90th percentile were also observed, especially amongst the subgroup of women with abnormally high glucose levels. Collectively, our results led us to speculate that fasting glucose might have a greater influence on excessive neonatal adiposity outcomes compared with post-challenge glucose, and that the influence of raised maternal fasting glucose levels on neonatal ∑SFT may be less pronounced for Indian mothers compared to Chinese mothers. We observed that amongst all

three ethnic groups, Malays formed the significantly smallest proportion (10.5%) that received management for gestational diabetes compared to Chinese and Indians, who appear to have relatively similar proportions that had received management (Chinese 19.3% vs. Indians 22.6%); this suggests that glucose management may not have confounded the ethnic difference between Indians and Chinese in the relationship between maternal glycemia and excessive neonatal adiposity outcomes that was observed in our study. The observed effect of ethnicity on the relationship between maternal glycemia and excessive neonatal adiposity may also imply the possibility of having different glucose management thresholds according to ethnicity.

There have been documented reports of fasting glucose better identifying the risk of macrosomia or excessive adiposity outcomes, as highlighted in recent studies by Ben Haroush et al(197) and Disse et al(198). In our study, though the variability in measurement for 2-hour glucose was larger, the strength of association between maternal glycemia with excessive adiposity outcomes was slightly greater for fasting glucose as compared to 2-hour glucose, when considered as SDS. Fasting glucose SDS was also significantly associated with all three measures of excessive neonatal adiposity; 2-hour glucose however showed no significant relationship with LGA. As such, our findings may highlight that fluxes of maternal glucose during the fasting state may have a slightly greater influence on excessive adiposity outcomes; nonetheless, our findings still illustrate that the dose-response effect of maternal glycemia on excessive neonatal adiposity is continuous across all glucose levels.

The classic Pedersen's hypothesis postulated that maternal hyperglycemia transmitted to the fetus would result in fetal hyperinsulinemia, which in turn can attribute to increased fetal fat accretion(199, 200). His findings however, were based on women with type-1 diabetes; over the years, there have been increases in the occurrences of GDM and type-2 diabetes(201). The underlying physiology of type-1 diabetes and type-2 diabetes/GDM are fundamentally different and as such, the metabolic environment that the developing fetus is exposed to would be different(202). Thus, the finding of a continuous association between maternal glycemia and neonatal adiposity provides us with a better understanding of the influence of maternal glycemia, with the effect not only restricted to maternal hyperglycemia, but extending throughout the range of glycemia. Other than the recent HAPO study, there are few published studies relating maternal metabolic factors with neonatal body composition. Past studies that have identified relationships between birth size and later adiposity are also based primarily on birthweight without any information regarding adiposity at birth(189). Thus, our study provides useful, informative data on the relationship between maternal glycemia and neonatal body composition in a multi-ethnic cohort independent from the HAPO study, and the consistency in the findings from both cohorts confirm the link between maternal glycemia and neonatal adiposity.

Our study has some limitations; glucose data was collected only at fasting and 2-hour post-challenge, but not at 1-hour post-challenge. Data on 1-hour glucose would have allowed us to better address the role of maternal glycemia with neonatal adiposity. Also, %BF was not measured directly, but

estimated using an equation based on the infant's gender, gestational age, weight and subscapular SFT, as described in Chapter 4. SFT is an indirect measure of adiposity; however the formula has been validated by infant body composition measurements with PEA POD.

In conclusion, our study involving an Asian population revealed a continuous dose-response relationship between maternal glycemia and neonatal adiposity which extends across the entire range of glycemia. The consistency of our findings with the HAPO study also confirms the link between maternal glycemia and neonatal adiposity. It remains to be seen however, if the observed association between maternal metabolic factors and neonatal body composition has long-term repercussions on the increasing prevalence of obesity and diabetes in adolescents as well as adults.

Chapter 6: Effect of maternal gestational glycemia and adiposity on early postnatal growth of offspring in the first three years of life

6.1 Summary

Background: Gestational hyperglycemia increases the risk of obesity and diabetes in offspring later in life. We examined the relationship between gestational glycemia and body mass index (BMI) on early postnatal growth of offspring in a multi-ethnic Asian birth cohort.

Methods: Pregnant mothers took 75g 2-hour oral glucose tolerance tests at 26-28 weeks gestation. In 1152 naturally-conceived singleton offspring, measurements included weight and length at birth, 3 weeks, and 3,6,9,12,15,18,24 and 36 months of age, and multivariable linear regression analysis was used to estimate the associations between gestational glycemia and BMI on offspring growth.

Results: Maternal fasting plasma glucose (FPG) was positively associated with birthweight [B(95%CI)=0.12(0.06,0.18)], birth BMI [0.19(0.13,0.25)], as well as length at 3 weeks [0.14(0.08,0.17)], but not from 3 months. 2-hour post-challenge glucose had little impact. Maternal BMI at 26-28 weeks gestation was positively associated with offspring weight and BMI throughout the first three years of life. Offspring born to mothers with higher FPG showed significant weight deceleration [B(95%C.I)=-0.23(-0.42,-0.04)] early in life (3 weeks-3 months), followed by statistically significant accelerated weight and BMI gain at 9-15 months. The effect of raised FPG on birth size was greater in obese women. The effect of raised maternal FPG on higher offspring weight, BMI and overweight status was significant for non-obese, multiparous and

Chinese women only at two-years of age (interaction $p<0.05$ for all), but not anymore at three years. The relationship between child overweight status and increasing maternal FPG in obese pregnant mothers has an unexpected pattern, where the risk of offspring being overweight is highest in those born to mothers who are obese and in the lowest fasting glucose category.

Conclusions: FPG is associated with growth deceleration over a short period after birth independent of maternal BMI, followed by transient growth acceleration between 9-15 months. The impact of maternal adiposity persists from birth into early childhood. Maternal obesity, ethnicity, and parity may confer different susceptibility to greater adiposity in response to maternal glycemia only at two years of age. Pregnancy fasting glycemia and obesity in tandem continues to exert an effect on risk of childhood overweight status at three years.

6.2 Introduction

Obesity and type 2 diabetes present massive health challenges as they rapidly become a worldwide epidemic(1), hence understanding the pathogenesis is important in order to formulate treatment and prevention strategies. Developmental influence on obesity risk originating from the maternal intrauterine environment has been put forth as one of the mechanisms which confer susceptibility to excessive adiposity(203). One of the earliest evidence of developmental plasticity conferring obesity susceptibility came from the Dutch famine study, which studied the offspring of women who conceived during the Dutch famine of 1944, when an embargo was placed on all food supplies to the Netherlands(3, 4). Studies have

documented that maternal obesity and hyperglycemia during pregnancy are associated with higher birth weight of offspring, and carried with it an inherently greater risk of diabetes and obesity in later life(204-207). There is also evidence that early postnatal growth pattern predicts subsequent adiposity and obesity risk in childhood, as demonstrated by the Quebec Longitudinal Study of Child Development (208) and the Fels Longitudinal Study (209), which highlighted that offspring with the highest quintiles of weight gain during early life had increased odds of being overweight at later ages.

It is believed that excessive exposure to increased glucose from the mother may contribute to excessive weight gain of offspring born to diabetic mothers(210). The influence of maternal glycemia during pregnancy on early postnatal growth has been described(80, 211), but can still be better defined, especially for the impact on postnatal growth pattern across the range of glucose levels, even for those that are below the diagnostic cutoff for gestational diabetes (GDM) (fasting glucose > 7.0 mmol/L or 2h glucose > 7.8 mmol/L)(120). In addition, studies on maternal glycemia during pregnancy influencing early postnatal growth in Asian populations are scarce, and since the Asian phenotype and susceptibility towards obesity and metabolic disease differs from that of Caucasians(115), further studies on the impact of maternal glycemia during pregnancy on offspring growth in Asian populations is merited. Thus in this study, we sought to examine the impact of maternal glycemia and body mass index (BMI) on birth measures and postnatal growth of offspring, and hypothesized that higher maternal glucose and adiposity

during pregnancy leads to increased adiposity and postnatal growth in the first three years of life.

6.3 Materials and Methods

6.3.1. Study population and assessment of gestational age

Details on the study population and assessment of gestational age have been described in Section 2.1 and Section 2.3.1.3.

6.3.2 Oral glucose tolerance testing and anthropometry measurements

Details regarding oral glucose tolerance testing and measurement of anthropometry in the first three years of life have been described in Section 2.3.1.2 and Section 2.3.2.1.

6.3.3 Statistical analysis

Descriptive statistics were reported as means and standard deviations for continuous variables and percents for categorical variables. Fasting plasma glucose (FPG) and 2-hour post-challenge glucose (2h-PG) measurements were divided into quartiles For FPG: 1st quartile (< 4.1mmol/l), 2nd quartile (4.1 - < 4.3), 3rd quartile (4.3 - < 4.6), 4th quartile (≥ 4.6). For 2h-PG: 1st quartile (< 5.5mmol/l), 2nd quartile (5.5 - < 6.3), 3rd quartile (6.3 - < 7.3), 4th quartile (≥ 7.3). Age- and gender-specific standard deviation scores (SDS) were calculated for weight, length and body mass index (BMI) for infants at all timepoints, referencing WHO Child Growth Standards(212). SDS was also produced for maternal glucose and BMI at 26-28 weeks gestation.

One-way analysis of variance (ANOVA) was used to assess the differences in child growth measures at each time point between the glucose

categories. Multiple linear regression analyses were used to estimate the association between maternal glucose level and offspring weight, length and BMI at birth to two years using SDS to enable comparison both between variables and across time points, in all cases adjusting for ethnicity, parity, maternal age, maternal education, maternal BMI at 26-28 weeks gestation, maternal height and breastfeeding duration. In view of mothers who received treatment for hyperglycemia, we additionally corrected for potential confounding by glucose management in the regression models. Potential effect modifications by ethnicity, parity and pregnancy obesity (BMI $\geq$ 30 kg m^{-2}) were also investigated, by adding the interaction term of maternal glucose with ethnicity/obesity/parity to the fully adjusted model.

Conditional growth models for weight, length and BMI SDS were built using linear regression analysis(213). Here, weight SDS is used as an example: conditional growth in weight SDS from birth to 3 weeks is equivalent to the standardised residuals resulting from the linear regression model of weight SDS at 3 weeks on weight SDS at birth. Accordingly, the conditional growth in weight SDS from 3 weeks to 3 months is given as the standardised residuals obtained from regressing weight SDS at 3 months on weight SDS at 3 weeks and at birth simultaneously. This process is continued for each subsequent time point, resulting in measures of growth that are uncorrelated. All analysis was performed using SPSS version 20.0 (IBM, SPSS Statistics, Armonk, NY) and Stata 13 (StataCorp, Texas).

6.4 Results

6.4.1 Demographics and clinical characteristics

Characteristics of the study participants were described in Table 6.1. 55.2% of the study participants were Chinese, 27.0% Malay and 17.8% Indian. Chinese mothers in our cohort tended to be slightly older (mean age = 31.4 years), and more educated (68% of them had at least 12 years of education). More Chinese mothers also breastfed their offspring for more than 4 months (27.8%) compared with other ethnicities. The mean maternal BMI at the time of OGTT was 26.1 kg m^{-2} and mean glucose levels for the participants were 4.4 mmol/L and 6.5 mmol/L for FPG and 2h-PG respectively. Mean gestational age at delivery was 38.3 weeks and the mean offspring birth weight, length and BMI were 3.09kg, 48.6cm and 13.0 kg m^{-2}, respectively.

Table 6.1: Demographics and clinical characteristics of study subjects

Mothers	**Chinese N = 561**	**Malay N = 274**	**Indian N = 181**	**Total N = 1016**	**P value#**
Age (yr)	31.4 ± 5.0	28.9 ± 5.4	29.8 ± 4.7	30.4 ± 5.2	**<0.001**
Marital Status (%)					0.225
Married	96.4	95.1	98.3	96.4	
Single	3.6	4.9	1.7	3.6	
No. of years of education(%)					**<0.001**
< 12 years	31.4	70.3	31.6	41.9	
≥ 12 years	68.6	29.7	68.4	58.1	
Type of housing (%)					**<0.001**
Government	88.7	99.3	91.5	92.0	
Private	11.3	0.7	8.5	8.0	
Breastfeeding Duration (%)					**<0.001**
Formula only	14.5	21.3	14.5	16.3	
Less than 4 months	57.7	68.6	63.5	61.6	
More than 4 months	27.8	10.0	22.0	22.1	
Parity (%)					**0.010**
Primiparious	52.5	59.7	64.2	56.5	
Multiparous	47.5	40.3	35.8	43.5	
Body Mass Index (kg/m2)	24.9 ± 3.4	28.0 ± 5.4	27.2 ± 4.5	26.1 ± 4.5	**<0.001**
Plasma Glucose (mmol/l)					
Fasting	4.3 ± 0.4	4.3 ± 0.5	4.5 ± 0.5	4.4 ± 0.5	**0.003**
2-hr	6.6 ± 1.4	6.2 ± 1.4	6.5 ± 1.5	6.5 ± 1.4	**0.001**
Received glucose management (%)					**0.001**
No	79.9	89.8	77.9	82.2	
Yes	20.1	10.2	22.1	17.8	
Gestational Age at time of OGTT	26.8 ± 1.1	26.1 ± 1.2	26.8 ± 1.3	26.8 ± 1.2	0.248

Table 6.1(continued): Demographics and clinical characteristics of study subjects

Offspring	Chinese N = 561	Malay N = 274	Indian N = 181	Total N = 1016	P value#
Gestational Age (weeks)	38.4 ± 1.5	38.2 ± 1.3	38.2 ± 1.5	38.3 ± 1.5	0.127
Gender (%)					0.664
Male	52.0	55.2	52.0	52.8	
Weight (kg)					
Birth (n=1005)	3.1 ± 0.4	3.1 ± 0.4	3.0 ± 0.5	3.1 ± 0.4	0.158
Week 3 (n=904)	4.0 ± 0.5	3.9 ± 0.5	3.8 ± 0.5	3.9 ± 0.5	**<0.001**
Month 3 (n=889)	6.3 ± 0.8	6.0 ± 0.8	5.8 ± 0.7	6.1 ± 0.8	**<0.001**
Month 6 (n=847)	7.8 ± 0.9	7.7 ± 0.9	7.5 ± 0.9	7.7 ± 0.9	**0.021**
Month 9 (n=808)	8.6 ± 1.0	8.6 ± 1.0	8.7 ± 1.0	8.6 ± 1.0	0.698
Month 12 (n=829)	9.4 ± 1.1	9.3 ± 1.1	9.5 ± 1.2	9.4 ± 1.1	0.138
Month 15 (n=835)	10.0 ± 1.1	10.0 ± 1.2	10.3 ± 1.3	10.1 ± 1.2	**0.020**
Month 18 (n=797)	10.7 ± 1.3	10.7 ± 1.4	11.1 ± 1.5	10.7 ± 1.3	**0.002**
Month 24 (n=803)	11.9 ± 1.5	12.0 ± 1.6	12.2 ± 1.7	12.0 ± 1.6	0.121
Month 36 (n=810)	14.1 ± 1.8	14.3 ± 2.3	14.6 ± 2.5	14.2 ± 2.1	**0.024**
Length (cm)					
Birth (n=1002)	48.8 ± 2.3	48.2 ± 2.0	48.7 ± 2.2	48.6 ± 2.2	**0.009**
Week 3 (n=902)	53.2 ± 2.2	52.2 ± 2.0	52.7 ± 2.0	52.9 ± 2.2	**<0.001**
Month 3 (n=889)	61.4 ± 2.5	60.0 ± 2.4	61.0 ± 2.2	60.9 ± 2.5	**<0.001**
Month 6 (n=851)	67.4 ± 2.7	66.1 ± 2.7	67.4 ± 2.5	67.1 ± 2.7	**<0.001**
Month 9 (n=809)	71.9 ± 3.0	70.6 ± 2.8	72.1 ± 2.5	71.6 ± 2.9	**<0.001**
Month 12 (n=830)	75.7 ± 3.1	74.1 ± 2.9	76.3 ± 2.8	75.4 ± 3.1	**<0.001**
Month 15 (n=828)	79.1 ± 3.2	77.4 ± 3.0	80.1 ± 3.0	78.8 ± 3.2	**<0.001**
Month 18 (n=694)	82.2 ± 3.4	81.1 ± 3.1	83.3 ± 3.4	82.1 ± 3.4	**<0.001**
Month 24 (n=708)	87.9 ± 3.7	86.3 ± 3.1	88.5 ± 3.7	87.6 ± 3.6	**<0.001**
Month 36 (n=804)	94.9 ± 3.8	93.5 ± 3.6	96.2 ± 3.8	94.8 ± 3.9	**<0.001**
BMI (kg/m^2)					
Birth (n=1002)	13.0 ± 1.3	13.3 ± 1.3	12.8 ± 1.3	13.0 ± 1.3	**<0.001**
Week 3 (n=901)	13.9 ± 1.2	14.2 ± 1.2	13.5 ± 1.3	13.9 ± 1.3	**<0.001**
Month 3 (n=889)	16.7 ± 1.5	16.7 ± 1.6	15.7 ± 1.4	16.5 ± 1.6	**<0.001**
Month 6 (n=847)	17.1 ± 1.6	17.6 ± 1.8	16.6 ± 1.5	17.1 ± 1.7	**<0.001**
Month 9 (n=808)	16.6 ± 1.4	17.2 ± 1.5	16.6 ± 1.5	16.8 ± 1.5	**<0.001**
Month 12 (n=827)	16.3 ± 1.3	16.9 ± 1.5	16.3 ± 1.5	16.4 ± 1.4	**<0.001**
Month 15 (n=828)	16.0 ± 1.3	16.6 ± 1.6	16.0 ± 1.5	16.2 ± 1.4	**<0.001**
Month 18 (n=692)	15.8 ± 1.3	16.1 ± 1.5	15.9 ± 1.5	15.9 ± 1.4	**0.032**
Month 24 (n=708)	15.4 ± 1.3	15.9 ± 1.5	15.5 ± 1.5	15.5 ± 1.4	**<0.001**
Month 36 (n=804)	15.6 ± 1.3	16.2 ± 1.8	15.7 ± 1.8	15.7 ± 1.6	**<0.001**

[#]p value across 3 ethnic groups, by Chi-square analysis (categorical) or one-way ANOVA (continuous)

6.4.2 Relationship of birth and early infant anthropometry with maternal glycemia and adiposity

Table 6.2 describes the association of maternal glycemia with offspring weight, length and BMI in the first three years of life. Maternal FPG SDS had significant positive associations with weight SDS [B(95%CI) = 0.12(0.06,0.18), p < 0.001)] and BMI SDS [B(95%CI) = 0.19 (0.13,0.25), p < 0.001)] at birth, and length SDS at 3 weeks of age conditional upon birth length SDS [B(95%CI) = 0.14(0.08,0.20), p <0.001], but no significant associations were observed from months 3 to 36, after adjusting for potential confounders. Maternal 2h-PG SDS also showed significant positive associations with infant weight SDS at birth only [B(95%CI) = 0.07(0.001,0.08), p = 0.03] (data not shown); no significant associations were observed between 2h-PG SDS and offspring's weight and BMI SDS from week 3 to month 36. Maternal BMI SDS showed significant association with infant weight at birth [B(95%CI) = 0.16 (0.10,0.22), p <0.001], and at 18, 24 and 36 months conditional upon birth weight SDS. Maternal BMI SDS also showed significant positive association with infant BMI SDS at birth, and at 9 to 36 months of life conditional upon BMI SDS at birth (Table 6.2), indicating that maternal BMI at 26-28 weeks of pregnancy is a strong correlate of offspring's growth during the first three years of life.

Table 6.2: Regression analysis with offspring weight, length and BMI SDS as the response variables, and maternal FPG SDS & maternal pregnancy BMI SDS, as the explanatory variables

	Birth	Week 3[+]	Month 3[+]	Month 6[+]	Month 9[+]	Month 12[+]	Month 15[+]	Month 18[+]	Month 24[+]	Month 36[+]
	B (95%CI)[a]	B (95%CI)[a]	B (95%CI)[a]	B(95%CI)[a]	B(95%CI)[a]	B(95%CI)[a]	B(95%CI)[a]	B(95%CI)[a]	B(95%CI)[a]	B(95%CI)[a]
Weight SDS										
Maternal FPG SDS	**0.12 (0.06,0.18)****	0.005 (-0.08,0.08)	-0.04 (-0.12,0.04)	-0.04 (-0.12,0.04)	-0.04 (-0.12,0.04)	-0.05 (-0.13,0.03)	-0.05 (-0.13,0.03)	0.005 (-0.75,0.85)	-0.01 (-0.09,0.07)	-0.05 (-0.13,0.03)
Maternal BMI SDS	**0.16 (0.10,0.22)****	0.003 (-0.08,0.08)	-0.02 (-0.03,0.01)	0.04 (-0.04,0.12)	0.04 (-0.04,0.12)	0.06 (-0.02,0.14)	0.04 (-0.04,0.12)	**0.09 (0.01,0.13)***	**0.09 (0.01,0.13)***	**0.17 (0.11,0.25)****
Length SDS										
Maternal FPG SDS	-0.03 (-0.11,0.05)	**0.14 (0.08,0.20)****	0.04 (-0.04,0.12)	0.03 (-0.05,0.11)	0.02 (-0.06,0.10)	0.01 (-0.07,0.09)	-0.005 (-0.09,0.08)	0.04 (-0.04,0.12)	0.005 (-0.08,0.09)	0.01 (-0.07,0.09)
Maternal BMI SDS	**0.13 (0.05,0.21)****	0.009 (-0.05,0.07)	0.03 (-0.05,0.11)	-0.02 (-0.06,0.04)	-0.003 (-0.08,0.08)	-0.04 (-0.12,0.04)	-0.03 (-0.11,0.05)	-0.01 (-0.09,0.08)	-0.02 (-0.10,0.06)	0.01 (-0.07,0.09)
BMI SDS										
Maternal FPG SDS	**0.19 (0.13,0.25)****	-0.05 (-0.13,0.03)	-0.02 (-0.10,0.06)	-0.04 (-0.12,0.04)	-0.02 (-0.10,0.06)	-0.04 (-0.12,0.04)	-0.02 (-0.10,0.06)	0.01 (-0.07,0.09)	0.03 (-0.05,0.11)	-0.05 (-0.13,0.03)
Maternal BMI SDS	**0.14 (0.06,0.20)****	**0.07 (0.001.0.15)***	-0.002 (-0.08,0.08)	0.08 (-0.001,0.16)	**0.08 (0.001,0.16)***	**0.12 (0.04,0.16)***	**0.09 (0.01,0.17)***	**0.16 (0.08,0.24)****	**0.16 (0.08,0.24)****	**0.23 (0.15,0.31)****

Variables also in model but not shown are gestational age, parity, ethnicity, maternal education, breastfeeding duration, glucose management, maternal height and maternal age

[+]SDS values for weight, length and BMI at week 3 – month 36 are conditional upon SDS values at birth

[a] B = beta coefficient; 95%CI = 95% confidence intervals

FPG = Fasting plasma glucose; BMI = Body Mass Index

* $p < 0.05$, ** $p < 0.001$

6.4.3 Relationship of infant conditional growth with maternal glycemia

Table 6.3 highlights the conditional gain in weight, length and BMI SDS of offspring during the first three years of life, according to FPG quartiles. Offspring born to mothers in highest FPG quartile showed significantly lower conditional gain in weight SDS between 3 weeks to 3 months of life [B(95%C.I)= -0.23(-0.42,-0.04)], but higher conditional gain in length SDS at 0-3 weeks of life [B(95%C.I) = 0.28(0.10,0.47)], BMI SDS at 9-15 months of life [B(95%C.I)= 0.23(0.01,0.46)] compared to those in the lowest FPG quartile. Offspring born to mothers in the second highest or 3rd FPG quartile also showed significantly higher conditional gain in weight SDS [B(95%C.I)= 0.26(0.05,0.48)] and BMI SDS [B(95%C.I)= 0.26(0.05,0.47)] at 9-15 months of life, compared to those in the lowest FPG quartile. Offspring born to mothers in 2nd FPG quartile demonstrated decelerated length gain at 9-15 months of life [B(95%CI) =-0.33(-0.55,-0.11)] and accelerated gain in BMI SDS [B(S.E) = 0.23(0.005,0.45)] compared to those in the lowest FPG quartile. For 2h-PG, the pattern of conditional weight SDS growth for offspring born to mothers in the highest 2h-PG quartile in the first three months of life showed no significant differences when compared with the lowest 2h-PG quartile. Offspring born to mothers in the second highest or 3rd 2h-PG quartile showed significantly higher conditional gain in weight SDS at 15-24 months of life [B(95%CI)=0.32(0.09,0.56)], compared to compared to those in the lowest 2h-PG quartile. No significant changes were observed at other time periods across all 2h-PG quartiles, and a similar observation was noted for length and BMI gain from birth till month 36 of age (Table 6.4).

Table 6.3: Conditional growth of offspring weight, length and BMI SDS at 0-3 weeks, 3weeks to 3 months, 3-9, 9-15, 15-24 and 24-36 months as the response variables and maternal FPG categories as the explanatory variable

	Conditional gain 0-3 weeks		Conditional gain 3 weeks - 3 months		Conditional gain 3-9 months		Conditional gain 9-15 months		Conditional gain 15-24 months		Conditional gain 24-36 months	
OGTT fast category	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**
1st Quartile	ref	-	ref	-	ref	-	ref	-	ref	-	ref	-
2nd Quartile	0.01(-0.18,0.20)	0.920	**-0.23(-0.42,0.03)**	**0.023**	-0.05(-0.25,0.16)	0.663	0.05(-0.18,0.27)	0.673	-0.003(-0.24,0.23)	0.983	0.07(-0.18,0.32)	0.558
3rd Quartile	0.15(-0.03,0.33)	0.093	-0.17(-0.35,0.02)	0.079	-0.02(-0.22,0.17)	0.810	**0.26(0.05,0.48)**	**0.015**	-0.03(-0.26,0.19)	0.788	0.09(-0.14,0.32)	0.451
4th Quartile	0.02(-0.17,0.20)	0.860	**-0.23(-0.42,-0.04)**	**0.018**	-0.04(-0.25,0.16)	0.691	0.21(-0.01,0.44)	0.067	-0.13(-0.37,0.11)	0.288	0.15(-0.10,0.40)	0.248
	#Length SDS B (95% CI)	**p value**	**#Length SDS B (95% CI)**	**p value**	**#Length SDS B (95% CI)**	**p value**	**#Length SDS B (95% CI)**	**p value**	**#Length SDS B (95% CI)**	**p value**	**#Length SDS B (95% CI)**	**p value**
1st Quartile	ref	-	ref	-	ref	-	ref	-	ref	-	ref	-
2nd Quartile	-0.06(-0.25,0.13)	0.556	-0.02(-0.22,0.18)	0.840	-0.13(-0.34,0.08)	0.232	**-0.33(-0.55,-0.11)**	**0.003**	0.08(-0.17,0.32)	0.535	-0.14(-0.42,0.14)	0.321
3rd Quartile	0.10(-0.08,0.28)	0.271	-0.01(-0.20,0.17)	0.886	-0.001(-0.20,0.20)	0.992	-0.19(-0.39,0.02)	0.079	0.004(-0.23,0.23)	0.971	-0.15(-0.41,0.11)	0.261
4th Quartile	**0.28(0.10,0.47)**	**0.003**	-0.09(-0.28,0.11)	0.384	-0.12(-0.33,0.09)	0.268	**-0.23(-0.45,-0.03)**	**0.047**	-0.01(-0.26,0.23)	0.907	0.11(-0.16,0.39)	0.417
	+BMI SDS B (95% CI)	**p value**	**+BMI SDS B (95% CI)**	**p value**	**+BMI SDS B (95% CI)**	**p value**	**+BMI SDS B (95% CI)**	**p value**	**+BMI SDS B (95% CI)**	**p value**	**+BMI SDS B (95% CI)**	**p value**
1st Quartile	ref	-	ref	-	ref	-	ref	-	ref	-	ref	-
2nd Quartile	0.13(-0.06,0.32)	0.174	-0.18(-0.38,0.02)	0.071	0.01(-0.20,0.23)	0.906	**0.23(0.005,0.45)**	**0.045**	-0.24(-0.49,0.02)	0.066	-0.04(-0.32,0.24)	0.789
3rd Quartile	0.13(-0.05,0.31)	0.155	-0.10(-0.29,0.09)	0.288	-0.07(-0.27,0.14)	0.526	**0.26(0.05,0.47)**	**0.015**	-0.05(-0.29,0.19)	0.678	-0.02(-0.29,0.24)	0.860
4th Quartile	-0.07(-0.25,0.12)	0.479	-0.13(-0.33,0.07)	0.196	-0.01(-0.23,0.20)	0.925	**0.23(0.01,0.46)**	**0.040**	-0.05(-0.31,0.21)	0.702	0.02(-0.27,0.30)	0.895

*,# Adjusted for parity, ethnicity, maternal education, glucose management, maternal age, maternal BMI SDS at 26-28 week gestation, maternal height and breastfeeding duration

+ Adjusted for parity, ethnicity, maternal education, glucose management, maternal age, maternal BMI SDS at 26-28 week gestation and breastfeeding duration

FPG Quartiles: 1st quartile (< 4.1mmol/L), 2nd quartile (4.1 - < 4.3mmol/L), 3rd quartile (4.3 - < 4.6mmol/L), 4th quartile (≥ 4.6mmol/L)

Table 6.4: Conditional growth of offspring weight, length and BMI SDS at 0-3 weeks, 3weeks to 3 months, 3-9, 9-15, 15-24 and 24-36 months as the response variables and maternal 2h-PG categories as the explanatory variable.

	Conditional gain 0-3 weeks		Conditional gain 3 weeks - 3 months		Conditional gain 3-9 months		Conditional gain 9-15 months		Conditional gain 15-24 months		Conditional gain 24-36 months	
OGTT 2h-PG category	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**	***Weight SDS B (95% CI)**	**p value**
1st Quartile	ref	-	ref	-	ref	-	ref	-	ref	-	ref	-
2nd Quartile	-0.05(-0.25,0.14)	0.574	-0.06(-0.26,0.13)	0.522	0.02(-0.19,0.22)	0.873	-0.13(-0.36,0.10)	0.258	0.10(-0.13,0.34)	0.383	-0.08(-0.33,0.16)	0.512
3rd Quartile	0.08(-0.11,0.26)	0.414	-0.15(-0.34,0.04)	0.131	0.08(-0.12,0.28)	0.447	0.007(-0.21,0.23)	0.954	**0.32(0.09,0.56)**	**0.006**	-0.01(-0.26,0.23)	0.912
4th Quartile	-0.12(-0.37,0.12)	0.330	-0.22(-0.48,0.04)	0.095	0.20(-0.07,0.48)	0.142	-0.02(-0.33,0.28)	0.877	0.08(-0.23,0.39)	0.619	-0.005(-0.33,0.32)	0.976
	#Length SDS B (95% CI)	**p value**	**#Length SDS B (95% CI)**	**p value**	**#Length SDS B (95% CI)**	**p value**	**#Length SDS B (95% CI)**	**p value**	**#Length SDS B (95% CI)**	**p value**	**#Length SDS B (95% CI)**	**p value**
1st Quartile	ref	-	ref	-	ref	-	ref	-	ref	-	ref	-
2nd Quartile	0.006(-0.18,0.20)	0.954	-0.21(-0.41,-0.009)	0.040	-0.12(-0.33,0.08)	0.245	-0.01(-0.23,0.21)	0.915	-0.11(-0.35,0.14)	0.393	-0.13(-0.40,0.15)	0.375
3rd Quartile	0.05(-0.14,0.23)	0.612	-0.11(-0.30,0.08)	0.266	-0.05(-0.25,0.15)	0.633	-0.06(-0.27,0.16)	0.594	0.13(-0.11,0.37)	0.295	-0.04(-0.31,0.23)	0.776
4th Quartile	0.07(-0.18,0.32)	0.587	-0.15(-0.41,0.11)	0.268	-0.18(-0.46,0.10)	0.207	0.06(-0.24,0.36)	0.697	0.12(-0.21,0.44)	0.479	0.16(-0.20,0.53)	0.381
	+BMI SDS B (95% CI)	**p value**	**+BMI SDS B (95% CI)**	**p value**	**+BMI SDS B (95% CI)**	**p value**	**+BMI SDS B (95% CI)**	**p value**	**+BMI SDS B (95% CI)**	**p value**	**+BMI SDS B (95% CI)**	**p value**
1st Quartile	ref	-	ref	-	ref	-	ref	-	ref	-	ref	-
2nd Quartile	0.02(-0.17,0.21)	0.837	0.08(-0.12,0.28)	0.443	0.07(-0.14,0.28)	0.521	-0.11(-0.33,0.11)	0.341	0.09(-0.16,0.35)	0.473	-0.06(-0.34,0.22)	0.685
3rd Quartile	0.11(-0.08,0.29)	0.254	-0.04(-0.24,0.15)	0.661	0.05(-0.16,0.28)	0.648	0.04(-0.18,0.25)	0.717	0.19(-0.06,0.44)	0.145	0.04(-0.24,0.32)	0.775
4th Quartile	-0.13(-0.37,0.12)	0.314	-0.16(-0.42,0.11)	0.244	0.26(-0.02,0.55)	0.070	0.04(-0.26,0.34)	0.775	0.007(-0.33,0.35)	0.966	-0.01(-0.38,0.35)	0.940

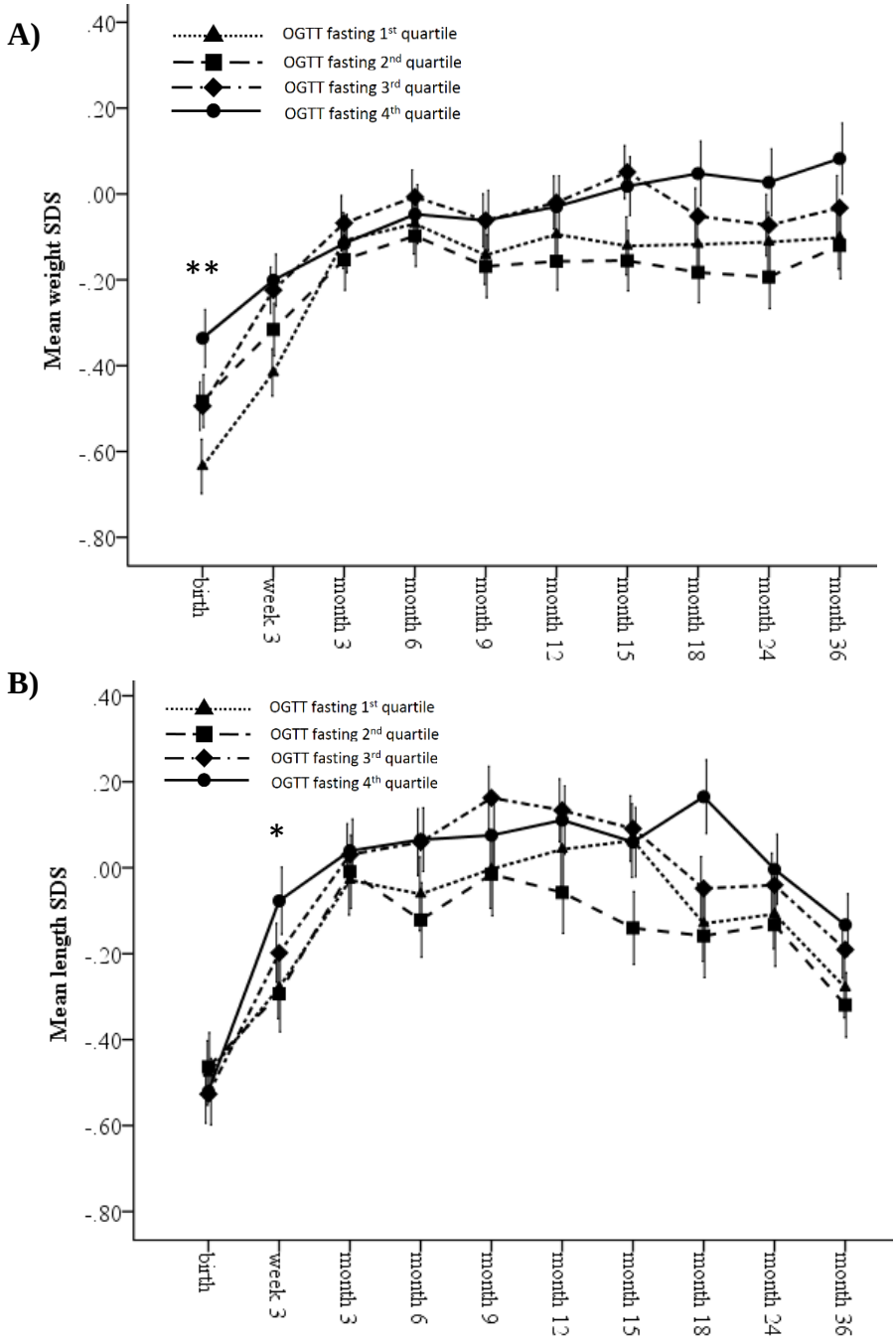
*,# Adjusted for parity, ethnicity, maternal education, glucose management, maternal age, maternal BMI SDS at 26-28 week gestation, maternal height and breastfeeding duration

+ Adjusted for parity, ethnicity, maternal education, glucose management, maternal age, maternal BMI SDS at 26-28 week gestation and breastfeeding duration

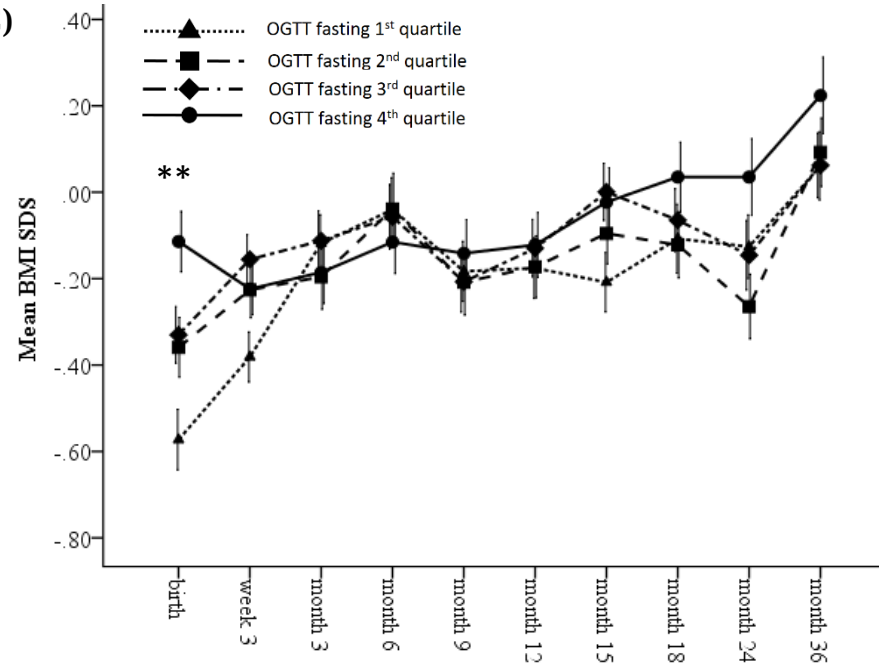
2h-PG Quartiles: 1st quartile (< 5.5mmol/l), 2nd quartile (5.5 - < 6.3), 3rd quartile (6.3 - < 7.3), 4th quartile (≥ 7.3)

The relationships between maternal glycemia with offspring weight, length and BMI were also highlighted graphically by subdividing the offspring according to FPG quartiles (Figures 6.1 A-C). We noted that offspring born to mothers of highest FPG quartile have significantly higher weight SDS at birth (Fig. 6.1A), higher length SDS at week 3 (Fig. 6.1B) and higher BMI SDS at birth (Fig. 6.1C), compared to infants born to mothers of lowest FPG quartile.

Figure 6.1: Offspring weight SDS (A), length SDS (B) and BMI SDS (C) trajectory in the first 3 years of life, shown according to categories of maternal fasting glucose, measured at 26-28 weeks of gestation



C)



Data are shown as mean SDS ± 1 S.E, corrected for gender (at all timepoints), gestation (birth only), and postnatal age. Differences between categories were assessed at each time point using ANOVA. For fasting glucose: 1st quartile (< 4.1mmol/l), 2nd quartile (4.1 - <4.3), 3rd quartile (4.3 - <4.6), 4th quartile (≥ 4.6). **p<0.01 for 4th quartile compared with 1st quartile, *p<0.05 for 4th quartile compared with 1st quartile

6.4.4 Effect of maternal obesity status, parity and ethnicity on relationship between maternal glycemia with early infant anthropometry and overweight status

We noted that the interaction between pregnancy obesity and maternal FPG SDS was significant only at birth for the outcome of weight SDS [β(S.E) = 0.19(0.07), p = 0.007] and length SDS [β(S.E) = 0.16(0.07), p = 0.024], and for the outcome of BMI SDS only at two years of age conditional upon birth [β(S.E) = -0.23(0.09), p = 0.010]. This implies that offspring of obese mothers with higher maternal FPG SDS have larger birth size and yet have lower BMI at two years of age, as compared to offspring of non-obese mothers of similarly elevated FPG. Parity and ethnicity (Malay compared with Chinese) were also observed to have significant interactions with FPG SDS on the outcome of BMI SDS only at two years of age conditional upon birth [β(S.E) = 0.20(0.08), p = 0.014 for parity; β(S.E) = -0.25(0.09), p = 0.007 for ethnicity]. As illustrated in Table 6.5, upon stratifying by ethnicity, obesity and parity, the effect of increased maternal FPG SDS on higher offspring BMI SDS at two years of age was observed to be significant amongst Chinese [β(S.E) = 0.15(0.06), p = 0.016], non-obese [β(S.E) = 0.11 (0.05), p = 0.026] and multiparous women [β(S.E) = 0.12(0.06), p = 0.036]. No significant interactions were observed for 2h-PG with maternal obesity, ethnicity and parity. No significant interactions were also observed for maternal FPG with maternal obesity, ethnicity and parity for the outcome of weight and BMI SDS at three years of age.

Furthermore, we noted that ethnicity and maternal obesity showed significant interactions with maternal FPG SDS on the outcome of overweight

status at two-years of age only. Chinese infants with higher maternal FPG had increased odds of being overweight at two-years of age [OR(95% CI): 3.70(1.49-9.20)] compared to Malay infants. Similarly, offspring of non-obese mothers with higher maternal FPG SDS were more likely to be overweight at two years of age [OR(95% CI): 3.19(1.27-7.97)] compared to offspring of obese mothers. The effect of maternal obesity on the relationship between maternal FPG and overweight status is further illustrated in Figures 6.2A-B. The association of increasing maternal FPG with higher proportion of offspring overweight is present only amongst non-obese women but only at two years, and disappears at three years. In obese mothers, the relationship between increasing maternal FPG and child overweight status is a reverse tick pattern, and unexpectedly the risk of child being overweight is highest in those born to mothers who are obese and in the lowest fasting glucose category.

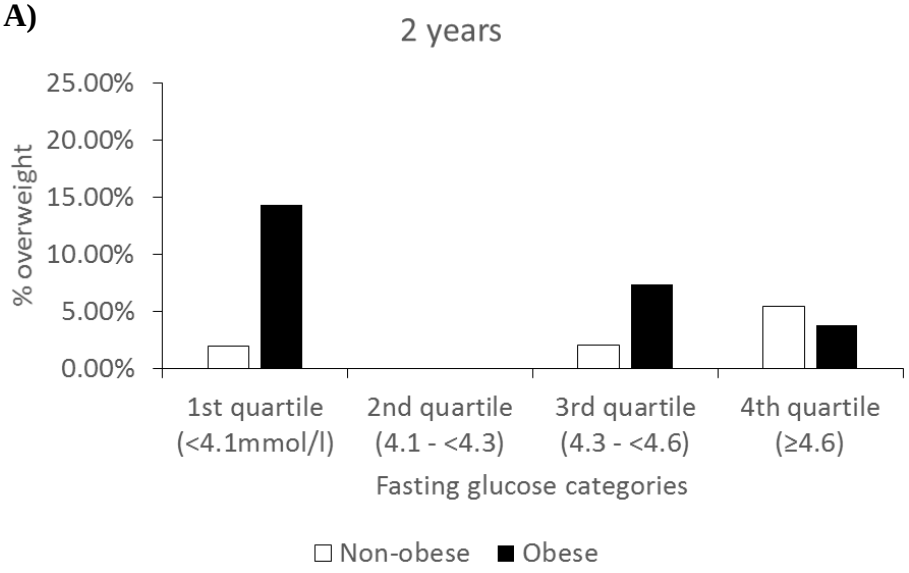
Table 6.5: Effect of maternal FPG SDS at 26-28 weeks gestation on BMI SDS at 2-years of age conditional upon birth BMI, stratified by ethnicity, parity and maternal obesity

Maternal FPG SDS and BMI SDS at 2-yr conditional upon birth				
	β	**S.E**	**p value**	**p for interaction with FPG SDS**
Ethnicity				**0.007**
Chinese	**0.15**	**0.06**	**0.016**	
Malay	-0.10	0.08	0.235	
Indian	0.02	0.11	0.825	
Parity				**0.014**
Primiparous	-0.11	0.07	0.11	
Multiparous	**0.12**	**0.06**	**0.036**	
Maternal Obesity				**0.010**
Non-obese	**0.11**	**0.05**	**0.026**	
Obese	-0.18	0.09	0.05	

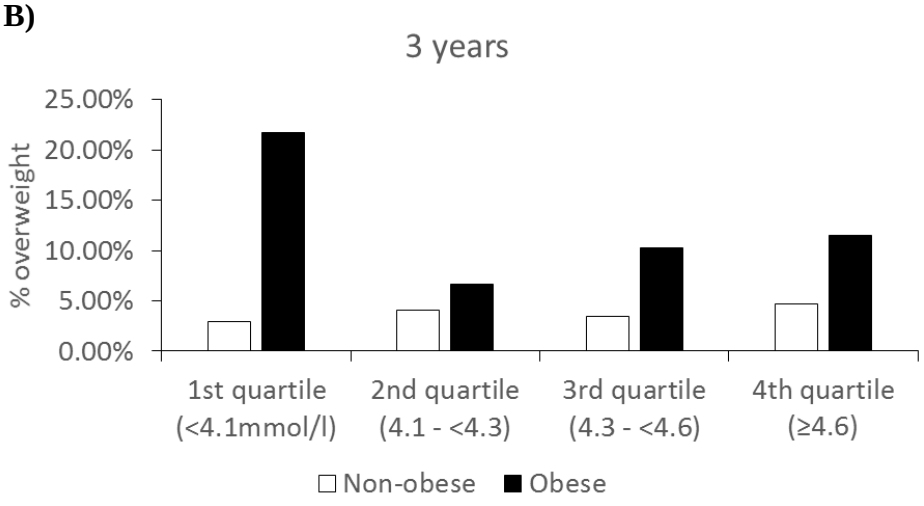
FPG: Fasting plasma glucose, S.E: Standard error

Figure 6.2: Association between offspring overweight status at two- (A) and three-years (B) of age with maternal FPG quartiles, according to maternal obesity

A)



B)



6.5 Discussion

This study on maternal gestational glycemia in a multi-ethnic Asian population showed the effect of maternal glycemia on fetal growth, which persisted for only a relatively short period postnatally after birth. Firstly, consistent with earlier studies done by Scholl et al(214) and Catalano et al(215), we showed that maternal FPG was associated with anthropometric parameters at birth, including weight and BMI, and this observed effect is most pronounced amongst mothers with higher FPG. In contrast, 2h-PG has little impact on postnatal growth, except at birth. Secondly, we also demonstrated that the association of maternal glycemia on postnatal weight and BMI showed no demonstrable difference by three months of age, in line with the findings of previous studies(79, 216). Thirdly, we have shown that offspring of mothers with higher FPG have a markedly different pattern of growth from their peers in early life. Whilst they have higher age-adjusted weight SDS at 3 weeks, there was a period of significant downward centile crossing for weight, but not length and BMI, between 3 weeks to 3 months after birth, followed by accelerated weight and BMI gain between 9-15 months of life when compared to those in the lowest FPG quartile. The novelty of this study is that we demonstrated the period of decelerated growth actually occurs over a shorter period earlier in life, which is possible due to the multiple follow-up anthropometric measurements at close 3-monthly intervals over the first three years of life, allowing us to demonstrate that the effect of maternal glycemia with greater granularity on postnatal weight, which persists up till the first 3 weeks of life only.

Our data are in line with a recent study by Crume et al(81), which highlighted that the growth of offspring exposed to diabetes *in-utero* demonstrated a slower rate of BMI gain [β(S.E) = -0.51(0.33)] over the first nine months of life, followed by a period of faster rate of BMI gain between 9-12 months, compared to offspring of non-diabetic pregnancies. A recent study by Liu et al also reported similar periods of rapid growth between 9-12 months of age amongst offspring of GDM mothers(217). We did note that increasing quartiles of maternal glycemia during pregnancy showed no significant associations with weight SDS, BMI SDS and overweight status at two- and three-years of age. However, earlier studies have documented the age-associated disappearance of the association between increasing maternal glycemia with higher child weight status at early ages, and its re-emergence at only at school-going age(22, 218, 219). Hence it is plausible that the observed accelerated weight and BMI gain between 9-15 months amongst offspring of mothers with high FPG might reflect the start of subsequent higher BMI beyond three years of age.

Our data also reaffirmed the association between maternal BMI during pregnancy with the offspring's weight and BMI, which persisted throughout the first three years of life, but no persistent association with length was documented. These findings, which included adjustment of maternal height, are in keeping with the work of Knight et al(79), who documented that the increase in offspring weight with maternal BMI persisted in the first two years of life and reflected an increase in BMI and not length. Our results are suggestive that maternal BMI during pregnancy has a greater influence on the childhood adiposity, and supports maternal adiposity as a strong determinant

of childhood growth compared to maternal glycemia in Asian mothers and offspring.

Interestingly, we noted in our cohort statistically significant interaction between maternal obesity with FPG for the outcome of size-at-birth, which implied that the combination of maternal obesity with raised glucose levels had greater influence on birth size than either factor acting alone. This finding is consistent with a recent study by Catalano et al(220), who documented that combination of gestational diabetes (GDM) with maternal obesity had far greater impact on neonatal size and adiposity than just GDM or maternal obesity alone. We also noted that the positive association of maternal FPG on BMI and overweight status at two-years of age was more pronounced for offspring of non-obese women. Similar findings were reported by Ehrlich et al on a cohort of Mexican-American women, where associations between increased levels of pregnancy plasma glucose with increased offspring BMI z-scores from 2-7 years of age were observed amongst non-obese women(221). It is plausible that the effects of maternal glycemia during pregnancy would be easier to detect in offspring of non-obese women, who are unexposed to excessive fuel substrates arising from maternal obesity. It is also interesting to note in this study that in obese women, the proportion of overweight children was highest for those in the lowest FPG quartile. We postulate that these obese women may exhibit compensatory hyperinsulinemia, as illustrated by Polonsky K who showed that insulin secretion rates were substantially higher in obese compared to normal weight subjects(222). There is evidence suggesting that relative hyperinsulinemia from mothers might predispose offspring to childhood obesity(223), which may explain the observation of

highest proportion of overweight children amongst obese mothers in the lowest FPG quartile.

We also noted that the positive association of maternal FPG on postnatal BMI at two years of age was present only amongst offspring of Chinese and multiparous women. Whilst the mechanism for this ethnicity-associated relationship between raised maternal glycemia and increased offspring weight status is unknown, we have previously shown in Chapter 5 that influence of raised maternal FPG on neonatal adiposity (as measured by ΣSFT) was more pronounced for Chinese mothers, indicating the possibility that this observation might extend into the postnatal period and later life. A recent study by Peters et al also highlighted that the effect of maternal glycemia on infant weight at two-years was found only amongst multiparous women, although the reported effect was small and in the negative direction (i.e. higher blood glucose levels were associated with lower weight at two-years). It is important to note that their observations were unadjusted correlations, which may have explained the inconsistency with our findings. Our findings have also been corrected for glucose management, highlighting that it may not have confounded our observations.

The prospective design of our cohort presents a clear strength in our study, as it is crucial for examining the effect of any *in-utero* exposure on the outcome of postnatal growth. To date, there are few published studies relating maternal metabolic factors with early postnatal growth in a multi-ethnic Asian cohort; thus our study provides useful, informative data on this relationship. Another strength of our study is the analysis of the growth data in a conditional manner. This method of analysis is important in growth, where

anthropometric measures are often related to the previous measures at earlier timepoints. The conditional SDS of growth are mutually uncorrelated, hence allowing the effects of growth to be distinguished from any statistical artifact attributable to regression to the mean. There are however, limitations to consider; glucose data was collected only at fasting and 2-hour post-challenge, but not at 1-hour post-challenge. Data on 1-hour glucose would have allowed us to better address the role of maternal glycemia with postnatal growth. This study also lacked measures of plasma insulin, insulin growth factor (IGFs), ghrelin, leptin and adiponectin, which would have been useful to mechanistically explain the observed associations between gestational glycemia with offspring weight and BMI.

In conclusion, we have demonstrated the association of maternal glycemia on postnatal growth as seen in our cohort is mainly limited to the first 3 weeks to 3 months (of the first three years of life) followed by transient growth acceleration between 9-15 months, with the exception for a subgroup of children born to non-obese, multiparous Chinese mothers only at two years of age, as compared to the association of maternal BMI on offspring weight and BMI, which persists into early childhood. It remains to be seen if our observation of maternal glycemia and postnatal growth have independent long-term effects on increasing the risk of subsequent obesity and impaired glucose tolerance in adolescence and adulthood in our cohort.

Chapter 7: Effect of infant milk feeding on early postnatal growth of offspring exposed to gestational diabetes *in-utero*

7.1 Summary

Background: Infants on prolonged breastfeeding are known to grow slower during the first year of life. It is still unclear if such effects are similar in offspring exposed to gestational diabetes (GDM) *in-utero.* We examined the effect of infant milk feeding on postnatal growth in the first three years of life amongst offspring exposed and unexposed to GDM.

Methods: In a prospective mother-offspring birth cohort, pregnant mothers took 75g 2-hour oral glucose tolerance tests at 26-28 weeks gestation. In 1152 singleton offspring, measurements included weight and length at birth, 3 weeks, and 3,6,9,12,15,18,24 and 36 months of age. Interviewer-administered questionnaires were used to ascertain the duration of breastfeeding. Conditional standard deviation score (SDS) growth models were used to assess the effect of breastmilk intake on early postnatal growth stratified by GDM status.

Results: Correcting for potential confounders, in offspring of mothers without GDM, greater breastmilk intake (≥ 4 milk-months) was associated with lower conditional weight [B(95%CI): -0.31(-0.49,-0.13)], length [-0.22(-0.40,-0.04)] and BMI [-0.22(-0.40,-0.04)] SDS gains in the first year of life. In contrast, in offspring of mothers with GDM, greater breastmilk intake was associated with greater conditional weight [0.45(0.07,0.83)] and BMI SDS gains [0.46(0.08,0.84)] during the first six months of life.

Conclusions: Infants of GDM mothers who had reduced breastmilk and more formula milk intake did not exhibit accelerated adiposity gain.

7.2 Introduction

The gestational and early postnatal periods have been identified as critical windows for risk of metabolic disorders later in life. Developmental influence on metabolic disease risk originating from the maternal intrauterine environment has been put forth as one of the mechanisms which confer susceptibility to excessive adiposity(203). Recent studies, including our findings as described in Chapter 5, have established that maternal hyperglycemia during pregnancy is associated with increased birth size and excessive neonatal adiposity(73, 74), and it is believed that prenatal exposure to increased fuel from the mother, namely glucose, may contribute to excessive weight gain of offspring born to diabetic mothers(210), thereby increasing the risk of overweight and obesity during childhood and adolescence(218).

In addition, infant nutrition during the early postnatal period has been identified as a critical window for later obesity risk(224). Many studies have extensively looked at the relationship between breastfeeding and long-term obesity risk, highlighting that periods of long and exclusive breastfeeding may have a protective effect on development of obesity later in life(225-228). Thus, breastfeeding has been recommended as a plausible solution to protect the offspring from the consequences of exposure to an adverse intrauterine environment, such as maternal diabetes(229, 230). Unfortunately, our current

understanding on how exposure to gestational diabetes (GDM) may influence the relationship between breastfeeding and postnatal infant growth is cluttered by the practice of combining variations of diabetes (Type I, Type II, GDM) into a single risk category(231, 232), despite the known differences in etiologies and pathophysiology of these variations of diabetic disease(202). Thus, it would be of particular interest to explore the effects of breastfeeding on growth amongst offspring who were exposed only to GDM *in-utero.* Emerging experimental evidence has also shown that exposure to over-nutrition *in-utero* (excessive maternal fat and glycemia) would lead to offspring that are hyperphagic(233, 234), suggesting that offspring of GDM mothers may have altered appetitive traits that might influence later postnatal growth. Additionally, few studies have looked if such effects exist in a multi-ethnic Asian population, where the Asian phenotype and susceptibility towards metabolic disease differs from Caucasians(115), and where different ethnic groups within the Asian population exhibit physiological differences in susceptibility to future metabolic risk. Thus in this study, we examined the effect of breastmilk intake on postnatal growth of offspring exposed and unexposed to GDM *in-utero* and hypothesized that reduced breastmilk intake may result in accelerated adiposity gain in infants of GDM mothers, and if these effects can be explained by infant appetitive traits.

7.3 Materials and Methods

7.3.1. Study population and assessment of gestational age

Details on the study population and assessment of gestational age have been described in Section 2.1 and Section 2.3.1.3.

7.3.2 Oral glucose tolerance testing and anthropometry measurements

Details regarding oral glucose tolerance testing and measurement of anthropometry in the first 3 years of life have been described in Section 2.3.1.2 and Section 2.3.2.1.

7.3.3 Infant feeding assessment

Details regarding infant feeding assessment has been described in Section 2.3.2.2.

Breastfeeding exclusivity weights were assigned to each feeding practice using weights from 0 and 1, with exclusive breastfeeding having a weight of 1 and exclusive formula feeding having a weight of 0. Infants who were on predominant breastfeeding were given a weight of 0.75, and infants who were on partial breastfeeding were given a weight of 0.5(81). The sum of months of exclusive breastfeeding and the weighted months of predominant and partial breastfeeding [duration of exclusive breastfeeding (months) + duration of predominant breastfeeding (months)*exclusivity weight + duration of partial breastfeeding (months)*exclusivity weight] was then calculated to estimate breastmilk intake received over a 12-month period as a breastmilk-month measure, divided into 2 categories (< 4 milk-months and ≥ 4 milk-months). We also calculated the sum of months of exclusive breastfeeding and

the weighted months of predominant breastfeeding [duration of exclusive breastfeeding (months) + duration of predominant breastfeeding (months)*exclusivity weight] to estimate breastmilk intake by only exclusive/predominant breastfeeding in a 12-month period as a milk-month measure, divided into 3 categories (< 4 milk-months, ≥ 4 milk-months, no exclusive/predominant breastfeeding).

7.3.3 Appetitive traits

Appetitive traits were measured using the self-administered Baby Eating Behavior Questionnaire (BEBQ) questionnaires(235). The BEBQ was handed out to the parents during the 3-month post-partum home visit and collected at the end of the visit. Questionnaires distributed were in English unless a preferred language of Mandarin, Malay or Tamil was requested. In this case, translated versions of the questionnaires were handed out.

The BEBQ relates to a period of exclusive milk feeding. Each item on the questionnaire was answered using a five-point Likert frequency scale (1=never, 2=rarely, 3=sometimes, 4=often and 5=always). The 18-item BEBQ in this study measured three appetitive trait subscales, food responsiveness, slowness in eating and enjoyment of food and each subscale measures an infant's response to exclusive milk feeding at 3 months of age. Satiety responsiveness and slowness in eating were combined under one subscale as slowness in eating as the items have been shown to have loaded into the same factor. Examples of items in the BEBQ are "My baby is always demanding a feed" (food responsiveness), "My baby finishing feeding quickly" (slowness in eating) and "My baby loves milk" (enjoyment of food).

7.3.4 Statistical analysis

Descriptive statistics were reported as means and standard deviations for continuous variables and percents for categorical variables. Age- and gender-specific standard deviation scores (SDS) were calculated for weight, length and body mass index (BMI) for infants at all timepoints, referencing WHO Child Growth Standards(212). Conditional growth models for weight, length and BMI SDS were built using linear regression analysis. Here, weight SDS is used as an example: conditional growth in weight SDS from birth to 6 months is equivalent to the standardised residuals resulting from the linear regression model of weight SDS at 6 months on weight SDS at birth. Accordingly, the conditional growth in weight SDS from 6 to 12 months is given as the standardised residuals obtained from regressing weight SDS at 12 months on weight SDS at 0, 3 and 6 months simultaneously. This process is continued for each subsequent time point, resulting in measures of growth that are uncorrelated. Multiple linear regression analyses were used to assess the association between estimated breastmilk intake with offspring weight, length and BMI conditional gain during the first 3 years of life, in all cases adjusting for ethnicity, parity, maternal age, maternal education, maternal BMI at 26-28 weeks gestation and gestational age at delivery. Associations between BEBQ scores with GDM status and estimated breastmilk intake were also tested using linear regression analysis. All analysis was performed using SPSS version 20.0 (IBM, SPSS Statistics, Armonk, NY).

7.4 Results

7.4.1 Demographic and clinical characteristics

Of the 1247 eligible pregnant mothers who were recruited to the study, 1152 subjects had naturally-conceived singleton pregnancies. Data on glucose levels were available for 1016 subjects, out of which 181 subjects (17.8%) were diagnosed with GDM at 26-28 weeks of gestation. Characteristics of the study participants were described in Table 7.1. There was a significant difference in the distribution of GDM across all 3 ethnicities, with more Chinese and Indians having GDM compared to Malays. Subjects with GDM were observed to be slightly older (32.3 vs. 30.0 years), more educated (70.2% vs. 55.5%), had higher BMI at 26-28 weeks of gestation (27.1 vs 25.9 kg m^{-2}) and shorter gestational age at delivery (38.0 vs 38.3 weeks) compared to their non-GDM counterparts (all $p < 0.05$). No significant differences in birth weight, length and BMI SDS, as well as breastmilk intake were observed for offspring of GDM and non-GDM mothers.

Table 7.1: Clinical characteristics and demographics of study subjects

Maternal characteristics	GDM (n = 181)		No GDM (n = 835)		P value[#]
	N	%/mean(SD)	N	%/mean(SD)	
Maternal age	181	32.3(4.8)	835	30.0(5.1)	**<0.001**
Maternal education					**<0.001**
< 12 years	54	29.8	366	44.5	
≥ 12 years	127	70.2	456	55.5	
Ethnicity					**0.001**
Chinese	113	62.4	448	53.7	
Malay	28	15.5	246	29.5	
Indian	40	22.1	141	16.9	
Parity					0.101
Primiparous	68	38.0	366	44.7	
Multiparous	111	62.0	453	55.3	
Maternal BMI	178	27.1(4.3)	815	25.9(4.5)	**<0.001**
Gestational age at delivery	181	38.0(1.7)	824	38.3(1.4)	**0.013**
Infant Characteristics					
Birth weight SDS	181	-0.50(1.17)	824	-0.48(0.95)	0.886
Birth length SDS	181	-0.39(1.37)	821	-0.52(1.14)	0.229
Birth BMI SDS	181	-0.47(1.20)	821	-0.31(1.08)	0.090
Breastmilk intake					0.141
< 4 milk-months	107	64.1	534	69.9	
≥ 4 milk-months	60	35.9	230	30.1	
Breastmilk intake by exclusive/predominant BF only					0.061
No exc/pred BF	99	59.3	511	66.9	
< 4 milk-months	26	15.6	120	15.7	
≥ 4 milk-months	42	25.1	133	17.4	

[#]p value by chi-square analysis (categorical) or two-sample t-test (continuous)
Exc/pred BF = exclusive/predominant breastfeeding; BMI = body mass index; SDS = standard deviation score

7.4.2 Effect of breastmilk intake on conditional growth in offspring exposed and unexposed to GDM *in-utero*

Table 7.2 describes the association of estimated breastmilk intake with the offspring conditional growth outcomes during the first three years of life. Amongst offspring of non-GDM mothers, those who had greater breastmilk intake (≥ 4 milk-months) had significantly decelerated conditional gain in weight SDS [B(95%CI) = -0.31(-0.49,-0.13)], length SDS [B(95%CI) = -0.22(-0.40,-0.04)] and BMI SDS [B(95%CI) = -0.22(-0.40,-0.04)] by the first year of life, compared to those who had reduced breastmilk intake (< 4 milk-months). Amongst offspring of GDM mothers however, we noted that those who had greater breastmilk intake showed significantly accelerated conditional gain in weight [B(95%CI) = 0.45(0.07,0.83)] and BMI SDS [B(95%CI) = 0.46(0.08,0.84)] in the first six months of life compared to those who had reduced breastmilk intake.

Table 7.2: Association between estimated breastmilk intake (< 4 and ≥ 4 milk-months) and conditional growth of offspring in the first three years of life for offspring exposed and not exposed to maternal gestational diabetes *in-utero*

	Conditional SDS gain B(95%CI)									
	Unexposed to GDM					Exposed to GDM				
	0-6 mth	**6-12 mth**	**12-18 mth**	**18-24 mth**	**24-36mth**	**0-6 mth**	**6-12 mth**	**12-18 mth**	**18-24 mth**	**24-36mth**
Weight SDS										
< 4 milk-mths			ref					ref		
≥ 4 milk-mths	-0.05 (-0.23,0.13)	**-0.31 (-0.49,0.13)**	-0.07 (-0.27,0.13)	0.16 (-0.04,0.36)	0.04 (-0.18,0.26)	**0.45 (0.07,0.83)**	-0.31 (-0.69,0.07)	-0.11 (-0.55,0.33)	0.13 (-0.35,0.61)	0.22 (-0.22,0.66)
Length SDS										
< 4 milk-mths			ref					ref		
≥ 4 milk-mths	**-0.21 (-0.39,-0.03)**	**-0.22 (-0.40,-0.04)**	0.06 (-0.16,0.28)	0.006 (-0.20,0.21)	0.05 (-0.19,0.29)	0.01 (-0.37,0.39)	-0.03 (-0.47,0.41)	0.07 (-0.45,0.59)	-0.36 (-1.00,0.28)	0.07 (-0.45,0.59)
BMI SDS										
< 4 milk-mths			ref					ref		
≥ 4 milk-mths	0.11 (-0.07,0.29)	**-0.22 (-0.40,-0.04)**	-0.28 (-0.48,0.08)	0.12 (-0.12,0.36)	0.08 (-0.16,0.32)	**0.46 (0.08,0.84)**	-0.16 (-0.54,0.22)	-0.23 (-0.75,0.29)	0.34 (-0.14,0.72)	0.22 (-0.24,0.68)

Adjusted for maternal age, ethnicity, maternal education, parity, maternal BMI at 26-28 weeks gestation, gestational age at delivery
Figures in bold indicate $p < 0.05$

7.4.3 Effect of breastmilk intake by only exclusive/predominant breastfeeding on conditional growth of offspring exposed and unexposed to GDM in-utero

We also assessed the association between estimated breastmilk intake by only exclusive/predominant breastfeeding with offspring conditional growth in the first three years of life (Table 7.3). Amongst offspring of non-GDM mothers, we observed that those with greater breastmilk intake by exclusive/predominant breastfeeding (≥ 4 milk-months) showed significantly decelerated conditional gain in weight SDS [B(95%CI) = -0.42(-0.64,-0.20)] and length SDS [B(95%CI) = -0.35(-0.57,-0.13)] by the first year of life, compared to those who were not on exclusive/predominant breastfeeding. Amongst offspring of GDM mothers however, those with greater breastmilk intake by exclusive/predominant breastfeeding showed significantly accelerated conditional gain in weight SDS [B(95%CI) = 0.52(0.08,0.96)] and BMI SDS [B(95%CI) = 0.73(0.29,1.17)] in the first six months of life, compared to those who were not on exclusive/predominant breastfeeding. No significant associations were observed between those who received breastmilk for less than 4 months by exclusive/predominant breastfeeding with growth outcomes during the first three years of life.

Table 7.3: Association between breastmilk intake by only exclusive/predominant (No exclusive/predominant breastfeeding, < 4 and ≥ 4 milk-months) and conditional growth of offspring in the first three years of life for offspring exposed and not exposed to maternal gestational diabetes *in-utero*

	Conditional SDS gain B(95%CI)									
	Unexposed to GDM					**Exposed to GDM**				
	0-6 mth	**6-12 mth**	**12-18 mth**	**18-24 mth**	**24-36 mth**	**0-6 mth**	**6-12 mth**	**12-18 mth**	**18-24 mth**	**24-36 mth**
Weight SDS										
No exc/pred BF			ref					ref		
< 4 milk-mths	-0.15 (-0.37,0.07)	-0.07 (-0.31,0.17)	0.17 (-0.11,0.45)	0.14 (-0.14,0.42)	0.16 (-0.14,0.46)	0.24 (-0.28,0.76)	-0.41 (-0.93,0.11)	-0.22 (-0.82,0.38)	0.24 (-0.40,0.88)	0.18 (-0.40,0.76)
≥ 4 milk-mths	-0.08 (-0.28,0.12)	**-0.42 (-0.64,-0.20)**	-0.17 (-0.41,0.07)	0.21 (-0.03,0.45)	0.04 (-0.20,0.28)	**0.52 (0.08,0.96)**	-0.35 (-0.77,0.07)	-0.19 (-0.69,0.31)	0.05 (-0.49,0.59)	0.30 (0.05,0.80)
Length SDS										
No exc/pred BF			ref					ref		
< 4 milk-mths	-0.02 (-0.26,0.22)	-0.21 (-0.47,0.05)	0.30 (-0.01,0.60)	0.32 (-0.001,0.68)	-0.04 (-0.38,0.30)	0.003 (-0.52,0.52)	0.18 (-0.42,0.78)	-0.40 (-1.12,0.32)	0.82 (-0.10,1.84)	-0.24 (-0.98,0.50)
≥ 4 milk-mths	**-0.38 (-0.58,-0.18)**	**-0.35 (-0.57,-0.13)**	-0.04 (-0.28,0.20)	0.07 (-0.17,0.31)	-0.02 (-0.30,0.26)	-0.41 (-0.01,0.83)	0.23 (-0.25,0.71)	0.32 (-0.26,0.90)	-0.11 (-0.81,0.59)	-0.40 (-0.98,0.18)
BMI SDS										
No exc/pred BF			ref					ref		
< 4 milk-mths	-0.18 (-0.40,0.04)	-0.03 (-0.29,0.23)	-0.09 (-0.37,0.19)	-0.05 (-0.39,0.29)	0.18 (-0.001,0.35)	0.24 (-0.28,0.76)	-0.42 (-0.96,0.12)	-0.14 (-0.86,0.58)	-0.34 (-1.06,0.38)	0.55 (-0.11,1.11)
≥ 4 milk-mths	0.18 (-0.02,0.28)	-0.18 (-0.40,0.04)	-0.34 (-0.58,-0.10)	0.11 (-0.17,0.39)	0.12 (-0.16,0.40)	**0.73 (0.29,1.17)**	-0.31 (-0.75,0.13)	-0.51 (-1.09,0.07)	0.12 (-0.42,0.68)	0.31 (-0.21,0.83)

Exc/pred BF: exclusive/predominant breastfeeding
Adjusted for maternal age, ethnicity, maternal education, parity, maternal BMI at 26-28 weeks gestation, gestational age at delivery
Figures in bold indicate $p < 0.05$

7.4.4 Differences in feeding behavior amongst GDM and non-GDM exposed infants

To evaluate if the observations of accelerated weight and BMI SDS gain amongst GDM-exposed infants who had greater breastmilk intake were driven by infant appetitve traits, we compared the infant BEBQ scores between GDM and non-GDM exposed infants who had greater estimated breastmilk intake. We noted that GDM-exposed infants who were on prolonged and exclusive breastmilk feeding exhibited lower food responsiveness and slowness in eating scores, and higher enjoyment of food scores (Table 7.4), although these differences are not statistically significant, indicating no observable relationship between infant appetitive traits with GDM status and breastmilk duration and exclusivity.

Table 7.4: Means and standard error for each subscale of BEBQ according to GDM and feeding type

	BEBQ appetitive traits [mean(SE)]		
	Food responsiveness	**Slowness in eating**	**Enjoyment of food**
GDM + breastmilk intake			
Non-GDM + ≥ 4 milk-mths (n = 134)	0.24 (0.09)	0.18 (0.09)	0.17 (0.09)
GDM + ≥ 4 milk-mths (n = 26)	-0.09 (0.19)	-0.06 (0.20)	0.21 (0.20)
P value*	0.687	0.271	0.855
GDM + breastmilk intake by only exclusive/predominant BF			
Non-GDM + ≥ 4 milk-mths (n = 82)	0.24 (0.11)	0.13 (0.12)	0.14 (0.11)
GDM + ≥ 4 milk-mths (n = 18)	-0.23 (0.23)	-0.04 (0.24)	0.30 (0.24)
P value*	0.063	0.520	0.540

Means adjusted for maternal age, ethnicity, education, BMI at 26-28 weeks gestation, gestational age at delivery and infant size at 3 months of age

7.5 Discussion

In this prospective Asian birth cohort study, we have demonstrated the varied effects of neonatal breastmilk intake on early postnatal growth in offspring who were exposed and unexposed to GDM *in-utero*. We noted that offspring of mothers without GDM who had greater breastmilk intake (i.e. ≥ 4 months) exhibited significantly decelerated growth within the first year of life. However, offspring of GDM mothers who were on reduced breastmilk intake (and more formula feeding) did not exhibit the hypothesized accelerated growth during this early postnatal period.

The findings amongst offspring of non-GDM mothers are consistent with that of the current literature. Griffiths et al reported that infants who did not receive breast milk grew faster than those whose mothers initiated breastfeeding, as did those who breastfed for 4 months or longer(87). A cohort study of randomly selected healthy newborns in Denmark and Iceland showed that exclusive breastfeeding beyond 2 months of age was related to lower weight gain from 2 to 6 months as well as from 6 to 12 months(236). A recent study on the Gemini cohort of 4680 infants also showed that infants breastfed for longer periods (>4 months) was independently associated with lower growth velocity by 6.8%(237). Not all studies have reported such similar results; a cluster randomized trial of a breastfeeding promotion intervention modelled on the WHO-UNICEF Baby-Friendly Hospital Initiative showed that prolonged and exclusive breastfeeding accelerated weight and length gain of the infants in the first few months with no detectable deficit by 12 months old(238).

Our findings amongst offspring of mothers with GDM are consistent with earlier animal studies which have suggested that milk derived from mothers with GDM may impart metabolic consequences to their offspring. It has been reported that control offspring who were fed milk from dams with GDM showed complex "malprogramming" of hypothalamic neural circuits that are critically involved in the regulation of food intake, body weight, and metabolism(239). Longitudinal studies in humans also echoed similar observations, where milk intake from diabetic mothers during the first week of life was associated with greater relative weight and risk of overweight at two years of age, compared to offspring of diabetic mothers who were fed banked donor breastmilk(229, 230). Other studies however, such as that by Crume et al(231), have reported contrasting findings where it was shown that adequate breastfeeding ($\geq$ 6 breast milk-months) reduces the overall body size and BMI growth velocity in the first nine months of life amongst offspring of diabetic pregnancies. Another study also reported that breastfeeding conferred similar protective effects against overweight at 9-14 years of age in offspring of both non-diabetic and diabetic women(232). It is important to note however, that these studies classified Type I diabetes as well as GDM into a single category, which may have explained the inconsistency in findings with our study due to the differences in etiologies of both diabetic sub-types. Our findings thus provides critical insights into this area of research, given the lack of understanding of the biochemical impact of breastmilk from GDM-only mothers on infant growth.

The mechanisms underlying the varied effects of neonatal breastmilk intake on early postnatal growth in offspring who were exposed and

unexposed to GDM *in-utero* are likely multiple. Researchers have postulated that there may be differences in breast milk constituents of diabetic and non-diabetic mothers, such as increased glucose or insulin concentrations in breast milk of diabetic mothers which may contribute to increased growth rates during early infancy. Concentration of ghrelins in milk of GDM-lactating women have also been reported to be lower when compared to non-diabetic control samples(240). Given the documented negative association between level of serum active ghrelin levels and BMI of infants(241), lower ghrelin concentrations in milk of GDM mothers might influence faster postnatal growth in their offspring. Milk from diabetic mothers may also contain more inflammatory cytokines (e.g. TNF-α, IL-6) which mimic signaling pathways characteristic of dysfunctional adipocytes of metabolic syndrome(242). Moreover, Kjos et al had earlier demonstrated that abnormal glucose metabolism still persists postpartum amongst women with GDM(243), further suggesting that continued exposure to altered fuels through breastmilk may bring about consequences to offspring growth. Plagemann et al had also proposed that milk originating from diabetic mothers may have an early obesogenic effect on infant weight gain that decreases with time, thus the positive effects of breastfeeding on reducing later adiposity may only be observed if breastfeeding is continued beyond a certain period where breast milk composition would have normalized over time(244). This begets the question whether proactive intervention to achieve better glycemic control in the early postpartum period of GDM mothers would help to accelerate the normalization of the breast milk and reduce the obesogenic effect, which poses

an interesting hypothesis that should be examined properly in an interventional trial.

Another potential mechanism could be that offspring of GDM mothers may have greater energy intake driven by intrauterine "metabolic programming" as a result of GDM exposure *in-utero*. In our study, we observed that offspring of GDM mothers had greater estimated breastmilk intake, compared to their non-GDM counterparts. Higher intake of breast milk from GDM mothers and hence higher energy intake, may contribute to increased growth rates during early infancy. We were however, unable to demonstrate that GDM-exposed infants with greater breastmilk intake had pro-feeding behavior, when comparing BEBQ appetitive trait scores between GDM and non-GDM exposed infants. As approximately 50% of the participants answered the BEBQ, this could have led to lack of power for detecting significant associations given the small effect sizes observed.

Strengths of this study include the prospective design with high follow-up rate, along with the study of Asian ethnic groups. To date, there are few published studies examining the effect of breastfeeding on early postnatal growth among offspring exposed to GDM *in-utero* in a multi-ethnic Asian cohort. Thus this study provides useful and informative data on this relationship. Another strength of the study is the analysis of the growth data in a conditional manner. This method of analysis is important in growth, where anthropometric measures are often related to the previous measures at earlier timepoints. The conditional SDS of growth are mutually uncorrelated, hence allowing the effects of growth to be distinguished from any statistical artifact attributable to regression to the mean. This study however, is not without

limitations. Due to the observational nature of our study, we cannot fully rule out the possibility that residual confounding by parental attributes or family environment may affect the observed associations, as breastfeeding is a behavior that is self-selected and women are usually not randomized to breastfeed. As with most studies on breastfeeding and infant growth, it is largely observational and hence subject to potential confounding. Socio-economic status presents as an important confounder, as mothers who are more educated tend to be more "nutrition-conscious", more likely to breastfeed and less likely to feed poor quality diets post-weaning(245, 246). Other important potential confounding factors include maternal BMI, which is generally associated with shorter durations of breastfeeding(247), as well as maternal age, which is generally associated with greater exclusive breastfeeding(248). In our study, we noted that mothers with GDM were observed to be older, more educated and had higher BMI at 26-28 weeks of gestation compared to their non-GDM counterparts, which presents as a potential bias. The associations observed in our study findings have been controlled for, and are independent of these potential confounders.

In conclusion, our study findings have demonstrated the varied effects of neonatal breastfeeding on early postnatal growth in offspring who were exposed and unexposed to GDM *in-utero*. Whilst offspring of mothers without GDM who had greater breastmilk intake exhibit decelerated weight and BMI gain in the first year of life, offspring of GDM mothers however, do not exhibit accelerated adiposity gain during the early postnatal period despite reduced breastmilk intake. It remains to be seen if our observations of effects of breastmilk on accelerated growth amongst offspring exposed to GDM *in-*

utero have independent long-term effects in adolescence and adulthood in our cohort.

Chapter 8: Identifying potential novel genetic markers of fetal growth and subsequent postnatal catch-up growth

8.1 Summary

Background: Fetal growth restriction (FGR) and accelerated postnatal catch-up growth often predisposes offspring to increased risk of metabolic disease later in life. However, the molecular mechanisms which entails FGR and subsequent catch-up growth is not well understood. We examined the transcriptomic profiles of umbilical cords of infants to identify potential novel genetic markers that are associated with fetal growth and subsequent postnatal growth.

Methods: The gene expression patterns of 80 umbilical cords from Singaporean newborns of Chinese ethnicity, who experienced poor fetal growth [with postnatal catch-up growth (n=20), without catch-up growth (n=20)], normal (n=20) and excessive fetal growth (n=20) were determined.

Results: The gene expression microarray data uncovered 19 genes which had expression levels significantly associated with fetal growth change between the 2nd (19-21 weeks of gestation) and 3rd trimester (32-34 weeks), and 29 genes that had significantly different expression levels between the catch-up and non-catch up growth groups, and all 48 genes were found to be significantly enriched in pathways related to immune response, apoptosis, nucleotide metabolism, DNA damage repair and angiopoietin signalling. Further validation of the array expression data using quantitative real-time PCR identified a total of 15 out of the 48 genes showing expression level differences similar to that observed in the microarray data, and were

associated with a variety of growth measures (fetal growth, birth weight, birth length and conditional postnatal growth).

Conclusion: This study has managed to uncover gene expression changes significant for fetal growth and subsequent postnatal growth, and provided insights into the transcriptomic profile of babies with differing fetal growth types. This may allow for the development of prognostic markers to predict FGR.

8.2 Introduction

Normal fetal growth represents a critical component of a healthy pregnancy and influences the long-term health of the offspring. In this aspect, fetal growth restriction (FGR) constitutes a major and important clinical problem, both in developed and developing countries. FGR often predisposes the offspring to increased risk of perinatal death, birth hypoxia, neonatal complications and impaired neurodevelopment(249-251). Additionally, an increasing body of evidence has shown that common adult diseases such as type 2 diabetes, coronary heart disease, hypertension and other manifestations of the metabolic syndrome are linked to abnormal fetal growth, particularly FGR(252). Hence, being able to predict a poor growing fetus *in-utero* would prove to be clinically valuable both in the short- and long-term because: (i) clinicians would be able to readily identify fetuses that require early referral to secondary care and closer surveillance, (ii) it would allow specific interventions to be tested on at-risk fetuses, and avoid the use of unnecessary interventions on low-risk fetuses, (iii) studying predictors of fetal growth would improve the understanding of biological and pathological mechanisms

of FGR, and (iv) accurate prediction of poor fetal growth at an early stage would play an important role in avoiding the adult consequences of FGR later in life(253).

Currently, the molecular mechanisms that underlie the pathology of FGR are not well-understood. Several biochemical markers for predicting FGR have been proposed in recent years(254-259), however none have been sufficiently accurate to recommend their use as predictors of FGR in routine clinical practice. With recent advances in genetic epidemiology such as high-throughput gene expression and genome-wide methylation microarrays, several groups have reported the association of transcriptomic and epigenetic marks, derived from umbilical cord tissue with adverse intrauterine experience(260-263). These studies however, use the common approach of defining FGR and small-for-gestational age (SGA) interchangeably (i.e. birthweight-for-gestational age < 10^{th} percentile). This is problematic, as SGA fetuses are not necessarily growth-restricted; they may be constitutionally small but healthy. Conversely, fetuses with weight above the 10^{th} percentile may not necessarily denote normal fetal growth. The birthweight of the newborn represents size-at-birth but does not necessarily reflect the actual growth of the fetus *in-utero*. The fetal growth rate may undergo a pathological decline during late gestation which incurs a risk of perinatal morbidity or mortality, even though the birthweight is still above the 10^{th} percentile(141). To overcome this, serial ultrasound measurements for the same fetus taken at different gestational periods, alongside birth anthropometric data may give a better indication of fetal growth velocity. Additionally, it may allow for a more direct examination of fetal growth by studying growth conditional on

earlier size, which enables growth at different gestational periods to be studied independently of earlier size and growth. This would identify if infants had consistently reduced fetal growth rates over the course of gestation, hence a better indication of FGR.

Majority (~two-thirds) of infants with FGR will tend to show catch-up growth, defined as height or weight growth above the statistical limits of normality for age(264, 265), in the first two to three years of life(266). This period of accelerated postnatal growth following FGR has been shown to be crucial in the programming of later metabolic disease risk(32, 264). However, a significant proportion (~20-30%) may still persist to remain small and failed to catch-up in their height and weight compared to their peers. Therefore identifying molecular signatures which are predictive of successful or failed catch-up growth following FGR will have potential clinical applications, such as determining which children should be considered for early growth hormone therapy, and also unravel biological pathways which determine catch-up growth. These molecular signatures would also be useful in understanding the associations between fetal growth, birth size and disease risk in later life. Thus in this study, we sought to examine the relationship between fetal growth (as measured by serial ultrasound measurements) with transcriptomic profiles of umbilical cords of GUSTO infants to identify potential novel genetic markers that are associated with fetal growth restriction and subsequent postnatal growth, and hypothesized that offspring with poor growth *in-utero* and subsequent catch-up growth have a unique gene expression profile which is predictive of catch-up growth

8.3 Materials and Methods

8.3.1 Study population, assessment of fetal biometry and gestational age

Details on the study population and assessment of fetal biometry and gestational age have been described in Section 2.1 and Section 2.3.1.3.

8.3.2 Criteria for the evaluation of fetal growth

All fetal growth characteristics for each subject, were converted to standard deviation scores (SDS) using internally derived gestational age-specific means and standard deviations.

SDS = (measurement – cohort mean)/cohort standard deviation).

Fetal growth was examined by calculating a change in SDS between the second trimester (19-21 weeks) and the third trimester (32-34 weeks) for biparietal diameter (BPD), femur length (FL) and abdominal circumference (AC). Fetal growth patterns was categorized by a change of more than 0.67 SDS, a well-recognized threshold value in studies on growth(267). "Poor fetal growth" infants were selected from those whose change in SDS was more than -0.67 SDS between the 2nd and 3rd trimester for AC, and in addition, either BPD or FL (which is, two criteria). Similarly, "excessive fetal growth" were selected from those whose change in SDS was more than +0.67 SDS between the 2nd and 3rd trimester for AC and either BPD or FL. Infant subjects whose change in SDS were between -0.67 or +0.67 SDS were classified as being "normal fetal growth".

8.3.3 Biospecimens – selection of umbilical cord samples

The microarray analysis sample set consisted of a total of 80 umbilical cords from babies of Chinese ethnicity. It was designed to include 20 "exceeding fetal growth", 20 "steady fetal growth" and 40 "poor fetal growth", of which 20 exhibited postnatal “catch-up growth” and the remaining 20 failed to exhibit catch-up growth. The samples were matched for gender and gestational age at delivery. Gestational ages were restricted to between 37-40 completed weeks. Mothers with underlying pregnancy complications with potential effects on fetal growth (gestational diabetes, hypertension, pre-eclampsia, anaemia) were excluded from this study. Mothers who smoked during the course of their pregnancy were also excluded in this study.

8.3.4 Infant anthropometry

Details regarding measurement of infant anthropometry have been described in Section 2.3.2.1.

8.3.5 Analysis of biospecimens and extraction of RNA

Details regarding biospecimen analysis and RNA extraction from umbilical cords have been described in detail in Section 2.4.1 and Section 2.4.2

8.3.6 Gene-expression microarray

HumanHT-12 v4 Expression BeadChips (cat#BD-103-0204, Illumina) with 47,231 transcript probes were used for gene expression analysis. Briefly, 500ng of total RNA was used for complementary RNA (cRNA) synthesis using Illumina® TotalPrep™-96 RNA Amplification Kit (cat# 4393543, Life

Technologies) according to manufacturer's instructions and recommendations. 750ng of cRNA was hybridized onto the array for 18 hours at 58°C. The arrays were scanned on Illumina iScan, and data was extracted by the Illumina Genome Bead Studio™ Software for further analysis. Background subtraction was performed on the extracted data, and probes with at least 3 beads ($N_{BEADS} \geq 3$) with significant detection value ($p < 0.05$) were selected. The data were then loaded into Arraystudio (Omicsoft), and all expression values were log transformed and normalised amongst samples using quantile normalisation, a technique for transforming data to have a common distribution of expression intensities across all samples(268), thus allowing the expression intensities to be compared between samples.

8.3.7 Pathway analysis

All genes that were significantly expressed were subjected to pathway enrichment and *de novo* network analysis. Pathway analysis was performed in GeneGo MetaCore™.

8.3.8 Quantitative Real Time-PCR (qRT-PCR)

To validate the gene expression levels observed from the microarray, qRT-PCR was performed. Total RNA (4ug) was reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems Inc, ABI, CA, USA). PCR reactions were prepared using 10ul of Power SyBr Green PCR 2X Master mix (Applied Biosystems Inc, ABI, Foster City, CA, USA), 1ul of each primer (2uM), and 20ng of cDNA in a total reaction volume of 20ul. PCR for each sample was done in triplicates in 384-well plates using the ABI 7900 HT Sequence Detection System. Cycle parameters used were 10 min at 95°C (1 cycle), then 15 s at 95˚C and 1 min at 60°C (40 cycles).

Targets genes and three endogenous control genes (RPL18, RPL19 and HSP90AB1) were analyzed. The threshold cycles (Ct) of samples provided from the equipment software were normalized by the average Ct of controls using $\Delta C_{t,Target} = C_{t,Target} - C_{t,AvgControl}$. Higher ΔCt value indicates lower gene expression level.

8.3.9 Statistical analysis

Descriptive statistics were reported as means and standard deviations for continuous variables and percents for categorical variables. As a measure of quality control for gene expression data, median absolute deviation (MAD) scores were calculated for the sample sets. MAD measures the variability in a univariate dataset and is a robust measure of statistical dispersion, defined as the median of absolute deviations from the data's median:

$$MAD = Median_i(|X_i - Median_j(X_j)|)$$

For each data point (X_i), individual deviations from the median of all data points (Median X_j) are first calculated [given by the formula $X_i - Median_j(X_j)$]. The absolute values of the individual deviations are taken [i.e. $|X_i - Median_j(X_j)|$]. The MAD would thus be the median of these absolute values. The MAD score would then be calculated using the following formula:

$$MAD\ Score = \frac{(CorrelationDifferenceSample - MedianCorrelationDifference)}{MAD * 1.46138189}$$

All samples with MAD scores < -5 were removed from the analysis(269). Principal component analysis and hierarchical clustering were performed to observe the clustering of technical replicates and discernible

batch effects. To identify probes that had significantly different expression levels, one-way ANOVAs (multiple testing correction: Benjamin- Hochberg) were performed. Linear regression analysis was applied on the delta Ct values of target genes against a variety of intrauterine, birth and postnatal growth measures in the first 2-years of life.

8.4 Results

8.4.1 Demographic and clinical characteristics

Baseline demographic characteristics of the study subjects were described in Table 8.1. No significant differences across the 3 fetal growth groups were observed for maternal education, housing type, parity, maternal age and BMI at 26-28 weeks gestation. Males and females were also equally distributed across the 3 fetal growth groups. Infants from the "poor fetal growth" group exhibited significantly lowest birth weight [mean(SD): 2.82(0.22) vs. 3.21(0.23) vs. 3.52(0.31)] and birth length [mean(SD): 48.1(2.2) vs. 49.4(1.9) vs. 50.1(1.8)] across the 3 groups.

Table 8.1: Comparison of baseline characteristics between "poor fetal growth", "normal fetal growth" and "excessive fetal growth".

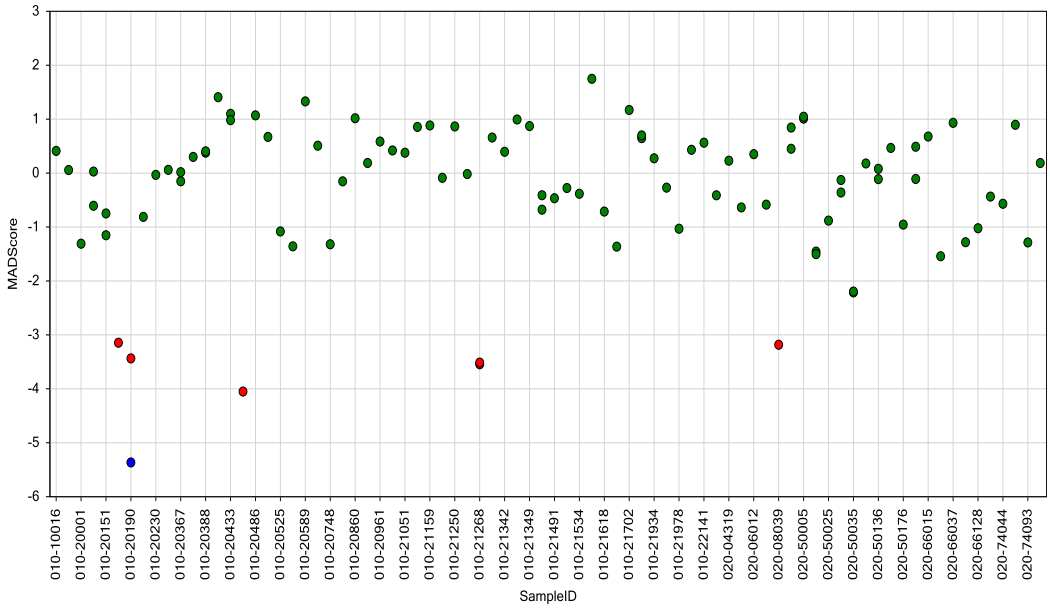
Mothers	"Poor growth" N = 40	"Normal growth" N = 20	"Excessive growth" N = 20	Total N = 80	P value [#]
Maternal education(%)					0.094
< 12 years	28.9	10.0	40.0	26.9	
≥ 12 years	71.1	90.0	60.0	73.1	
Type of housing (%)					0.895
Government	84.2	80.0	85.0	83.3	
Private	15.8	20.0	15.0	16.7	
Parity (%)					0.419
Primiparious	62.5	70.0	50.0	61.3	
Multiparous	37.5	30.0	50.0	38.8	
Body Mass Index (kg/m^2)	24.1 ± 3.2	23.9 ± 2.5	25.0 ± 3.5	24.3 ± 3.1	0.452
Age (years)	31.1± 5.0	30.1 ± 4.1	31.4 ± 4.5	30.9 ± 4.7	0.621
Offspring					
Gender (%)					0.759
Male	55.0	45.0	50.0	51.2	
Female	45.0	55.0	50.0	48.8	
Gestational age (wks)	38.4 ± 0.9	38.7 ± 1.0	38.6 ± 1.0	38.5 ± 1.0	0.513
Birth weight (kg)	2.82 ± 0.22	3.21 ± 0.23	3.52 ± 0.31	3.10 ± 0.38	**<0.001**
Birth length (cm)	48.1 ± 2.2	49.4 ± 1.9	50.1 ± 1.8	48.9 ± 2.2	**0.002**

[#]p value across 3 groups, by Chi-square analysis (categorical) or one-way ANOVA (continuous)

8.4.2 Microarray analysis and quality control

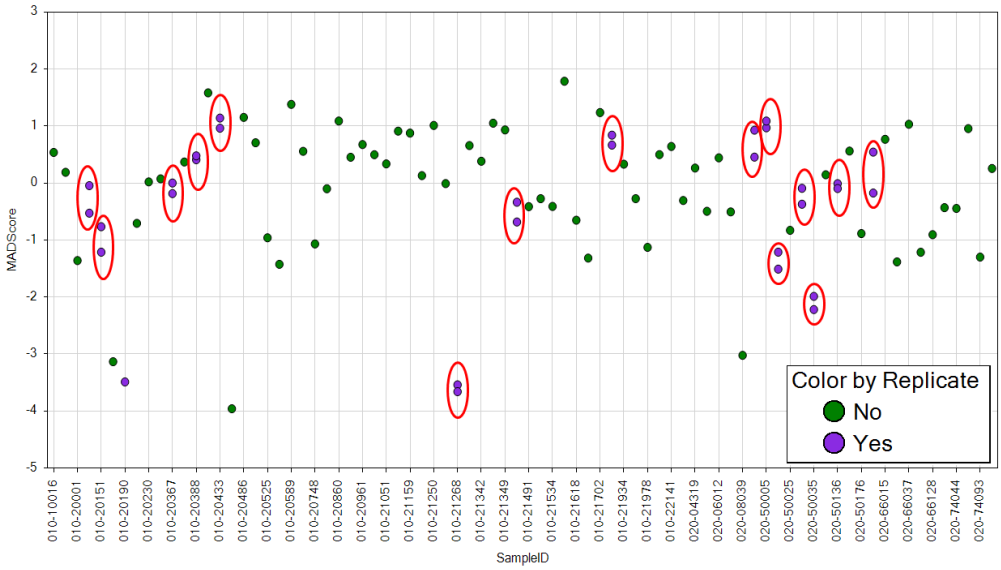
All samples were subjected to expression microarray analysis and the expression values were log transformed. Of the 47221 probes that are present on the microarray chip, 14281 probes were found to have at least 3 beads with significant detection p value < 0.05. After probe quality control and inter-sample quantile normalisation of the 80 RNA samples interrogated on the array, the data for one sample failed quality control (MAD scores < -5); this sample was a technical replicate and was subsequently removed from analysis (Fig 8.1). Sixteen series of technical replicates were included in the experiment, of which they clustered together in 15 out of 16 cases, highlighting that intra-sample variation was lower than the inter-sample variation. Hence the data was deemed of acceptable quality and the technical replicates were combined (Fig 8.2).

Figure 8.1: Plot of MAD scores for each sample ID



Each point represents MAD score for 1 sample

Fig 8.2 Plot of MAD scores for each sample ID highlighting clustering of sample replicates

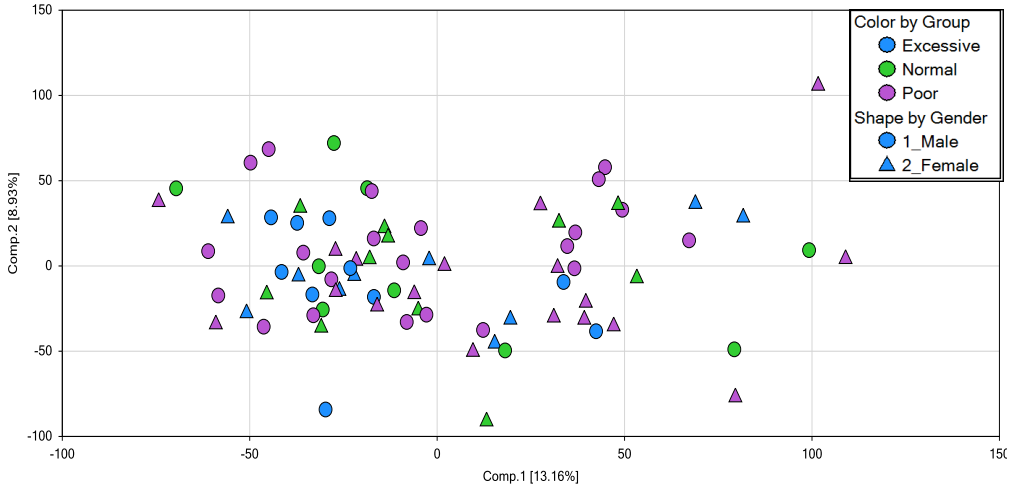


Each point represents MAD score for 1 sample. Green points represent non-replicates, purple points represent technical replicates within each microarray chip. Red circles indicate clustering of replicates.

8.4.3 Identifying potential underlying associations between individual transcriptomes and fetal growth

Figure 8.3 illustrates the pattern of relatedness between individual transcriptomes and potential underlying associations with fetal growth using principal components analysis. No significant relationship with fetal growth type (i.e. poor vs. normal vs. excessive growth) was observed and the samples did not appear to separate according to the fetal growth type. This may be indicative that the fetal growth type may not be a strong driver of umbilical cord transcriptomes.

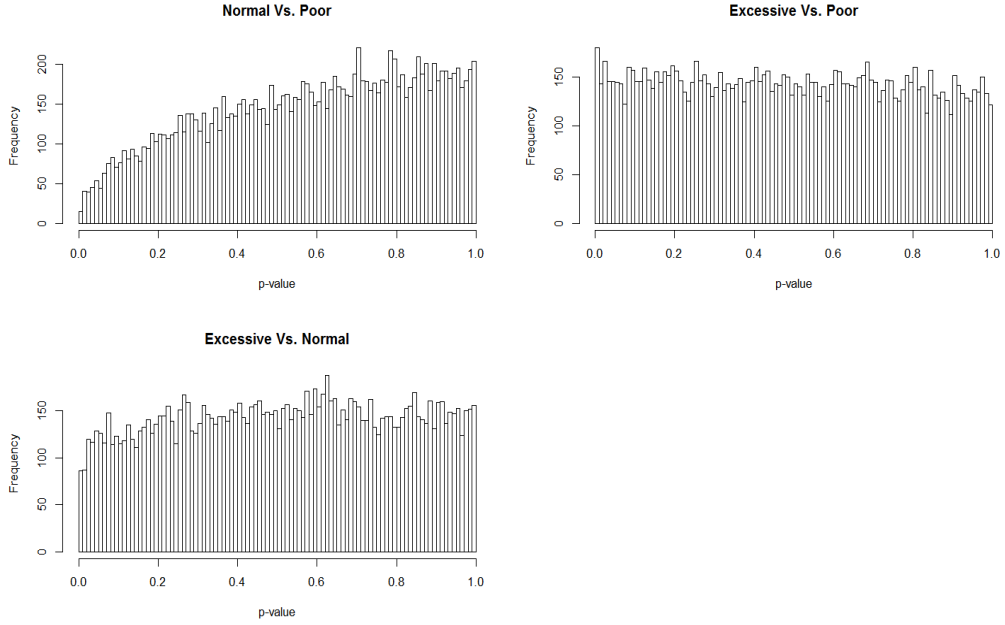
Fig 8.3: Prinicipal component analysis using the RNA expression microarray data across fetal growth type



Samples are classified as excessive (blue), normal (green) and poor fetal growth (purple). Circles represent males while triangles represent females

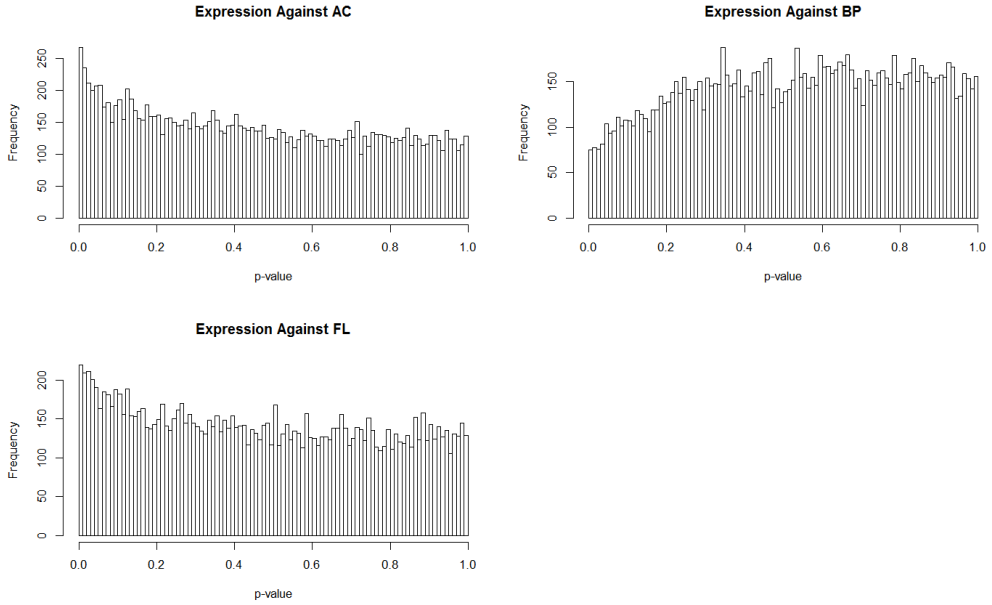
We performed 2-sample group tests to identify genes for which transcript levels were differential for fetal growth type, by comparing poor vs. normal, poor vs. excessive and normal vs. excessive fetal growth. Figure 8.4 illustrates the p-value distribution of all the genes for the 3 group tests. The distribution is relatively uniform and there are no more p-values < 0.05 than would be expected by chance, indicating that there is possibly no association between the gene expression level with the fetal growth type.

Fig 8.4: The distribution of p-values for mean transcript level differences between normal vs. poor (top left), excessive vs. poor (top right) and excessive vs. normal (bottom left)



In addition to this, linear regression analysis was performed to identify which gene expression levels were most highly correlated with fetal growth change between 2nd to 3rd trimester (i.e. ΔAC, ΔBP, ΔFL between 2nd and 3rd trimester). The distribution of p-values obtained for the regression coefficients is shown in Fig 8.5. There were more p values < 0.05 than would be expected by chance for the outcome of change in AC and FL, which suggests that some genes were more significantly associated with AC and FL change between the 2nd and 3rd trimester. A total of 104 common genes were found to be significantly associated with change in AC, BP and FL, and 87 common genes were significantly associated with change in AC and FL.

Fig 8.5: The distribution of p-values for regression coefficients of association between transcript levels with ΔAC (top left), ΔBP (top right) and ΔFL (bottom left) between 2nd and 3rd trimester



Pathway analysis was then performed on the 87 genes which had significant associations with ΔAC, ΔBP and ΔFL between 2nd and 3rd trimester. The most enriched pathways were related to immune response (Table 8.2); Immune response IFN alpha/beta signaling pathway (p = 6.87 x 10-10, FDR = 4.26 x 10-8) and Immune response antiviral actions of interferons (p = 2.02 x 10-7, FDR = 6.28 x 10-6), with 11 out of the 87 genes related to the aforementioned pathways (*AVP, STAT1, IRF9, ISG15, IFI6, IFIT2, OAS1, OAS2, MX1, MX2*). An additional 8 probes (*DHX58, IFIH1, SET, HMGB2, XPC, RAD1, LAP3, PREB*) were found to be enriched in other pathways with a p-value < 0.05.

Table 8.2: Pathway enrichment analysis of genes significantly associated with fetal growth

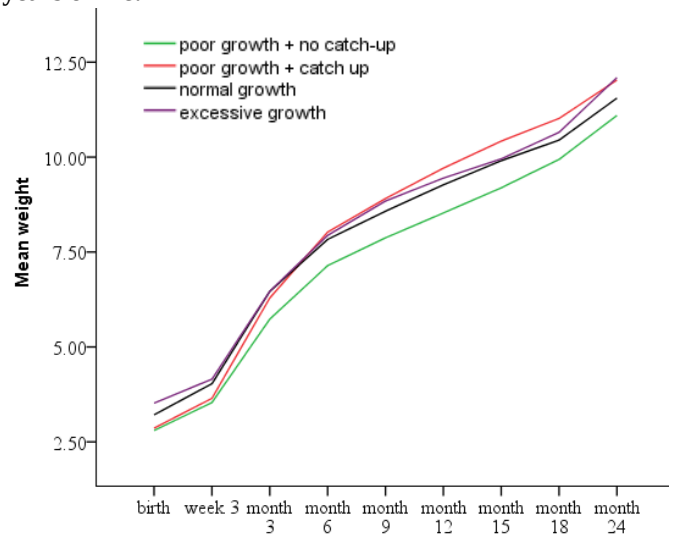
#	Maps	pValue	FDR	Genes
1	Immune response_IFN alpha/beta signaling pathway	6.870E-10	4.259E-08	AVP, STAT1, IRF9, ISG15, IFI6, IFIT2
2	Immune response_Antiviral actions of Interferons	2.024E-07	0.0000063	AVP, OAS1, OAS2, STAT1, IRF9, MX1, MX2
3	Development_Angiotensin signaling via STATs	3.670E-03	0.07584	STAT1, IRF9
4	Immune response_Innate immune response to RNA viral infection	6.916E-03	0.09184	DHX58, IFIH1
5	Apoptosis and survival_Granzyme A signaling	7.548E-03	0.09184	SET, HMGB2
6	DNA damage_Nucleotide excision repair	8.888E-03	0.09184	XPC, RAD1
7	DNA damage_Brca1 as a transcription regulator	1.266E-02	0.1122	STAT1, XPC
8	Development_G-CSF signaling	1.702E-02	0.1237	STAT1, LAP3
9	Immune response _IFN gamma signaling pathway	1.796E-02	0.1237	STAT1, IRF9
10	Development_c-Kit ligand signaling pathway during hemopoiesis	2.403E-02	0.1416	STAT1, LAP3
11	Development_Prolactin receptor signaling	2.512E-02	0.1416	STAT1, OAS1
12	Delta508-CFTR traffic / ER-to-Golgi in CF	3.990E-02	0.1903	PREB
13	Normal wtCFTR traffic / ER-to-Golgi	3.990E-02	0.1903	PREB

FDR: false discovery rate

8.4.4 Identifying potential underlying associations between individual transcriptomes with postnatal and catch-up growth

Figure 8.6 illustrates the postnatal growth trajectory in the first two years of life of the 80 infants from the three fetal growth types. We noted that amongst the "poor fetal growth" infants, there was a subgroup (n=20) who exhibited catch-up growth, and another subgroup that remained consistently small (i.e. non catch-up, n=20) during the first two years of life. We also performed 2-sample group tests to identify the genes for which transcript levels were differential between catch-up and non catch-up groups. A total of 208 genes were observed to have significantly different expression levels between the catch-up and non catch-up group. Pathway enrichment was also performed for the 208 genes identified to have significantly different expression levels between catch-up and non catch-up group. The most enriched pathways were related to dATP metabolism (p = 0.00288) and NFAT immune response (p = 0.00336). A total of 29 out of the 208 genes were found to be enriched in pathways with a p-value < 0.05.

Figure 8.6: Weight trajectory of poor, normal and excessive fetal growth infants during the first 2 years of life.



8.4.5 Experimental validation of identified genes associated with fetal and postnatal growth

Given that microarray data is inherently "noisy" and to rule out any artefacts of false positive results, we performed an experimental validation by assaying the expression levels of the 48 genes that were significantly enriched in pathway analysis using quantitative real-time PCR (qPCR). A total of 15 out of 48 genes (*AVP, IFI6, SET, HMGB2, RAD1, AIFM1, APAF1, CALM2, NUDT1, IKBKG, CCNL1, LIG1, PITX, TNIP2, RBP*) were validated to have the expression level differences similar to that observed in the microarray data, between the fetal growth types and the catch-up vs. non catch-up group.

We next enhanced the number of umbilical cord samples (n=200) to ascertain whether the expression levels of the 15 experimentally-validated genes identified above were associated with fetal growth characteristics, birth outcomes as well as postnatal growth characteristics of the GUSTO infant subjects. First, we conducted qRT-PCR experiments using umbilical cord specimens from the 200 individuals. Linear regression analysis was then applied on the delta Ct values of target genes against the variety of clinical measures. We noted that *AIFM1* expression levels had a positive significant association with fetal AC SDS [B(95%CI) = 0.18(0.03,0.33)] and BPD SDS at 26-28 weeks gestation [0.13(0.006,0.25)]. *TNIP2* expression levels also showed a positive significant association with fetal AC SDS at 26-28 weeks [0.53(0.23,0.83)], and with FL SDS at 19-21 weeks [0.42(0.13,0.71)], 26-28 weeks [0.41(0.11,0.70)] and 32-34 weeks gestation [0.35(0.03,0.67)]. For birth outcome characteristics, *AIFM1* and *CCNL1* showed a positive significant association with birth weight SDS [0.24(0.03,0.46) and 0.14(0.02,0.27)

respectively]. *APAF1* and *CCNL1* expression levels also showed positive significant association with birth length SDS [0.24(0.05,0.42) and 0.36(0.14,0.58) respectively]. For postnatal growth characteristics, only *AIFM1* expression levels showed a positive association with conditional growth in length SDS between 0-6 months of age [0.23(0.05,0.41)].

8.5 Discussion

A period of sub-optimal fetal growth carries an increased susceptibility for a range of diseases later in life, although this may vary from one individual to another. Prognostic markers, such as gene expression levels, may be able to predict individuals who are on the trajectory of increased risk of disease. In this study, we were able to assess the gene expression patterns of umbilical cords across groups of infants with differing fetal growth types between the 2^{nd} and 3^{rd} trimester of pregnancy using birth tissue specimens collected by GUSTO cohort study, and associate the expression levels with birth as well as postnatal growth outcomes.

Our study was designed to examine the molecular correlates of fetal growth during pregnancy across three groups, namely poor, normal and excessive fetal growth, with very little emphasis on birthweight. One of the stumbling blocks in other studies is the problematic approach of defining FGR and SGA interchangeably. Such a definition does not clearly distinguish those who are pathologically growth-restricted from those who are constitutionally small and healthy. The novelty of this study was the use of serial ultrasound measurements for the same fetus taken at different gestational periods to give a better indication and definition of fetal growth velocity. To our knowledge, few studies have utilized such a measure when examining molecular correlates of FGR. Our minimal emphasis on birthweight is also in-line with recent evidence that birthweight is not a crucial factor in driving the transcriptome. An earlier study conducted by Stunkel W et al on the GUSTO cohort highlighted that gestational age rather than birthweight, even at extremes, was the important factor in driving the transcriptome(269), a finding that was also

supported by the work of Cohen et al(270). Another study utilizing umbilical cord blood from Caucasian and African-American mothers in the Conditions affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) cohort also failed to find significant relationships with birth weight at the transcript level(113). Given these findings, birth measurements may not be entirely useful when studying molecular correlates of fetal growth.

The gene expression microarray data obtained after comparison between subjects of differing fetal growth types revealed a putative list of 15 genes that were differentially expressed. These genes were also observed to be significantly enriched in pathways related to immune response (*AVP, IFI6, CALM2, IKBKG),* apoptosis and survival (*AIFM1, APAF1, SET, HMGB2*). Immune response is known to be modulated in FGR infants. Mukhopadhyay D et al documented that FGR is correlated with fewer circulating as well as decreased functioning of T-regulatory cells compared to normal infants(271). Troger B et al also reported significantly lower white blood cell and platelet count amongst SGA infants compared to normal infants(272), highlighting how immune response may be modulated by intrauterine growth. FGR has also been reported to be associated with increased incidence of apoptosis; Murthi P et al documented increased apoptosis in FGR-affected fetal membranes, with the apoptotic cells restricted primarily to the chorionic trophoblast layer of the fetal membranes, which can impair normal fetal development and growth(273). Similarly, Whitehead CL et al reported an increased expression of genes regulating intrinsic apoptosis amongst FGR infants(274), though this was observed in the placenta rather than the umbilical cord. With the knowledge that FGR may play a role in modulating

genes in immune response and apoptotic pathways, these genes may serve to be useful prognostic markers to predict FGR individuals.

In addition, we observed differential expression for genes enriched in pathways related to nucleotide metabolism (*NUDT1*), DNA damage repair (*RAD1*), and angiopoietin signalling (*TNIP2*). *NUDT1* is a deoxyinosine diphosphatase, and studies have shown that its deficiency induces accumulation of single-strand breaks in nuclear DNA, which can lead to growth arrest. *RAD1* is a gene that encodes a component of a heterotrimeric cell cycle checkpoint complex, known as the 9-1-1 complex, which stops cell cycle progression in response to DNA damage or incomplete DNA replication(275, 276). In our expression microarray and qRT-PCR data, we noted lower expression levels for these two genes in the poor fetal growth group and also in the subgroup that exhibits non catch-up growth in the first 2 years of life. Reduced expression of these 2 genes might lead to growth arrest due to accumulation of DNA single-strand breaks and reduced ability to repair the damaged DNA. *TNIP2* is a protein-encoding gene which inhibits activation of NF-κB in the angiopoeitin signalling pathway. This signalling pathway is crucial for angiogenesis and vascular maintenance(277), and inhibition of NF-κB activity in this pathway is known have anti-apoptotic action(278). We also noted reduced expression of *TNIP2* in the poor fetal growth subgroup that exhibits non catch-up growth, hinting increased apoptotic activity, which is in line with existing evidence of increased apoptotic activity amongst FGR infants. Again, these genes may serve to be useful prognostic markers to predict FGR individuals

This study is not without limitations; we assayed the genomics of umbilical cord tissue as the only available somatic tissue. While the umbilical cord may represent a suitable "snapshot" of the gene expression patterns associated with fetal growth, such patterns may not be truly reflective of differences in expression levels in true target tissues that give rise to diseases with developmental origins (e.g. liver, pancreas or other endocrine tissues), and thus it would be difficult to extrapolate the molecular patterns observed in umbilical cord to later disease risk. Our approach is built on the premise that gene expression differences in the true target tissues might be reflected in the umbilical cord, although any observed strength of association may be much weaker. Given that we could only at best detect indirect associations, there might be some merit to more closely examining the top ranked associations in our data set.

In conclusion, our study has managed to uncover gene expression changes significant for fetal growth and subsequent postnatal growth. Previous studies have examined the association between retarded fetal growth with gene expression patterns in a variety of pathological states (e.g. Beckwith-Wiedemann or Russell-Silver Syndromes)(112, 279, 280). By contrast, our study is one of the few to determine if variation in gene expression is associated with fetal growth as measured by ultrasound among a set of newborns of from low-risk pregnancies. Our results may have provided insights into the transcriptomic profile of babies with differing fetal growth types. This may allow for the development of prognostic markers to predict FGR. Future research should focus on the functional characterization of these genes to explore if the expression differences are associated with

characteristics related to metabolic disease (e.g. insulin sensitivity and resistance, glucose uptake, adipogenesis).

Chapter 9: The contrasting effects of melanocortin-3-receptor (*MC3R*) and fat-mass and obesity associated gene (*FTO*) polymorphisms on adiposity in early childhood

9.1 Summary

Background: Polymorphic variants within the melanocortin-3 receptor gene (*MC3R*) and the fat-mass and obesity-associated gene (*FTO*) have been associated with adult obesity. However, their influence on early childhood adiposity is unclear. We assessed the association between genotype at polymorphic sites within the *MC3R* and *FTO* genes with overweight status during early childhood, and determined if this was mediated by early childhood feeding behaviour.

Methods: One thousand and ninety singleton offspring in a prospective birth cohort genotyped for *MC3R* (rs3746619, rs3827103) and *FTO* (rs9939973, rs1421085, rs1121980, rs9939609, rs17817449, rs8050136) variants were studied, as well as in a subgroup (n=422) with completed childhood appetitive trait scores at 1-year of age. Overweight status at 1-, 2- and 3-years of age was defined as having a body mass index z-score more than 2, following the World Health Organization 2006 Child Growth Standards. Associations between *MC3R* and *FTO* genotype with overweight status was assessed using multivariable logistic regression.

Results: Independent of potential confounders, each additional *MC3R* variant minor allele was associated with a linear increase in the proportion of overweight children at 2- and 3-years of age, but not at 1-year of age, and with an increased odds of overweight at ages 2- and 3-years. In contrast, no

significant associations were observed between *FTO* variants and overweight at 1-, 2- and 3-years of age. Children homozygous for the minor allele in *MC3R* variants had the highest scores for "slowness in eating" appetitive trait.

Conclusion: The findings suggest that *MC3R*, but not *FTO* genetic variants are associated with early childhood adiposity, and this relationship might be mediated by childhood appetitive traits at 1-year. The relative effects of various susceptibility genetic variants may differ at different ages and stages of life.

9.2 Introduction

Overweight and obesity are commonly associated with increased risk of chronic diseases including type 2 diabetes and cardiovascular disease, and present a massive public health challenge as they rapidly become a worldwide epidemic(1, 281). Non-syndromic or common obesity is often viewed as a complex and multifactorial condition, where exposure to an "obesogenic" environment, coupled with an underlying genetic susceptibility to excessive weight gain causes an obese phenotype (282, 283). In recent years, genome-wide association studies (GWAS) have succeeded in identifying genetic variants that were associated with body mass index (BMI) and risk of obesity. An example is the fat-mass and obesity-associated gene (*FTO*)(284-286). The association between *FTO* variants with BMI and obesity risk has been confirmed in many populations (287-290), though the effect is modest, with the minor (risk) allele increasing BMI by 0.39 kg/m^2 (or ~1 kg in body weight) and increasing obesity risk by 1.2 fold (291). A recent study conducted on 4,298 multi-ethnic Singaporean adults highlighted nine *FTO* variants commonly reported in such studies of European populations were also

associated with increased BMI amongst ethnic Chinese and Malays in Singapore, and also contributed to increased risk of type 2 diabetes amongst Malays (292).

Linkage studies by Fox et al (293) and Lembertas et al (294) reported that a polymorphism within the melanocortin-3-receptor (*MC3R*) locus is associated with susceptibility for obesity. Mice models with genetic alterations which disrupt *MC3R* exhibited hypophagia, higher energy efficiency, reduced locomotor activity, hyperleptinemia and reduced linear growth accompanied by higher percentage of body fat without increased body weight, compared with the wildtype mice (295, 296). Furthermore, two case-control studies have independently demonstrated two missense *MC3R* variants, Thr6Lys (rs3746619) and Val81Ile (rs3827103) which are in near complete linkage disequilibrium, were significantly associated with increased adiposity in childhood, and exhibited reduced *in-vitro* activity compared to wild-type *MC3R* (297, 298). It has been previously described that Singaporean obese children with the Thr6Lys/Val81Ile variants exhibited significantly higher leptin levels, percentage body fat and insulin sensitivity (298). Thus, the published evidence supports the role of *MC3R* as well as *FTO* in human weight regulation, and the effects of *MC3R* and *FTO* variants on childhood adiposity are mediated by increased energy intake and possibly altered sensitivity to satiety (299-302). Data on the influence of *MC3R* and *FTO* variants on adiposity during the first few years of life is fragmentary, and it is unclear when and at what age do such susceptibility variants start to exert effects on phenotype. Thus in this study, we assessed the association between genetic variants within the *MC3R* and *FTO* loci with overweight status at early

childhood, and hypothesized that offspring with polymorphic variants of known adiposity-associated genes (*MC3R* and *FTO*) would be predisposed to overweight during early childhood, and determined if these effects were mediated by childhood appetitive traits.

9.3 Materials and Methods

9.3.1 Study population, antenatal and infant anthropometry measurements

Details on the study population, antenatal and infant anthropometry measurements have been described in Section 2.1, Section 2.3.1 and Section 2.3.2.1

9.3.2 Illumina Omniexpress + exome genotyping

Genotyping was performed on DNA extracted from frozen umbilical cords using the Illumina omniexpress + exome array platform. These arrays genotype the most frequent SNPs genome wide (omniexpress) and those discovered within exons (exome). DNA hybridization to arrays and scanning was performed by the service provider Expression Analysis Inc. Data was processed in GenomeStudio Genotyping Module™. Genotyping calls were made by the GenCall software which incorporates a clustering algorithm (GenTrain) and a calling algorithm (Bayesian model). GenCall score of each SNP probe and call rate of each sample are generated. Genotypes with a GenCall score less than 0.15 were considered missing. There were no poorly performing samples as defined by low sample call rates or low GC Scores. Nine variants within the *MC3R* gene (rs3746619, rs3827103, exm1551534, exm1969518, exm1551535, exm1969522, exm1551559, exm1551560,

exm1551565) were genotyped on the arrays. However, only two variants (rs3746619, rs3827103) were polymorphic in our study population. The minor alleles of these two variants represented missense polymorphisms 17C > A (Thr6Lys) and 241 G >A (Val81Ile) respectively. Nine *FTO* variants were previously reported to show significant association with obesity in ethnic Chinese and Malay adults in Singapore Nine *FTO* variants were previously reported to show significant association with obesity in ethnic Chinese and Malay adults in Singapore (292). Of those nine variants, six were genotyped on the arrays and analyzed in this report (rs9939973, rs1421085, rs1121980, rs9939609, rs17817749, rs8050136).

9.3.3 Infant feeding and appetitive traits

Details regarding infant feeding assessment has been described in Section 2.3.2.2.

Early childhood appetitive traits were measured using the Child Eating Behaviour Questionnaire (CEBQ) completed by the mothers. The CEBQ was sent by postal service to the participants home prior to the 1-year visit and collected during the 1-year home visit. Questionnaires distributed were in English unless a preference for another language (Mandarin, Malay or Tamil) was expressed. For these cases, translated versions of the questionnaires were distributed. The CEBQ relates to a period in which the child was predominatly fed solid food. Each item on the questionnaire was answered using a five-point Likert frequency scale (1=never, 2=rarely, 3=sometimes, 4=often and 5 =always). Principal component analysis (PCA) was performed to reduce the appetitive trait items into robust components, with Varimax normalized rotation applied on all items of the CEBQ. Questions with reverse scales were

first reverse scored, and a factor loading cut-off of 0.5 was applied before running the analysis. The 35-item CEBQ in this study was reduced to seven components, four of which measured food approach appetitive traits: food esponsiveness, enjoyment of food, emotional over eating and desire to drink. Food fussiness and enjoyment of food were combined as one subscale under enjoyment of food as they have been shown to load into the same factor. Examples of items that fall under the category of food approach behavior are "My child is always asking for food" (food responsiveness), "My child loves food" (enjoyment of food), "My child eats more when worried" (emotional over eating), "My child is always asking for a drink" (desire to drink). The three other subscales of the CEBQ measure food avoidant behaviors: Emotional under eating, satiety responsiveness and slowness in eating. Examples of items that fall under the category of food avoidant appetitive traits are "My child eats less when angry" (emotional under eating), "My child gets full up easily" (satiety responsiveness) and "My child eat slowly" (slowness in eating).

9.3.4 Statistical analyses

Descriptive statistics were reported as means and standard deviations for continuous variables and percentages for categorical variables. Infant BMI was calculated as infant weight/(infant height)^2. Age- and gender-specific standardized z-scores were calculated for BMI, referencing the WHO Child Growth Standards (212). Overweight status at 1-, 2- and 3-years of age was defined as having a BMI z-score of more than 2 as defined by WHO (212). Linkage disequilibrium (LD), in the form of r^2, for the two *MC3R* and six *FTO* variants respectively were estimated using Haploview (303). For analyses of

associations of *MC3R* and *FTO* variants with overweight status at 1, 2- and 3-years of age, each variant was considered as a continuous variable. Individuals were assigned as 0, 1, or 2 according to their number of minor alleles. Logistic regression models were used to calculate odds ratios for each variant, higher by one minor allele, with Model I adjusting for ethnicity, Model II including additional adjustment of birth factor i.e.birthweight-for-gestational age and Model III including additional adjustment for postnatal factor i.e. breastfeeding duration. To test the hypothesis that appetitive traits may mediate the relationship between *MC3R* or *FTO* variants and overweight status, we analysed the association between *MC3R* or *FTO* variants with CEBQ scores at 1-year of age using multivariate linear regression. All analyses were performed using SPSS version 20.0 (IBM, SPSS Statistics, Armonk, NY).

9.4 Results

9.4.1 Demographics and clinical characteristics

Complete genotype data for the two and six polymorphic variants within *MC3R* and *FTO* loci respectively, were available for 1090 study participants. Demographic and clinical characteristics, stratified by *MC3R* genotype, are shown in Table 9.1. No significant differences were observed in maternal characteristics (education, BMI at 26-28 weeks gestation, parity, gestational diabetes) and offspring characteristics (gender, gestational age at delivery, breastfeeding duration, birth weight, length, BMI) across the *MC3R* genotypes. Indian infants were more likely to carry two copies of the minor allele compared to Chinese and Malay infants, although this difference is not statistically significant. No significant differences in parity, gestational diabetes, infant gender, gestational age, birth weight, length and BMI were observed across the *FTO* genotypes (Tables 9.2-9.4). Infants who carried the minor allele had higher maternal BMI at 26-28 weeks gestation ($p<0.001$ for all six *FTO* variants), were more likely to be of Indian ethnicity ($p<0.001$ for all six *FTO* variants), tended to breastfeed for less than 6 months ($p < 0.05$ for rs9939609, rs17817449, rs8050136) and were more likely to have less than 12 years of education ($p < 0.05$ for rs1421085, rs9939609, rs17817449, rs8050136). Figures 9.1A-B illustrates the LD of the two *MC3R* and six *FTO* variants respectively. The two *MC3R* variants, rs3746619 and rs3827103, were in near perfect LD in our study population ($r^2 = 0.98$). The six *FTO* variants were also in strong LD ($r^2 = 0.73$-0.99).

Table 9.1: Demographic and clinical characteristics of study participants by *MC3R* genotype

	rs3746619				rs3827103			
Mothers	**CC N = 639**	**AC N = 385**	**AA N = 66**	**P value[#]**	**CC N = 644**	**AC N = 380**	**AA N = 66**	**P value[#]**
Education level				0.111				0.092
< 12 years	242 (55.4)	162 (37.1)	33 (7.6)		243 (55.6)	161 (36.8)	33 (7.6)	
≥ 12 years	389 (60.8)	218 (34.1)	33 (5.2)		393 (61.4)	214 (33.4)	33 (5.2)	
Ethnicity				0.069				0.137
Chinese	385 (62.4)	199 (32.3)	33 (5.3)		385 (62.4)	199 (32.3)	33 (5.3)	
Malay	150 (54.3)	108 (39.1)	18 (6.5)		150 (54.3)	108 (39.1)	18 (6.5)	
Indian	104 (52.8)	78 (39.6)	15 (7.6)		109 (55.3)	73 (37.1)	15 (7.6)	
Parity				0.755				0.661
Primiparous	286 (57.7)	181 (36.5)	29 (5.8)		287 (57.9)	180 (36.3)	29 (5.8)	
Multiparous	353 (59.4)	204 (34.3)	37 (6.2)		357 (60.1)	200 (33.7)	37 (6.2)	
Body Mass Index (kg/m^2) at 26-28 weeks pregnancy	26.1 ± 4.3	26.5 ± 4.6	25.7 ± 4.7	0.305	26.2 ± 4.3	26.5 ± 4.6	25.7 ± 4.7	0.375
Gestational diabetes				0.854				0.855
No	480 (58.0)	294 (35.6)	53 (6.4)		483 (58.4)	291 (35.2)	53 (6.4)	
Yes	110 (58.5)	68 (36.2)	10 (5.3)		111 (59.0)	67 (35.6)	10 (5.3)	
Offspring								
Gestational age (weeks)	38.3 ± 1.4	38.3 ± 1.6	38.3 ±1.2	0.935	38.3 ± 1.4	38.3 ± 1.6	38.3 ± 1.2	0.909
Gender				0.611				0.633
Male	308 (60.2)	175 (34.2)	29 (5.7)		310 (60.5)	173 (33.8)	29 (5.7)	
Female	331 (57.3)	210 (36.3)	37 (6.4)		334 (57.8)	207 (35.8)	37 (6.4)	
Breastfeeding duration				0.062				0.072
< 6 months	277 (56.2)	182 (36.9)	34 (6.9)		280 (56.8)	179 (36.3)	34 (6.9)	
≥ 6 months	204 (63.9)	101 (31.7)	14 (4.4)		205 (64.3)	100 (31.3)	14 (4.4)	
Birth weight	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.4	0.545	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.4	0.485
Birth length	48.7 ± 2.3	48.6 ± 2.3	48.6 ± 1.9	0.764	48.7 ± 2.3	48.6 ± 2.3	48.6 ± 1.9	0.719
Birth BMI	13.1 ± 1.3	13.1 ± 1.4	13.0 ± 1.4	0.670	13.1 ± 1.3	13.1 ± 1.4	13.0 ± 1.4	0.615

Numbers represent mean ± SD or n(%)

[#] P value for continuous variables by One-Way ANOVA; for categorical variables by chi-square analysis

Table 9.2: Demographic and clinical characteristics of study participants by *FTO* rs9939973 and rs1421085 genotype

	rs9939973				rs1421085			
Mothers	**GG N = 615**	**AG N = 387**	**AA N = 87**	**P value[#]**	**TT N = 684**	**CT N = 346**	**CC N = 59**	**P value[#]**
Education level				0.243				**0.044**
< 12 years	235 (53.9)	161 (36.9)	40 (9.2)		256 (58.7)	151 (34.6)	29 (6.7)	
≥ 12 years	375 (58.6)	219 (34.2)	46 (7.2)		422 (65.9)	188 (29.4)	30 (4.7)	
Ethnicity				**<0.001**				**<0.001**
Chinese	415 (67.4)	175 (28.4)	26 (4.2)		463 (75.2)	139 (22.6)	14 (2.3)	
Malay	128 (46.4)	124 (44.9)	24 (8.7)		134 (48.6)	121 (43.8)	21 (7.6)	
Indian	72 (36.5)	88 (44.7)	37 (18.8)		87 (44.2)	86 (43.7)	24 (12.2)	
Parity				0.196				0.117
Primiparous	289 (58.4)	174 (35.2)	32 (6.5)		325 (65.7)	149 (30.1)	21 (4.2)	
Multiparous	326 (54.9)	213 (35.9)	55 (9.3)		359 (60.4)	197 (33.2)	38 (6.4)	
Body Mass Index (kg/m^2) at 26-28 weeks pregnancy	25.6 ± 4.0	26.9 ± 4.8	27.7 ± 4.9	**<0.001**	25.7 ± 4.0	27.0 ± 4.8	28.0 ± 5.2	**<0.001**
Gestational diabetes				0.267				0.595
No	463 (56.1)	302 (36.6)	61 (7.4)		516 (62.5)	266 (32.2)	44 (5.3)	
Yes	115 (61.2)	57 (30.3)	16 (8.5)		122 (64.9)	54 (28.7)	12 (6.4)	
Offspring								
Gestational age (weeks)	38.4 ± 1.4	38.2 ± 1.4	38.2 ± 1.9	0.246	38.4 ± 1.4	38.2 ± 1.4	38.0 ± 2.1	0.083
Gender				0.531				0.634
Male	280 (54.7)	189 (36.9)	43 (8.4)		314 (61.3)	169 (33.0)	29 (5.7)	
Female	335 (58.1)	198 (34.3)	44 (7.6)		370 (64.1)	177 (30.7)	30 (5.2)	
Breastfeeding duration				0.466				0.087
< 6 months	265 (53.8)	182 (36.9)	46 (9.3)		293 (59.4)	164 (33.3)	36 (7.3)	
≥ 6 months	182 (57.2)	113 (35.5)	23 (7.2)		208 (65.4)	97 (30.5)	13 (4.1)	
Birth weight	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.5	0.939	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.5	0.712
Birth length	48.7 ± 2.2	48.6 ± 2.2	48.4 ± 3.0	0.591	48.7 ± 2.2	48.6 ± 2.2	48.2 ± 3.2	0.186
Birth BMI	13.0 ± 1.3	13.1 ± 1.3	13.1 ± 1.4	0.568	13.0 ± 1.3	13.1 ± 1.3	13.1 ± 1.4	0.471

Numbers represent mean ± SD or n(%)

[#] P value for continuous variables by One-Way ANOVA; for categorical variables by chi-square analysis

Table 9.3: Demographic and clinical characteristics of study participants by *FTO* rs1121980 and rs9939609 genotype

	rs1121980				rs9939609			
Mothers	**CC N = 623**	**CT N = 383**	**TT N = 83**	**P value[#]**	**TT N = 698**	**AT N = 337**	**AA N = 54**	**P value[#]**
Education level				0.167				**0.025**
< 12 years	236 (54.1)	162 (37.2)	38 (8.7)		261 (59.9)	147 (33.7)	28 (6.4)	
≥ 12 years	382 (59.7)	214 (33.4)	44 (6.9)		431 (67.3)	183 (28.6)	26 (4.1)	
Ethnicity				**<0.001**				**<0.001**
Chinese	421 (68.3)	172 (27.9)	23 (3.7)		466 (75.6)	138 (22.4)	12 (1.9)	
Malay	129 (46.7)	123 (44.6)	24 (8.7)		137 (49.6)	118 (42.8)	21 (7.6)	
Indian	73 (37.1)	88 (44.7)	36 (18.3)		95 (48.2)	81 (41.1)	21 (10.7)	
Parity				0.153				0.170
Primiparous	294 (59.4)	171 (34.5)	30 (6.1)		329 (66.5)	147 (29.7)	19 (3.8)	
Multiparous	329 (55.4)	212 (35.7)	53 (8.9)		369 (62.1)	190 (32.0)	35 (5.9)	
Body Mass Index (kg/m^2) at 26-28 weeks pregnancy	25.6 ± 4.0	26.9 ± 4.8	27.6 ± 5.0	**<0.001**	25.7 ± 4.0	27.0 ± 4.9	27.9 ± 5.4	**<0.001**
Gestational diabetes				0.324				0.841
No	470 (56.9)	298 (36.1)	58 (7.0)		526 (63.7)	258 (31.2)	42 (5.1)	
Yes	116 (61.7)	57 (30.3)	15 (8.0)		124 (66.0)	55 (29.3)	9 (4.8)	
Offspring								
Gestational age (weeks)	38.4 ± 1.4	38.2 ± 1.5	38.2 ± 1.8	0.159	38.4 ± 1.4	38.1 ± 1.6	38.3 ± 1.1	0.086
Gender				0.667				
Male	286 (55.9)	187 (36.5)	39 (7.6)		319 (62.3)	167 (32.6)	26 (5.1)	
Female	337 (58.4)	196 (34.0)	44 (7.6)		379 (65.7)	170 (29.5)	28 (4.9)	
Breastfeeding duration				0.332				**0.034**
< 6 months	266 (54.0)	183 (37.1)	44 (8.9)		297 (60.2)	161 (32.7)	35 (7.1)	
≥ 6 months	187 (58.8)	109 (34.3)	22 (6.9)		214 (67.3)	93 (29.2)	11 (3.5)	
Birth weight	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.5	0.977	3.1 ± 0.4	3.1 ± 0.4	3.1 ± 0.4	0.964
Birth length	48.7 ± 2.2	48.6 ± 2.2	48.4 ± 2.9	0.509	48.7 ± 2.2	48.5 ± 2.4	48.4 ± 2.5	0.408
Birth BMI	13.0 ± 1.3	13.1 ± 1.3	13.1 ± 1.3	0.621	13.0 ± 1.3	13.1 ± 1.3	13.3 ± 1.1	0.398

Numbers represent mean ± SD or n(%)

[#] P value for continuous variables by One-Way ANOVA; for categorical variables by chi-square analysis

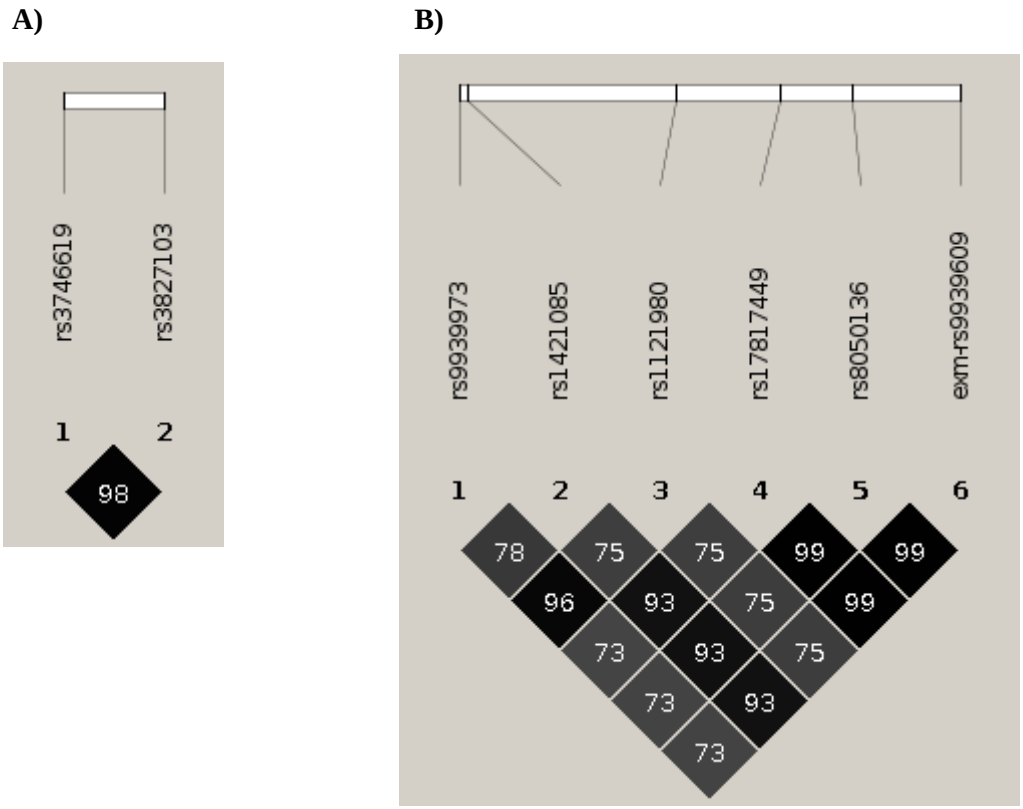
Table 9.4: Demographic and clinical characteristics of study participants by *FTO* rs17817449 and rs8050136 genotype

	rs17817449				rs8050136			
Mothers	**TT N = 700**	**GT N = 335**	**GG N = 54**	**P value[#]**	**TT N = 700**	**AT N = 335**	**AA N = 54**	**P value[#]**
Education level				**0.020**				**0.020**
< 12 years	261 (59.9)	147 (33.7)	28 (6.4)		261 (59.9)	147 (33.7)	28 (6.4)	
≥ 12 years	433 (67.7)	181 (28.3)	26 (4.1)		433 (67.7)	181 (28.3)	26 (4.1)	
Ethnicity				**<0.001**				**<0.001**
Chinese	468 (76.0)	136 (22.1)	12 (1.9)		468 (76.0)	136 (22.1)	12 (1.9)	
Malay	137 (49.6)	118 (42.8)	21 (7.6)		137 (49.6)	118 (42.8)	21 (7.6)	
Indian	95 (48.2)	81 (41.1)	21 (10.7)		95 (48.2)	81 (41.1)	21 (10.7)	
Parity				0.144				0.144
Primiparous	331 (66.9)	145 (29.3)	19 (3.8)		331 (66.9)	145 (29.3)	19 (3.8)	
Multiparous	369 (62.1)	190 (32.0)	35 (5.9)		369 (62.1)	190 (32.0)	35 (5.9)	
Body Mass Index (kg/m^2) at 26-28 weeks pregnancy	25.7 ± 4.0	27.0 ± 4.9	27.9 ± 5.4	**<0.001**	25.7 ± 4.0	27.0 ± 4.9	27.9 ± 5.4	**<0.001**
Gestational diabetes				0.857				0.785
No	527 (63.8)	257 (31.1)	42 (5.1)		527 (63.8)	257 (31.1)	42 (5.1)	
Yes	124 (66.0)	55 (29.3)	9 (4.8)		125 (66.5)	54 (28.7)	9 (4.8)	
Offspring								
Gestational age (weeks)	38.4 ± 1.4	38.1 ± 1.6	38.3 ± 1.1	0.091	38.4 ± 1.4	38.2 ± 1.6	38.3 ± 1.1	0.100
Gender				0.583				0.583
Male	321 (62.7)	165 (32.2)	26 (5.1)		321 (62.7)	165 (32.2)	26 (5.1)	
Female	379 (65.7)	170 (29.5)	28 (4.9)		379 (65.7)	170 (29.5)	28 (4.9)	
Breastfeeding duration				**0.026**				**0.032**
< 6 months	297 (60.2)	161 (32.7)	35 (7.1)		298 (60.4)	160 (32.5)	35 (7.1)	
≥ 6 months	216 (67.9)	91 (28.6	11 (3.5)		215 (67.6)	92 (28.9)	11 (3.5)	
Birth weight	3.1 ± 0.4	3.1 ± 0.5	3.1 ± 0.4	0.964	3.1 ± 0.4	3.1 ± 0.5	3.1 ± 0.4	0.965
Birth length	48.7 ± 2.2	48.5 ± 2.4	48.4 ± 2.5	0.411	48.7 ± 2.2	48.5 ± 2.4	48.4 ± 2.5	0.411
Birth BMI	13.0 ± 1.3	13.1 ± 1.3	13.3 ± 1.1	0.401	13.0 ± 1.3	13.1 ± 1.3	13.3 ± 1.1	0.399

Numbers represent mean ± SD or n(%)

[#] P value for continuous variables by One-Way ANOVA; for categorical variables by chi-square analysis

Figure 9.1: Linkage disequilibrium analysis for *MC3R* (A) and *FTO* variants (B)



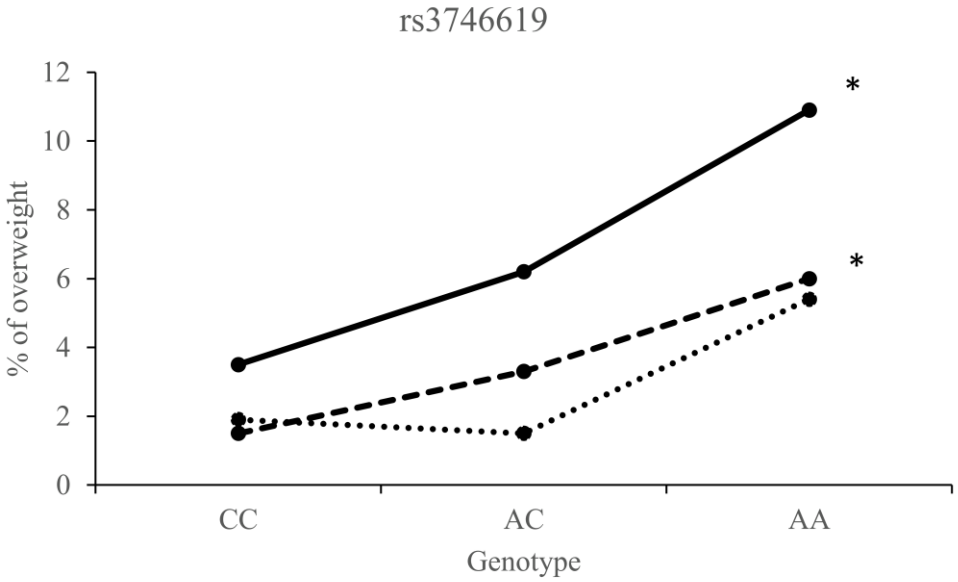
9.4.2 Relationship between *MC3R* and *FTO* variants with frequency of overweight at 1-, 2- and 3-years of age

We next examined the association between *MC3R* and *FTO* variants with overweight status at 1-, 2- and 3-years of age. Due to the strong LD between the two genetic variants within the *MC3R* locus, we discuss the analysis results for *MC3R* rs3746619 and note that similar results are obtained for *MC3R* rs3827103. Likewise, we discuss results for *FTO* rs9939973 since similar conclusions are obtained for the other *FTO* variants. We selected rs3746619 and rs9939973 as representative variants to cover the *MC3R* and *FTO* haploblocks respectively, as these variants exhibited the highest minor allele frequency. Overall, we observed a significant increase in the proportion

of overweight infants at 2- and 3-years of age (1.5% to 6.0% at 2-years; 3.5% to 10.9% at 3-years, $p<0.05$ by Chi-square test for trend), but not at 1-year of age, for every minor allele increase for *MC3R* rs3746619 (Figure 9.2A), highlighting the positive relationship between *MC3R* risk variants with overweight status during early childhood. No obvious linear relationship with increasing frequency of overweight status at 1- and 2-years of age was noted for *FTO* rs9939973 (Figure 9.2B). A chi-square test revealed no significant linear trend for overweight status at 1- and 2-years of age for *FTO* rs9939973 Our findings in Figure 9.2B also seems to suggest that there is an increase in risk of overweight only for the *FTO* heterozygote variants at 3-years of age, implying a plausible co-dominance genetic model for *FTO* and overweight status at 3-years of age. A sub-analysis using a co-dominant model of *FTO* (heterozygote vs. the two homozygotes) with outcome of overweight status at 3-years however, showed no significant associations (Table 9.5). The proportion of overweight infants at 1-, 2- and 3-years of age according to the other *MC3R* and *FTO* variants are illustrated in Figures 9.3A-F.

Figure 9.2: Frequency of overweight status at 1-, 2- and 3-years of age according to *MC3R* rs3746619 (A) and FTO rs9939973 (B) genotype. Dotted line = overweight at 1-year, Dashed line = overweight at 2-years, Solid line = overweight at 3-years. *p<0.05 for chi-square test of linear trend.

A)



B)

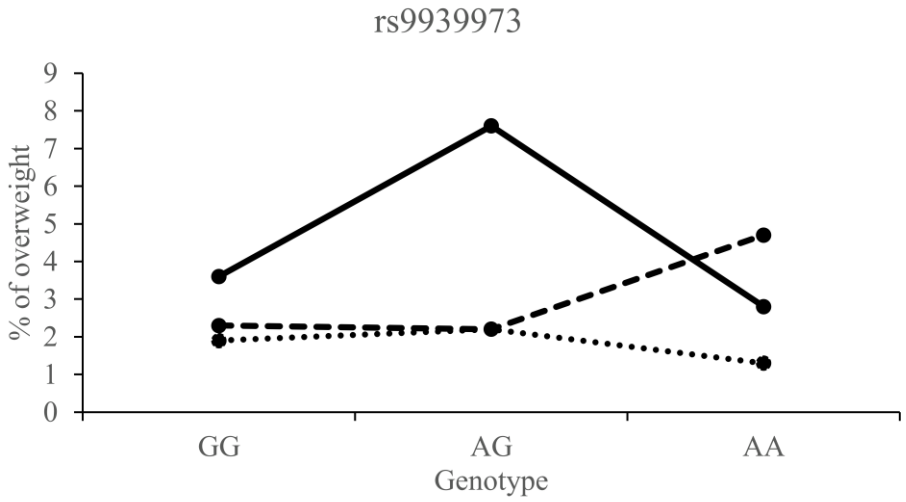


Figure 9.3: Frequency of overweight status at 1-, 2- and 3-years of age according to *MC3R* rs3827103 (A), FTO rs1421085 (B), FTO rs1121980 (C), FTO rs9939609 (D), FTO rs17817449 (E), FTO rs8050136 (F). Dotted line = overweight at 1-year, Dashed line = overweight at 2-years, Solid line = overweight at 3-years. *p<0.05 for chi-square test of linear trend.

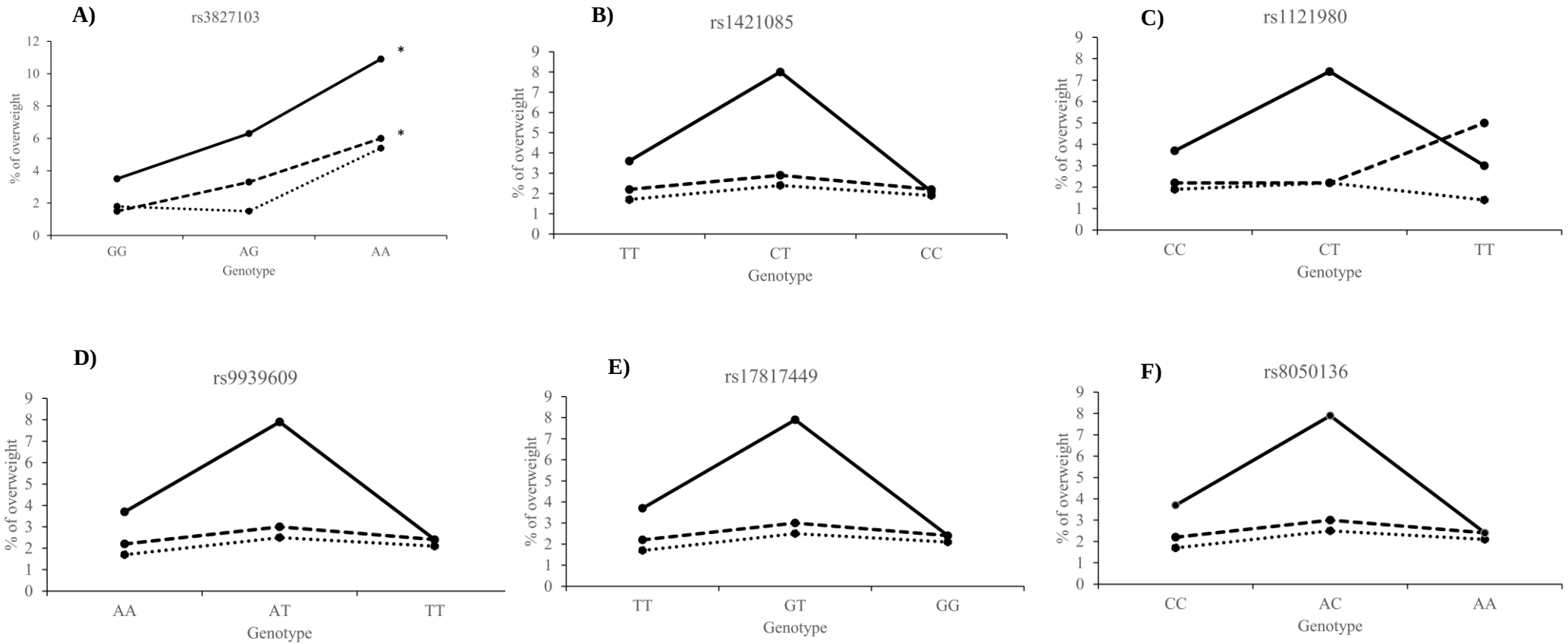


Table 9.5: Association between *FTO* genetic variants with overweight status at 3-years of age using a co-dominant genetic model

Genetic variants	Overweight at 3-years	
	OR	**95% CI**
FTO		
rs9939973 AG vs. (AA/GG)	2.09	0.97-4.50
rs1421085 CT vs. (CC/TT)	1.71	0.79-3.70
rs1121980 CT vs. (CC/TT)	2.08	0.97-4.47
rs9939609 AT vs. (AA/TT)	1.82	0.84-3.91
rs17817449 GT vs. (GG/TT)	1.82	0.84-3.92
rs8050136 AT vs. (AA/TT)	1.82	0.84-3.92

Odds ratios represent odds of being overweight for heterozygote vs. two homozygotes
Adjusted for ethnicity, birthweight-for-gestational age, breastfeeding duration

9.4.3 Association between *MC3R* and *FTO* variants with overweight status at 1-, 2- and 3-years of age

The associations of *MC3R* and *FTO* with overweight status at 1-, 2- and 3-years of age are given in Table 9.6, including odds ratios (OR) and 95% confidence intervals (CI). The minor allele for *MC3R* rs3746619 increased the risk of being overweight at 2-years (p=0.031) and 3-years of age (p=0.012), with a consistent albeit not statistically significant trend at 1-year. Each additional copy of the risk allele for *MC3R* rs3746619 increased the odds of being overweight at 2- years by 2.23 times (95% CI: 1.08-4.61), after adjustment for potential confounders. Similar results were noted for overweight status at 3-years of age (OR = 2.05, CI: 1.17-3.58). In addition, *FTO* rs9939973 variant was not significanly associated with overweight status at 1-, 2- and 3-years of age. The associations of the other *MC3R* and *FTO* variants with overweight status at 1-, 2- and 3-years of age are also illustrated in Table 9.6.

Table 9.6: Association between *MC3R* and *FTO* genetic variants with overweight status at 1-, 2- and 3-years of age.

Genetic variants	Overweight at 1-year						Overweight at 2-years						Overweight at 3-years					
	Model 1[a]		Model 2[b]		Model 3[c]		Model 1[a]		Model 2[b]		Model 3[c]		Model 1[a]		Model 2[b]		Model 3[c]	
	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI	OR	95% CI	OR	95% CI	OR	95% CI
MC3R																		
rs3746619[1]	1.33	0.64-2.78	1.45	0.70-3.10	1.59	0.71-3.57	**2.00**	**1.02-3.94**	**2.16**	**1.09-4.28**	**2.23**	**1.08-4.61**	**1.77**	**1.12-2.79**	**1.82**	**1.14-2.89**	**2.05**	**1.17-3.58**
rs3827103	1.34	0.64-2.79	1.49	0.71-3.11	1.60	0.71-3.58	**2.01**	**1.03-3.95**	**2.17**	**1.10-4.29**	**2.24**	**1.09-4.63**	**1.79**	**1.13-2.82**	**1.84**	**1.16-2.91**	**2.08**	**1.19-3.61**
FTO																		
rs9939979[2]*	0.78	0.36-1.69	0.80	0.37-1.75	0.76	0.31-1.82	1.20	0.59-2.43	1.27	0.62-2.60	1.10	0.51-2.40	1.12	0.70-1.79	1.12	0.70-1.79	0.86	0.48-1.56
rs1421085[+]	0.91	0.42-1.99	0.92	0.42-2.04	0.83	0.34-2.02	0.93	0.43-2.01	0.96	0.44-2.11	0.90	0.39-2.05	1.17	0.71-1.92	1.17	0.70-1.93	0.85	0.45-1.59
rs1121980*	0.79	0.37-1.72	0.81	0.37-1.77	0.76	0.32-1.83	1.23	0.60-2.50	1.30	0.63-2.68	1.12	0.51-2.45	1.09	0.68-1.75	1.08	0.67-1.74	0.88	0.48-1.59
rs9939609[+]	0.95	0.44-2.06	0.95	0.43-2.08	0.85	0.35-2.05	0.98	0.45-2.11	0.99	0.45-2.18	0.94	0.41-2.13	1.19	0.72-1.96	1.16	0.70-1.92	0.89	0.48-1.67
rs17817449[+]	0.95	0.44-2.06	0.95	0.43-2.08	0.85	0.35-2.05	0.98	0.45-2.12	1.00	0.45-2.19	0.94	0.41-2.13	1.19	0.72-1.96	1.16	0.70-1.92	0.89	0.48-1.67
rs8050136[+]	0.95	0.44-2.06	0.95	0.43-2.08	0.85	0.35-2.05	0.98	0.45-2.12	1.00	0.45-2.18	0.94	0.41-2.13	1.19	0.72-1.96	1.16	0.70-1.92	0.89	0.48-1.67

OR represents the odds ratios of being overweight associated with for every additional copy of minor allele
[1] Representative variant for *MC3R* haploblock
[2] Representative variant for *FTO* haploblock
[a] Model 1: Adjusted for ethnicity
[b] Model 2: Adjusted for Model 1 + birthweight-for-gestational age
[c] Model 3: Adjusted for Model 1 + Model 2 + breastfeeding duration
*Additionally adjusted for maternal BMI at 26-28 weeks gestation
[+]Additionally adjusted for maternal BMI at 26-28 weeks gestation and maternal education

9.4.4 Association between *MC3R* and *FTO* variants with childhood appetitve traits at 1-year of age

To test the hypothesis that appetitive traits may mediate the relationship between *MC3R* and *FTO* variants and overweight status at 2- and 3-years of age, we analysed the association of each variant with each subscale of the CEBQ appetitive trait scores within a subgroup (n = 422) that had completed the CEBQ questionnaires. Characteristics of participants who completed the CEBQ questionnaires tended to be Chinese, had at least 12 years of education, breastfed their infants for at least 6 months, had lower BMI at 26-28 weeks of gestation, had shorter duration of gestation and had higher birth weight, length and BMI compared to those who did not complete the questionnaire (Table 9.7). We observed a significant positive relationship between *MC3R* with only the "slowness in eating" subscale, (p=0.019, Table 9.8). Those who were homozygous for the minor allele had the highest scores for "slowness in eating" (mean z-score ± SD: 0.53 ± 1.16 for rs3746119) compared to those who were homozygous for the protective allele (mean z-score ± SD: -0.11 ± 1.00 for rs3746119). However, this finding does not pass strict multiple testing correction. No significant associations were observed between *FTO* variants with all CEBQ subscales (Tables 9.9-9.11).

Table 9.7: Characteristics of participants who completed the CEBQ questionnaire, compared to those who did not complete the CEBQ questionnaire

	Completed CEBQ (n = 422)	Did not complete CEBQ (n = 678)	P value
Maternal education			**0.022**
< 12 years	152 (36.3)	285 (43.3)	
≥ 12 years	267 (63.7)	373 (56.7)	
Ethnicity			**<0.001**
Chinese	268 (63.5)	349 (52.2)	
Malay	101 (23.9)	175 (26.2)	
Indian	53 (12.6)	144 (21.6)	
Parity			0.452
Primiparous	186 (44.1)	310 (46.4)	
Multiparous	236 (55.9)	358 (53.6)	
BMI at 26-28 weeks pregnancy	25.8 ± 4.1	26.5 ± 4.6	**0.011**
Gestational Diabetes			0.041
No	335 (84.6)	492 (79.5)	
Yes	61 (15.4)	127 (20.5)	
Gestational age at delivery	38.6 ± 1.0	38.0 ± 1.6	**<0.001**
Gender			0.879
Male	225 (53.3)	353 (52.8)	
Female	197 (46.7)	315 (47.2)	
Breastfeeding duration			**0.015**
< 6 months	206 (56.1)	287 (64.5)	
≥ 6 months	161 (43.9)	158 (35.5)	
Birth weight (kg)	3.2 ± 0.4	3.0 ± 0.5	**<0.001**
Birth length (cm)	49.0 ± 2.0	48.4 ± 2.4	**<0.001**
Birth BMI (kg/m^2)	13.3 ± 1.2	12.9 ± 1.3	**<0.001**

Table 9.8: Childhood eating behaviour (CEBQ) scores by *MC3R* and *FTO* genotypes

	MC3R rs3746619				*FTO* rs9939973			
	AA (n=24)	**AC (n=150)**	**CC (n=248)**	**[+]P value**	**AA (n=27)**	**AG (n=149)**	**GG (n=245)**	**[+]P value**
CEBQ								
Enjoyment of food	-0.15 ± 1.26	0.07 ± 0.99	-0.02 ± 0.96	0.883	-0.01 ± 1.24	0.02 ± 1.00	-0.008 ± 0.95	0.922
Food responsiveness	0.24 ± 0.94	-0.04 ± 0.95	-0.004 ± 1.03	0.440	-0.20 ± 0.94	-0.08 ± 1.07	0.07 ± 0.95	0.257
Emotional under eating	-0.05 ± 1.02	-0.02 ± 0.98	-0.004 ± 1.00	0.592	-0.23 ± 1.10	-0.11 ± 0.98	0.07 ± 0.99	0.525
Slowness in eating	0.53 ± 1.16	0.10 ± 0.91	-0.11 ± 1.00	**0.019**	0.07 ± 1.09	-0.02 ± 0.96	0.008 ± 1.00	0.659
Emotional over eating	0.14 ± 1.19	0.04 ± 0.98	-0.04 ± 0.98	0.120	-0.16 ± 0.97	0.08 ± 1.04	-0.04 ± 0.96	0.542
Desire to drink	-0.02 ± 0.90	0.12 ± 1.01	-0.06 ± 1.01	0.364	-0.01 ± 0.98	-0.04 ± 0.96	0.03 ± 1.04	0.598
Satiety responsiveness	-0.14 ± 0.85	0.01 ± 0.98	0.05 ± 1.02	0.123	0.06 ± 1.12	0.06 ± 1.00	-0.06 ± 0.98	0.347

CEBQ scores are computed as z-scores relative to the GUSTO cohort
Numbers represent mean z-score ± S.D
[+]Adjusted for gender, ethnicity, birthweight-for-gestational age and breastfeeding duration

Table 9.9: Childhood eating behaviour (CEBQ) scores by *MC3R* and *FTO* genotypes

	MC3R rs3827103				*FTO* rs1421085			
	AA (n=24)	**AG (n=149)**	**GG (n=249)**	**[+]P value**	**CC (n=20)**	**CT (n=131)**	**TT (n=270)**	**[+]P value**
CEBQ								
Enjoyment of food	-0.15 ± 1.26	0.07 ± 1.00	-0.02 ± 0.96	0.931	-0.06 ± 1.31	0.05 ± 1.00	-0.02 ± 0.96	0.901
Food responsiveness	0.24 ± 0.94	-0.04 ± 0.96	-0.003 ± 1.02	0.485	-0.10 ± 0.84	-0.10 ± 1.10	0.05 ± 0.96	0.529
Emotional under eating	-0.05 ± 1.02	-0.01 ± 0.98	-0.007 ± 0.94	0.557	-0.23 ± 1.26	-0.20 ± 0.92	0.09 ± 1.00	0.261
Slowness in eating	0.53 ± 1.17	0.11 ± 0.91	-0.12 ± 1.00	**0.013**	0.13 ± 1.24	-0.03 ± 0.98	0.006 ± 0.99	0.485
Emotional over eating	0.14 ± 1.19	0.05 ± 0.98	-0.04 ± 0.98	0.112	-0.15 ± 0.83	0.07 ± 1.08	-0.03 ± 0.95	0.626
Desire to drink	-0.02 ± 0.90	0.12 ± 1.01	-0.06 ± 1.01	0.366	-0.002 ± 1.02	0.0001 ± 0.94	0.007 ± 1.04	0.876
Satiety responsiveness	-0.14 ± 0.85	-0.09 ± 0.99	0.04 ± 1.01	0.133	0.19 ± 1.17	-0.03 ± 0.99	-0.02 ± 0.99	0.622

CEBQ scores are computed as z-scores relative to the GUSTO cohort
Numbers represent mean z-score ± S.D
[+]Adjusted for gender, ethnicity, birthweight-for-gestational age and breastfeeding duration

Table 9.10: Childhood eating behaviour (CEBQ) scores by *FTO* genotypes

	***FTO* rs1121980**				***FTO* rs9939609**			
	TT (n=26)	**CT (n=147)**	**CC (n=248)**	**[+]P value**	**AA (n=19)**	**AT (n=129)**	**TT (n=273)**	**[+]P value**
CEBQ								
Enjoyment of food	-0.01 ± 1.26	0.04 ± 1.00	-0.02 ± 0.96	0.814	-0.06 ± 1.35	0.06 ± 0.98	-0.02 ± 0.97	0.851
Food responsiveness	-0.23 ± 0.95	-0.08 ± 1.08	0.07 ± 0.95	0.250	-0.13 ± 0.85	-0.09 ± 1.10	0.05 ± 0.95	0.642
Emotional under eating	-0.23 ± 1.11	-0.13 ± 0.95	0.08 ± 1.01	0.425	-0.23 ± 1.30	-0.21 ± 0.89	0.10 ± 1.01	0.195
Slowness in eating	0.09 ± 1.11	-0.006 ± 0.95	-0.008 ± 1.01	0.874	0.16 ± 1.27	-0.005 ± 0.97	-0.007 ± 0.99	0.722
Emotional over eating	-0.13 ± 0.97	0.09 ± 1.04	-0.05 ± 0.96	0.630	-0.11 ± 0.83	0.07 ± 1.08	-0.04 ± 0.95	0.747
Desire to drink	-0.03 ± 1.00	-0.04 ± 0.96	0.03 ± 1.04	0.557	-0.03 ± 1.04	-0.002 ± 0.94	0.01 ± 1.04	0.815
Satiety responsiveness	0.05 ± 1.14	0.06 ± 1.01	-0.06 ± 0.98	0.435	0.18 ± 1.20	-0.04 ± 0.98	-0.008 ± 0.99	0.625

CEBQ scores are computed as z-scores relative to the GUSTO cohort
Numbers represent mean z-score ± S.D
[+]Adjusted for gender, ethnicity, birthweight-for-gestational age and breastfeeding duration

Table 9.11: Childhood eating behaviour (CEBQ) scores by *FTO* genotypes

	***FTO* rs17817449**				***FTO* rs8050136**			
	GG (n=19)	**GT (n=129)**	**TT (n=273)**	**[+]P value**	**AA (n=19)**	**AT (n=129)**	**TT (n=273)**	**[+]P value**
CEBQ								
Enjoyment of food	-0.06 ± 1.35	0.06 ± 0.98	-0.02 ± 0.97	0.851	-0.06 ± 1.35	0.06 ± 0.98	-0.02 ± 0.97	0.851
Food responsiveness	-0.13 ± 0.85	-0.09 ± 1.10	0.05 ± 0.95	0.642	-0.13 ± 0.85	-0.09 ± 1.10	0.05 ± 0.95	0.642
Emotional under eating	-0.23 ± 1.30	-0.21 ± 0.89	0.10 ± 1.01	0.195	-0.23 ± 1.30	-0.21 ± 0.89	0.10 ± 1.01	0.195
Slowness in eating	0.16 ± 1.27	-0.005 ± 0.97	-0.007 ± 0.99	0.722	0.16 ± 1.27	-0.005 ± 0.97	-0.007 ± 0.99	0.722
Emotional over eating	-0.11 ± 0.83	0.07 ± 1.08	-0.04 ± 0.95	0.747	-0.11 ± 0.83	0.07 ± 1.08	-0.04 ± 0.95	0.747
Desire to drink	-0.03 ± 1.04	-0.002 ± 0.94	0.01 ± 1.04	0.815	-0.03 ± 1.04	-0.002 ± 0.94	0.01 ± 1.04	0.815
Satiety responsiveness	0.18 ± 1.20	-0.04 ± 0.98	-0.008 ± 0.99	0.625	0.18 ± 1.20	-0.04 ± 0.98	-0.008 ± 0.99	0.625

CEBQ scores are computed as z-scores relative to the GUSTO cohort
Numbers represent mean z-score ± S.D
[+]Adjusted for gender, ethnicity, birthweight-for-gestational age and breastfeeding duration

9.5 Discussion

In this longitudinal study, we unravelled the novel associations between *MC3R* variants with early childhood adiposity and overweight status. To our knowledge, this is the first study to assess the relationship between *MC3R* variants and overweight status in children at such an early age. The effect of *MC3R* minor alleles on overweight status was present at both 2- and 3-years of age, but not at 1-year, after adjusting in the regression models for factors which are known to influence size and adiposity during early childhood, suggesting that the influence of *MC3R* variants on childhood adiposity may manifest clinically from two years of age. A possible explanation is that the child might only start to exercise autonomy in self-feeding between one and two years of age, while during the first year of life feeding is still very much dependent on what the mother provides. Earlier reports by Feng N et al(297), Zegers D et al(304) and Lee YS et al(298) have documented associations of these *MC3R* missense polymorphisms with adiposity in older obese children, in later childhood. Other studies that have identified significant relationships between *MC3R* variants and obesity were conducted in adult populations (305-307). Unlike the *MC3R* variants, the *FTO* variants showed no significant associations with overweight status at both two and three years of age despite its established status as the most prominent BMI susceptibility locus at later ages. Taken together, our findings support the role of *MC3R* minor allele variants in determining early childhood adiposity, and suggests effects at a much earlier ages as compared to *FTO* variants.

Our findings are consistent current reports that the *MC3R* risk variants could be contributing to common obesity (283). Studies done on obese

children from different populations have independently described the association between *MC3R* minor allele variants with childhood adiposity. Feng N et al(297) showed that those who were homozygous for Thr6Lys and Val81Ile variants were significantly heavier, had more body fat, greater plasma leptin and insulin concentrations, and greater insulin resistance than children who were wild-type or heterozygous for *MC3R* variants. These homozygous variants were also only observed amongst children who were at risk of overweight (BMI $\geq$ 85th percentile), or who were considered overweight (BMI $\geq$ 95th percentile). Similar observations in a population of obese Singaporean Asian children have also been described, whereby obese children with the Thr6Lys and Val81Ile variants exhibited significantly higher leptin levels, and percentage body fat, highlighting that *MC3R* variants may be a predisposing factor for excessive body weight gain in children (298). However, other studies have reported null findings between *MC3R* risk variants with childhood obesity. A study by Obregón AM et al(301) on 229 obese Chilean children found insufficient evidence of significant association between childhood obesity and the common *MC3R* variants, including the Val81Ile variant. Another study by Cieslak J et al(308) on a group of Polish children showed that the common *MC3R* polymorphism, Val81Ile, was widely distributed amongst obese and control cohorts, suggesting that the predisposing effect of the Val81Ile polymorphic variant to obesity may be rather unlikely.

We also reported null associations between the *FTO* variants with overweight status at 1-, 2- and 3-years of age, which are in line with previous reports. A recent study by Mook-Kanamori et al involving 703 infants from

the Generation R cohort highlighted no association between the *FTO* rs9939609 polymorphism and body composition at the age of six months(309). Another study involving 2732 full-term neonates of the German GINI-plus and LISA-plus birth cohorts also reported no evidence for BMI differences between genotypes of *FTO* variants during the first three years of life(310). A recent Fels Longitudinal Study involving 534 subjects also reported no association between *FTO* genotype with early growth in the first three years of life(311). Only one study thus far has shown that *FTO* gene polymorphism might associate with adipose tissue accumulation and weight gain, at least temporarily, during the neonatal period, although the authors noted that the genetic associations were shown without correcting for multiple comparisons(312). Interestingly, our findings suggested that there was an increase in risk of overweight only for the *FTO* heterozygote at three-years of age, implying a plausible co-dominance genetic model for *FTO* and overweight status. A sub-analysis of a co-dominant genetic model of *FTO* with the outcome of overweight at three-years of age however, showed no significant associations. Taken together, it seems to suggest that the effect of *FTO* genotype on adiposity does not appear to exert its influence during infancy and early childhood, unlike the *MC3R* variants in our study where the effect was observed as early as two-years of age.

Mouse studies have shown that the mechanism in which *MC3R* variation leads to increased body fat was not increased food intake, but increased feed efficiency. These mice exhibited hypophagia compared to wildtype littermates, and were unusually susceptible to high fat diet-induced obesity, partly explained by physical inactivity(295, 296). Interestingly, our

study findings also reported a significant positive relationship between *MC3R* with the "slowness in eating" subscale of childhood appetitive traits at 1-year of age, indicating that subjects with the *MC3R* minor allele were perceived by their mothers to be eating more slowly compared to those without the risk allele. Although our finding does not pass strict multiple testing correction, it is strikingly similar with an earlier study conducted on a Chilean cohort, where obese boys carrying the minor allele for rs3746619 and rs3827103 variants also showed higher scores for "slowness in eating" subscale for CEBQ compared to those without the minor allele(301). However, it was reported that older children with the risk *MC3R* variants had higher energy intake(302). Taken together, a possible explanation is that these children were eating more and therefore taking longer to complete meals, and mothers perceive this behaviour as "slow eating". However, we cannot exclude other explanations for instance the possibility that the child's adiposity influences the mother's perception of their eating behaviour. To our knowledge, there are currently no studies on *MC3R* variants and energy intake in subjects younger than 6 years of age.

Strengths of our study include the prospective design with high follow-up rate, along with the study of Asian ethnic groups. To date, there are no published studies relating *MC3R* genetic variants with overweight status at one to three years of age, thus our study provides useful and informative data on this relationship. There are however limitations to consider. Although we have adjusted all analyses for potential confounders of adiposity, we could not rule out the possibility of confounding by population stratification at higher resolution than ethnicity. Furthermore, this study primarily used

anthropometry as indicators of adiposity, but lacks more detailed measures of body composition, such as dual x-ray absorptiometry (DXA) or air displacement plethysmography (BOD POD), at two- and three-years of age. Hence we were unable to further distinguish if these *MC3R* genetic variants were associated with fat mass or fat-free mass.

In summary, this study has provided evidence of significant association between *MC3R*, but not *FTO*, genetic variants with early childhood overweight status at two and three years of age in a multi-ethnic Asian population. Our study highlighted the relative effects and roles of various susceptibility alleles may differ at different stages of life, and the implication is that different weight regulation pathways may assume varying temporal importance at various ages. Follow-up studies would be necessary to examine if these *MC3R* variants would continue to influence overweight status and adiposity at later ages, and with more detailed measures of body composition.

Chapter 10: Conclusions and future directions

The primary aim of this dissertation is to examine the developmental factors that can predict patterns of size, growth and body composition in infancy and early childhood in a multi-ethnic Asian cohort. One of the first key objectives was to establish new references for size-at-birth based on a recent cohort of near-term and term Singapore infants, for the purposes of evaluating birth size correctly. Our study provided new reference values (percentiles and z-scores) for birth weight, length and head circumference for newborns 35-41 weeks of gestational age, allowing for more suitable classification of infants as small-, appropriate- or large-for-gestational age. In addition, we also sought to establish and validate a fat-mass estimation formula specific for the GUSTO cohort during the early postnatal period, using PEA POD® body composition measurements as reference. Our study also provided a new fat mass prediction reference model for use in Asian neonates, with weight, gender, gestational age and subscapular SFT as significant predictors of neonatal fat mass. This equation would be useful as a non-invasive method to obtain quick *in-vivo* estimates of fat mass in groups of infant subjects. More importantly, both references were important in establishing norms and a way to predict fat mass to explore our hypotheses on *in-utero*, postnatal, as well as genetic risk factors that would predispose an individual to adverse adiposity and growth outcomes

The second objective was to examine the associations of maternal/*in-utero* factors with size and adiposity of Singapore infants at birth. More specifically for this dissertation, we hypothesized on whether higher maternal glucose levels during pregnancy is associated with higher neonatal adiposity at

birth, utilizing the new birth size and adiposity reference models derived from our two earlier studies. Key findings were that maternal glucose levels during pregnancy measured at a single time point was effective in identifying excessive neonatal adiposity outcomes, and this dose-response relationship was graded across the range of maternal glucose levels, even at those that were below the diagnostic criteria for gestational diabetes, thereby confirming the link between maternal glycemia and neonatal adiposity. Furthermore, we noted that fluxes of maternal glucose during the fasting state showed slightly greater influence on excessive adiposity outcomes, and that the influence of raised maternal fasting glucose levels on neonatal ∑SFT were less pronounced for Indian mothers compared to Chinese mothers, highlighting the influence of ethnicity on the relationship between maternal glycemia and neonatal adiposity.

We extended the findings of the above-mentioned study and proceeded to examine the relationship between maternal glycemia (fasting and post-challenge glucose levels) and adiposity (BMI) during pregnancy on early postnatal growth of offspring during the first three years of life. Findings from this study demonstrated that maternal glycemia was associated with decelerated postnatal growth limited to the first 3 weeks to 3 months of life, followed by a transient period of accelerated growth between 9-15 months of life. Unlike maternal glycemia, the association between maternal adiposity during pregnancy with offspring adiposity persisted into early childhood till three years of age. In addition, the effect of increasing maternal glucose with increased size-at-birth was more pronounced in obese mothers compared to non-obese mothers. The association of increasing maternal glucose with

increased offspring BMI and overweight status however, was present only amongst non-obese women two years, and disappears at three years, whilst in obese mothers, the risk of offspring being overweight was unexpectedly highest in those who were in the lowest fasting glucose category. Furthermore for a subgroup of children born to multiparous and Chinese mothers, raised maternal glycemia levels was associated with greater weight and BMI at two years of age, highlighting the plausibility of ethnic and parity differences in the relationship between maternal glycemia and adiposity at later childhood. Taken together, findings from these two studies added significant, useful and informative data relating the maternal metabolic environment with neonatal body composition and early postnatal growth in a multi-ethnic Asian cohort.

Another key objective of this study involved examining postnatal factors that might influence early postnatal growth and adiposity in the first three years of life. More specifically, we hypothesized that reduced breastmilk intake may result in accelerated adiposity gain in infants of GDM mothers. Interestingly, we demonstrated varied effects of breastmilk on early postnatal growth between offspring of non-GDM and GDM mothers. Whilst offspring of mothers without GDM who had greater breastmilk intake exhibited decelerated growth in the first year of life, offspring of GDM mothers however, do not exhibit accelerated adiposity gain during the early postnatal period despite reduced breastmilk intake as hypothesized, and we postulated that differences in breast milk constituents of GDM and non-GDM mothers, such as increased glucose or insulin concentrations, may have contributed to these observed differences.

Finally, we proceeded to examine potential predisposing genetic factors that may influence growth and adiposity in children. Firstly, we hypothesized that offspring with growth restriction *in-utero* and subsequent catch up growth have unique gene expression profile which is predictive of catch up growth, and did this by examining the transcriptomic profiles of umbilical cords of infants in the GUSTO cohort. Using a discovery-based genome-wide approach, we have uncovered gene expression changes that are significantly associated with fetal growth as well as subsequent postnatal growth. Utilizing a pathway analysis approach, these genes were also found to be significantly enriched in immune response, nucleotide metabolism, apoptotic as well as angiogenic pathways. The novelty of this study, unlike other studies, was the use of serial ultrasound measurements for the same fetus taken at different gestational periods to give a better indication and definition of fetal growth velocity, with minimal emphasis on birthweight to define a growth-restricted infant. Additionally, our study is one of the few to determine if variations in gene expression is associated with fetal growth among a set of newborns from low-risk pregnancies, hence providing greater insights into the transcriptomic profile of babies with differing fetal growth types.

We also hypothesized that polymorphic variants of known adiposity-associated genes *MC3R* and *FTO* would increase the risk of being overweight or obese during early childhood. It was observed that the effect of *MC3R* minor alleles on overweight status was present at both 2- and 3-years of age, but not at 1-year of age, whereas the *FTO* polymorphic variants showed no significant associations with overweight status at 1-, 2- or 3-years of age, despite its established status as the most prominent BMI susceptibility locus at

later ages. Whilst other studies have documented associations of *MC3R* with adiposity in older obese children as well as adults, this study is the first to show that the effects of *MC3R* on adiposity occurs at a much earlier age as compared to *FTO* variants, highlighting how the relative effects and roles of various susceptibility alleles may differ at different stages of life, with the implication that different weight regulation pathways may assume varying temporal importance at various ages.

The findings in this dissertation can have significant impact on clinical practices and recommendations, and pave the way for interventions at early stages. As illustrated in Chapter 5, the findings on maternal glycemia during pregnancy and neonatal adiposity demonstrated a dose-response effect across the range of maternal glucose levels, even at levels below the diagnostic criteria for gestational diabetes. Currently, all obstetric clinics in Singapore make use of the 2-hour 75-gram OGTT to screen at-risk pregnant mothers based on a set of pre-defined risk factors (e.g. obese mothers, mothers with GDM history etc). Our study findings however, highlight the importance of universal OGTT screening as a possible intervention measure to identify pregnant mothers who would be at risk of having an infant with an excessive adiposity outcome, even for those with normal glucose levels. Results from our gene expression microarray study may also pave the way for development of prognostic markers to predict growth-restriction *in-utero*. Accurate prediction of poor fetal growth at an early stage would play an important role in avoiding the cardio-metabolic consequences of growth-restriction later in life. Our results on the effects of *MC3R* on early childhood overweight and adiposity provided better insights into the molecular circuitry governing

weight regulation during early childhood, which in turn can be targets for drug development for early prevention of overweight and obesity. In conclusion, the findings in this dissertation have unravelled the developmental risk factors that influence infant size, adiposity and growth during the first three years of life in a multi-ethnic Asian population. Given the scarcity of data on early-life outcomes in Asian populations, these results have helped to fill this gap in knowledge, providing new insights into growth and development during the early part of the human life course. These findings have also provided a stepping stone for hypotheses-generation for future studies, which would involve examining whether these risk factors that operate during early growth and development would have long-term repercussions on increasing prevalence of later obesity, diabetes and other cardio-metabolic disorders. Currently the infants in the GUSTO cohort would be followed-up till 9 years of age, with various cardio-metabolic measures taken at these later ages. This gives us ample opportunity to examine if the same risk factors would influence cardio-metabolic disorders at early adolescent stage.

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1) **Aris, I. M.**, S. E. Soh, M. T. Tint, S. Liang, A. Chinnadurai, S. M. Saw, K. Kwek, K. M. Godfrey, P. D. Gluckman, Y. S. Chong, F. K. Yap and Y. S. Lee (2013). "*Body fat in Singaporean infants: development of body fat prediction equations in Asian newborns*." Eur J Clin Nutr. 67(9): 922-927. (Chapter 4)

2) **Aris, I. M.**, S. E. Soh, M. T. Tint, S. Liang, A. Chinnadurai, S. M. Saw, V. S. Rajadurai, K. Kwek, M. J. Meaney, K. M. Godfrey, P. D. Gluckman, F. K. Yap, Y. S. Chong and Y. S. Lee (2014). "*Effect of maternal glycemia on neonatal adiposity in a multiethnic Asian birth cohort*." J Clin Endocrinol Metab. 99(1): 240-247 (Chapter 5)

3) **Aris, I. M.**, M. Gandhi, Y. B. Cheung, S. E. Soh, M. T. Tint, P. D. Gluckman, Y. S. Lee, F. K. Yap and Y. S. Chong (2014). "*A New Population-based Reference for Gestational Age-specific Size-at-birth of Singapore Infants*." Ann Acad Med Singapore. 43(9): 439-447. (Chapter 3)

4) **Aris, I.M**, M.T. Tint, S.E. Soh, S.M. Saw, V.S. Rajadurai, K.M. Godfrey, P.D. Gluckman, Y.S. Chong, F. Yap, Y.S. Lee (2015). *Associations of gestational glycemia and pre-pregnancy adiposity with offspring growth and adiposity in an Asian population*. Am J Clin Nutr. In press (Chapter 6)

Submitted/Under review/Undergoing revision

1) **Aris, I.M**, M.T. Tint, S.E. Soh, S.M. Saw, V.S. Rajadurai, K.M. Godfrey, P.D. Gluckman, Y.S. Chong, F. Yap, Y.S. Lee. *Associations of infant milk feed type on early postnatal growth of offspring exposed and unexposed to gestational diabetes in-utero.* (Chapter 7) Undergoing 1st revision in European Journal of Nutrition.

2) **Aris, I.M**, J.H. Tan, M.T. Tint, S.E. Soh, S.M. Saw, K. Kwek, K.M. Godfrey, P.D. Gluckman, Y.S. Chong, N. Lek, F. Yap, W. Stunkel, Y.S. Lee. *Identifying potential novel genetic markers of fetal growth and subsequent postnatal catch-up growth*. (Chapter 8) Submitted

3) **Aris, I.M**, M.T. Tint, A.L. Teh, J.D. Holbrook, P.L. Quah, M.F.F. Chong, X. Lin, S.E. Soh, S.M. Saw, K. Kwek, K.M. Godfrey, P.D. Gluckman, Y.S. Chong, N. Lek, F. Yap, Y.S. Lee. *MC3R gene polymorphisms are associated with early childhood adiposity gain and infant appetite in an Asian population.* (Chapter 9) Submitted to Pediatric Obesity.

RECRUITMENT VISIT
ELIGIBILITY QUESTIONNAIRE

Study ID : _____ Date of interview : _____

Interviewer code : _____ Interview start time : _____

Date of Birth: ☐☐ ☐☐ ☐☐☐☐ Age: ☐☐ years
D D M M Y Y Y Y

Status: ☐ 1. Singapore citizen ☐ 2. Singapore PR ☐ 3. Foreigner

Address:
Block/House no/Building Name/Street: _____

Unit no:_____ Postal Code: _____

Do you intend to reside in Singapore for the next 5 years? ☐ 0: No ☐ 1: Yes

Do you intend to deliver in KKH or NUH? ☐ 0: No ☐ 1: Yes

Do you intend to donate cord, cord blood and placenta?
☐ 0: No ☐ 1: Yes ☐ 99: Don't know

Race:

Baby's mother
☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4 : Others

Baby's maternal grandfather (mother's father)
☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Baby's maternal grandmother (mother's mother)
☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Baby's father
☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Baby's paternal grandfather (father's father)
☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Baby's paternal grandmother (father's mother)
☐ 1: Chinese ☐ 2: Malay ☐ 3: Indian ☐ 4: Others

Are the grandparents of the baby of homogenous ethnicity?
☐ 0: No
☐ 1: Yes

1. OBSTETRIC HISTORY:

1.1. When was the first day of your last menstrual period (LMP)?

☐☐ D D ☐☐ M M ☐☐☐☐ Y Y Y Y ☐ 99: Don't know

1.2. How many weeks is your pregnancy (based on LMP)?

☐☐ weeks

1.3. Is this current pregnancy conceived through IVF?

☐ 0: No ***go to question 2.1***

1: Yes ***Please refer to IVF study team***

1.4. Have you enrolled for an IVF study?

☐ 0: No

1: Yes

2. CANCER

2.1. Have you ever been told by a doctor that you have cancer?

☐ 0: No ***go to question 3.1***

1: Yes

2.2. When did the doctor first tell you that you had cancer? *(Fill in one of the options below)*

☐☐ Age ☐☐☐☐ Year ☐ 99: Don't know

2.3. Are you currently on chemotherapy?

☐ 0: No

1: Yes

3. MENTAL ILLNESSES

3.1. Have you ever been told by a doctor that you are suffering from a mental or psychological condition?

☐ 0: No ***go to question 4.1***

1: Yes, please specify: _____

3.2. When the diagnosis was first made? ***(Fill in one of the options below)***

Age ☐☐ (or) Year ☐☐☐☐

3.3. Are you currently taking any medication for mental or psychological condition?

☐ 0: No

1: Yes, please specify: _____

4. OTHER LONG TERM ILLNESSES

4.1. Have you ever been told by a doctor that you have other long term illnesses?

☐ 0: No ***go to question 5.1***

1: Yes

If yes, please specify:

S/N	Type of illness	Age (yr)	OR	Year (YYYY)	99:Don't know
1	Type 1 diabetes		OR		
If others, please specify:					
			OR		
			OR		
			OR		

5. MEDICATION

5.1. In the past year (before this pregnancy), did you take any regular medications, supplements and/or traditional medicine?

☐ 0: No ***End of eligibility questionnaire***

1: Yes

ELIGIBILITY: ☐ **0: NO** ☐ **1: YES**

Interview end time: _____

THANK YOU VERY MUCH FOR YOUR TIME !

RECRUITMENT VISIT
1ST CLINIC VISIT QUESTIONNAIRE

Study ID : _____ Date of interview : _____

Interviewer code : _____ Interview start time : _____

1. DEMOGRAPHY

I would like to start by asking you some questions about yourself.

1.1. How old were you when you left long term full time education?
(enter current age if still studying) ☐☐ years

1.2. What is the highest level of education that you have attained?

☐ 1: None
2: Primary (PSLE)
3: Secondary (GCE 'O'/ 'N' levels)
4: ITE/NTC
5: GCE 'A' levels/Polytechnic/diploma
6: University
7: Others, specify:_____

1.3. What is your marital status?

☐ 1: Single and living with the baby's father
2: Single and **not** living with the baby's father
3: Married (living with husband)
4: Married but **not** living with husband
5: Separated
6: Divorced
7: Widowed
8: Others, specify:_____

1.4. What is your religion?

☐ 1: No religion
2: Buddhism
3: Christianity
4: Islam
5: Taoism
6: Hinduism
7: Others, specify:_____

1.5. Where were you born?

☐ 1: Singapore ***go to question 2.1***
2: Malaysia
3: China
4: India
5: Others, specify:_____

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

1.6. When did you move to Singapore?

M	M		Y	Y	Y	Y

2. OCCUPATION

2.1. What is your current job?

- 1: Legislator/senior official
- 2: Professional
- 3: Technician & associated professional
- 4: Clerical worker
- 5: Service worker
- 6: Agricultural worker
- 7: Production craftsman
- 8: Plant and machine operator
- 9: Homemaker
- 10: Retired
- 11: Student
- 12: Unemployed
- 13: Others, specify:_____
- 14: Refused

3. HOUSING AND HOUSEHOLD COMPOSITION

3.1. What type of accommodation do you live in?

- 1: 1-2 room HDB flat
- 2: 3 room HDB flat
- 3: 4-5 room HDB flat
- 4: HUDC/executive flat
- 5: Condominium
- 6: Landed property
- 7: Others, specify:_____

3.2. Does anyone else live together with you?

- 0: No *go to question 4.1*
- 1: Yes *please specify in the following table (next page)*

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

For each person living in the household (apart from the woman herself), complete one line.
A household is defined as a group of people who share a living room or eat together for at least one meal a day.

CHILDREN

*For **all children**, record date of birth (or age if D.O.B. not available).*
*For the **woman's own children**, give the child's birth weight.*

S/N	Relationship to woman	Sex		D.O.B			Age (yrs)	Child's birth weight (Specify in gm or lb.oz)
		M	F	DD	MM	YYYY		
1								
2								
3								
4								
5								

ADULT

S/N	Relationship to woman	Sex		Age (yrs)	Smoker (Yes=1, No=0)
		M	F		
6					
7					
8					
9					
10					

4. CHILDCARE ARRANGEMENTS

4.1. Do you have your own child or children at home under the age of 12 years?

☐ 0: No, ***go to question 5***
1: Yes

4.1.1 If yes, you are:

☐ 1: Working part time, ***go to question 4.2***
2: Working full time, ***go to question 4.2***
3: Stay home mother, ***go to question 5***

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

4.2. Which of the following best describes the way you arrange for your child/children aged 12 or under to be looked after while you are at work?
Please fill in numbers of relevant choices in boxes on right. You can select up to 3 choices.

1: I work only while they are at school.
2: They look after themselves until I get home.
3: I work from home.
4: My husband/partner looks after them.
5: A nanny/grandparent/relative looks after them at home
6: They go to a workplace nursery.
7: They go to a day nursery.
8: They go to a child minder.
9: A relative looks after them.
10: A friend or neighbour looks after them.
11: Others, specify_____

1st choice ☐
2nd choice ☐ ☐ No further choices
3rd choice ☐ ☐ No further choices

5. PERSONAL HEALTH

Now, I would like to ask you about your personal health and about the stress level you face.

5.1. How is your health in general? Would you say it is:

☐ 1: Very good
2: Good
3: Fair
4: Bad
5: Very bad

5.2. Do you have any long term illness or disability? By long term, I mean anything that has troubled you over a period of time**?**

☐ 0: No ***go to question 5.4***
1: Yes

5.3. What is the illness/disability? _____

_____

(Do not record headaches, indigestion, aches and pains. We are interested in major problems such as diabetes, multiple sclerosis, rheumatoid arthritis, muscular dystrophy – anything which might affect growth or body composition.)

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

5.4. To what extent do you feel that the stress or pressure you have experience in your life has affected your health?

☐ 1: None
2: Slightly
3: Moderately
4: Quite a lot
5: Extremely

5.5. In general, how much stress or pressure have you experienced in your daily living in the last 4 weeks?

☐ 1: None
2: Just a little
3: A good bit
4: Quite a lot
5: A great deal

5.6. Were you part of a multiple birth (twins, triplets etc.)?

☐ 0: No
1: Yes

5.7. Were you born early, late or when your maternal mother was expecting you?

☐ 1: Early
2: When expected***, go to question 5.9***
3: Late
99: Don't know***, go to question 5.9***

5.8. How early/late were you?

☐☐ Wks ☐ Days ☐ 99: Don't know

5.9. How many children did your mother have before you were born? (including stillbirths)

☐☐ ☐ 99: Don't know

5.10. Approximately what was your weight before this pregnancy?

☐☐☐ kg ☐ 99: Don't know

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

6. ASTHMA

6.1. Have you ever suffered from asthma, either as a child or an adult?

☐ 0: No ***go to question 6.3***
1: Yes
99: Don't know ***go to question 6.3***

6.1.1. If yes, was this confirmed by a doctor?

☐ 0: No
1: Yes
99: Don't know

6.2. How many attacks of wheezing have you had in the last 12 months?

☐ 0: None
1: 1-3
2: 4-12
3: More than 12

6.3. Did you suffer from eczema (recurrent itchy skin) in childhood?

☐ 0: No ***go to question 6.5***
1: Yes
99: Don't know

6.4. Have you had eczema (recurrent itchy skin) affecting the creases of your elbows or knees in the last year?

☐ 0: No
1: Yes

6.5. Have you ever had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or flu?

☐ 0: No ***go to question 6.7***
1: Yes
99: Don't know ***go to question 6.7***

6.5.1. If "YES", is the nose problem usually accompanied by itchy-watery eyes?

☐ 0: No
1: Yes
2: Sometimes
99: Don't know

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

6.6. In the last 12 months, have you had a problem with sneezing, or a runny, or blocked nose when you did not have a cold or the flu?

☐ 0: No
1: Yes

6.7. In the last 12 months, have you used any medicines to treat hay fever, rhinitis, or any other nasal problems, at any time (including sprays, solutions, pills, capsules or tablets)?

☐ 0: No
1: Yes

7. HIGH BLOOD PRESSURE (HYPERTENSION)

7.1. Has a doctor, a nurse or other healthcare professional ever told you that you have high blood pressure?

☐ 0: No ***go to question 8.1***
1: Yes

7.2. At what age were you diagnosed to have high blood pressure? *(Fill in one of the options below)*

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

8. DIABETES MELLITUS

8.1. Has a doctor ever told you that you have diabetes?

☐ 0: No ***go to question 9.1***
1: Yes

8.2. How old were you when the doctor first told you that you had diabetes? *(Fill in one of the options below)*

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

9. MYOPIA

9.1. Have you ever been told by a doctor or an optometrist that you need to wear glasses or contact lenses?

☐ 0: No ***go to question 10.1***
1: Yes

9.2. Did you get the glasses / contact lenses?

☐ 0: No ***go to question 10.1***
1: Yes

9.3. When did you first begin wearing glasses or contact lenses?

Age ☐☐ (or) Year ☐☐☐☐ ☐ 99: Don't know

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

9.4. What is the purpose for the glasses / contact lenses?

☐ 1: Seeing far ± Astigmatism
2: Seeing near ± Astigmatism
3: Seeing both far and near
4: Astigmatism only
99: Don't know

10. FAMILY HISTORY

10.1. Do you have a history of one of the following diseases in your first degree biological relatives (immediate family members)?

☐ 0: No, ***go to question 11***
1: Yes, ***specify in the following table.***

Code	First degree relatives
1:	Father
2:	Mother
10-19:	Sisters
20-29:	Brothers
30-39:	Sons
40-49:	Daughters

Code	Site of cancer
1:	Breast
2:	Ovarian
3:	Colorectal
4:	Others, specify _____ _____
99:	Don't know

Please use multiple rows if multiple diseases per individual
Pre-eclampsia = high blood pressure in pregnancy
Code Yes=1, No=0, Don't know=99 and N.A. for Not Applicable

First degree relative	Cancer		High blood pressure	Diabetes mellitus	Myopia	Cardio-vascular disease	Pre-eclampsia
	Yes=1 No=0 Don't know =99	Site	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99	Yes=1 No=0 Don't know=99

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

11. MENSTRUAL CYCLES AND PREGNANCIES

11.1. Is your usual cycle regular, or has it varied by more than 5 days between periods in the last 6 months?

☐ 1: Regular ***go to question 11.2***
2: Varied by more than 5 days ***go to question 11.3***
3: Don't know ***go to question 11.3***

11.2. How long is your usual menstrual cycle between the start of one period and the start of the next period?

☐☐ days ☐ 99: Don't know

11.3. How old were you when you had your first period?

☐☐ years ☐ 99: Don't know

11.4. **IF THIS IS YOUR FIRST PREGNANCY, PLEASE GO TO QUESTION 12.1**

Next, would you please tell me the ending date(s) and outcome(s) of each of your pregnancy in sequence?

1: Live birth – Normal vaginal delivery
2: Live birth – Assisted delivery (Forceps/vacuum)
3: Live birth – Caesarean section
4: Abortion
5: Miscarriage
6: Stillbirth
7: Premature birth – Normal vaginal delivery
8: Premature birth – Assisted delivery
9: Premature birth – Caesarean section
10: Ectopic pregnancies
11: Others, please specify: _____

S/N	Pregnancy outcome	Year of start of pregnancy	Total weeks of pregnancy	Baby's weight (Specify in gm or in lb.oz)	If live birth, breastfed or not?		If breastfed, how long?			Pregnancy related complications						
										Hypertension		Diabetes Mellitus		Anaemia		Others (Please specify)
					0: No	1: Yes	Year(s)	Mth(s)	Wk(s)	0: No	1: Yes	0: No	1: Yes	0:No	1: Yes	
1																
2																
3																
4																
5																

11.5. Were you anaemic after the birth of any of your previous babies?

☐ 0: No
1: Yes
99: Don't know

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

12. MEDICATION

The questions below ask about REGULAR consumption of medications, supplements and traditional medicine in the past year BEFORE THIS PREGNANCY.

Regular refers to more than once a week for at least 1 month in past1 year.

12.1. Have you been taking any medications regularly before this pregnancy?

☐ 0: No ***go to question 12.2***
1: Yes, please specify in table below

S/N	Name of Medication
1	
2	
3	
4	
5	

12.2. Have you been taking folic acid supplement before your current pregnancy?

☐ 0: No ***go to question 12.3***
1: Yes

12.2.1. How many weeks before pregnancy have you been taking folic acid supplement?

☐☐ weeks

12.3. Are you still taking folic acid supplement **NOW**?

☐ 0: No
1: Yes

12.4. Have you been taking any fortified milk supplement (e.g. Anlene, Anmum) regularly before this pregnancy?

☐ 0: No
1: Yes

12.5. Have you been taking any probiotics (e.g. Yakult, Vitagen, Yoghurt) regularly before this pregnancy?

☐ 0: No
1: Yes

12.6. Have you been taking any other vitamins or supplements regularly before this pregnancy?

☐ 0: No
1: Yes

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

12.7. Have you been taking any traditional medicines regularly before this pregnancy?

☐ 0: No
1: Yes

13. INCOME

13.1. What is your personal monthly income?

☐ 1: $0 - $999
2: $1000 - $1999
3: $2000 - $3999
4: $4000 - $5999
5: more than $6000
6: Refuse to answer
99: Don't know

13.2. What is the monthly income of your household?

☐ 1: $0 - $999
2: $1000 - $1999
3: $2000 - $3999
4: $4000 - $5999
5: more than $6000
6: Refuse to answer
99: Don't know

Interview end time: _____

THANK YOU VERY MUCH FOR YOUR HELP.

NOTE TO RECRUITERS WHEN FILLING IN QUESTIONNAIRE SETS:
(1) PLEASE USE THE CAPITAL LETTER.
(2) PLEASE WRITE CLEARLY.

Week 26-28 clinic visit
Interviewer-administered questionnaire (Mother)

Study ID: _____ Date of interview: _____

Interviewer code: _____ Interview start time: _____

Have you changed your address or telephone number since you were seen in early pregnancy?

☐ 0: No
1: Yes: Please specify_____

Address:
Block/House no./Building Name/Street:

_____

Unit no:_____ Postal Code: _____

CONFIDENTIAL
PLEASE USE CAPITAL LETTER AND WRITE CLEARLY.

1. OCCUPATIONAL ACTIVITY

1.1. Have you had any jobs at any time since you became pregnant?

☐ 0: No *go to Section 2*

1: Yes

1.2. Would you please tell me your jobs during pregnancy and the weeks of your pregnancy in which you have done them?
If started before pregnancy, week started = 0
If job is still ongoing, week finished = 88

Occupation	Week started	Week finished
1.		
2.		
3		
4.		

1.3. How many hours in total did you work during an average week?

☐☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

1.4.Did this include working night shifts?
Night shift means "working at least once a week or more from 12 midnight to 6:00am"

☐ 0: No

1: Yes

1.5. At around this time, did your paid work involve any of the following activities in an average day at work?
i) Standing or walking for more than **four** hours in total?

☐ 0: No

1: Yes

ii) Kneeling or squatting for more than an hour in total?

☐ 0: No

1: Yes

iii) Standing or sitting with your trunk bent forward for more than an hour in total?

☐ 0: No

1: Yes

iv) Lifting or carrying weight of 25kg (56lbs) or more by hand (equivalent to a sack of potatoes, a nine year old child, a very heavy suitcase)

☐ 0: No

1: Yes

CONFIDENTIAL
PLEASE USE CAPITAL LETTER AND WRITE CLEARLY.

1.6. Have you at any time during your pregnancy left a job or changed the type of work that you were doing because of a health problem?

☐ 0: No
1: Yes

1.6a. If yes, give details of health problems_____
_____

1.6b. and the stage of pregnancy ☐☐ weeks

2. ACTIVITY AND EXERCISE– BEFORE THIS PREGNANCY

Now I'm going to ask you about your activity and exercise patterns during the 1 year before your pregnancy. We would like you to divide up a "typical" day into three types of activities. These are:
(1) sleeping or lying, (2) sitting, (3) standing or walking.

2.1. Over a typical 24 hour day, how many hours do you generally spend sleeping or lying with your feet up?
(ask what time she usually goes to bed & wakes up, including any at work!)

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

2.2. How many hours on a typical day do you spend sitting down?
(e.g. includes sitting at work, mealtimes, driving, reading, watching TV)

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

2.3. This would mean that you spend about xx hours a day on your feet.
Does this sound about right?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

Sum of hours reported in Q2.1, 2.2 and 2.3 should total up to 24 hours

Total hours: _____
Checked and signed: _____

2.4. Out of these xx hours spent on your feet, about how much of the time are you actively on the move (rather than standing fairly still)?

☐	1: Very little	10%
	2: Some	30%
	3: About half	50%
	4: Most	70%
	5: Almost all	90%

2.5. During the 1 year before your pregnancy, how often have you done the following kind of exercises or activities?

a) **Strenuous exercise** which normally makes your heart beats rapidly AND leaves you breathless e.g. jogging, vigorous swimming or cycling, aerobics

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

b) **Moderate exercise** which normally leaves you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

c) **Gentle exercise** which normally leaves you tired but not exhausted, e.g. walking, driving, housework (including washing windows and polishing), gardening, DIY, golf.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

2.6.On a typical day, how many hours do you generally spend watching television?

☐ 1: More than 5 hours
2: 4-5 hours
3: 3-4 hours
4: 2-3 hours
5: 1-2 hours
6: Less than one hour
7: None

2.7. Which of the following best describes your walking speed?

☐ 1: Very slow
2: Stroll at an easy pace
3: Normal speed
4: Fairly brisk
5: Fast

3. ACTIVITY AND EXERCISE –DURING THIS PREGNACY

Can I now ask you about your activity and exercise patterns over the last 6 months?
As before, we would like you to divide up a "typical" day into three types of activities. These are:
(1) sleeping or lying, (2) sitting, (3) standing or walking.

3.1. Over a typical 24 hour day, how many hours do you generally spend sleeping or lying with your feet up?
(ask what time she usually goes to bed & wakes up, including any at work!)

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

3.2. How many on a typical day do you spend sitting down?
(e.g. includes sitting at work, mealtimes, driving, reading, watching TV)

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

3.3. This would mean that you spend about xx hours a day on your feet.
Does this sound about right?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

Sum of hours reported in Q3.1, 3.2 and 3.3 should total up to 24 hours

Total hours: _____
Checked and signed: _____

3.4. Out of these xx hours spent on your feet, about how much of the time are you actively on the move (rather than standing fairly still)?

☐ 1: Very little 10%
2: Some 30%
3: About half 50%
4: Most 70%
5: Almost all 90%

CONFIDENTIAL
PLEASE USE CAPITAL LETTER AND WRITE CLEARLY.

3.5. During the past six months, how often have you done the following kinds of exercise or activities?

a) **Strenuous exercise** which normally makes your heart beat rapidly AND leaves you breathless e.g. jogging, vigorous swimming or cycling, aerobics

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

b) **Moderate exercise** which normally leaves you exhausted but not breathless, e.g. brisk walking, dancing, easy swimming or cycling, badminton, sailing.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

c) **Gentle exercise** which normally leaves you tired but not exhausted, e.g. walking, driving, housework (including washing windows and polishing), gardening, DIY, golf.

☐ 1: Never
2: Once every 2-3 months
3: Once a month
4: Once a fortnight
5: 1-2 times per week
6: 3-6 times per week
7: Once a day
8: More than once a day

and **on average** about how long does each period of activity last?

☐☐ . ☐ hrs *(round to nearest 0.5 hr)*

3.6.On a typical day, how many hours do you generally spend watching television?

☐ 1: More than 5 hours
2: 4-5 hours
3: 3-4 hours
4: 2-3 hours
5: 1-2 hours
6: Less than one hour
7: None

3.7. Which of the following best describes your walking speed?

☐ 1: Very slow
2: Stroll at an easy pace
3: Normal speed
4: Fairly brisk
5: Fast

4. CONTRACEPTION

4.1.How many weeks pregnant were you when you first found out that you were pregnant?

☐☐ wks

4.2.Was this pregnancy planned?

☐ 0: No *Go to question 4.4*
1: Yes: *Go to question 4.3*

4.3 If YES, did you change your diet when you were planning to be pregnant?

☐ 0: No *Go to question 5.1*
1: Yes *Go to question 5.1*

4.4.If NO, this pregnancy is due to

☐ 1: No contraception: *Go to question 5.1*
2: Failure of contraceptive methods

4.5.If NO, which was the main contraceptive method used which failed?

☐ 1. Safe period
2. Barrier e.g. condom, diaphragm
Hormones
3.a. Pills
3.b. Patch
3.c. Injection
3.d. Implants
4. Intrauterine contraceptive device
5. Withdrawal
6. Others: specify _____

5. DIET DURING PREGNANCY

5.1 Are you following any special diet?

☐ 0: No *go to question 5.3*
1: Yes

5.2 If yes, what is your special diet?

☐ 1: Vegetarian (Eggs and milk allowed)
2: Vegan (No eggs or milk allowed)
3. Diabetic diet
4. Low fat diet
5. Others, specify _____

5.3 How often do you eat eggs?

☐ 1: More than one egg a day
2: One egg a day
3. 4 to 6 eggs a week
4. 1 to 3 eggs a week
5. Less than one egg a week
6. Do not eat eggs at all

5.4 How often do you eat liver (any type e.g. chicken, beef, pork)?

☐ 1: Every day
2: 4 to 6 times a week
3. 1 to 3 times a week
4. Less than once a week but more than once a month
5. Less than once a month
6. Do not eat liver at all

5.5 How often do you eat out or purchase take-away foods?

☐ 1: Two meals a day or more
2: One meal a day
3. 4 to 6 meals a week
4. 1 to 3 meals a week
5. Less than once a week
6. Never/ rarely

5.6 I would like to find out more about your diet during pregnancy compared to what you usually ate before you were pregnant. I will be asking you about your eating habit for a list of foods during pregnancy. Please tell me if you ate more, less or similar amount of the food during pregnancy compared to your usual diet.

	Types of Food	**Change in amount**
1.	Chicken	
2.	Fish	
3.	Meat (beef / mutton / pork)	
4.	Organ meats (e.g. liver, kidney, heart, brain)	
5.	Seafood (e.g. prawn, crab, mussels, clams)	
6.	Egg	
7.	Vegetables (all types)	
8.	Fruits (all types)	
9.	Red, orange, yellow fruits and vegetables (e.g. carrots, papaya)	
10.	Rice, noodles, breads	
11.	Cheese, yogurt	
12.	Chocolates, sweets, biscuits, cakes	
13.	Milk	
14.	Chocolate drinks (Milo, Ovaltine)	
15.	Soft drinks (e.g. Coke, sprite, 7-up, Pepsi)	
16.	Tea	
17.	Coffee	
18.	Wine/alcohol (including tonic wine)	

Key
1: More
2: Less
3. Same as before
9. Don't usually eat

6. APPETITE AND NAUSEA DURING PREGNANCY

6.1. Have you experienced any nausea or sickness since becoming pregnant?

☐ 0: No *go to question 6.5*
1: Yes

6.2. If yes, has this been:

☐ 1: Mild (nausea only)
2: Moderate (sometimes sick, vomiting)
3: Severe (regularly sick, vomiting, can't retain meals)

6.3. If yes, were you admitted to the hospital because of nausea?

☐ 0: No *go to question 6.5*
1: Yes

6.4. If yes, how were you treated?

☐ 1: Fasting, then slowly introducing food
2: Intravenous fluid treatment
3: Medication *(Note: Refer to medical records/CPSS)*

6.5. **Compared with BEFORE you were pregnant**, are you eating:

☐ 1: More *go to question 6.5a*
2: The same *go to question 7.1*
3: Less in amount *go to question 6.5b*
99: Don't know

6.5a. If **more**, is this:

☐ 1: Because you feel more hungry
2: To prevent from feeling sick
3: Because you feel it is best for the baby
4: Other reasons; specify: _____

6.5b. If **less**, is this:

☐ 1: Because you feel less hungry
2: Because of nausea/sickness
3: Don't want to put on too much weight
4: Other reasons; specify: _____

7. DIETING

7.1 Which of the following describes you best?

☐ 1: I have NEVER been on a diet to lose weight.
2: I have ONLY ONCE been on a diet to lose weight.
3: I USED TO diet REGULARLY to lose weight but NOT ANYMORE
4: I go on a diet to lose weight EVERY NOW AND AGAIN.
5: I am USUALLY on a diet to lose weight.

If answered 2, 4, 5, please ask question 7.2; otherwise go to next section.

7.2 Are you currently trying to lose weight by dieting?

☐ 0: No
1: Yes

8. ALCOHOL CONSUMPTION – BEFORE THIS PREGNANCY

I'd like to ask you a few questions about your drinking and smoking habits.

8.1 Did you ever drink alcohol before this pregnancy?

☐ 0: No *go to section 9*
1: Yes
99: Don't know

8.2 How often did you drink the following alcoholic beverages in the 1 year before you became pregnant? Please select the category that best describes how often and how much you drank during the past year.

Alcoholic beverages	Average consumption in past year	Usual serving size
Beer	☐ 1. Never or hardly ever 2. Once a month 3. 2-3 times a month 4. Once a week 5. 2-3 times a week 6. 4-6 times a week 7. Once a day 8. 2 or more times a day	☐ 1. One small bottle (375ml) or less 2. One large bottle (750ml) 3. Two large bottles 4. Three large bottles or more
Wine (eg. red wine)	☐	☐ 1. One wine glass (118ml) or less 2. Two wine glasses 3. Three wine glasses 4. Four wine glasses or more
Traditional wine (eg. DOM)	☐	☐ 1. One wine cup (30ml) or less 2. Two wine cups 3. Three wine cups 4. Four wine cups or more
Hard liquor (eg. brandy)	☐	☐ 1. One drink (30ml) or less 2. Two drinks 3. Three drinks 4. Four drinks or more

9 ALCOHOL CONSUMPTION – DURING THIS PREGNANCY

Did you ever drink alcohol during this pregnancy?

☐ 0: No *go to section 10*
1: Yes
2: Refuse to answer

9.1 During the past 6 months, how often did you drink the following alcoholic beverages? Please select the category that best describes how often and how much you drank.

Alcoholic beverages	Average consumption past 6 mth	Usual serving size
Beer	☐ 1. Never or hardly ever 2. Once a month 3. 2-3 times a month 4. Once a week 5. 2-3 times a week 6. 4-6 times a week 7. Once a day 8. 2 or more times a day	☐ 1. One small bottle (375ml) or less 2. One large bottle (750ml) 3. Two large bottles 4. Three large bottles or more
Wine (eg. red wine)	☐	☐ 1. One wine glass (118ml) or less 2. Two wine glasses 3. Three wine glasses 4. Four wine glasses or more
Traditional wine (eg. DOM)	☐	☐ 1. One wine cup (30ml) or less 2. Two wine cups 3. Three wine cups 4. Four wine cups or more
Hard liquor (eg. brandy)	☐	☐ 1. One drink (30ml) or less 2. Two drinks 3. Three drinks 4. Four drinks or more

10. PERSONAL VIEWS ON BREAST FEEDING

10.1 Have you breastfed before?

☐ 0: No, *go to question 10.3*
1: Yes

10.1.1 If "YES", how many children have you breastfed before?

☐☐ Number of children

10.1.2 If YES, please describe your type of breastfeeding for your last child:

☐ 1. Exclusive breastfed (Only breast milk with no water)
2. Predominant breastfed (Breast milk and liquids (including water) other than formula)
3. Partial breastfed (Breast milk, formula and liquids)

10.1.3 How long did you breastfeed your last child?

☐ Year ☐☐ Months ☐☐ weeks

10.2 Are you still breastfeeding during this pregnancy?

☐ 0: No
1: Yes but I stopped at ☐☐ weeks of pregnancy
2: Yes, I am still continuing breastfeeding

10.3 Do you know people who have successfully breastfed their babies?

☐ 0: No
1: Yes

10.4 Did you receive advice from family or friends about breastfeeding?

☐ 0: No
1: Yes

10.5 Have you read books or watched programs on breastfeeding?

☐ 0: No
1: Yes

10.6 Are you currently attending antenatal classes?

☐ 0: No
1: Yes

10.7 Do you plan to breastfeed?

☐ 0: No
1: Yes, for how long ☐☐ months , *go to question 10.8*
99: Don't know

10.7.1 If No, please specify reason

☐ 1: Underlying medical problems
2: Painful
3: Troublesome
4: Inconvenient
5: Formula more nutritious
6: No reason
7: Others, specify _____

10.8 Who will be the main person helping you with the baby after delivery?

☐ 1. Confinement nanny
2. Mother / Mother-in-law
3. Husband
4. Other relatives
5. Others, specify_____

11. SMOKING – <u>BEFORE</u> THIS PREGNANCY

11.1 Have you ever smoked regularly (at least once a day for a year or more)?

☐ 0: No *go to question 11.5*
1: Yes
2: Refuse to answer

11.2 How old were you when you first smoked regularly?

☐☐ yrs

11.3 Did you smoke during the 1 year before you became pregnant?

☐ 0: No *go to question 11.5*
1: Yes

11.4 If yes, how many sticks per day? *Record maximum stated.*

☐☐

<u>Note to interviewer:</u> You may want to explain to the participant that even though she does not smoke, there is some evidence of health implications from second-hand smoke exposure. The following questions are to capture information on second-hand smoke exposure, i.e. where the participant was close enough to the smoker(s) to smell the smoke.

11.5 Did anyone living in your home smoke at home on a daily basis for 6 months or longer?

☐ 0: No *go to question 11.7*
1: Yes

11.6 For how many years did at least 1 person living in your home smoke daily at home?

☐ 1: 1 year or less
2: 2-5 years
3: 5-14 years
4: 15-24 years
5. 25+ years

11.7 Have you ever had a job in which, on a daily basis, you were exposed to cigarette smoke from others?

☐ 0: No
1: Yes

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PLEASE USE CAPITAL LETTER AND WRITE CLEARLY.

12. SMOKING – DURING THIS PREGNANCY

12.1 Are you currently smoking?

☐ 0: No *go to question 12.3*
1: Yes

12.2 If yes, how many sticks per day? *Record maximum stated.*

☐☐

12.3 During your pregnancy, did anyone living in your home smoke at home on a daily basis?

☐ 0: No
1: Yes

12.4 During your pregnancy, have you ever had a job in which, on a daily basis, you were exposed to cigarette smoke from others?

☐ 0: No *go to section 13*
1: Yes

12.5 On average, how many hours were you exposed to cigarette smoke at work?

☐ 1: 1 hour or less
2: 1-3 hours
3: More than 3 hours

12.6 Are you currently exposed to cigarette smoke at work on a daily basis?

☐ 0: No
1: Yes

13. MEDICATION

13.1 Are you taking any medications / supplements / traditional medicine regularly DURING this pregnancy?

Regular refers to more than once a week

☐ 0: No *END OF QUESTIONNAIRE*
1: Yes, please specify in table below

S/N	Name of Medication
1	
2	
3	
4	
5	
S/N	**Name of Supplement**
1	
2	
3	
4	
5	
S/N	**Name of Traditional Medicine**
1	
2	
3	
4	
5	

THANK YOU VERY MUCH FOR YOUR HELP!

Interview end time: _____

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

26-28 Week clinic visit
Mother's Case Report Form

Study ID: _____ Date of interview: _____

Interviewer code: _____ Interview start time: _____

		Tick when completed	Interviewer code
1.	**Registration**	☐	☐☐
2.	**26-28 wk visit questionnaires**		
2.1.	**Mother's questionnaire**	☐	☐☐
2.2.	**Mother's self administered questionnaires**		
	• STAI	☐	NA
	• EPDS	☐	NA
	• BDI-II	☐	NA
	• LYDON Maternal	☐	NA
	• LYDON Domestic Helper	☐	NA
	• Pittsburgh Sleep Quality Index	☐	NA
3.	**Anthropometric measurements**	☐	☐☐
3.1.	Weight		
3.2.	Height		
3.3.	Mid-upper arm circumference		
3.4.	Triceps skinfold		
3.5.	Biceps skinfold		
3.6.	Subscapular skinfold		
3.7.	Suprailiac skinfold		
4.	**Collection of hair**	☐	☐☐
5.	**Collection of buccal swab**	☐	☐☐
6.	**Pulse wave velocity**	☐	☐☐
7.	**Auto Refraction**	☐	☐☐
8.	**Fundus photography**	☐	☐☐
9.	**Case file completed/checked**	☐	☐☐

3. Anthropometric Measurements

3.1. Weight

			.		**kg**
			.		**kg**
			.		**kg**

should be taken only if the first 2 measurements differed by >200gm.

3.2. Height

			.		**cm**
			.		**cm**
			.		**cm**

should be taken only if the first 2 measurements differed by >1.0cm.

3.3. Mid-upper arm circumference

		.		**cm**
		.		**cm**
		.		**cm**

should be taken only if the first 2 measurements differed by >1.0cm.

3.4. Triceps skinfold

			.		**mm**
			.		**mm**
			.		**mm**

3.5. Biceps skinfold

			.		**mm**
			.		**mm**
			.		**mm**

3.6. Subscapular skinfold

			.		
			.		**mm**
			.		**mm**
			.		**mm**

3.7. Suprailiac skinfold

			.		
			.		**mm**
			.		**mm**
			.		**mm**

Skin fold calipers used _____

4. Hair Collection

Taken ☐ Not taken ☐ Refused ☐

Number of hair strands ☐

5. Collection of buccal swab

Done ☐ Not done ☐ Refused ☐

Number of swabs ☐

6. Pulse wave velocity

Done ☐ Not done ☐ Refused ☐

(Please attach report)

7. Fundus Photography:

7.1. Fundus Photo

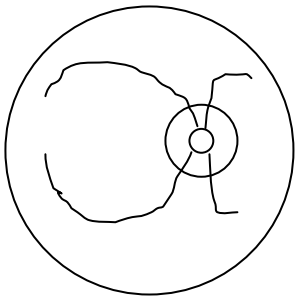
Taken ☐ Not taken ☐ Refused ☐ Unable ☐

Comments_____

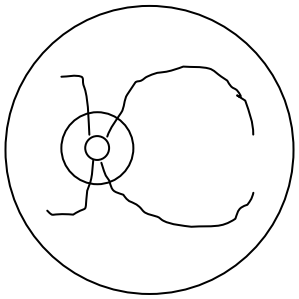
Right Eye _____

Left Eye _____

7.2. Fundus



(RE)



(LE)

	1. Normal	2. Abnormal	3. Unable	1.Normal	2. Abnormal	3. Unable
Macular [macular]	☐	☐	☐	☐	☐	☐
Disc [Discr]	☐	☐	☐	☐	☐	☐
Media [mediar]	☐	☐	☐	☐	☐	☐
Posterior Pole of retina [postretr]	☐	☐	☐	☐	☐	☐
Peripheral retina[periretr]	☐	☐	☐	☐	☐	☐
Describe lesion [desbr]	_____			_____		

8. Auto-Refraction(Undilated with Table-Mounted)

Taken ☐ Not taken ☐ Refused ☐ Unable ☐

Comments_____

Right Eye _____

Left Eye _____

PLEASE WRITE THE STUDY ID AND MAKE A COPY OF THE AUTO REFRACTION REPORT AS THE DATA FADES AWAY WITHIN ONE WEEK

PLEASE PASTE THE COPIED REPORT PAGE HERE

Check the following:

- Ensure best readings
- Cross-out readings with *and extra readings in excess of 5
- Retake if more than 1*or SD.+/- 0.25
- Write down comments for any rejection or unsuccessful attempts

Right Eye _____

Left Eye _____

DELIVERY CASE REPORT FORM

Study ID: _____ Date: _____

Interviewer code: _____

I. INFANT DATA

1. Date of birth ☐☐ / ☐☐ / ☐☐ DDMMYY
2. Time of delivery ☐☐ : ☐☐ Hr
3. Gender ☐ 1. Male 2. Female
4. Birth order ☐☐
5. Gestational age at delivery ☐☐ Wks+ ☐ Days
6. Birth weight ☐☐☐☐ gm
7. Birth length ☐☐ · ☐ cm (to nearest 1 decimal point)
8. Head circumference ☐☐ · ☐ cm (to nearest 1 decimal point)
9. APGAR SCORE (***Enter 99 if not recorded***)
   1 ☐☐ at 1 minute 2 ☐☐ at 5 minutes
   3 ☐☐ at 10 minute
10. Was resuscitation needed? ☐ 0. No 1. Yes

    10.1. If yes, specify specific action taken:

    ☐
    1. O_2 free flow
    2. Bag and Mask
    3. Intermittent positive pressure ventilation (IPPV)
    4. External Cardiac Massage (ECM)
    5. Adrenalin No of doses_____
       Route of administration_____
    6. Other resuscitative measures:_____

11. Placental weight ☐☐☐☐ gm (to nearest gm)

II. NEONATAL PROBLEMS

1. Neonatal Jaundice:

☐ 0. No significant jaundice (not requiring phototherapy)

1. Jaundice requiring phototherapy (Peak bilirubin_____at_____hr of age)

2.Jaundice requiring exchange transfusion

2. Had the newborn have any other neonatal complication? ☐ 0. No 1. Yes

2.1. If yes, please specify : ☐

1. Secondary to transient tachypnoea of new born
2. Meconium aspiration
3. Congenital Pneumonia
4. Hypoglycemia
5. Feeding related disorders
6. Perinatal stress
7. Congenital malformation
8. GBS-related
9. Other perinatal infection
10. Others, specify _____

3. Highest level of neonatal stay ☐

1. ICU
2.Special Care nursery
3. Well baby nursery

4. Date of discharge ☐☐ / ☐☐ / ☐☐

5. Final Diagnosis: ☐

1. Well baby
2.Others: Please specify:_____

III. DAY 1 ASSESSMENT

A. ANTHROPOMETRIC MEASUREMENTS

Interviewer code: _____

Date performed: _____ Start time: _____

1. Weight

				gm
				gm
				gm

3rd measurement if the first 2 differ >100 grams

2. Length

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >1.0 cm

3. Head circumference

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >1.0cm

4. Abdominal circumference

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >0.5cm

5. Mid-arm circumference

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >0.5cm

6. Foot Length

	.		cm (to nearest 1 decimal point)
	.		cm (to nearest 1 decimal point)
	.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >0.5cm

For each of the skinfold site, take 3 measurements and record to last completed 0.2 mm.

If any of repeated tests varies by more than 1mm, repeat the measurement.

7. Triceps skinfold

		.		mm
		.		mm
		.		mm
		.		mm
		.		mm

8. Subscapular skinfold

		.		mm
		.		mm
		.		mm
		.		mm
		.		mm

Other remarks:

End time: _____

B. BIOELECTRICAL IMPEDANCE ANALYSIS

Interviewer code: _____

Date performed: _____ Start time: _____

Test 1	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes
Test 2	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes
Test 3	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes
Test 4	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes
Test 5	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes
Test 6	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes

Test 7	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes
Test 8	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes
Test 9	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes
Test 10	☐	1. Performed 2. Not performed 3. Refused 4. Unable	Crying	☐	0. No 1. Yes	Movement	☐ 0.No 1. Yes

Time of last feed: _____

Other comments:

End time: _____

C. PEAPOD MEASUREMENT

☐ 1. Performed
2. Not performed
3. Refused
4. Unable

Interviewer code: _____

Date Performed: _____

Start time: _____

Other comments:

_____

_____

_____

_____

_____

End time: _____

Study ID: _____ Date: _____

Interviewer code: _____

IV. DETAILS OF LABOUR AND DELIVERY

1. Gravida ☐☐ Para ☐☐

2. Presentation ☐
   1. Cephalic
   2.Breech
   3.Compound
   4. Other _____

3. Antepartum Haemorrhage ☐
   1. No
   2.Abruptio placenta
   3. Placenta previa
   4. Other_____

4. Onset of labour (For spontaneous and induced labour)

	TIME		**DATE** (DDMMYY)		
4.1. 1st stage (Active Phase)	☐☐ : ☐☐ Hr		☐☐ / ☐☐ / ☐☐		
4.2. 2nd stage (10cm to delivery of baby) (for vaginal delivery only)	☐☐ : ☐☐ Hr		☐☐ / ☐☐ / ☐☐		
4.3. 3rd stage (delivery of baby→placenta)	☐☐ : ☐☐ Hr		☐☐ / ☐☐ / ☐☐		
4.4 End of stage 3	☐☐ : ☐☐ Hr		☐☐ / ☐☐ / ☐☐		

5. State of liquor ☐
   1. Clear
   2. Light meconium stained liquor (LMSL)
   3. Medium meconium stained liquor (MMSL)
   4. Thick meconium stained liquor (TMSL)
   5. Blood stained

6. Prolonged rupture of membranes >24 hrs: ☐ 0. No 1. Yes

7. Maternal pyrexia > 37.5 ˚C: ☐ 0. No 1. Yes

8. Chorioamnionitis: ☐ 0. No 1. Yes

8.1. If "yes" ☐ 1. Clinical
2. Bacteriological/Histopathological
3. Clinical and histopathological

9. Antibiotics during labour: ☐ 0. No 1. Yes

9.1. If "Yes", please specify:
☐ 1. Ampicillin
☐ 2. Augmentin
☐ 3. Erythromycin
☐ 4. Others, specify_____

10. Tocolysis: ☐ 0. No 1. Yes

10.1. If "Yes", please specify the medication (Tocolytics):
☐ 1. IV Salbutamol
☐ 2. Oral Nifedipine
☐ 3. Glyceryl Trinitrate (GTN)
☐ 4. Indomethacin
☐ 5. Others, specify_____

11. Maternal Steroids: ☐
1. No
2. Incomplete (<24hrs before birth)
3. Complete, 1 course
4. Two courses or more

11.1 Date of dexmethasone injection.

Date of 1st course ☐☐ / ☐☐ / ☐☐ DDMMYY
Date of 2nd course ☐☐ / ☐☐ / ☐☐ DDMMYY
Date of 3rd course ☐☐ / ☐☐ / ☐☐ DDMMYY

12. Analgesia/sedation during labour ☐
Please fill up the respective numbers in more than one box if necessary.
0. None
1. Entonox
2. Pethidine
3. Regional (Epidural / Spinal)
4. General anaesthesia
5. Non-pharmacological (e.g . hydrotherapy)

13. Mode of delivery: ☐
1. Normal Vaginal delivery
2. Emergency LSCS
3. Elective LSCS
4. Assisted Breech
5. Vacuum
6. Forceps

14. Main indication for operative delivery (instrumental or caesarean) ☐
1. Abnormalities of labour progress
2. Fetal distress or other fetal indications
3. Maternal distress or other maternal indications
4. Social
5. Others, specify: _____

15. Was the labour spontaneous or induced? ☐ 1. Spontaneous 2. Induced 3. N.A.

15.1 If induced, please specify indication (s) for induction

Please fill up the respective numbers in more than one box if necessary.

☐ 1. Hypertension
☐ 2. Diabetes
☐ 3. IUGR
☐ 4. Impending hypoxia
☐ 5. Pre-existing maternal condition
☐ 6. Post date (>40-41 weeks)
☐ 7. Post-term (>41 weeks)
☐ 8. Social
☐ 9. Others (Fetal factors), please specify: _____
☐ 10. Others (Maternal factors), please specify:_____

15.2 If induced, was prostaglandin used? ☐ 0. No 1. Yes

If yes, please specify

15.2.1 Misoprostol ☐ 0. No 1. Yes

15.2.1.1 dose (mcg) ☐☐

15.2.1.2 number of doses ☐☐

15.2.2 Prostin ☐ 0. No 1. Yes

15.2.2.1 dose (mg) ☐☐

15.2.2.2 number of doses ☐☐

15.3 If induced, was I/V oxytocin used? ☐ 0. No 1. Yes

If yes, please specify

15.3.1 Maximum dose (units) ☐☐ • ☐

15.3.2 Duration ☐☐ hours ☐☐ mins

16. Was augmentation conducted? ☐ 0. No 1. Yes

16.1 If yes, was I/V oxytocin used? ☐ 0. No 1. Yes

If yes, please specify

16.1.1 Maximum dose (units) ☐☐ • ☐

16.1.2 Duration ☐☐ hours ☐☐ mins

17. Estimated blood loss (ml) at delivery ☐☐☐☐ ml ☐ Please tick if Not Recorded

V. PREGNANCY DATA

Blood pressure recordings

During Antenatal period

1. Blood pressure (mmHg) recording at 1st earliest clinic visit
2. Blood pressure (mmHg) recording at 26wk clinic visit
3. Blood pressure (mmHg) recording at last antenatal clinic visit

During labour

1. 1st blood pressure (mmHg) recording in labour ward/OT
2. Highest blood pressure (mmHg) during labour/OT

Upon discharge

1. Blood pressure (mmHg) recording before discharge

Systolic BP	Diastolic BP	Date (DD/MM/YY)	Not recorded
			☐
			☐
			☐
Systolic BP	Diastolic BP	Date (DD/MM/YY)	Not recorded
			☐
			☐
Systolic BP	Diastolic BP	Date (DD/MM/YY)	Not recorded
			☐

Weight

1. 1st recorded weight

Weight (Kg)	Date (DD/MM/YY)	Weeks of Gestation
Weight (Kg)	Date (DD/MM/YY)	Not recorded
		☐
		☐
		☐
		☐
		☐
		☐
		☐

2. Weight at 1st antenatal clinic visit
3. Weight at 12±1wk gestation
4. Weight at 20-22wk gestation
5. Weight at 26-28wk gestation
6. Weight at 32-34wk gestation
7. Weight at 36-38wk gestation
8. Weight at last antenatal clinic visit

VI. PREGNANCY COMPLICATION

During 1st trimester (from conception to week 12)

1. History of any bleeding during 1st trimester.

☐ 0. No
1. Yes

2. If "yes", was admission required?

☐ 0. No
1. Yes

3. If "yes", was any treatment given?

☐ 0. No
1. Yes, please specify: _____

During 2nd and 3rd trimester (from week 13 till delivery)

1. History of any bleeding during 2nd and 3rd trimester.

☐ 0. No
1. Yes

2. If "yes", was admission required?

☐ 0. No
1. Yes

3. If "yes", was any treatment given?

☐ 0. No
1. Yes, please specify: _____

2. Were there admissions for other reasons? **Please tick more than one box if necessary.**

	No	Yes
1. Preterm labour	☐	☐
2. APH	☐	☐
3. Hypertension	☐	☐
4. Diabetes	☐	☐
5. Premature Rupture of Membrane (Leaky liquor)	☐	☐
6. Others; please specify_____	☐	☐

3. Any other medications given?

☐ 0. No
1. Yes

3.1. If yes, what was the medication? *(Supplement will be recorded separately)*

			Date Started (DD/MM/YY)
Preterm labour			
1. Dexamethasone	☐	**0. No**	/ /
2. Nifedipine	☐	**1. Yes**	/ /
3. I/V Salbutamol	☐		/ /
4. I/V Terbutaline	☐		/ /
5. GTN patch	☐		/ /
6. Indomethacin	☐		/ /
Anaemia			
1. Oral Iron, specify_____	☐		/ /
2. I/V Iron	☐		/ /
Antibiotics, specify 1)_____	☐		/ /
Antibiotics, specify 2)_____	☐		/ /
Antibiotics, specify 3)_____	☐		/ /
Others			
1. Aspirin	☐		/ /
2. Warfarin	☐		/ /
3. Thyroxine	☐		/ /
4. PTU	☐		/ /
5. Methyldopa	☐		/ /
6. Labetalol	☐		/ /
7. Others, specify _____	☐		/ /
8. Others, specify _____	☐		/ /
9. Others, specify _____	☐		/ /
10. Others, specify _____	☐		/ /
11. Others, specify _____	☐		/ /
12. Others, specify _____	☐		/ /

VII. LABORATORY INVESTIGATIONS

Blood tests:

1. Full Blood Count

	Date : _____ (DD/MM/YY)	Date : _____ (DD/MM/YY)	Date : _____ (DD/MM/YY)
White Blood Cell			
Red Blood Cells			
Haemoglobin			
MCV			
MCH			
MCHC			
Haematocrit			
Platelets			
MPV			
RDW			
Differential Counts			
Neutrophils%			
Neutrophils			
Lymphocytes %			
Lymphocytes			
Monocytes %			
Monocytes			
Eosinophil %			
Eosinophils			
Basophils%			
Basophils			
LUC%			
LUC			

2. Hepatitis B sAg Date of test (DD/MM/YY)_____

Result ☐
1. Reactive
2. Non reactive
3. Not done

3. Anti-HBs Date of test (DD/MM/YY): _____

Result ☐☐ IU/L ☐ Not done

☐
1. Positive
2. Negative

4. HIV testing Date of test (DD/MM/YY)_____

Result ☐ 1. Reactive
2. Non reactive
3. Not done

5. GroupB Strept Vaginal Swab: 1. Isolated 2. Not isolated 3. Not done

Result ☐ Date of test (DD/MM/YY)_____
Result ☐ Date of test (DD/MM/YY)_____
Result ☐ Date of test (DD/MM/YY)_____

6. High Vaginal Swab: 1. Positive 2. Negative 3. Not done

i. Tricho. vaginalis ☐ Date of test (DD/MM/YY)_____
ii. Gard. vaginalis ☐
iii. Candida species ☐

i. Tricho. vaginalis ☐ Date of test (DD/MM/YY)_____
ii. Gard. vaginalis ☐
iii. Candida species ☐

i. Tricho. vaginalis ☐ Date of test (DD/MM/YY)_____
ii. Gard. vaginalis ☐
iii. Candida species ☐

If mother or baby had any of the further complication, the relevant Annex forms need to be filled in.

	No	Yes	
1. Hypertension	☐	☐	**ANNEX-1**
2. Preeclampsia	☐	☐	**ANNEX-2**
3. Gestational Diabetes/Type 2 Diabetes	☐	☐	**ANNEX-3**
4. IUGR	☐	☐	**ANNEX-4**
5. Multiple Pregnancy	☐	☐	**ANNEX-5**
6. Preterm (< 37 gestation week) OR babies in NICU	☐	☐	**ANNEX-6**

NOTE: ALL CASES WITH COMPLICATIONS SHOULD REFER TO OBSTETRICIANS FOR CHECKING OF THE ENTRIES

Questionnaires are similar for months 3 to 36

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

Appendix C

WEEK 3 INFANCY CRF

Study ID: _____ Date: _____

Examiner code: _____ Start time: _____

1. ANTHROPOMETRIC MEASUREMENTS

1. Weight

				gm
				gm
				gm

3rd measurement if the first 2 differ >100 grams

2. Length

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >1.0 cm

3. Head circumference

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >1.0cm

4. Abdominal circumference

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >0.5cm

5. Mid-arm circumference

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >0.5cm

6. Foot Length

		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)
		.		cm (to nearest 1 decimal point)

3rd measurement if the first 2 differ >0.5cm

2. BIOELECTRICAL IMPEDANCE ANALYSIS

Test 1 Z= ☐☐☐.☐ Ω Crying ☐ 0=No Movement ☐ 0=No
Ph= ☐.☐ ° 1=Yes 1=Yes
R= ☐☐☐.☐ Ω
Xc= ☐☐.☐ Ω

Test 2 Z= ☐☐☐.☐ Ω Crying ☐ 0=No Movement ☐ 0=No
Ph= ☐.☐ ° 1=Yes 1=Yes
R= ☐☐☐.☐ Ω
Xc= ☐☐.☐ Ω

Test 3 Z= ☐☐☐.☐ Ω Crying ☐ 0=No Movement ☐ 0=No
Ph= ☐.☐ ° 1=Yes 1=Yes
R= ☐☐☐.☐ Ω
Xc= ☐☐.☐ Ω

Test 4 Z= ☐☐☐.☐ Ω Crying ☐ 0=No Movement ☐ 0=No
Ph= ☐.☐ ° 1=Yes 1=Yes
R= ☐☐☐.☐ Ω
Xc= ☐☐.☐ Ω

Test 5 Z= [ ][ ][ ].[ ] Ω Crying [ ] 0=No Movement [ ] 0=No

Ph= [ ].[ ] ° 1=Yes 1=Yes

R= [ ][ ][ ].[ ] Ω

Xc= [ ][ ].[ ] Ω

Test 6 Z= [ ][ ][ ].[ ] Ω Crying [ ] 0=No Movement [ ] 0=No

Ph= [ ].[ ] ° 1=Yes 1=Yes

R= [ ][ ][ ].[ ] Ω

Xc= [ ][ ].[ ] Ω

Test 7 Z= [ ][ ][ ].[ ] Ω Crying [ ] 0=No Movement [ ] 0=No

Ph= [ ].[ ] ° 1=Yes 1=Yes

R= [ ][ ][ ].[ ] Ω

Xc= [ ][ ].[ ] Ω

Test 8 Z= [ ][ ][ ].[ ] Ω Crying [ ] 0=No Movement [ ] 0=No

Ph= [ ].[ ] ° 1=Yes 1=Yes

R= [ ][ ][ ].[ ] Ω

Xc= [ ][ ].[ ] Ω

Test 9 Z= [ ][ ][ ].[ ] Ω Crying [ ] 0=No Movement [ ] 0=No

Ph= [ ].[ ] ° 1=Yes 1=Yes

R= [ ][ ][ ].[ ] Ω

Xc= [ ][ ].[ ] Ω

Test 10 Z= [ ][ ][ ].[ ] Ω Crying [ ] 0=No Movement [ ] 0=No

Ph= [ ].[ ] ° 1=Yes 1=Yes

R= [ ][ ][ ].[ ] Ω

Xc= [ ][ ].[ ] Ω

Time of last feed: _____

Other remarks:

End time: _____

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

MONTH 3 INFANCY QUESTIONNAIRES

Appendix D

Study ID: _____ Date of interview: _____

Interviewer code: _____ Interview start time: _____

1. YOUR CHILD'S HEALTH

The following questions refer to the period between the last three months, or from the last home visit to the current visit

1.1. At any time, has your child had running nose, blocked or congested nose, snoring or noisy breathing during sleep or when awake that has **lasted for 2 or more weeks duration**

☐ 0: No

1: Yes

1.1.1 If yes, will you give permission for a nurse to call you for more details of your child's nose problem? It would take about 5mins.

☐ 0: No

1: Yes

1.2 Has your child at any time had an itchy rash that is coming and going, other than nappy rash? *(Refer to photo)*

☐ 0: No ***Go to Question 1.9***

1: Yes

1.3 Has this itchy rash at any time affected any of the following places: folds of the elbows, behind the knees, in front of the ankles, on the cheeks, or around the neck, ears, or eyes?

☐ 0: No

1: Yes

1.4 How often, on average, has your child been kept awake at night by this itchy rash?

☐ 1: Never

2: About once a week

3: Almost every night

1.5 Did you use ORAL medicine to treat it?

☐ 0: Never

1: Yes, specify _____

99: Don't know

1.6 Have you ever used creams or moisturizers on your child's skin?

☐ 0: No

1: Yes, specify name of product _____

99: Don't know

1.7 Have you ever used topical steroids on your child's skin?

☐ 0: No

1: Yes

99: Don't know

1.8 Has your child ever been diagnosed with eczema?

** Eczema – a medical condition where the skin is red, dry, scaly, itchy and sore*

☐ 0: No

1: Yes , specify age of diagnosis ☐☐ months

99: Don't know

1.9 Has your child ever wheezed?

** Wheeze – Noisy breathing with a high-pitch, whistling sound heard from the chest, not the mouth*

☐ 0: No

1: Yes , specify no. of wheezing episodes ☐☐

99: Don't know

1.9.1 If yes, how old was your child at the start of the first episode of wheeze?

☐ Month ☐ Week

1.10 Has your child ever been diagnosed with bronchiolitis /bronchitis?

** Bronchiolitis/Bronchitis – respiratory infection causing wheeze, cough, fever, runny nose and breathing difficulty*

☐ 0: No

1: Yes , specify no. of episodes ☐☐

99: Don't know

1.11 Has your child ever been prescribed with nebulizer/inhaler treatment?

☐ 0: No

1: Yes, specify _____

99: Don't know

1.12 Has your child had a cough for a long period of time (e.g. 1 month)?

☐ 0: No

1: Yes

99: Don't know

1.13 Has your child ever had any episodes of croupy cough or been diagnosed with croup?

** Croup – respiratory infection causing barking cough, hoarse voice, runny nose, fever, loud high-pitched hoarse noise when breathing in, and breathing difficulty*

☐ 0. No

1: Yes , specify no. of episodes ☐☐

99: Don't know

1.14 Has your child ever been diagnosed with pneumonia?

** Exclude bronchiolitis/bronchitis*

☐ 0. No

1: Yes , specify no. of episodes ☐☐

99. Don't know

1.15 Has your child had any bouts of vomiting lasting 2 days or longer?

** Do not include possetting or regurgitation*

☐ 0. No
1. Yes, specify number of bouts ☐☐
99. Don't know

1.16 Has your child had any bouts of diarrhoea lasting 2 days or longer?

** Diarrhoea – frequent unformed stools, not including breastfed stools*

☐ 0. No
1. Yes, specify number of bouts ☐☐
99. Don't know

1.17 Has your child ever been diagnosed by a doctor as having an ear infection?

☐ 0. No
1. Yes, specify number of times ☐☐
99. Don't know

1.18 Has your child had any episodes of fever measuring more than 38.0 degrees Celsius?

** Include fever episodes associated with above problems*

☐ 0. No
1. Yes, specify number of episodes ☐☐
99. Don't know

1.19 Has your child had any admission to a hospital? ** Including admission for above problems*

☐ 0. No
1. Yes, specify number of times *(please fill in table below)* ☐☐

S/N	Age (month) admitted	Duration admitted (days)	Diagnosis	Level of care *Indicate 1=General/2=HD/3=ICU*
1				
2				
3				

1.20 Has your child ever had antibiotics?

☐ 0: No

1: Yes *(please fill in table below)*

99: Don't know

S/N	Age (month) prescribed	Duration taken (days)	Name of antibiotics *Indicate 99 if unknown*	Indications
1				
2				
3				

1.21 Has your child been diagnosed with any other medical conditions in the last three months?

☐ 0. No

1. Yes *(please fill in table below)*

S/N	Age (month) diagnosed	Diagnosis	Treatment details
1			
2			
3			

2. FEEDING PRACTICES

2.1 Please indicate your baby's type of feed in the last 3 months.
Code 0= No, 1= Yes

Time (Week/Mth)	Exclusive Breastfeeding	Predominant breastfeeding	Partial Breastfeeding	Formula only
Week 4				
2nd Month				
3rd Month				

2.2. Are you currently still breastfeeding?

☐ 0. No

1. Yes ***Go to Question 2.4***

2.3 If currently you are no longer breastfeeding, when did you stop breastfeeding?

Please indicate End date of Breastfeeding ***Go to Question 2.12***

Age stopped or Date stopped

☐☐	☐☐	☐☐		☐☐	☐☐	☐☐
Mths	Wks	Days		M M	D D	Y Y

2.4. If you are breastfeeding, how would you rate the volume of milk you are producing for your baby?

☐ 1: More than enough

2: Just enough

3: Not enough

2.5. If given a choice, how would you like to change the volume of milk you are producing?

☐ 1: Produce more

2: Produce less

3: No change

2.6. If you are breast feeding, how is your baby breast fed? **Please choose one.**

☐ 1: Direct breast feeding

2: Expressed breast feeding and feed from bottle

3: Mixed (Direct and expressed breast feeding)

2.7. If you are breastfeeding directly, how many times do you breastfeed your baby in 1 day (24 hour)?

☐☐ Number of times

2.8. If you are expressing breast milk, what is the main reason? **Please choose one**.

☐ 1: Excess breast milk after direct feeds

2: Want to store breast milk for future use

3: Need to go back to work

4: Prefer bottle feeding to direct breast feeding

5: Bottle feeding is more comfortable than breast feeding

6: Know how much milk you produce

7: Problem with latching

8: Convenient to express.

2.9. If you are expressing breast milk, what are the other reasons? **Tick as many as applicable.**

☐ 1: Excess breast milk after direct feeds
☐ 2: Want to store breast milk for future use
☐ 3: Need to go back to work
☐ 4: Prefer bottle feeding to direct breast feeding
☐ 5: Bottle feeding is more comfortable than breast feeding
☐ 6: Know how much milk you produce
☐ 7: Problem with latching
☐ 8: Convenient to express.

2.10. What proportion of expressed breast milk fed to your baby is from frozen milk?

☐ 1: None or hardly (0 – 10%)
2: Some (11 – 40 %)
3: About half (41 – 60%)
4: Most (61 - 90%)
5: All or almost all (91-100%)

2.11 Did you have any illnesses during breastfeeding?

Breastfeeding –related infections: mastitis	☐ 0.No	1.Yes
Abscess	☐ 0.No	1.Yes
Respiratory tract infections	☐ 0.No	1.Yes
Gastrointestinal infections	☐ 0.No	1.Yes
Others,	☐ 0.No	1.Yes

specify_____

2.12. How much expressed breast milk/formula is given to the baby during last week?

Day	Volume of express breast milk fed to baby per day (ml)	Volume of formula fed to baby per day (ml)
Day 1		
Day 2		
Day 3		
Day 4		
Day 5		
Day 6		
Day 7		

2.13. Are you going back to work?

☐ 0: No *Go to question 2.15*
1: Yes

2.14. If yes, what changes have you planned for feeding your baby?

☐ 1: Stop breastfeeding
2: Expressed breast milk and feed from bottle only
3: Expressed breast milk and direct breastfeeding
4: Switch to formula partially
5: Switch to formula completely

2.15. Since our last visit, which types of milk or formula are you giving your baby?

(Refer list for formula code) *If still using formula, age/date stopped = 88*

Formula code	Age started			Age stopped			Date started (dd/mm/yy)	Date stopped (dd/mm/yy)
	Mths	wks	days	Mths	wks	days		

If formula is not found in the list, please specify name: _____

2.16 Does your baby take any powder / drops / supplement / medicine containing <u>probiotics</u> (good bacteria)?

☐ 0. No
1. Yes, please specify

Supplements	Total daily dose & unit	Age started			Age stopped			Date started (dd/mm/yy)	Date stopped (dd/mm/yy)
		Mths	wks	days	Mths	wks	days		

PLEASE USE CAPITAL LETTER AND WRITE CLEARLY.

2.17 Does your baby take any other dietary supplements (e.g. vitamins, minerals, iron, fish oil, etc)?

☐ 0. No

1. Yes, please specify

Supplements	Total daily dose & unit	Age started			Age stopped			Date started (dd/mm/yy)	Date stopped (dd/mm/yy)
		Mths	Wks	Days	Mths	Wks	Days		

THANK YOU VERY MUCH FOR YOUR HELP

Interview end time: _____

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

Study ID: _____ Date: _____

BABY EATING BEHAVIOUR QUESTIONNAIRE (BEBQ)

These questions are about your baby's appetite over his/her first few months of life. We are specifically interested in the period during which your baby is fed milk only, i.e. no solid foods or pre-prepared baby food yet.

How would you describe your baby's feeding style at a typical daytime feed?

	Never	Rarely	Sometimes	Often	Always
1. My baby seems contented while feeding	☐	☐	☐	☐	☐
2. My baby frequently wants more milk than I provide	☐	☐	☐	☐	☐
3. My baby loves milk	☐	☐	☐	☐	☐
4. My baby has a big appetite	☐	☐	☐	☐	☐
5. My baby finishes feeding quickly	☐	☐	☐	☐	☐
6. My baby becomes distressed while feeding	☐	☐	☐	☐	☐
7. My baby gets full up easily	☐	☐	☐	☐	☐
8. If allowed to, my baby would take too much milk	☐	☐	☐	☐	☐
9. My baby takes more than 30 minutes to finish feeding	☐	☐	☐	☐	☐
10. My baby gets full before taking all the milk I think he/she should have	☐	☐	☐	☐	☐
11. My baby feeds slowly	☐	☐	☐	☐	☐
12. Even when my baby has just eaten well, he/she is happy to feed again if offered	☐	☐	☐	☐	☐
13. My baby finds it difficult to manage a complete feed	☐	☐	☐	☐	☐
14. My baby is always demanding a feed	☐	☐	☐	☐	☐
15. My baby sucks more and more slowly during the course of a feed	☐	☐	☐	☐	☐
16. If given the chance, my baby would always be feeding	☐	☐	☐	☐	☐
17. My baby enjoys feeding time	☐	☐	☐	☐	☐
18. My baby can easily take a feed within 30 minutes of the last one	☐	☐	☐	☐	☐

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

Study ID: _____

Date: _____

Child Eating Behaviour Questionnaire (CEBQ) [12 months]

Please read the following statements and tick the boxes most appropriate to your child's eating behaviour.

	Never	Rarely	Some-times	Often	Always
My child loves food	☐	☐	☐	☐	☐
My child eats more when worried	☐	☐	☐	☐	☐
My child has a big appetite	☐	☐	☐	☐	☐
My child finishes his/her meal quickly	☐	☐	☐	☐	☐
My child is interested in food	☐	☐	☐	☐	☐
My child is always asking for a drink	☐	☐	☐	☐	☐
My child refuses new foods at first	☐	☐	☐	☐	☐
My child eats slowly	☐	☐	☐	☐	☐
My child eats less when angry	☐	☐	☐	☐	☐
My child enjoys tasting new foods	☐	☐	☐	☐	☐
My child eats less when s/he is tired	☐	☐	☐	☐	☐
My child is always asking for food	☐	☐	☐	☐	☐
My child eats more when annoyed	☐	☐	☐	☐	☐
If allowed to, my child would eat too much	☐	☐	☐	☐	☐
My child eats more when anxious	☐	☐	☐	☐	☐
My child enjoys a wide variety of foods	☐	☐	☐	☐	☐
My child leaves food on his/her plate at the end of a meal	☐	☐	☐	☐	☐
My child takes more than 30 minutes to finish a meal	☐	☐	☐	☐	☐

	Never	Rarely	Some -times	Often	Always
Given the choice, my child would eat most of the time	☐	☐	☐	☐	☐
My child looks forward to mealtimes	☐	☐	☐	☐	☐
My child gets full before his/her meal is finished	☐	☐	☐	☐	☐
My child enjoys eating	☐	☐	☐	☐	☐
My child eats more when she is happy	☐	☐	☐	☐	☐
My child is difficult to please with meals	☐	☐	☐	☐	☐
My child eats less when upset	☐	☐	☐	☐	☐
My child gets full up easily	☐	☐	☐	☐	☐
My child eats more when s/he has nothing else to do	☐	☐	☐	☐	☐
Even if my child is full up s/he finds room to eat his/her favourite food	☐	☐	☐	☐	☐
If given the chance, my child would drink continuously throughout the day	☐	☐	☐	☐	☐
My child cannot eat a meal if s/he has had a snack just before	☐	☐	☐	☐	☐
If given the chance, my child would always be having a drink	☐	☐	☐	☐	☐
My child is interested in tasting food s/he hasn't tasted before	☐	☐	☐	☐	☐
My child decides that s/he doesn't like a food, even without tasting it	☐	☐	☐	☐	☐
If given the chance, my child would always have food in his/her mouth	☐	☐	☐	☐	☐
My child eats more and more slowly during the course of a meal	☐	☐	☐	☐	☐