Metabolic phenotypes of infants with normal birth weight, small-for-gestational-age, or after maternal gestational diabetes mellitus

Laurentya Olga

Department of Paediatrics

University of Cambridge

This thesis is submitted for the degree of

Doctor of Philosophy

Peterhouse

January 2021

"By the end of the first 1000 days the body is almost complete. So, what you have on your 1000th day is what you will ever have."
(David Barker, 1938-2013)

Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration, except as declared in the Preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit for the relevant Degree Committee.

Metabolic phenotypes of infants with normal birth weight, small-for-gestational-age, or after maternal gestational diabetes mellitus

Laurentya Olga

Summary

Numerous studies have associated both under- and overnutrition during early life with long-term metabolic outcomes. Those conditions are typically represented by two groups of infants in animal and human studies: infants born small-for-gestational-age (SGA; reflecting intrauterine undernutrition) and offspring of mothers with gestational diabetes mellitus (OGDM; reflecting intrauterine overnutrition and hyperglycaemia). However, the underlying mechanism behind this phenomenon is still unknown: how these distinct groups can end up with similar metabolic risks, despite having opposite *in utero* nutritional conditions.

This thesis aims to characterise biological similarities and differences across SGA, OGDM, and a control population from the Cambridge Baby Growth Study (CBGS). The CBGS, set up in 2001, is an ongoing longitudinal cohort aiming to examine the ante- and postnatal determinants of infant growth and body composition, including genetic and environmental factors.

While SGA infants in CBGS showed typical rapid postnatal growth patterns, the contemporary OGDM cohort showed a distinct trend to that in earlier cohorts, with normal birth weights but reduced adiposity, which was sustained from birth to 24 months. Preliminary analyses of infant capillary blood spot profiles suggested that pre- and postnatal exposures reflected in SGA and OGDM may share common hormonal and lipidomic signatures during early infancy, independent of feeding practice and other confounding factors. In a CBGS breastmilk (BM) study, higher BM intake volume at 6 weeks conferred protection against subsequent rapid weight gain. Analyses of BM macronutrients also suggested that carbohydrate and protein intakes may have functional relevance to later infant growth and adiposity.

This work has characterised in detail the effects of antenatal and postnatal nutritional factors on infant growth, body composition and biochemical profiles. The early infancy metabolic signatures identified here may reflect the continuum of early programming from pre- to early postnatal and might be potentially linked to future metabolic risks.

Acknowledgements

I thank Lord Jesus, my God, whose grace and mercy have enabled me to start and finish this important battle of my life; and to Mother Mary and St Joseph for being faithful intercessors to my prayers.

And of course, for precious people whom God has placed in my life, who are worth mentioning for their support during my study.

First and foremost, I would like to thank Professor David Dunger and Professor Ken Ong for their guidance, knowledge, mentorship, support, and patience throughout my PhD. I was puzzled at times by the process and also the results of the study and without their willingness to help, I would not be able to well interpret the data. I also need to put forward Dr Clive Petry for offering his help since the early stage of this thesis writing, and how his kind gesture help reducing my anxiety and lack of confidence along the way.

This study (Cambridge Baby Growth Study, CBGS) in general would not be possible without our brilliant and dedicated paediatric research nurses whom I am eternally grateful: Ann-Marie Wardell, Karen Forbes, Suzanne Smith. Their tremendous dedication in recruiting and visiting the babies and the families, as well as their companionship as true friends have kept me going through my PhD.

To Karen Whitehead and Dianne Wingate, for dealing with the diverse range of biological samples collected in the study and for having a solution to every problem.

To Mark Wilson, for all his work in managing CBGS databases tidy, structured, and accurate, plus for his responsiveness to my (often too last minute) data requests.

To Jane Horsford, who never minds being disturbed by my annoying procrastination habit, who keeps checking in to ensure my well-being during this pandemic.

To Dr. Philippa Prentice, who keeps being my inspiration, who always puts total dedication in everything she does. The CBGS has become a fruitful study because of her.

To the collaborators, especially Dr. Albert Koulman (NIHR BRC Metabolomics and Lipidomics Facility, University of Cambridge), Dr. Jacques Vervoort (Wageningen University), and Dr. Daniela Barile (University of California, Davis) for processing the samples as well as allowing generous time to discuss the results with a total amateur like me. To Dr. Emanuella de Lucia Rolfe (MRC Epidemiology Unit), for training me to review the ultrasounds data and writing a paper together. To Dr. Janna van Diepen from Mead Johnson Nutrition, for all those important discussions and sharing of ideas to keep the collaboration going. To Dr. Robert Kleemann and team from TNO, the Netherlands, who have introduced me to the beauty and usefulness of animal studies.

As a member of Peterhouse, I also realise how my college has always put much faith and support in me, especially via my graduate tutor, Dr. Christopher Lester, who had always said 'yes' every single time I asked for help in any aspects, financially and well-being related matters. Our college's master, Bridget Kendall, is always happy and enthusiastic in commenting the graduate students' work in the regular graduate symposium. Peterhouse has also funded me to go present my work in an international conference in 2018, accommodated me with an affordable and comfortable graduate residence for the whole period of my study, and even granted me with study funds to sit the medical exams to pursue a medical career after my PhD.

I should not forget how privilege I am to be able to grasp graduate studies and research experience in Cambridge, all because of the scholarship I received from Jardine Foundation and Cambridge Trust.

To my beloved family, especially my mother, for being all ears, for their never-ending love and prayers.

To dearest friends, too many to individually name to whom I appreciate their encouragement and friendship, who have supported me during this long marathon of PhD, especially David and Yosephine Revell for all those nutritious meals, for reading the whole thesis excitedly, for treating me like more than a family; Kevin Dialdestoro for all your kindness and for teaching me statistics patiently and free-of-charge; Michael Prasetya for his incessant effort to put a smile on my face (sometimes with terrible jokes) and for continuously instilling me with positive and happy thoughts day and night; Hannah Harberd for your cheery messages and smile; Angelina Morelos for being a sister from another mister; Theresa Bartels for having such huge confidence in me; Ingrid Cizaite, Vanessa Karbach, and Victoria Stevens for our Fabulous Fun Friday coffee routines; to WiFi Family Band who have produced lovely music with me, which brightened up my day many times during storms from PhD and thesis; to my housemates, especially the lovely couple Antoine Koen and Carolina Orozco; and to fellow Indonesian PhD students: Dewi Safitri, Chakrita Saulina, and Rangga Dachlan, thanks for telling me many times that hard work will pay off eventually.

Last but actually the most important person in my life: my late father, who always lives in my heart, whose sincere smile and kind heart are the only reasons I rise again every time I fall.

Things are looking up. I know above the clouds the sun is shining. (Jason Mraz)

Contents

List of Figures

List of Tables

List of Abbreviations

ADA	American Diabetes Association
ADP	air-displacement plethysmography
AGA	appropriate-for-gestational age
AGD	Anogenital distance
ALSPAC	the Avon Longitudinal Study of Parents and Children
AUC	area under the curve
BM	breast milk
BMI	body mass index
bp	base pair
CA	conceptual age
CBGS	Cambridge Baby Growth Study
CBGOS	Cambridge Baby Growth Outcome Study
CE	cholesteryl ester
CHD	coronary heart disease
CI	confidence interval
CNS	central nervous system
CVD	cardiovascular disease
DOHaD	developmental origins of health and disease
DBS	dried blood spots
DI	disposition index
DMS	dried milk spots
DXA	dual-energy X-ray absorptiometry
EASD	European Association for the Study of Diabetes
FADS	fatty acid desaturase
FFA	free-fatty acids
FFM	fat-free mass
FM	fat mass
FL	fucosyllactose
FUT2	fucosyltransferase 2
GA	gestational age
GDM	gestational diabetes mellitus
GH	growth hormone
GHIH	growth hormone inhibiting hormone
GHRH	growth hormone releasing hormone
GL	glycerolipids
GP	glycerophospholipids
GWAS	genome-wide association studies
HAPO	Hyperglycaemia and Adverse Pregnancy Outcome
HMO	human milk oligosaccharides
IADPSG	International Association of the Diabetes and Pregnancy Study Group
IGF	insulin-like growth factor
IMD	index of multiple deprivation
IODM	infants of diabetic mothers
IUGR	intrauterine growth restriction

LGA	large-for-gestational-age
LMIC	low- and middle-income countries
LNFP	lacto-N-fucopentaose
LNnT	lacto-N-neotetraose
LNT	lacto-N-tetraose
MSD	Meso Scale Discovery
MUFA	monounsaturated fatty acids
NCD	non-communicable disease
NDDG	the National Diabetes Data Group
OGDM	offspring of mothers with gestational diabetes mellitus
OGTT	oral glucose tolerance test
PC	phosphatidylcholine
PCR	polymerase chain reaction
PI	ponderal index
PUFA	polyunsaturated fatty acids
QC	quality control
RFLP	restriction fragment length polymorphism
RIA	radioimmunoassay
rRNA	ribosomal RNA
SA	sialic acid
SCD	stearoyl-CoA desaturase
SCFA	short-chain fatty acids
SD	standard deviation
SDS	standard deviation score
SE	standard error
SEM	standard error of mean
SES	socioeconomic status
SGA	small-for-gestational age
SL	sialyllactose
SM	sphingomyelin
SNP	single nucleotide polymorphism
SP	sphingolipids
ST	sterol lipids
TBW	total body water
TG	triglycerides
T1D	type 1 diabetes mellitus
T2D	type 2 diabetes mellitus
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

Chapter 1 Introduction

Since the introduction of developmental origins of health and diseases (DOHaD) theory, early life nutrition and growth have been considered more important than before, not only for immediate infancy outcomes, but also childhood, adolescence, and even adulthood. Proposing this theory in the 1990s, David Barker postulated that fetal nutritional provision *in utero* could determine an individual's susceptibility to chronic metabolic diseases later in life[1]. This was aligned with what Geoffrey Rose had reported more than 25 years earlier: compared to their healthy counterparts, myocardial infarct survivors had higher infant death rates among their siblings, suggesting adverse events in childhood could influence disease risk later in life[2].

Through a trio of influential publications in The Lancet[3–5], Barker and colleagues showed that geographical areas with the highest infant mortality in the 1910s in England and Wales also had the highest rate of cardiovascular disease (CVD) in the 1970s; suggesting an adverse environment in the womb and during infancy could be casually linked to chronic disease risk later in life, and lower birth weight and poor infancy growth increase an individual's susceptibility to CVD, hypertension, and diabetes in middle age. Although initially considered controversial, this fetal-origins-of-adult-diseases concept is now widely accepted with established supporting theories and numerous replicated studies in animals and humans. Early life is now viewed as a critical period to influence later health and thus is included

in the World Health Organization (WHO) Global Action Plan for the prevention and control of non-communicable diseases (NCD) 2013-2020[6].

As the major link with many NCDs, obesity, defined in adults as having body mass index (BMI) greater than 30.0 kg/m^2, has become a major public health problem. This effects both developing and developed countries with the WHO reporting a two-fold increase in the global prevalence of obesity between 1980 and 2014[7]. Moreover, obesity is widespread across all age groups, including children[8]. In 2019, more than 38 million children under 5 years old worldwide were either obese or overweight[9]. In England, at least 12% and 9.7% of children at reception (age 4-5 years old) are overweight or obese, respectively[10].

Obesity has been linked to the increased risks of type 2 diabetes mellitus (T2D)[11], CVD[12], cancer[13], and overall mortality[14]. Consequently, the rising prevalence of obesity has been mirrored by those diseases. For example, the number of people living with diabetes mellitus quadrupled globally between 1980 and 2014, 90% of whom had T2D[15]. In the UK alone, the number of people with diabetes is expected to reach 5 million by 2025[16].

Given the large burden of obesity and its comorbidities, a substantial number of studies have attempted to find effective treatment modalities. However, tackling obesity is not easy as the aetiology is multifaceted: it is a combination of genetic susceptibility, sedentary lifestyle, and a high calorific diet. Comprehensive lifestyle intervention is considered as the most effective modality, but the results are quite modest and variable, affected by gender, ethnicity, and other factors, and the sustainability is questionable due to the high risk of failure and weight regain[17,18].

Therefore, it is important to investigate if new strategies during early life could provide a 'window of opportunity' with greater prevention and continuing benefit later in life, both in child- and adulthood. Despite being inconsistent in the study designs, mainly empirical and observational, and having residual confounding factors, early infancy weight gain and feeding are considered promising and biologically plausible targets for preventing later obesity[19,20].

It is now widely accepted that both overnutrition and undernourishment in the fetal or early life have long-term metabolic consequences. This has led to the observation that two groups of infants are the most affected: infants born small-for-gestational-age (SGA) and offspring of mothers with gestational diabetes mellitus (OGDM); this has been confirmed by replication studies in humans and animals. Postnatally, SGA infants tend to catch-up in both weight and height, especially if postnatally exposed to plentiful nutrition[21–23]. 'Catch-up' is defined as a gain in SDS greater than 0.67 SDS, representing the width of each percentile band on standard growth charts, while 'catch down' is a reduction in SDS by more than -0.67[24]. As opposed to SGA, OGDM infants with typical bigger size at birth tend to catch-down in weight[25] initially but may gain excessive weight later in childhood.

SGA and OGDM with their particular postnatal growth patterns appear to cause similar metabolic risks later in life. However, the underlying mechanism behind this phenomenon is still unknown. We hypothesise that this could be due to common metabolic changes early in life. This thesis aims to characterise those changes by capturing and comparing the biological similarities and differences across SGA,

OGDM, and a control group consisting of normal birth weight infants, in order to identify any potential biomarkers of later disease risk.

This chapter will begin by further describing how early growth and nutrition could impact later life diseases risk, the DOHaD concept and its supporting theories, infancy growth patterns and corresponding consequences, before focusing on SGA and OGDM specifically and discussing experiments conducted in this study. The research questions and aims of the study will be described in more detail at the end of this chapter.

1.1 Early growth and later life implications

1.1.1 The DOHaD hypothesis and metabolic programming

According to the DOHaD hypothesis, disruptions during critical periods (pre- and postnatal) can permanently affect organ structures and body metabolism[26]. An example of this is the association between perinatal adverse exposures and CVD risk in adulthood, which has now been robustly replicated around the world. Some examples of the first epidemiological studies reporting this association are listed in Table 1.1.

This association offers an alternative to the commonly held doctrine that CVD is caused by the combination of unfavourable genetics and Westernised unhealthy adult lifestyles.

Although having been around for many decades, this belief has yet several inexplicable phenomena: 1) in many Western countries, for example in the USA, the

drastic rise of CVD has been followed by a fall in the last decades *but* no parallel changes in adult lifestyles seem to be able to explain it[27]; 2) in Britain, despite lifestyle changes during World War II, the rate of CVD was increasing throughout and post-war[28]; 3) although adult biochemical and physiological properties, e.g. serum cholesterol and blood pressure, are associated with CVD, these factors combined with adult lifestyle have only limited ability to predict CVD[29], 4) the rise in CVD prevalence over the last century has occurred faster than can be explained solely by genetics[30].

Table 1.1 Example of pioneering studies associating early life exposures and adulthood CVD risks, implying the DOHaD concept

Investigator	Setting	Key results
Barker[3]	England and Wales	Geographical association between rates of death from CVD and death rates among neonates 50-70 years earlier
Forsdahl[31]	Norway	Geographical link between arteriosclerotic CVD and past infant mortality
Notkola[32]	Finland	Poor living conditions in childhood, including bad housing, recurrent exposures to infection, were associated with CVD
Buck[33]	USA (17 states)	Geographical link between infant mortality due to diarrhoeal disease and mortality from CVD
Rose[2]	USA	Siblings of CVD patients had twice higher stillbirth and infant mortality rates
Marmot[34]	London (civil servants)	Link between short stature (reflecting unsupportive environment in early life) and higher death rate
Hinkle[35]	USA (Bell System Company)	Lower death from CVD rate among men from 'white collar' (indicating higher socio-economic status) compared to 'blue-collar' parents

The relationship between early and later life health implications was actually first examined through animal studies. In 1933, restricting calorie intake during early life in rats was reported to have immediate and long-term effects by reducing postnatal

weight gain and adult life morbidity as well as expanding the animal's life span[36]. Similarly, a rat study in 1962 demonstrated that early postnatal overfeeding would result in higher risks of obesity and its corresponding morbidities[37].

However, the positive association between the amount of early life nutrition and later obesity risk found from animals was not exactly similar to Barker's observation in human epidemiology.

After showing an inverse relationship between birth weight and adult death from CVD in the Hertfordshire birth cohort[4], Barker and colleagues published similar inverse associations between birth weight and later glucose intolerance[38], as well as with hypertension and metabolic syndrome[39]. These data suggest infants born small have the highest risk of future metabolic diseases, a finding which has been replicated in many parts of the world and confirmed by meta-analyses (Table 1.2).

Table 1.2. Meta-analyses associating lower birth weight and adverse metabolic risks in the future

Investigator, year	Outcomes (increased risks of adult diseases due to being born small)	
Tian *et al*, 2019[40]	Risk of T2D	12% reduced risk of T2D per 500-g increment in birth weight (<5000 g)
Mu *et al*, 2012[41]	Later hypertension	21% higher risk of later hypertension among individuals born with birth weight <2.5 kg
Silveira and Horta, 2008[42]	Metabolic syndrome	Low birth weight increases the risk of adulthood metabolic syndrome (compared to normal birth weight)
Wang *et al*, 2014[43]	CHD	19% increased risk of CHD among infants born with low birth weight (<2500 g) compared to those with birth weight ≥2500 g
Risnes *et al*, 2011[44]	Mortality	6% lower risk of all-cause mortality and 12% lower risk of CVD mortality per kg higher birth weight

Gestational age, socioeconomic and environmental factors did not seem to confound these results[39]. Barker then proposed that "the nourishment a baby receives from its mother and its exposure to infection after birth determine its susceptibility to chronic disease in later life"[5].

In addition to these studies, several other studies reported U- or J- shaped, rather than inverse relationships between birth weight and later risk of T2D[45], CVD[46], and all-cause mortality[47]. This is supported by a Finnish retrospective cohort involving 290 adults with T2D. In this study, 66% of the population were born small and showed rapid weight gain during the first two years and beyond whereas the remainder were found to have large birth weight with initial weight loss but gained much more weight from 2 years onwards and became obese[48]. This implies that higher birth weight, which usually resulted from maternal obesity or gestational diabetes mellitus (GDM), also confers similar increased adverse metabolic risks later in life.

Moreover, before Barker's studies, the Dutch famine studies provided intriguing insights into how the effect of undernutrition during pregnancy to later life obesity risk is modified by timing in pregnancy. If the fetus had been exposed to the famine during the first weeks of pregnancy, they had a higher risk of adulthood obesity. But, if the famine exposure happened in the last trimester of pregnancy, there was a reduced risk of later obesity on the offspring, observed until 19 years of age[49].

Not only birth weight, early postnatal weight gain seems to substantially affect later life disease risk. The Avon Longitudinal Study of Parents and Children (ALSPAC) has demonstrated the association between early infancy weight gain, irrespective of

birth weight, with markers of later disease risk. In this study, rapid infancy weight gain was positively associated with later adiposity, and it was also linked to intrauterine growth constraint[50].

Subsequently, numerous trials have consistently reported similar associations with an emphasis on infants born SGA experiencing catch-up growth[21,51–53]. Furthermore, there were also contemporaneous prospective birth cohorts that showed that rapid infancy weight gain could relate to later childhood adiposity, central adiposity gains, and insulin resistance not only in SGA but also in AGA populations[24].

Related to OGDM, the effect of gestational diabetes mellitus on offspring health and later disease risk has been studied for many years. A large body of research was based on the Pima Indians, a population with one of the highest prevalence of T2D in the world (diabetes occurs in 38% of the population) and studies on this population have been continuing since 1965[25].

Pima Indian infants born to mothers with diabetes were heavier both at birth and 7.7 years of age, although their weight normalised at 18 months old. *In utero* high glucose exposure has been identified as the strongest single risk factor for childhood obesity in this study[25]. Interestingly, a recent study in this high-risk population discovered a U-shaped effect of birth weight on diabetes incidence in adolescence (10-19 years old, $p<0.001$). However, a negative linear effect was observed in young adulthood, meaning only low birth weight was associated with T2D risk in this age group ($p<0.001$ for 20-29 years and $p=0.003$ for 30-39 years old). Children's BMI, maternal diabetes, and higher genetic risk score for T2D showed additive effects to these associations but did not confound or interact with

the effect of birth weight on diabetes incidence at all age intervals[54], suggesting the relationship between birth weight and T2D risk seemed to be independent of genetic risk in this population.

Those epidemiological studies have at least shown associations between neonatal measures (both small and large) and infant growth with later metabolic outcomes. This "slow journey" of increasing metabolic risk should leave metabolic signatures along the way, especially during early childhood, which this study attempted to capture.

The most plausible mechanistic pathway being proposed is 'metabolic programming', defined by Lucas as 'a process whereby a stimulus or insult at a critical or sensitive period of development in early life has permanent effects on structure, physiology, and metabolism, thus resulting in lifelong significance'[55]. The main two hypotheses supporting this principle are thrifty phenotype and match-mismatch theories.

First, the thrifty phenotype theory[39] by Hales and Barker, which declared that decreased fetal growth and subsequent low birth weight caused by *in utero* poor nutrition would lead to increased later disease risk *if* these individuals were exposed to plentiful nutrition during postnatal life. This is supported by evidence of permanent changes in organ structure and metabolic pathways due to antenatal poor nutrition (to allow fetal survival), for example: 1) reduced number of pancreatic beta-cell mass and function, resulting in altered glucose-insulin metabolism, 2) reduced nephron number in developing kidney. All of these would increase the vulnerability of those individuals to adult diseases, such as T2D and hypertension.

Equivalent to that, Gluckman and Hanson and their match-mismatch theory[56] suggested discrepancy between *in utero* nutritional milieu and later life nutritional provision as the main suspect. According to this theory, inadequate maternal nutrition *in utero* would be interpreted as a signal of a poor environment by the developing fetus. This would stimulate physiological changes in offspring phenotypes to promote survival. However, this early advantage might become detrimental when the postnatal environment appears to be much more supportive. These inaccurate (mismatched) developmental cues could result in an increased risk of NCD.

Epigenetics has been considered as the plausible mechanism behind those theories[57,58]. Epigenetics elucidates the environmental influence in linking early-life exposures with later/transgenerational disease risk. According to epigenetics, the interaction between fetal genes and maternal uterine environment would result in permanent epigenetic changes and this has been observed across multiple populations, regardless of birth weights and early weight gain trajectories. As one of the major epigenetic modifications, DNA methylation could change homeostatic control systems, including hormones, and appetite, neuronal signalling, gut microbiota, mitochondrial function, and other early structural development of organs and tissues[57,58]. One source of evidence comes from individuals whose mothers' were exposed to the Dutch Hunger Winter (1944-1945) during pregnancy. Their epigenomes were found to have lower DNA methylation of the *IGF2* gene, 60 years later.[58]

Alternative to DOHaD and its supporting hypotheses, several genetic theories have also been postulated. The thrifty genotype hypothesis proposed by Neel[59] argued

that instead of phenotypic programming, those adverse outcomes could have been due to common genetic variations, which are advantageous in times of lack but become detrimental in times of plenty. For example, genes that maximise fat storage during intermittent food supply will predispose to adverse metabolic phenotypes in times of plenty[59].

Similarly, Hattersley and Tooke[60] with their fetal insulin theory suggested that instead of intrauterine programming in response to maternal malnutrition, the link between low birth weight and future insulin resistance and other metabolic derangements could be due to the same insulin-resistant genotype that manifested as all of those phenotypes throughout different periods of life: in early life as impaired fetal growth, abnormal vascular development, and lower birth weight; later in life as genetically-impaired insulin resistance, affected by environmental factors.

1.1.2 Growth and its determinants

The importance of the first 1,000 days

Early life, defined as the first 1,000 days starting from conception until 2 years of postnatal age, reflects a unique and important period because of two interconnected reasons. First, there is remarkable rapid physical growth and rapid development of organs. Second, due to the rapid growth and development during this period, any environmental insult affecting this critical stage could potentially cause long-term or permanent health consequences[61]. This has been evidenced

through numerous studies, both of animals and human, suggesting this period as a window of opportunity for early intervention.

Adequate nutritional provision in the first 1,000 days of life, starting from good maternal nutritional status during pregnancy and proper infant feeding practice postnatally, is essential for optimal growth and lifelong health[62]. This is because this period of rapid growth has specific nutritional requirements that small nutritional insufficiency, especially when persistent, could adversely impact contemporaneous later growth and overall health[63].

1.1.2.1 Prenatal period

Pregnancy plays a pivotal role in shaping the offspring's future health. Any compromise during pregnancy, especially in the first 10 weeks of gestation (time of transition between the embryo and fetal periods[63]), can cause intrauterine growth changes of specific tissues and organs and lead to irreversible functional consequences[64].

Rapid growth and development occur during early gestation. Approximately 1 week after conception, the embryo begins to implant in the uterus and the placenta starts to form[65]. During the developmental and embryonic stages, both embryo and placenta go through rapid cell multiplication and differentiation to provide the basis for organ formation, as well as to prepare maternal-fetal interface in the placenta[65]. The fetal stage commences 8 weeks after conception or at around the 10th week of pregnancy, indicated by a fully-formed and functional placenta[65]. Rapid fetal growth

is observed between weeks 22 to 40 of gestation leading to a six-fold increase in fetal weight[66].

The development of adipose tissue begins at 25 weeks' gestation and fat deposition starts progressively until full term[63]. A normal full-term neonate has approximately 17% body fat at birth, with female infants having slightly higher adiposity than males[67]. This is substantially higher in the case of maternal obesity, excess gestational weight gain, or GDM[67,68]. On the contrary, intrauterine growth restriction (IUGR) leads to lower total body adiposity[69].

Factors affecting fetal growth

There is a positive association between neonatal birth weight and parental height[70], and hence short maternal stature increases the risk of low birth weight and stunting[71]. Meanwhile, a higher pre-pregnancy maternal BMI is positively associated with both offspring birth weight and neonatal adiposity[70,72]. However, not all infants born to obese mothers are large-for-gestational-age (LGA), reflecting the involvement of other factors in the regulation of placental nutrient transfer[72]. Beyond infancy, higher maternal pre-pregnancy BMI was associated with an increased risk of childhood overweight or obesity[73]. In addition, paternal BMI has also been demonstrated to have a modest positive association with infant BMI at birth[74].

Other maternal factors affecting fetal growth are age, hypertension, prenatal psychiatric conditions, parity, and inter-pregnancy interval. Younger maternal age, existing hypertensive disorder and psychiatric conditions before pregnancy

(including depression, anxiety, and obsessive-compulsive disorders), nulliparity, and short inter-pregnancy interval (<18 months) have been associated with a higher incidence of poor fetal growth and SGA births[75–77]. Nulliparous status and young maternal age are considered to cause placental nutritional restraint and lead to higher rate of SGA delivery[77].

Other various environmental factors can also influence fetal growth and development, with the placenta playing a major role. Placental size and function affect birth outcomes because fetal growth is vitally driven by nutrition and oxygen supply via the placenta[78]. Rather than being a passive channel, the placenta is an active organ which responds efficiently to maternal and fetal signals that regulate its transport and metabolic function[65,72]. Nutrient uptake and distribution are controlled by placental and fetal hormones[78], including insulin, insulin-like growth factors (IGFs), thyroid hormones, leptin, and cortisol.

More specifically on maternal nutrition, pre-conception and pregnancy dietary pattern has been linked to early placental development and thus perinatal outcomes[72,79]. Undernourishment during pregnancy, which can be due to inadequate food quantity and/or quality or persistent hyperemesis, can cause both fetal insufficient growth and compensatory placental overgrowth with a constrained nutrient transfer interface[80]. On the contrary, maternal overnutrition leading to excessive pregnancy weight gain can cause glycaemic dysregulation and GDM, leading to increased neonatal adiposity[81] and LGA birth[82] or macrosomia (birth weight of >4 kg regardless of gestational age or GA)[83]. Postnatally, excessive gestational weight gain is also associated with childhood overweight or obesity, although the effect is not as strong as maternal pre-pregnancy BMI[73].

Both maternal macro-[84] and micronutrient[85] intake could directly influence fetal growth and infant birth weight. Maternal anaemia during pregnancy has been associated with lower birth weight, although only limited in some populations[62]. Other maternal micronutrient intake being studied include magnesium, selenium, vitamin D, B12, and E, and retinol, all during third trimester. From the ROLO (Randomised cOntrol trial of LOw glyaemic index diet versus no dietary intervention to prevent recurrence of fetal macrosomia) study in Ireland, infant birth weight was found to be negatively associated with maternal vitamin D but positively with B12 intakes; magnesium was positively associated with birth length; and neonatal central adiposity was positively associated with retinol, but negatively with vitamin E and selenium intake[85]. However, all these associations were resulted from an observational study and still need further mechanistic study.

Considering the crucial role of maternal nutrition before and during pregnancy, the International Federation of Gynaecology and Obstetrics (FIGO) has released a report on the importance of balanced nutrition and specific nutrients for each stage of fetal development as well as recommendations on preconception and maternal nutrition[86]. Throughout gestation, high consumption of fruits, vegetables, poultry, fish, and low-fat dairy product is advised in the form of whole food; whereas red and processed meats, high-fat dairy products, and refined carbohydrates are not recommended and could increase the risk of SGA birth[84]. Moreover, a high saturated fat diet could lead to increased neonatal adiposity[87,88]. Regarding protein, animal studies have found that both low and high protein intakes were associated with fetal growth restriction[88,89].

Another important lifestyle-related factor is exercise. There have been several studies linking maternal exercise and pregnancy outcomes. Increased physical activity during pregnancy is recommended to prevent maternal glycaemic dysregulation[90], and reduce the adverse effect of maternal obesity on infant birth size[91], improve blood flow and placental functional capacity which then increase nutrient delivery, and most importantly, improve maternal well-being during pregnancy[90]. Those beneficial effects supported the proposal that exercise could help in preventing both SGA and LGA deliveries, although direct effect cannot be claimed[90]. Moreover, in recent animal studies, exercise during pregnancy among obese mothers could improve both maternal and offspring insulin sensitivity, placental insufficiency, and offspring cardiovascular outcomes[92,93].

Other environmental factors are parental (especially maternal) tobacco smoking, alcohol, and drugs consumption. Tobacco smoking has been strongly associated with IUGR, preterm birth, and SGA with a possible dose-dependent correlation[94]. It is also similar to alcohol and drugs, which have been associated with placental insufficiency and decreased birth size[95]. Furthermore, there have been studies reporting endocrine disrupting chemicals, such as bisphenol-A, to adversely affect fetal growth[96]. There is also evidence that environmental exposures during oogenesis and spermatogenesis might have an effect on fetal growth and development[72].

There are several other factors that can affect the growth of the fetus. Gender is the most determinant factor, with higher birth weight among males[97] and higher adiposity among female neonates[97,98]. Other factors positively associated with fetal

growth include whether the infant is a singleton (rather than a twin, triplet, etc)[99], Caucasian ethnicity (versus Asian or other ethnicity)[98], and higher GA[98].

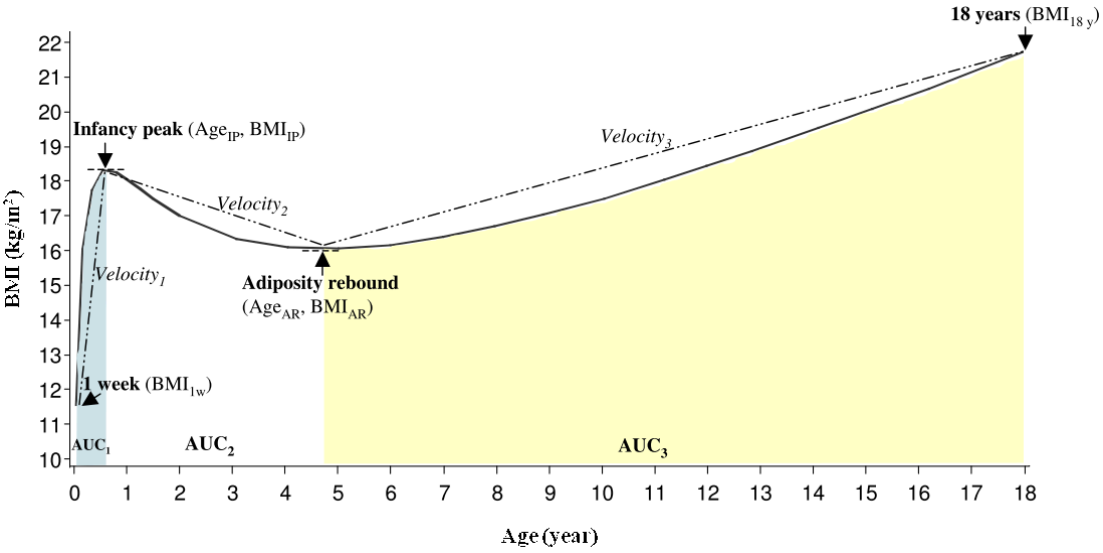
1.1.2.2 Postnatal (infancy and childhood) period

A large number of studies have reported the long-term effect of early growth on health both in child- and adulthood[5,61]. Growth monitoring during infancy and early childhood is therefore important to detect any significant growth deviations, be it rapid weight gain[64] and its increased later cardiometabolic risks, or stunting and the risk of reduced cognitive capacity[100]. However, to define the most desirable growth pattern is not easy for at least three reasons: 1) widely inter-individual variations between infants[67], 2) during infancy, there are also some rapid changes in body composition[67], and 3) there are diverse growth parameters and standards[101]. Besides that, although curves from the WHO growth standards and other national growth references represent the common growth patterns, the cut-off points used are based on statistical calculations of normality rather than evidence of adverse effects of growth deviations[102].

During the first few days after birth, infants will lose between 3-6% of their weight due to water loss and transient catabolism, which will then be regained within 10 days postnatally[103]. Afterwards until 4 months of age, infants will experience rapid growth velocity in both weight and length gains, although less pronounced in length. The rapid weight velocity is reflected by the peak of weight gain reaching 30-35 g/day between 1-2 months old[64,104]. This velocity then falls significantly and

the growth continues at a slower pace, steadily gaining 5-6 g/day until 5 years of age[104].

Figure 1.1. Typical childhood body mass index (BMI) trajectory
Retrieved under the Creative Commons Attribution licence from BMC Med Res Methodol (Wen X *et al*. *Childhood body mass index trajectories: Modeling, characterizing, pairwise correlations and socio-demographic predictors of trajectory characteristics*. BMC Med Res Methodol 2012[105])
AUC = area under the curve



As a result of those weight and length gains, there will be a rapid body mass index (BMI) gain resulting in BMI peak at 6-9 months of age[104,105], as shown in Figure 1.1. As length gain is more pronounced than weight after 12 months of age, BMI declines over the next few years and remains stable until reaching its lowest point at around 5-7 years of age. This point will quickly be followed by another period of rapid adiposity gain, termed as the adiposity rebound, and continue to increase further at puberty. The adulthood BMI is reached at around 18 years of age[105], although it does not necessarily remain stable throughout the adult life.

However, BMI is not an ideal marker of adiposity due to both fat and lean mass contributing to it. This limitation is particularly pronounced over the first year of life

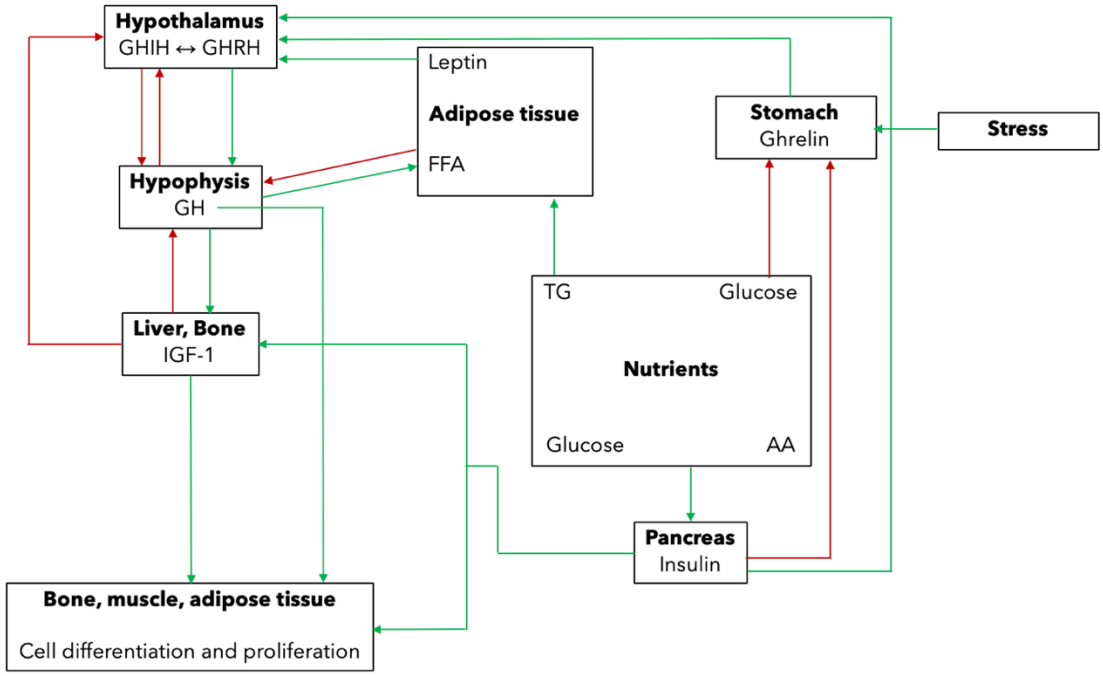
due to the large magnitude of changes in both fat and lean mass over this period of time. For example, fat mass contributes almost half the proportion of weight gain between birth to 4 months, but then falls to less than 10% by the age of 2 years[106]. This rapid fat deposition during early infancy may be due to high energy demands that happen shortly following birth, which aim to support the infant's survival and stable thermal regulation[107].

Endocrine factors also play an important role in infancy growth. The rapid growth observed during infancy is dependent on nutrition, which drives insulin and IGF-1 production. Afterwards, GH starts to regulate IGF-1 production and growth from 1 to 2 years of age[108]. Other hormones affecting growth include leptin and ghrelin (Figure 1.2).

Two hypothalamic hormones, growth hormone-releasing hormone (GHRH) and growth hormone-inhibiting hormone (GHIH, also called somatostatin), control the secretion of GH from the pituitary/hypophysis. Circulating GH stimulates IGF-1 production, mainly from the liver as the source of circulating IGF-1. Besides that, GH can also have direct effects on many target tissues, independent of IGF-1 action. Together with IGF-1, GH regulates GHRH concentration through negative feedback mechanisms. Ghrelin and leptin also act centrally to stimulate GH release. Macronutrient deficiencies cause lower circulating levels of ghrelin, leptin, IGF-1, and other related hormones, which affect linear growth directly[108].

In summary, although affected by the genetic constitution, optimal postnatal growth is also a product of adequate nutrition, good endocrine function, and the supporting environment.

Figure 1.2. Schematic representation of hormonal regulation on childhood growth
Summarised from Murray PG and Clayton PE. *Endocrine control of growth*. Am J Med Genet Part C Semin Med Genet 2013[108]
GHIH=growth hormone-inhibiting hormone, GHRH=growth hormone-releasing hormone, GH=growth hormone, IGF-1=insulin growth factor-1, FFA=free fatty acids, TG=triglycerides, AA=amino acids. Green and red lines represent positive and negative feedback, respectively.



Factors affecting postnatal growth

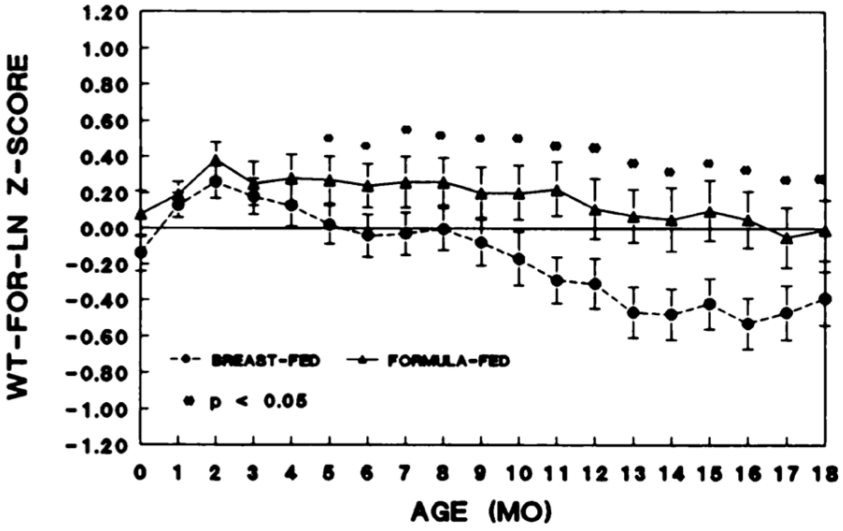
Birth weight (adjusted for GA) is a robust determinant of subsequent growth rate, at least during early postnatal period: smaller infants tend to catch-up[23,64,109], while heavier infants tend to catch-down[110]. However, this association is strongly affected by postnatal environment, i.e. in the case of poor postnatal nutritional provision, low birth weight is a strong predictor of stunting in the first 2 years due to unsupportive environment for a catch-up growth[111]. Similar to birth weight, fetal growth during the last trimester of pregnancy is also negatively associated with immediate postnatal weight gain[64,109,111,112].

GA and sex are important to consider in infancy growth evaluation. GA influences birth weight, neonatal adiposity, and postnatal growth evidenced by distinct features between term and pre-term infants. The gender effect on linear growth is also apparent as female infants grow more slowly than males in the first 6 months[109]. Male infants have higher fat-free mass (FFM) and lower fat mass (FM) compared to female infants[106,113]. Moreover, sexual dimorphism has also been observed to affect other environmental factors on growth, especially feeding mode. To those children born from overweight mothers, breastfeeding was reported to protect boys from childhood overweight more strongly than girls[114]. The greater infancy length gain due to infant formula feeding compared to breastfeeding was also more apparent in girls compared to boys[115].

Acknowledging the benefits of breastfeeding, WHO recommends infants to be exclusively breastfed for 6 months. Introduction of appropriate complementary food should follow after that with continued breastfeeding until 24 months[116]. Breastfed infants display remarkably different growth trajectories compared to formula-fed infants[112], with apparently faster weight and length gains in the first months[102,109], an earlier infant BMI peak[117], and then slower growth gains up to 2-3 years[102,109,112]. Formula-fed infants, on the contrary, perform slower growth gains in the beginning but with a constantly increasing pace, resulting in higher weight (both weight-for-age and weight-for-length z scores) compared to breastfed infants, observed as early as 6 months of age (Figure 1.3)[109,118]. Besides being heavier, infants fed with formula are also significantly fatter, characterised by larger skinfold thicknesses and body fat percentage from 9 months, persisting until 2 years of

age[118,119]. The typical growth performed by breastfed infants is considered as the healthier growth pattern.

Figure 1.3 Weight-for-length trajectories between breastfed and formula-fed infants
Reproduced with permission from Dewey KG *et al*. *Growth of breast-fed and formula-fed infants from 0 to 18 months: the DARLING study*. Pediatrics 1992[118]
This study was conducted among infants in California, the USA between the period of 1986-1991 and the result is still used as a classical reference to illustrate the distinguishing growth trajectories between breast- and formula-fed infants in the current literature.



The effect of moderation of growth rate provided by breastfeeding (especially prolonged and exclusive breastfeeding) seems to last for a long period[118–120] and protects against childhood overweight/obesity (Figure 1.4)[121], particularly among those with higher risks, e.g. born SGA/LGA and rapid weight gain in early infancy[122].

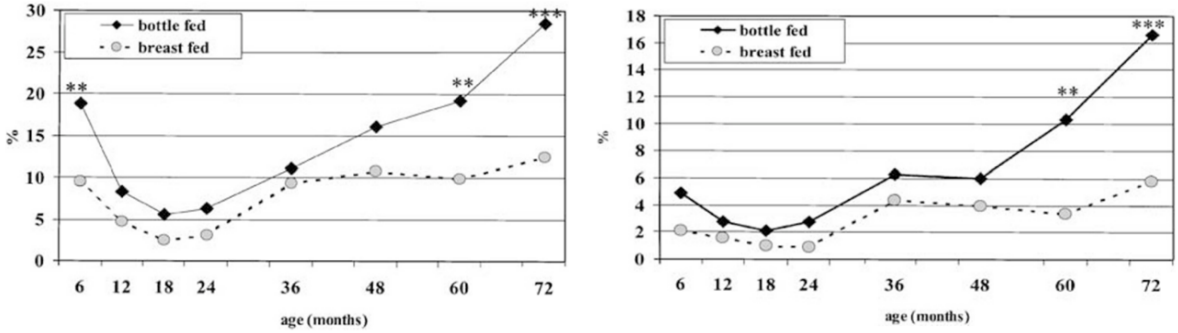
However, studies associating breastfeeding and infant growth are usually observational, relying mainly on maternal recall, and are confounded by many factors, including feeding behaviours, weaning, nutritional composition of complementary feeding, comorbidities, and maternal factors[61].

Figure 1.4 Prevalence of overweight (left) and obesity (right) from 6 months-6 years of age between breast- and formula-fed infants

Reproduced with permission from Bergmann KE *et al*. *Early determinants of childhood overweight and adiposity in a birth cohort study: role of breast-feeding*. Int J Obes 2003[121]

Overweight was defined as BMI >90th percentile of the reference values while obesity as >97th percentile of the reference values

Total number of subjects=480, all with complete measurements between 0-6 years. **p<0.01, ***p<0.001



Parental factors influencing infant growth including height, BMI, and ethnicity. Maternal height and BMI and paternal height are positively associated with weight velocity in early infancy[123]. In many cases, the influence of parental ethnicity on infancy growth disappears after controlling for socioeconomic factors[104]. For that reason, there is a high degree of similarity in linear growth across ethnically diverse populations seen in the large multicentre study which the WHO Multicentre Growth Standard was constructed from[124,125]. Maternal low birth weight[126], high plasma glucose level during pregnancy[123], and multiparity[109,123] are also associated with lower weight gain velocity during infancy.

1.2 What has been learnt from animal models of compromised *in utero* conditions

Studies in both small and large animal models have confirmed that compromised early nutrition increases long-term risks of obesity, insulin resistance and T2D, and other metabolic diseases[127]. In those animal studies, the role of nutrition was shown to be critical in all three developmental stages: early (implantation, placentation, and embryogenesis), mid (organogenesis), and late gestation (acceleration of fetal growth and adiposity)[128].

1.2.1 Animal models of SGA

In rodents, both moderate (50%) and severe (70%) calorie restriction during pregnancy lower offspring birth weight. Moderately calorie-restricted pups, with or without excessive postnatal feeding, demonstrated rapid catch-up growth that persisted in adulthood with greater fat mass compared to the non-restricted pups. Moreover, severely-restricted pups nursed by adequately-fed dams showed the signs of hyperphagia, which were linked to hyperinsulinaemia, hyperleptinaemia, hypertension, and obesity in adulthood[129].

Furthermore, studies on rats have also supported that a low nutrient environment during fetal development predetermine endocrine pancreas and insulin-sensitive tissues. Regardless of the type of diet, malnourished rat progenies were born with a defective beta cell population: fewer number of cells with inadequate insulin secretion and increased vulnerability to oxidative stress[130].

In sheep, early gestational undernutrition induced lower hepatic expression of gluconeogenic factors in the fetus and reduced in vivo adipose tissue insulin sensitivity in adulthood which might increase later-life obesity and dyslipidaemia risk[127]. This result was in line with McMillen and Robinson who discovered an association between inadequate gestational nutrition and disease vulnerability and decreased lifespan[131].

1.2.2Animal models of OGDM

To examine neonatal adiposity among OGDM, Oliveira *et al*. induced diabetes in pregnant rats using streptozotocin, which is toxic to pancreatic beta cells. Having excised and weighed the epididymal and subcutaneous adipose fat of the male offspring of diabetic mother rats, they found that offspring body weight, fat mass, and adipocytes diameter were higher than normal[132]. This study suggested that OGDM experienced metabolic programming in the adipose tissue, resulting in increased capacity to store lipids.

A recent animal study discovered that offspring of GDM rats were heavier, hyperglycaemic, and hyperinsulinaemic, compared to controls at term pregnancy. This finding suggested that glucose and insulin were important for fetal overgrowth in GDM. Interestingly, although increased triglycerides and cholesterol levels were found in the maternal circulation, fetal circulating triglyceride and cholesterol concentrations were unaltered in GDM rats[133]. This again suggests increased fat deposition in OGDM is an issue that requires future studies.

1.3 Being born SGA and its consequences

First-trimester fetal ultrasound is essential to obtain accurate GA as the basis of detecting IUGR, the most common cause of SGA[134]. However, it is important to note that not *all* IUGR will end up as SGA birth, as well as not *all* SGA infants may have experienced IUGR. SGA is diagnosed postnatally based on a specified cut-off point for each birth anthropometric measurement (hence SGA classifications include SGA for weight only, SGA for length only, or SGA for both), against reference data from a relevant general population[134]. This way of categorising SGA causes some growth-restricted infants who are born to large parents not to meet the SGA criteria at birth. In contrast, non-IUGR infants can be classified as SGA simply because they are constitutionally small at birth[134].

Moreover, cut-off points used vary between studies or populations. The European Society of Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) define SGA infants as infants with birth anthropometric measurements falling below the 10th percentile for their GA and gender[135]. Other SGA definitions use the 3rd percentile as the cut-off or more than 2 standard deviations below the mean[136,137].

1.3.1 Epidemiology and risk factors for SGA

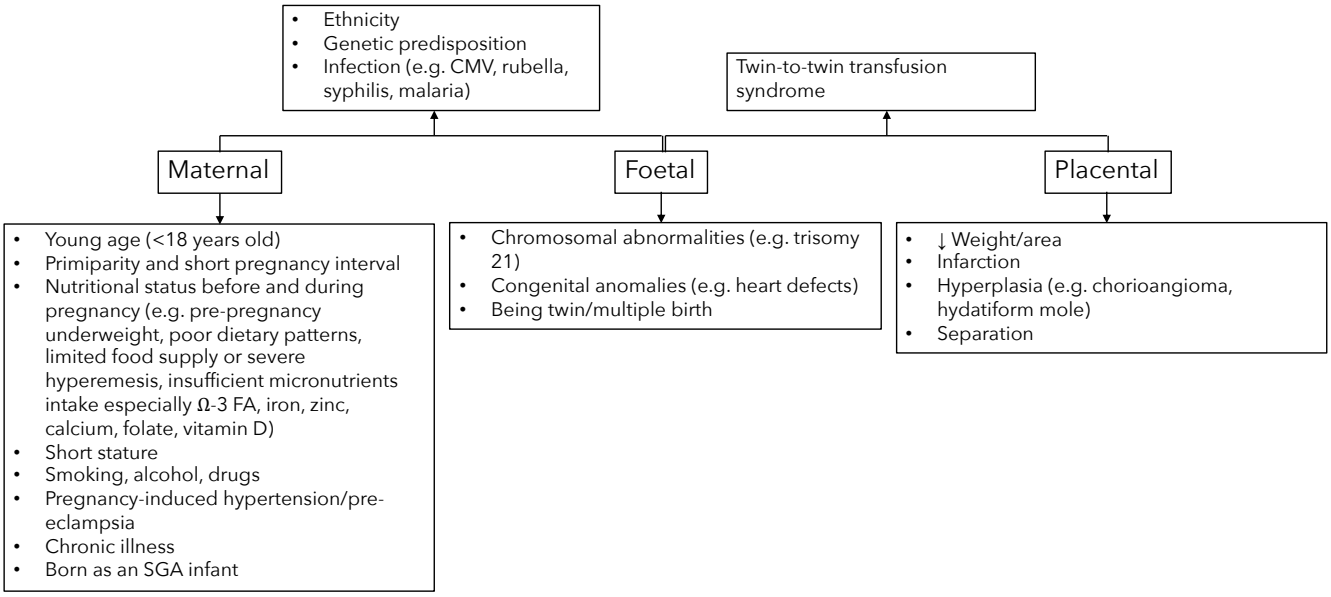
SGA is defined as being born smaller in size than normal for the gestational age. As mentioned previously, there are many cut-offs to determine SGA with the most common using birth weight below the 10th percentile for sex and GA[138]. Using that definition, SGA is more prevalent in low-to-middle income countries (LMIC) where its incidence is approaching 20%[138] compared to 10% or less in high-income

countries. Among the latter, the national SGA incidence is estimated to be 6% in the UK, 7-8% in the USA, and 10% in Australia[63]. In LMIC, SGA occurs in 1 every 5 live births with total number of 23.3 million infants, the majority of them are SGA at term. Of this number, up to two-thirds are born in sub-Saharan Africa and Asia, mainly in Nigeria, India, Pakistan, and Bangladesh[138].

Physically, SGA is considered to be symmetrical if the standardised values of weight, length, and head circumference all fall below the cut-off, or asymmetrical if the compromised value is only weight with normal length and head circumference values, thus represent preserved brain size and development. Consequently, the outcome of symmetrical SGA is generally poorer, due to failed brain-sparing[139]. While symmetrical SGA is more likely caused by inherent genetic/chromosomal factors or first-trimester placental insufficiency, asymmetrical SGA is mainly related to any interference to placental function or maternal health at later gestation[134].

The recognised aetiology and maternal, fetal, or placental risk factors for SGA delivery are listed in Figure 1.5.

Figure 1.5 Currently known SGA aetiology and risk factors
Summarised from Finken MJJ *et al*. *Children born small for gestational age: differential diagnosis, molecular genetic evaluation, and implications*, Endocr Rev 2018[134]



1.3.2 Negative implications of being born SGA

There are several adverse effects caused by being born SGA, both in the short term (childhood) and long term (adulthood), which may be influenced by GA (term- vs pre-term) and degree of IUGR experienced. In general, term SGA infants possess lower adverse health consequences compared to pre-term SGA infants[140].

Although the majority will catch-up, at least 10% of SGA infants display persistent sub-optimal growth until late childhood. These individuals are at increased risk of permanent short stature (adult height 1 SD below general population mean) most will not achieve their potential final height[134].

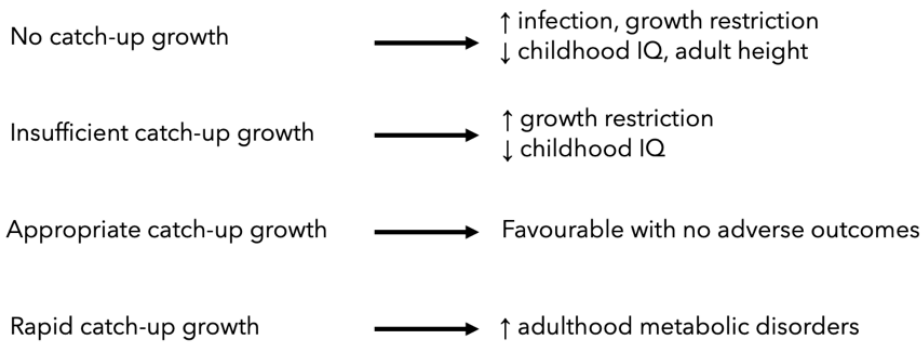
Children born SGA are also at higher risk of having impaired neurodevelopment and cognitive functions[135,141], especially among those who fail to catch-up in height and/or head circumference[142]. In the long term, this might affect their working performance leading to lower wages and lower likelihood to hold managerial positions. These individuals with poor catch-up growth are also more susceptible to infections in infancy and early childhood which are strongly intercorrelated with permanent growth restriction[141].

There are many studies associating SGA delivery and later adiposity outcomes. Kramer *et al* reported short stature and lower childhood adiposity among SGA compared to children born appropriate-for-gestational age (AGA), measured by BMI and total body fat percentage at age 6 and 11 years of age[53]. Interestingly, SGA with catch-up growth in the first 3-6 months had growth and adiposity measurements lying between SGA without catch-up and AGA children[53]. In contrast, Biosca found higher regional adiposity, both truncal and abdominal, measured by dual-energy X-ray absorptiometry (DXA) at age 6-10 years among individuals born SGA, compared to AGA[143]. These differences are most likely to be explained by differences in numbers of SGA infants who showed rapid catch-up growth as early as 4-6 weeks after birth[64]. Correspondingly, SGA, in particular those with rapid postnatal weight gain, would also possess a higher risk of adverse cardiometabolic outcomes, including cardiac remodelling[144] and insulin resistance leading to T2D risk[145,146]. This is evidenced by studies linking SGA with adulthood metabolic disorders, including obesity, T2D, CHD, and stroke, with higher risk among those who displayed rapid catch-up growth in the first months after birth[51,140,145,146].

Among girls, being born SGA followed by rapid catch-up growth increases their likelihood to have earlier puberty[146]. They also have a higher probability of giving birth to SGA infants themselves[134]. Another health challenge that has been linked to SGA is lower mineral bone content that can potentially lead to a higher risk of osteoporosis in the future[143].

In conclusion, although the definition of desirable growth trajectory in infants born SGA has not been yet established, it should consider *both* neurodevelopmental impairments caused by poor catch-up and long-term adverse cardiometabolic implications caused by excessive catch-up weight gain, and aim to find a good balance to achieve both favourable short- and long-terms outcomes (Figure 1.6).

Figure 1.6 All possible outcomes based on early infancy growth patterns among SGA infants



1.3.3 SGA, catch-up growth, and putative risk of T2D

As mentioned in the previous section, the prenatal and early postnatal periods are both critical in determining the long-term risk of T2D: *both* birth size and early-life weight gain pattern play a major role[147]. Combining both, the highest risk is found among infants who experience a history of IUGR *and* gain weight rapidly during early infancy[24]. This catch-up trend, which is experienced by 90% of infants born

SGA, usually begins by increasing subcutaneous fat deposition and may be linked to higher central adiposity[145].

Compared to preterm SGA, SGA at term are more likely to perform catch-up growth, especially in early infancy[21], and achieve normal-range anthropometric measurements. Ethnicity[141], genetic constitution, chromosomal abnormalities, inherent birth defects[23], the timing of onset of IUGR[139], and postnatal feeding history[23,141] will also affect an SGA individuals chances to develop catch-up growth postnatally.

This catch-up growth pattern is strongly related to endocrine function, especially leptin and IGF-1[145]. Low cord blood leptin measured at birth could provide an accurate prediction for catch-up growth postnatally[23]. Of all hormones, IGF-1 is the most studied hormone in association with SGA.

As shown in Figure 1.7, rapid catch-up growth during infancy will alter infant body composition by increasing fat deposition and body fat percentage, and this can continue through childhood. At 5 years old, such SGA children were found to have higher waist circumference, BMI, body fat percentage, fat mass, and insulin secretion[51] compared to the non-catch-up counterparts. Moreover, the link between early catch-up with later adiposity is also existent among AGA. The Stockholm Weight Development Study reported that infants with rapid weight gain during the first 6 months, had greater body fat percentage at age 17 years, independent of childhood weight gain, maternal size, and social factors[148].

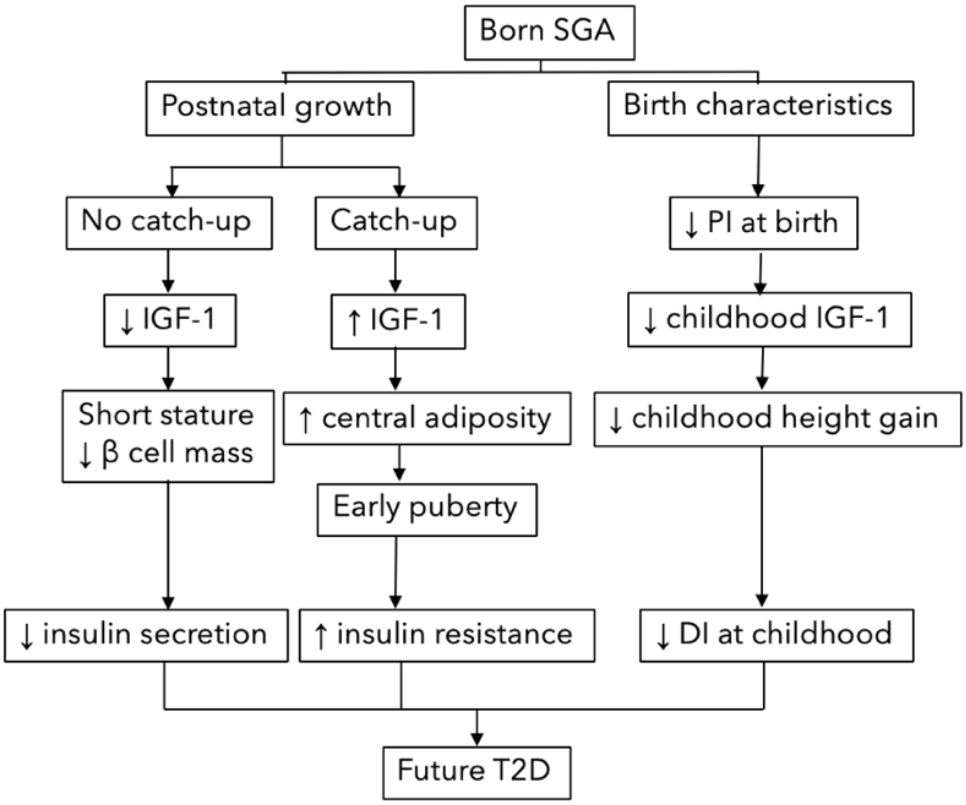
Previously, the ALSPAC study also reported that rapid catch-up weight gain in the first year of life was strongly related to higher insulin resistance and central

abdominal fat mass at the age of 8 years[50]. However, insulin secretion was more strongly and positively associated with birth ponderal index (PI) than the first-year weight gain. PI is considered as a more accurate body composition measure than BMI in infants, calculated by dividing body weight by the third power of body height. Disposition index (DI) is a marker of the amount of insulin secretion in response to the increased insulin resistance (IR). Among 8-year old children, those with the lowest PI at birth had also the lowest DI[22], possibly mediated by low height gain and IGF-1 levels (Figure 1.7).

Despite the substantial evidence linking catch-up among SGA and insulin resistance, it does not mean that non-catch-up SGA infants have lower risk of T2D. Among these individuals who do not display catch-up growth but remain small with reduced statural growth, later adverse metabolic consequences still exist if they become obese during adult life, possibly due to reduced pancreatic beta cell mass and insulin secretion (Figure 1.7). Studies on children and adults have found that beta cell mass was more related to height rather than adiposity gains[149,150].

With regard to feeding, breastfeeding is reported to lead to better neurodevelopmental outcomes, with head circumference gain catch-up at 3 months and higher cognitive scores at 18 months[151], compared to formula feeding. Breastfeeding for at least 24 weeks has also been linked with lower risk of impaired neurodevelopment in SGA infants[152].

Figure 1.7 The putative pathway between SGA and later T2D risk
Summarised from Dunger DB *et al*. *Early childhood contributions to insulin resistance*. Humana Press 2008[22]



Moreover, breastfed term SGA infants were reported to have more normal fat deposition and insulin sensitivity compared to their formula-fed counterparts, especially if the formula was protein-, fat-, and energy-enriched. High-protein and/or high-fat formula contributed to faster weight gain among term SGA infants and this would lead to both increased fat mass and blood pressure during childhood[153,154]. This may suggest that it is better to use the standard formula to feed term SGA infants, rather than the nutrient-enriched formula, if breastfeeding is not possible.

1.4 Gestational diabetes mellitus

It is established that insulin sensitivity decreases during pregnancy and is accompanied by compensatory increased insulin secretion from maternal pancreatic beta cells. As the pregnancy develops, reduction in insulin action also deteriorates, with insulin sensitivity declining up to 80% by the end of the third trimester[155].

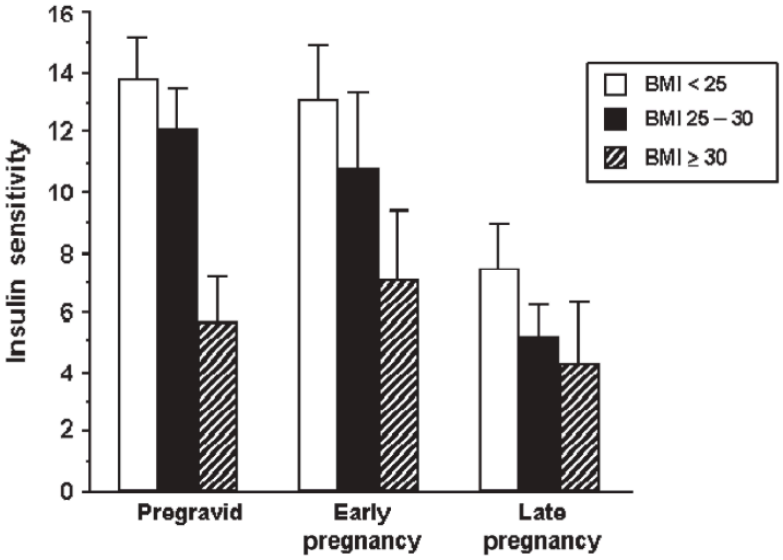
This phenomenon is exacerbated by maternal overweight and obesity (Figure 1.8). With this insulin-resistant state, there are changes in maternal energy metabolism and placental hormonal secretion. The ultimate goal is to enable more maternal use of lipids than carbohydrates to assure fetal-sufficient glucose supply[156]. Insulin action typically normalises after the baby is born[156].

In GDM, in contrast to normal pregnancy, insulin resistance is not followed by sufficient increased compensatory insulin secretion. By definition, GDM is "any degree of glucose intolerance that first recognised during pregnancy", although women with undiagnosed, pre-existing diabetes should be excluded from this definition[157], which is not always possible. Abnormal insulin action among GDM mothers usually normalises following delivery. However, GDM women retain a higher risk of developing T2D compared to those women without GDM in pregnancy[158].

Figure 1.8 Progressively declining insulin sensitivity before and during pregnancy across BMI categories

Reproduced with permission from Catalano PM and Ehrenberg HM. *The short- and long-term implications of maternal obesity on the mother and her offspring*. BJOG 2006[159]

Pregravid=before conception, early pregnancy=12-14 weeks, late pregnancy=34-36 weeks



As far as we are aware, the first case of GDM, recorded in 1824 in Berlin, was described as a 22-year old pregnant woman with an insatiable thirst who strenuously delivered a 12-pound stillbirth baby[160]. More than a century later, in 1954 Hoet studied impaired glucose tolerance during pregnancy, which started to resolve one month after delivery, as well as the outcomes of offspring born of diabetic mothers (OGDM), resulting in miscarriages, stillbirth, or large-born infants[161]. The first GDM diagnostic criteria were formulated around a decade later by O'Sullivan *et al* who conducted a 3-hour 100 g oral glucose tolerance test (OGTT) on 752 subjects at various stages of pregnancy (Figure 1.9 and Table 1.3)[162].

There are many negative implications of GDM, both to mothers and infants. Although OGDM typically have large birth weight and subsequently display catch-

down weight gain during infancy, OGDM with normal birth weight are still prone to having poor glycaemic control later in life. This suggests that body composition, rather than birth weight alone, may better predict growth patterns, biochemical changes, or the observed metabolic abnormalities. Furthermore, numerous studies have associated GDM with subsequent obesity and T2D risks in the offspring[163–165] although the mechanism is not yet completely understood. Glucose exposure during pregnancy, transfer of genetic predisposition to T2D, or transmission of unhealthy lifestyles are plausible pathways.

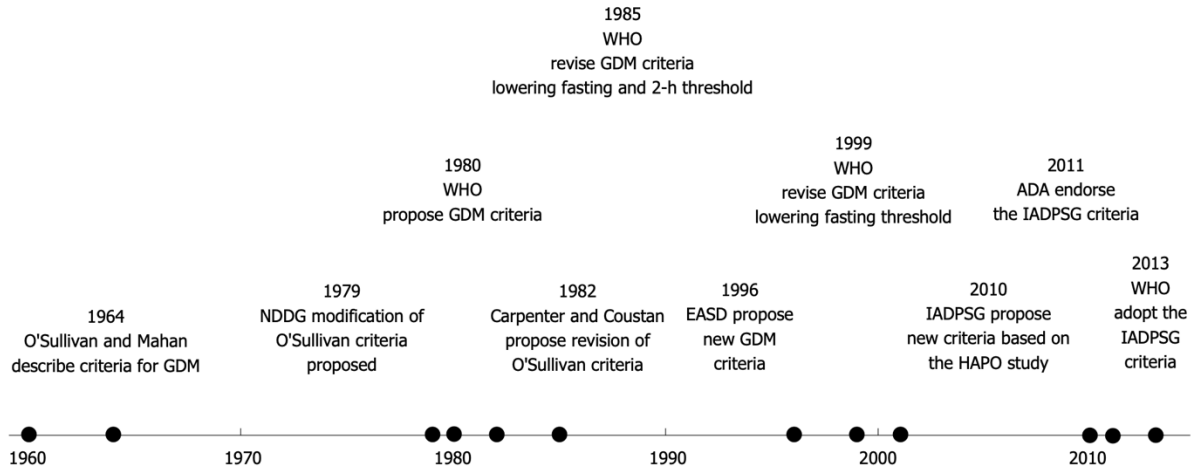
1.4.1 GDM screening and diagnostic criteria

Although being regarded as one of the most common complications during pregnancy, international agreement on GDM screening and diagnostic criteria are still subject of debate. There has been argument regarding whether OGTT screening should be universally applied to all pregnant women or only to the high-risk group[166]. Yet there has been no consensus on the methods or the timing of screening including one-step OGTT versus a two-step procedure with an initial 50 g oral glucose loading, OGTT dose (75 versus 100 g), and OGTT duration[166]. Besides, GDM diagnostic criteria have undergone several alterations over the years (Figure 1.9), attempting to improve the identification of diabetes in early pregnancy[167,168].

As shown in Figure 1.9, the first criteria were proposed by O'Sullivan *et al*[169]. These criteria were derived mathematically and were validated against mother's future diabetes risk rather than pregnancy outcomes[162]. Using the same OGTT duration (3

hours) and amount of glucose load (100 g), the National Diabetes Data Group (NDDG) proposed different thresholds in 1979 after converting O'Sullivan criteria from whole blood to plasma values[157]. Carpenter and Coustan[170] did the same in 1982, additionally allowing for the enzymatic methods[162]. The comparison of these two modified versions of O'Sullivan criteria resulted in 50% higher GDM prevalence if the Carpenter and Coustan criteria were used[162].

Figure 1.9 Changing criteria used to diagnose GDM from 1964-present



In addition to the commonly used OGTT method at the time, the American Diabetes Association (ADA) recommended a 75 g glucose load for 2 hours using the same thresholds as the longer 3 hours method, endorsing the Carpenter and Coustan criteria (Table 1.3). However, it was discovered that the use of lower glucose load resulted in 0.9 and 0.5 mmol/L lower 1-h and 2-h glucose levels, respectively[171].

Meanwhile, WHO in 1980 proposed GDM diagnostic criteria to follow the diabetes criteria used in the non-pregnant population, which were later revised into more stringent thresholds in 1985 and 1999[162]. In 1996, having studied these criteria in

1,000 Caucasian women, the European Association for the Study of Diabetes (EASD) proposed its own criteria, consisting of fasting and 2-h post-prandial glucose levels only[162].

Aside from the criteria shown in Table 1.3, there are other different criteria used either by adopting older criteria or from studies conducted locally. There can even be more than one criterion being applied in the same region or even the same country. However, none of the criteria had been designed specifically to predict the adverse pregnancy outcomes in the offspring.

Table 1.3 Different criteria and thresholds used to diagnose GDM

NDDG=National Diabetes Data Group, WHO=World Health Organization, EASD=European Association for the Study of Diabetes, ADA=American Diabetes Association; IADPSG=International Association of Diabetes and Pregnancy Study Group, HAPO=Hyperglycaemia And Pregnancy Outcomes Study

Criteria	Glucose load	Fasting glucose mmol/L (mg/dL)	1-h glucose mmol/L (mg/dL)	2-h glucose mmol/L (mg/dL)	3-h glucose mmol/L (mg/dL)	No. of criteria required
O'Sullivan *et al*[4]	100 g	5 (90)	9.2 (165)	8.1 (145)	6.9 (125)	⩾ 2
NDDG	100 g	5.8 (105)	10.6 (190)	9.2 (165)	8.1 (145)	⩾ 2
WHO 1980	75 g	8 (144)	N/A	8 (144)	N/A	⩾ 1
Carpenter and Coustan	100 g	5.3 (95)	10 (180)	8.6 (155)	7.8 (140)	⩾ 2
ADA	75 g or 100 g	5.3 (95)	10 (180)	8.6 (155)	7.8 (140)	⩾ 2
WHO 1985	75 g	7.8 (140)	N/A	7.8 (140)	N/A	⩾ 1
EASD	75 g	6 (108)	N/A	9 (162)	N/A	⩾ 1
WHO 1999	75 g	7 (126)	N/A	7.8 (140)	N/A	⩾ 1
IADPSG GDM	75 g	5.1 (92)	10 (180)	8.5 (153)	N/A	⩾ 1
IADPSG overt diabetes	[a]None/75 g	7 (126)	N/A	11.1 (200)	N/A	⩾ 1

Figure 1.9 and Table 1.5 were retrieved under the Creative Commons Attribution Non Commercial licence from Noctor E and Dunne FP. *Type 2 diabetes after gestational diabetes: the influence of changing diagnostic criteria.* World J Diabetes 2015[162]

A large multicentre study, the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO), was conducted to address this issue. Involving more than 23,000 pregnant women across nine countries, the HAPO study showed that maternal glycaemia was linearly related to adverse neonatal outcomes, including higher birth weight, cord blood C-peptide level, risk of Caesarean section delivery, and neonatal hypoglycaemia[81]. Based on those adverse outcomes, HAPO resulted in new and more stringent criteria for universal GDM screening endorsed by the International

Association of the Diabetes and Pregnancy Study Group (IADPSG). These guidelines recommend glucose testing in early pregnancy to detect overt pre-existing diabetes, followed by a 75 g OGTT at 24-28 weeks among those with normal initial screening. GDM is diagnosed if a single glucose value exceeds 5.1, 10.0, or 8.5 mmol/L at 0, 60, or 120 minutes, respectively[172]. Although not substantially different from the ADA guidelines[166], the prevalence of GDM is higher if IADPSG criteria were used. This is because ADA uses a 100 g OGTT and requires 2 elevated readings with higher threshold, whereas IADPSG uses the 75 g load and requires only one abnormal value with lower threshold[168].

Because of its lower threshold for fasting glucose level, the application of IADPSG criteria has resulted in expectedly increased prevalence rates of GDM. In parallel, treatment for GDM has also become more aggressive over recent years, including diet and lifestyle modification with/without medication agents, insulin and metformin. This is also because several recent randomised trials reported decreased risk of perinatal complications of offspring born of pregnant mothers with hyperglycaemia not meeting GDM and T2D diagnostic criteria[173].

Although the application of IADPSG thresholds has been reported to be cost-effective in improving pregnancy outcomes[174], there have been some concerns that these more stringent recommendations are potentially harmful to the offspring by increasing the risk of maternal hypoglycaemia and poor fetal growth[175].

1.4.2 Epidemiology and risk factors of GDM

The global prevalence of obesity and T2D have increased in the recent decades[176]. This also applies in the UK with the number of obese adults estimated to reach 26 million in the next 15 years while the prevalence of diabetes would rise to 5 million by 2025 from 3.8 million in 2018[177]. Mirroring these phenomena, the rate of diabetes during pregnancy is also rising. Of the 5% of pregnant mothers with diabetes in the UK, 87.5% have GDM, 7.5% have type 1 diabetes (T1D), and 5% have pre-existing T2D[178]. GDM prevalence in the UK used to be much lower but doubled between 1996 to 2004[179]. Historically the prevalence was between 1-3% but since 2010, it ranges between 8-24%, presumably affected in part by the application of the new guidelines[180].

GDM prevalence varies worldwide and depends on ethnicity and female obesity rate, the GDM diagnostic criteria, and the OGTT screening strategy being applied[181]. However, since there are no uniform diagnostic criteria applied internationally, it is difficult to estimate the global incidence of GDM[166]

Zhu and Zhang in 2016 reported the estimated GDM prevalence of country-specific and WHO region by using the median values of available sources[182]. As illustrated in Figure 1.10, GDM was most prevalent in the Middle East and North Africa region at 13%, followed by Southeast Asia, Western Pacific, South and Central America, Africa, and North America and the Caribbean. The GDM prevalence in Europe was found to be the lowest with a median estimate of 6%[182].

Figure 1.10 GDM prevalence (%) in 2005-2015 based on WHO region
Values are in median(interquartile range)

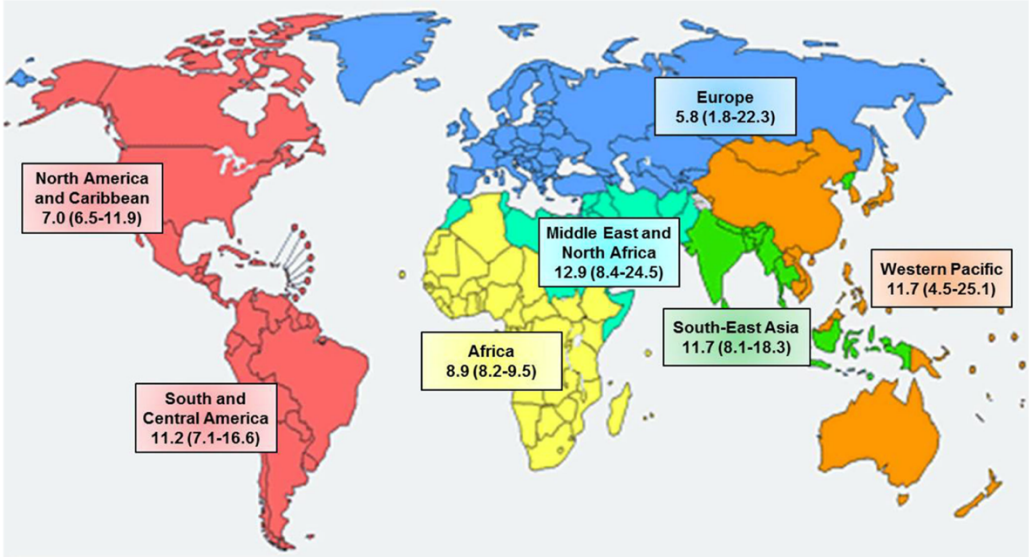


Figure 1.11 GDM prevalence based on diagnostic criteria applied
WHO=World Health Organization, NDDG=National Diabetes Data Group, C&C=Carpenter and Coustan, IADPSG=International Association of Diabetes and Pregnancy Study Group

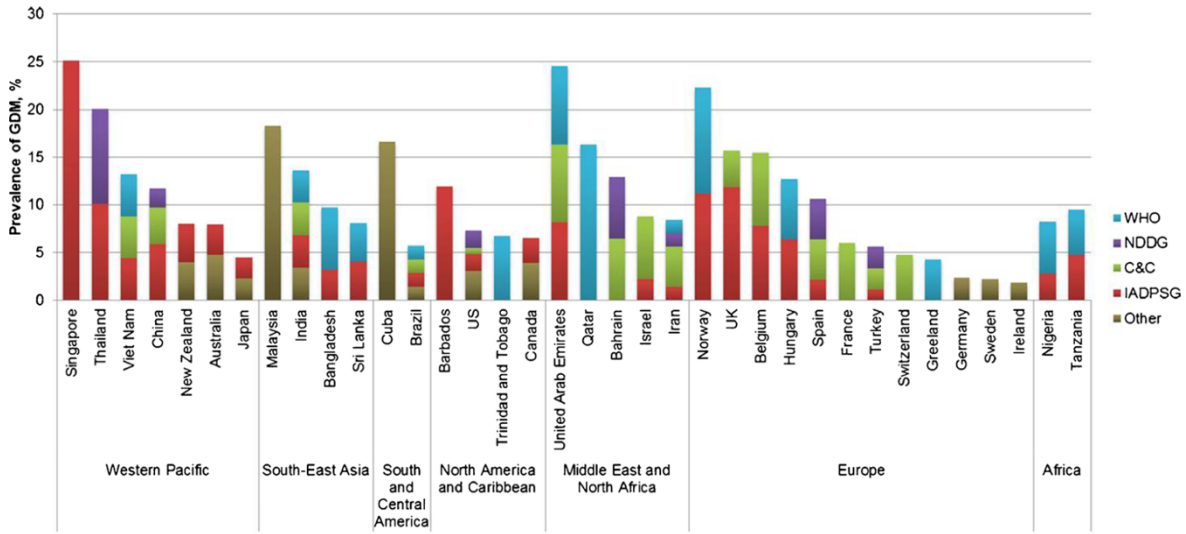


Figure 1.10 and Figure 1.11 were reproduced with permission from Zhu Y and Zhang C. *Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective*. Curr Diab Rep 2016[182]

Given that GDM prevalence is contingent upon the diagnostic criteria applied, Figure 1.11 shows the potential variations in GDM prevalence between countries in the same region, as well as within countries applying different criteria. Therefore, interpreting national or regional GDM prevalence should take its applied criteria into consideration[182].

As mentioned in the previous section, GDM is caused by an inadequate pancreatic beta cells response to increased insulin requirements during pregnancy. This key pathophysiological feature resembles T2D and therefore GDM and T2D share several inherent risk factors, including older age, diabetes family history, and ethnic origin (higher rates in South Asian, Middle Eastern, and black Caribbean)[165]. Genome-wide association studies (GWAS) have identified some candidate genes for GDM which are also associated with T2D. This shared common genetic architecture between GDM and T2D could plausibly explain the higher T2D risk among women with GDM history[183].

Obesity is the most prominent modifiable risk factor for GDM. A meta-analysis involving 20 studies conducted internationally reported that overweight, obese, and severely obese women had an over two-fold, three-fold, and eight-fold higher of risk of developing GDM, respectively, compared with normal weight pregnant mothers[184]. A large-scale population-based questionnaire study involving more than 23,000 people in the US illustrated how GDM risk rose as pre-pregnancy BMI increased (Figure 1.12)[185]. In addition, gestational weight gain of more than 0.27 kg per week was also associated with increased GDM risk (odds ratio 1.43 for 0.27-0.4 kg/week and 1.74 for $\geq$0.41 kg/week), especially if obtained in the first trimester, independent of age at delivery, ethnicity, and pre-pregnancy BMI[186].

Cigarette smoking is another modifiable GDM risk factor, relating to not only the individual's smoking history, but also to their parents'. Bao *et al*. from the large Nurses' Health Study II reported that maternal, but not paternal, heavy smoking history (at least 25 cigarettes a day) during pregnancy increased GDM risk among their daughters[187]. Other environmental factors are listed in Table 1.4.

Figure 1.12 GDM probability by maternal pre-pregnancy BMI

Reproduced with permission from Kim SY *et al*. *Percentage of gestational diabetes mellitus attributable to overweight and obesity*. Am J Public Health 2010[185]

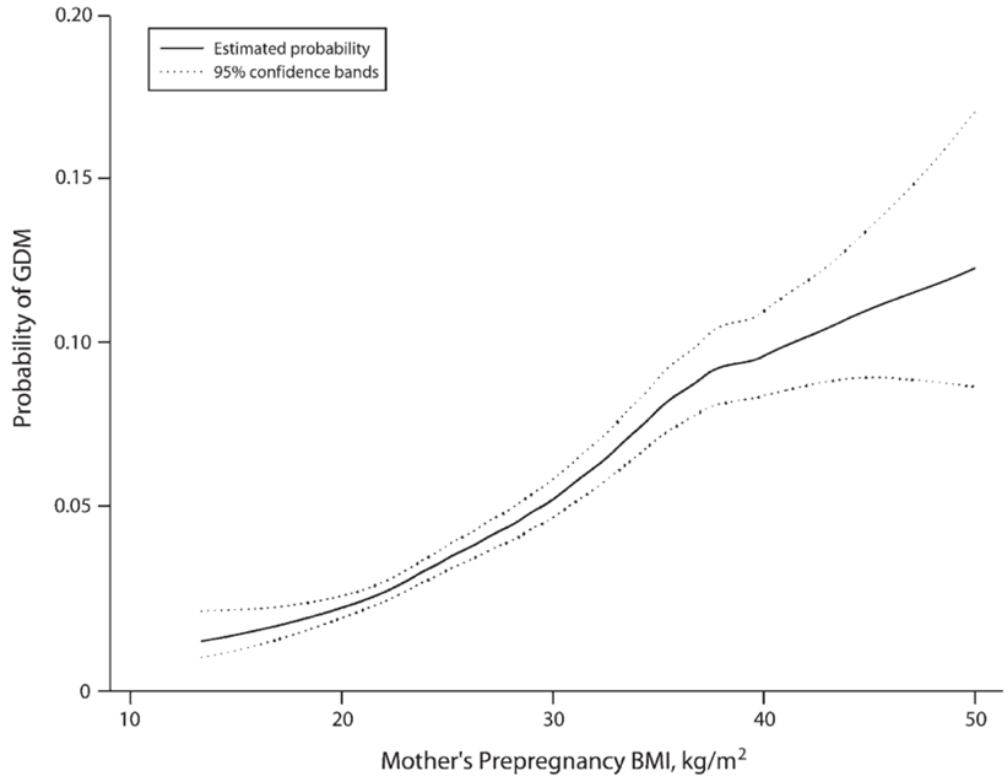


Table 1.4 Environmental risk factors of GDM

Summarised from Zhang C *et al*. *Risk factors for gestational diabetes: is prevention possible?* Diabetologia 2016[181] and Zhang C and Ning Y. *Effect of dietary and lifestyle factors on the risk of gestational diabetes: review of epidemiologic evidence*. Am J Clin Nutr 2011[188]

Environmental factors	Description	Association with GDM risk
Physical activity	↑ Frequency of mild or recreational physical exercise before and/or during pregnancy	↓
	Duration of physical exercise, especially vigorous activity	↓
Diet	↑ Plasma vitamin D and C concentrations in early pregnancy and dietary fat intake during pregnancy	↓
	↑ Sugar intake; highly processed food for example fried foods, processed meat, refined grain products, fast food; high intake of red meat; great potato consumption	↑
	↑ Intake of fruit, green leafy vegetables, poultry, fish, Mediterranean diet, nut, fibre	↓
Others		
PFOA	↑ Endocrine disruptor, found in cooking utensils, microwave bags, cleaning liquids	↑
Cigarette smoking	↑ Individual and maternal smoking history	↑

1.4.3 Negative implications of GDM

As a growing public health concern, GDM has been associated with short- and long-term adverse health events for both mothers and offspring. For the mothers, GDM has been linked with a higher risk of preeclampsia and polyhydramnios as pregnancy comorbidities, and higher metabolic risks after pregnancy, including T2D and cardiovascular diseases[165,181]. Bellamy *et al* reported in their meta-analysis that women with GDM had seven times higher subsequent T2D risk compared to those with normoglycaemic pregnancy[189]. In addition, their likelihood to develop recurrent GDM in the next pregnancy was as high as 48%[190].

Similarly, GDM can cause immediate and long-term negative implications for infants. The OGDM is typically macrosomic and this can lead to various birth complications, for example, preterm birth, birth injury, shoulder dystocia, higher

chance of Caesarean section, neonatal hypoglycaemia and hyperbilirubinaemia, neonatal respiratory distress and admission to the neonatal intensive care unit[165].

Later in life, OGDM potentially develop obesity, both in child- and adulthood, impaired glucose tolerance, insulin resistance, and T2D, hypertension and other cardiovascular disorders[165,181]. GDM has also been associated with autism and lower cognitive function in the offspring, although the evidence is inconclusive[181].

Despite all these studies, much is still to be discovered about infancy growth patterns in OGDM, as well as how feeding practice and breast milk (BM) composition impact their growth and the underlying hormonal and metabolic changes.

1.5 The importance of biomarkers in infant growth study

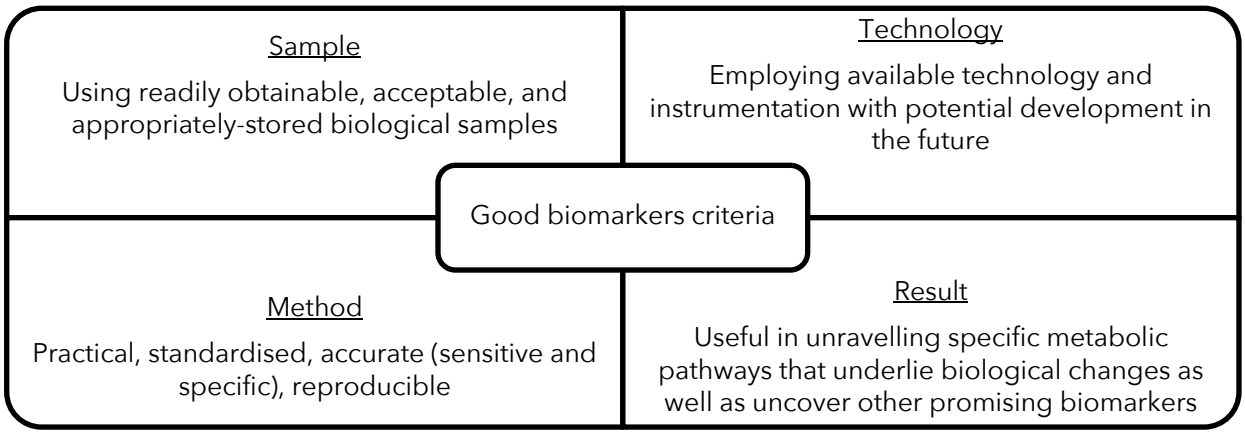
The robust link between early life and later health has provided a window of opportunity to detect individuals at risk of later adverse metabolic outcomes as early as possible, for example by capturing biomarkers during infancy that could predict future growth and body composition trajectories. These potential biochemical markers can also identify early nutritional variation and therefore can help in the development of either future targeted nutritional-based intervention for high-risk infants or general primary prevention used for public health policy. Therefore, studies to investigate or identify biomarkers are scientifically, clinically, and epidemiologically important.

By definition, a biomarker is defined as 'a biological characteristics that can be objectively measured and that serves as an indicator of normal biological processes, or responses to therapeutic intervention'[191]. There are several different types of biomarkers as listed in Table 1.5. Across all these types, to be considered good and valid, biomarkers need to fulfil some criteria from the perspectives of 1) sample source, 2) technology employed, 3) measurement method, and 4) the quality of the results (Figure 1.13)[192].

Table 1.5 Biomarker studies in this thesis based on its type[191]

Type	Example	Used in this study	Resulting publications
Physical measures	Anthropometric indices	Anthropometry: weight, length, head circumference Body composition: BMI, PI, skinfold thickness, estimated fat- and fat-free mass (general and abdominal) (Chapter 3 and 4 of this thesis)	Reduced size at birth and persisting reductions in adiposity in recent, compared with earlier, cohorts of OGDM[193]
Genetic traits	Gene polymorphisms	*Fucosyltransferase 2 (FUT2)* polymorphism (Chapter 8)	
Biochemical analytes	Hormonal measurements, metabolomics	• Hormones: IGF-1, C peptide (Chapter 5) • Lipidomics (Chapter 6) • Breastmilk study: macronutrient, butyrate (representing short-chain fatty acids), human milk oligosaccharides; all as concentration and intake (Chapter 7)	Evidence from 3-month-old infants shows that a combination of postnatal feeding and exposures in utero shape lipid metabolism[194] Lipid ratios representing SCD1, FADS1, and FADS2 activities as candidate biomarkers of early growth and adiposity[195]
Physiological functions	Cognitive assessment, morbidity records	Antenatal history (used as covariates in all chapters), infancy morbidity records (used as covariates in Chapter 7 and 8), infant development monitoring (as focusing more on infant growth and body composition, development assessment is not included in the scope of this thesis)	

Figure 1.13 Criteria of good biomarkers[192]



1.5.1 Biomarker studies involved in this thesis

1.5.1.1 Anthropometric measurements

Measures of infant anthropometry have been used traditionally as biomarkers for later growth. Inadequate weight gain among children with nutritional deficiency, for instance, can be used as a proxy to indicate later retarded statural growth[196]. In this study, weight, height, and weight for height indices (BMI and PI) are measured at each visit, along with head circumference that is representative of brain development[197].

Since those measurements are unable to distinguish between fat- and lean mass, several procedures were conducted to estimate body composition, including skinfold thickness, subcutaneous and medial abdominal fat thickness via ultrasound, and general fat- and fat-free mass based on air-displacement plethysmography (ADP) system. Detailed anthropometric measurements are displayed in Chapter 2.

However, complex body composition assessment in childhood is problematic because the reference is scarce and the resulting value needs to be standardised by age, gender, and size (weight or height). Besides, although the ADP system is an appealing method to estimate fat- and fat-free mass during infancy, it can only calculate body composition for infants up to 8 kg, and therefore, longitudinal evaluation is not possible. For that reason, reliance on simple and repeated assessment, eg skinfold thickness, is necessary and important for longitudinal studies. Abdominal ultrasound can also produce valuable approximation for visceral and subcutaneous abdominal fat mass. This method has also been validated and shown a good correlation with MRI[198].

1.5.1.2 Biochemical analyses

In this study, serial measurement of anthropometry and body composition were linked to blood biomarkers. Thus far, there are limited biomarkers investigated to reflect metabolic, growth, and nutritional status during early life as well as to understand the mechanistic pathways between nutrition and growth. These available biomarkers are also considered not sensitive to rapid change in growth and development, which is the hallmark of infancy period[199].

Biochemical assays run in this study included hormones (IGF-1 and C-peptide) and lipidomics from dried blood spots (DBS) samples. DBS is superior in research involving paediatric population since it only requires collecting some drops of blood onto filter paper, can be conducted by heel-prick, and therefore could minimise distress to children and parents.

IGF-1 is the major mediator of pre- and postnatal growth and therefore was reported higher among infants who gained greater weight, length, and adiposity between 3-12 months of age[200]. Meanwhile, C-peptide is a connecting polypeptide between insulin chains in the proinsulin molecule. C-peptide has been associated with birth weight and early infancy weight gain[201].

Lipidomics was chosen across other -omics experimentation for several reasons. First, variation in lipid metabolism during infancy has been associated with subsequent weight gain[202]. Second, from the previous study, lipidomics provided a more valid assay and more significant associations to infant growth parameters than metabolomics[203]. Third, lipidomics is superior in this case compared to proteomics because proteins are highly modifiable and therefore difficult to be identified.

1.5.1.3 Breast milk study

If anthropometric and biochemical analytes were compared across 3 groups of infants (SGA, OGDM, and controls) in this thesis, breast milk analyses were conducted among controls only due to sampling availability (more details in Chapter 2).

Numerous studies have reported different growth trajectories between breast- and formula-fed infants with growth patterns performed by breastfed infants considered as desirable, with more gain during early infancy period, followed by slower gain during later infancy period[153,154]. However, these breastfed infant growth patterns, while widely considered to be optimal, show wide inter-individual differences that presumably are influenced by macronutrient content of human breast milk[153,154].

However, findings between studies are inconsistent[204–206], possibly because macronutrient content may not reflect the real amount consumed by infants (intake).

Breast milk study in this thesis was conducted to examine the associations between both breast milk macronutrient content as well as intake with infant growth and adiposity.

1.5.1.4 *FUT2* genotyping exploratory study

In the past few decades, studies have been conducted to investigate the influence of *Fucosyltransferase 2* (*FUT2*) gene polymorphism on many diseases[207]. This polymorphism results in homo/heterozygous *FUT2 secretor* and homozygous *FUT2 non-secretor*. While secretors (*Se*) carry at least one functional allele of *FUT2* to enable ABH expression on body secretions, non-secretors (*se*) carry two non-functional *FUT2* alleles and therefore are unable to present ABH antigens in secretions and on epithelial cells[208].

FUT2 status determines an individual's susceptibility to several infections[209–211] and autoimmune diseases[212,213], affects gut microbiota[214], and is involved in defining human milk oligosaccharides (HMO) among lactating mothers[215]. HMO are the third-largest solid constituent in breast milk, consisting of indigestible complex carbohydrates that may possibly act as prebiotics for infant gut microbiota[216].

FUT2 studies in this thesis focused on exploring the associations between the gene polymorphism, both maternal (via HMO) and infant, with early life outcomes including overall infant's health, growth, and body composition.

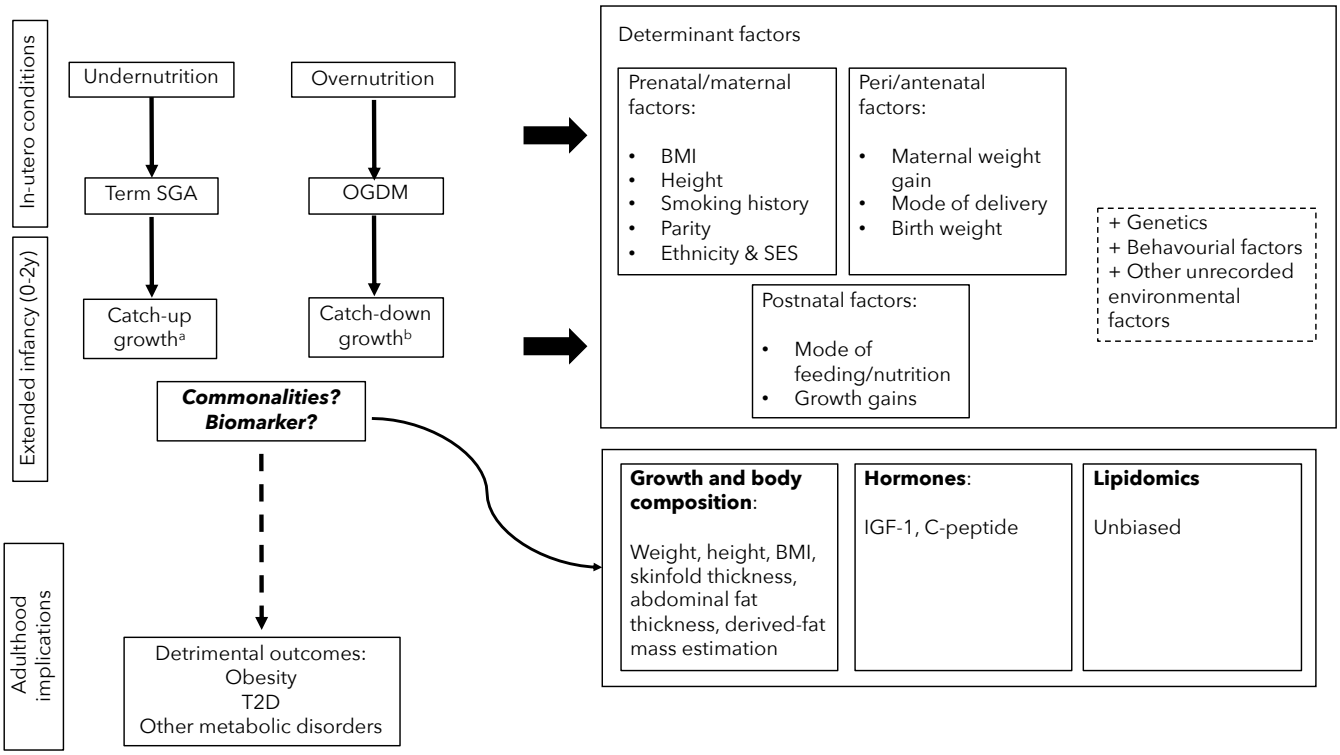
1.6 Aims and questions

Despite having distinct almost opposing *in utero* conditions, both SGA and OGDM acquire similar increased risks for future cardio-metabolic disease. In contrast to the well-established SGA growth trajectory, i.e. undernutrition during pregnancy followed by rapid weight gain during infancy leading to obesity and related adverse metabolic consequences in child- and adulthood, the pathway of OGDM to obesity and T2D is not well-understood. In both cases, longitudinal studies can help to provide a clearer understanding of plausible pathways and underlying mechanisms, and therefore, to inform strategies to prevent later-life metabolic adversities.

Since both SGA and OGDM have relatively higher risks of similar metabolic outcomes in the future, it is plausible to hypothesis that they might also share similar metabolic derangements early in life. Understanding of these early life changes will help in discovering potential shared biomarkers of future metabolic disease and in informing interventions during critical early windows of developmental plasticity.

Figure 1.14 describes the focus of this thesis. SGA and ODGM were recruited at birth and then were monitored and were separately compared to the same control group. Three major comparative factors were studied: growth and body composition, the circulating hormonal milieu, and unbiased lipidomics. The 'determinant factors' boxes list the potential confounding factors that were included in the analyses in this thesis, collected directly or from parental questionnaires. Factors listed in the dashed box are also plausible factors but were not collected and therefore were not included in the analysis.

Figure 1.14 Conceptual framework of the main objective of this thesis
a=typical growth of SGA infants based on literature[24], b=typical growth trend of OGDM based on literature[25], SES=socioeconomic status



The main objectives of this thesis were:

1. To identify early patterns of growth and adiposity of SGA and OGDM compared to control infants (Chapter 3 and 4)
2. To investigate candidate biomarkers that can differentiate between infants in high metabolic risk groups (SGA and OGDM) versus controls (Chapter 5 and 6)

The hypotheses that were tested in this thesis included:

1. There might be similar metabolic derangements shared among SGA and OGDM, including growth patterns, circulating IGF-1 and C-peptide levels, and lipidomic signatures (Chapter 3-6)
2. Those shared disrupted metabolic markers could be captured between birth and 12 months of age (infancy period)

Additionally, this thesis also aimed to:

1. Investigate the effect of changing GDM diagnosis and management on the offspring (Chapter 3)
2. Discover candidate nutritional biomarkers for infant growth and adiposity among control infants (Chapter 5, 6, and 7)
3. Conduct an in-depth study on breastmilk in a control population, including its intake volume and macronutrient concentration, in relation to infant growth and adiposity outcomes (Chapter 7)
4. Explore the influence of both maternal and infant *FUT2 gene* on infant growth, adiposity, and general health, in relation to human milk oligosaccharides and other biochemical analytes measured in the study (Chapter 8)

The summary of each study plan is described in Table 1.6.

Table 1.6 Hypothesis and methods of each study

Results chapter	**Hypothesis (H)/Aims (A)**	**Methods to address the hypothesis**
Chapter 3: Growth trends in offspring of mothers with GDM	H: Changes in GDM diagnosis criteria and treatment modalities in the last decade would have impacted growth outcomes among offspring of mothers with GDM	Comparing growth and adiposity outcomes between 2 groups of offspring of mothers with GDM born in non-overlapping years
Chapter 4, 5, 6: Investigating early life physical and biochemical similarities between SGA and OGDM, in separate comparison to controls	H: There should be metabolic commonalities shared during early life between SGA and OGDM, affected by antenatal/maternal factors and early postnatal exposures	Comparing physical and biochemical markers between SGA and OGDM, separately against controls
Chapter 7 and 8: Breast milk and *FUT2* exploratory study	A: To explore: • how much exclusive breastfeeding duration could influence infant growth and adiposity development • the associations between both breast milk macronutrient *concentrations* and *intake* with infant growth and adiposity • the associations between both maternal and infant *FUT2* polymorphisms and infant growth, adiposity, and overall health	• Measuring macronutrient and HMO concentrations • Measuring breast milk intake volume • Maternal and infant *FUT2* genotyping

Chapter 2 Population and Methods

2.1 Cohort profiles

The Cambridge Baby Growth Study (CBGS) is an ongoing prospective observational birth cohort with longitudinal growth assessments in infancy and childhood. Mother-infant pairs are recruited from a single centre in Cambridge, the Rosie Maternity Hospital. The main goal of CBGS is to examine antenatal and early postnatal determinants of infancy growth[217].

The CBGS consists of sub-studies, CBGS1, CBGS2, and CBGS-breastfeeding study (CBGS-BF), which are outlined concisely in Table 2.1 and described in detail in the next sub-sections. All studies were approved by the Cambridge Local Research Ethics Committee and informed consent was given by all mothers during recruitment.

Table 2.1 Study design of each CBGS cohorts

CBGS=Cambridge Baby Growth Study, HC=head circumference, SFT=skinfold thickness, WC=waist circumference, AGD=anogenital distance, US=ultrasound, DBS=dried blood spot, BM=breast milk, SGA=small-for-gestational age, IODM=infants of diabetic mothers, DMS=dried milk spot, GA=gestational age, T1D=type 1 diabetes, T2D=type 2 diabetes, GDM=gestational diabetes mellitus

Study	Visits (months)	Year of recruitment	Number of subjects at birth	Measurements	Biological samples
CBGS 1	0, 3, 12, 18, 24	2001-2009	1661	Weight, length/height, HC, SFT, WC, AGD, abdominal US	Blood (DBS and plasma), liquid BM, saliva
	Inclusion criteria: expectant mothers aged ≥16 years old Exclusion criteria: unable to give informed consent Recruitment: first trimester of pregnancy				
CBGS 2	0, 3, 6, 12, 24, 36	2011-2015	144 SGA 181 IODM 10 SGA/IODM	Weight, length/height, HC, SFT, WC, AGD, abdominal US	Blood (DBS and plasma), BM (liquid and DMS), saliva
	Inclusion criteria: • IODM: GA≥34 weeks, born to mothers with T1D/T2D/GDM • SGA: GA≥34 weeks with low birth weight (defined as ≤-1.5 SD based on UK 1990 UK growth reference) Exclusion criteria: • Syndromal/known genetic causes for SGA • Severe congenital malformations • Maternal age <16 years old • GA <34 weeks Recruitment: at birth				
CBGS-BF	0, 0.5 (2 weeks), 1.5 (6 weeks), 3, 6, 12, 24, 36	2015-2019	173	Weight, length/height, HC, SFT, WC, AGD, abdominal US, ADP-Pea Pod	Blood (DBS and plasma), BM (liquid and DMS), saliva, stool
	Inclusion criteria: • Healthy term, vaginally delivered singletons with birth weight >-1.5 SD based on UK 1990 UK growth reference • The family intended to give exclusive breastfeeding from birth until at least 6 weeks of age Exclusion criteria: • Maternal age <16 years old or those unable to give informed consent • Maternal pre-pregnancy BMI >30 kg/m^2 • Any significant maternal illness or pregnancy comorbidity • Use of antibiotics or steroids in 30 days before delivery or regular probiotics consumption Recruitment: at birth				

2.1.1 Cambridge Baby Growth Study 1

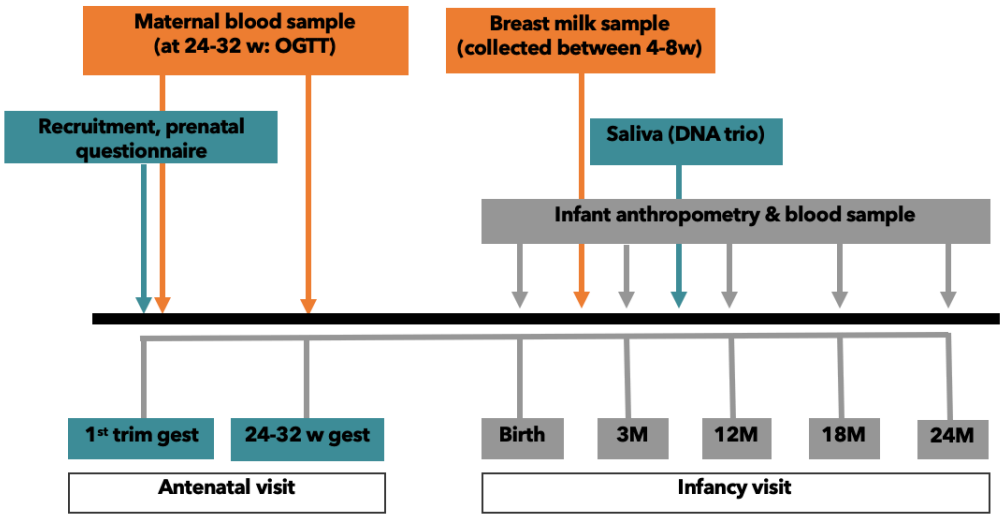
The CBGS1 or the original study was established to investigate how environmental exposures during pregnancy could affect male offspring reproductive development thus including the measurement of testicular descent, penile length, and anogenital distance (AGD) in the study protocol. From the outset, this remit was extended to include infant growth and body composition and therefore also included female infants[217].

Recruitment took place between 2001-2009 targeting pregnant mothers from the general population aged over 16 years old with wide inclusion criteria. They were recruited in the first trimester (approximately 12 weeks) when attending their first ultrasound clinic for that pregnancy. As part of the study, a 75 g OGTT was conducted at 28 weeks gestation to examine maternal glycaemic control and following that, mothers with GDM were treated according to the hospital protocol. Mothers were also asked to fill out questionnaires of maternal baseline demographics and environmental exposures during the antenatal period. (Appendix 1a)

At birth, cord blood and placental samples were collected if possible. Infant visits were conducted at birth, and then 3, 12, 18, and 24 months, consisting of anthropometric measurement and biological sample collection (Figure 2.1).

Figure 2.1 CBGS1 study design

w=weeks, gest=gestation, M=months



At all infant visits, weight, height, and head circumference (HC), and AGD were measured (Table 2.1). Waist circumference (WC) and abdominal ultrasound were added into the visit protocol in 2006 to measure abdominal fat thickness representing central adiposity at 3, 12, and 24 months. Besides anthropometry and biological specimens, several infant questionnaires were also administered, including items on infant feeding practice (Appendix 1b) and infant behaviour/temperament at 3 months (Appendix 1c), and a 3-day food diary at 12 and 24 months (Appendix 1d).

Normal birth weight infants born of generally fit and healthy non-GDM mothers from the CBGS1 serve as a control group in the data analyses of the subsequent results chapters of this thesis, unless otherwise stated. OGDM arising in CBGS1 will be compared with the more recent OGDM cases from the CBGS2 in chapter 3. CBGS1 GDM cases were clinically diagnosed using the criteria in routine use at that time: venous fasting glucose > 6.0 mmol/L or 120-minute glucose > 7.7 mmol/L, or

capillary fasting glucose > 7.0 mmol/L or 120-minute capillary glucose > 8.8 mmol/L but were further classified retrospectively using IADPSG criteria and recorded OGTT data.

2.1.2 Cambridge Baby Growth Study 2

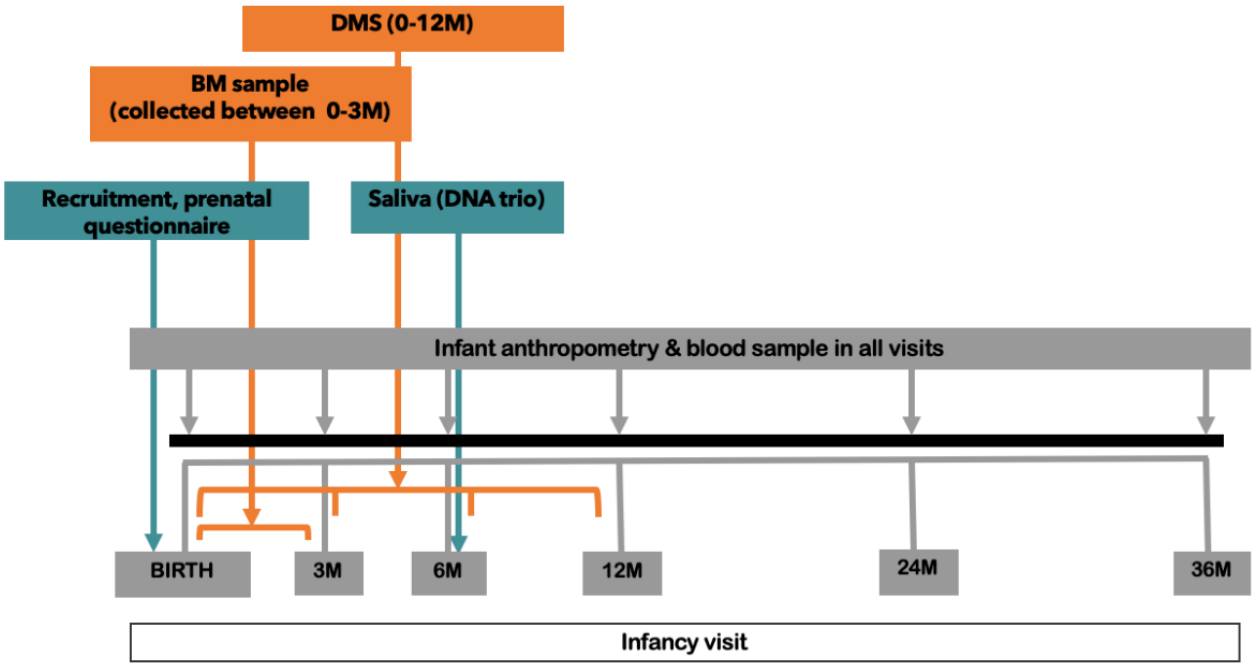
To augment what had been found from the CBGS1, the Cambridge Baby Growth Study 2 (CBGS2) was designed to focus on the 'high risk' groups, infants born SGA and/or infants of diabetic mothers (IODM). In order to enhance subject recruitment as well as to allow a dose-dependent analysis of the effect of small birth weight to later outcomes, SGA in this cohort was liberally defined as birth weight $\leq$ -1.5 SDS using the 1990 UK growth reference[218]. IODM recruited to the study comprised infants born to mothers with T1D, T2D, or GDM. Subsequent analyses reported in this thesis only include infants born to mothers with GDM (OGDM). To eliminate the major confounding effect of prematurity, gestational age in this cohort was limited to those more than 34 weeks. Infants with genetic syndromes were also excluded.

The CBGS2 infants were recruited at birth from the postnatal ward, thus there was no antenatal sample collection. Throughout recruitment, GDM definitions and treatments were decided by the clinical team. GDM-related information was extracted from the medical records. During the CBGS2 study period, the hospital OGTT criteria for GDM were: glucose value ≥5.3 at 0 minute, ≥10.0 at 60 minutes, or ≥8.5mmol/l at 120 minutes, in line with the IADPSG/WHO guidelines. For treatment, all women were given standardised dietary and lifestyle advice, and seen in clinic regularly. Additionally, metformin and/or insulin were prescribed when

recommended by the clinical team, guided by regular fasting and post-prandial monitoring of glucose levels.

The CBGS2 used an identical infant protocol and similar study schedule as the CBGS1, with the addition of 6- and 36-months visits (Figure 2.2, Table 2.1). Apart from abdominal ultrasound, which was conducted from 3 months onwards, all anthropometry measurements and blood sampling were performed at each visit by the same paediatric research nurses as in CBGS1. Moreover, CBGS2 mothers were asked to complete the same CBGS1 questionnaire at birth or 3 months visit to collect their detailed baseline demographics and environmental exposures during the antenatal period. Pooled liquid BM between 0-3 months and dried milk spots (DMS) were also collected (detailed procedure in 2.3.2).

Figure 2.2 CBGS2 study design
M=months, BM=breast milk, DMS=dried milk spots



2.1.3 Cambridge Baby Growth and Breastfeeding Study (CBGS-BF)

To identify factors in human breast milk that may confer a slower pattern of infant growth and hence reduce obesity risk later in life, the CBGS-BF was established in 2015. In this study, parameters of breast milk intake and composition were studied more extensively, including BM intake volume using a deuterium-labelled water technique, longitudinal BM collection and a more detailed BM composition including macronutrients, butyrate and human milk oligosaccharides (HMOs), and explorative analyses of microbiota in BM and infant gut.

The CBGS-BF was also conducted to provide contemporaneous control infants to compare with the SGA and IODM groups in CBGS2, rather than solely using CBGS1. This study recruited normal birth weight infants at delivery and used similar design and protocol as the previous cohorts with extra visits and biological samples collection (Table 2.1, Figure 2.3). Additional infant visits were conducted at 2 and 6 weeks of age because the very early infancy period (before 3-6 months) has been reported to be critical for future metabolic outcomes[219]. Extra biological samples included infant stool, maternal milk, and both maternal and infant urine to estimate BM intake volume.

BM nutritional composition analysis was performed more thoroughly in the CBGS-BF from each individual visit time points until 12 months of age if mothers were still breastfeeding. At 6 weeks when all mothers were still breastfeeding, there was also a sterile collection of a full breast expression via pumping for the microbiome study (detailed procedure in section 2.3.2). Analysis of BM composition included macronutrients (carbohydrate, fat, protein), short-chain fatty acids (SCFA), and

human milk oligosaccharides (HMOs). DMS was also collected in each visit until mothers stopped breastfeeding for the future study of lipid profiles.

Actual volume of BM received by the infant was estimated between 4-6 weeks of age among exclusively breastfed infants using deuterium-enriched water. This was done by collecting daily maternal and infant urine samples for 14 days. The procedure is explained in section 2.4.5.

Moreover, to more accurately assess infant body composition, the measurement of fat- and fat-free mass using air-displacement plethysmography (ADP) Pea Pod system was added into the study protocol at 6 weeks and 3 months from 2016. ADP Pea Pod is a safe and non-invasive procedure and can provide reliable body composition measurement in infancy.

Figure 2.3 CBGS-BF study design

M=months, BM=breast milk, DMS=dried milk spots

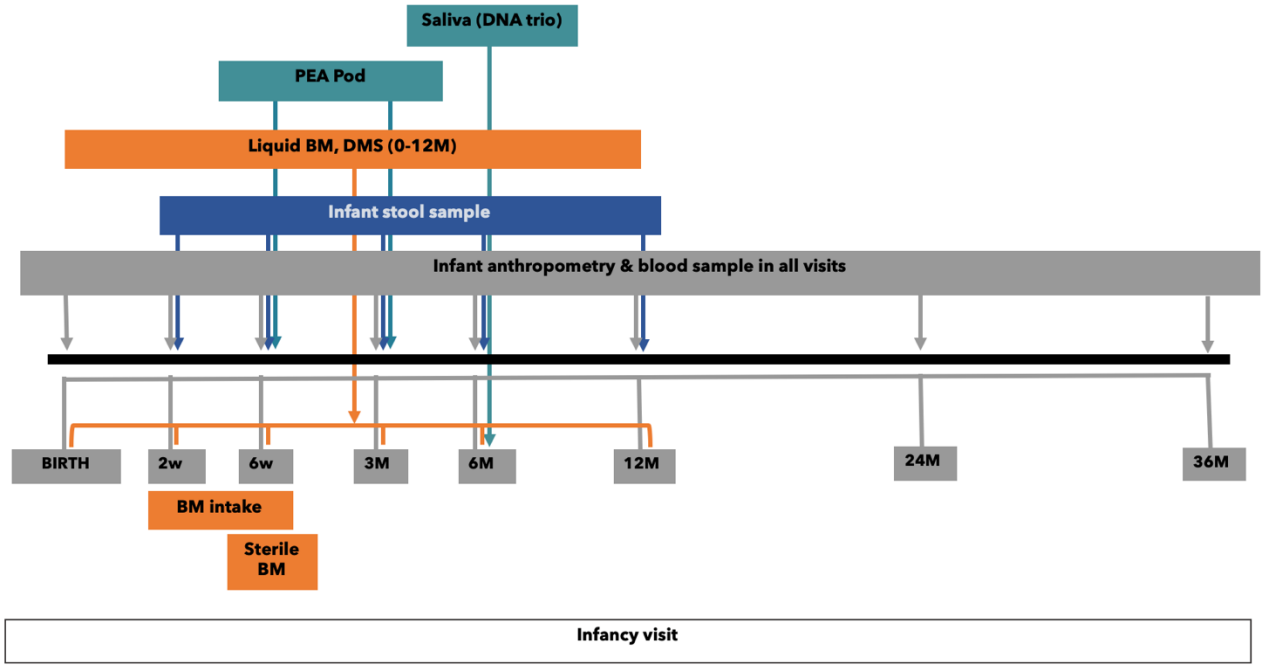


Table 2.2 CBGS-BF data collection at each individual study visit

	Birth	2w	6w	3M	6M	12M	24M	36M
Recruitment	+							
Collection of pregnancy/demographics data	+							
Growth measurement (weight, length, head circumference, waist circumference, AGD)	+	+	+	+	+	+	+	+
Skinfold thicknesses	+	+	+	+	+	+	+	
Abdominal ultrasound			+	+	+	+	+	
Pea Pod			+	+				
Allergy, infection/antibiotics exposure, probiotic exposure, feeding history	+	+	+	+	+	+		
Food diary					+	+	+	+
Stool sample for gut microbiome		+	+	+	+	+		
Sterile BM for microbiome			+					
Other (non-sterile) BM liquid sample and DMS	+	+	+	+	+	+		
BM intake volume (tracer water)			+ (4-6w)					
Blood sample (DBS and small amount of plasma)	+	+	+	+	+	+		
Saliva (DNA)					+			

w=weeks, M=months, BM=breastmilk, ADP=air displacement plethysmography, DMS=dried milk spot, DBS=dried blood spot

2.2 Physical measurements

All anthropometry and body composition measurements in the CBGS were performed by three trained paediatric research nurses. To ensure the quality of data produced, each infant clinic visit was scheduled based on the exact age of infants

with +7 days tolerance for birth, 2-, and 6-weeks visits, and ±28 days for 3 months onwards. The list of anthropometric measurements in all CBGS cohorts is displayed in Appendix 2.

2.2.1 Weight and length/height

In all cohorts, birth weight was recorded from the medical records of values measured by the clinical staff immediately post-delivery. Other infant anthropometry at birth (Table 2.2) were mostly measured within the first 7 days of life. Weight and length were measured at all visits in all of the CBGS cohorts to evaluate physical growth. Weight and length measurements were used to calculate BMI and PI (see 2.2.6).

A Seca 757 electronic baby scale was used to measure weight of 0-24 months infants to the nearest 1 g. Sitting or standing scales were sometimes used for toddlers older than 2 years old. Infants and toddlers were weighed nude without nappies, or alternatively the weight of the diaper was subtracted from the resulting weight. Weight measurement was made before feeding.

A Seca 416 infantometer was used to measure the supine length to the nearest 0.1 cm. Standing height was measured using a stadiometer if subjects could stand without assistance, typically from 2 years old onwards. Measurement of length/height was strictly performed without foot- and headwear.

2.2.2 Head and waist circumference

Head circumference (HC) is a standard routine measurement in paediatrics, especially from birth until 5 years old, to monitor growth, nutritional status, and brain development, especially among SGA children[220]. To measure HC, Seca 212 measuring tape was circled around the largest area of the head, i.e. from above the eyebrows and around the back of the head. HC was measured in all visits of all CBGS cohorts.

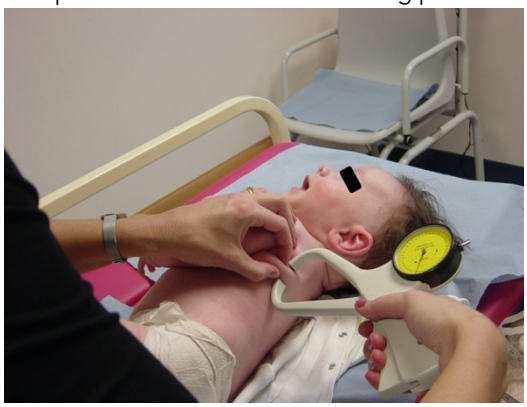
Waist circumference (WC) was measured to assess central adiposity. This was conducted using Seca 201 ergonomic circumference measuring tape. WC measurement was taken at the end of a normal expiration midway between the lowest rib and the iliac crest as the minimum diameter, preferably before feeding. In the CBGS1, WC measurement was measured on a subgroup of infants at 3- and 12 months (N=510 and 595, respectively). In the CBGS2 and CBGS-BF, WC was measured on all infants at all visits.

2.2.3Skinfolds thickness

Skinfold thickness (SFT) measurement is a simple and useful clinical method and has produced valuable information in the study of infant growth and body composition. SFT reflects subcutaneous fat folds and can be used to assess subcutaneous fat at various regions of the body or summed to estimate relative total body fatness[220]. SFT has also been shown to correlate with body fat assessed by DXA in different age groups. Several anthropometric equations including SFT have

also been published to estimate body fat mass. The use of these equations are common in large epidemiological or multi-centre cohort studies[221,222].

Figure 2.4 Subscapular skinfold measurement in CBGS
This picture was taken after obtaining parental written consent



SFT was measured at 4 sites (triceps, subscapular, flank, and quadriceps) in triplicate on the left-hand side of the body using a Holtain Tanner/Whitehouse Skinfold Caliper (Holtain Ltd). The locations of each skinfold measurement were: triceps at the posterior surface of the arm, halfway between the acromial process (shoulder) and the olecranon (elbow); subscapular at the oblique angle below the scapula (upper back, Figure 2.4); flank in the posterior axillary line immediately posterior to the iliac crest; quadriceps in the midline and halfway between the top of the patella and the inguinal crease.

2.2.4Abdominal ultrasound

A standard ultrasound machine (Logiq Book XP ultrasound with 3C MHZ-RS abdominal curved array transducer, GE Healthcare, Bedford, UK) was used to assess intra-abdominal (visceral/medial) and abdominal subcutaneous depth as parameters of abdominal fat deposition. The participants were lying in the supine

position on a flat surface and the ultrasound probe was placed at a point where the midline of the transverse plane used for WC measurement intercepts with the xiphoid line. To measure intra-abdominal depth, the probe was placed on the longitudinal plane and was set to a depth of 6 or 7 cm. Intra-abdominal depth was defined as the distance between the peritoneal boundary and the lumbar vertebrae (Figure 2.5). Meanwhile, the abdominal subcutaneous thickness was measured on the transverse plane with a probe depth of 4 or 5 cm. It was defined as the distance between a point underneath the cutaneous layer and the linea alba, a fibrous sheath lining the anterior abdominal wall (Figure 2.5 and 2.6).

In CBGS1 abdominal ultrasound was only measured on a subgroup of infants at 3- and 12 months (N=498 and 582, respectively). In the CBGS2 and CBGS-BF, it was performed on all infants from the 6 week-visit onwards.

Figure 2.5 Measurement of abdominal fat thickness using ultrasound
Reproduced with permission from *Diet, Anthropometry and Physical Activity (DAPA) Measurement Toolkit* website (https://dapa-toolkit.mrc.ac.uk/anthropometry/objective-methods/ultrasound)[220]

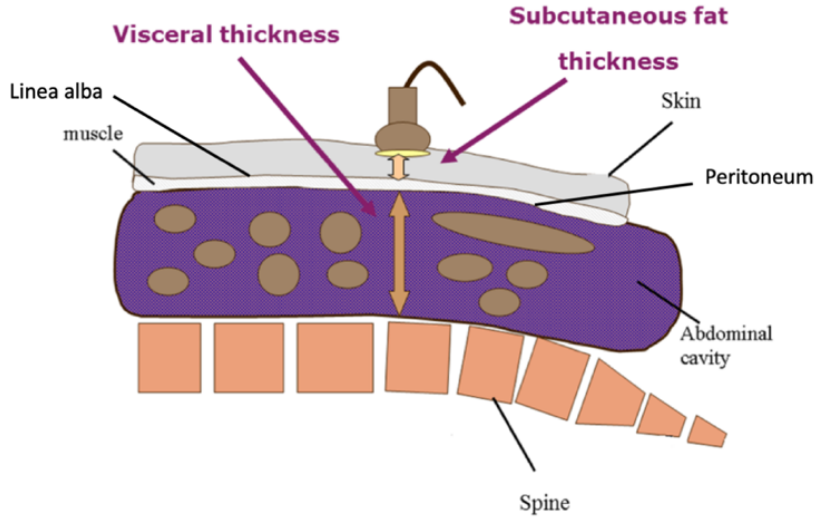
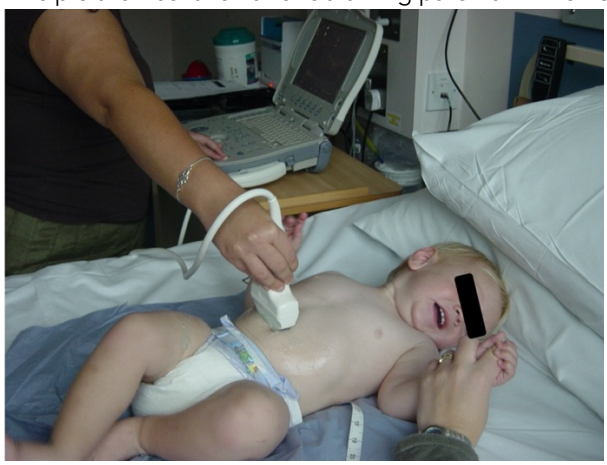


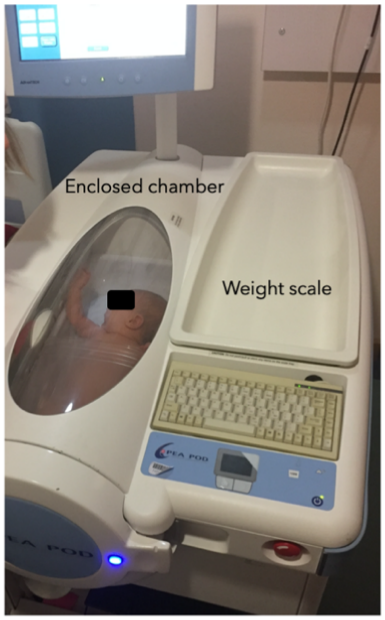
Figure 2.6 Measurement of abdominal subcutaneous fat thickness in CBGS
This picture was taken after obtaining parental written consent



2.2.5 Air-displacement plethysmography (ADP)

ADP uses two-component model of body composition dividing the body into fat- and fat-free components. The commercial names for the devices are Bod Pod for adults and Pea Pod for infants (Life measurement, Inc, Concord, CA). ADP-Pea Pod was used in the CBGS-BF at 6 week and 3 month visits.

Figure 2.7 Accurate infant body composition measurement using ADP-Pea Pod in CBGS
This picture was taken after obtaining parental written consent



To measure infant body composition using ADP-Pea Pod, the infant is required to lie down inside an enclosed chamber (Figure 2.7). Thereafter, by changing the chamber's volume, the volume of the body (i.e. the volume of the displaced air) can be determined from the changes in air pressure. An external integrated electronic scale is used to measure body mass precisely (Figure 2.7). Dividing body mass by volume results in body density which can be used to calculate fat and fat-free mass due to their difference in density (0.9 kg/L and 1.2 kg/L, respectively). This device only measures overall body density, total body fat and lean mass but not their regional distributions[223].

ADP-Pea Pod provides a non-invasive, accurate, and reliable body composition assessment in infants[222,224]. However, the use of this instrument is limited to those weighing between 1 and 8 kg, thus it cannot be used for most infants older than 6 months. The equipment is also bulky, expensive, not portable and impractical to use in large population studies[220]. Another limitation is a particular level of anxiety experienced by parents as their infants stay inside the closed system ADP-Pea Pod for around 2 minutes[220].

2.2.6 Calculations

BMI (kg/m2) was calculated by dividing weight by the square of length/height. PI (kg/m^3) was calculated by dividing the length by the height cubed. Age and sex-appropriate standard deviation scores (SDS) were calculated for weight, length/height, and BMI, additionally adjusting for GA for 0-3 months measurements, by comparison to the UK 1990 growth reference[218]. The reference

was used for analyses as well as recruitment criteria in the CBGS2. This was considered appropriate since the CBGS is a UK setting cohort with mainly Caucasian subjects and commenced in 2001, contemporaneous with the launching of the reference. LMS growth software designed by Cole *et al*[225] and installed as an add-in to Microsoft® Excel® v 16.34 was used to derive the SDS values.

As a parameter for family socio-economic status, as one of the important baseline demographics, index of multiple deprivation (IMD) was assessed using an integrated index based on residential postcodes[226].

2.2.7 Anthropometric data quality control

CBGS data quality was maintained by involving the same paediatric research nurses throughout the study. Regular machine calibration and personnel training were also conducted periodically. To exclude implausible data values from analyses, data cleaning was applied in all anthropometric measurements and invalid data values were excluded.

For the ultrasound measurements, all the images saved on the machine were reviewed separately by an independent researcher, with regular training and monitoring by Dr. Emanuella De Lucia Rolfe, Anthropometry Specialist in the MRC Epidemiology Unit, University of Cambridge. CBGS1 ultrasound images were reviewed by Dr. Philippa Prentice, a former clinical research fellow in the Department of Paediatrics. I reviewed all ultrasound images taken in the CBGS2 and CBGS-BF. The review process included examining the ultrasound images' quality,

checking measurement accuracy, and re-measuring or excluding incorrect data points.

To determine the relative intra-observer technical error of measurement (TEM), validation measurements were performed on 14 volunteers. The relative TEM for length ranged between 0.1-0.5%; for skinfold measurement between 2.9-4.3%; for ultrasound medial measurement between 0.5-3.5%; and for ultrasound subcutaneous measurement between 1.0-3.2%.

2.3 Biological sample collections

2.3.1 Bloods

In all CBGS cohorts, dried blood spot (DBS) samples and small amounts of plasma were collected at every clinic visit from capillary heel-prick blood sampling. DBS was obtained by dropping single drops of blood onto Whatman™ 903® untreated filter paper (Ahlstrom 226, ID Biological Systems), similar to the method employed in the UK newborn routine screening programme. The samples were then air dried at ambient temperature overnight and were stored frozen at -20^{0}C for CBGS1 and -80^{0}C for CBGS2. When needed for analysis, 3.2 mm diameter single aliquot spots were punched from the larger DBS spots. DBS samples were used for lipidomic and endocrine measurements.

2.3.2 Breast milk

In both CBGS1 and CBGS2, lactating mothers were asked to provide hindmilk samples collected by hand expression after feeding their infants and pooled into one sample between 4-8 weeks (CBGS1) or 0-3 months (CBGS2). CBGS2 mothers were also asked to provide DMS by collecting a drop of their BM onto Whatman™ 903® untreated filter paper (Ahlstrom 226, ID Biological Systems), before and after breastfeeding.

In CBGS-BF, to examine how BM composition varies over time, liquid hindmilk sample was collected at each visit time point until 12 months if mothers were still breastfeeding. This sampling method aimed to provide a single and standardised BM sample for each time point, rather than as a pooled sample as in CBGS1 and CBGS2. All liquid BM samples were kept frozen at -20^{0}C (CBGS1) or -80^{0}C (CBGS2) until the time of analysis.

Moreover, sterile BM was collected in the CBGS-BF for microbiome study at 6 weeks of age. Complete milk expression took place from one breast using a breast pump and sterile milk collection tubes provided by the research team. The breast was first cleaned using antiseptic liquid and dried with sterile paper towels.

2.4 Laboratory analyses

2.4.1 Lipidomics

Lipid profiling was conducted on DBS samples. Koulman *et al*. have reported acceptable and reproducible lipidomics results on DBS compared to the other

methods with larger sample volumes[227]. Assays were carried out by Dr. Koulman's group at the Institute of Metabolic Science, Metabolic Research Laboratories. I was involved in sorting and preparing the samples as well as in data analysis.

The studies were conducted using benchtrop orbitrap high-resolution mass spectrometry (HRMS) using on 3- and 12- months DBS samples.

All experiments were run with blank controls, 2 different quality control (QC) samples, and 6 internal standards (0.6 μM 1,2-Di-o-octadecyl-sn-glycero-3-phosphocoline, 1,2 μM 1,2-di-O-Phytanyl-sn-glycero-3-phosphoethanolamine, 0.6 μM C8-cerramide, 0.6 μM N-heptadecanoyl-D-erythro-sphingosylphosphoryl choline, 6.2 μM undecanoic acid, 0.6 μM trilaurin). While QC1 spots were made from mixed anonymised adult human venous blood samples, QC2 spots were obtained from commercially available horse blood. A punched-out 3.2 mm DBS was eluted in methanol containing those 6 internal standards in 1.2 mL cryovials. This process results in lipids being partitioned into methyl tertiary butyl ether . The sample was then centrifugated and the separated organic layer was concentrated and used for lipid analysis. Samples were infused into a Thermo Exactive benchtop orbitrap (Hemel Hampstead UK) using an Advion Triversa Nanomate (Ithaca US) and data were acquired from both positive and negative modes (+1.2 kV and -1.5 kV voltage, respectively).

The data obtained from this study were semi-quantitative in the form of signal intensity of each lipid expressed, relative to the total lipid signal acquired for each individual, per thousand ($^0/_{00}$). Due to the spotting and drying process used to obtain DBS samples, several lipid species were oxidised, mainly unsaturated fatty

acid-containing lipids across all lipid classes. Signals from these lipid species were therefore not used in further analyses and any lipid species where more than 30% of subjects had a value of zero were excluded. Those signal values were processed using XCMS and Peakpicker (in-house R script) and data were normalised using log transformation. Lipidomic profiles were compared between SGA and OGDM, together or separately against the control group, adjusted for feeding mode as the most important confounder.

2.4.2 Hormone measurements

IGF-1 and C-peptide were measured using DBS from 3 groups of infants: SGA, OGDM, and controls. IGF-1 measurement was carried out by Markus Langkamp, Mediagnost, Tübingen, Germany, while C-peptide level was measured at the NIHR Core Biochemical Assay Laboratory (CBAL) Cambridge. I was involved in selecting the samples at 3 months of age and analysing the results. Each group of high-risk infants (SGA and OGDM) was represented by 50 CBGS2 subjects and their hormone results were compared with the control group from CBGS1, whose IGF-1 and C-peptide concentrations had been measured previously. Selection of the 50 subjects in each SGA/OGDM group was based on infants with the smallest birth weight (SGA) and infants born of mothers (OGDM) with the highest fasting glucose level during pregnancy (from the hospital-recorded OGTT).

To measure IGF-1 concentrations, capillary blood from DBS was extracted using 400 µL of acidifying buffer and IGF-1 measured with a specific radioimmunoassay (Mediagnost, Tübingen, Germany). C-peptide concentrations were measured using CBAL's in-house developed Meso Scale Discovery (MSD) assay.

2.4.3 BM macronutrients concentrations

BM macronutrients concentrations were measured from CBGS1 and CBGS-BF as part of a collaboration with Mead Johnson Nutrition and Wageningen University, the Netherlands. I was responsible for data handling and analysis of BM macronutrients concentrations in the CBGS-BF, under the supervision of Professor Jacques Vervoort, Wageningen University.

BM samples were defrosted and thoroughly homogenised before assays. 400 μL of this homogenate was mixed with 400 μL $CDC1_3$ solvent for 10 minutes before being centrifuged for 30 minutes at 10,000 rpm. The resulting non-polar fraction was utilised to measure lipid concentrations using ^{1}H-Nuclear magnetic resonance (NMR) spectra. Triglyceride (TG) served as a surrogate for total fat content since it contributes to 95-98% of total BM lipid content. Meanwhile, the polar fraction of the BM was used to measure lactose, the most abundant BM carbohydrate, by ^{1}H 1D nuclear Overhauser effect spectroscopy (NOESY). For protein, total nitrogen level was measured by the DUMAS method[228], and the protein factor conversion of 6.25 was used to calculate crude protein content.

Atwater conversions were used to calculate the metabolisable energy content of BM, taking energy contents of 4, 9, and 4 kcal/g for lactose, fat, and protein, respectively[229]. Total calorie content (TCC) of BM was calculated in kcal/100 mL. Nutrient density was presented as percentages of macronutrient contents of TCC, i.e. %carbohydrate, %fat, %protein.

2.4.4 Human milk oligosaccharides (HMO) measurement

HMO concentrations were measured and quantified among liquid BM samples in the more recent control group (CBGS-BF) at 2 weeks, 6 weeks, 3 months, 6 months, and 12 months postpartum. The experiment was part of the collaboration with Mead Johnson Nutrition and the University of California Davis, USA (Dr. Daniela Barile). My contribution to this project was to further calculate HMO intakes and analyse their associations with *FUT2* genotypes, growth, and adiposity outcomes.

Each milk sample was diluted and filtered, and its oligosaccharide concentrations were quantified by high-pH anion-exchange chromatography using a Thermo Scientific Dionex ICS-5000+ system with a pulsed amperometric detector. The most abundant HMO were chosen for analysis, including 2'-fucosyllactose (2'FL), 3-fucosyllactose (3'FL), lacto-N-fucopentaose I (LNFPI), lacto-N-tetraose (LNT), lacto-N-neotetraose (LNNT), 3'-sialyllactose (3'SL), and 6'-sialyllactose (6'SL).

2.4.5BM intake volume

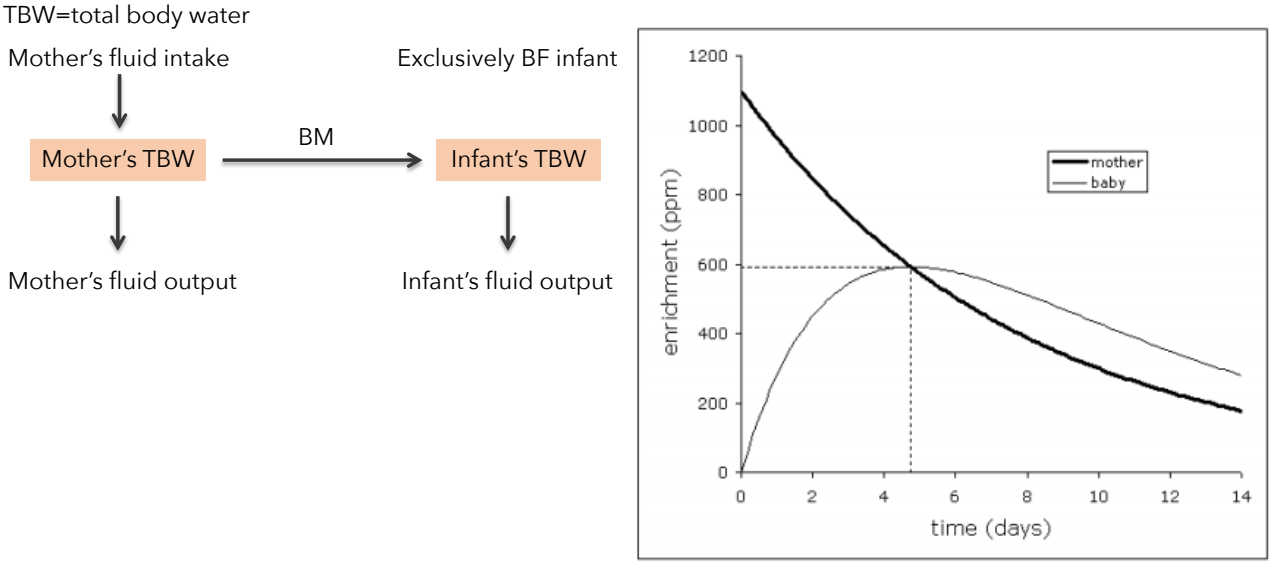
BM intake volume was estimated in CBGS-BF in 67 exclusively breastfed infants between 4-6 weeks of age employing the mother-infant deuterium-oxide (^2H_2O) turnover technique.[230,231].

Baseline urine samples from both mother and infant were collected on day 0, after which the mother consumed an oral dose of 50 g of deuterated water. Further daily urine samples from mother and baby were collected over a 14-day period. To obtain infant urine samples, cotton wool was placed in a clean nappy, which was then checked every hour to see if it had been saturated with urine. If wet, urine was

collected from the cotton wool by mothers by compressing it in a syringe. If contaminated with stool, the cotton wool was discarded, and the procedure was restarted. Urine samples were stored in the home fridge and were transported to the hospital on ice. The day and time of collections were carefully recorded.

BM intake volume was determined by measuring isotope enrichment from mother to her baby. After being administered, the deuterium-enriched tracer water is incorporated into the mother's total body water (TBW) pool and passed onto her baby in BM. The amount of BM consumed by the baby can be calculated by analysing the rate of deuterium (^{2}H) appearance in the baby's urine and disappearance from the mother's urine (Figure 2.4). ^{2}H enrichment in the urine samples was measured by isotope ratio mass spectrometry. The formulas and assumptions used for calculating BM intake were those of Haisma *et al*[231]. The procedure was conducted in a collaboration with the MRC Elsie Widdowson Laboratory, Cambridge (Dr. Michelle Venables). Values are displayed in kg/day unit.

Figure 2.8 Isotope enrichment of maternal and infant TBW for an exclusively BF infant
TBW=total body water



2.4.6 *FUT2* genotyping study

Fucosyltransferase 2 (FUT2) genotyping was performed on DNA extracted from maternal and infant saliva samples obtained from a subgroup of subjects from all cohorts (total N=749). I carried out this procedure under the supervision of Dr. Clive Petry from the Department of Paediatrics in Cambridge. The process consisted of DNA extraction, DNA quantification, PCR, DNA fragmentation by applying a restriction enzyme (restriction fragment length polymorphism (RFLP)), and gel electrophoresis. The single nucleotide polymorphism (SNP) targeted was rs516246 because it is in complete linkage disequilibrium with rs601338, which is the most common SNP used in *FUT2* genetic studies[210,213], but with a better and available restriction enzyme. The secretor phenotype is indicated by homozygous G/G or heterozygous A/G genotypes, whereas the homozygous A/A genotype represents the non-secretor phenotype.

Saliva samples were collected using the Oragene.DNA kit (OG-500 for mothers and OG-575 for infants (DNA Genotek, Ottawa, Canada). Manual DNA purification from the sample was carried out using prepIT.L2P kit (DNA Genotek, Ottawa, Canada). The procedure began with inversing and shaking the sample gently to mix the viscous sample properly. The sample was then incubated in the air incubator at 50^0C for at least 2 hours to maximise DNA yield and inactivate nucleases. Afterwards, 500 μL sample was transferred to a fresh tube and mixed with 20 μL (1/25th of the sample volume) PT-L2P reagent to precipitate impurities and inhibitors. The next process was ice incubation for 10 minutes, followed by 10

minutes centrifugation at room temperature at a minimum speed of 3,500 x *g*. Higher centrifugal force and longer centrifugation period minimise the amount of turbid material in the purified DNA. The clear supernatant was then transferred carefully into a new tube and the pellet (containing impurities) was discarded. To precipitate the DNA, 1.2x volume of room temperature 95-100% ethanol was added, and the solution was inversed gently 10 times. DNA may be visible as a clot of fibres after this step. The sample was then incubated for 10 minutes at room temperature to allow fully DNA precipitation. After that, the sample was centrifuged at room temperature for 10 minutes at the highest speed, minimum 3,500 x *g*. Following this process, supernatant (now containing impurities) was very carefully discarded without disturbing the DNA pellet. Next I performed a washing step using 1 mL of 70% ethanol, followed by 1-minute incubation at room temperature and short centrifugation (<1 minute) to remove the supernatant. DNA was then rehydrated by adding 200 μL of TE solution (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), vortexing the sample for 30 seconds, and incubating it overnight at room temperature.

DNA quantification was performed by the absorbance method at 280 nm and 260 nm (NanoDrop 3300 Fluorospectrometer, Thermo Fisher, Massachusetts, US). PCR was performed by incubating 10 ng genomic DNA in a 10 μL solution containing 1 μL NH_4 buffer, 0.5 μL of 4mM dNTP, 0.3 μL of 50 mM $MgCl_2$, 0.3 μL of 20 pmol/UL forward (5'-CGCTGCTGAACGTGAAATATAG-3') and reverse (5'- AGCACACACA-CACCCACACT-3') oligonucleotide primers, 0.5 μL of 10 ng Taq DNA polymerase (Bioline Ltd, London, UK), and 7 μL of nuclease-free water. This PCR mix was incubated at 94°C for 5 minutes followed by 20 cycles of 94°C for 45 seconds, 65°C

for 45 seconds (dropping 0.5°C per cycle) and 72°C for 45 seconds. After this the PCR mix was incubated for 15 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 45 seconds, followed by a final incubation at 72°C for 10 minutes. Following this, RFLP was conducted by incubating the sample at 37°C for at least 4 hours with 2 units of *Ava*I (New England Biolabs, Hitchin, UK).

The final products were separated on a 3% agarose gel, resulting in the A allele producing a single 202-base pair band and the G allele producing two bands, at 125- and 77- base pairs.

2.5 Statistical analysis

Unless otherwise stated, all descriptive data are presented as mean ± standard deviation (SD) for continuous variables or as percentages (%) for categorical variables.

Univariate analyses to test differences in continuous variables between 2 groups were performed by unpaired t-test for normally distributed data and Mann Whitney-U for non-normally distributed data. Differences among more than 2 groups were tested using ANOVA (with Bonferroni correction) or Kruskal-Wallis for non-parametric data. Differences in categorical variables were tested using Chi-squared test.

Pearson's correlation coefficient and Spearman's as the non-parametric alternative were used to test correlations between predictor(s) and outcome variables. Afterwards, multiple regression models were run to adjust for confounding factors using a priori approach. Commonly used covariates included maternal pre-

pregnancy BMI, parity, maternal height, pregnancy glucose concentrations, ethnicity, smoking history during pregnancy, and socioeconomic status based on the index of multiple deprivation (IMD, postcode based). Variables with positively skewed distributions were log-transformed before regression analyses.

For lipidomic analyses, data were normalised using log transformation and unit variance scaled. Principle component analysis was used first to observe general patterns and exclude outliers, followed by partial least squares-discriminant analysis (PLS-DA) using Q^2 as cross-validation parameter. Data analysis for lipidomics was mainly run on Metaboanalyst version 4.0.

Statistical analyses were carried out using SPSS version 25.0 (IBM) and R version 1.0.136. Statistical significance was achieved if *p values* <0.05.

Chapter 3 Growth trends in offspring of mothers with GDM

3.1 Introduction

Due to being exposed to high glucose environment *in utero*, many studies have reported that offspring of mothers with gestational diabetes mellitus (OGDM) are more likely to be macrosomic[232], with increased body fat (independent of birth weight)[233], and have elevated risk of neonatal complications, such as prematurity, shoulder dystocia, hypoglycaemia and jaundice[234]. In addition, these infants have increased risk of later overweight, metabolic and cardiovascular disease risk, related to maternal glycaemia and increased maternal BMI[164,235,236].

Diagnostic criteria for gestational diabetes (GDM) vary within and between countries. However, there has been a recent shift to using lower glucose thresholds, mainly influenced by the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study's findings that maternal glycaemia was linearly related to risk of neonatal adverse outcomes, including higher birth weight, cord blood C-peptide level, risk of Caesarean section delivery and neonatal hypoglycaemia, across multiple populations (over 23,000 pregnant women across nine countries)[237]. Subsequent International Association of Diabetes and Pregnancy Study Groups (IADPSG) guidance in 2010 suggested more stringent GDM diagnostic criteria than previously proposed, using a single glucose value > 5.1 mmol/L at 0 minutes, >10.0

mmol/L at 60 minutes or >8.5 mmol/L at 120 minutes, during a 75-g oral glucose tolerance test (OGTT)[172]. Consequently, treatment for GDM has also become more intensive over recent years, and several randomised trials have shown decreased perinatal complications after treatment in mild GDM or the condition of pregnancy hyperglycaemia but not meeting GDM or T2D criteria. In those studies, treatment consisting of dietary advice, glucose monitoring, and insulin as needed, resulted in reductions in rates of macrosomia, Caesarean delivery rates and shoulder dystocia, compared to routine pregnancy care[173,238,239].

Following those changes to diagnostic and treatment criteria, there have also been a few studies describing a more normal size of OGDM, at least at birth. An Australian study, including 599 infants, 67 of which were born to mothers with GDM, showed for the first time no difference in birth weight, as well as in neonatal body fat (assessed by air-displacement plethysmography) in OGDM compared to controls[240]. In that population, a diagnosis of GDM was confirmed based on the Australian Diabetes in Pregnancy Society criteria: a 75-g OGTT with a venous plasma glucose level at 0 hours of $\geq$5.5 mmol/L and/or 2 hours of $\geq$8.0 mmol/L at 2 hours [241].

The CBGS has recruited two groups of infants affected by GDM, born in non-overlapping years, 2001-2009 and 2011-2013. This chapter investigated whether the 'recent' OGDM still exhibited an increased size at birth as observed in earlier cohorts, and also explored their subsequent growth trajectories from birth until 2 years of age. The 2010 IADPSG criteria were applied retrospectively in both cohorts in order to compare cohorts with identical severity of GDM. Birth size and early

childhood anthropometry in each OGDM group were compared separately against the same control group unaffected by GDM.

This chapter has been published *in part* previously[193], where I shared co-first authorship with Dr. Philippa Prentice. I was responsible for the whole data analyses and was involved in writing as well as revising the manuscript.

3.2 Population and methods

As described in 2.1, a general CBGS1 population (2001-2009) was recruited antenatally and a 75g OGTT at 28-week gestation was performed as part of the research protocol. The control population (N=876) and the 'earlier' OGDM in this analysis (N=98) were part of the CBGS1, comprising of mother-infant dyads with normal and abnormal glycaemia on OGTT, respectively. The fasting and 2h-glucose results from OGTT were fed back to guide clinical management and the 'earlier' GDM women were treated based on WHO 1999 guideline[242]. Based on available records and information from treating clinicians, GDM was mostly treated with diet and lifestyle modification, with or without insulin. Metformin was not routinely used to treat GDM during that period.

The 'recent' OGDM (2011-2013, N=122) cohort was recruited at birth as part of the CBGS2. The 'recent' GDM mothers were diagnosed in a specialist antenatal GDM clinic following routine practice (a 75g formal OGTT in high-risk women or in those with hyperglycaemia following a 50g glucose challenge test as universal screening at 24-26 weeks). All women received standardised dietary and lifestyle advice and seen in clinic regularly (on average every 2 weeks). Additionally, metformin and/or

insulin were prescribed as required, guided by regular fasting and post-prandial glucose monitoring.

To reduce any bias in GDM severity resulting from changing diagnostic criteria, IADPSG criteria[168] were uniformly applied to both OGTT collected as part of the CBGS1 research protocol (2001-2009) and also to those OGTT carried out as part of the clinical diagnostic procedures in the CBGS2 (2011-2013), allowing comparable severity of 'earlier' and 'recent' GDM groups. Based on the IADPSG criteria[168], GDM is diagnosed if at least one glucose concentration on a 75g OGTT at around 28-week gestation $\geq$5.1 mmol/L at 0 minute, $\geq$10.0 mmol/L at 60 minutes, or $\geq$8.5 mmol/L at 120 minutes. Since IADPSG criterion for fasting glucose level is more stringent than the WHO 1999 criteria, a small subset of mothers from the 'earlier' OGDM were not clinically identified and treated as GDM at the time (Table 1.5).

The control population fulfilled the criteria of normal pregnancy glucose based on both WHO 1999 and IADPSG criteria. The additional criteria applied to all subgroups for this analysis included singleton pregnancy, no significant maternal comorbidity (such as pre-eclampsia, hypertension, antiphospholipid syndrome, ankylosing spondylitis, lupus, ulcerative colitis), and gestational age at delivery ≥ 36 weeks. Cases of pre-existing maternal type 1 or type 2 diabetes and infants with a genetic or syndromal disease were also excluded.

Infants' anthropometry was measured at birth, 3, 12, and 24 months using the identical study protocol (Section 2.2) and performed by the same paediatric

research nurses across all studies. All calculations, including BMI, PI, SDS values derivations, and IMD were based on those described in 2.2.7.

Maternal and birth characteristics were compared between groups using ANOVA with Bonferroni post-hoc analysis for continuous variables, and X^2 tests for categorical outcomes. Unless otherwise stated, all data are presented as means $\pm$ SDs.

Multiple linear regression was used to investigate the effect of GDM on birth outcomes, allowing adjustment for potential confounders, including infant sex, postnatal age at visit, gestational age, pre-pregnancy maternal BMI, maternal height, parity, breastfeeding history at 3 months, delivery method, maternal ethnicity, socioeconomic status indicated by IMD, and maternal smoking history during pregnancy. All confounders were chosen *a priori* through the extensive previous study of CBGS and ALSPAC[23].

In all 3 groups, at least 30% of the subjects had incomplete covariate data, primarily due to missing perinatal questionnaire responses. Covariates with the most missing values were maternal pre-pregnancy BMI for controls and 'earlier' OGDM, and smoking history for 'recent' OGDM. Assuming that they are missing at random, incomplete covariates including index of multiple deprivation (N=3), parity (N=4), maternal ethnicity (N=8), smoking history during pregnancy (N= 39), maternal pre-pregnancy BMI (N=185), maternal height (N=148), delivery method (N=27), and infant feeding history (N=189) were imputed using the R package "Multiple Imputations via Chained Equations (MICE)". Twenty imputed datasets were generated, using linear regression for continuous variables and logistic linear regression for binary variables. Analyses run on each dataset were pooled

according to Rubin's rules[243]. Imputed values were reasonably comparable to observed values, and the outcomes obtained from listwise deletion were similar to those with imputed values, therefore imputed values were presented in the subsequent analyses.

After birth, missing anthropometric measurements were commonly due to loss-to-follow-up. To capitalize on the longitudinal growth data with robust handling of missing values, linear mixed-effects models were used to relate the weight, height, and skinfolds (as continuous variables) to visit time point, cohort group, and their interaction with infant age, taking into account the same confounders as in the linear regression models for birth measurements. Time was modelled using linear splines with knots at ages 3 and 12 months due to non-linear relationships with age (indicated by significant estimates for age-squared). Models were fitted to the data by restricted maximum likelihood (REML).

3.3 Results

3.3.1 Baseline demographics and birth data

The demographics of 122 'recent' GDM mothers are shown in Table 3.1, along with those of the control population (876 women) and 'earlier' GDM (98 women). 'Earlier' GDM and control groups had comparable height, ethnicity proportion, and IMD levels. In contrast, compared to the other two groups, women from 'recent' GDM group were more ethnically diverse (76% Caucasian, 13% Asian, 3% African, and 8% mixed/other ethnic categories), more deprived (higher IMD level), and shorter.

Both GDM groups had higher BMI, higher smoking rate, and higher C-section deliveries compared to controls.

Between the two GDM groups, 'recent' GDM mothers were more likely to be primiparous, delivered at an earlier GA, and had lower fasting but higher OGTT 60- and 120-minute venous glucose concentrations.

Table 3.1 Maternal baseline demographics and offspring birth characteristics

Values are mean ± standard deviation (SD) or %

[a]SDS, standard deviation score (for weight, length, head circumference, and BMI are calculated using the UK 1990 reference, for skinfolds using internal references). All SDS values are adjusted for GA, sex and postnatal age at measurement

IMD, index of multiple deprivation; GA, gestational age; SGA, small for gestational age; SFT, skinfolds thicknesses

*p<0.05 vs. control group; **p<0.005 vs. control group

	Control (Total N=876)	**'Recent' OGDM (Total N=122)**	**'Earlier' OGDM (Total N=98)**
Maternal demographics			
Age at birth (years)	33.4 ± 4.2	33.6 ± 5.1	33.4 ± 4.4
Caucasian ethnicity	96%	76%**	98%
IMD	8.9 ± 3.3	11.3 ± 6.8**	9.1 ± 3.6
Primiparous pregnancy	48%	52%	37%*
Height (cm)	166.1 ± 7.2	162.7 ± 6.8**	165.8 ± 6.9
Pre-pregnancy BMI (kg/m^2)	24.0 ± 4.4	27.0 ± 6.3**	26.6 ± 5.6**
OGTT gestational age (weeks)	28.5 ± 0.7	28.9 ± 5.6	28.5 ± 1.5
Fasting venous glucose (mmol/l)	4.2 ± 0.3	4.8 ± 0.8**	5.3 ± 1.1**
60 minutes venous glucose (mmol/l)	6.5 ± 1.4	10.6 ± 1.5**	9.2 ± 2.1**
120 minutes venous glucose (mmol/l)	6.0 ± 1.0	8.5 ± 1.6**	6.7 ± 1.9**
Smoking during pregnancy	4%	9%*	8%*
Offspring birth characteristics			
GA (weeks)	40.0 ± 1.3	38.9 ± 0.9**	39.5 ± 1.4**
Caesarean delivery	28%	42%**	40%*
Male infant sex	52%	54%	53%
Weight (kg)	3.523 ± 0.481	3.303 ± 0.472**	3.632 ± 0.588
Weight SDS[a]	0.07 ± 0.93	0.10 ± 1.01	0.55 ± 1.13**
Length (cm)	51.5 ± 2.4	50.0 ± 2.0**	51.3 ± 2.7
Length SDS[a]	-0.05 ± 0.93	-0.07 ± 0.94	0.22 ± 0.97*
Head circumference (cm)	34.9 ± 1.3	34.6 ± 1.2	34.7 ± 1.4
Head circumference SDS[a]	-0.14 ± 0.94	0.05 ± 0.88	0.04 ± 1.01
BMI (kg/m^2)	13.7 ± 1.3	13.2 ± 1.4	13.9 ± 1.6
BMI SDS[a]	0.38 ± 1.06	0.15 ± 1.11	0.75 ± 1.12**
Ponderal Index (kg/m^3)	25.9 ± 3.2	26.3 ± 2.7	26.7 ± 3.2

Triceps SFT (mm)	5.2 ± 1.2	4.5 ± 0.8**	5.3 ± 1.1
Subscapular SFT (mm)	5.0 ± 1.2	4.8 ± 1.0	5.3 ± 1.1
Flank SFT (mm)	5.7 ± 1.6	4.8 ± 1.1**	6.9 ± 2.0**
Quadriceps SFT (mm)	7.2 ± 1.8	5.9 ± 1.3**	6.9 ± 1.8
Sum skinfolds thicknesses (mm)	24.6 ± 6.0	20.0 ± 3.6**	26.0 ± 6.3
Triceps SFT SDS	0.04 ± 1.03	-0.42 ± 0.65**	0.30 ± 0.97*
Subscapular SFT SDS	0.02 ± 1.02	-0.22 ± 0.87*	0.17 ± 1.01
Flank SFT SDS	0.05 ± 0.97	-0.59 ± 0.64**	0.45 ± 1.26**
Quadriceps SFT SDS	0.04 ± 1.03	-0.40 ± 0.66**	0.32 ± 1.01*
Mean SFT SDS[a]	0.03 ± 0.86	-0.41 ± 0.61**	0.31 ± 0.85*
Macrosomia (birth weight > 4.0 kg)	15%	7%*	27%**
SGA (birth weight ≤ -1.5 SDS)	5%	4%	2%

To examine participation bias, demographics of the recruited 'recent' GDM group were also compared to the wider hospital GDM population, using routine data available for all women with GDM seen in the hospital clinic who gave births in 2011 (Table 3.2). The total 2011 GDM population was similar to the study cohort, with no large differences in maternal age (32.6±5.6 years), gestational age (38.8±1.0 weeks), and birth weight SDS (0.25±1.0)

Table 3.2 'Recent' OGDM study cohort vs. 2011 Hospital GDM statistics

Values are mean or %

GA, gestational age

[a]SDS, standard deviation score (calculated using the UK 1990 reference, adjusted for GA, sex and postnatal age at measurement)

	'Recent' OGDM cohort N=122	**2011 Hospital GDM N=231**
Maternal age (years)	33.6	32.6
GA (weeks)	38.9	38.8
Birth weight (kg)	3.3	3.3
Birth weight SDS[a]	0.1	0.25
Induced (%)	80%	83%
Emergency C-section	22%	15%
Elective C-section	20%	26%

Furthermore, to evaluate if the control group used in the analysis was appropriate, this group was compared with a smaller more recent CBGS control group, born in

2015-2018 (Table 3.3). From birth to 24 months, there were no significant difference in anthropometric measurements between these two control groups apart from a marginal difference in weight SDS at 3 months. Since the number of subjects of the original CBGS cohort ('earlier' control) is much bigger than the more recent control group, the earlier control group was used for analyses in this chapter.

Table 3.3 Comparison of cross-sectional growth trajectories between 'earlier' versus 'recent' controls

Values are mean ± SD
SDS, standard deviation score (for weight and length are calculated using the UK 1990 reference, for skinfolds using internal references)
Significant p is typed in bold
Weight-, length-, and mean skinfolds-SDS values are adjusted for gestational age, sex and postnatal age at measurement

	'Earlier' Control (Born in 2001-2009)	**'Recent' Control (Born in 2015-2018)**	***p***
Birth	**Total N=874**	**Total N=118**	
Weight SDS	0.07 ± 0.93	0.19 ± 0.85	*0.198*
Height SDS	-0.05 ± 0.93	-0.14 ± 0.89	*0.358*
Mean skinfolds SDS	0.03 ± 0.86	-0.07 ± 0.70	*0.216*
3 months	**Total N=710**	**Total N=107**	
Weight SDS	-0.05 ± 1.03	0.22 ± 1.22	***0.034***
Height SDS	0.14 ± 0.94	0.22 ± 1.09	*0.429*
Mean skinfolds SDS	-0.01 ± 0.81	-0.11 ± 0.70	*0.233*
12 months	**Total N=621**	**Total N=86**	
Weight SDS	0.08 ± 1.07	0.07 ± 1.14	*0.957*
Height SDS	0.36 ± 1.09	0.24 ± 1.05	*0.311*
Mean skinfolds SDS	0.02 ± 0.78	-0.01 ± 0.68	*0.714*
24 months	**Total N=710**	**Total N=38**	
Weight SDS	0.19 ± 1.04	0.44 ± 0.96	*0.152*
Height SDS	0.43 ± 1.05	0.50 ± 0.94	*0.691*
Mean skinfolds SDS	0.02 ± 0.83	-0.04 ± 0.60	*0.716*

The 'recent' GDM study group consisted of 71 women who received nutrition/lifestyle advice alone during pregnancy, and 51 additionally prescribed medication (27 insulin, 9 metformin, 15 both). Comparing these treatment

subgroups, women needing metformin and/or insulin during pregnancy had higher BMI (29.7 vs. 25.1, p<0.0005), were diagnosed with GDM earlier (27.5 vs. 29.9 weeks, p=0.02), and had higher fasting (5.2 vs. 4.5, p<0.0005) and 60 minute blood glucose (11.1 vs. 10.3, p=0.006), with no differences in glucose levels at 120 minutes (Table 3.4). Moreover, they were also more likely to deliver earlier (38.5 vs. 39.2 weeks gestation, p<0.0005), with higher Caesarean delivery rate (53% vs. 32%). There was no difference in the incidence of neonatal hypoglycaemia or in infant anthropometry at birth. There was no detailed treatment information available from the 'earlier' GDM group.

Table 3.4 'Recent' OGDM subgroup: comparison between mothers given dietary & lifestyle advice alone vs. dietary, lifestyle advice & medication

Values are mean ± SD or %
GA, gestational age; SFT, skinfolds thicknesses
[a]SDS, standard deviation score (for weight and length are calculated using the UK 1990 reference, for skinfolds using internal references). All SDS values are adjusted for GA, sex and postnatal age at measurement. SFT were measured from 4 sites: triceps, subscapular, flank, and quadriceps.
*p<0.05, **p<0.0005

	Lifestyle only (N=71)	**Medication (N=51)**
Demographics		
GA (weeks)	39.2±0.9	38.5±0.6**
Maternal BMI (kg/m^2)	25.1±5.0	29.7±6.9**
Maternal age (years)	33.4±5.1	33.8±5.2
Caucasian ethnicity	73%	75%
Primiparous pregnancy	62%	45%
GA when diagnosed with GDM (weeks)	29.9±5.6	27.5±5.4
Venous glucose 0 min (mmol/l)	4.5±0.4	5.2±0.9**
Venous glucose 60 min (mmol/l)	10.3±1.3	11.1±1.7*
Venous glucose 120 min (mmol/l)	8.4±1.2	8.7±2.0
Caesarean section delivery	34%	57%*
Infant sex (% male)	52%	55%
Birth anthropometry		
Weight SDS[a]	-0.04±1.04	0.28±0.96
Length SDS[a]	-0.13±0.94	0.02±0.94
Mean SFT SDS[a]	-0.52±0.64	-0.5±0.65
Neonatal hypoglycaemia	28%	31%

Birth anthropometry

As expected, 'earlier' OGDM were significantly heavier at birth than controls (Table 3.1), even after further adjustment for all covariates (Table 3.6, *p=0.002*). Furthermore, fully adjusted birth weight and adiposity of 'earlier' OGDM were significantly greater than those of 'recent' OGDM (Table 3.6).

In contrast, the 'recent' OGDM had similar birth weight (0.10±1.01) and length (-0.07±0.94) SDS to the control population (Table 3.1). Of interest, the mean skinfold thickness measurements were lower in 'recent' OGDM than controls (-0.40 vs.0.03, p<0.005, Table 3.1). The triceps, flank and quadriceps skinfold thickness (SFT) measurements were particularly lower, with less difference for the subscapular skinfold (Table 3.1).

Since the 'recent' GDM mothers were more ethnically diverse than the other groups, a sensitivity analysis of solely Caucasian mother-infant dyads was performed (Table 3.5). The Caucasian babies from the 'recent' OGDM subgroup again had birth weight and length SDS not differing to control infants with much reduced SFT measurements (p<0.0005 against both control and 'earlier' groups). A significant difference between SFT measurements of 'recent' OGDM and control infants was again observed when included in a multivariate analysis, with maternal ethnicity, BMI, height, age, parity, and other covariates (p<0.0001, Table 3.6).

Table 3.5 Birth anthropometry comparison between Caucasian-only populations

Values are mean ± SD
[a]SDS, standard deviation score (for weight and length are calculated using the UK 1990 reference, for skinfolds using internal references). All SDS values are adjusted for GA, sex and postnatal age at measurement. SFT were measured from 4 sites: triceps, subscapular, flank, and quadriceps.
**p<0.005 vs. control group
Weight-, length-, and mean skinfolds-SDS values are adjusted for GA, sex and postnatal age at measurement

	Control (Total N=836)	**'Recent' OGDM (Total N=90)**	**'Earlier' OGDM (Total N=88)**
Weight SDS[a]	0.09±0.94	0.14±1.03	0.49±1.11**
Length SDS[a]	-0.01±0.91	-0.05±0.97	0.18±0.93
Mean SFT SDS[a]	0.31±1.06	-0.56±0.6**	0.52±1.2

Predictors of neonatal anthropometry in GDM groups

Among the 'recent' GDM group, maternal height and pre-pregnancy weight were positively related to offspring birth weight SDS (maternal weight *p=0.005*, height *p=0.012*). Similarly, maternal pre-pregnancy weight and BMI positively related with infant BMI at birth (maternal weight *p=0.004*, BMI *p=0.01*). The only associations found between maternal factors and infant SFT at birth were between pregnancy fasting glucose level with flank SFT (R=0.26, *p=0.004*) and mean SFT (R=0.19, *p=0.038*). Other OGTT timepoints, 60- or 120 minutes, did not correlate with infant SFT at birth in this group.

Among the 'earlier' OGDM, maternal height showed a positive association with infant birth weight (*p=0.002*). Pregnancy glucose level at 60 minutes was positively related with infant mean SFT at birth (*p=0.044*, Figure 3.1), but not with any infant individual SFT measurements.

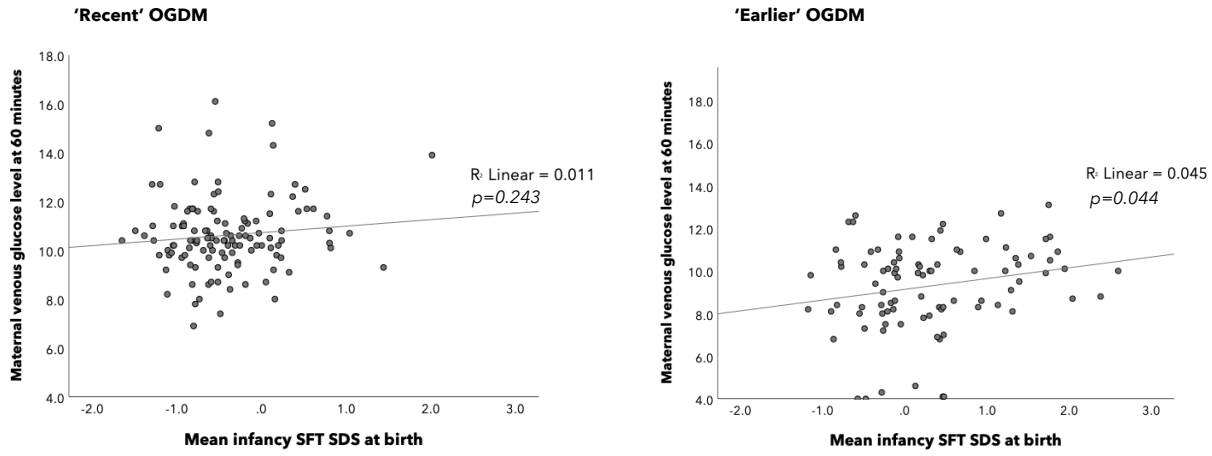
Table 3.6 Linear regression comparison of infant growth parameters at birth between groups
Model 1: adjusted for GA, sex, and age at measurement
Model 2: Model 1 + adjusted for pre-pregnancy maternal BMI, maternal height (for height gain only), parity (primiparous, yes/no), feeding history (exclusively breastfed at 3 months, yes/no; except for birth anthropometry), maternal ethnicity (Caucasian decent, yes/no), IMD, delivery method (Caesarean delivery, yes/no), maternal smoking history during pregnancy (yes/no).

B (regression coefficients) ± standard error (SE) are displayed
p^1 = *p* values for with predictor: 'Recent' OGDM vs 'Earlier' OGDM groups comparison

Outcomes: Birth anthropometry		**Predictor: Infant groups in comparison to controls (controls as reference)**				
		'Recent' OGDM vs. Controls		**'Earlier' OGDM vs. Controls**		
		B±SE	***p***	**B±SE**	***p***	***p*[1]**
Weight SDS	Model 1	-0.11±0.09	*0.24*	**0.21±0.05**	***<0.0001***	***0.001***
	Model 2	-0.05±0.1	*0.629*	**0.16±0.05**	***0.002***	***0.01***
Length SDS	Model 1	-0.01±0.11	*0.925*	0.08±0.06	*0.192*	*0.157*
	Model 2	-0.11±0.11	*0.306*	0.05±0.06	*0.347*	*0.567*
Skinfolds SDS	Model 1	**-0.51±0.09**	***<0.0001***	0.07±0.05	*0.167*	***<0.0001***
	Model 2	**-0.53±0.09**	***<0.0001***	0.05±0.05	*0.385*	***<0.0001***

There was a strong positive association between neonatal skinfolds and maternal glycated haemoglobin concentrations at diagnosis among the 'recent' OGDM (B=0.04, *p<0.0005*), but no relationship with birth weight. There was no available data on glycated haemoglobin from the 'earlier' GDM group.

Figure 3.1 Correlation between maternal 60-minute glucose level and mean SFT at birth among GDM groups



Although there was a trend for 'recent' GDM mothers who were prescribed medication during pregnancy to deliver larger babies, as well as those who had poorer glucose control, these associations did not reach statistical significance. It was not possible to separately analyse results for metformin vs. insulin treatment, due to the small numbers available in each subgroup.

As a comparison, among the controls, there were strong positive correlations between maternal factors, including parity, maternal pre-pregnancy BMI and weight, maternal height, and pregnancy glucose levels, with infant weight and adiposity at birth.

3.3.2 Postnatal growth trajectories

After birth, the 'earlier' OGDM displayed the expected downwards growth trajectories. Weight and length gains were significantly lower in this group from 3 and 12 months compared to controls, adjusting for all covariates (Figure 3.2, Table 3.7). Between 12 and 24 months, there were no significant difference observed in weight, height and skinfold thicknesses between 'earlier' OGDM and control infants. However, at all time-points, 'earlier' OGDM maintained slightly higher subcutaneous adiposity (reflected by mean SFT) compared to controls.

In contrast, 'recent' OGDM showed different postnatal growth trajectories to the 'earlier' OGDM and controls. In this group, there was an upward trend for weight and adiposity between birth and 3 months (Figure 3.2) compared to the other 2 groups, but with reduced length gain only when compared to controls. In the mixed

models, these differences remained after adjustment for covariates (Table 3.8). This trend was then followed by lower weight, length and adiposity gains, between 3 and 12 months, compared to the steady growth of control infants (Figure 3.2). Weight and length SDS for the 'recent' OGDM only became comparable to the control infants at 24 months, showing significant gains in weight and length (especially length) between 12 and 24 months. Meanwhile, their mean SFT was consistently lower than controls until 24 months (Table 3.7 and Figure 3.2). Even after adjusting for potential confounding factors, including maternal BMI, height, parity, ethnicity, and IMD, these unusual growth patterns of 'recent' OGDM persisted (Table 3.8).

Figure 3.2 a-f. Weight, length, and skinfolds growth trajectories of 'recent' or 'earlier' OGDM compared to controls from birth to 2 years

Plotted values are mean ± SE of SDS values, adjusted for sex, gestational age (birth and 3 months only), and age at measurement. Comparisons are adjusted for sex, postnatal age at measurement, pre-pregnancy maternal BMI, maternal height (for length only), parity, 0-3 months feeding history, delivery method, maternal ethnicity, socioeconomic status reflected by index of multiple derivation, and pregnancy smoking history. Comparisons from 0-3 months are additionally adjusted for gestational age at birth.

Horizontal bars indicate statistically significant differences between OGDM and control groups for the displayed growth periods (* and dotted bar: p<0.05; ** and solid bar: p<0.001). Significance is based on linear mixed-effect models of infant growth parameters between groups, with time modelled using linear splines (Table 3.8).

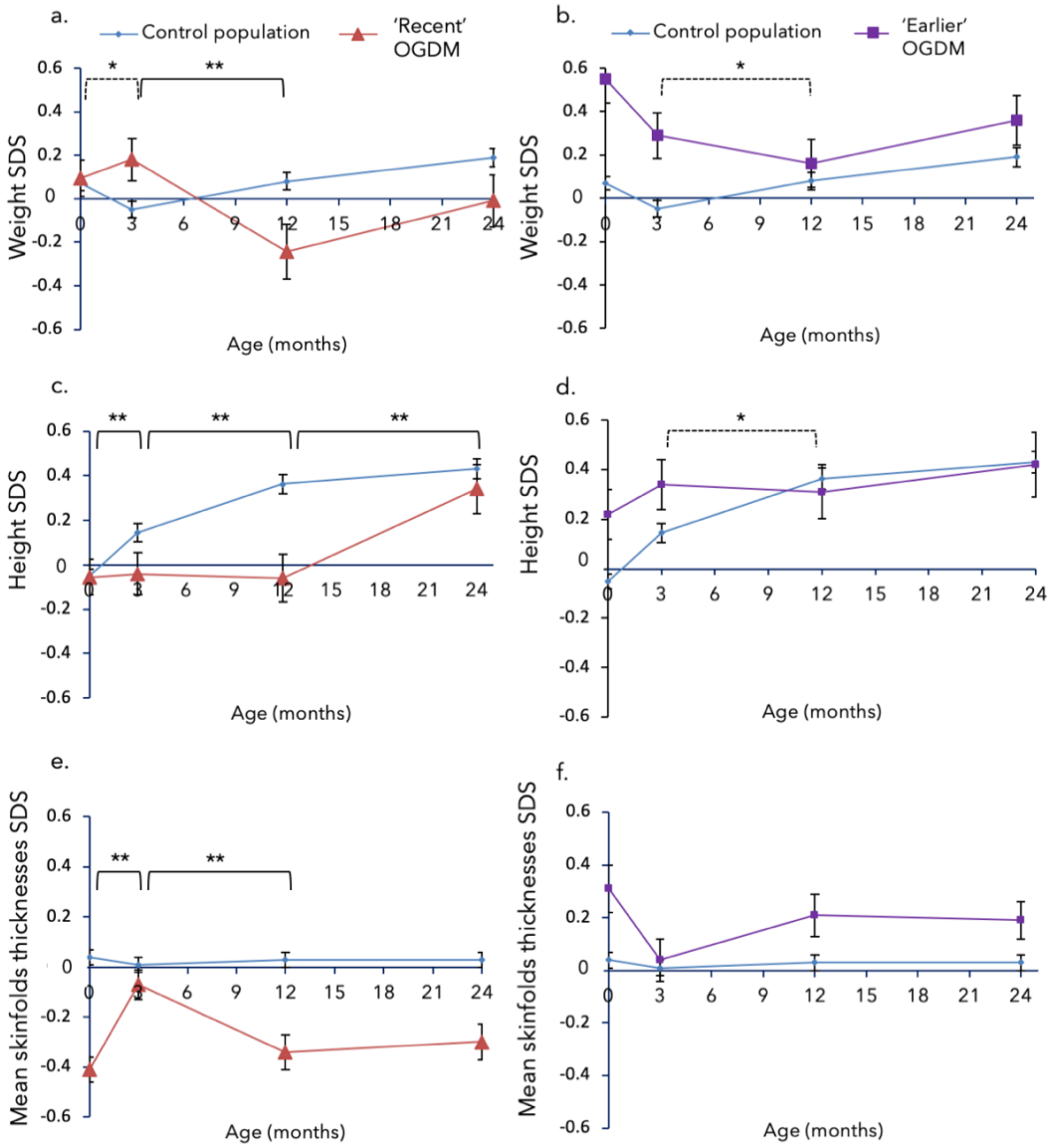


Table 3.7 Cross-sectional comparisons of postnatal infant growth parameters at 3, 12, and 24 months

Values are mean ± SD, or %
SDS, standard deviation score (for weight and length are calculated using the UK 1990 reference, for skinfolds using internal references). Weight-, length-, and mean skinfolds-SDS values are adjusted for gestational age, sex and postnatal age at measurement
*p<0.05 vs. control group; **p<0.005 vs. control group
Comparisons are adjusted for sex, postnatal age at measurement, pre-pregnancy maternal BMI, maternal height (for length only), parity, and 0-3 months feeding history. Comparisons at age 3 months are additionally adjusted for gestational age at birth.

	Control	**'Recent' OGDM**	**'Earlier' OGDM**
3 months	**Total N=710**	**Total N=102**	**Total N=91**
Nutrition (% of 0-3 months exclusively breastfed)	45%	46%	38%
Weight (kg)	6.134 ± 0.784	6.069 ± 0.808	6.285 ± 0.853
Weight SDS	-0.05 ± 1.03	0.18 ± 1.04	0.29 ± 1.02
Length (cm)	61.1 ± 2.3	59.9 ± 2.4**	61.2 ± 2.4
Length SDS	0.14 ± 0.94	-0.03 ± 0.98	0.34 ± 0.96
Head circumference (cm)	40.7 ± 1.3	40.4 ± 1.2*	40.7 ± 1.3
Head circumference SDS	-0.21 ± 0.97	-0.45 ± 0.85	-0.18 ± 0.86
BMI (kg/m^2)	16.4 ± 1.5	16.9 ± 1.4*	16.9 ± 1.02
BMI SDS	-0.25 ± 1.12	0.11 ± 1.01*	0.1 ± 0.85
Ponderal Index (kg/m^3)	26.8 ± 2.4	28.2 ± 2.3**	27.4 ± 2.4
Triceps SFT SDS	0.02 ± 1.04	-0.27 ± 0.75*	0.18 ± 0.92
Subscapular SFT SDS	-0.01 ± 1.00	0.14 ± 0.98	-0.09 ± 1.01
Flank SFT SDS	0.001 ± 1.03	-0.03 ± 0.83	0.01 ± 0.92
Quadriceps SFT SDS	0.01 ± 1.03	-0.13 ± 0.77	0.09 ± 0.97
Mean SFT SDS	-0.01 ± 0.81	-0.07 ± 0.65	0.04 ± 0.78
12 months	**Total N=624**	**Total N=86**	**Total N=77**
Weight (kg)	9.99 ± 1.159	9.711 ± 1.42	10.076 ± 1.158
Weight SDS	0.08 ± 1.07	-0.25 ± 1.26**	0.16 ± 1.00
Length (cm)	75.9 ± 2.8	75.0 ± 3.0*	75.7 ± 2.6
Length SDS	0.36 ± 1.09	-0.01 ± 1.07	0.31 ± 0.98
Head circumference (cm)	46.4 ± 1.4	46.4 ± 1.5	46.5 ± 1.2
Head circumference SDS	-0.62 ± 1.08	-0.68 ± 1.06	-0.58 ± 0.97
BMI (kg/m^2)	17.3 ± 1.4	17.2 ± 1.7	17.5 ± 1.3
BMI SDS	-0.16 ± 1.02	-0.3 ± 1.23	-0.02 ± 0.92
Ponderal Index (kg/m^3)	22.9 ± 1.9	23.0 ± 2.2	23.1 ± 1.7
Triceps SFT SDS	0.03 ± 1.02	-0.47 ± 0.85**	0.27 ± 0.88
Subscapular SFT SDS	0.01 ± 1.02	-0.09 ± 0.97	0.05 ± 0.90
Flank SFT SDS	0.03 ± 1.01	-0.38 ± 0.80**	0.24 ± 1.04
Quadriceps SFT SDS	0.04 ± 1.00	-0.50 ± 0.90**	0.29 ± 0.98
Mean SFT SDS	0.02 ± 0.78	-0.36 ± 0.74**	0.21 ± 0.69

24 months	**Total N=611**	**Total N=83**	**Total N=76**
Weight (kg)	12.596 ± 1.441	12.297 ± 1.591	12.837 ± 1.44
Weight SDS	0.19 ± 1.04	-0.03± 1.10	0.36 ± 0.96
Length (cm)	87.7 ± 3.3	87.3 ± 3.6	87.8 ± 3.5
Length SDS	0.43 ± 1.05	0.34 ± 1.11	0.42 ± 1.11
Head circumference (cm)	48.7 ± 1.4	49.0 ± 2.8	48.9 ± 2.6
Head circumference SDS	-0.76 ± 1.04	-0.52 ± 2.04	-0.66 ± 0.93
BMI (kg/m^2)	16.3 ± 1.2	16.1 ± 1.3	16.6 ± 1.0
BMI SDS	-0.2 ± 0.94	-0.42 ± 0.99	0.16 ± 1.05*
Ponderal Index (kg/m^3)	18.6 ± 1.5	18.4 ± 1.5	19.0 ± 1.2
Triceps SFT SDS	0.02 ± 1.03	-0.28 ± 0.87*	0.16 ± 0.88
Subscapular SFT SDS	0.01 ± 1.04	-0.13 ± 0.82	0.07 ± 0.81
Flank SFT SDS	0.03 ± 1.04	-0.43 ± 0.68**	0.26 ± 0.79
Quadriceps SFT SDS	0.03 ± 1.02	-0.44 ± 0.78**	0.25 ± 0.92
Mean SFT SDS	0.02 ± 0.83	-0.31 ± 0.65**	0.19 ± 0.64

Table 3.8 Linear mixed-effect models of infant growth parameters between groups

Smoothing splines were added to the models with knots at 3 and 12 months
Model 1: adjusted for gestational age (birth and 3 months growth outcomes only), sex, and age at measurement
Model 2: Model 1 + adjusted for pre-pregnancy maternal BMI, maternal height (for height gain only), parity (primiparous, yes/no), feeding history (exclusively breastfed at 3 months, yes/no; except for birth anthropometry), maternal ethnicity (Caucasian decent, yes/no), index of multiple deprivation, delivery method (Caesarean delivery, yes/no), maternal smoking history during pregnancy (yes/no).

Fixed effect estimates (visit period and group interaction) ±SE are displayed

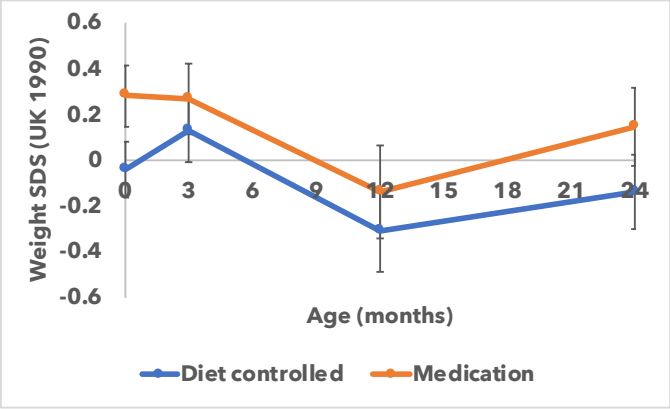
p^1 = *p* values for models with predictor: 'Recent' OGDM vs 'Earlier' OGDM

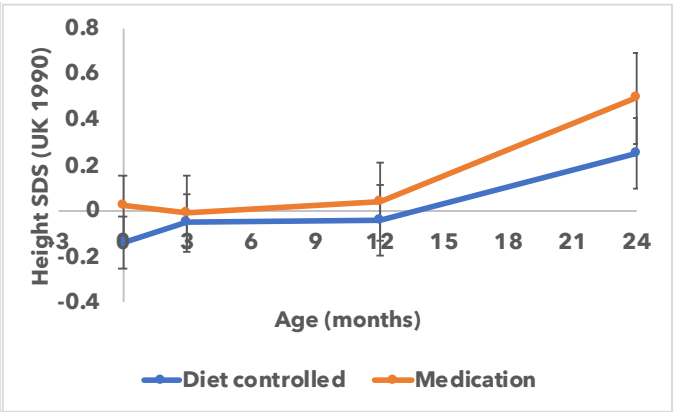
Outcomes: Growth gains		**Predictor: Infant groups in comparison to controls (controls as reference)**				
		'Recent' OGDM vs controls		**'Earlier' OGDM vs controls**		
		Estimate±SE	***p***	**Estimate±SE**	***p***	***p*[1]**
0-3 months						
Weight SDS	Model 1	**0.07±0.03**	***0.03***	-0.05±0.04	*0.18*	***0.0096***
	Model 2	**0.07±0.03**	***0.039***	-0.05±0.04	*0.172*	***0.01***
Length SDS	Model 1	**-0.06±0.03**	***0.04***	-0.03±0.03	*0.372*	*0.477*
	Model 2	**-0.06±0.03**	***0.035***	-0.03±0.04	*0.36*	*0.467*
Skinfolds SDS	Model 1	**0.14±0.03**	$***1.37*10^{-5}***$	-0.04±0.04	*0.277*	$***8.43*10^{-5}***$
	Model 2	**0.14±0.03**	$***1.54*10^{-5}***$	-0.05±0.04	*0.235*	$***6.45*10^{-5}***$
3-12 months						
Weight SDS	Model 1	**-0.06±0.01**	$***1.39*10^{-7}***$	**-0.03±0.01**	***0.01***	***0.06***
	Model 2	**-0.06±0.01**	$***1.42*10^{-7}***$	**-0.03±0.01**	***0.01***	*0.057*
Length SDS	Model 1	**-0.03±0.01**	***0.004***	**-0.03±0.01**	***0.009***	*0.943*
	Model 2	**-0.03±0.01**	***0.005***	**-0.03±0.01**	***0.01***	*0.969*
Skinfolds SDS	Model 1	**-0.03±0.01**	***0.009***	0.02±0.01	*0.15*	***0.0025***
	Model 2	**-0.03±0.02**	***0.008***	0.02±0.01	*0.146*	***0.002***
12-24 months						
Weight SDS	Model 1	0.015±0.01	*0.123*	0.003±0.01	*0.768*	*0.368*
	Model 2	0.01±0.01	*0.127*	0.003±0.01	*0.79*	*0.361*
Length SDS	Model 1	**0.029±0.005**	$***2.78*10^{-5}***$	0.004±0.01	*0.638*	***0.007***
	Model 2	**0.035±0.008**	$***2.95*10^{-5}***$	0.004±0.009	*0.688*	***0.006***
Skinfolds SDS	Model 1	0.004±0.01	*0.638*	-0.005±0.01	*0.602*	*0.455*
	Model 2	0.004±0.01	*0.649*	-0.006±0.01	*0.578*	*0.446*

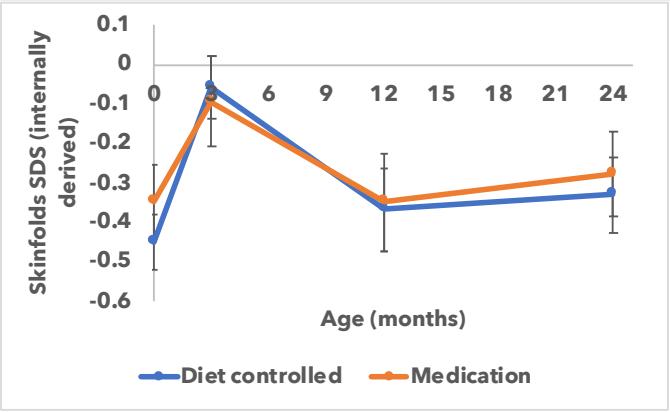
As mentioned before, out of 122 mothers of 'recent' GDM group with medication history, 71 received lifestyle modification only, while the remaining 51 were also treated with insulin with or without metformin. When analysed based on the treatment modalities received, there were no differences observed in postnatal growth trajectories among 'recent' OGDM (Figure 3.3).

Figure 3.3 Weight, length, and skinfolds growth trajectories between lifestyle-modified vs. medication groups of 'recent' OGDM from birth to 2 years

Plotted values are mean ± SE of SDS values, adjusted for sex, gestational age (birth and 3 months only), and age at measurement.







Additionally, as many as 19% women in the 'earlier' GDM group were identified retrospectively and thus did not receive any GDM treatment. However, excluding these women from the analyses gave similar findings (Table 3.9).

Table 3.9 Linear regression comparison of infant growth parameters between 'Earlier' OGDM and controls, with/without untreated subjects

Model 1: adjusted for gestational age (birth and 3 months growth outcomes only), sex, and age at measurement
Model 2: Model 1 + adjusted for pre-pregnancy maternal BMI, maternal height (for length and height gain only), parity (primiparous, yes/no), feeding history (exclusively breastfed at 3 months, yes/no; except for birth anthropometry), maternal ethnicity (Caucasian descent, yes/no), index of multiple deprivation, delivery method (Caesarean delivery, yes/no), maternal smoking history during pregnancy (yes/no).

B (regression coefficients) ±SE are displayed

Outcomes: Growth parameters			**Predictor: 'Earlier' OGDM vs Controls (controls as reference)**	
			B±SE	***p***
Birth anthropometry				
Weight SDS	Model 1	Include all subjects	**0.27±0.06**	***<0.0001***
		Without 19% untreated subjects	**0.26±0.06**	***<0.0001***
	Model 2	Include all subjects	**0.15±0.06**	***0.01***
		Without 19% untreated subjects	0.12±0.07	*0.064*
Length SDS	Model 1	Include all subjects	**0.14±0.06**	***0.015***
		Without 19% untreated subjects	**0.13±0.06**	***0.048***
	Model 2	Include all subjects	0.08±0.06	*0.201*
		Without 19% untreated subjects	0.05±0.07	*0.462*
Skinfolds SDS	Model 1	Include all subjects	**0.13±0.05**	***0.015***
		Without 19% untreated subjects	**0.16±0.06**	***0.005***
	Model 2	Include all subjects	0.02±0.06	*0.699*
		Without 19% untreated subjects	0.05±0.06	*0.382*
Growth gains between 0-3 months				
Weight SDS	Model 1	Include all subjects	-0.10±0.06	*0.085*
		Without 19% untreated subjects	-0.12±0.07	*0.079*
	Model 2	Include all subjects	-0.06±0.07	*0.338*
		Without 19% untreated subjects	-0.05±0.07	*0.529*
Length SDS	Model 1	Include all subjects	-0.06±0.05	*0.2*
		Without 19% untreated subjects	-0.085±0.05	*0.117*
	Model 2	Include all subjects	-0.07±0.05	*0.205*
		Without 19% untreated subjects	-0.097±0.06	*0.107*
Skinfolds SDS	Model 1	Include all subjects	-0.12±0.07	*0.073*

		Without 19% untreated subjects	**-0.16±0.07**	***0.024***
	Model 2	Include all subjects	0.003±0.07	*0.967*
		Without 19% untreated subjects	-0.03±0.08	*0.671*
Growth gains between 3-12 months				
Weight SDS	Model 1	Include all subjects	-0.1±0.06	*0.07*
		Without 19% untreated subjects	-0.11±0.06	*0.079*
	Model 2	Include all subjects	-0.07±0.06	*0.234*
		Without 19% untreated subjects	-0.08±0.07	*0.251*
Length SDS	Model 1	Include all subjects	**-0.14±0.06**	***0.015***
		Without 19% untreated subjects	**-0.15±0.06**	***0.017***
	Model 2	Include all subjects	-0.09±0.06	*0.13*
		Without 19% untreated subjects	-0.07±0.07	*0.31*
Skinfolds SDS	Model 1	Include all subjects	**0.13±0.06**	***0.023***
		Without 19% untreated subjects	**0.14±0.06**	***0.034***
	Model 2	Include all subjects	0.1±0.07	*0.123*
		Without 19% untreated subjects	0.11±0.07	*0.147*
Growth gains between 12-24 months				
Weight SDS	Model 1	Include all subjects	-0.01±0.04	*0.8*
		Without 19% untreated subjects	-0.01±0.04	*0.803*
	Model 2	Include all subjects	-0.02±0.04	*0.702*
		Without 19% untreated subjects	-0.01±0.04	*0.758*
Length SDS	Model 1	Include all subjects	0.01±0.04	*0.9*
		Without 19% untreated subjects	0.01±0.04	*0.779*
	Model 2	Include all subjects	0.03±0.04	*0.541*
		Without 19% untreated subjects	0.03±0.05	*0.524*
Skinfolds SDS	Model 1	Include all subjects	-0.03±0.05	*0.5*
		Without 19% untreated subjects	-0.04±0.06	*0.486*
	Model 2	Include all subjects	-0.05±0.06	*0.379*
		Without 19% untreated subjects	-0.05±0.06	*0.453*

3.4 Discussion

There were significant differences in birth size and postnatal infancy growth trajectories observed between the 'recent' and 'earlier' OGDM, when separately compared to the control group. While the 'earlier' OGDM were consistent with the classical features of OGDM in the literature by being born heavier, longer, and more

adipose at birth[57,233], the 'recent' OGDM group had comparable birth weights and lengths SDS to controls, but with lower SFT, indicating reduced adiposity. Interestingly, triceps, flank and quadriceps skinfold thicknesses were reduced more than subscapular skinfold thickness (Table 3.7), suggesting that peripheral adiposity may be particularly reduced at birth in these infants. This needs to be further investigated with more detailed body composition measures.

Although the control infants and the 'recent' OGDM were born in non-overlapping years, comparison between these two groups was still considered appropriate. This was because the anthropometric measures between two control groups in the CBGS born in similar timeframes as those of the two OGDM groups did not differ substantially (Table 3.3). Moreover, hospital demographic data (Table 3.2) for all GDM mother-infant dyads over a year period during study recruitment were also similar to the study population. Therefore, the unexpected anthropometric findings of 'recent' OGDM at birth were unlikely to be due to study participation bias.

Reduced subcutaneous adiposity among 'recent' OGDM in the CBGS might be novel finding but this supported a previous report by Au *et al*. in an Australian cohort[240], who reported similar birth weights for OGDM and control infants. They also demonstrated reduced total body fat in OGDM, measured using ADP, although this difference did not reach significance [mean±SD neonatal body fat %: 7.9±4.5% in OGDM vs. 9.5±4.3% in controls]. Of note, Au *et al*. (2013) reported findings in only 67 OGDM and it has been speculated as to whether this study was sufficiently powered to detect significant difference in body fat percentage[244]. This finding conflicted with a recent systematic review and meta-analysis (2017), including Au *et al*. and all other relevant studies published before 1 February 2014, which

concluded that neonatal adiposity is still increased in OGDM[245]. However, although in that study the overall numbers were large, the individual studies were generally very small, and many combined infants of type 1, type 2 and GDM mothers in the same analysis[245]. Furthermore, two of the most recent studies included actually showed no difference in body fat percentage of OGDM compared to controls. One of them was a UK study by Logan *et al*. that demonstrated no difference in total adipose tissue mass measured by MRI in OGDM born between 2011-2014 compared to controls at 11 days of life[179]. Moreover, this study also reported lower weights and lengths among OGDM compared to controls, measured in the first 2 weeks of life.[179]

Another study with similar findings was a recent randomised controlled trials in 'mild' GDM (no treatment vs. dietary and lifestyle advice with/without insulin) which have suggested a shift towards normal birth weights among OGDM whose mothers received treatment for GDM[173,239]. Therefore, the CBGS findings and the recent literature suggest that OGDM diagnosed and treated in the recent decade may result in offspring birth size comparable to the general population with reduced adiposity.

After demonstrating normal weight and reduced subcutaneous adiposity at birth, the 'recent' OGDM cohort showed significantly higher adjusted gains in weight and skinfold thickness compared to controls from birth to 3 months (despite similar breastfeeding rates) and ended up having comparable skinfold thickness with controls at 3 months. Logan *et al*.'s UK cohort (2011-14) also reported greater weight and adiposity gains from birth to 2.5 months of age in OGDM compared

with controls. However, in their study, this resulted in significantly greater total adipose tissue at 2.5 months, adjusting for infant sex and maternal pre-pregnancy BMI[179].

Interestingly, in the current study, after increased growth trajectories from birth to 3 months, the 'recent' OGDM showed subsequent reduced gains in weight, length and skinfold thicknesses from 3 to 12 months, making them significantly lighter with lower adiposity at 12 months compared to controls. The level of adiposity measured by skinfolds in this group remained lower than controls until the age of 24 months. By contrast, the 'earlier' macrosomic OGDM maintained higher subcutaneous adiposity during infancy than controls, despite showing an expected postnatal trend towards reduced gains in weight and skinfold thickness from birth to 3 months, and significantly lower gains in weight and length between 3 and 12 months

In the CBGS population, 'recent' OGDM have very different growth trajectories to 'earlier' OGDM over the first two years of life, despite having higher maternal BMI and higher OGTT 60-minute glucose concentrations. Both OGDM groups were defined using the same IADPSG criteria yet the observed differences persisted. Therefore, it can be speculated that the normalisation of birth anthropometry seen in 'recent' OGDM is likely due to an intensification of GDM monitoring and treatment, rather than inclusion of 'milder' GDM cases. This could potentially be due to tighter glycaemic control *per se*, direct effects of medication (metformin) transported across the placenta, or interactions between these environmental factors, genetic predisposition, and epigenetic modulation. On the contrary, 'earlier' OGDM showed the typical finding of higher body weight at birth,

presumably due to greater nutrient supply in pregnancy and fetal hyperinsulinism. Unfortunately, there were no detailed information on glucose variability, maternal treatments, and HbA1c levels, especially among the 'earlier' OGDM, to further support this hypothesis.

The reduced length and skinfold gains from 3 to 12 months in 'recent' OGDM may reflect an endogenous predisposition to lower insulin secretion, possibly due to genetic or *in utero* factors. This may also explain the differences in subcutaneous fat deposition at birth. The subsequent normalisation of weight and length in 'recent' OGDM by 24 months may reflect the opposite effects of endogenous predisposition to obesity, possibly due to genetic or other familial factors.

Amelioration of the classic macrosomic phenotype is likely to be associated with fewer adverse outcomes, especially at birth; however, the long-term metabolic effect of early reduced infancy weight and subcutaneous fat is unclear and could potentially be associated with higher risks, particularly if then leading to catch-up growth, which would increase future risk of obesity and T2D[23,51]. The finding that tight glucose control can normalise birth weight but also be associated with reduced body size has been previously reported. Langer *et al*. investigated three groups in GDM pregnancies and showed that the group with lowest glucose values had a higher proportion of infants born small-for-gestational-age (SGA) than control pregnancies[246]. Treatment timing could also play a role as a recent study reported that early GDM treatment was associated with a higher rate of SGA-related NICU admissions, whereas later treatment resulted in more LGA infants[247]. Therefore,

whilst there are clear advantages of intensive multi-disciplinary GDM management, there may also be negative implications for some OGDM.

In later childhood, recent HAPO data on the follow up of infants born to mothers with a wide range of glucose values at 28-week gestation confirm a positive relationship between those levels and offspring adiposity at 10-14 years of age, measured by skinfolds thicknesses and ADP (BOD POD machine)[248]. As well as linking high antenatal glucose exposures to childhood overweight/obesity, we could also infer from these data that lower glucose exposures might result in persisting lower offspring adiposity.

Treatment of GDM has changed significantly over the last decade, including more extensive and stringent diet and lifestyle advice to achieve tighter glucose targets[179]. In particular, the use of metformin, which crosses the placenta in significant amounts, has become more common. Whilst detailed medication use could not be included in the analyses due to lack of data, approximately 20% of women were treated with metformin during the recruitment time for 'recent' OGDM, compared to near zero during the 'earlier' OGDM recruitment period. Other recent studies also describe a higher proportion of GDM women who received metformin, for example, 50% in the study by Logan *et al*[179].

It has been suggested that metformin use itself may affect later infancy fat deposition. In the Metformin in gestational diabetes (MiG) trial, women with GDM were randomised to metformin (+/- insulin if needed) or insulin and they observed no difference in birth anthropometry. However, at 2 years of age, children whose mothers had received metformin had increased subscapular and biceps skinfold thicknesses, despite no difference in overall fat, suggesting a more favourable fat

distribution. In our 'recent' OGDM, there was a preferentially increased gain in subscapular skinfold thickness at 3 months of age but decreased until 24 months of age compared to the 'earlier' OGDM, which was assumed to have little to no metformin exposure (Table 3.7).

Moreover, out of 122 mothers of 'recent' group with medication history, 24 were treated with metformin (+/- insulin) and 27 with insulin only. Both subgroups showed similar weight, height, and adiposity measured by skinfolds thickness from 0-24 months. The remaining 71 mothers were treated with diet and exercise only. As seen in Figure 3.2, both treatment modalities subgroups (lifestyle only and lifestyle + medication groups) showed comparable birth anthropometry and postnatal growth trajectories until 24 months. As expected, women treated with no medication had significantly lower pre-pregnancy BMI and lower glucose levels, with higher primiparous and lower C-section rates.

At 7-9 years the children of mothers randomised to metformin compared with insulin in MiG trial had similar total body fat, as well as metabolic measures, although the 9-year olds from metformin group were larger by measures of weight, arm and waist circumferences, and waist/height ratio[249]. Metformin effects may also differ depending of maternal BMI and weight gain, supporting interaction of treatment effects, glycaemic control and other environmental factors. A study in polycystic ovary syndrome (PCOS) suggested a growth restriction effect of metformin among infants of normal-weight mothers, resulting in reduced length and weight at birth[250]. Further studies are therefore needed to elucidate the effect

of metformin itself, effects on maternal calorie intake and weight gain, and its interaction with other environmental factors.

Different from many other epidemiological studies on OGDM, the analyses above also involved neonatal length and skinfold thickness reflecting adiposity, in addition to birth weight. Additional adjustment for maternal baseline characteristics was also possible due to collection of detailed maternal and demographic data. A uniform application of the IADPSG diagnostic criteria was used in this study, and so results found are likely to be relevant to many populations worldwide, where GDM is now diagnosed with lower thresholds and treated more intensively. Despite those strengths, no available details of glycaemic control after GDM diagnosis made it difficult to test the hypothesis if the intensive GDM treatment had led to tight glycaemic control leading to birth size normalisation and secular postnatal growth trajectories.

Another limitation was, although the study population was large compared to most in the literature, there were insufficient numbers to investigate the specific effect of treatment and the differences between insulin and metformin as there was no access to detailed medication history among the 'earlier' OGDM. Detailed medication history, especially with or without metformin, should have been recorded to analyse the extent to which it associates with the offspring's growth outcomes. Besides that, in this study, only SFT was used to reflect adiposity. More detailed body composition measures, for example by MRI or ADP, are needed to provide more accurate estimation of adiposity. Pregnancy weight gain should also be carefully recorded to compare 'recent' and 'earlier' GDM cohorts.

It can be debated whether reduced body size in OGDM following intensified therapy is likely to have positive or negative implications, particularly on longer-term health. In the neonatal period it may be advantageous, especially in contributing to a normal birth weight and reduced pregnancy complications, although this may potentially increase the number of SGA deliveries. However, whether reduced adiposity at birth will encourage subsequent 'catch up' growth, and potentially lead to later increased metabolic disease risk[251] as is seen in other populations showing rapid infancy weight or adiposity gains, is yet to be established.

3.5 Conclusions and future recommendations

Current GDM diagnosis and management, consisting of dietary modification and frequent medication use in pregnancy, has resulted in better glycaemic control, as reported by recent studies, and comparable birth size to control infants, as reported in this study. This normalisation of birth weight in 'recent' OGDM, compared to the classical 'heavier and fatter at birth' displayed by 'earlier' OGDM, may possibly indicate improved later-life metabolic adverse outcomes[252]. However, there is also evidence from this study and the literature that the 'recent' OGDM rapid weight and SFT gain between 0-3 months postnatally could be more reflective of SGA infants, which with its subsequent catch-up growth trend may have its own metabolic risk[51]. Follow-up of these cohorts and replication in other populations are now needed, as well as studies to understand the mechanisms responsible for anthropometric outcomes in OGDM and ideal GDM management going forward. It may be that

there is a trade-off between ideal birth size and infancy growth trajectories. In addition, further research on adiposity distribution and fat deposits in OGDM is also of interest to investigate favourable adiposity gains.

Chapter 4 Growth and adiposity trajectories of infants born SGA versus infants of mothers with GDM

4.1 Introduction

Significant disruptions in nutritional provision occurring during the first 1,000 days of life, manifesting in size at birth and postnatal growth deviations, have been associated with adulthood metabolic derangements[150]. The majority of infants born small-for-gestational age (SGA) infants were exposed to placental insufficiency leading to intrauterine undernutrition and growth retardation[134], while offspring born to mothers with gestational diabetes mellitus (OGDM) was typically exposed to intrauterine overnutrition and hyperglycaemia[155].

Although the exact aetiology has not been elucidated yet in all cases, there are several inextricable maternal-fetal-antenatal predisposing factors for SGA as illustrated in Figure 1.5 (Section 1.2). Those factors would also affect the subsequent postnatal growth trajectories of infants born SGA, thus need to be taken into consideration when analysing such data.

Furthermore, as explained in Section 1.2, being born SGA is related to several short- and long-term negative consequences. The risk of respiratory problems, necrotizing enterocolitis, thermal dysregulation, hypoglycaemia, and overall

mortality is higher among SGA infants, especially during neonatal period[136]. SGA delivery also relates to long-term sequelae, including metabolic derangements and neurodevelopmental impairments[136].

With regard to metabolic derangements, postnatal growth trajectory may be a stronger determinant of future outcomes rather than being SGA itself[145,146]. While failing to catch-up is associated with poor cognitive functions with a higher risk of infections[135,141], rapid weight gains might lead to future obesity and its complications[145,146]. Therefore, growth trend of SGA after birth has to be closely monitored to achieve the trade-off between achieving optimal neurocognitive function and minimal metabolic consequences in the future (Figure 1.6, Section 1.2).

Moreover, timing and composition of gains (fat- vs fat-free masses) are also considered important, especially when analysing the detrimental effect of catch-up growth in relation to future metabolic risks. Regarding timing, the risks are higher among those who displayed catch-up growth as early as in the first 4-6 weeks of life[64]. SGA infants are also reported to be more likely to gain fat rather than lean mass during the period of rapid weight gain[21].

As well as total fat mass, later abdominal adiposity has also been linked to both spectrums of *in utero* under- and overnutrition[143]. In adults and children, abdominal adiposity is more important than BMI or overall adiposity as a predisposing factor for unfavourable metabolic profiles, including hypertension, insulin resistance, and dyslipidaemia. To assess internal and subcutaneous abdominal adipose tissue in infancy (IA-AT and SCA-AT, respectively), the use of ultrasound (US) as an alternative

non-invasive method to MRI has been validated[198]. US has also been previously evidenced to produce reliable estimates of abdominal adipose tissue distribution, compared to CT or MRI, in adults and adolescents[253,254].

This chapter aimed to display the longitudinal postnatal growth and adiposity trajectories of SGA infants and OGDM in comparison to controls. Abdominal adiposity parameters assessed in this study included waist circumference (WC) and US measures consisting of IA-AT and SCA-AT. The analyses *only* included the 'recent' OGDM group as these infants performed distinct growth trajectories to the 'earlier' group, which exhibited classical OGDM characteristics. As OGDM infancy growth outcomes have been discussed in the previous chapter, this chapter puts more emphasis on comparing SGA versus controls.

4.2 Methods

There are multiple criteria used to define SGA in the existing literature. Some studies defined SGA if the chosen anthropometry parameters fell more than 2 SD below the mean (< 2.3rd percentile), while some others used the 10th, 5th, or 3rd percentile as the cut-off[136]. In view of the lack of a definitive criterion, SGA in the CBGS was defined as birth weight $\leq$ -1.5 SDS (7th percentile) using the 1990 UK growth reference, as explained in Section 2.1.2. This decision was mainly to increase the numbers of subjects eligible for the study as well as to observe if the use of different SGA cut-offs would lead to different growth trajectories from birth to late infancy. To take out the effect of prematurity, only subjects born after full 36 week-gestation were included in the analyses. In addition, SGA infants with genetic or

syndromal disorders or born to mothers with major comorbidities before or during pregnancy, were excluded from the analyses.

The OGDM group used in this chapter was the 'recent OGDM' from CBGS2 with criteria described in Chapter 3.

To compare with SGA and OGDM, the control group in this chapter consisted of infants whose birth weight was more than -1.5 SDS from both CBGS1 and 2, born of non-diabetic mothers without any significant pregnancy comorbidities. Only singletons were included in the analyses of OGDM and controls, however, since the rate of twin pregnancy in the SGA group is high (11%; 23 infants), SGA twins were retained to avoid excluding too many subjects in the analyses. All SGA statistical models were adjusted for twin pregnancy.

Infant's anthropometry including weight, height, and skinfolds thicknesses were measured by CBGS paediatric research nurses, with details explained in Section 2.2. Postnatal weight, height, and adiposity gains were treated as continuous or categorical variables by categorising them into catch-up, no change, and catch-down groups. Catch-up is defined if delta growth gain between 0-12 months more than +0.67 SDS while catch-down if it is less than -0.67 SDS.

Seca 201 ergonomic circumference measuring tape was used to measure WC, ideally before feeding, at the end of a normal expiration midway between the lowest rib and the iliac crest. IA-AT and SCA-AT were assessed using a standard ultrasound machine (Logiq Book XP ultrasound with 3C MHZ-RS abdominal curved array transducer, GE Healthcare, Bedford, UK). IA-AT was defined as the distance

between the peritoneal boundary and the lumbar vertebrae, while SCA-AT was the distance between a point underneath the cutaneous layer and the linea alba. SD score was internally derived for WC and US measures to be able to compare both groups separately with controls.

Independent T- and X^2-tests were used to compare baseline demographics, birth characteristics, and unadjusted cross-sectional growth parameters for continuous and categorical variables, respectively. Multiple linear regression was used to compare growth parameters at each time point, adjusting for all recorded covariates, including infant sex, GA (only for birth and 3 months data), postnatal age at visit, prematurity, twin pregnancy, parity, maternal height and pre-pregnancy BMI, maternal age at delivery, maternal ethnicity, IMD representing socioeconomic status, delivery method, and smoking history during pregnancy. For longitudinal growth models, linear mixed-effects models were used to take into consideration the interaction between growth outcomes, visit time point, and infant group, adjusting for the same set of confounders used in the linear regression models.

4.3 Results

4.3.1 Baseline demographics and birth data

From both CBGS1 and 2 cohorts, there were in total 205 infants categorised as SGA. The demographics of both SGA and OGDM are shown in Table 4.1, compared to controls.

As displayed in Table 4.1, mothers of SGA infants and OGDM were from more diverse ethnic backgrounds compared to controls while maternal age at delivery was similar between the three groups. Both groups of infants were born of shorter mothers and GDM mothers were more obese. SGA infants were more likely to be first-born compared to the other 2 groups. The rate of maternal smoking during pregnancy in the SGA group was almost 4 times higher compared to controls, while among GDM mothers, the rate of smoking was more than twice higher to controls. Both SGA and OGDM were from more deprived households.

Table 4.1 Baseline demographics

Values are mean ± SD or %
IMD=index of multiple deprivation; BMI=body mass index
*p<0.05, **p<0.005 between SGA or 'Recent' OGDM against controls

	Control (Total N=1000)	**SGA (Total N=205)**	**'Recent' OGDM (Total N=122)**
Maternal demographics			
Age at birth (years)	33.3 ± 4.4	32.9 ± 4.9	33.6 ± 5.1
Caucasian ethnicity	95%	89%**	76%**
IMD	9.2 ± 4.0	10.2 ± 6.0*	11.3 ± 6.8**
Primiparous pregnancy	46%	67%**	52%
Height (cm)	166.5 ± 7.0	162.7 ± 7.0**	162.7 ± 6.8**
Pre-pregnancy BMI (kg/m^2)	23.7 ± 4.3	23.7 ± 4.2	27.0 ± 6.3**
Smoking during pregnancy	4%	15%**	9%*

Table 4.2 summarises the birth anthropometry of the 3 groups, measured in the first 8 days of life. As predicted, there was a higher proportion of C-section delivery among SGA and OGDM compared to the control group (Table 4.2). At birth, all anthropometric measurements of infants born SGA were reduced compared to controls and OGDM, including weight, length, head circumference, adiposity parameters, and AGD (Table 4.2). As mentioned previously, the 'recent' OGDM

were born with similar weight and length SDS to controls, but with reduced skinfold thickness representing subcutaneous adiposity.

Recorded maternal baseline demographics and perinatal characteristics, including maternal pre-pregnancy BMI, height, age, smoking history, parity, IMD, ethnicity, and GA, could not predict birth weight among SGA infants. In this group, only twin pregnancy and delivery method were correlated significantly with birth weight (non-twin and vaginally born individuals were heavier than C-section delivered twins). Furthermore, 8% of SGA mothers were diagnosed with pre-eclampsia. The birth outcomes of the SGA infants born of these mothers were comparable to the other SGA infants.

Table 4.2 Birth anthropometry of 3 groups of infants

Values are mean ± SD or %
[a]SDS, standard deviation score (for weight, length, head circumference, and BMI are calculated using the UK 1990 reference, for skinfolds using internal references). All SDS values are adjusted for GA, sex and postnatal age at measurement
GA, gestational age; SFT, skinfolds thicknesses
*p<0.05, **p<0.005

	Control (Total N=1000)	**SGA (Total N=205)**	**'Recent' OGDM (Total N=122)**
GA (weeks)	40.0 ± 1.2	39.6 ± 1.7**	38.9 ± 0.9**
Caesarean delivery	24%	37%**	42%**
Male infant sex	54%	43%*	53%
Weight (kg)	3.568 ± 0.439	2.483 ± 0.336**	3.303 ± 0.472**
Weight SDS[a]	0.15 ± 0.83	-2.04 ± 0.43**	0.10 ± 1.01
Length (cm)	51.6 ± 2.3	47.8 ± 2.5**	50.0 ± 2.0**
Length SDS[a]	-0.02 ± 0.86	-1.61 ± 0.86**	-0.07 ± 0.94
Head circumference (cm)	35.5 ± 1.5	33.0 ± 1.5**	34.6 ± 1.2
Head circumference SDS[a]	-0.08 ± 0.9	-1.63 ± 0.78**	0.05 ± 0.88
BMI (kg/m^2)	13.4 ± 1.4	10.8 ± 1.0**	13.2 ± 1.4
BMI SDS[a]	0.07 ± 1.1	-2.18 ± 0.98**	0.15 ± 1.11
Ponderal Index (kg/m^3)	26.0 ± 3.1	22.7 ± 2.4**	26.3 ± 2.7
Triceps SFT (mm)	5.5 ± 1.4	3.9 ± 1.0**	4.5 ± 0.8**
Subscapular SFT (mm)	5.4 ± 1.3	3.9 ± 1.0**	4.8 ± 1.0**
Flank SFT (mm)	6.1 ± 1.7	4.2 ± 1.1**	4.8 ± 1.1**
Quadriceps SFT (mm)	7.9 ± 2.4	4.9 ± 1.6**	5.9 ± 1.3**
Sum skinfolds thicknesses (mm)	24.9 ± 5.9	16.9 ± 4.2**	20.0 ± 3.6**
Triceps SFT SDS	0.06 ± 0.96	-0.9 ± 0.64**	-0.42 ± 0.65**
Subscapular SFT SDS	0.08 ± 0.96	-1.04 ± 0.55*	-0.22 ± 0.87*
Flank SFT SDS	0.06 ± 0.98	-0.88 ± 0.63**	-0.59 ± 0.64**
Quadriceps SFT SDS	0.09 ± 0.94	-1.05 ± 0.5**	-0.40 ± 0.66**
Mean SFT SDS[a]	0.07 ± 0.81	-0.97 ± 0.48**	-0.41 ± 0.61**

4.3.2 Postnatal growth trajectories

Figure 4.1 illustrates the changes in weight, height, and skinfolds trajectories from birth to 24 months between the 3 groups of infants. There were significant weight and subcutaneous adiposity gains immediately after birth (between 0-3 months)

among both groups of infants born after abnormal uterine conditions, especially among SGA. When looking at abdominal adiposity, both SGA and OGDM had persistently visceral abdominal fat thickness or IA-AT (Figure 4.2). Interestingly, OGDM had even lower IA-AT measures than SGA, especially at 6 months.

Over the first year of life (between 0-12 months), almost 57% of SGA showed 'catch-up' weight gain, compared to 25% among controls and 20% among OGDM (Table 4.4). Therefore, although the mean weight SDS of SGA infants was -2.04 SDS at birth, it increased to -1.3 SDS at 3 months, -1.16 at 12 months, and -0.86 at 24 months, as shown in Table 4.3. Similarly, height and subcutaneous adiposity rapidly increased among SGA with 56% and 51% of them showing 'catch-up' in these parameters, respectively. Meanwhile, the proportion of OGDM catching down in weight and height in the first year of life was higher than of both SGA and controls.

Despite the overall 'catch-up' tendency in all growth parameters, SGA infants remained significantly lighter, shorter, and less adipose by 24 months of age than controls (Table 4.3), even after adjustment for confounding factors (Table 4.5).

Figure 4.1 Birth-24 months growth trajectories among 3 groups of infants

Values are mean±SEM
Weight and height SDS are based on UK 1990 growth reference, whilst skinfold thickness SDS are internally derived. All SDS values are adjusted for infant sex, GA (birth and 3 months only), and postnatal age at visit. The circle denotes significance of cross-sectional growth parameters of SGA/OGDM against controls at the corresponding time point (adjusted for confounders on multiple regression models, Table 4.5)

Horizontal bars indicate statistically significant differences between SGA (*) or OGDM (#) versus controls for the displayed growth periods. Significance is based on linear mixed-effect models of infant growth parameters between groups, with time modelled using linear splines (Table 3.8 for OGDM versus controls and Table 4.6 for SGA versus controls).

SFT=skinfold thickness

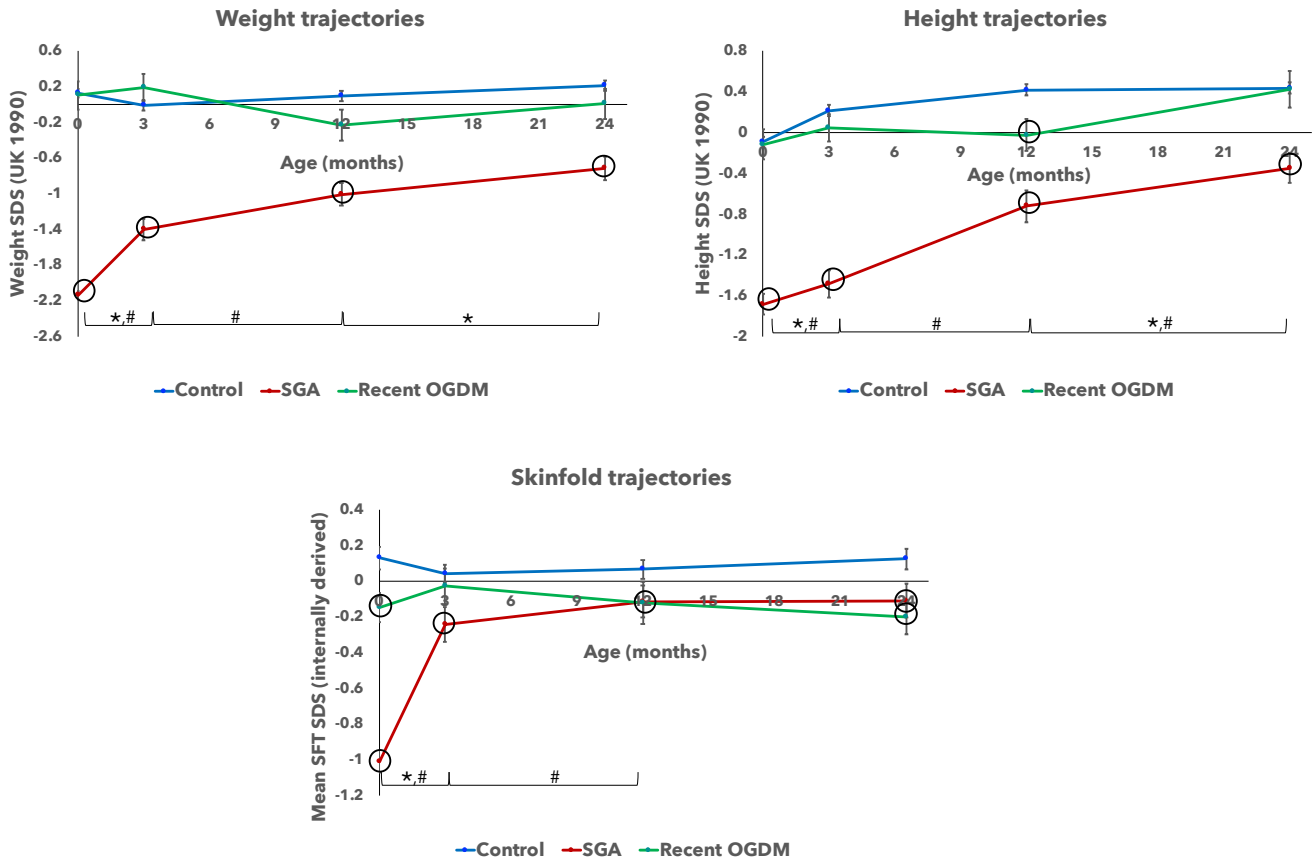


Figure 4.2 Adiposity parameters of 3 infant groups

Values are mean±SEM

The circle denotes strong significance (*p<0.005*) of cross-sectional corresponding adiposity parameters of SGA/OGDM against controls at the corresponding time point (adjusted for confounders on multiple regression models, Table 4.5)

IA-AT= internal abdominal adipose tissue; SCA-AT = subcutaneous abdominal adipose tissue

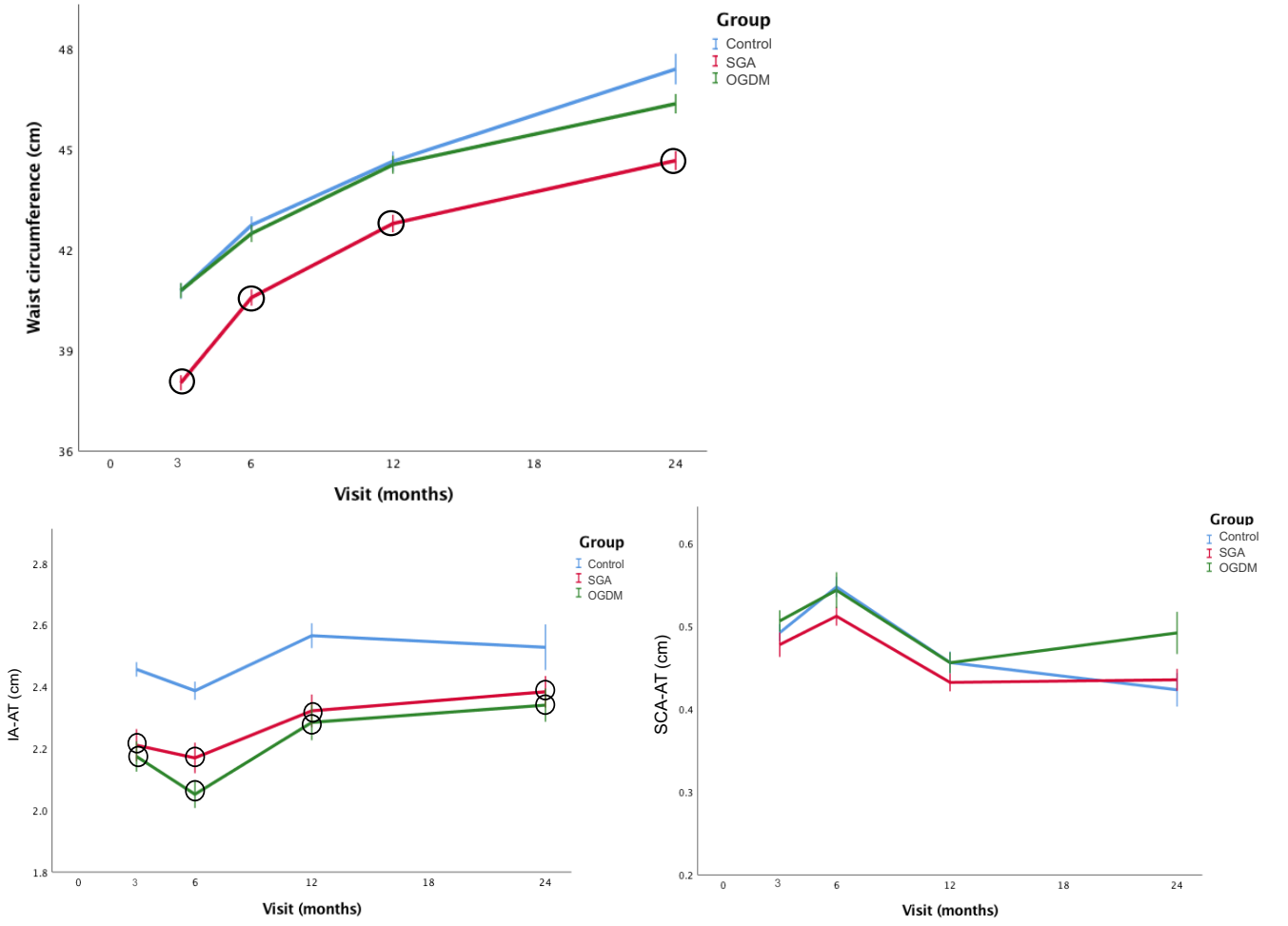


Table 4.3 Cross-sectional comparisons of postnatal infant growth parameters

Values are mean ± SD, or %
SDS=standard deviation score (for weight and length are calculated using the UK 1990 reference, for skinfolds using internal references). All SDS values are adjusted for gestational age (at 3 months only), sex and postnatal age at measurement.
WC=waist circumference; IA-AT=internal abdominal-adipose tissue; SCA-AT=subcutaneous abdominal-adipose tissue
*$p<0.05$, **$p<0.005$ (unadjusted)

	Control	**SGA**	**OGDM**
3 months			
Nutrition (% of 0-3 months exclusively breastfed)	43%	39%	46%
Weight (kg)	6.138 ± 0.788	5.108 ± 0.707**	6.069 ± 0.808
Weight SDS	0.01 ± 1.0	-1.3 ± 0.87**	0.18 ± 1.04
Length (cm)	61.0 ± 2.4	57.3 ± 2.5**	59.9 ± 2.4**
Length SDS	0.18 ± 0.95	-1.29 ± 0.91**	-0.03 ± 0.98
Head circumference (cm)	40.7 ± 1.3	34.8 ± 3.5**	40.4 ± 1.2*
Head circumference SDS	-0.19 ± 0.96	-1.13 ± 0.85**	-0.45 ± 0.85
BMI (kg/m^2)	16.4 ± 1.4	15.5 ± 1.4**	16.9 ± 1.4*
BMI SDS	-0.21 ± 1.07	-0.83 ± 1.07**	0.11 ± 1.01*
Triceps SFT SDS	0.01 ± 1.04	-0.18 ± 0.8**	-0.27 ± 0.75*
Subscapular SFT SDS	0.01 ± 0.99	-0.08 ± 0.99	0.14 ± 0.98
Flank SFT SDS	0.01 ± 1.03	-0.12 ± 0.91	-0.03 ± 0.83
Quadriceps SFT SDS	0.03 ± 1.03	-0.36 ± 0.83**	-0.13 ± 0.77
Mean SFT SDS	0.01 ± 0.79	-0.19 ± 0.71**	-0.07 ± 0.65
WC (cm)	41.2 ± 2.9	38.2 ± 2.6**	40.8 ± 2.8
IA-AT (cm)	2.36 ± 0.6	2.28 ± 0.49*	2.13 ± 0.33**
SCA-AT (cm)	0.43 ± 0.1	0.45 ± 0.11	0.5 ± 0.11**
12 months			
Weight (kg)	9.974 ± 1.165	8.733 ± 1.049**	9.711 ± 1.42
Weight SDS	0.05 ± 1.07	-1.16 ± 1.1**	-0.25 ± 1.26**
Length (cm)	75.8 ± 2.9	73.0 ± 3.0**	75.0 ± 3.0*
Length SDS	0.33 ± 1.1	-0.74 ± 1.13**	-0.01 ± 1.07
Head circumference (cm)	46.5 ± 1.4	45.3 ± 1.5**	46.4 ± 1.5
Head circumference SDS	-0.58 ± 1.08	-1.51 ± 1.16**	-0.68 ± 1.06
BMI (kg/m^2)	17.3 ± 1.4	16.4 ± 1.2**	17.2 ± 1.7
BMI SDS	-0.17 ± 1.02	-0.9 ± 1.03**	-0.3 ± 1.23
Triceps SFT SDS	0.02 ± 1.04	-0.25 ± 0.79**	-0.47 ± 0.85**
Subscapular SFT SDS	0.01 ± 1.02	-0.14 ± 0.91*	-0.09 ± 0.97

Flank SFT SDS	0.02 ± 1.03	-0.21 ± 0.79**	-0.38 ± 0.80**
Quadriceps SFT SDS	0.02 ± 1.03	-0.29 ± 0.84**	-0.50 ± 0.90**
Mean SFT SDS	0.02 ± 0.8	-0.22 ± 0.65**	-0.36 ± 0.74**
WC (cm)	45.9 ± 3.0	42.8 ± 4.7**	44.7 ± 3.3**
IA-AT (cm)	2.76 ± 0.55	2.52 ± 0.57**	2.21 ± 0.46**
SCA-AT (cm)	0.43 ± 0.09	0.43 ± 0.1	0.45 ± 0.12
24 months			
Weight (kg)	12.646 ± 1.424	11.246 ± 1.305**	12.297 ± 1.591
Weight SDS	0.19 ± 1.01	-0.86± 1.1**	-0.03± 1.10
Length (cm)	87.8 ± 3.4	85.1 ± 3.4**	87.3 ± 3.6
Length SDS	0.43 ± 1.06	-0.42 ± 1.07**	0.34 ± 1.11
Head circumference (cm)	48.8 ± 1.5	47.6 ± 1.5**	49.0 ± 2.8
Head circumference SDS	-0.71 ± 1.04	-1.6 ± 1.15**	-0.52 ± 2.04
BMI (kg/m^2)	16.4 ± 1.2	15.5 ± 1.1**	16.1 ± 1.3
BMI SDS	-0.16 ± 0.93	-0.87 ± 0.93**	-0.42 ± 0.99
Triceps SFT SDS	0.02 ± 1.04	-0.21 ± 0.83**	-0.28 ± 0.87*
Subscapular SFT SDS	0.01 ± 1.05	-0.15 ± 0.81*	-0.13 ± 0.82
Flank SFT SDS	0.02 ± 1.04	-0.28 ± 0.88**	-0.43 ± 0.68**
Quadriceps SFT SDS	0.03 ± 1.04	-0.35 ± 0.79**	-0.44 ± 0.78**
Mean SFT SDS	0.02 ± 0.83	-0.24 ± 0.67**	-0.31 ± 0.65**
WC (cm)	47.4 ± 3.1	44.7 ± 2.8	45.6 ± 6.3*
IA-AT (cm)	2.75 ± 0.5	2.44 ± 0.48	2.37 ± 0.42**
SCA-AT (cm)	0.42 ± 0.1	0.43 ± 0.1	0.46 ± 0.12*

Table 4.4 0-12 months infancy growth gains patterns among infant groups

Catch-up is defined if delta growth gain is ≥+0.67 SDS, whilst catch-down if it is ≤-0.67 SDS
Growth SDS values are based on UK 1990 growth reference, adjusted for infant sex, GA (at birth only), and postnatal age at visit.
p^1 SGA vs controls, p^2 OGDM vs controls, p^3 SGA vs OGDM

0-12 months growth gain patterns	**Control**	**SGA**	***p1***	**OGDM**	***p2***	***p3***
Weight			*<0.0001*		*0.049*	*<0.0001*
Catch up	25%	57%		20%		
No change	50%	33%		43%		
Catch down	25%	10%		37%		
Height			*0.01*		*0.01*	*<0.0001*
Catch up	39%	56%		23%		
No change	46%	36%		54%		
Catch down	15%	8%		23%		
Skinfold thickness			*<0.0001*		*0.26*	*<0.0001*
Catch up	27%	51%		27%		
No change	46%	48%		54%		

Catch down	27%	1%	19%

Table 4.5 Linear regression comparison of infant growth parameters between groups at each time point

Model 1: adjusted for sex, GA (for outcomes at birth and 3 months only), prematurity (yes/no), twin pregnancy (yes/no), and age at measurement (except for birth weight models)
Model 2: Model 1 + adjusted for parity (primiparous, yes/no), pre-pregnancy maternal BMI, maternal height (for height gain only), feeding history (exclusively breastfed at 3 months, yes/no; except for birth anthropometry), maternal ethnicity (Caucasian decent, yes/no), maternal age at delivery, IMD, delivery method (vaginal delivery, yes/no), maternal smoking history during pregnancy (yes/no).

B (regression coefficients) ±SE, *p*, and adjusted R^2 are displayed

Outcomes: Growth parameters	**Predictor: SGA vs controls (Controls as reference)**						**Significant covariates**
	Model 1			**Model 2**			
	B±SE	***p***	**R^2**	**B±SE**	***p***	**R^2**	
Birth							
Weight SDS	-2.1±0.08	***<0.0001***	0.43	-2.1±0.08	***<0.0001***	0.45	GA, prematurity, twin pregnancy, parity, maternal pre-pregnancy BMI, delivery method
Height SDS	-1.42±0.1	***<0.0001***	0.24	-1.3±0.1	***<0.0001***	0.3	Maternal pre-pregnancy BMI, maternal height
SF SDS	-1.04±0.09	***<0.0001***	0.17	-1.03±0.09	***<0.0001***	0.19	Infant sex, parity, maternal pre-pregnancy BMI, delivery method
3 months							
Weight SDS	-1.23±0.11	***<0.0001***	0.16	-1.22±0.11	***<0.0001***	0.17	GA, parity, maternal pre-pregnancy BMI
Height SDS	-1.27±0.1	***<0.0001***	0.18	-1.14±0.1	***<0.0001***	0.25	Twin pregnancy, maternal pre-pregnancy BMI, maternal height
SF SDS	-0.19±0.09	***0.027***	0.002	-0.18±0.09	***0.048***	0.003	-
12 months							
Weight SDS	-1.11±0.12	***<0.0001***	0.13	-1.16±0.12	***<0.0001***	0.18	Prematurity, maternal pre-pregnancy BMI, infant feeding
Height SDS	-0.9±0.12	***<0.0001***	0.1	-0.9±0.12	***<0.0001***	0.23	Prematurity, maternal height., infant feeding
SF SDS	-0.21±0.09	***0.015***	0.01	-0.22±0.09	***0.014***	0.04	Prematurity, infant feeding
24 months							
Weight SDS	-0.85±0.12	***<0.0001***	0.09	-0.87±0.12	***<0.0001***	0.12	Prematurity, maternal pre-pregnancy BMI, infant feeding
Height SDS	-0.63±0.13	***<0.0001***	0.05	-0.53±0.12	***<0.0001***	0.17	Prematurity, maternal pre-pregnancy BMI, maternal height, infant feeding
SF SDS	-0.22±0.09	***0.022***	0.01	-0.24±0.1	***0.014***	0.03	Maternal pre-pregnancy BMI, delivery method

Table 4.6 Linear mixed-effect models of infant growth parameters between SGA and controls

Model 1: adjusted for gestational age (birth and 3 months growth outcomes only), sex, age at measurement, and twin pregnancy
Model 2: Model 1 + adjusted for pre-pregnancy maternal BMI, maternal height (for height gain only), parity (primiparous, yes/no), feeding history (exclusively breastfed at 3 months, yes/no; except for birth anthropometry), maternal ethnicity (Caucasian decent, yes/no), index of multiple deprivation, delivery method (Caesarean delivery, yes/no), maternal smoking history during pregnancy (yes/no).

Fixed effect estimates (visit period and group interaction) ±SE are displayed

Outcomes		**SGA vs. controls (Controls as reference)**	
		Estimate±SE	***p***
0-3 months			
Weight SDS	Model 1	0.3±0.03	***<2*10^{-16}***
	Model 2	0.3±0.03	***<2*10^{-16}***
Length SDS	Model 1	0.04±0.03	*0.241983*
	Model 2	0.04±0.03	*0.255259*
Skinfolds SDS	Model 1	0.28±0.04	***4.79*10^{-15}***
	Model 2	0.14±0.04	***7.36*10^{-15}***
3-12 months			
Weight SDS	Model 1	0.01±0.01	*0.5029*
	Model 2	0.01±0.01	*0.47415*
Length SDS	Model 1	0.03±0.01	***0.002255***
	Model 2	0.03±0.01	***0.002005***
Skinfolds SDS	Model 1	-0.01±0.01	*0.5439*
	Model 2	-0.01±0.01	*0.569*
12-24 months			
Weight SDS	Model 1	0.02±0.01	***0.0171***
	Model 2	0.02±0.01	***0.01494***
Length SDS	Model 1	0.03±0.01	***0.000361***
	Model 2	0.03±0.01	***0.000325***
Skinfolds SDS	Model 1	0.003±0.01	*0.73685*
	Model 2	0.003±0.01	*0.70242*

Moreover, as demonstrated in Figure 4.3, SGA infants who received solely breastmilk between 0-3 months caught-up less in height over the first year compared to mixed-fed SGA infants.

As most previous studies used birth weight < -2SD to define SGA[136], Figure 4.4 sub-classified CBGS SGA infants into 2 groups: infants whose birth weight fell between -1.5 to -2 SDS and the smaller infants born with weight less than -2 SDS. Cross-sectionally, from 3 months onwards there was no difference between these 2 subgroups, although longitudinally, the smaller SGA infants showed more catch-up in weight and height between 0-3 months (*p values 0.001 and* 0.002, respectively) and showed subsequent lower adiposity gain between 3-12 months.

Figure 4.3 Growth trajectories among SGA infants based on feeding history

EBF=0-3 months exclusive breastfeeding
No significant difference between cross-sectional growth parameters at all time points
***p=0.005* (multiple linear regression; fully adjusted for all confounding factors)

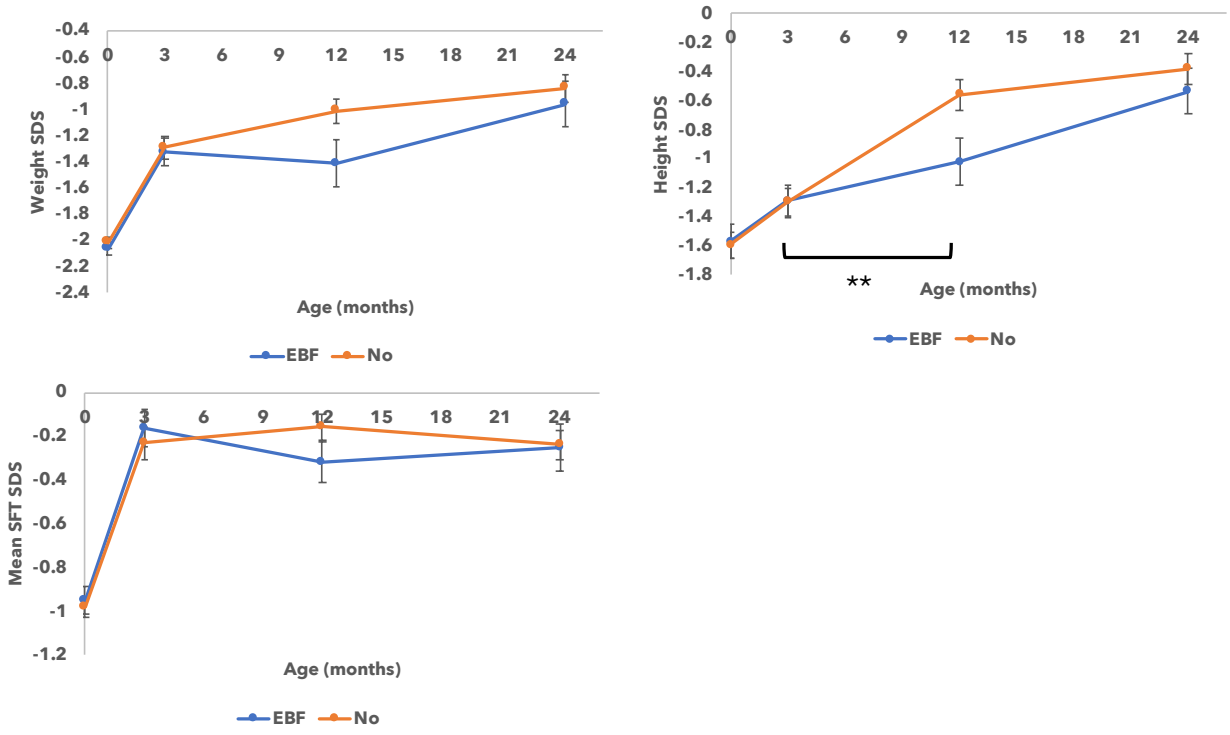
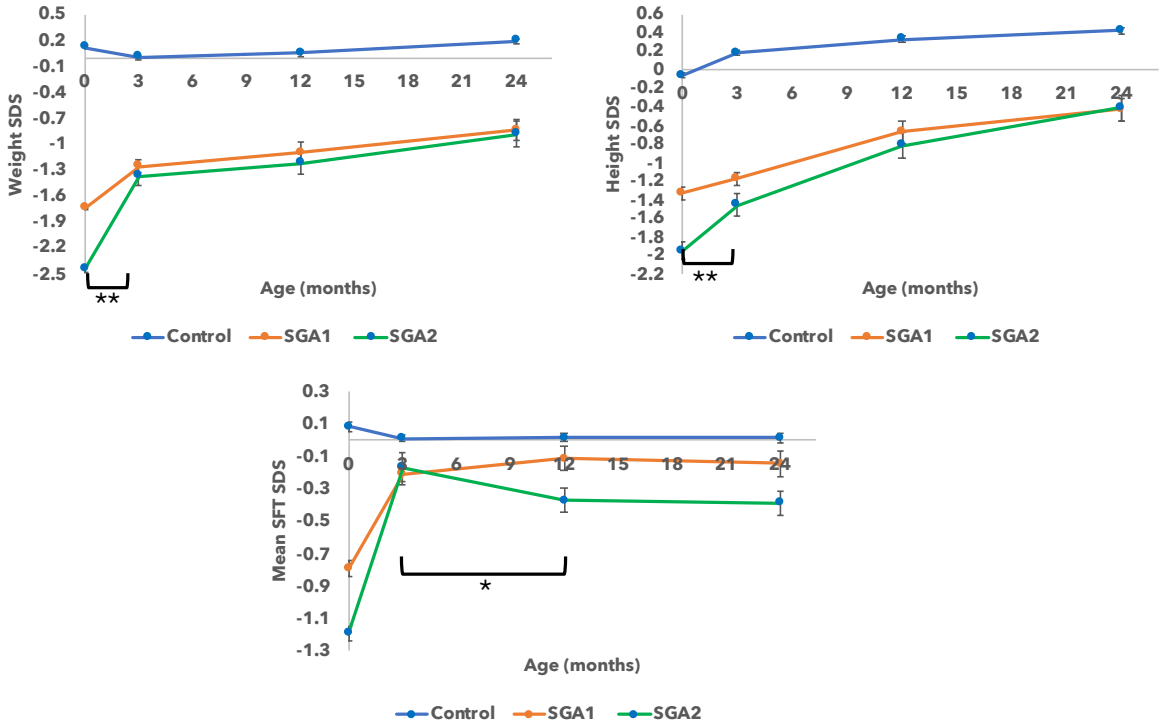


Figure 4.4 Growth trajectories between 2 subgroups of SGA and controls

SGA1=birth weight SDS between -1.5 to -2.0 (total N at birth 125 and by 24 months N=79); SGA2=birth weight SDS ≤ -2.0 SDS (total N at birth 88 and by 24 months N=62);
No significant difference between cross-sectional growth parameters at 3, 12, and 24 months
**p=0.027; **p<0.005* of corresponding growth gains between SGA1 vs SGA2 (multiple linear regression; fully adjusted for all confounding factors)



4.4 Discussion

The characteristics of women who delivered SGA infants in this study were similar to those described in the literature[134] (Section 1.2.1, Figure 1.5) with shorter stature and a higher proportion of primiparity, twin pregnancies, and maternal smoking history. SGA deliveries in the CBGS were also associated with maternal ethnicity, with more diverse ethnicities among the SGA group. However, mothers who gave

birth to SGA babies did not have lower BMI compared to controls, and unlike control groups, neonatal anthropometric measurements were not predicted by any maternal or pregnancy-related environmental data collected in the study. The rates of pregnancy comorbidities, such as pre-eclampsia, among mothers who gave birth to SGA babies were low (8%) and possibly underpowered to identify their associations with birth outcomes. The majority of these women also had normal pregnancy scans at both 12 and 20 weeks of gestation. It could be speculated, however, that there were undiagnosed aetiologies of placental insufficiency leading to infant's low birth weight.

Most SGA infants in this study had symmetrical SGA (details on Section 1.2.1), reflected by all anthropometric measures at birth, including weight, length, and head circumference, all falling below the cut-off. As predicted, SGA infants caught-up during infancy, especially between 0-3 months. The rapid weight gain in this period seemed to be originated from an increase in fat mass, as shown in Figure 4.1. This study supports evidence from previous observations reporting rapid weight gain during infancy among SGA to be more associated with increased fat mass rather than lean mass[21,23,24]. It is interesting to speculate that a period of undernourishment *in utero* among these infants has led to several organs compromise, altered metabolism, and eventually gain in fat mass following the thrifty phenotype theory, which perhaps mainly due to the need for thermogenesis.

The similarities between SGA and OGDM in terms of growth trajectories were faster weight and adiposity gains in the first 3 months of life, compared to controls. This is a novel finding as no study has compared SGA and OGDM in the same longitudinal

cohort. In addition, both infant groups had also persistently lower visceral abdominal thickness from 3-24 months, measured by ultrasound. Unfortunately, observation of changes in abdominal adiposity parameters during early infancy (0-3 months) was not possible since it was not performed at birth.

Sub-analyses on feeding history showed that exclusive breastfeeding between 0-3 months seemed to alleviate subsequent 'catch-up' weight and height gains between 3-12 months (unadjusted *p values 0.031* for weight gain and *<0.0001* for height gain; fully adjusted height gain model remained significant but weight gain did not). Regarding subcutaneous adiposity trajectories, there was no significant difference between those who were exclusively breastfed for the first 3 months compared to the mixed-/formula-feeders (Figure 4.3).

As shown in Figure 4.4, there was no cross-sectional difference in any growth parameter from 3 months onwards between the stricter and more lenient definitions of SGA, due to greater rates of early catch-up growth between 0-3 months among the 'smaller' SGA infants.

4.5 Conclusions and further recommendations

In the CBGS, infants born SGA displayed classical catch-up growth trajectories, especially in weight and subcutaneous adiposity gains in the first 3 months of life. Similarly, the 'recent' OGDM also showed faster gains in weight and subcutaneous adiposity during this early infancy period, suggesting either shared metabolic disruption or adaptation. Further follow-up of these infants is of interest to

investigate the detrimental metabolic effect of intrauterine nutritional insults projected into later childhood and eventually adulthood.

Chapter 5 Infancy IGF-1 and C-peptide levels among SGA and OGDM

5.1 Introduction

The robust associations between *in utero* exposure, infancy growth, and later health outcomes may relate to the early hormonal milieu. Therefore, circulating hormone levels early in life could act as biomarkers for later phenotype or disease risk. Two hormones of interest are insulin-like growth factor 1 (IGF-1) and C-peptide.

IGF-1 is a pleiotropic growth factor involved in various aspects of both normal and pathological growth. Originally called somatomedin C, IGF-1 is the major mediator of pre- and postnatal growth. Circulating levels of this hormone were reported to be higher in infants who gained greater weight, length, and adiposity between 3-12 months of age[200]. IGF1 could also link the mechanistic pathway of higher growth rates caused by formula feeding as it is present at higher concentration in the circulation of formula-fed babies[200]. Later during childhood, as reported in The Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, lower IGF1 concentrations are associated with shorter stature and lower insulin secretion at age 8 years[50]. This suggests that IGF-1 levels may mediate the positive relationship between height and pancreatic beta cell function.

IGF-1 shares nearly 50% amino acid sequence homology with proinsulin[255] and is primarily produced in liver and kidney under the control of growth hormone (GH)[256]. IGF-1 mainly serves as an endocrine hormone mediating the action of GH in peripheral tissues, such as muscle, cartilage, bone, kidney, nerves, skin, lungs, and the liver itself[257]. It regulates cell proliferation, apoptosis, migration, and differentiation[258]. A family of IGF binding proteins (IGFBPs) modulate the effects of IGF-1 by carrying the ligand in the circulation and extracellular fluids. In the circulation, IGF1 exists in a complex with its binding protein (mainly IGFBP3) and an acid-labile subunit (ALS)[257].

Similar to insulin, C-peptide is formed from proinsulin molecules and is co-secreted with insulin from pancreatic beta cells. This 31-amino acid peptide was once thought biologically inactive but now has been considered necessary in facilitating the correct folding of insulin, although studies are still ongoing to discover the entire physiological significance of this hormone[259].

In relation to infant growth and adiposity, the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study reported a continuous positive relationship between cord blood C-peptide concentrations and adiposity, in almost 20,000 neonates[163]. The EDEN cohort (French longitudinal study on pre- and early postnatal determinants of child health and development) also found that higher cord blood C-peptide concentrations were associated with higher birth weight and slower subsequent weight gain from birth to 3 months, although this was only observed in girls[201].

The observation that SGA and OGDM could end up with the same increased metabolic risks despite having completely-opposite *in utero* conditions could relate to common metabolic adaptations early in life. In this chapter, circulating IGF-1 and C-peptide concentrations in SGA and OGDM are compared with those of controls to characterise any common insulin-related metabolic changes during early life.

5.2 Methods

5.2.1 Cohorts description

The control infants for the analyses in this chapter were of CBGS1 (2001-2009) participants who met these maternal criteria: normal glycaemia on oral glucose tolerance test (OGTT) at 28 weeks, no record of diabetes diagnosis, no significant medical history. In addition, all infants (N=314 for IGF-1 measurement and N=122 for C-peptide measurement, Table 5.1) were singletons, born after full 36 weeks or longer gestational age with birth weight more than -1.5 SDS according to the UK 1990 growth reference.

Meanwhile, SGA and 'recent' OGDM subsets in this hormonal study were obtained from CBGS2, plus a small group of SGA from CBGS1 who had available measurements (N=14). As mentioned in Section 2.4.2, in CBGS2, this hormonal analysis was carried out on 50 subjects per group (Table 5.1). The selection was based on the condition severity, i.e. SGA infants with the lowest birth weights and 'recent' OGDM born of mothers with the highest fasting glucose levels during pregnancy. It is important to emphasise that all 'OGDM' involved in this hormonal

study were of the ***'recent' OGDM*** (CBGS2) group and none of the 'older' OGDM (CBGS1).

Table 5.1 Number of subjects per subgroup in this study

Subset	IGF-1 (Assay: RIA-Mediagnost)		C-peptide (Assay: MSD-CBAL)	
	CBGS1 (2009)	CBGS2 (2019)	CBGS1 (2009)	CBGS2 (2019)
Control	314	-	122	-
SGA	14	50	14	50
'Recent' OGDM	-	50	-	50

5.2.2Sample handling and assays

Dried blood spots (DBS) from capillary heel-prick sampling were obtained from CBGS1 and CBGS2 infants at 12 months of age. The detailed sampling procedure is explained in Section 2.3.1.

IGF-1 and C-peptide were measured in 2009 for CBGS1 and in 2019 for CBGS2, using the same corresponding assay procedures. To ensure that both timepoints produced comparable results, assay reproducibility was assessed (Section 5.2.3).

For IGF-I measurement in both CBGS1 and CBGS2, 2 blood-spot disks (diameter 3.2 mm) were punched out from the DBS cards, extracted with 400 μL of acidifying buffer, and then measured using a specific radioimmunoassay or RIA (Mediagnost, Tübingen, Germany) according to the manufacturer's instructions[200] (Section 2.4.2). C-peptide measurement was carried out in the Core biochemical assay laboratory (CBAL), Cambridge University Hospital using an in-house developed Meso Scale Discovery (MSD) assay (Maryland, USA).

5.2.3 Statistical analyses

Continuous variables were presented as mean±SD for normally distributed data, otherwise as median(IQR) or log-transformed values. Categorical data were displayed as proportion or percentage.

Chi-square was used to compare the proportion of categorical variables across each 2 infant groups, e.g. catch-up rate between SGA versus controls, OGDM versus controls, and SGA versus OGDM. Analysis of variance was used to test differences in IGF-1 and C-peptide concentrations across infant groups, by sex and type of milk feeding, or Kruskal-Wallis test as an alternative if data were not normally distributed. Correlation analyses and multiple linear regression were used to examine significant determinant factors of capillary hormone concentrations, as well as to test associations between hormone concentrations and growth outcomes.

Assay reproducibility was assessed in the next section using correlation analysis, linear regression, and Bland-Altman plot. Correlation and regression models were used to evaluate the linearity between the old and recent measurement values, while Bland-Altman plots analysed the agreement between the same assays used to measure IGF-1 (RIA) and C-peptide (MSD) but conducted at different times. Wilcoxon signed-rank test (alternative to paired t-test) was used as the non-parametric univariate analysis to compare the values of old and recent measurements.

5.2.4 Assay reproducibility

As capillary levels of IGF-1 and C-peptide from 3 infant groups in this study were not all measured at the same time (*all* controls and 14 SGA were measured in 2009, while the remaining SGA and all 'recent' OGDM subset were in 2019, Table 5.1), the assay reproducibility needs to be assessed. To do this, as many as 20 DBS of control group with previous IGF1 and C-peptide results (measured in 2009) were remeasured in 2019.

The recently measured IGF-1 and C-peptide results were lower than the previous values (Figure 5.1, Table 5.2), which could reflect assay differences but also could be due to prolonged storage. However, both recent IGF-1 and C-peptide results showed good correlations with the previous measurements, displayed by the positive and linear association between each paired sample, all mean biases were in the limits of agreement±95% confidence interval, and *relatively small* normalised RMSE (nRSME) values (Figure 5.2 and Table 5.2).

Therefore, it was considered reasonable to combine both previous and recent measurements in the subsequent analyses.

Figure 5.1 Scatter plots comparing recent vs previous IGF1 (A) and C-peptide (B) measurements of CBGS1 samples

N=20 paired samples
Values are log-transformed; R^2 values are shown with line of best fit

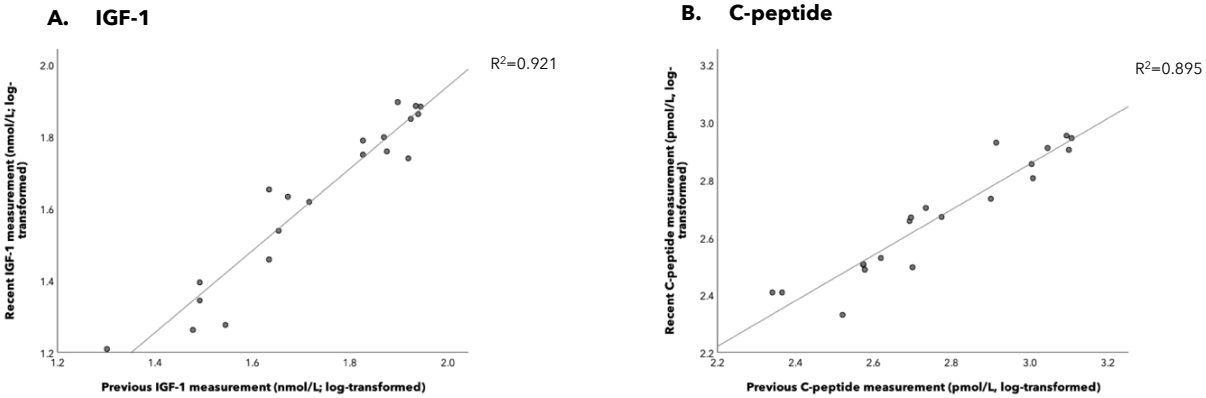


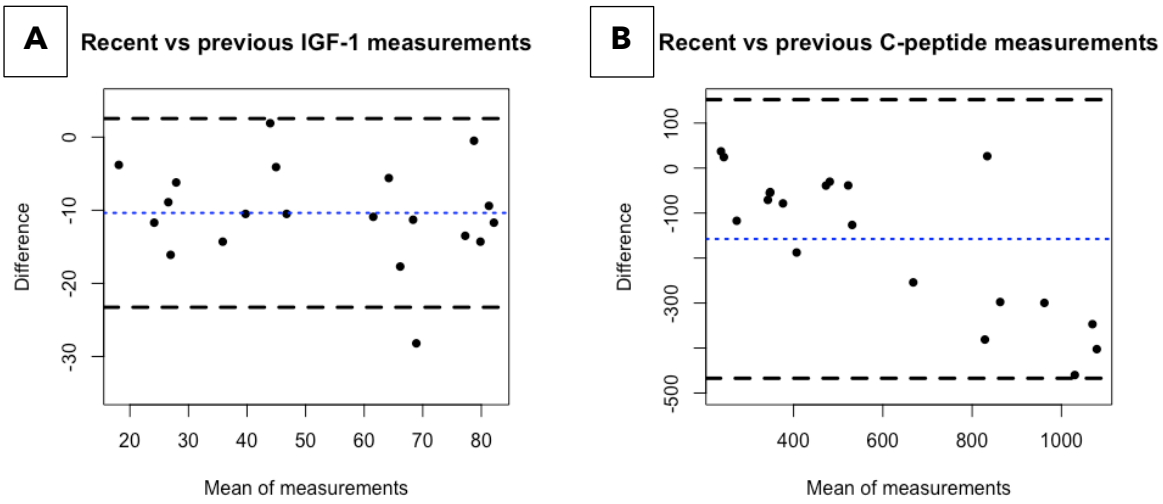
Table 5.2 Summary of assay reproducibility

IQR=interquartile range, Pearson R=Pearson correlation coefficient, LoA=limits of agreement, RMSE=root mean square error, nRMSE=normalised RMSE (RMSE/IQR)

Descriptive	**IGF-1 (nmol/L)**	**C-peptide (pmol/L)**
Number of paired samples	20	20
Median(IQR) of previous results	59.5(45.0)	521.16(642.0)
Median(IQR) of recent results	49.85(42.78)	467.0(464.0)
p (Wilcoxon rank test)	*<0.0001*	*<0.0001*
Pearson R	0.96	0.95
RMSE	6.04	72.94
nRMSE	0.14	0.16
Bland-Altman		
Mean bias±SD	-10.37±6.58	-157.57±157.98
95% LoA	-23.27 to 2.54	-467.21 to -152.07
The line of equality is in the LoA interval	Yes	Yes

Figure 5.2 Bland-Altman plots of recent versus previous measurements of IGF1 (A) and C-peptide (B)

Paired measurements were conducted on CBGS1 samples (N=20). Each plot shows mean bias (blue dashed line) and limit of agreement (LOA, represented by 95% confidence interval; black dashed line).



5.3 Results

5.3.1 Baseline characteristics and growth trajectories

IGF-1 and C-peptide were measured in the subsets of controls (total N for IGF-1=314 and for C-peptide 122), SGA (N=64), and OGDM (N=50). Maternal demographics and birth characteristics of these 3 subsets are displayed in Appendix 3. Compared to control subset, SGA and OGDM subsets were of more diverse ethnicities. SGA mothers were shorter and had a higher proportion of first pregnancy (primiparity), smoking history and C-section delivery. There were no differences in 0-3 months exclusive breastfeeding rates across these 3 subsets.

Although there were some differences in maternal baseline characteristics between subsets and the whole groups, for example, higher proportion of White Caucasian among OGDM subset (86%) compared to the 'recent' OGDM whole group (76%), the comparisons observed among 3 subsets versus 3 whole groups were similar. For instance, in both among subsets (Appendix 3) and whole groups (Table 4.1), SGA and OGDM were born of more ethnically-diverse and shorter mothers, with higher proportion of primiparous and active smokers, compared to controls. Therefore, these subsets could be representative of the whole groups described in the previous chapters.

With regard to growth patterns, there was no significant difference between 'subset' and 'whole group' inter-subgroup comparisons in general, i.e. 'recent' OGDM subset vs control subset was comparable to all 'recent' OGDM vs all controls; SGA subset vs control subset was comparable to all SGA vs all controls.

Similar to the whole 'recent' OGDM group (Figure 4.1)[193], the 'recent' OGDM subjects in this hormonal study were not heavier, taller, and fatter at birth, unlike the classical features in the literature[232] and even showing persistent trends towards reduced adiposity until 24 months.

Although the SGA subset had higher 0-12 months weight gain SDS compared to the whole group (mean$\pm$SD 1.12$\pm$1.07 vs 0.87$\pm$1.14, respectively, *p=0.03*), this result was not unexpected. SGA subset used in this hormonal study was selected from the smallest SGA infants in the whole cohort and it might reflect the 'dose-dependent' catch-up growth trend among low birth weight infants[21]. From 12-24 months, the SGA subset exhibited a similar growth pattern to the whole SGA group. As shown in Figure 4.1, despite having higher rates of weight and height gain catch-

up (defined by delta growth gain >0.67 SDS), SGA infants were still significantly smaller by the age of 24 months compared to controls. The same result was also observed when comparing SGA subset vs control subset in this hormonal study.

5.3.2 Infant feeding history and its relation to growth outcomes

It was previously reported that among control infants in the CBGS, the growth parameters of infants who received formula feeding did not differ with their breastfed counterparts at 3 months; however, all SDS values became significantly greater by 12 months of age[200].

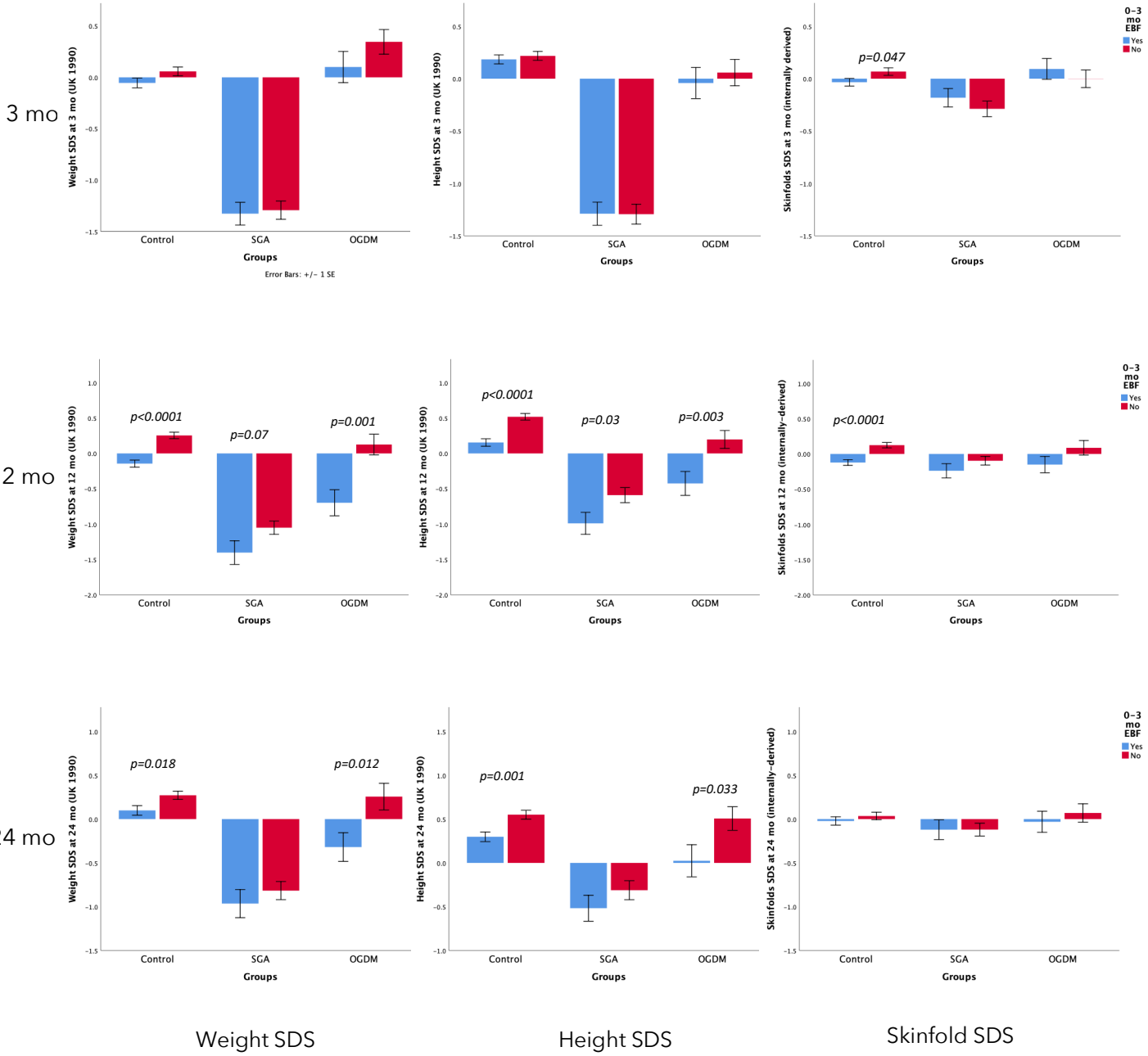
In this study, the same analysis was repeated across ***all*** controls, SGA, and OGDM, (with or without available hormone data), to compare the effect of feeding to infant's growth in each group. Infant feeding history was categorised into 2 groups based on exclusive breastfeeding history from birth to 3 months (yes vs no).

As seen in Figure 5.3, across all groups (SGA, OGDM, and controls), mixed- or formula-fed infants were overall heavier, taller, and fatter at 12 and 24 months, although not all comparisons were statistically significant. Of note, there was no observable difference in any birth size parameter between these feeding groups in each *in utero* group. The same analyses on the subsets (controls, SGA, and OGDM with hormone data, which would be included in the subsequent models) did not produce statistically different results.

The relationship between IGF-1 and C-peptide levels with these outcomes is described in the next section.

Figure 5.3 Weight-, height-, and skinfolds thicknesses-SDS at 3, 12, and 24 months between exclusively breastfed and mixed-/formula-fed infants among SGA, OGDM, and controls

Values are mean±SE. SDS values are adjusted for sex, gestational age (3 months only), and postnatal age at visit. *p* values are for comparisons between feeding groups in each infant group (SGA/OGDM/Control, unadjusted). Blue: infants who received exclusive breastfeeding from 0-3 months, red: infants who received formula or mixed-feeding from 0-3 months. mo=months



5.3.3 Hormones and infant growth

5.3.3.1 IGF-1

In non-parametric unadjusted Kruskal-Wallis test with post-hoc analysis, the levels of capillary blood spot IGF-1 of both SGA and OGDM at 12 months were lower than controls (median(IQR) of controls 48.0(32.0) nmol/L, vs SGA 41.5(25.9) *p=0.056*; vs OGDM 40.1(29.3) *p=0.045*, Figure 5.4).

To investigate which factors influence this outcome, bivariate correlation analysis was run to examine the relationship between IGF-1 level and maternal and infant factors. Table 5.3 lists all correlations with significant *p* values. Across all recorded maternal characteristics, only parity appeared to have any correlation. There was no significant correlation between IGF-1 level at 12 months with maternal age, height, pre-pregnancy BMI, and pregnancy glucose concentration (OGTT).

IGF-1 level correlated with all contemporaneous infant growth parameters at 12 months, as well as with 0-12 month weight and height gains (Table 5.3). There was also a positive association between IGF-1 and C-peptide level in all groups pooled together, but when groups were considered separately this was only significant among OGDM and controls.

Using a pragmatic approach and adjusting for identified significant covariates, capillary IGF-1 concentration at 12 months was compared between SGA and OGDM separately with controls. As seen in Table 5.4, SGA infants had lower IGF-1 level at 12 months in a fully adjusted model (*p=0.028*). OGDM also showed lower IGF-1 level although not significant.

Table 5.3 Ante- and postnatal factors that correlated significantly with capillary blood spots IGF-1 level at 12 months

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

Parameters	Pearson correlation coefficient (R)
Sex (M/F)	0.217**
Maternal parity	0.104*
Infant feeding history	0.154**
C-peptide level at 12 mo	0.346**
0-12 mo growth gains SDS	
Weight	0.235**
Height	0.244**
12 months anthropometry	
Weight-SDS	0.267**
Height-SDS	0.195**
BMI-SDS	0.213**
Mean SF-SDS	0.11*
24 months anthropometry	
Weight-SDS	0.231**
Height-SDS	0.225**
BMI-SDS	0.136**

Figure 5.4 12-month capillary IGF-1 levels between SGA, 'Recent' OGDM, and controls
Values are median, error bars are representing 95% CI

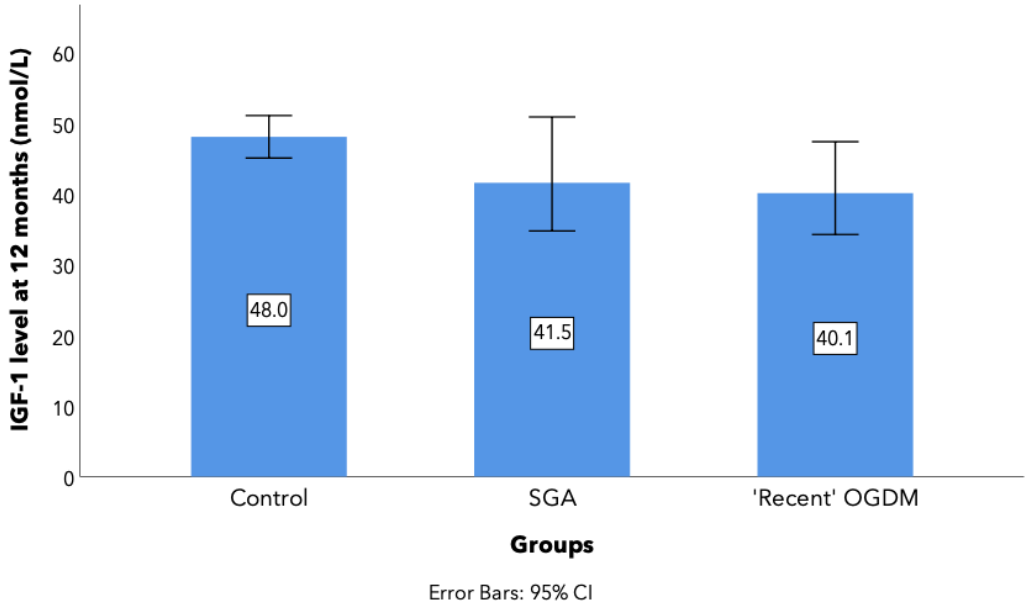


Table 5.4 Comparison of IGF-1 level at 12 months in SGA and OGDM groups separately against controls

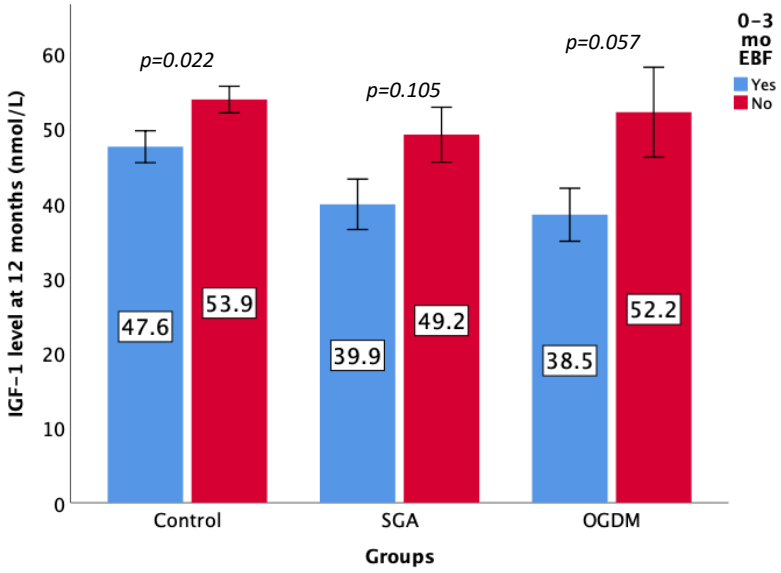
Linear regression analysis. IGF-1 values in nmol/L are log-transformed.
B=unstandardised coefficient, SE=standard error.
Model 1 = unadjusted. Model 2 = adjusted for infant sex, postnatal age at visit, and infant feeding history (0-3 mo EBF vs mixed-/formula-fed). Model 3 = Model 2 + adjusted for maternal pre-pregnancy BMI and parity (primiparous vs multiparous).

	SGA vs controls		OGDM vs controls	
	B±SE	*p*	B±SE	*p*
Model 1	-0.04±0.03	*0.146*	-0.03±0.02	*0.077*
Model 2	-0.05±0.03	*0.069*	-0.03±0.02	*0.1*
Model 3	-0.07±0.03	***0.028***	-0.03±0.02	*0.088*

Infant sex and type of milk feeding were significant correlates of IGF-1 level at 12 months (both *p* values *0.001*). Across all groups, mixed-fed infants had generally higher IGF-1 levels at 12 months compared to their breastfed counterparts, although significance was only observed among controls (Figure 5.5, Table 5.5).

Figure 5.5 IGF-1 level measured at 12 months between exclusively breastfed and mixed-fed infants in SGA, OGDM, and controls

Values are mean, error bars are representing ±1SE. *P* values are for comparisons between feeding groups in each infant group (SGA/OGDM/Control, unadjusted). Blue: infants who received exclusive breastfeeding from 0-3 months, red: infants who received formula or mixed-feeding from 0-3 months.



Moreover, as seen in Table 5.5, female infants had higher IGF-1 concentrations at 12 months than males across all groups (SGA, OGDM, and controls).

Table 5.5 Capillary blood-spot IGF-1 concentrations (nmol/L) at age 12 months by infant groups, sex, and type of milk feeding

[a]Significantly different from breastfed, *p<0.05*
[a]Significantly different from boys, *p<0.05*

Groups	Boys		Girls	
	Breastfed	Mixed-/formula-fed	Breastfed	Mixed-/formula-fed
Control	39.1±15.8	49.2±21.6[a]	55.3±26.7[b]	59.3±25.5[b]
SGA	40.5±13.3	38.5±17.2	39.1±19.2	57.1±24.2[a,b]
OGDM	33.9±17.4	48.8±31.2	43.1±13.4	61.5±29.2

In relation to weight gain pattern in the first year of life, as expected, the proportion of the SGA subset who caught-up was higher compared to both controls and OGDM (Table 5.6), similar to that observed across the whole groups (Chapter 4, Table 4.3). Across groups, infants who caught-up had higher capillary blood spots IGF-1 concentrations measured at 12 months compared to their counterparts (significantly different among controls and OGDM, Figure 5.6).

Furthermore, IGF-1 level at 12 months could predict growth parameters at 24 months (Table 5.7) with significant associations among controls and OGDM, or in the whole sample pooled together. However, when looking at growth gains between 12-24 months, the IGF-1 level at 12 months was positively associated with subsequent height gain from 12-24 months, but inversely with adiposity gain among SGA and controls, but *not* 'recent' OGDM (Table 5.8).

Table 5.6 Proportion of each growth pattern (catch-down, no-change, catch-up) infants who performed catch-up weight gain in the first year of life

Catch-up is defined if growth gain between 2 periods >+0.67 SDS while catch-down is defined if it is <-0.67 SDS[23]
All 2 by 2 Chi-square tests *p values* between SGA vs controls and SGA vs OGDM *<0.0001*; between OGDM vs controls *<0.05*

A. 0-12 months

	Catch-down	No-change	Catch-up
Weight gain			
Control	28%	46%	26%
SGA	5%	33%	62%
'Recent' OGDM	37%	43%	20%
Height gain			
Control	11%	46%	43%
SGA	5%	38%	57%
'Recent' OGDM	10%	67%	23%
Skinfold gain			
Control	30%	41%	29%
SGA	2%	41%	57%
'Recent' OGDM	20%	54%	26%

B. 12-24 months

	Catch-down	No-change	Catch-up
Weight gain			
Control	6%	84%	10%
SGA	4%	75%	21%
'Recent' OGDM	5%	79%	16%
Height gain			
Control	6%	84%	10%
SGA	4%	75%	21%
'Recent' OGDM	5%	79%	16%
Skinfold gain			
Control	18%	61%	21%
SGA	10%	76%	14%
'Recent' OGDM	15%	80%	5%

Figure 5.6 IGF-1 concentrations among SGA, OGDM, and controls based on 0-12 months weight gain pattern

Values are mean±SE. *P* values are for comparisons between catch-up vs non- in each infant group (SGA/OGDM/Control, unadjusted)

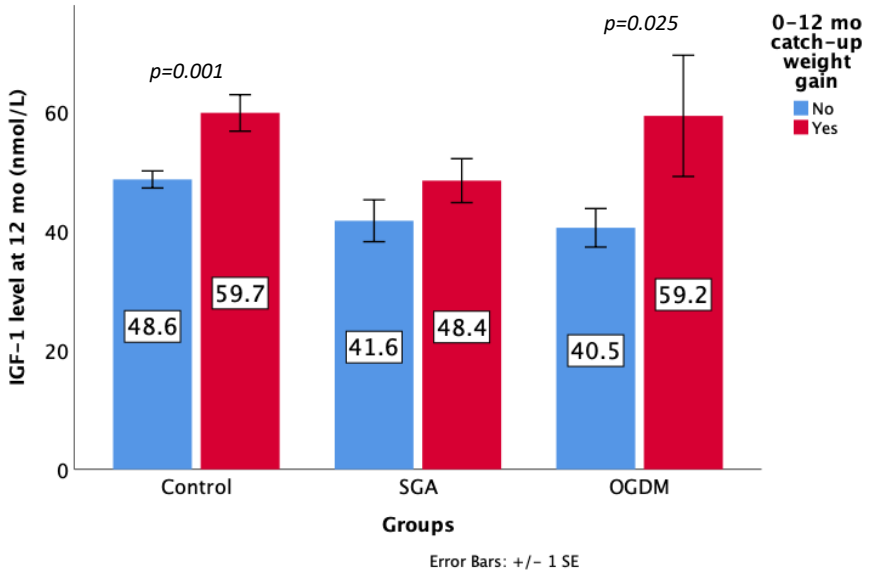


Table 5.7 Associations between capillary blood spots IGF-1 level and growth parameters-SDS at 24 months

Multiple linear regression

Models are adjusted for infant sex, postnatal age at visit, feeding history, and maternal parity

*Models are additionally adjusted for groups (as binary variable: controls vs SGA/OGDM)

#Unadjusted model has significant *p* value (*<0.05*)

Outcomes: Anthropometry at 24 months	**Predictor: IGF-1 level at 12 months**							
	All*		Controls only		SGA only		OGDM only	
	B±SE	*p*	B±SE	*p*	B±SE	*p*	B±SE	*p*
Weight SDS	0.01±0.002	***<0.0001***	0.01±0.003	***<0.0001***	0.005±0.007	*0.44*	0.02±0.007	***0.005***
Height SDS	0.012±0.002	***<0.0001***	0.009±0.003	***0.002***	0.015±0.008	*0.059#*	0.017±0.007	***0.024***
BMI SDS	0.006±0.002	***0.009***	0.006±0.003	***0.019***	-0.004±0.005	*0.447*	0.013±0.006	***0.043***
SF SDS	0.001±0.002	*0.479*	0.002±0.003	*0.422*	-0.006±0.005	*0.221*	0.009±0.004	***0.012***

Table 5.8 Associations between capillary blood spots IGF-1 level and 12-24 months growth gains

Multiple linear regression
IGF-1 concentrations in log-transformed values
Model 1 is adjusted for infant sex, postnatal age at visit, feeding history, and maternal parity
Model 2 is model 1 + adjustment for baseline growth parameter at 12 months
*Models are additionally adjusted for groups (as binary variable: controls vs SGA/OGDM)
#Unadjusted model has significant *p* value (*<0.05*)

Outcomes: Growth gains	**Predictor: IGF-1 level at 12 months**							
	All*		Controls only		SGA only		OGDM only	
	B±SE	*p*	B±SE	*p*	B±SE	*p*	B±SE	*p*
12-24 mo Weight gain								
Model 1	-0.17±0.13	*0.194*	-0.15±0.16	*0.329*	-0.29±0.39	*0.464*	-0.25±0.34	*0.466*
Model 2	-0.01±0.13	*0.926*	-0.02±0.16	*0.908*	-0.17±0.37	*0.644*	0.09±0.36	*0.809*
12-24 mo Height gain								
Model 1	0.41±0.14	***0.005***	0.39±0.17	***0.021***	0.58±0.44	*0.19*	0.32±0.36	*0.377*
Model 2	0.5±0.14	***<0.0001***	0.46±0.16	***0.005***	0.78±0.4	*0.057*	0.34±0.38	*0.369*
12-24 mo BMI gain								
Model 1	-0.62±0.19	***0.001***	-0.58±0.22	***0.008***	-0.97±0.52	*0.071*	-0.58±0.51	*0.261*
Model 2	-0.27±0.17	*0.11*	-0.27±0.2	*0.178*	-0.76±0.42	*0.074*	0.18±0.46	*0.694*
12-24 mo SF gain								
Model 1	-0.57±0.22	***0.011***	-0.68±0.28	***0.017***	-0.62±0.44	*0.163*	-0.6±0.55	*0.283*
Model 2	-0.32±0.2	*0.102*	-0.33±0.26	*0.197*	-0.75±0.37	***0.047***	0.17±0.44	*0.705*

5.3.3.2 C-peptide

SGA infants had higher C-peptide levels than controls, while levels in OGDM infants were lower than controls: (median(IQR) of SGA 548.5(458.3) vs controls 526.1(346.3) vs OGDM 435.0(359.8)), although none of these differences reached statistical significance (Figure 5.7).

From all recorded maternal data, only maternal height displayed a significant positive correlation with infant C-peptide level at 12 months (Table 5.9). There was, however, a positive relationship with both contemporaneous and later (at 24

months) weight and height-SDS. Interestingly, a moderate positive correlation was observed with abdominal intra-abdominal fat thickness at 12 months (Table 5.9 and Figure 5.8).

Figure 5.7 C-peptide concentrations between SGA, 'recent' OGDM, and controls
Values are median, error bars are representing 95% CI

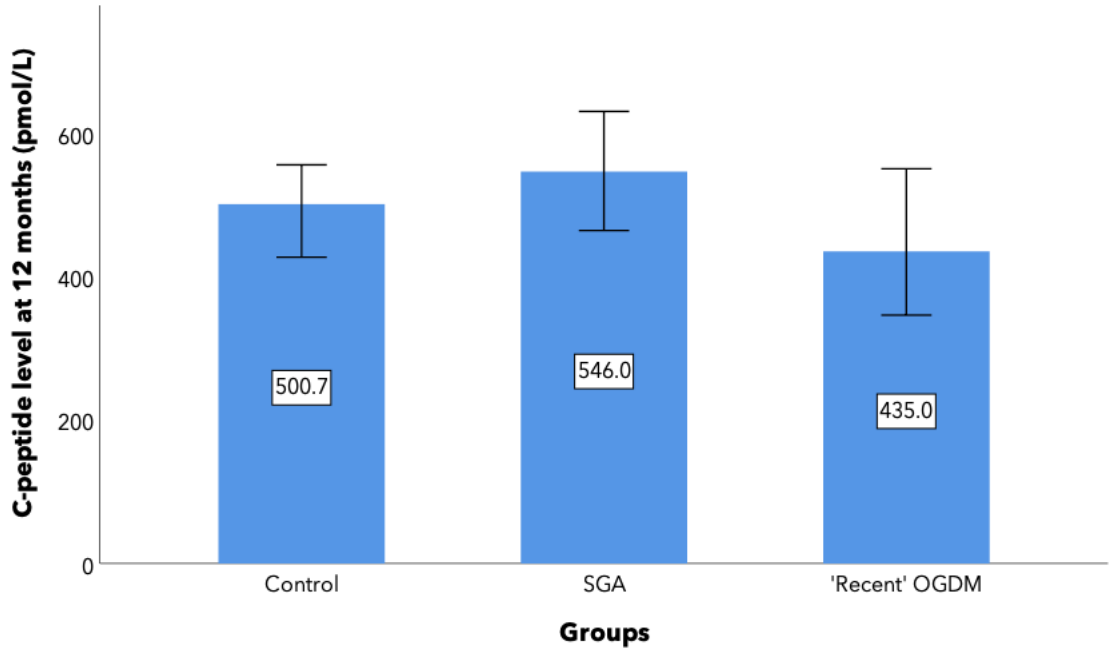


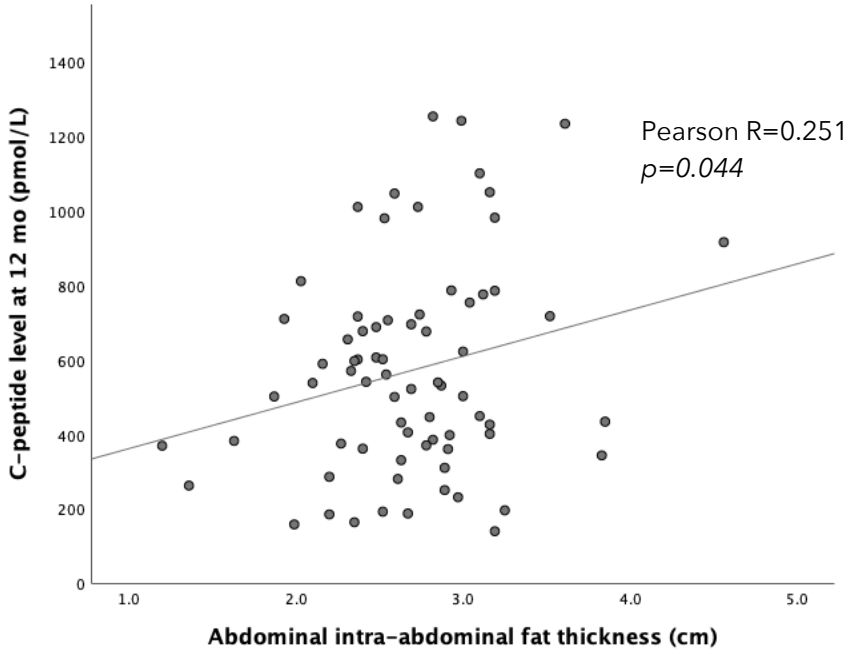
Table 5.9 Ante- and postnatal factors that correlated significantly with capillary blood spots C-peptide level at 12 months

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
US=ultrasounds

Parameters	Pearson correlation coefficient (R)
Maternal height	0.151*
0-12 mo height gains SDS	0.128*
12 months anthropometry	
Weight-SDS	0.164*
Height-SDS	0.141*
Abdominal intra-abdominal fat thickness (US)	0.251*
24 months anthropometry	
Weight-SDS	0.184**

Height-SDS	0.164*

Figure 5.8 Positive correlation between capillary blood spots C-peptide level at 12 months with contemporaneous abdominal intra-abdominal fat thickness among control infants



Among controls, C-peptide concentrations at 12 months were comparable across feeding groups. SGA/OGDM mixed-/formula-feeders had slightly higher C-peptide level, but the difference was not statistically significant (Figure 5.9). As seen in Figure 5.7, there was no significant difference in C-peptide level between SGA or OGDM when independently compared against the control group. However, when stratified based on weight gain pattern in the first year of life, control infants who caught-up had higher capillary blood spot C-peptide levels at 12 months compared to those controls without catch-up weight gain (Figure 5.9).

Figure 5.9 C-peptide concentrations measured at 12 months between exclusively breastfed and mixed-fed infants in SGA, OGDM, and controls

Total N=119 for controls, 58 for SGA, and 46 for OGDM
Values are mean ± SE

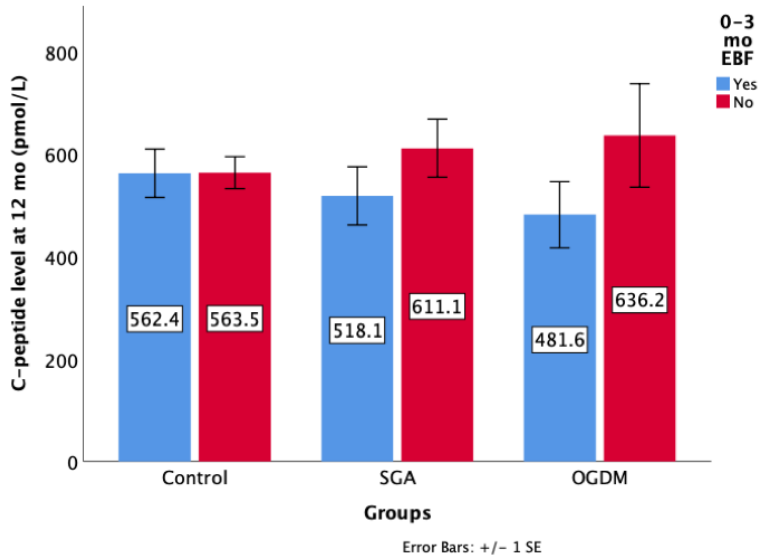
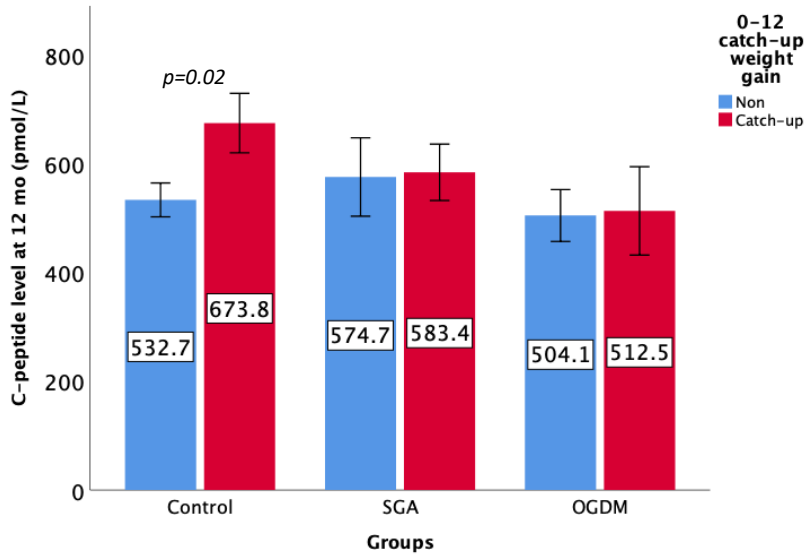


Figure 5.10 C-peptide concentrations among SGA, OGDM, and controls based on 0-12 months weight gain pattern

Values are mean ± SE. *P* values are for comparisons between catch-up vs non- in each infant group (SGA/OGDM/Control, unadjusted)



With regard to subsequent growth, associations between C-peptide level at 12 months and cross-sectional growth parameters (weight and height) at 24 months were significant among controls, but not among SGA or OGDM (Table 5.10). There were no associations between the capillary level of this hormone with growth gains between 12-24 months across all infant groups.

Table 5.10 Associations between capillary blood spots C-peptide level and growth parameters-SDS at 24 months

Multiple linear regression
Models are adjusted for infant sex, postnatal age at visit, feeding history, and maternal parity
*Models are additionally adjusted for groups (as binary variable: controls vs SGA/OGDM)

Outcomes: Anthropometry at 24 months	**Predictor: C-peptide level at 12 months**			
	All*		Controls only	
	B±SE	*p*	B±SE	*p*
Weight SDS	0.1±0.02	***0.016***	0.1±0.03	***0.043***
Height SDS	0.1±0.02	***0.028***	0.1±0.04	***0.032***
BMI SDS	0.02±0.02	*0.254*	0.02±0.03	*0.497*
SF SDS	0.01±0.01	*0.439*	0.01±0.03	*0.671*

5.4 Discussion

Control and OGDM subsets in this analysis displayed similar growth trajectories to the corresponding overall CBGS groups. However, as expected, SGA extremes born with the lowest birth weights in the group gained more weight from 0-12 months although no significant difference observed in the subsequent growth to 24 months.

With regard to IGF-1, both SGA and OGDM infants had lower hormone concentrations at 12 months, particularly after adjustment for maternal pre-pregnancy BMI and parity among SGA infants. When pooled together, the

significant determinants of IGF-1 level at this age included infant sex, feeding type, and maternal parity. The significant effect of sex on IGF-1 levels in this study, with female infants having higher IGF-1 values, corroborates earlier findings[200,260].

Meanwhile, apart from maternal height, there was no correlation with other maternal or infant factors, including sex and feeding type, on C-peptide levels at 12 months.

In relation to feeding type, across all 3 infant groups (both in the whole groups with or without available hormone data and also in subsets), mixed-/formula-fed infants had generally greater growth parameters-SDS at 12- and 24- months, including weight, height, and skinfolds reflecting subcutaneous adiposity, although not all reached statistical significance. The body size of these differently-fed infants was comparable at birth and 3 months and this result was in line with previous findings in the CBGS[200]. Those breastfed infants also had lower IGF-1 concentrations at 12 months compared to their mixed/formula-fed counterparts, although it was only statistically significant among controls. This, with other previous reports, have provided more information to the involvement of IGF-1 in the mechanistic pathway of formula feeding exposure causing greater growth gains during infancy, at least among AGA infants[200,260]. This link has also been attributed to increased overall intake in mixed-/formula-fed infants, particularly protein, leading to increased insulin, growth factors, and IGF-1 secretion. Correspondingly, infants fed with protein-reduced formula displayed lesser gains that were more comparable to the growth trajectories of breastfed infants[261].

However, there was no link between feeding type and C-peptide concentration in this study across all 3 infant groups, unlike a Swedish study reporting higher urinary

C-peptide levels in 3-6 months old formula-fed infants[262]. This may be due to the type of biological samples used to measure C-peptide (DBS) or timing of measurement (12 months of age). The latter also appeared in a more recent study reporting near marginal significance of higher serum C-peptide concentration among formula feeders compared to breastfed infants at 6 months (*p=0.07*), but this became insignificant at 12 months[263].

Furthermore, catch-up growth was also related to higher IGF-1 concentrations at 12 months of age and this hormone level was subsequently associated with cross-sectional growth outcomes at 24 months. This also occurred with C-peptide, but only among controls. In the previous CBGS publication[200], capillary IGF-1 level at 3 months was reported to be able to predict greater gains of length but lesser gains of adiposity from 3-12 months in control infants. Similar trends were evident in the present study among control and SGA infants (although not as strongly as in controls), but not among OGDM. This strengthens the evidence that IGF-1 could be involved in subsequent weight gain regulation with more favour to linear growth (height) rather than adiposity in infants across all birth weight range.

Data from ALSPAC have also reported a positive relationship between IGF-1 levels and greater height gains until later childhood, between age 5-10 years old of age[264]. This was plausible because IGF-1 plays an essential role in stimulating bone and muscle growth, chondrocyte proliferation, and growth plate maturation. Locally produced IGF-1 may be more important than the hepatic-derived hormone evidenced from the observation that 75% reduction in serum IGF-1 concentration

in mice due to selective hepatic deletion of *Igf1* in mice does not result in significant growth disruption[108].

It is still questionable whether IGF-1 has little to no contribution to subsequent overall weight gain across infant groups, although the current data confirmed the previous CBGS findings which was conducted on control infants[200]. At least 2 existing studies reported a positive association between IGF-1 concentration and earlier weight gain[200,260], which was also found in this study. Other studies reported that IGF-1 concentration could predict subsequent growth gains much later in life, e.g. within age range 5-10 years old[264,265].

Specific to the SGA vs control comparison, IGF-1 concentrations in both groups at 12 months were higher (103.75 ng/mL SGA and 120 ng/mL AGA) than that reported by Chellakooty *et al*.[260] (88 ng/mL SGA and 92 ng/mL AGA). This Danish study involved a much larger population and the results have been used to generate infancy IGF-1 reference values. The higher IGF-1 levels in our analysis could be due to different sources of sampling (capillary whole blood vs serum) and age at measurement (3 vs 12 months). However, significantly lower IGF-1 concentration among SGA compared to AGA at 3 months found by Chellakooty *et al* was consistent with the similar observation with 12 months IGF-1 found in this study.

Although these conclusions might be limited to AGA or control infants, the positive associations between capillary C-peptide concentration at 12 months with prior, contemporaneous, and subsequent body size and adiposity, especially with abdominal intra-abdominal fat thickness, are new findings. They are consistent with HAPO study reports of a positive association between cord blood C-peptide

concentrations and adiposity at birth[163], and EDEN cohort findings with higher birth weight[201].

The main limitation of this study was the hormone measurements were not conducted at the same period, but with a ±10 year-gap (controls and 14 SGA were measured in 2009, the remaining SGA and all 'recent' OGDM were in 2019). Although both measurements used the same corresponding assays, were performed by the same laboratories (RIA-Mediagnost for IGF-1 and MSD-CBAL for C-peptide), and all analyses conducted in this chapter have taken into account the batch-difference effect, any distinctive features between 3 infant groups observed in this chapter cannot confidently reflect the 'real' difference between infant groups (SGA vs control, 'recent' OGDM vs control, or SGA vs 'recent' OGDM). This is because the possibility of technical issues (i.e. assay differences and prolonged storage) causing/affecting the results cannot be excluded. Therefore, the potential explanation that IGF-1 levels were lower among controls than SGA and OGDM could be attributed to those technical factors, rather than differences between the groups themselves.

However, having acknowledged this issue, the influence of hormones being measured in this study (IGF-1 and C-peptide) on contemporary and subsequent growth deserves further attention and replication in a larger cohort with longer growth monitoring. This is because the analyses between hormones and growth parameters were conducted separately and independently between infant groups and thus the results should be minimally affected by different periods in measuring both hormone levels. Detailed nutritional intake, especially protein in relation to

IGF-1 level, could also be beneficial in explaining the effect of hormones in driving growth outcomes during infancy period.

5.5 Conclusions and future recommendations

12-month IGF-1 concentrations were positively related to prior, contemporaneous, and subsequent body size, and were strongly affected by early postnatal nutrition. However, the limited evidence found in this study that they might essentially drive later growth needs further confirmation. To provide stronger evidence for the association between IGF-1, amino acids, and infant growth, data on protein intake during infancy would be of importance in a longitudinal study on infant growth like this.

The associations between C-peptide and infant growth and adiposity are new findings but associations were weak and require confirmation. However, the link between the hormone level at 12 months and the contemporaneous abdominal adiposity parameter deserves further investigation.

Chapter 6 Lipidomic signatures of SGA and OGDM, in comparison to controls

6.1 Introduction

The importance of the prenatal period in defining later metabolic diseases risks[5,38] is supported by the match-mismatch theory[56] which has proposed that over or under nutrition *in utero*, could lead to metabolic disruption in the offspring. However, apart from birth size (usually, but not always, reflecting *in utero* nutritional availability), an emerging body of literature has pointed out that postnatal growth trajectories are also fundamental in shaping those associations. For example, SGA infants who caught up after being exposed to plentiful postnatal nutrition[21] would possess greater metabolic risks later in life[5,61]. Unfortunately, the evidence is still sparse regarding how infancy metabolic profiles change over in the early postnatal period in relation to dietary exposures and growth deviations.

To better understand how these distinct groups *in utero*, SGA and OGDM develop similar metabolic risks in the future, this chapter characterises their metabolic signatures via unbiased lipidomics. The rationale being that variation in lipid metabolism during infancy has been associated with subsequent weight gain[202] and lipidomics provides comprehensive profiles of lipid molecules[266].

From the earlier study[203] involving CBGS1 subjects, lipidomics provided more valid assay and more significant associations than metabolomics, especially when the amount of samples is limited due to being obtained from DBS. Although newborn blood spot test (Guthrie card) is helpful in diagnosing rare inborn errors of metabolism, the large defects in those metabolites of interest are far more obvious in magnitude than the likely differences between the infant groups in this study. Lipidomics is also preferable to proteomics since proteins are highly modifiable (e.g. by glycosylation or phosphorylation) thus often difficult to be captured and identified by the relevant software. Proteomics assays are also more expensive and time-consuming than lipidomics.

This study aimed to distinguish lipid profiles of SGA and OGDM, separately with a control population. As infancy lipidomic profiles have been reported to change over the first year of life, in association with early dietary exposures (breast- versus formula- versus mixed-feeding) as described in the previous publication of the CBGS[202], it is also intriguing to observe such associations in infant groups at high metabolic risk: SGA and OGDM.

In addition, this chapter also explored the link between lipid ratios representing key enzymes in lipid metabolism and subsequent growth and adiposity in the 3 groups of infants. The lipid ratios examined in this study were translated from mice studies conducted by the Netherlands Organisation for Applied Scientific Research (Toegepast Natuurwetenschappelijk Onderzoek or TNO)[195].

Some parts of this chapter have been published previously in 2 publications. The first one by Furse *et al* reported the combination of *in utero* conditions (intrauterine growth retardation or maternal hyperglycaemia) and postnatal feeding in shaping

the lipidomic profiles across SGA, OGDM, and controls[194]. This study involved small subsets of each infant group (40 controls, 34 SGA, and 59 OGDM). I was involved in the clinic visits and DBS collections, preparing the samples for the lipidomic experiments, revising the manuscript as well as dealing with the reviewers' comments. The second one was about the associations between lipid ratios representing desaturase enzymes activities and infant growth and adiposity[195], of which I am the first author and was involved in the whole process of analysing the human data and writing the manuscript. The animal studies were conducted by TNO and are not discussed in this thesis. The human study was performed on CBGS samples in collaboration with Dr. Albert Koulman, NIHR BRC Metabolomics and Lipidomics Facility, University of Cambridge.

6.2 Methods

6.2.1 Research subjects

An unbiased lipidomic approach was performed to identify similarities and differences between SGA, OGDM, and a control group of infants (N of each group=99), all from the CBGS2, were investigated at 2 time points, age 3 and 12 months. Infants were randomly selected from each corresponding group. This study approach was designed to enable the samples to be processed together within the same experiment and avoid inter-batch imprecision.

The results were then validated in the larger and existing dataset involving control infants from CBGS1 and additional SGA and OGDM subjects from CBGS2.

6.2.2 Lipid profiling

The experiment was conducted on DBS samples as detailed in Section 2.4.1. This was built upon the methodology employed in our previous studies[194,202,203,227] developed in collaboration with Dr Albert Koulman's group from the NIHR BRC Metabolomics and Lipidomics Facility, University of Cambridge.

To isolate the lipid fraction, blood spots/analytes were placed in the wells of a glass coated 2.4 ml plate and then 100 µl of ultrapure water, 250 µl methanol, and 500 µl methyl tertiary butyl ether were added. The plates were centrifuged for 10 minutes at 6,000 rpm after being shaken for 10 minutes at 600 rpm. This resulted in two layers in the plate: an aqueous layer at the bottom and an organic layer on top. The organic layer was then transferred, dried down, reconstituted, and used for lipid analysis. The next process was direct infusion high-resolution mass-spectrometry[227].

Lipid identification was performed by injecting each sample onto a column, followed by eluting the lipids with a gradient of MeOH/H_2O and MeOH/isopropanol. Selected masses were isolated, and all spectra were recorded.

Untargeted mass spectrometry was utilised to capture all potential lipid species since there were no existing lipidomic database of infants born SGA and OGDM. Three lipid species which were discovered previously in CBGS[202] as important nutritional biomarkers, PC (35:2), SM (36:2), SM (39:1), were prioritised. In addition, several other lipids were quantified that had been identified from previous experiments[202,227], as markers of desaturases activities, including stearoyl-coA desaturase-1 (SCD-1) and fatty acid desaturase (FADS) 1 and 2.

6.2.3 Statistical analyses

Multivariate analyses, consisting of principal component analysis (PCA) and partial least squares-determinant analysis (PLS-DA), were run to examine and compare highly interrelated lipid species across infant groups and to identify lipid candidate biomarkers.

PCA was used to observe general trends and to detect outliers, while the sparse PLS-DA (sPLS-DA) was used to reduce the number of metabolites in high-dimensional lipidomics data in order to produce robust and interpretable models. These models were assessed and cross-validated based on goodness-of-fit (R^2Y) and goodness-of-prediction (Q^2Y) metrics.

The groups were compared 2x2 separately, i.e. SGA versus controls, OGDM versus controls, and SGA versus OGDM. Significance in multivariate space was assessed using permutation tests[267], with permutation numbers set to be 2000 tests. Unpaired T-test was used for univariate analyses, comparing shortlisted lipid species across 2 groups, i.e. SGA versus controls, OGDM versus controls, and SGA versus OGDM. Adjusting significance threshold by number of variables or lipid species and false discovery rate (FDR) were used to minimise error caused by multiple testing.

Associations between infant groups and lipid ratios representing desaturases activities were assessed using multiple linear regression analyses.

Multivariate analyses were performed using Metaboanalyst version 4.0[268] and other analyses using SPSS version 26 and R version 4.0.2.

6.3 Results

6.3.1 Baseline characteristics and growth trajectories

Baseline maternal and birth characteristics of the SGA infants, OGDM, and controls included in this lipidomic study are shown in Appendix 4. Similar to the larger groups (Table 4.1), the OGDM mothers in this study had higher pre-pregnancy BMI compared to other 2 groups; SGA and GDM mothers were shorter, ethnically diverse, and more likely to be primiparous compared to the control population.

Also as in the larger groups (Table 4.2), both SGA and OGDM groups studied here were delivered significantly earlier than controls. Among infants involved in this study, the rate of exclusive breastfeeding from 0-3 months was significantly lower among OGDM (57%) compared to controls (76%). Apart from the lower exclusive breastfeeding rate in the current OGDM subset, no other difference was found between SGA/OGDM/control subsets and their corresponding larger groups in terms of baseline characteristics and postnatal growth trajectories.

Appendix 5 and 6 exhibit postnatal growth trajectories between the 3 groups in the first year of life. At all visit timepoints, SGA infants were smaller and shorter while OGDM were comparable to controls. Infants born SGA also demonstrated the greatest weight gain during 0-3 and 3-12 months compared to the other 2 groups. In contrast to the postnatal growth trajectories of the larger groups of SGA, OGDM,

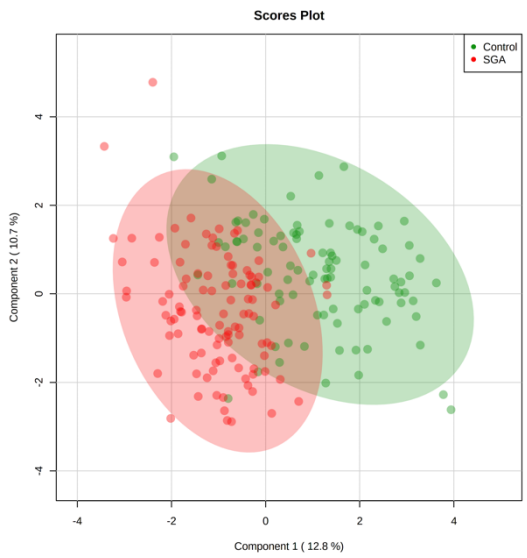
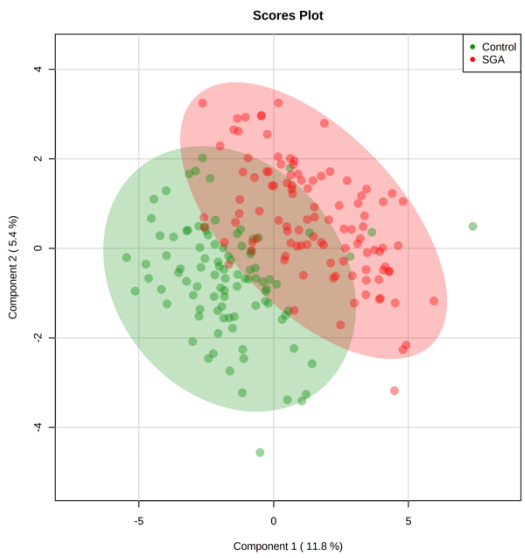
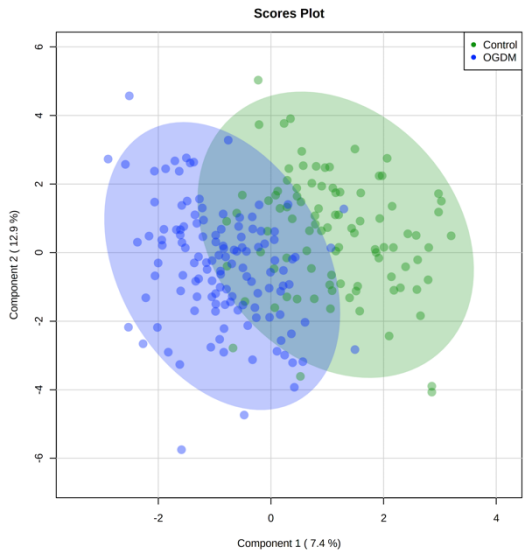
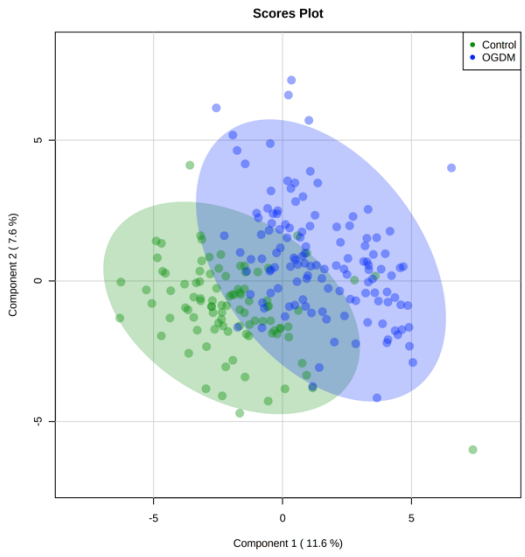
and controls (Chapter 4, Table 4.4 and Figure 4.1), the current OGDM subset showed almost identical growth pattern to controls from birth to 12 months.

6.3.2Lipid profiling analyses across CBGS2 infant groups

There were 65 and 299 lipid species captured from positive and negative modes, respectively. At both 3 and 12 months, the sPLS-DA models show a clearer separation of SGA and OGDM from controls, than SGA from OGDM (Figure 6.1 and 6.3). Comparing SGA or OGDM to controls resulted in significant features, even after adjusting the significance threshold (α=0.05) for the numbers of captured lipid species, as seen in Figures 6.2 and 6.4. Meanwhile, comparing lipid profiles of SGA with OGDM infants did not display any significant differences.

Figure 6.1 sPLS-DA models comparing capillary lipid profiles of each infant group at 3 months

sPLS-DA is a supervised multivariate regression model, used to extract via linear combination of lipid species the information that can predict the class membership (SGA/OGDM versus control). Permutation test is performed to assess the significance of class discrimination. In each permutation, a model is built between the data and permuted class labels.

	Positive mode	**Negative mode**
SGA vs Control	 Permutation test *p<0.0005* (0/2000)	 Permutation test *p<0.0005* (0/2000)
OGDM vs Control	 Permutation test *p<0.0005* (0/2000)	 Permutation test *p<0.0005* (0/2000)

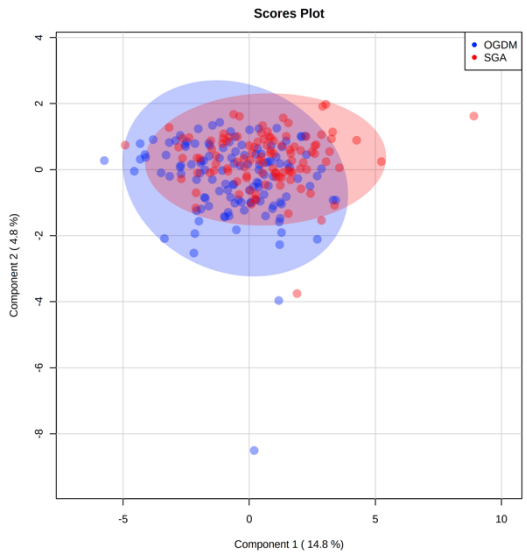
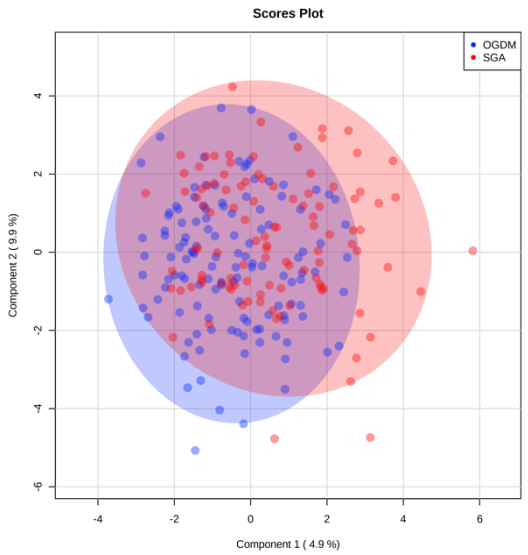
SGA vs OGDM	 Permutation test *p=0.001* (2/2000)	 Permutation test *p=0.0135* (27/2000)

Figure 6.2 Comparison of individual lipid species abundance between infant groups at 3 months

Each graph represents important determining features picked up by t-tests with adjusted significant threshold of 0.00077 (0.05/65 captured lipids) and 0.00017 (0.05/299 captured lipids) for positive and negative modes, respectively. *p values* are -log10 transformed thus the more significant values (the lower *p values*) are plotted higher on the graph. Purple dots represent lipid species with significant *p values*.

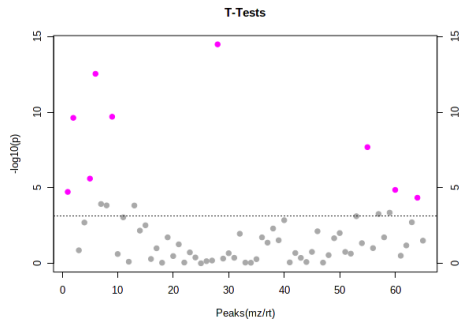
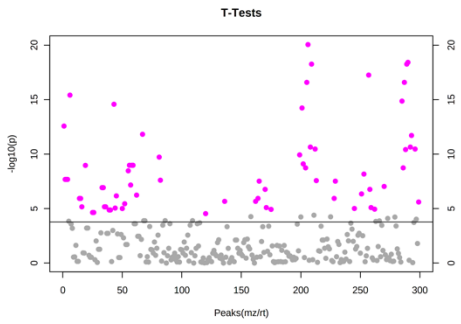
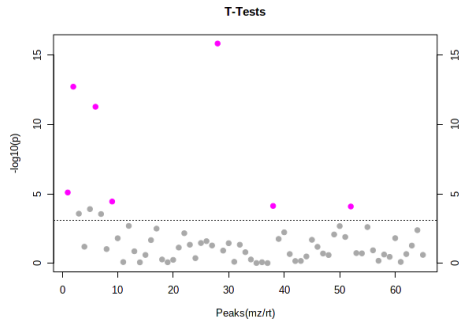
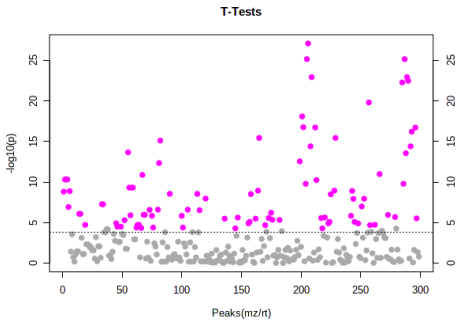
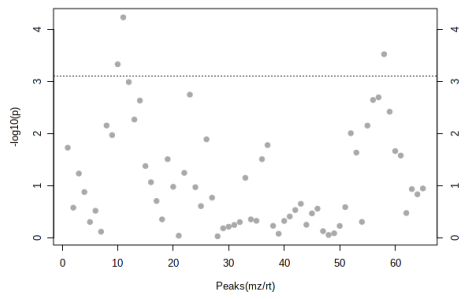
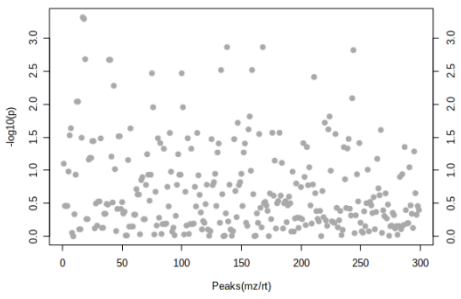
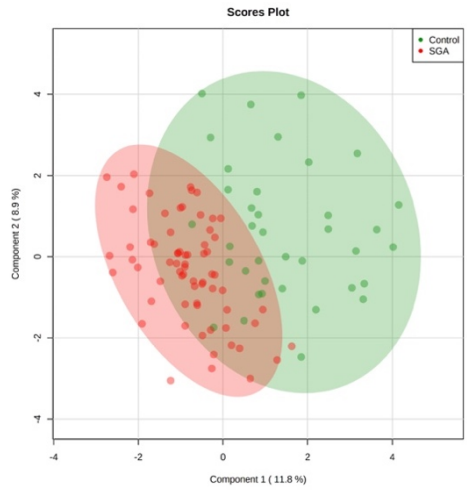
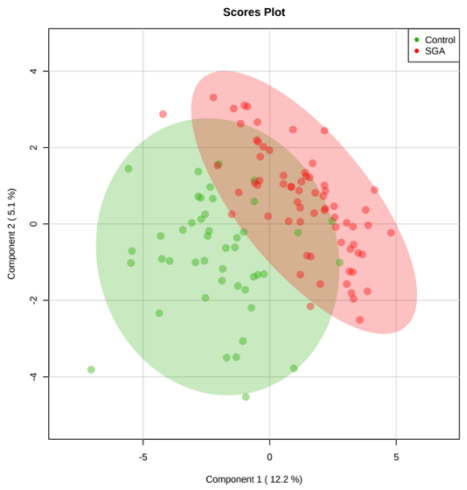
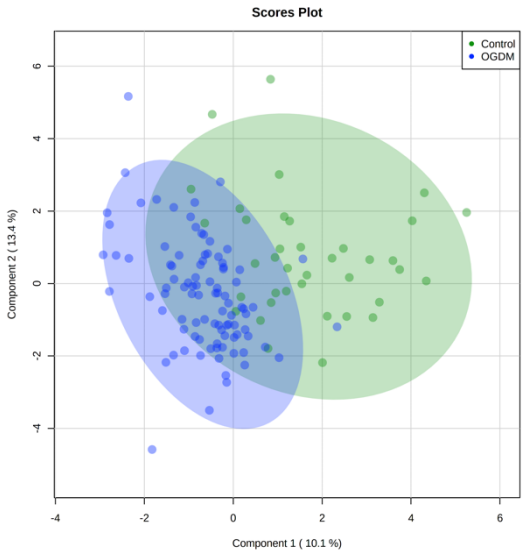
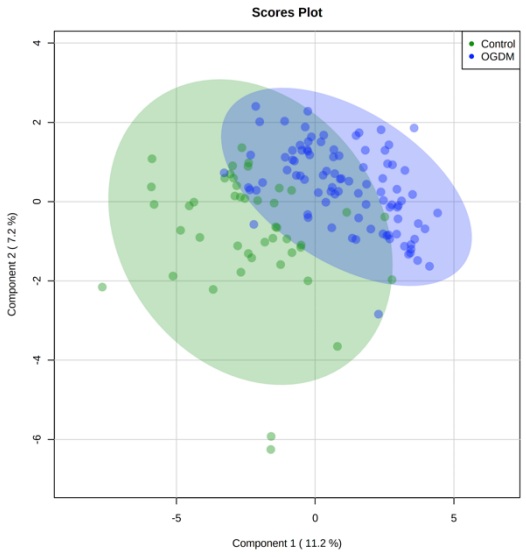
	Positive mode	**Negative mode**
SGA vs Control	**9 significant features** 	**69 significant features** 
OGDM vs Control	**7 significant features** 	**95 significant features** 
SGA vs OGDM	**No significant features** 	**No significant features** 

Figure 6.3 sPLS-DA models comparing capillary lipid profiles of each infant group at 12 months

sPLS-DA is a supervised multivariate regression model, used to extract via linear combination of lipid species the information that can predict the class membership (SGA/OGDM versus control). Permutation test is performed to assess the significance of class discrimination. In each permutation, a model is built between the data and permuted class labels.

	Positive mode	**Negative mode**
SGA vs Control	 Permutation test *p<0.0005* (0/2000)	 Permutation test *p<0.0005* (0/2000)
OGDM vs Control	 Permutation test *p<0.0005* (0/2000)	 Permutation test *p<0.0005* (0/2000)

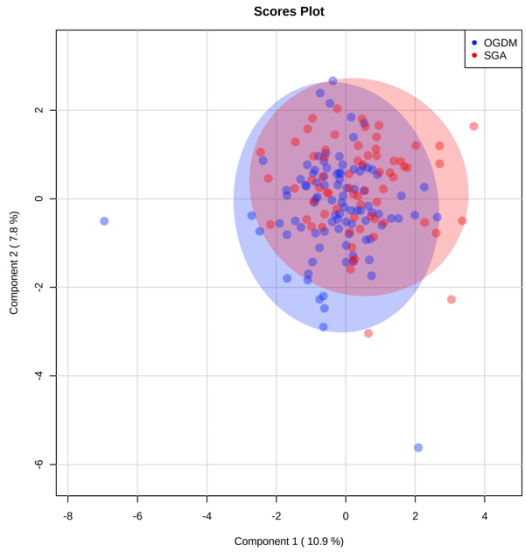
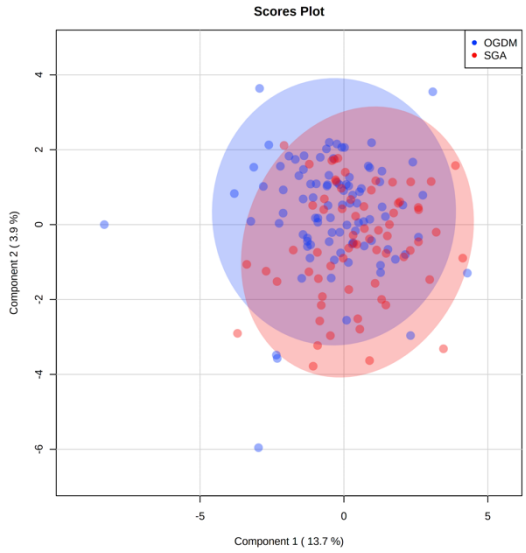
SGA vs OGDM	 Permutation test *p=0.4305* (861/2000)	 Permutation test *p=0.5305* (1061/2000)

Figure 6.4 Comparison of individual lipid species abundance between infant groups at 12 months

Each graph represents important determining features picked up by t-tests with adjusted significant threshold of 0.00077 (0.05/65 captured lipids) and 0.00017 (0.05/299 captured lipids) for positive and negative modes, respectively. *p values* are -log10 transformed thus the more significant values (the lower *p values*) are plotted higher on the graph. Purple dots represent lipid species with significant *p values*.

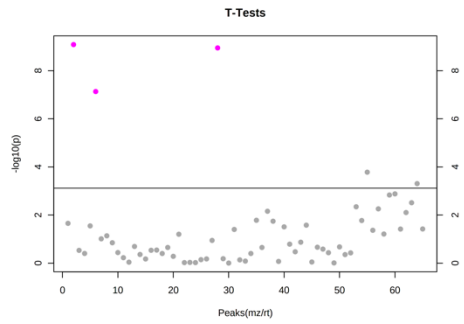
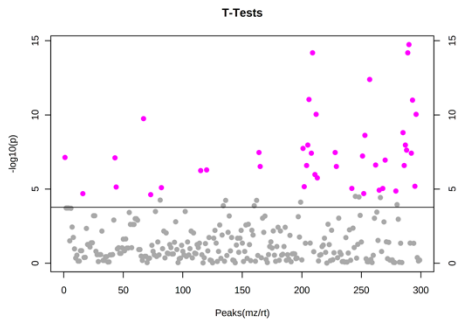
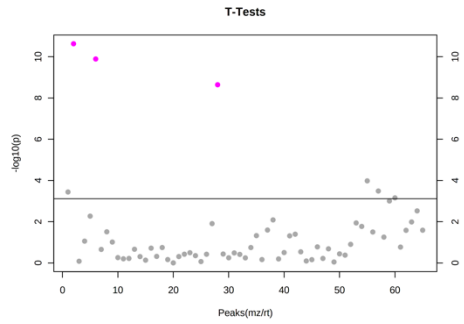
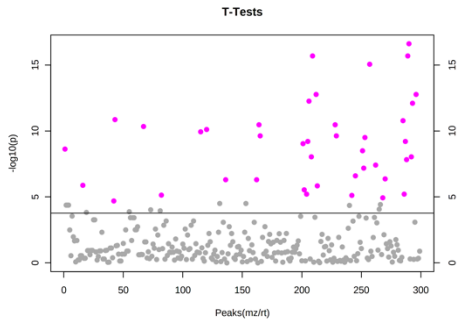
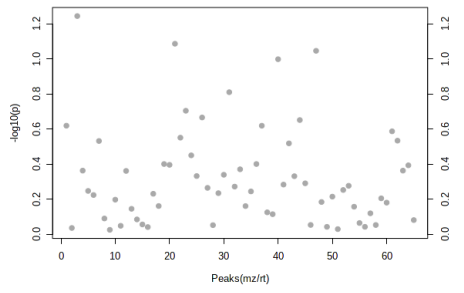
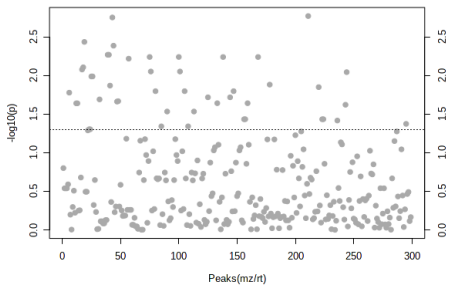
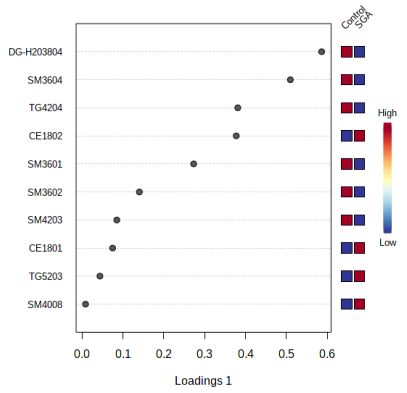
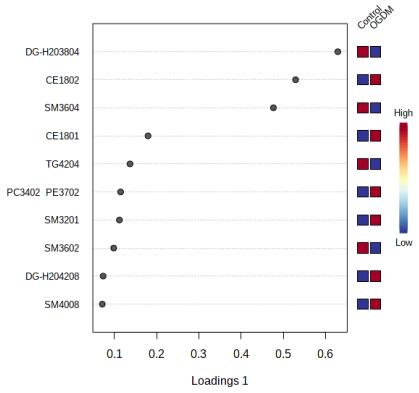
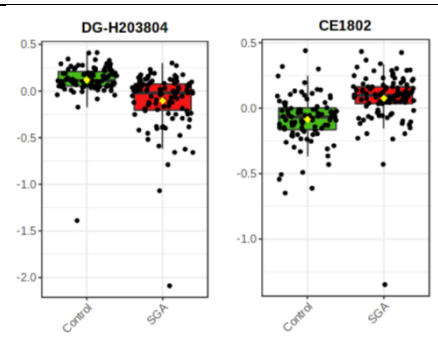
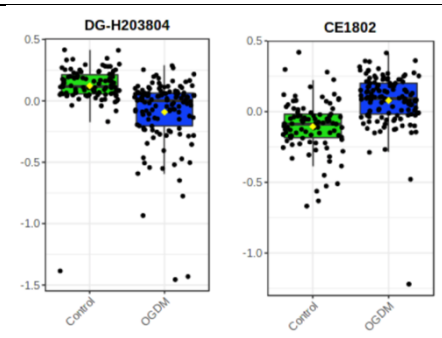
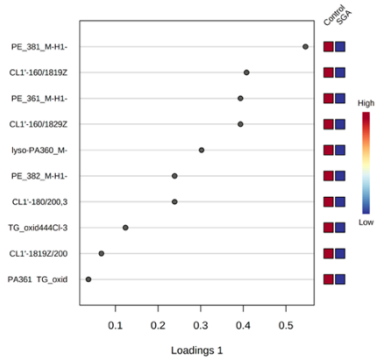
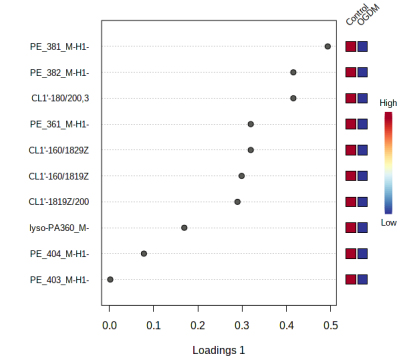
	Positive mode	**Negative mode**
SGA vs Control	**3 significant features** 	**43 significant features** 
OGDM vs Control	**3 significant features** 	**41 significant features** 
SGA vs OGDM	**No significant features** 	**No significant features** 

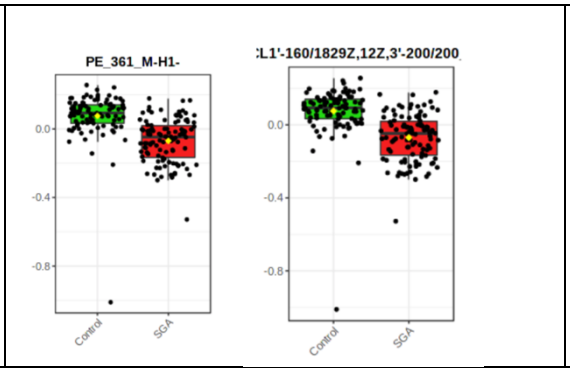
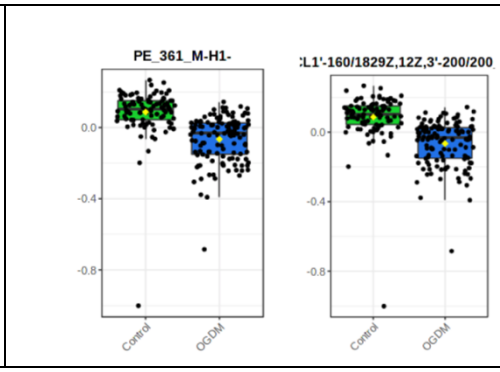
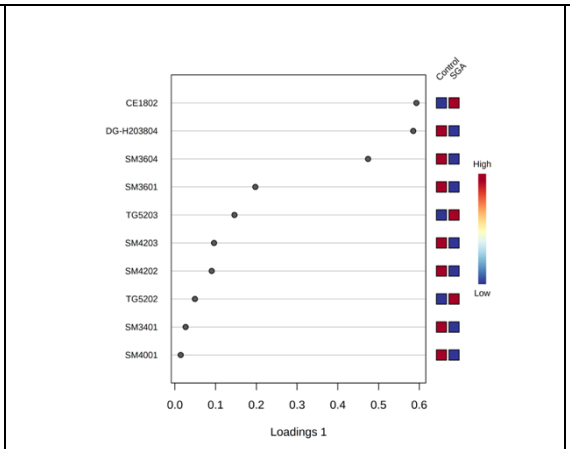
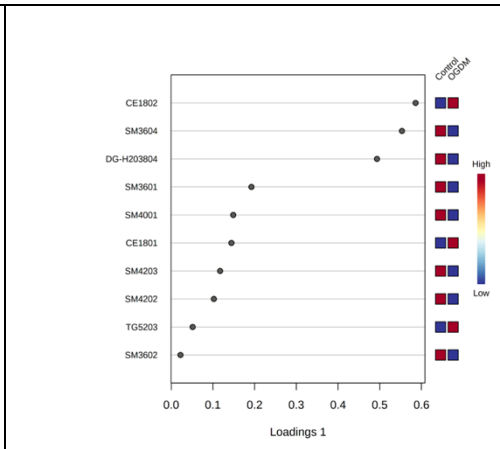
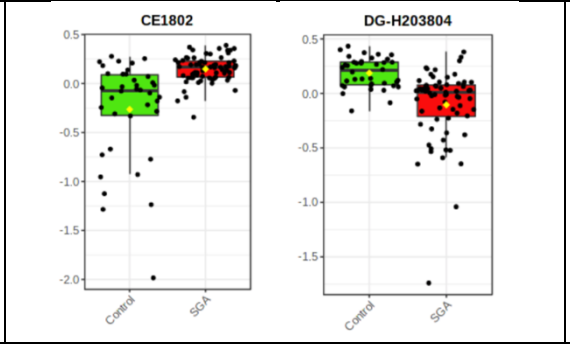
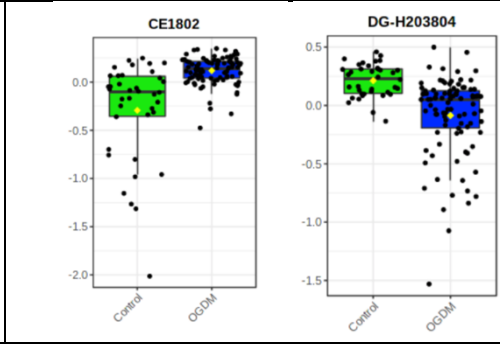
Figure 6.5 presents the key lipid species driving the differences between SGA and OGDM versus controls at 3 and 12 months. There were several identical lipids whose circulatory concentrations at 3 months captured the main distinguishing features when comparing SGA and OGDM separately to controls as the reference group. Moreover, some of these lipids persisted to be the main defining factors between SGA and OGDM versus controls at 12 months with the same relative trends, e.g. from positive mode, the levels of DG-H_2O (38:04) and SM (36:04) were lower but CE (18:02) was higher among SGA or OGDM compared to controls, at both 3 and 12 months (Table 6.1).

Figure 6.5 Important lipid species identified by PLS-DA driving differences between SGA/OGDM and control population

In graphs showing important features, the coloured boxes (on the right of each picture) indicate the relative concentrations of the corresponding metabolite in each group under study. In each graph, lipid species are ranked by their level of importance in driving the separations between groups (models in Figure 6.1 and 6.3).

Some examples of univariate analyses comparing lipid species between groups are also displayed.

	SGA vs Control	**OGDM vs Control**
	3 months	
	Positive mode	
Important features		
Examples of univariate analysis		
	Negative mode	
Important features		

Examples of univariate analysis		
12 months		
Positive mode		
Important features		
Examples of univariate analysis		

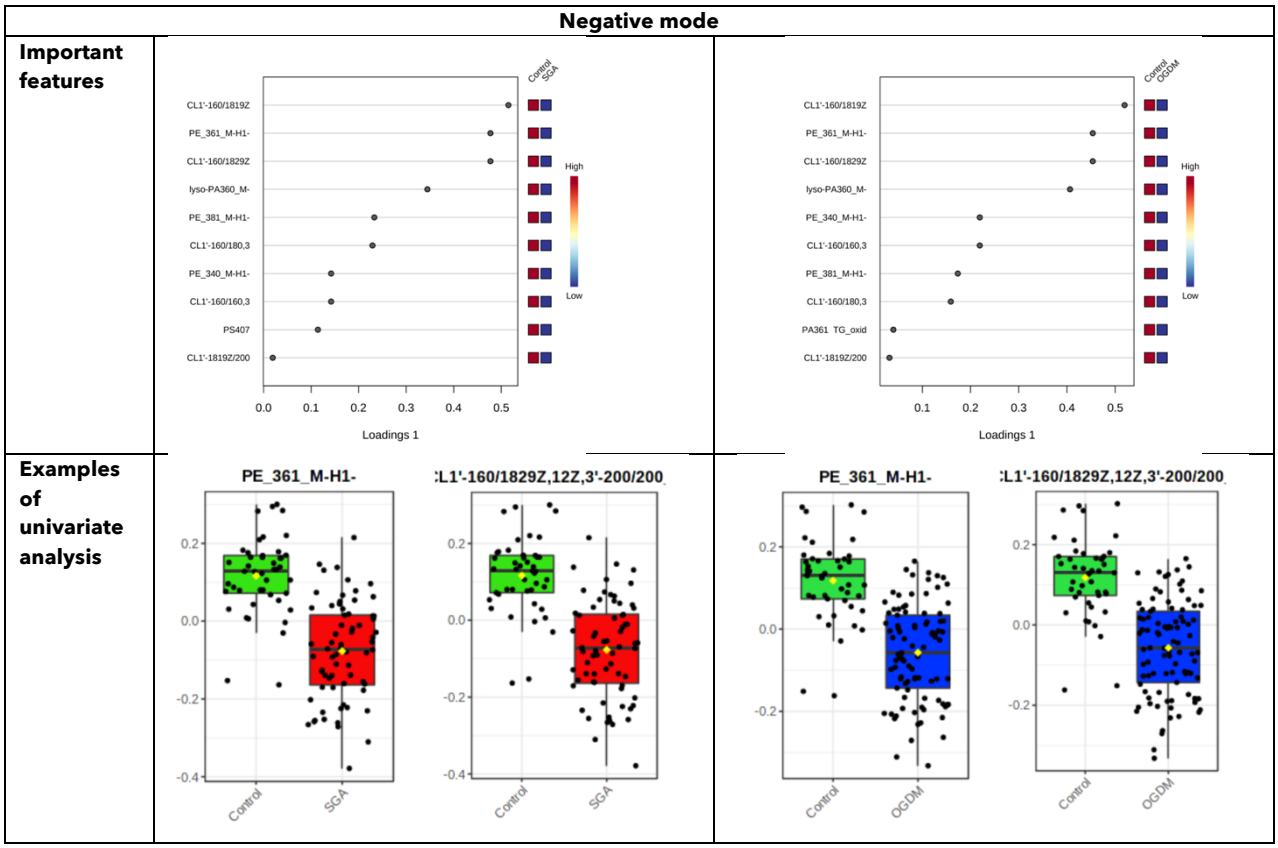


Table 6.1 Identical distinguishing lipid features of SGA and OGDM comparisons to controls, detected at 3 months and persisted at 12 months

	Lipid species	Lipid class	Lipid sub-category	Among SGA and OGDM, compared to controls
Positive mode	DG-H_2O (38:4)	Glycerolipids	Diradylglycerolipids	Lower
	SM (36:4)	Sphingolipids	Sphingomyelins	Lower
	CE (18:2)	Sterol lipids	Cholesterol esters	Higher
Negative mode	PE (38:1)	Glycerophospholipids	Glycerophosphoethanolamines	Lower
	CL(1'-[16:0/18:1(9Z)],3'-[20:0/20:0])	Glycerophospholipids	Cardiolipins	Lower
	PE (36:1)	Glycerophospholipids	Glycerophosphoethanolamines	Lower
	CL(1'-[16:0/18:2(9Z,12Z)],3'-[20:0/20:0])	Glycerophospholipids	Cardiolipins	Lower
	lysoPA(36:0)	Glycerophospholipids	Glycerophosphates	Lower

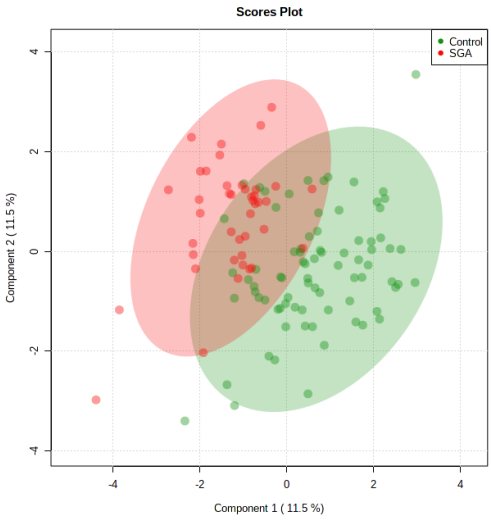
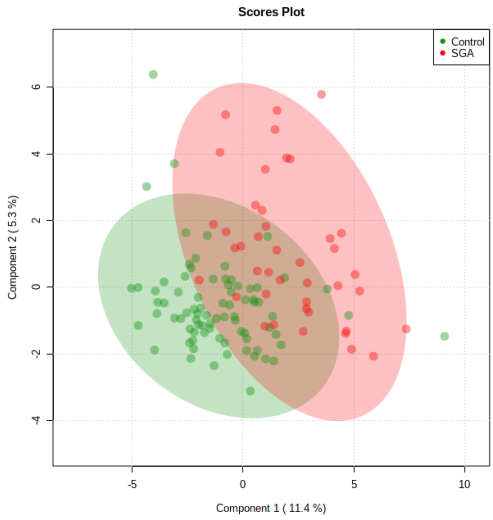
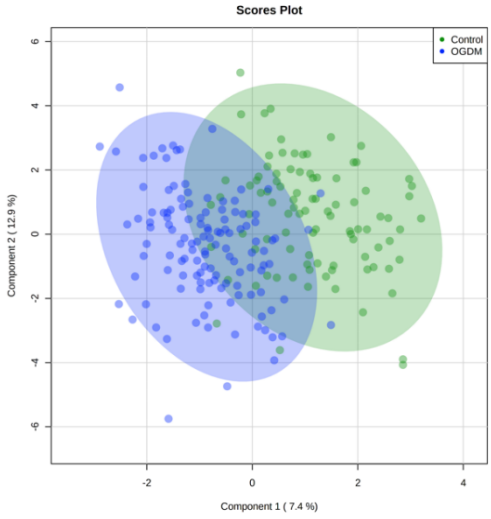
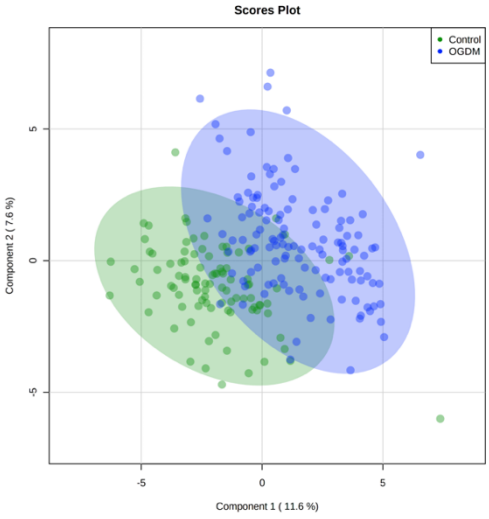
	CL(1'-[18:1(9Z)/20:0],3'-[20:0/20:0])	Glycerophospholipids	Cardiolipins	Lower

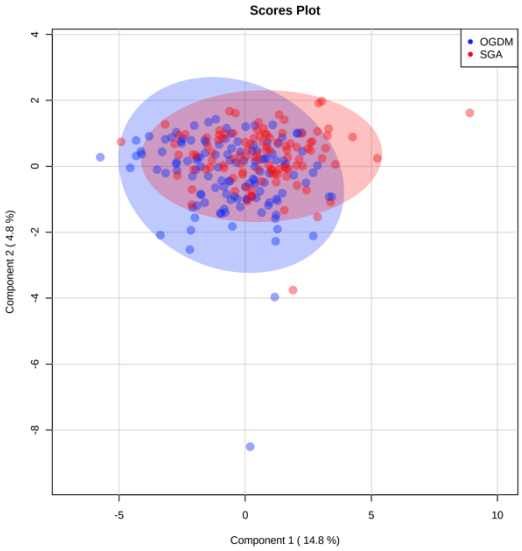
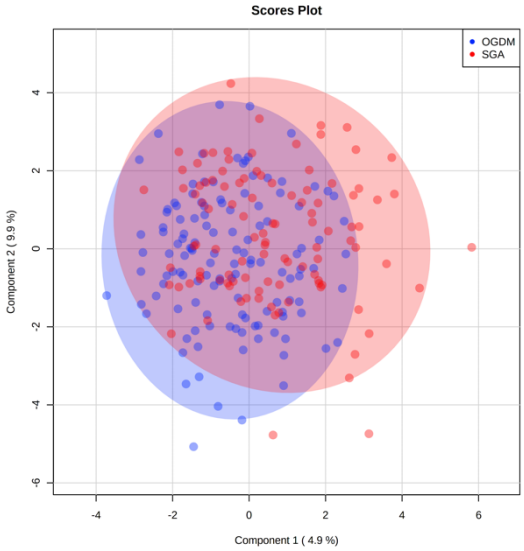
When sub-analysed in only breastfed infants, there remained more noticeable separation between SGA or OGDM compared to controls, than SGA versus OGDM (Figure 6.6). The 3 identified lipid determining drivers from positive mode, DG-H_2O (38:4), SM (36:4), and CE (18:2), remained as the important features, whereas results from negative mode produced a different set of lipid species. Unfortunately, sensitivity analyses among mixed-feeders between groups were not possible due to the small number of mixed-feeders among CBGS2 controls.

Figure 6.6 sPLS-DA models comparing 3 months capillary lipid profiles between infant groups, involving only exclusively breastfed infants

sPLS-DA is a supervised multivariate regression model, used to extract via linear combination of lipid species the information that can predict the class membership (SGA/OGDM versus control). Permutation test is performed to assess the significance of class discrimination. In each permutation, a model is built between the data and permuted class labels.

All infants consumed solely breastmilk in the first 3 months of life.

	Positive mode	**Negative mode**
SGA vs Control	 Permutation test *p<0.0005* (0/2000)	 Permutation test *p<0.0005* (0/2000)
OGDM vs Control	 Permutation test *p<0.0005* (0/2000)	 Permutation test *p<0.0005* (0/2000)

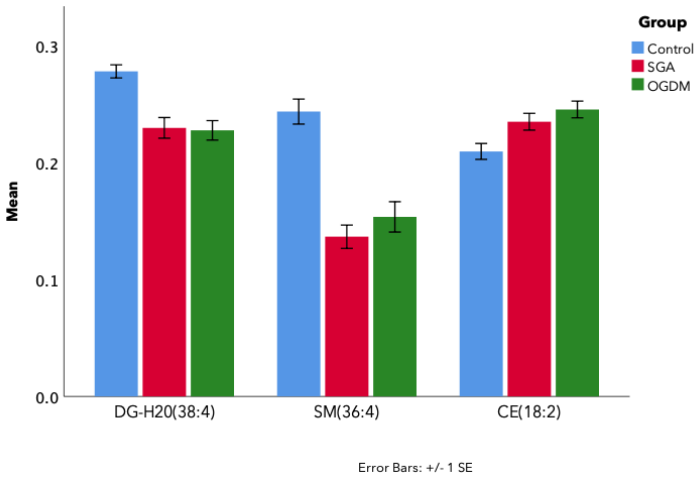
SGA vs OGDM	 Permutation test *p<0.11* (220/2000)	 Permutation test *p<0.042* (84/2000)

6.3.3 Combining recent and previous lipidomics data of CBGS

To validate the shortlisted distinguishing lipid features from CBGS2 subset (Table 6.1), those identified lipids or closely related lipids were tested in combined datasets, to also involve control and SGA infants from CBGS1. In these analyses, most identified lipids from positive and negative modes in CBGS2 experiment still appeared significant in discriminating SGA (from CBGS1 and 2) and OGDM ('recent' OGDM from CBGS2 only) versus all control infants from CBGS1 and CBGS2 after batch adjustment, with the same directions of associations (Figure 6.7 and 6.8).

Figure 6.7 Comparison of the abundance of shortlisted lipid species discovered from CBGS2 analyses in the whole dataset from positive mode

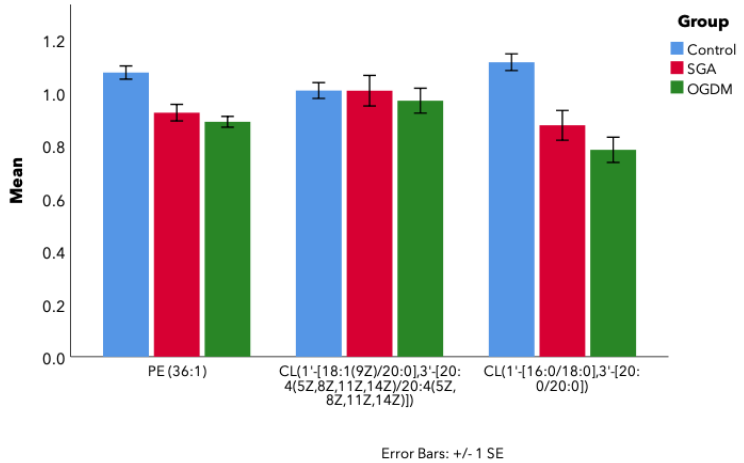
Y axis displays mean of relative abundance (arbitrary unit)
Control and SGA infants are from CBGS1 and CBGS2, OGDM are only the 'recent' ones from CBGS2



Comparison	*p values*		
	DG-H2O(38:4)	**SM(36:4)**	**CE(18:2)**
SGA vs Control	*<0.0001*	*<0.0001*	*0.107*
OGDM vs Control	*<0.0001*	*<0.0001*	*0.009*
SGA vs OGDM	*1.0*	*1.0*	*1.0*

Figure 6.8 Comparison of the abundance of shortlisted lipid species discovered from CBGS2 analyses in the whole dataset from negative mode

Y axis displays mean of relative abundance (arbitrary unit)
Control and SGA infants are from CBGS1 and CBGS2, OGDM are only the 'recent' ones from CBGS2



Comparison	*p values*		
	PE (36:1)	**CL(1' -[18:1(9Z)/20:0],3'-(20:4(5Z,8Z,11Z,14Z)/20:4(5Z,8Z,11Z,14Z)])**	**CL(1'-[16:0/18:0],3'-[20:0/20:0])**
SGA vs Control	*0.002*	*1.0*	*0.001*
OGDM vs Control	*<0.0001*	*1.0*	*<0.0001*
SGA vs OGDM	*1.0*	*1.0*	*0.707*

When analysing this larger dataset, similar to comparisons of CBGS2 subjects, sPLS-DA models comparing infant groups resulted in clearer separations between SGA or OGDM separately versus controls, but not between SGA and OGDM. The important lipid features driving those separations are shown in Figure 6.9.

Since most CBGS2 control infants were breastfed, involving more control infants from the CBGS1 allowed the addition of more mixed feeders to the analysis and thus enabled more thorough comparisons between subgroups based on the type of feeding. Interestingly, this sensitivity analysis highlighted a single lipid species, PS (29:0) as the most distinctive biomarker of risk groups (SGA or OGDM), regardless of the type of feeding (Figure 6.10).

Figure 6.9 Important lipid species identified by PLS-DA driving differences between SGA/OGDM and control population from CBGS1 and 2

The coloured boxes (on the right of each picture) indicate the relative concentrations of the corresponding metabolite in each group under study. In each graph, lipid species are ranked by their level of importance in driving the separations between groups in PLS-DA models.

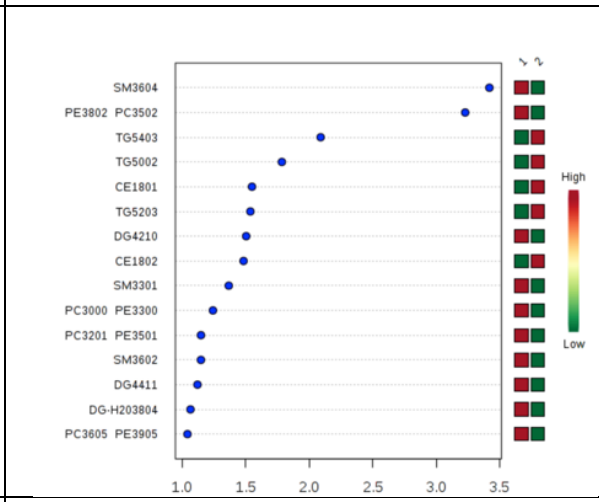
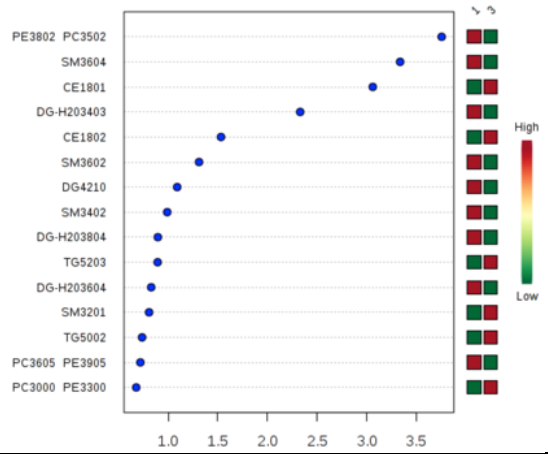
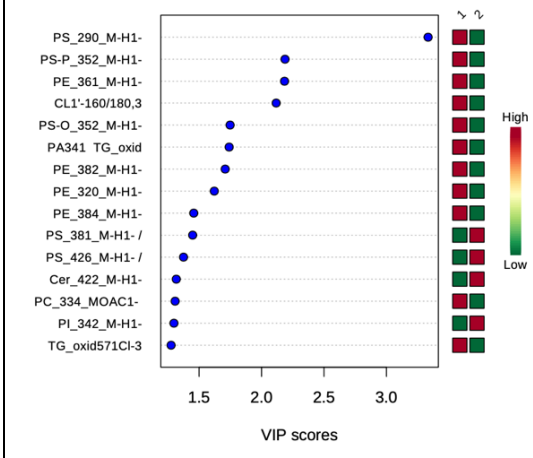
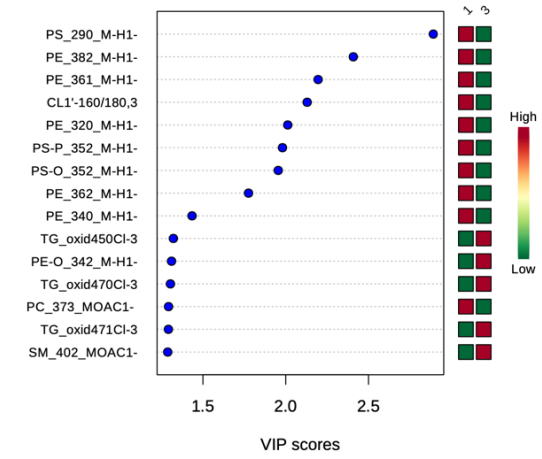
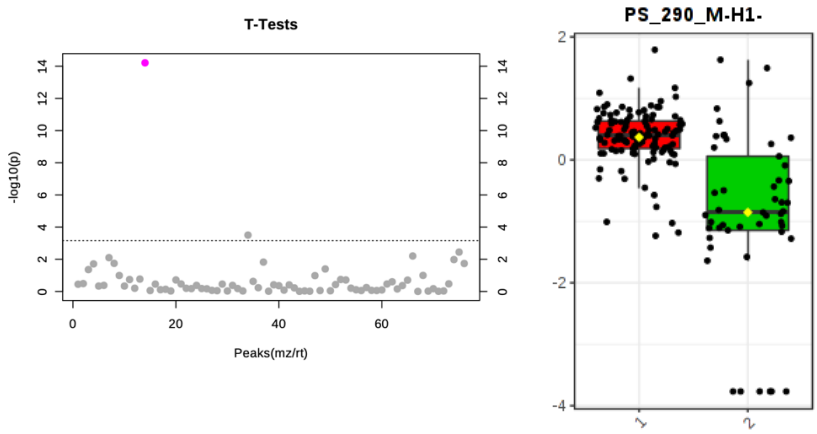
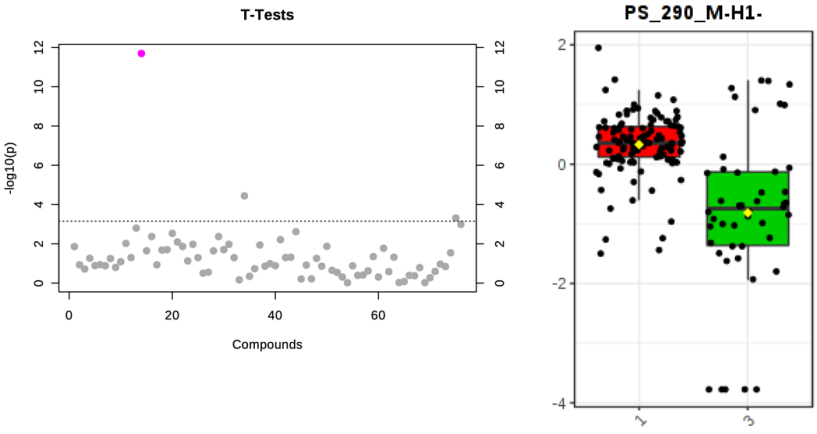
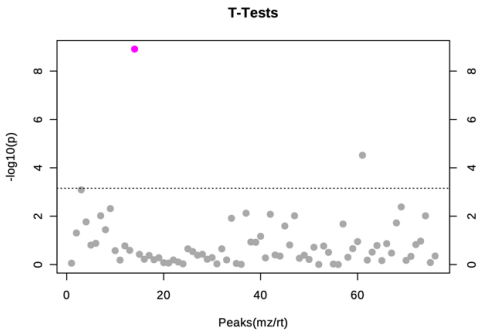
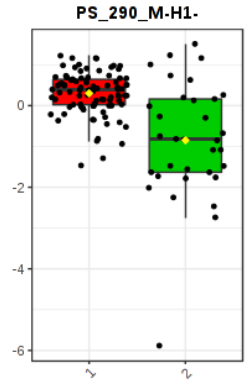
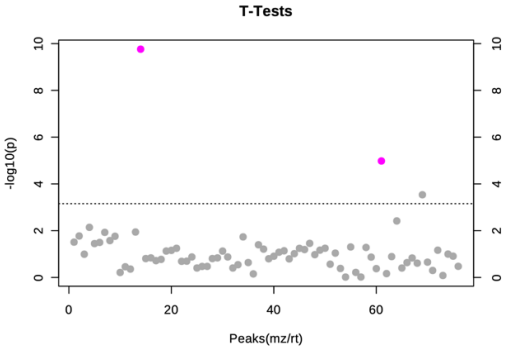
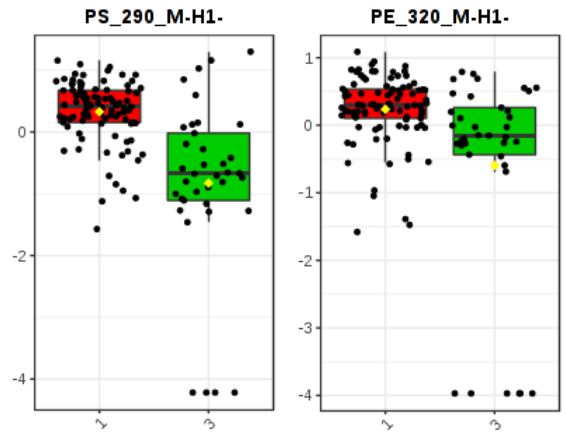
	SGA vs Control	**OGDM vs Control**
	1=Control, 2=SGA, 3=OGDM	
Positive mode		
Negative mode		

Figure 6.10 Comparisons of lipid abundance from negative mode between infant groups based on their *in utero* exposures and feeding history

Each T-test graph represents important determining features picked up by t-tests with adjusted significant threshold of 0.00017 (0.05/299 captured lipids), respectively. *p values* are -log10 transformed thus the more significant values (the lower *p values*) are plotted higher on the graph. Purple dots represent lipid species with significant *p values*.

FDR=false discovery rate

<table>
<tr><td colspan="2">1=Control, 2=SGA, 3=OGDM</td></tr>
<tr><td colspan="2">Among breastfed infants <u>only</u></td></tr>
<tr><td rowspan="2">SGA vs Control</td><td> 
</td></tr>
<tr><td>T stat = 8.6143, p = 6.176*10⁻¹⁵, FDR = 4.6938*10⁻¹³</td></tr>
<tr><td rowspan="2">OGDM vs Control</td><td> 
</td></tr>
<tr><td>T stat = 7.6339, p = 2.031*10⁻¹², FDR = 1.5436*10⁻¹⁰</td></tr>
</table>

<table>
<tr><td colspan="3">Among formula-fed infants <u>only</u></td></tr>
<tr><td rowspan="2">SGA vs Control</td><td> 
</td><td> 
</td></tr>
<tr><td colspan="2">T stat = 6.5723, p = 1.2334*10⁻⁹ , FDR = 9.3739*10⁻⁸</td></tr>
<tr><td rowspan="2">OGDM vs Control</td><td> 
</td><td> 
</td></tr>
<tr><td colspan="2">PS (29:0)
T stat = 6.9425, p = 1.7288*10⁻¹⁰ , FDR = 1.3139*10⁻⁸

PE (32:0)
T stat = 4.5896, p = 1.046*10⁻⁵ , FDR = 0.00039747</td></tr>
</table>

6.3.4Associations between 3-month lipids and infant growth among SGA and OGDM

In the previous publication from CBGS[202] involving control infants and capturing lipids from positive mode only (because positive mode is considered more robust in lipid profiling experimentation), several lipids have been found to associate with contemporaneous weight SDS as well as subsequent weight gain (Table 6.2). The correlation was re-analysed among SGA and OGDM infants.

Table 6.2 Associations between lipid abundance at 3 months and infancy weight
Retrieved under Creative Commons licence from Prentice P *et al*. *Lipidomic analyses, breast- and formula-feeding, and growth in infants*. J Pediatr 2015[202]
*Lower values in exclusively breast-fed
†Higher values in exclusively breast-fed

	3-mo weight SDS		12-mo weight SDS		3- to 12-mo weight gain SDS	
Lipid	Spearman	*P* value	Spearman	*P* value	Spearman	*P* value
PC-O 34:1*	0.2	.003	0.2	.009	−0.03	.7
PC 34:1*	0.2	<.0005	0.2	.003	0.01	.9
PC 38:4†	−0.2	<.0005	−0.2	.002	−0.04	.6
SM 34:2†	0.06	.4	−0.07	.3	−0.2	.005
PC-O 36:4†	−0.1	.1	−0.2	.02	−0.2	.004

Apart from lipid species enlisted in Table 6.5, the correlation analysis was also run on the lipids that distinguished SGA/OGDM versus controls at 3 months and persisted until 12 months (Table 6.1), including DG-H_2O (38:4), SM (36:4), and CE (18:2).

Among SGA, there was no significant association observed between all those lipids and any contemporaneous/subsequent growth parameters. On the contrary, several triglycerides and other diglyceride-waters were associated with 3-12 month growth gains among this infant group (Table 6.3), although with only moderate correlations.

Similar to SGA, there was almost no similarities between OGDM and controls with regard to lipid species associating with later growth (Table 6.4). However, more significant correlations between lipids and growth were displayed among OGDM, compared to SGA, especially from the sphingomyelin class.

Table 6.3 Correlations between 3-month capillary lipid abundance and subsequent growth gains among SGA

Significant correlations are in bold
SP=sphingolipids, GP=glycerophospholipids, GL=glycerolipids

Lipid species	Lipid class	3-12 months growth gains (total N=113)					
		Weight gain SDS		Height gain SDS		Skinfolds gain SDS	
		Spearman R	*p value*	Spearman R	*p value*	Spearman R	*p value*
SM (36:1)	SP	-0.06	*0.526*	-0.11	*0.26*	-0.2	***0.045***
PC (38:5) & PE (41:5)	GP	-0.1	*0.28*	-0.23	***0.016***	-0.09	*0.395*
LysoPC (20:4)	GP	-0.17	*0.072*	-0.2	***0.039***	-0.06	*0.561*
DG-H2O (30:1)	GL	0.25	***0.008***	0.31	***0.001***	0.12	*0.216*
DG-H2O (34:1)	GL	0.13	***0.164***	0.18	*0.054*	0.24	***0.013***
DG-H2O (36:2)	GL	0.19	***0.042***	0.17	*0.069*	0.24	***0.013***
TG (50:2)	GL	0.23	***0.016***	0.14	*0.135*	0.19	*0.054*
TG (52:2)	GL	0.18	*0.06*	0.19	***0.049***	0.22	***0.028***
TG (54:3)	GL	0.26	***0.005***	0.21	***0.025***	0.2	***0.041***

Table 6.4 Correlations between 3-month capillary lipid abundance and subsequent growth gains among OGDM

Significant correlations are in bold
[a]denotes the same growth-associating lipid found among controls (Table 6.8)
[a]denotes the same growth-associating lipid found among SGA (Table 6.9)
SP=sphingolipids, GP=glycerophospholipids, GL=glycerolipids, ST=sterol lipids

Lipid species	Lipid class	3-12 months growth gains (total N=113)					
		Weight gain SDS		Height gain SDS		Skinfolds gain SDS	
		Spearman R	*p value*	Spearman R	*p value*	Spearman R	*p value*
SM (34:1)	SP	-0.15	*0.109*	-0.21	***0.028***	-0.16	*0.138*
SM (34:2)[a]	SP	-0.07	*0.44*	-0.2	***0.034***	-0.19	*0.094*
SM (36:1)[b]	SP	-0.21	***0.025***	-0.22	***0.02***	-0.24	***0.029***
SM (36:2)	SP	-0.05	*0.63*	-0.2	***0.038***	-0.06	*0.583*
SM (40:2)	SP	-0.1	*0.275*	-0.19	***0.041***	-0.09	*0.438*
SM (42:1)	SP	-0.14	*0.15*	-0.22	***0.021***	0.02	*0.833*
SM (42:2)	SP	-0.13	*0.18*	-0.31	***0.001***	0.02	*0.882*
SM (42:3)	SP	-0.22	***0.017***	-0.34	***0.0002***	-0.21	*0.056*
PC (34:3) & PE (37:3)	GP	-0.18	*0.061*	-0.1	*0.299*	-0.26	***0.019***
Cholesterol-loss OH	ST	-0.21	***0.024***	0.004	*0.97*	-0.3	***0.006***
DG-H2O (30:1)[b]	GL	0.12	*0.201*	0.1	*0.319*	0.29	***0.008***
DG-H2O (34:1)	GL	0.18	*0.051*	0.11	*0.23*	0.318	***0.003***
DG-H2O (36:1)[b]	GL	0.08	*0.405*	0.01	*0.902*	0.22	***0.041***
DG-H2O (36:2)[b]	GL	0.19	***0.049***	0.13	*0.182*	0.31	***0.005***
DG-H2O (38:4)	GL	-0.102	***0.283***	-0.21	***0.024***	-0.14	*0.22*
TG (48:4)	GL	-0.09	*0.365*	-0.22	***0.018***	0.02	*0.888*
TG (50:1)	GL	0.2	***0.03***	0.17	*0.077*	0.33	***0.002***
TG (50:2)[b]	GL	0.16	*0.083*	0.1	*0.305*	0.32	***0.003***
TG (52:2)[b]	GL	0.24	***0.012***	0.19	***0.04***	0.36	***0.001***
TG (52:3)	GL	0.17	*0.081*	0.23	***0.015***	0.21	*0.06*
TG (54:3)[b]	GL	0.26	***0.006***	0.15	*0.108*	0.35	***0.001***

6.3.5 Lipid ratios representing desaturase activities and infant growth

Among transgenic mice models (in collaborative studies by TNO Netherlands), several lipid ratios representing activities of key enzymes in lipid metabolism (stearoyl-CoA desaturase-1/SCD1 and fatty acid desaturase/FADS 1 and 2), were discovered to associate with early weight gain and other obesity parameters. These findings were then translated to our human cohort by finding associations between

these lipid biomarkers and weight and adiposity gains, first among control infants (total N=201) up to 24 months. Lipid ratios at age 3 months indicating SCD1, FADS1, and FADS2 activities that were examined in this study and each corresponding molecular mass are listed in Table 6.5.

Table 6.5 Lipid ratios representing similar enzyme activities used in the CBGS

Enzyme	Lipid ratio	Molecular mass	
SCD1	CE(16:1) / CE(16:0)	640.6027	642.6183
	PC(32:1) / PC(32:0)	732.5543	734.57
FADS1	PC(38:4) / PC(38:3)	810.6013	812.6169
	TG(54:4) / TG(54:3)	900.8019	902.8176
FADS2	PC(36:3) / PC(36:2)	784.5856	786.6013
	TG(50:3) / TG(50:2)	846.755	848.7706

SCD= stearoyl Co-A desaturase, FADS= fatty acid desaturase

Linear regression models were employed to demonstrate the associations between lipid ratios representing enzyme activities with growth gains during infancy, with early (3-12 months) and late infancy (12-24 months) being analysed separately. In these analyses, first models were unadjusted while the second models were adjusted for significant confounding factors[193,202], including maternal parity, maternal pre-pregnancy BMI, and 0-3 months infant feeding history.

In fully adjusted models, PC ratio reflecting SCD1 activity was positively associated with weight gain between 3-12 months of age (*p=0.012*, Table 6.6 a). Meanwhile, CE ratio reflecting SCD1 activity showed negative associations with 3-12 months weight and BMI gains (*p=0.027* and *0.033*, respectively, Table 6.6 a and c). The association between SCD1 activity represented by CEs and growth gains became positive at 12-24 months (fully adjusted, *p=0.02* with weight and *p=0.009* with height, Table 6.6 a and b). Similarly, SCD1 PC ratio was also positively associated with 12-24 month-skinfolds gain reflecting adiposity (Table 6.6 d). FADS1 PC ratio

was inversely-, while FADS2 PC ratio was positively associated with subsequent 3-12 months height gain. The latter lipid ratio was inversely associated with adiposity gain from 3-12 months.

Table 6.6 Regression models associating lipid ratios at 3 months and subsequent growth among control infants

B: unstandardised beta, SE B: the standard error for the unstandardised beta
All lipid ratios are log-transformed
Weight, height, BMI SDS values are based on UK 1990 growth reference, adjusted for sex, gestational age (at 3 months only), and postnatal age at measurement. Skinfolds SDS values are internally-derived, adjusted for sex, gestational age, and postnatal age at measurement.
Age at visit is limited to 70-112 days for 3 months, 337-393 days for 12 months, and 702-758 days for 24 months visits
[a]Model 2 is adjusted for maternal parity (primiparous vs non), maternal pre-pregnancy BMI, and infant feeding history at 3 months

a. Lipid ratios and infant weight gain

Predictor: Lipid ratios	**Outcome: Weight gain SDS**											
	3-12 months						**12-24 months**					
	Model 1			Model 2[a]			Model 1			Model 2[a]		
	B	SE B	*p*	B	SE B	*p*	B	SE B	*p*	B	SE B	*p*
SCD1												
CE(16:1)/CE(16:0)	-0.28	0.13	***0.034***	-0.31	0.14	***0.027***	0.2	0.07	***0.005***	0.17	0.07	***0.02***
PC(32:1)/PC(32:0)	0.7	0.33	***0.035***	0.86	0.34	***0.012***	-0.01	0.17	*0.942*	-0.1	0.18	*0.577*
FADS1												
PC(38:4)/PC(38:3)	-0.99	0.57	*0.083*	-1.02	0.56	*0.069*	0.01	0.3	*0.988*	0.06	0.3	*0.847*
TG(54:4)/TG(54:3)	-0.57	0.47	*0.228*	-0.82	0.48	*0.085*	0.25	0.25	*0.304*	0.19	0.25	*0.45*
FADS2												
PC(36:3)/PC(36:2)	0.66	1.45	*0.651*	1.21	1.45	*0.405*	-0.52	0.75	*0.492*	-0.38	0.76	*0.624*
TG(50:3)/TG(50:2)	0.01	0.28	*0.973*	-0.01	0.28	*0.959*	0.15	0.15	*0.3*	0.1	0.15	*0.514*

b. Lipid ratios and infant height gain

Predictor: Lipid ratios	**Outcome: Height gain SDS**											
	3-12 months						**12-24 months**					
	Model 1			Model 2[a]			Model 1			Model 2[a]		
	B	SE B	*p*	B	SE B	*p*	B	SE B	*p*	B	SE B	*p*
SCD1												
CE(16:1)/CE(16:0)	-0.08	0.13	*0.552*	-0.09	0.13	*0.502*	0.23	0.08	***0.003***	0.21	0.08	***0.009***
PC(32:1)/PC(32:0)	0.35	0.32	*0.278*	0.49	0.33	*0.135*	-0.1	0.2	*0.603*	-0.15	0.21	*0.472*
FADS1												
PC(38:4)/PC(38:3)	-1.45	0.54	***0.007***	-1.53	0.53	***0.005***	0.04	0.34	*0.901*	0.07	0.34	*0.826*
TG(54:4)/TG(54:3)	0.02	0.45	*0.966*	-0.19	0.45	*0.67*	0.53	0.27	***0.05***	0.46	0.28	*0.101*
FADS2												
PC(36:3)/PC(36:2)	2.06	1.37	*0.135*	2.74	1.38	***0.048***	-0.12	0.84	*0.888*	0.15	0.86	*0.861*
TG(50:3)/TG(50:2)	0.32	0.27	*0.231*	0.3	0.27	*0.271*	-0.02	0.16	*0.911*	-0.08	0.17	*0.641*

c. Lipid ratios and infant BMI gain

Predictor: Lipid ratios	Outcome: BMI gain SDS											
	3-12 months						12-24 months					
	Model 1			Model 2[a]			Model 1			Model 2		
	B	SE B	*p*	B	SE B	*p*	B	SE B	*p*	B	SE B	*p*
SCD1												
CE(16:1) /CE(16:0)	-0.3	0.15	***0.042***	-0.33	0.15	***0.033***	0.07	0.1	*0.5*	0.05	0.11	*0.652*
PC(32:1)/PC(32:0)	0.57	0.37	*0.122*	0.66	0.38	*0.085*	0.05	0.25	*0.832*	-0.04	0.26	*0.884*
FADS1												
PC(38:4)/PC(38:3)	0.05	0.63	*0.942*	0.03	0.64	*0.966*	-0.04	0.42	*0.932*	0.01	0.43	*0.986*
TG(54:4)/TG(54:3)	-0.54	0.52	*0.305*	-0.72	0.53	*0.179*	-0.14	0.35	*0.696*	-0.15	0.36	*0.671*
FADS2												
PC(36:3)/PC(36:2)	-1.64	1.6	*0.305*	-1.33	1.63	*0.418*	-0.63	1.07	*0.558*	-0.68	1.09	*0.533*
TG(50:3)/TG(50:2)	-0.21	0.31	*0.491*	-0.25	0.32	*0.426*	0.22	0.21	*0.296*	0.19	0.21	*0.363*

d. Lipid ratios and infant skinfolds gain

Predictor: Lipid ratios	Outcome: Skinfolds gain SDS											
	3-12 months						12-24 months					
	Model 1			Model 2[a]			Model 1			Model 2[a]		
	B	SE B	*p*	B	SE B	*p*	B	SE B	*p*	B	SE B	*p*
SCD1												
CE(16:1) /CE(16:0)	0.12	0.13	*0.363*	-0.14	0.14	*0.305*	0.05	0.1	*0.621*	-0.07	0.11	*0.534*
PC(32:1)/PC(32:0)	-0.03	0.33	*0.94*	-0.01	0.34	*0.985*	0.73	0.26	***0.005***	0.53	0.26	***0.04***
FADS1												
PC(38:4)/PC(38:3)	-0.42	0.56	*0.463*	-0.41	0.56	*0.471*	-0.45	0.45	*0.322*	-0.33	0.43	*0.447*
TG(54:4)/TG(54:3)	-0.38	0.46	*0.407*	-0.59	0.47	*0.214*	-0.16	0.37	*0.676*	-0.3	0.37	*0.41*
FADS2												
PC(36:3)/PC(36:2)	-3.49	1.39	***0.013***	-3.32	1.42	***0.021***	1.65	1.11	*0.139*	1.99	1.08	*0.067*
TG(50:3)/TG(50:2)	-0.18	0.28	*0.517*	-0.21	0.28	*0.456*	0.09	0.23	*0.705*	-0.04	0.22	*0.864*

Table 6.7 Summary of results of the association analyses between infant growth/adiposity outcomes and desaturase activities among control infants

(+) in green blocked boxes reflect positive associations while (-) in red blocked boxes reflect negative associations

	SCD1		FADS1	FADS2
3-12 months				
Weight gain	+ (PC)	- (CE)		
Height gain			- (PC)	+ (PC)
Adiposity gain	- (CE)			- (PC)
12-24 months				
Weight gain	+ (CE)			
Height gain	+ (CE)		+ (TG)	
Adiposity gain	+ (PC)			

The same lipid ratios were then tested among SGA and OGDM in relation to infancy growth parameters. As there were not many significant associations observed in the models, Table 6.8 only displays lipid ratios with significant associations with growth gains between 3-12 or 12-24 months (fully adjusted). As seen in Table 6.8, the results are more sporadic than the controls regression models.

Table 6.8 Regression models associating lipid ratios at 3 months and subsequent growth among SGA and OGDM

This table only shows lipid ratios if they have at least one significant association with any growth parameters between 3-12 or 12-24 months, adjusted for same covariates as the control models (Table 6.6). Significant associations are typed in bold.

Predictor: Lipid ratios	**Outcome: Growth parameters in each group, analysed separately**							
	SGA				**OGDM**			
	Weight gain				**Weight gain**			
	3-12 months		**12-24 months**		**3-12 months**		**12-24 months**	
	B±SE	*p*	B±SE	*p*	B±SE	*p*	B±SE	*p*
SCD1 CE(16:1)/CE(16:0)	1.53±0.55	***0.015***	-0.18±0.57	*0.75*	-0.73±0.31	***0.024***	0.31±0.16	*0.065*
SCD1 PC(32:1)/PC(32:0)	1.77±0.6	***0.01***	-0.11±0.62	*0.857*	-0.39±0.27	*0.156*	-0.13±0.14	*0.334*
	Height gain				**Height gain**			
	3-12 months		**12-24 months**		**3-12 months**		**12-24 months**	
	B±SE	*p*	B±SE	*p*	B±SE	*p*	B±SE	*p*
FADS2 TG(50:3)/TG(50:2)	-0.36±1.92	*0.853*	-3.48±1.51	**0.036**	-0.29±0.41	*0.483*	-0.07±0.29	*0.801*
	SF gain				**SF gain**			
	3-12 months		**12-24 months**		**3-12 months**		**12-24 months**	
	B±SE	*p*	B±SE	*p*	B±SE	*p*	B±SE	*p*
FADS1 TG(54:4)/TG(54:3)	0.86±0.56	*0.151*	-0.05±1.45	*0.972*	1.1±0.41	***0.011***	0.7±0.69	*0.314*
FADS2 TG(50:3)/TG(50:2)	0.88±1.0	*0.387*	-1.52±2.38	*0.533*	0.35±0.24	*0.16*	0.84±0.37	***0.028***

6.4 Discussion

6.4.1 Multivariate lipid profiling analyses across infant groups and their association with growth

From multivariate analyses, distinct separation could be observed when comparing lipid profiles of SGA or OGDM infants from those of controls. Since this clear separation remained even after controlling for feeding, i.e. when the analysis was performed on breastfed infants only, it can be speculated that the results were more caused by *in utero* rather than early infancy nutritional exposures. Unfortunately, the existing knowledge of how lipidomic profiles vary among infants with different intrauterine exposures is still sparse.

From the positive mode, there were 3 main lipids driving the differences between SGA or OGDM *independently* against controls that identified at 3 months and persisted until 12 months: DG-H2O(38:4) and SM(36:4) which were lower among SGA and OGDM, as opposed to CE(18:2), which was higher among those 2 groups compared to controls. DG-H20 is classified as glycerolipids (GL), SM is from sphingolipids (SP) group, and CE is sterol lipids (SL)[266]. Meanwhile, the negative mode identified 6 lipid species that were lower among SGA and OGDM compared to controls, both at 3 and 12 months. All these lipids (Table 6.1) are categorised as glycerophospholipids (GP) with 2 glycerophosphoethanolamines (PE), 1 lyso glycerophosphates (lysoPA), and 4 cardiolipins (CL)[266].

Apart from that, it is intriguing to notice different lipid species correlating with growth between control and the other 2 high-risk infant groups, although there

were still some similarities across all infant groups. In all 3 groups, lipids from SM classes correlated negatively with growth, but among OGDM, the correlations were mainly observed with height gain. Unlike lipid profiles from controls, more lipids from DG and TG classes were generally correlated with growth among both SGA and OGDM infants. With regard to OGDM, this phenomenon could relate to pre- and antenatal maternal TG profiles that significantly define maternal risk to develop obesity and GDM[269].

A recently published lipidomic analysis from the SCreening fOr Pregnancy Endpoint study (SCOPE) reported associations between a set of lipids measured antenatally with SGA birth. In this study, lipids were captured from plasma at 20 weeks' gestation from nulliparous women. All participants were followed until delivery and women who delivered SGA infants were matched to controls (each N=40). Most (22 out of 33) lipids detected in this study were from GP class and all of them were in higher levels among women who delivered SGA, suggesting its role in SGA pathophysiology. The remaining lipids were from GL, SP, SL, and fatty acyl (FA). Some lipids identified in the SCOPE study are closely related to this study, such as PE(36:4), SM(34:1), and CE(17:0)[270].

Differences in lipidomic profiles between OGDM and controls could be influenced by the hyperlipidaemic *in utero* environment. There is evidence that GDM mothers experienced a more intense level of physiological dyslipidaemia compared to normal pregnancy and it is caused by disturbance in lipid metabolism[271]. Moreover, a 2015 study in Brazil compared GDM and normal pregnancies. Of the distinguishing patterns between the 2 groups captured by lipidomics, most of them were driven by the GP and sterol lipid classes[272]. The study reported that GDM

group had less abundant GP class (e.g. $C_{42}H_{72}NO_8P$, mass 772.4843 m/z) but more of sterol lipids (e.g. $C_{27}H_{48}O_8S$, mass 555.2986 m/z)[272], similar to the lipidomic feature of both OGDM and SGA in this study. Moreover, GP was apparently the most common lipid class identified in the last 20 years of metabolomics studies of women with GDM and their offspring[273].

GPs are essential components of a cell's lipid bilayer[266], they possess anti-inflammatory and immunomodulatory properties, and may be useful in alleviating dyslpidemia[272]. Similarly, sterol lipids are also key elements of cell membrane. These lipids are constituents of bile acids and steroid hormones and thus play an important role in cell growth and proliferation[272].

Shared lipidomic features between SGA and OGDM shown in this study might indicate similar signatures in lipid metabolism pathways and this could be affected by peroxisome proliferator-activated receptor (PPAR) activities. PPARs are ligand-activated transcription factors of nuclear hormone receptor superfamily that play an important role in energy homeostasis and metabolism. There are 3 subtypes of PPARs: PPARα, which is involved in energy regulation and whose activation decreases TG level; PPARγ, whose activation augments glucose metabolism and induces insulin sensitisation; and PPARβ/δ, which enhances fatty acid metabolism[274,275]. Importantly, many studies have reported PPARs as a candidate gatekeeper pathway of fetal metabolic and developmental programming[275-277]. PPARs are involved in the pathophysiology of *in utero* nutritional insults, including caloric restriction and maternal diabetes, causing later detrimental effects in the offspring, including hypertension and metabolic syndrome[275,276].

In this study, the similar lipid abundance patterns observed between SGA and OGDM, in independent comparisons to control infants, seemed to be most influenced by PPARα since lipids from GP class are ligands for this transcription factor[278].

As PPAR isoforms are highly expressed in reproductive tissues and developing fetus, they have been implicated to mediate fetal adaptive responses to inappropriate maternal diet during pregnancy through their interactions with fatty acids[276]. PPAR-γ and PPAR-β/δ play pivotal role during placentation and development of skeletal muscle, adipose cells, and other important metabolic tissues. Although being expressed at low level in the fetal liver, PPAR-α is important because changes in the methylation of DNA in its promoter region occur during fetal development. Therefore, two stand-alone PPAR-dependent mechanisms might be involved in the fetal adaptations to inadequate maternal diet: 1) via regulation of cell growth and differentiation by PPAR-γ and PPAR-β/δ, and 2) via alteration to long-term lipid metabolism due to epigenetic changes in PPAR-α; both to optimise postnatal survival[276,279].

Of note, a large variety of lipid classes contributed to the total lipidomic signal in this study, and the experiments were conducted using DBS samples which presumably would produce lower signals compared to serum or plasma. However, DBS samples have been shown to produce reliable results relative to those of other methods requiring larger sample volumes in lipid profiling analysis[227].

The differences found between subset (CBGS2 only) and whole group (CBGS1 and CBGS2) analyses were likely due to 2 major reasons. First, although the analyses were statistically adjusted for batch differences, it could be speculated that there

might be a large disparity in infant nutritional exposures during different decades of studies conduction (CBGS1 from 2001-2009 and CBGS2 from 2011-present). This affected both mixed-/formula-fed and breastfed infants. Among mixed-/formula-feeders, there have been significant changes in the nutritional content of formula in the last decades in order to mimic the nutritional compound of breastmilk[280]. Among breastfed infants, this could be influenced by the recent modification of diet, exercise, and other lifestyle-related factors among adults[281], including pregnant and lactating women. The second speculation is related to technological issues, i.e. different temperatures used to store the samples and varying durations between sampling and lipidomic experiments, which could affect lipid abundance captured by high-resolution mass spectrometry (HRMS). DBS samples from CBGS1 were stored at -20^{0}C while CBGS2 samples were stored at -80^{0}C. Some lipids are also reported to be susceptible to oxidation and hydrolysis but how storage time affects the recovery of lipids from DBS samples has never been studied thus far[227]. Although these factors could be a limitation of the current study, batch differences were controlled throughout to minimise such effects.

The outstanding appearance of PS (29:0) as single lipid driving differences among infant groups deserves further investigation. From another ongoing study of Dr. Koulman's group on non-alcoholic fatty liver disease (NAFLD) in adults, this lipid also distinctly emerged as the most important driver of adiposity, although this signal could reflect another lipid with similar mass: PC (26:0)[282]. More controlled study is needed to validate this result.

6.4.2 Lipid ratios representing desaturase activities and infant growth

Differences in lipid ratios reflecting desaturase activity have previously been associated with obesity and the metabolic syndrome[283]. Several studies have also associated polyunsaturated fatty acids (PUFA) intake and its related enzyme activities (SCD1, FADS1, and FADS2) with childhood metabolic parameters[284]. For example, Wolters *et al* reported that a FADS1 polymorphism influenced blood pressure and body mass index (BMI) in a multinational European study involving children aged 2-10 years[285]. Another study from the same group reported positive associations between fatty acid ratios representing SCD1 and FADS2 activities at baseline with BMI and TG levels both at baseline and 2 years later[286]. In contrast, FADS1 activity, estimated by measuring fatty acid ratios at baseline, was inversely associated with both measures at the two time points[286]. Similar to this particular study, in our current study a positive association was also observed between SCD1 indices at 3 months with later adiposity gains between 12-24 months, as well as with weight and height gains (Table 6.7). There was also a contemporaneous positive association between FADS2 activity and infant BMI at 3 months ($B \pm SE = 3.74 \pm 1.61$, $p = 0.021$), and a positive association with height gain between 3-12 months (Table 6.6 b). Meanwhile, a negative association between FADS1 activity (with PC ratio) was not observed with adiposity but with height gain between 3-12 months. As shown in the summary Table 6.10, from all lipid ratios employed as proxies of enzyme activities, SCD1 activity appeared to be the most significant marker of growth and adiposity development during early life.

Among the high-risk infant groups, the associations between those lipid ratios and later gains in growth and adiposity were more sporadic. However, SCD1 also seemed as the most promising key enzyme compared to FADS1 and 2, especially with regard to weight gain.

SCD1 plays a pivotal role in fat storage, lipid homeostasis, and energy metabolism. This enzyme is involved in monounsaturated fatty acid (MUFA) biosynthesis via introduction of a double bond into saturated fatty acids that can come from lipogenesis or from the diet. Increased hepatic SCD1 activity, measured as fatty acid ratio, has been linked to obesity and its comorbidities in both animals and humans[283,287]. In a population study involving more than 1800 elderly participants, Vinkness reported positive associations between plasma SCD1 indices, CE 16:1/16:0 and 18:1/18:0, and adiposity parameters, including BMI and body fat mass measured by dual X-ray absorptiometry (DXA). In contrast, those markers showed inverse associations with polyunsaturated fatty acids (PUFA) and exercise[287].

It is interesting to speculate on the origin of the identified lipids and the underlying mechanism for their predictive value for obesity later in life. In infants, the demand for PUFA is different from adults. Moreover, the genetic makeup may contribute to differences in plasma lipid profiles, since single nucleotide polymorphisms (SNPs) in FADS genes can modulate desaturase activity[288]. Furthermore, pre- (maternal) and postnatal feeding could influence circulating desaturase activity markers.

Gene expression of the desaturase enzymes *FADS1*, *FADS2* and *SCD1* is regulated by the lipogenic transcription factors SREBP1c and PPARs, predominantly in the

liver[289], but also in adipose tissue[279]. Interestingly, studies focusing on mechanisms of metabolic programming have implicated PPARs as candidate gatekeepers of pathways of developmental programming[275] and adipose tissue expansion during obesity development[290]. Notably, the PPARα gene has shown sensitivity for epigenetic changes[277], which may account for long-term changes in PPARα and its target genes.

From the CBGS data, of all 3 enzymes activities measured as lipid ratios, only SCD1 differed between 0-3 months infant feeding history, with breastfed infants had higher levels of CE and PC ratios (both *p values <0.0005*). The differences in direction of the associations between lipid ratios and growth parameters in the early (3-12 months) versus late infancy period (12-24 months) demonstrate how dynamic lipid metabolism is and thus affecting its potential use as candidate biomarkers. Of note, studies in adults have shown that the activity of those enzymes is strongly driven by dietary FA composition[291]. Therefore, the effects of FA enzymatic activity on growth and metabolism might also change with the large variations in diet over time in infants.

6.5 Conclusions

Pattern recognition of lipid profiling analysis could serve effectively to capture useful candidate lipid biomarkers of early growth gains among different groups of infants, especially when comparing those at relatively higher risks versus controls. Whether those significant associations more relate to the causes or the consequences of being SGA/OGDM deserves further investigations.

Several lipid species have been correlated with subsequent growth during infancy with different kinds of lipid although with similar directions of correlations among infant groups. More studies, especially mechanistic ones, are needed to confirm these results and understand the involved metabolic pathways.

Lipid ratios potentially reflecting SCD1, FADS1, and FADS2 activities were associated with subsequent body size. While these lipids are promising as candidate biomarkers, further longitudinal cohorts are required to confirm these findings.

Chapter 7 Breast milk study among control population

7.1 Introduction

As reflected in many parts of this thesis, early postnatal nutrition strongly affects or mediates the connection between early life and risk of long-term health outcomes[154]. With regard to this, breastfeeding has been associated with slower subsequent growth and adiposity gains in infancy and childhood compared to formula feeding[204,292], thus help to achieve desirable infancy growth and reducing risk for later obesity[121]. These potential benefits may be attributed to nutrient contents in human breast milk (BM). From previous studies, higher fat, lower protein, and higher short-chain fatty acids (SCFA) contents in BM have been considered beneficial for optimal subsequent infant weight and adiposity gains, with an aim to prevent childhood obesity[204,261,293].

The growth of breastfed infants is widely considered to be optimal, however there is much heterogeneity between individual infants[119]. Moreover, evidence for associations between macronutrient concentrations of BM and infant growth are inconsistent, possibly because BM macronutrient concentrations may not reflect macronutrient intakes consumed by infants. The measurement of BM nutritional intake would, therefore, potentially provide a better mechanistic link between breastfeeding and infancy growth and adiposity.

The aim of this study was to examine the associations between both BM macronutrient concentrations and BM intakes with growth and adiposity among control infants. In this study, BM nutritional intake was measured directly by examining the concentration of each macronutrient in BM and estimating the volume of BM consumed by infants. The product of macronutrient concentrations and BM intake volume resulted in the estimation of infant's intake of BM specific nutrients.

7.2 Methods

7.2.1 Study participants

In total 94 mother-infant pairs of the CBGS-BF study (Section 2.1.3) were included in this study. All infants were born vaginally at term and exclusively breastfed until at least 6 weeks of age. They also met other stringent inclusion criteria, including being singleton pregnancies, no exposure to antibiotics or pharmaceutical steroids for at least 30 days before delivery, no significant maternal illness or pregnancy comorbidities, no regular use of probiotics during pregnancy, and normal maternal pre-pregnancy BMI. As described in Section 2.2, anthropometry measurements and biological sampling of the subjects were performed by the trained CBGS paediatric research nurses.

7.2.2 Samples collections and laboratory assays

Details of BM collection and assays are reported in Section 2.3.2 and 2.4.3, respectively. In brief, breastfed mothers were asked to hand express their hindmilk samples after feeding their infants at 2 weeks, 6 weeks, 3 months, and 6 months of age. Expression was done from the same breast last used to feed their infants. Samples were kept frozen at -20^{0}C until being processed at a single time point.

The samples were thoroughly mixed before macronutrient analyses, which consisted of the measurement of lactose (reflecting carbohydrate), triglyceride (surrogate for total fat)[204,205], and protein concentrations. In addition, butyrate was also quantified as this SCFA was previously identified as having the strongest association with infant's later adiposity[293]. Lactose, triglyceride, and butyrate concentrations were measured from ^{1}H-nuclear magnetic resonance (NMR) spectra. The DUMAS method was employed to measure total nitrogen to calculate crude protein content[228].

As described in Section 2.4.5, BM intake volume was measured using the dose-to-the-mother deuterium-oxide (^2H_2O) turnover technique[231]. The mothers were given deuterium-enriched (tracer) water when the babies were approximately 4 weeks old. The tracer water would be incorporated into BM and passed to the infant during breastfeeding. Urine samples were collected from both mothers and babies for a period of 2 weeks. ^{2}H enrichment in the urine samples was measured by isotope ratio mass spectrometry [231].

7.2.3 Calculations and statistical analyses

Atwater conversions, which were developed by Atwater and his colleagues from large experimental studies, were used to calculate the metabolisable energy content of BM, taking energy contents of 4, 9, and 4 kcal/g for lactose, fat, and protein, respectively[229]. Weight, height, and BMI were computed into sex- and age-specific z-scores based on UK 1990 growth reference using LMS Growth[225]. BM macronutrient intake was calculated as the multiplication product between each macronutrient content (kcal/100mL) and BM intake volume (L/day).

Continuous descriptive data are displayed as mean $\pm$ standard deviation or median (interquartile range), while categorical data were presented as number (%). Multiple linear regression models were performed to test the associations between infancy growth (as the outcome) against exclusive breastfeeding duration, BM intake volume, BM macronutrient content, and BM macronutrient intake. Exclusive breastfeeding duration was categorised into 3 groups: 1) more than 6 weeks but less than 12 weeks (6-12 weeks), 2) at least for full 12 weeks or 3 months but less than 26 weeks or 6 months (12-26 weeks), and 3) full 26 weeks or more ($\geq$26 weeks). The use of 6 weeks as the cut-off value for the exclusive breastfeeding period was due to the significant drop of exclusive breastfeeding rate among mothers in the UK after this age[294].

7.3 Results

7.3.1 Baseline and infant growth characteristics

The baseline characteristics of mother-infant dyads involved in this study are shown in table 8.1. All mothers were in healthy BMI range (mean + SD: 22.27 + 2.55). More than 91% mothers were of Caucasian ethnicity and almost 40% of them had delivered their first baby. Out of a total of 94 infants (61% male), 80 continued receiving solely breastmilk until age 3 months and 28 infants maintained exclusive breastfeeding until age 6 months. Growth measurements were recorded from birth to 12 months (Table 7.1).

As illustrated in Figure 7.1, infants who had the shortest exclusive breastfeeding (EBF) period showed distinct growth patterns compared to the other two groups with longer duration of EBF. This group gained least weight in the first 3 months but then exhibited an upward trend for weight gain, (while the other groups showed the opposite trend), thus ending up heavier than longer exclusively breastfed infants by 12 months (Table 7.2, Figure 7.1). A similar trend, although not statistically significant, was also observed in height and skinfolds trajectories (with a turning point in skinfold thicknesses at 6 weeks of age, rather than 3 months; Figure 7.2 and 7.3).

Table 7.1 Baseline characteristics and cross-sectional growth data

Total N=94. Values are mean±SD or median(IQR) or n(%)
All birth measurements were taken 8 days after delivery, except weight which was taken from hospital records.
SDS values are based on UK 1990 Growth Reference, adjusted for sex, gestational age for birth until 3 months measurements, and postnatal age.
[a]Skinfolds were measured at 4 sites: triceps, subscapular, flank, and quadriceps; [b]Some infants were too upset during the ultrasounds procedure thus not all subjects had these results; [c]PEAPod was not carried out since the beginning of the study thus not all subjects had this measurement; [d]Catch-up and catch-down are defined if weight/height gain ≥0.67 or ≤-0.67 SDS, respectively. US: Ultrasounds; SC: subcutaneous

Characteristics	N	Values
Maternal		
Age at delivery, years	94	33.25±4.68
Pre-pregnancy BMI, kg/m^2	92	22.27±2.55
Height, cm	91	166.74±6.21
Primiparous rate	94	37(39.4%)
White/European ethnicity	94	86(91.5%)
Birth		
Male sex	94	57(60.6%)
Gestational age, weeks	94	40.26±1.06
Weight-SDS	94	0.12±0.75
Length-SDS	94	-0.2±0.74
BMI-SDS	94	0.08±0.87
Head circumference-SDS	94	-0.17±0.92
Sum skinfolds[a], mm	94	23.83±4.76
Waist circumference, cm	94	33.35(2.5)
6 weeks		
Weight-SDS	94	0.27±0.94
Height-SDS	94	0.12±0.88
US-visceral depth[b], cm	57	2.57(0.28)
US-SC-abdo depth[b], cm	84	0.41±0.11
% Body fat from PEA Pod[c]	29	20.15±5.14
3 months		
Rate of exclusive breastfeeding	94	80(85%)
Weight-SDS	93	0.09±1.03
Height-SDS	93	0.09±0.89
US-visceral depth[b], cm	82	2.45±0.25
US-SC-abdo depth[b], cm	92	0.49±0.12
% Body fat from PEA Pod	38	23.46±5.14
12 months		
Weight-SDS	88	-0.3±1.0
Height-SDS	88	-0.02±0.98
US-visceral depth[b], cm	70	2.58(0.34)
US-SC-abdo depth[b], cm	78	0.46±1.13
Growth		
0-3 months weight gain-SDS	93	-0.03±0.85
0-3 months height gain-SDS	93	0.29±0.65
0-3 months catch-up[d] weight gain	93	19(20.2%)
0-3 months catch-up[d] height gain	93	23(24.5%)
3-12 months weight gain-SDS	87	-0.41±0.82
3-12 months height gain-SDS	87	-0.13±0.77
3-12 months catch-up[d] weight gain	87	8(8.5%)
3-12 months catch-up[d] height gain	87	12(12.8%)

Figure 7.1 Infant weight trajectories based on EBF duration
Values are mean±SEM. All SDS values are based on UK 1990 growth reference.

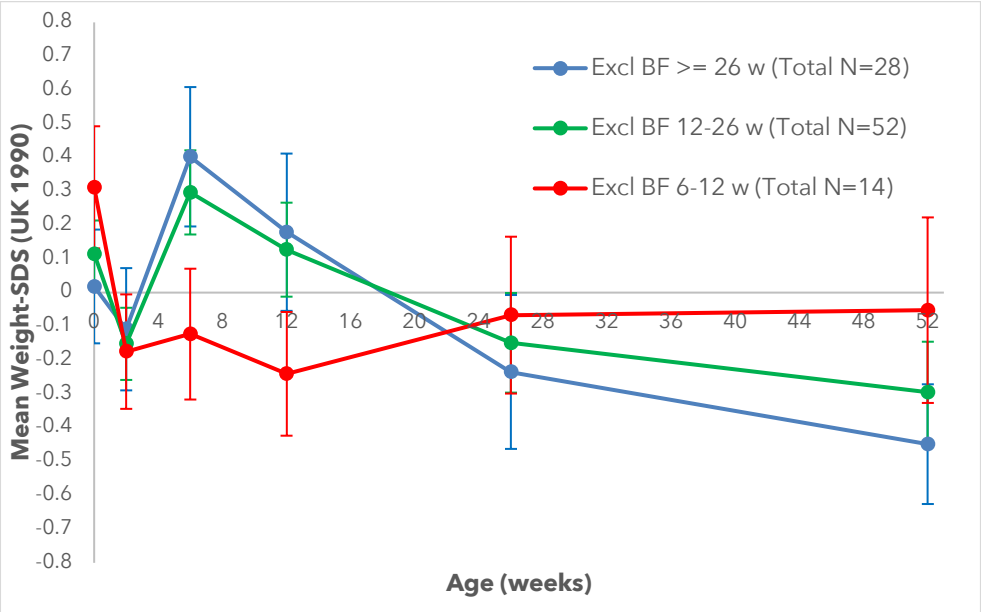


Figure 7.2 Infant height trajectories based on EBF duration
Values are mean±SEM. All SDS values are based on UK 1990 growth reference.

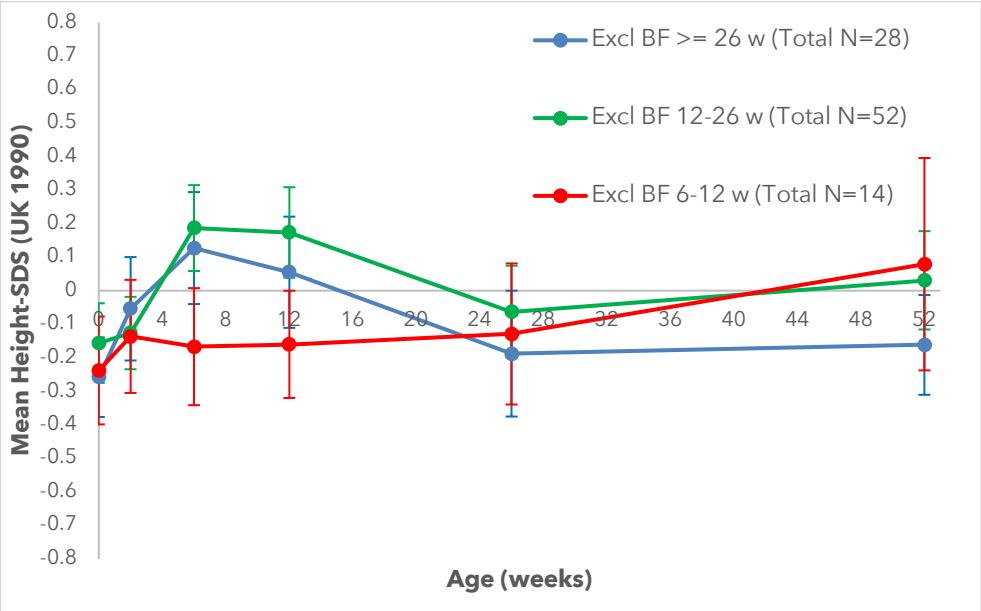


Figure 7.3 Infant skinfold trajectories (reflecting adiposity) based on EBF duration
Values are mean±SEM. SDS values are internally-derived.

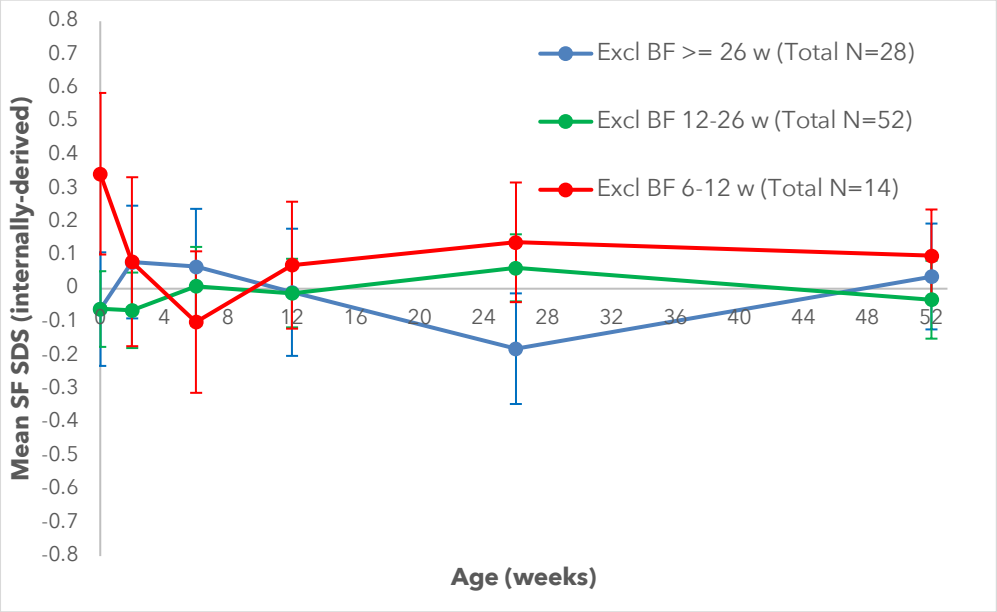


Table 7.2 Longitudinal growth gains according to duration of EBF
Weight, height, and BMI SDS values are based on UK 1990 growth reference. Skinfolds SDS values are internally-derived
**p<0.05 and **p≤0.005* against 6-12 wk group (unadjusted)

Growth gain SDS	Duration of exclusive breastfeeding		
	6-12 wk (N=14)	**12-26 wk (N=52)**	**≥26 wk (N=28)**
0-6 weeks			
Weight	-0.43±0.68	0.19±0.71*	0.36±0.67**
Height	0.07±0.73	0.35±0.69	0.39±0.54
BMI	-0.42±0.98	0.22±1.08	0.38±0.83*
Skinfolds	-0.44±0.88	0.06±0.97	0.16±0.99
6 weeks-3 months			
Weight	-0.12±0.5	-0.16±0.3	-0.22±0.4
Height	0.01±0.58	-0.01±0.51	-0.07±0.39
BMI	-0.2±0.6	-0.27±0.51	-0.31±0.59
Skinfolds	0.17±0.5	0.02±0.52	-0.04±0.51
3-6 months			
Weight	0.17±0.54	-0.28±0.4*	-0.43±0.79**
Height	0.03±0.62	-0.21±0.56	-0.23±0.6
BMI	0.25±0.59	-0.15±0.60	-0.33±1.07
Skinfolds	-0.03±0.64	0.06±0.57	-0.19±0.91
6-12 months			
Weight	0.01±0.38	-0.14±0.53	-0.29±0.44**
Height	0.12±0.71	0.08±0.58	-0.04±0.61
BMI	-0.05±0.91	-0.21±0.6	-0.31±0.48
Skinfolds	-0.02±0.6	-0.09±0.61	0.16±0.63

7.3.2BM nutritional content and intake

The analyses of BM macronutrient concentrations were carried out on samples collected from 2 weeks until 6 months (Table 7.3). The macronutrient composition of human milk varied across lactation period. Table 8.4 shows BM macronutrient contents at 6 weeks of age (expressed as calories per 100 mL and percentages of total calorie content (TCC)) compared to the previous measurements from CBGS1[204]. On average, compared to CBGS1 results[204], BM in this study contained lower lactose with higher fat and similar protein concentrations. However, the total calorie content (kcal/100mL) of BM in this study (mean±SD 65.0±19.5) was comparable with the literature (range 65-70 kcal/100mL).

When divided according to EBF history in the first 3 months, BM from mothers who EBF for > 3 months tended to have lower butyrate, higher protein, and lower triglycerides contents, with comparable lactose content. However, these results did not reach statistical significance.

Table 7.3 BM macronutrient contents across time

BM macronutrient composition				
Macronutrient	**Age**			
	2 weeks	**6 weeks**	**3 months**	**6 months**
	N=37	**N=59**	**N=37**	**N=27**
Lactose (g/100mL)	6.19(0.52)	6.28±0.37	6.4(0.42)	6.45±0.22
Triglyceride (g/100mL)	4.13(3.65)	3.92±2.11	4.21(4.83)	3.24(3.75)
Protein (g/100mL)	1.28(0.41)	1.14±0.22	1.1(0.39)	0.9(0.38)
Butyrate (mg/100mL)	0.82±0.45	0.91±0.49	1.13(1.11)	0.99(1.17)

Table 7.4 BM macronutrient content at 6 weeks in comparison to the previous CBGS results

Values are median(IQR)

[a]Atwater conversions were used to calculate the metabolisable energy content of BM, taking energy contents of 4, 9, and 4 kcal/g for lactose, fat, and protein, respectively.

[b]% macronutrient content was calculated as macronutrient energy/total energy content

BM macronutrient contents		
Macronutrient	**Current study**	**Previous study**[204]
	6 weeks	**4-8 weeks**
	N=59	**N=614**
Lactose (kcal/100mL)[a]	25.1(24.0-26.2)	34.3(32.9-35.3)
% Carbohydrate content[b]	41.7(31.0-51.6)	55.2(47.6-62.9)
Fat/Triglyceride (kcal/100mL)[a]	31.9(20.7-49.6)	23.1(15.4-32.4)
% Fat content[b]	51.5(39.8-62.1)	37.3(28.4-48.9)
Protein (kcal/100mL)[a]	4.4(3.9-5.1)	4.6(4.2-5.1)
% Protein content[b]	7.2(6.2-8.4)	7.5(6.4-9.0)

Table 7.5 BM composition based on exclusive breastfeeding history in the first 3 months

Values are mean±SD or median(IQR)

Atwater conversions were used to calculate the metabolisable energy content of BM, taking energy contents of 4, 9, and 4 kcal/g for lactose, fat, and protein, respectively.

	0-3 months exclusive breastfeeding		***p***
	Yes	**No**	
Butyrate (mg/100 mL)	0.85±0.41	1.17±0.75	*0.226*
Lactose (kcal/100 mL)	25.22±1.44	24.65±1.61	*0.273*
Triglyceride (kcal/100 mL)	34.54±19.2	39.01±18.33	*0.502*
Protein (kcal/100 mL)	4.63±0.85	4.23±0.96	*0.183*
Total calorie content (kcal/100 mL)	64.39±19.53	67.89±19.99	*0.609*

BM intake volume (L/day) was measured in 70 infants between 4-6 weeks. Compared to infants who were introduced mixed feeding between 6-12 weeks, infants who were EBF for at least 3 months had higher daily BM intake volume (*p=0.017*, Figure 7.4).

Figure 7.4 Daily BM intake volume (L/day) based on 0-3 months EBF history

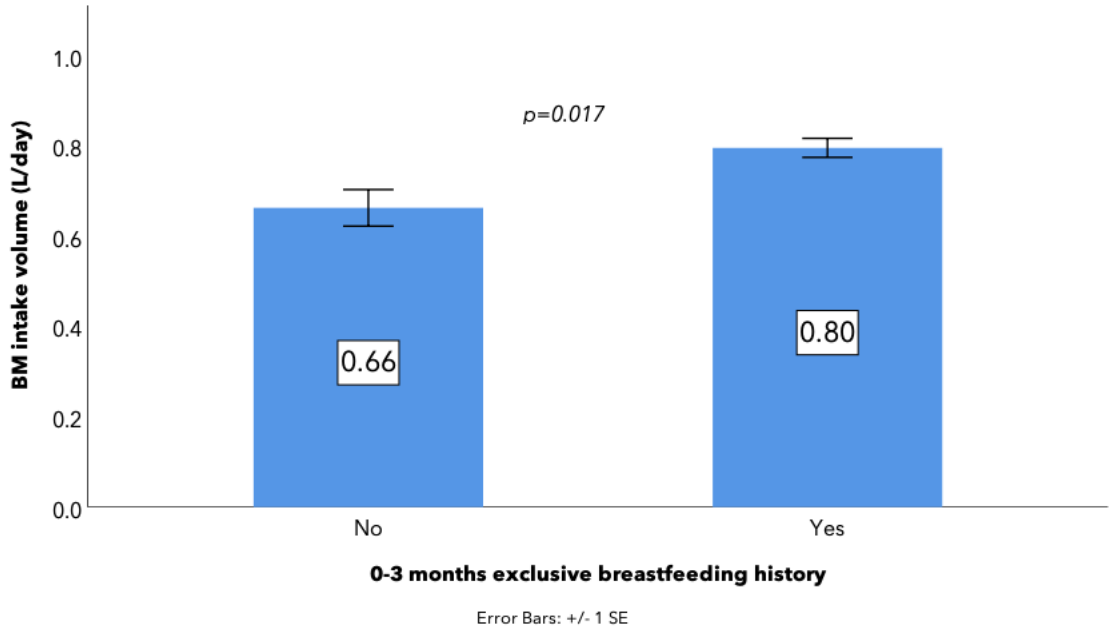


Table 7.6 shows the correlations between BM intake volume and BM nutrient concentrations. BM butyrate and protein contents were both negatively associated with BM intake volume (Figure 7.5). In addition, protein content was also highly correlated with TG and both of them were highly correlated with total BM energy content in BM (Table 7.6).

In this study, 47 out of 94 mother-infant pairs had both BM intake volume and macronutrient results at 6 weeks. Table 7.7 displays the Intake volume of each macronutrient which was calculated by multiplying each macronutrient content (kcal/100 mL) by BM intake volume (L/day) and by 10.

Table 7.6 Correlations between BM intake volume (measured between 4-6 weeks) and macronutrient concentrations (measured at 6 weeks)
BM=breast milk, TG=triglycerides, TCC=total calorie content

		BM intake volume (L/day)	BM butyrate content (mg/100mL)	BM lactose content (g/100mL)	BM TG content (g/100mL)	BM protein content (g/100mL)	BM TCC (kcal/100mL)
BM intake volume (L/day)	R	1	**-0.29**	0.14	-0.19	**-0.29**	-0.19
	p		***0.047***	*0.339*	*0.195*	***0.048***	*0.202*
BM butyrate content (mg/100mL)	R		1	0.03	0.19	0.01	0.13
	p			*0.867*	*0.212*	*0.964*	*0.337*
BM lactose content (g/100mL)	R			1	-0.14	-0.13	0.02
	p				*0.353*	*0.387*	*0.888*
BM TG content (g/100mL)	R				1	**0.66**	**0.997**
	p					***<0.0001***	***<0.0001***
BM protein content (g/100mL)	R					1	**0.69**
	p						***<0.0001***
BM TCC (kcal/100mL)	R						1
	p						

Figure 7.5 Inverse correlations between BM intake volume with butyrate (left) and protein (right) concentrations in BM

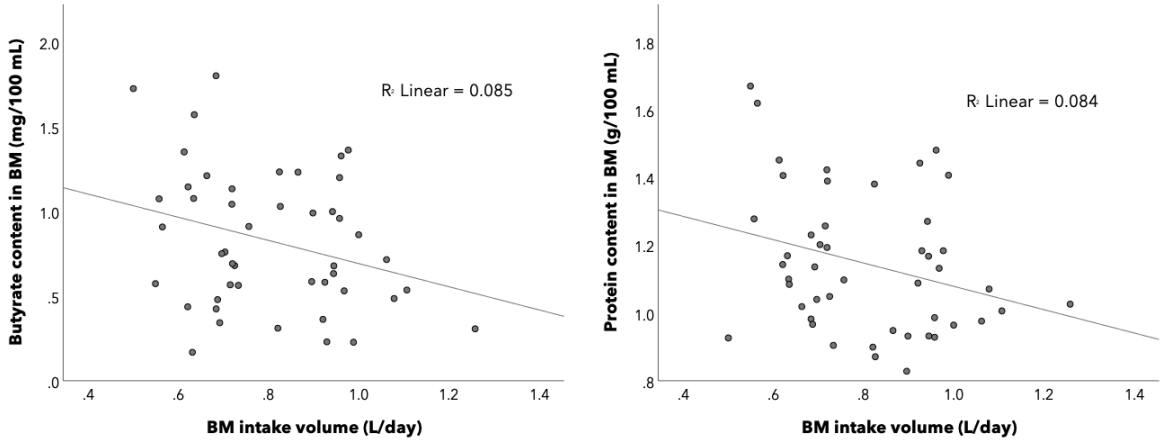


Table 7.7 BM intake at 6 weeks
Values are mean±SD or median(IQR)
[a]Atwater conversions were used to calculate the metabolisable energy content of BM, taking energy contents of 4, 9, and 4 kcal/g for lactose, fat, and protein, respectively.
[b]% macronutrient content was calculated as macronutrient energy/total energy content
[c]% macronutrient intake was calculated as macronutrient intake/total energy intake

	Age: 6 weeks
	N=47
BM intake volume (L/day)	0.78±0.16
Lactose (g/day)	50.82±11.65
Triglyceride (g/day)	24.65(24.89)
Protein (g/day)	9.13±2.14
Butyrate (mg/day)	6.44±3.04
Total energy intake (kcal/day)	506.37±175.54
% Energy intake from carbohydrate[b]	41.3±13.24
% Energy intake from lipid[b]	49.03±14.66
% Energy intake from protein[b]	7.61±1.73

With regard to maternal baseline characteristics, both BM macronutrient contents and intake were unrelated to maternal age, ethnicity, pre-pregnancy BMI, height, and parity, thus these factors were not included in the further regression models. GA was associated with BM protein content at 2 weeks (Pearson R=0.36, *p=0.028*, unadjusted) and butyrate at 6 months (R=-0.45, *p=0.018*, unadjusted). Male infants had a higher volume of BM intake than female infants (*p=0.044*, unadjusted). Therefore, multiple linear regression models were adjusted for infant feeding history at 3 months (exclusive breast feeding versus mixed feeding), birth weight SDS, and postnatal age at visit, GA, and infant sex.

7.3.3 BM nutritional content/intake and infant growth

To examine if differences in growth patterns, especially weight gain (Figure 7.1) across EBF duration groups could be explained by BM nutrient concentrations

and/or intake, multiple linear regression models were performed. The models only included subjects with results for both BM macronutrient contents and intake (N=47). Table 7.8 shows the associations between BM macronutrient concentrations and infant growth, while table 7.10 shows the associations between BM macronutrient intake and infant growth.

As shown in Table 7.8, BM butyrate concentration was inversely associated at borderline significance with early infancy weight gain (0-3 months, *p=0.05*), as well as with weight, BMI, and sum of skinfold thicknesses at 6 weeks. However, these associations were no longer observed with butyrate intake instead of concentration (Table 7.10).

Cross-sectionally, carbohydrate and protein intakes at 6 weeks were positively correlated with all growth parameters at both 6 weeks and 3 months. Longitudinally, carbohydrate and protein intakes were also positively associated with gains in weight, BMI, and adiposity between 0-3 months. In general, the amount of BM consumed by infant was positively correlated to all growth measures until 3 months, but was inversely correlated with gains in weight, BMI, and adiposity from 3-12 months.

Sensitivity analyses were carried out, allowing separate examinations to infants who were EBF for at least 3 months (Table 7.9 and 7.11). In the BM macronutrient intake models (Table 7.11), these showed the same directions as the total population, but with stronger *p values*. Since BM butyrate and protein contents were highly-intercorrelated, Table 7.12 shows regression models with further adjustment for other macronutrient intakes. The changes in the significance level of each macronutrient intake model after this further adjustment are summarised in Table 7.13.

Table 7.8 Association between BM macronutrient concentration and infancy growth/adiposity (in subjects who have both BM intake volume and macronutrient content results)

All subjects were exclusively breastfed until at least 6 weeks old (total N=47)

[a]SDS values are based on UK 1990 growth reference, adjusted for infant sex, gestational age for birth until 3 months measurements, and postnatal age at visit

[b]Skinfolds measurements were done on 4 sites: triceps, subscapular, flank, quadriceps

Models were adjusted for infant sex, exclusive breast vs. mixed feeding at 3 months, birthweight SDS, GA, and postnatal age at visit

B=unstandardised B coefficient; TCC=total calorie content

*associations approached the borderline of significance (*p<0.1*); associations with *p<0.05* are highlighted in bold

Outcomes: Growth and adiposity parameters	**Predictors: BM nutrient concentration (measured at 6 weeks)**									
	Butyrate (mg/100mL)		**Carbohydrate (kcal/100 mL)**		**Fat (kcal/100 mL)**		**Protein (kcal/100 mL)**		**TCC (kcal/100 mL)**	
	B	***p***	**B**	***p***	**B**	***p***	**B**	***p***	**B**	***p***
Weight-SDS[a]										
6 weeks	**-0.68**	***0.007***	-0.03	*0.713*	-0.01	*0.212*	-0.2	*0.125*	-0.01	*0.19*
3 months	**-0.63**	***0.05***	-0.12	*0.192*	-0.003	*0.728*	-0.1	*0.563*	-0.003	*0.644*
6 months	-0.32	*0.349*	-0.18	*0.058**	-0.001	*0.935*	0.002	*0.989*	-0.001	*0.832*
12 months	-0.09	*0.797*	-0.18	*0.073*	<0.0001	*0.996*	-0.06	*0.744*	-0.001	*0.891*
Δ Weight SDS										
0-3 months	**-0.63**	***0.05***	-0.1	*0.322*	-0.01	*0.439*	-0.1	*0.559*	-0.01	*0.392*
3-12 months	**0.66**	***0.017***	-0.07	*0.39*	0.01	*0.456*	0.12	*0.399*	0.004	*0.486*
Height-SDS[a]										
6 weeks	-0.24	*0.364*	-0.06	*0.452*	-0.01	*0.279*	-0.05	*0.706*	-0.01	*0.259*
3 months	-0.28	*0.285*	-0.02	*0.802*	<0.0001	*0.99*	0.03	*0.807*	<0.0001	*0.997*
6 months	-0.38	*0.161*	-0.14	*0.083**	0.002	*0.702*	0.11	*0.414*	0.002	*0.777*
12 months	-0.3	*0.285*	-0.1	*0.233*	-0.002	*0.767*	-0.01	*0.948*	-0.002	*0.705*
Δ Height SDS										
0-3 months	-0.47	*0.077**	-0.02	*0.83*	-0.004	*0.468*	0.08	*0.581*	-0.004	*0.485*
3-12 months	0.06	*0.805*	-0.09	*0.171*	0.001	*0.866*	0.03	*0.799*	<0.0001	*0.943*
BMI-SDS[a]										
6 weeks	**-0.74**	***0.021***	0.01	*0.902*	-0.01	*0.497*	-0.24	*0.142*	-0.01	*0.474*
3 months	-0.62	*0.082**	-0.15	*0.152*	-0.004	*0.667*	-0.16	*0.385*	-0.01	*0.572*
6 months	-0.11	*0.763*	-0.15	*0.153*	-0.002	*0.841*	-0.08	*0.662*	-0.002	*0.751*
12 months	0.12	*0.763*	-0.16	*0.157*	<0.0001	*0.956*	-0.07	*0.732*	-0.001	*0.949*
Δ BMI SDS										
0-3 months	-0.62	*0.289*	-0.22	*0.181*	<0.0001	*0.974*	-0.27	*0.37*	-0.002	*0.863*
3-12 months	0.12	*0.763*	-0.01	*0.953*	0.01	*0.466*	0.19	*0.413*	0.01	*0.459*
Sum skinfolds[b] (cm)										
6 weeks	**-6.75**	***0.003***	-0.52	*0.457*	-0.09	*0.076**	**-2.55**	***0.03***	-0.1	*0.06**
3 months	-4.34	*0.096**	-1.01	*0.179*	-0.03	*0.654*	-0.96	*0.474*	-0.03	*0.569*
6 months	0.69	*0.784*	-0.84	*0.239*	-0.02	*0.705*	-0.68	*0.575*	-0.03	*0.633*
12 months	0.04	*0.987*	-0.95	*0.204*	0.01	*0.822*	-0.29	*0.832*	0.01	*0.905*
Δ Sum skinfolds										
0-3 months	-0.85	*0.735*	-0.78	*0.277*	-0.01	*0.903*	-0.3	*0.816*	-0.01	*0.833*
3-12 months	**5.44**	***0.031***	0.12	*0.878*	0.05	*0.373*	1.15	*0.4*	0.05	*0.362*

Table 7.9 Association between BM macronutrient <u>concentrations</u> and infancy growth and adiposity (only on subjects who were exclusively breastfed for ≥3 months)

All subjects were exclusively breastfed until at least 3 months old (total N=49)

[a]SDS values are based on UK 1990 growth reference, adjusted for infant sex, gestational age for birth until 3 months measurements, and postnatal age at visit

[b]Skinfolds measurements were done on 4 sites: triceps, subscapular, flank, quadriceps

Models were adjusted for infant sex, birthweight SDS, GA, and postnatal age at visit

B=unstandardised B coefficient

*associations approached the borderline of significance ($p<0.1$); associations with $p<0.05$ are highlighted in bold

Outcomes: Growth and adiposity parameters	**Predictors: BM nutrient concentration (measured at 6 weeks)**									
	Butyrate (mg/100mL)		**Carbohydrate (kcal/100 mL)**		**Fat (kcal/100 mL)**		**Protein (kcal/100 mL)**		**TCC (kcal/100 mL)**	
	B	***p***	**B**	***p***	**B**	***p***	**B**	***p***	**B**	***p***
Weight-SDS[a]										
6 weeks	-0.25	*0.354*	-0.03	*0.659*	-0.002	*0.665*	-0.18	*0.145*	-0.003	*0.603*
3 months	-0.42	*0.201*	-0.1	*0.298*	-0.001	*0.936*	-0.05	*0.734*	-0.001	*0.865*
6 months	0.02	*0.951*	**-0.18**	***0.05***	0.01	*0.17*	0.15	*0.336*	0.01	*0.213*
12 months	0.2	*0.525*	**-0.18**	***0.045***	0.002	*0.754*	0.01	*0.933*	0.001	*0.865*
Δ Weight SDS										
0-3 months	-0.42	*0.201*	-0.1	*0.298*	-0.001	*0.936*	-0.05	*0.734*	-0.001	*0.865*
3-12 months	**0.63**	***0.02***	-0.09	*0.265*	0.01	*0.424*	0.12	*0.363*	0.004	*0.46*
Height-SDS[a]										
6 weeks	-0.07	*0.777*	-0.07	*0.335*	-0.004	*0.501*	-0.12	*0.332*	-0.004	*0.44*
3 months	-0.16	*0.533*	-0.02	*0.814*	0.002	*0.739*	0.01	*0.945*	0.002	*0.756*
6 months	-0.1	*0.713*	-0.15	*0.055**	0.004	*0.546*	0.1	*0.428*	0.003	*0.624*
12 months	-0.17	*0.516*	-0.1	*0.197*	0.001	*0.827*	0.02	*0.902*	0.001	*0.899*
Δ Height SDS										
0-3 months	-0.29	*0.258*	0.02	*0.829*	-0.002	*0.78*	0.15	*0.212*	-0.001	*0.84*
3-12 months	-0.04	*0.844*	-0.08	*0.201*	0.001	*0.882*	0.03	*0.778*	0.0003	*0.95*
BMI-SDS										
6 weeks	-0.25	*0.446*	0.02	*0.865*	-0.001	*0.905*	-0.17	*0.274*	-0.001	*0.879*
3 months	-0.4	*0.292*	-0.11	*0.313*	-0.003	*0.76*	-0.09	*0.624*	-0.003	*0.692*
6 months	0.13	*0.731*	-0.12	*0.232*	0.01	*0.2*	0.12	*0.498*	0.01	*0.231*
12 months	0.41	*0.236*	-0.17	*0.076**	0.002	*0.804*	0.01	*0.971*	0.001	*0.905*
Δ BMI SDS										
0-3 months	-0.41	*0.49*	-0.21	*0.208*	0.001	*0.948*	-0.26	*0.354*	-0.001	*0.944*
3-12 months	**1.32**	***0.002***	-0.06	*0.634*	0.01	*0.314*	0.17	*0.433*	0.01	*0.325*
Sum skinfolds[b] (cm)										
6 weeks	-2.52	*0.341*	-0.64	*0.395*	-0.05	*0.395*	-2.06	*0.094**	-0.05	*0.333*
3 months	-2.78	*0.318*	-0.78	*0.321*	0.01	*0.877*	0.12	*0.928*	0.01	*0.935*
6 months	1.88	*0.371*	-0.61	*0.315*	0.07	*0.095**	1.0	*0.311*	0.07	*0.108*
12 months	1.87	*0.793*	-1.03	*0.145*	0.03	*0.555*	-0.08	*0.952*	0.02	*0.636*
Δ Sum skinfolds										
0-3 months	0.18	*0.949*	-0.52	*0.506*	0.02	*0.693*	0.49	*0.707*	0.02	*0.724*
3-12 months	5.18	*0.057**	-0.05	*0.954*	0.04	*0.54*	0.16	*0.909*	0.03	*0.549*

Table 7.10 Association between BM macronutrient <u>intake</u> and infancy growth/adiposity (on subjects who have <u>both</u> BM intake volume and macronutrient content results)

All subjects were exclusively breastfed until at least 6 weeks old (total N=47)

[a]The dose-to-mother method using a deuterium-rich water is employed to determine the volume of BM received by breastfed infants age 4-6 weeks

[b]SDS values are based on UK 1990 growth reference, adjusted for infant sex, gestational age for birth until 3 months measurements, and postnatal age at visit

[c]Skinfolds measurements were done on 4 sites: triceps, subscapular, flank, quadriceps

Models were adjusted for infant sex, exclusive breast vs. mixed feeding at 3 months, birthweight SDS, GA, and postnatal age at visit

B=unstandardised B coefficient

*associations approached the borderline of significance ($p<0.1$); associations with $p<0.05$ are highlighted in bold

Outcomes: Infant growth and adiposity	**Predictors**									
	BM macronutrient Intake (measured at 6 weeks)								**BM intake volume[a] (4-6 weeks; L/day)**	
	Butyrate (mg/day)		**Carbohydrate (g/day)**		**Fat (g/day)**		**Protein (g/day)**			
	B	***p***	**B**	***p***	**B**	***p***	**B**	***p***	**B**	***p***
Weight-SDS[b]										
6 weeks	-0.03	*0.427*	**0.05**	***<0.0001***	0.004	*0.569*	**0.2**	***0.001***	**3.91**	***<0.0001***
3 months	-0.02	*0.609*	**0.05**	***<0.0001***	0.008	*0.296*	**0.22**	***0.001***	**4.09**	***<0.0001***
6 months	-0.01	*0.834*	0.02	*0.087**	0.007	*0.424*	**0.15**	***0.039***	**2.4**	***0.009***
12 months	0.04	*0.388*	0.02	*0.121*	0.005	*0.566*	0.11	*0.129*	1.77	*0.052**
Δ Weight SDS										
0-3 months	-0.02	*0.609*	**0.05**	***<0.0001***	0.008	*0.296*	**0.22**	***0.001***	**4.09**	***<0.0001***
3-12 months	**0.08**	***0.038***	**-0.03**	***0.013***	-0.001	*0.916*	-0.09	*0.139*	**-2.31**	***0.002***
Height-SDS[b]										
6 weeks	0.004	*0.92*	**0.02**	***0.045***	-0.003	*0.617*	0.1	*0.084**	**2.03**	***0.003***
3 months	-0.001	*0.988*	**0.03**	***0.006***	0.005	*0.386*	**0.15**	***0.005***	**2.23**	***0.001***
6 months	-0.04	*0.275*	0.01	*0.475*	0.003	*0.641*	0.1	*0.101*	1.18	*0.107*
12 months	-0.03	*0.506*	0.01	*0.371*	0.0003	*0.969*	0.06	*0.342*	1.0	*0.169*
Δ Height SDS										
0-3 months	-0.05	*0.165*	0.01	*0.201*	-0.002	*0.744*	0.09	*0.101*	1.38	*0.054**
3-12 months	-0.02	*0.628*	-0.02	***0.035***	-0.003	*0.632*	*-0.08*	*0.113*	-1.22	*0.056**
BMI-SDS[b]										
6 weeks	-0.03	*0.458*	**0.05**	***<0.0001***	0.01	*0.419*	**0.2**	***0.003***	**3.73**	***<0.0001***
3 months	-0.02	*0.678*	**0.05**	***0.001***	0.01	*0.542*	**0.19**	***0.008***	**3.61**	***<0.0001***
6 months	0.02	*0.654*	0.02	*0.089**	0.01	*0.511*	0.12	*0.11*	**2.07**	***0.027***
12 months	0.08	*0.165*	0.02	*0.301*	0.01	*0.43*	0.09	*0.273*	1.59	*0.126*
Δ BMI SDS										
0-3 months	0.03	*0.734*	**0.07**	***0.002***	0.01	*0.308*	**0.29**	***0.012***	**5.52**	***<0.0001***
3-12 months	**0.16**	***0.014***	**-0.04**	***0.018***	0.003	*0.772*	-0.14	*0.142*	**-3.07**	***0.011***
Sum skinfolds[c] (cm)										
6 weeks	-0.39	*0.244*	**0.46**	***<0.0001***	0.01	*0.851*	**1.54**	***0.002***	**33.14**	***<0.0001***
3 months	-0.21	*0.568*	**0.32**	***0.002***	0.06	*0.391*	**1.34**	***0.013***	**23.68**	***<0.0001***
6 months	0.35	*0.326*	0.14	*0.17*	0.04	*0.508*	0.67	*0.198*	11.89	*0.075**
12 months	0.36	*0.301*	0.17	*0.121*	0.04	*0.503*	0.65	*0.223*	11.14	*0.088**
Δ Sum skinfolds										
0-3 months	0.28	*0.418*	**0.22**	***0.031***	0.05	*0.381*	**1.11**	***0.033***	**18.09**	***0.004***
3-12 months	**0.74**	***0.038***	-0.17	*0.12*	-0.004	*0.948*	-0.58	*0.285*	**-13.49**	***0.035***

Table 7.11 Association between BM macronutrient intake and infancy growth/adiposity (only on subjects who were exclusively breastfed for ≥3 months)

All subjects were exclusively breastfed until at least 3 months old

[a]The dose-to-mother method using a deuterium-rich water is employed to determine the volume of BM received by breastfed infants age 4-6 weeks

[b]SDS values are based on UK 1990 growth reference, adjusted for infant sex, gestational age for birth until 3 months measurements, and postnatal age at visit

[c]Skinfolds measurements were done on 4 sites: triceps, subscapular, flank, quadriceps

Models were adjusted for infant sex, birthweight SDS, GA, and postnatal age at visit

B=unstandardised B coefficient

*associations approached the borderline of significance ($p<0.1$); associations with $p<0.05$ are highlighted in bold

Outcomes: Infant growth and adiposity	**Predictors**									
	BM macronutrient Intake (measured at 6 weeks)								**BM intake volume[a] (4-6 weeks; L/day)**	
	Butyrate (mg/day)		**Carbohydrate (g/day)**		**Fat (g/day)**		**Protein (g/day)**			
	B	***p***	**B**	***p***	**B**	***p***	**B**	***p***	**B**	***p***
	Total N=40[c]								**Total N=60[b]**	
Weight-SDS[b]										
6 weeks	-0.02	*0.524*	**0.04**	***<0.0001***	0.003	*0.632*	**0.19**	***<0.0001***	**3.53**	***<0.0001***
3 months	-0.03	*0.492*	**0.04**	***0.001***	0.011	*0.172*	**0.25**	***<0.0001***	**3.75**	***<0.0001***
6 months	-0.01	*0.806*	0.02	*0.217*	0.01	*0.118*	**0.2**	***0.006***	**2.47**	***0.004***
12 months	0.05	*0.311*	0.02	*0.332*	0.01	*0.367*	0.14	*0.067**	1.4	*0.104*
Δ Weight SDS										
0-3 months	-0.03	*0.492*	**0.04**	***0.001***	0.011	*0.172*	**0.25**	***<0.0001***	**3.75**	***<0.0001***
3-12 months	**0.09**	***0.025***	**-0.03**	***0.019***	0.001	*094*	-0.09	*0.2*	**-2.27**	***0.002***
Height-SDS[b]										
6 weeks	-0.003	*0.934*	0.02	*0.088**	-0.003	*0.651*	0.1	*0.11*	**2.1**	***0.001***
3 months	-0.01	*0.804*	**0.03**	***0.014***	0.01	*0.358*	**0.16**	***0.004***	**2.19**	***<0.0001***
6 months	-0.04	*0.345*	0.01	*0.648*	0.004	*0.561*	**0.13**	***0.048***	**1.58**	***0.027***
12 months	-0.04	*0.334*	0.01	*0.243*	0.002	*0.785*	0.11	*0.075**	1.26	*0.103*
Δ Height SDS										
0-3 months	-0.05	*0.173*	0.011	*0.38*	-0.003	*0.7*	0.1	*0.109*	1.09	*0.069**
3-12 months	-0.02	*0.475*	**-0.03**	***0.019***	-0.002	*0.73*	-0.05	*0.359*	-0.95	*0.145*
BMI-SDS[b]										
6 weeks	-0.02	*0.592*	**0.04**	***0.001***	0.01	*0.45*	**0.17**	***0.014***	**3.25**	***<0.0001***
3 months	-0.03	*0.538*	**0.04**	***0.019***	0.01	*0.271*	**0.2**	***0.009***	**3.43**	***<0.0001***
6 months	0.02	*0.765*	0.02	*0.222*	0.01	*0.114*	**0.16**	***0.04***	**2.04**	***0.042***
12 months	0.1	*0.059**	0.01	*0.607*	0.01	*0.318*	0.11	*0.212*	0.85	*0.345*
Δ BMI SDS										
0-3 months	0.01	*0.93*	**0.06**	***0.013***	0.02	*0.134*	**0.33**	***0.009***	**5.35**	***<0.0001***
3-12 months	**0.2**	***0.002***	**-0.04**	***0.042***	0.002	*0.88*	-0.13	*0.223*	**-3.5**	***0.002***
Sum skinfolds[c] (cm)										
6 weeks	-0.31	*0.337*	**0.42**	***<0.0001***	0.004	*0.948*	**1.58**	***0.002***	**35.48**	***<0.0001***
3 months	-0.19	*0.613*	**0.29**	***0.008***	0.09	*0.181*	**1.7**	***0.002***	**25.22**	***<0.0001***
6 months	0.36	*0.227*	0.09	*0.339*	**0.11**	***0.03***	**0.9**	***0.048***	8.85	*0.108*
12 months	0.48	*0.206*	0.13	*0.276*	0.06	*0.406*	0.67	*0.261*	5.38	*0.437*
Δ Sum skinfolds										
0-3 months	0.29	*0.421*	0.2	*0.072**	0.08	*0.23*	**1.4**	***0.012***	**19.81**	***0.001***
3-12 months	**0.83**	***0.031***	-0.18	*0.148*	-0.01	*0.847*	-0.89	*0.145*	**-19.17**	***0.008***

Table 7.12 Table 7.10 with further adjustment for other macronutrient intake values (eg. Butyrate models are adjusted for CHO, protein, and TG intake values)

All subjects were exclusively breastfed until at least 6 weeks old

[a]SDS values are based on UK 1990 growth reference, adjusted for infant sex, gestational age for birth until 3 months measurements, and postnatal age at visit

[b]Skinfolds measurements were done on 4 sites: triceps, subscapular, flank, quadriceps

Each model was adjusted for infant sex, exclusive breast vs. mixed feeding at 3 months, birthweight SDS, GA, postnatal age at visit, and other macronutrient intake values

B=unstandardised B coefficient

*associations approached the borderline of significance ($p<0.1$); associations with $p<0.05$ are highlighted in bold; associations with worsened *p values* (from significant become not significant) are highlighted in red; ssociations with improved *p values* (from not significant become significant) are highlighted in green

Outcomes: Infant growth and adiposity	Predictors: BM macronutrient Intake (measured at 6 weeks)							
	Butyrate (mg/day)		Carbohydrate (g/day)		Fat (g/day)		Protein (g/day)	
	B	*p*	B	*p*	B	*p*	B	*p*
Weight-SDS[a]								
6 weeks	-0.03	*0.34*	**0.04**	***<0.0001***	-0.003	*0.61*	0.09	*0.171*
3 months	-0.02	*0.679*	**0.03**	***0.027***	-0.004	*0.673*	0.14	*0.172*
6 months	0.01	*0.798*	0.01	*0.579*	-0.003	*0.803*	0.17	*0.194*
12 months	0.06	*0.244*	0.01	*0.584*	-0.005	*0.722*	0.15	*0.285*
Δ Weight SDS								
0-3 months	-0.01	*0.764*	**0.03**	***0.05***	-0.005	*0.632*	0.14	*0.169*
3-12 months	**0.09**	***0.027***	**-0.03**	***0.032***	-0.002	*0.84*	0.03	*0.787*
Height-SDS[a]								
6 weeks	0.03	*0.504*	0.006	*0.684*	-0.02	*0.083**	0.17	*0.073**
3 months	0.01	*0.758*	0.01	*0.299*	-0.006	*0.449*	0.14	*0.103*
6 months	-0.01	*0.812*	-0.003	*0.851*	-0.003	*0.79*	0.17	*0.15*
12 months	-0.006	*0.906*	0.006	*0.734*	-0.0003	*0.98*	0.095	*0.459*
Δ Height SDS								
0-3 months	-0.04	*0.369*	0.001	*0.967*	-0.01	*0.183*	0.16	*0.109*
3-12 months	-0.02	*0.544*	-0.02	*0.266*	0.003	*0.706*	-0.06	*0.504*
BMI-SDS[a]								
6 weeks	-0.05	*0.187*	**0.05**	***0.001***	0.009	*0.386*	-0.02	*0.878*
3 months	-0.03	*0.555*	0.04	*0.058**	-0.0002	*0.987*	0.07	*0.58*
6 months	0.03	*0.604*	0.02	*0.434*	-0.002	*0.854*	0.09	*0.492*
12 months	0.09	*0.125*	0.01	*0.681*	-0.006	*0.659*	0.12	*0.402*
Δ BMI SDS								
0-3 months	0.02	*0.817*	0.06	*0.09**	0.005	*0.82*	0.09	*0.664*
3-12 months	**0.18**	***0.003***	-0.04	*0.051**	-0.007	*0.63*	0.1	*0.5*
Sum skinfolds[b] (cm)								
6 weeks	-0.06	*0.082*	**0.06**	***<0.0001***	-0.001	*0.916*	0.04	*0.634*
3 months	-0.009	*0.823*	**0.04**	***0.012***	-0.002	*0.836*	0.06	*0.542*
6 months	0.05	*0.209*	0.002	*0.87*	0.003	*0.77*	0.11	*0.299*
12 months	0.05	*0.292*	0.01	*0.478*	0.001	*0.943*	0.03	*0.764*
Δ Sum skinfolds								
0-3 months	0.08	*0.07**	0.03	*0.073**	-0.003	*0.797*	0.11	*0.289*
3-12 months	0.06	*0.134*	**-0.03**	***0.031***	0.004	*0.687*	0.003	*0.972*

Table 7.13 Summary of associations with infant growth/adiposity gains

	Before adjusting for other macronutrient intakes	**After adjusting for other macronutrient intakes**	**Comment**
Carbohydrate intake	Strong positive association with 0-3 months weight and adiposity gains	Persisting positive association with 0-3 months weight gain with weaker *p value* Persisting strong positive association with contemporaneous BMI and skinfolds SDS	
Protein intake	Strong positive association with 0-3 months growth and adiposity gains	No significant association	The disappearance of the previously significant association was presumably because of the strong correlation between protein and TG contents (Pearson R 0.66, *p<0.0001*; Figure 7.5) since TG did not correlate with any growth/adiposity parameters
Fat intake	No significant association	No significant association	
When being additionally adjusted for BM intake volume, almost all significant associations between macronutrient intake and growth/adiposity disappeared			

7.4 Discussion

Compared to both previous CBGS reports[204] and other literature[205], BM in this study contained lower lactose, higher fat, but with comparable protein concentrations (term BM macronutrient concentrations according to Ballard and Morrow[205] are as follows: 0.9-1.2 g/100mL protein, 3.2-3.6 g/100mL fat, and 6.7-7.8 g/100mL

lactose). BM TCC was also similar to the literature, e.g. the 6 weeks BM has 64.98 + 19.48 kcal/100mL (reference population had BM TCC ranging from 65-70 kcal/100mL[205]).

The protective role of breastfeeding against increased adiposity during early childhood may be dose-dependent, as infants with the longest duration of EBF ($\geq$ 6 months) had the lowest weight gains by at 12 months of age (Figure 7.1). These infants displayed particular growth trajectories with an initial upturn in weight gain (0-3 months) followed by a decline in the later period (3-12 months). On the contrary, the individuals who were introduced to formula before 3 months old ended up heavier and fatter after showing early downward growth trend in the first weeks of life. The initial difference may also reflect 'reverse causality' in that infants showing the poorest growth gains in the first months of life could lead to parents introducing infant formula which consequently resulted in greater weight gains. Consistent with this view, the early introduction of mixed feeding was associated with a lower BM intake volume at 6 weeks compared to the longer EBF infants. BM of mothers who stopped EBF after 6 weeks and before 3 months contained a trend towards more butyrate, more fat, less lactose and less protein although not statistically significant.

The effect of each BM nutrient content and intake on infancy growth and adiposity was then explored. In this population, BM butyrate content was inversely associated with immediate subsequent measures of infant weight and adiposity and this result was similar to the previous study in CBGS1[293]. Butyrate is a SCFA detected in gut, as final products of bacterial fermentation of undigested dietary fibres[295]. From both animal and human studies, butyrate and its producing bacteria (phylum Firmicutes)

have been linked to lower risk of obesity and its metabolic complications, including liver fibrosis[296] and insulin resistance[297,298], and may function as an anti-inflammatory agent in metabolic diseases[299]. This is mainly via host energy metabolism with butyrate serving as a signalling molecule in de novo lipids production[297]. Butyrate is also the source of energy for large intestine epithelium thus it may affect energy intake and energy balance, as 10% of energy intake may be attributed to dietary residues entering the large intestine[300].

However, when looking at butyrate intake rather than concentrations, the same associations were no longer observed. This implies that the associations between butyrate content and infant growth were either mediated or confounded by lower BM intake. As BM butyrate content was inversely correlated with BM intake volume, it could be then hypothesised that the high butyrate concentration in BM might be the reason for low BM intake in some infants. A recent animal study has reported that butyrate reduced appetite and decreased food intake in mice, presumably due to its odour and/or taste[301]. In addition, SCFAs have also been reported to be key molecules governing signalling pathway within the gut-brain axis and influencing appetite by modulating the secretion of regulatory neuropeptide XY[300]. It is then plausible to hypothesise that the interplay between butyrate odour and/or taste in BM and its effect on appetite regulation had lowered infants milk intake could contribute to poor intake and early infant weight gain.

In CBGS1, it was reported that BM fat and carbohydrate contents predicted changes in infancy weight and adiposity gains up to age 12 months, with protein content positively correlated to 12-month BMI[204]. In that study, there were no associations

found between BM macronutrient content and length gains and no data on milk intakes were available. In this current study, BM intake volume was measured and positive associations were displayed between daily BM carbohydrate and protein intakes at 6 weeks with all growth parameters, including weight, length, BMI, and skinfolds at 6 weeks and 3 months. Moreover, BM carbohydrate and protein intake were also positively related to weight and adiposity gains from birth to 3 months. However, no associations were found between BM fat intake and early infant growth.

The findings of carbohydrate and protein intakes being positively associated with later growth gains were similar to the existing literature[204,302]. The positive associations between BM lactose intake and weight/adiposity gains could be explained by two possible mechanisms. First, carbohydrate excess from BM is stored as glycogen and fat. Therefore, the higher the lactose consumed, the more adipose tissue the infants would gain. Secondly, greater early BM intake with higher carbohydrate concentrations with consequently lower fat would increase infant's appetite, reduce satiety, and finally enhance milk consumption.

The inverse association between protein content and BM intake found in this study was similar to the previous observations[206,303]. In the Davis Area Research on Lactation, Infant Nutrition and Growth (DARLING) study, milk protein concentration was negatively correlated to milk volume at 6 and 9 months, while milk lactose was positively correlated[303]. Milk energy density was also highly correlated with lipid concentration, which was similar to the finding in this study.

Lack of associations between BM fat content/intake with infant's weight/adiposity gains was unexpected and this finding is contrary to previous studies which have

suggested that greater BM fat may benefit later infant body composition[304,305]. The standardised single collection (not pooled) of hindmilk sample per visit in this study might be the reason behind this unanticipated result.

The strength of the current study included the measurement of BM intake alongside with BM nutrient concentration, allowing analyses of infant BM nutrient intakes. The longitudinal study design was also valuable in understanding the results in relation to infant growth and adiposity parameters. However, the small number of subjects in the study, especially those with complete measurements, limited power in many statistical analyses thus the interpretation of the results should be taken with caution.

7.5 Conclusions and future recommendations

From this study, exclusive breastfeeding duration could affect immediate and subsequent infancy growth. The effect was dose-dependent and correlated with BM intake volumes. BM macronutrient intake, especially lactose and protein, may have functional relevance to infant growth and adiposity. The possible role of BM butyrate in limiting appetite and BM intakes deserves further investigation. Validating the results of this study in a larger longitudinal cohort would be of pivotal importance.

Chapter 8 Exploratory analysis of *Fucosyltransferase 2* gene polymorphism and its association with infant growth and adiposity

8.1 Introduction

Fucosyltransferase 2 (*FUT2*) gene has been studied extensively in the last decades, especially in relation to gastrointestinal and autoimmune diseases. *FUT2* gene encodes for enzyme α(1,2) fucosyltransferase which is involved in the H antigen synthesis. This H antigen is essential for the synthesis and secretion of soluble A and B antigens of ABO histo-blood group[207] in other body fluids as well as intestinal mucosa.

FUT2 polymorphisms result in homo/heterozygous *secretor* and homozygous *non-secretor* status. Secretors (*Se*) carry at least one functional allele of *FUT2* to enable ABH expression on body secretions, while non-secretors (*se*) carry two non-functional *FUT2* alleles. As a result, non-secretors fail to present ABH antigens in secretions and on epithelial cells[208]. It is estimated that 80% of the global population

are secretors (homozygote *SeSe* or heterozygote *Sese*)[306], with an approximate range between 65-98% due to substantial ethnic and geographic variation[216].

Both secretor and non-secretor status confer some advantages and disadvantages. The saliva of the secretors contains additional carbohydrate compounds in the mucin that aggregate certain bacteria and decrease their activity. This makes secretors less susceptible to some infections, for example, *Candida albicans*, *Streptococcus pneumonia*, and *Haemophilus influenza*[211]. However, a study in Japan found that the *FUT2* secretors are more prone to *Helicobacter pylori*[307]. Other studies also found other benefits of being non-secretors such as having higher circulating serum vitamin B12 levels[308], slower progression to HIV[309], and being resistant to Norovirus infection[310]. The latter is because *Norovirus* Gene Group (GG) II, similar to *H. pylori*, attach to the gastric epithelium through H and Lewis b antigens. Children of secretor (*FUT2*-positive) mothers were also reported to have a 38% increased adjusted risk of all-cause diarrhoea and significantly reduced time to first diarrhoeal episode[311].

From genome-wide association studies (GWAS), the *FUT2* region has been identified as a susceptibility locus for Crohn's disease and several single nucleotide polymorphisms (SNPs) with a high degree of linkage disequilibrium, including rs601338, are associated with Crohn's disease[212]. Moreover, having 2 copies of the non-secretor allele at this SNP (rs601338) confers susceptibility to T1D and this may relate to non-secretor's resistance to some strains of Norovirus. Conversely, having a single functional copy of the *FUT2* gene and subsequent Norovirus and/or some

other infection provides protection against T1D[213,312]. In the European population, having one functional *FUT2* allele provides about 30% protection against T1D[312].

Since *FUT2* determines if ABH antigens are secreted into intestinal mucosa, studies have been conducted to explore the link between *FUT2* and the gut microbiota[207,313,314]. Different bacteria compositions are observed between secretors and non-secretors[313,314], mainly because H antigen acts as both attachment site and carbon source for certain intestinal bacteria[313]. This was speculated to outline the increased susceptibility of Crohn's disease among non-secretors. Metagenomic analysis on the mucosal ecosystem of healthy non-secretor and heterozygote individuals has revealed perturbed energy metabolism, involving carbohydrate, lipid, cofactor, vitamin, and glycan, showing that their colonic microbiota was altered at both compositional and functional levels[313].

More specifically in the perinatal period, maternal secretor status contributes to the significant microbiota changes observed during pregnancy. While in general pregnant women have altered intestinal bacteria composition compared to non-pregnant counterparts, non-secretors appear to have decreased *C. coccoides* group, *Clostridium histolyticum*, *Lactobacillus-Enterococcus* groups, and Actinobacteria with increased Proteobacteria[214]. Non-secretors also exhibit more pronounced adverse effects of C-section on infant gut microbiota. Among infants born by C-section, those who are born of non-secretor mothers have more depleted Bifidobacteria and increased enterococci and Akkermansia compared to those born of secretor mothers[315].

The main interest of *FUT2* studies in relation to early life is how the secretor status of lactating mothers could impact on the human milk oligosaccharides (HMO) composition and abundance[216,316], breastmilk and infant gut microbiota[317], and essentially overall infant's health, growth, and body composition[211,216]. HMO are complex carbohydrates that are abundant in breastmilk, being the third-largest constituent after lactose and lipid[216]. HMO are indigestible or non-nutritive and act as soluble prebiotics for the gut microbiota[216,316].

Secretor and non-secretor mothers have distinct HMO profiles, with predominant 2'fucosyllactose (2'FL) and lacto-N-fucopentaose I (LNFPI) among secretors. Since FUT2 and 3 determine the synthesis of fucosylated HMO, 2'FL is little or undetected in the milk of non-secretors[216,318]. 2'FL presence in the secretor's milk promotes the growth of *B. bifidum* and therefore suppressing the growth of the undesirable bacteria. On the contrary, non-secretor's milk would have less prevalent Bifidobacterium and delayed establishment of bifidobacterial-laden microbiota and relatively higher abundance of other bacteria, such as Enterobacteriaceae and Staphylococcae[215,319].

Although the link between maternal FUT2 secretor status and infant growth via HMO and BM and infant gut microbiota is biologically plausible, studies associating *FUT2* and growth are scarce. In animal studies, 3 fucosyltransferases, FUT8, FUT12/POFUT1 and FUT13/POFUT2, are essential for proper development in mice[320]. In humans, a study in Singapore although found substantial variation in HMO profiles between secretor and non-secretor mothers, but failed to correlate the findings with offspring growth up to 4 months of age[316].

Since both maternal and infant *FUT2* secretor status have implications on infant's growth and adiposity, this chapter first investigates the prevalence of infant secretor status in the 3 groups of infants in CBGS1 and CBGS2 and then explores the associations between both maternal and infant *FUT2* status with early growth and adiposity.

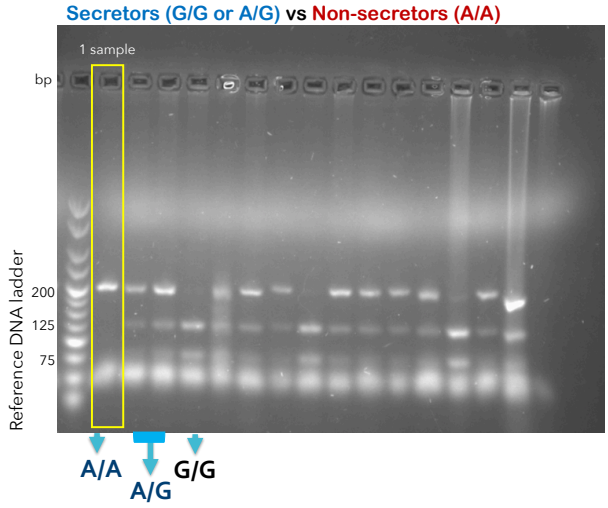
8.2 Methods

Saliva was collected from both mothers and infants during 6 month-visit. DNA extraction and quantification were done with the standard protocol as described in the Section 2.4.6. Thereafter, DNA was fragmented by applying a restriction enzyme or via restriction fragment length polymorphism (RFLP). Maternal and infant FUT2 genotypes were determined via agarose gel electrophoresis based on the identification of SNP at rs516246. The particular SNP was chosen because it is in complete linkage disequilibrium with the most studied SNP which distinguished secretors and non-secretors, rs610338, but was more amenable to restriction digestion. Homozygote GG and heterozygote AG (producing 125 and 77 base pair bands, respectively) correspond to the secretor phenotype while homozygote AA (202 base pair) indicate non-secretors (Figure 8.1).

HMO were quantified in CBGS liquid BM samples obtained from 2 weeks to 12 months of age by collaboration with the University of California Davis, USA (Barile's group-https://barilelab.ucdavis.edu/). The method is described in the Section 2.4.4. The most abundant and represented HMOs analysed in this study included: 2'-

fucosyllactose (2-FL), 3-fucosyllactose (3-FL), lacto-N-fucopentaose I (LNFP I), lacto-N-tetraose (LNT), and lacto-N-neotetraose (LNnT) as neutral HMO, and 3'-sialyllactose (3SL) and 6'-sialyllactose (6SL) as acidic HMO.

Figure 8.1 Gel electrophoresis results of *FUT2 rs516246* secretor and non-secretor genotypes
Gel electrophoresis is a technique used to separate DNA fragments according to their size. Small fragments move through the gel faster than the large ones. Each ladder represents 1 sample, to compare with DNA ladder on the left as reference. bp=base pair



8.3 Results

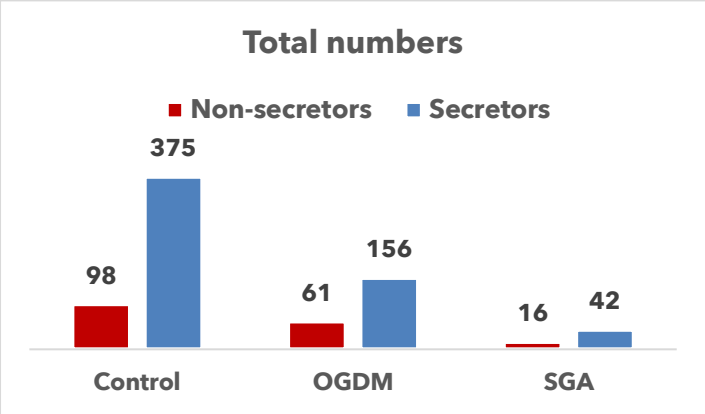
8.3.1 Infant FUT2 secretor status

8.3.1.1 In relation to growth and adiposity

In the combined CBGS sample, the ratio between secretors and non-secretors was approximately 3:1. Both SGA and OGDM had higher proportions of non-secretor infants, with the proportion in OGDM being significantly different from controls (Figure 8.2). The genotypes in each subgroup were in Hardy-Weinberg equilibrium.

Among controls only, there was a non-significant higher proportion of exclusively breastfed infants from birth to 3 months among non-secretor infants compared to the secretors (54% vs 45%, *p=0.066*).

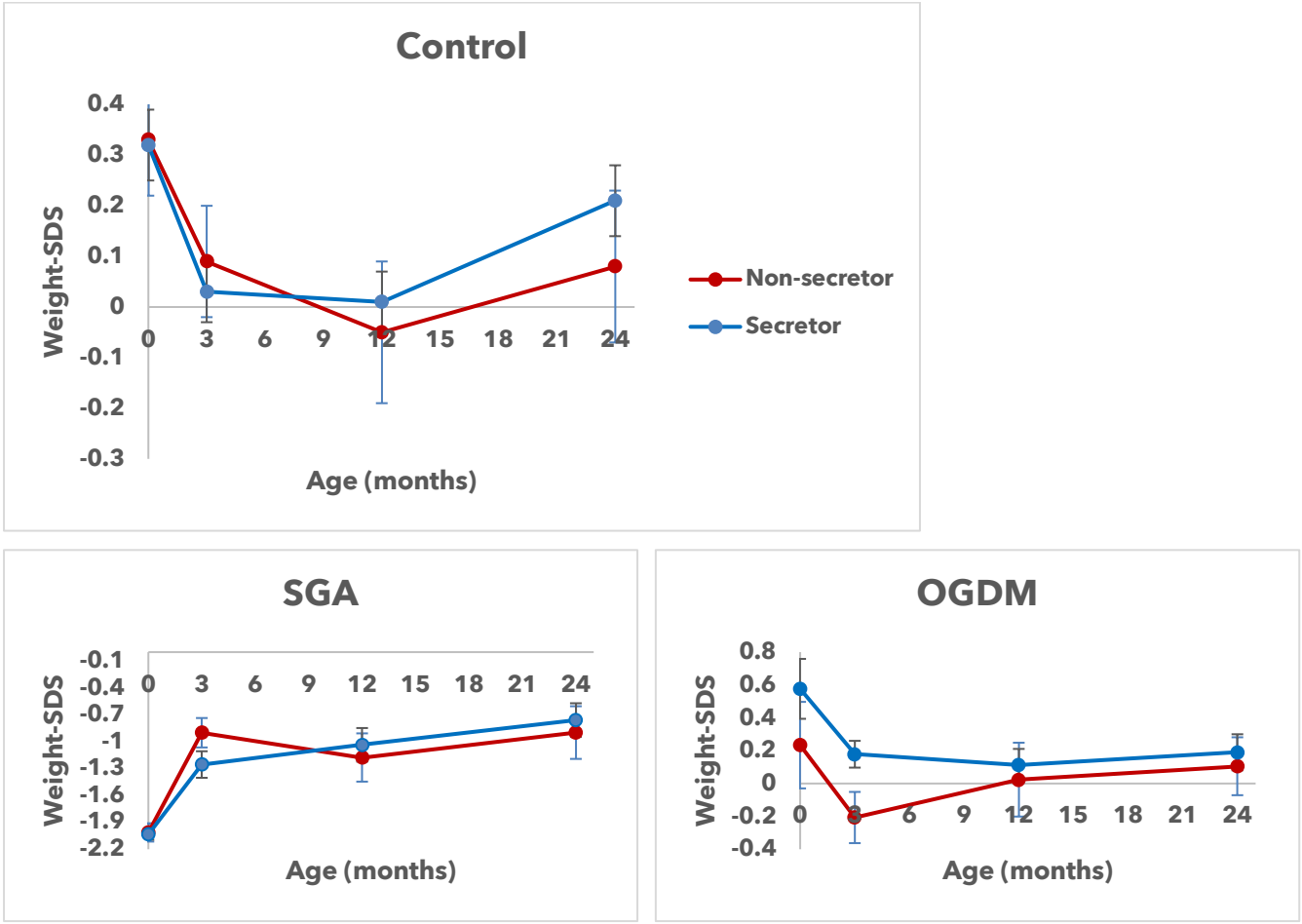
Figure 8.2 Prevalence of FUT2 secretor and non-secretor among CBGS infant groups
Total N=749



Group	Non-secretors (%)	Secretors (%)	*p (against controls)*
Control	20.7	79.3	
OGDM	28.1	71.9	***0.032***
SGA	27.6	72.4	*0.229*
All	23.4	76.6	

With regard to weight trajectories, as shown in Figure 8.3, among controls, secretors and non-secretors started from the birth weight, initially diverged but crossed lines between 7-8 months, and subsequently secretors were slightly heavier than non-secretors. Among OGDM, non-secretors showed more prominent early downward trend from birth to 3 months followed by an upward weight gain from 3-12 months. Among SGA, non-secretors caught-up more in weight between birth to 3 months, followed by a slower trend to secretors. Consequently, across all 3 groups, secretors ended up being slightly heavier at 24 months than non-secretors, and OGDM secretors were always heavier than OGDM non-secretors at all time points.

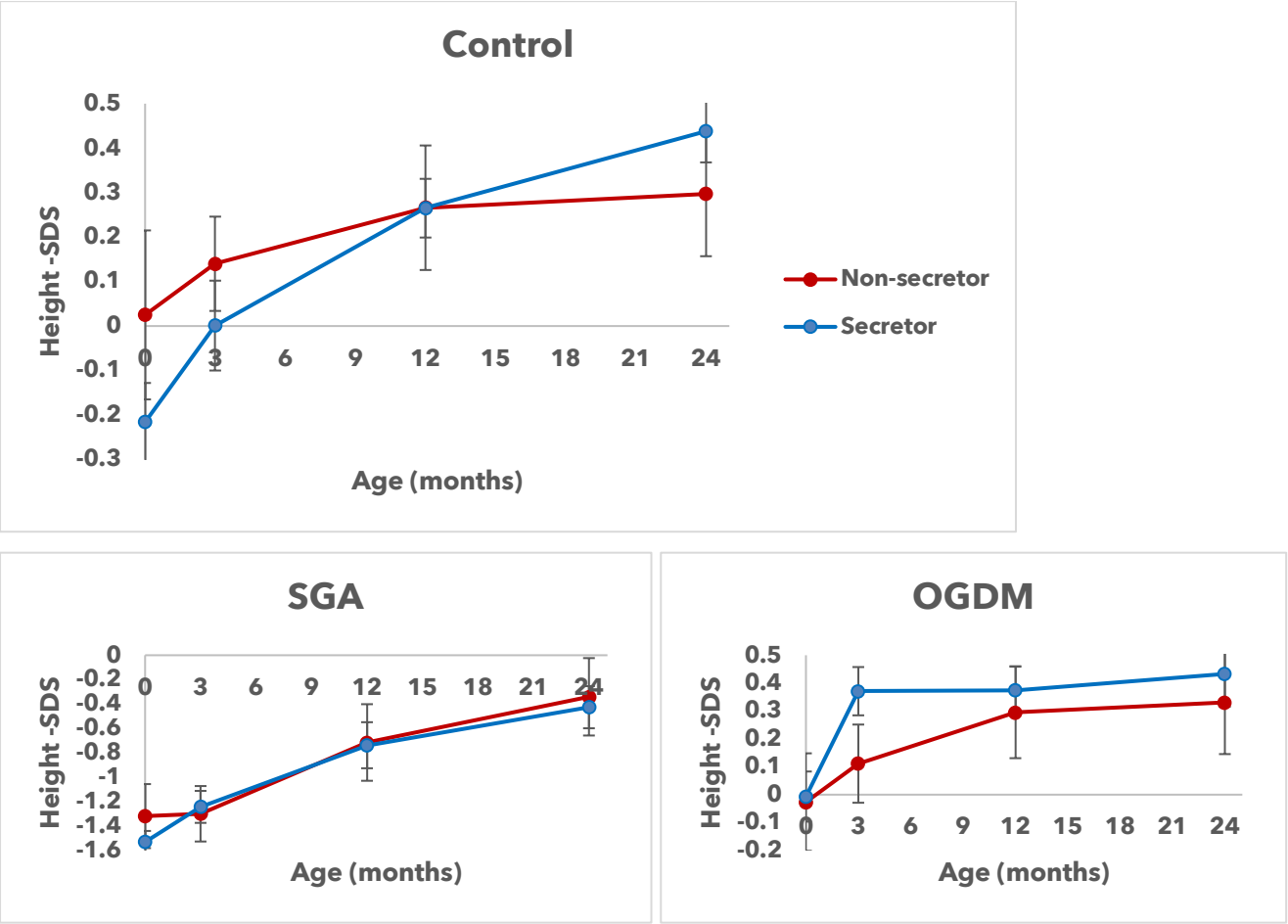
Figure 8.3 Infancy weight trajectories between secretors and non-secretors
Values are mean±SEM. All SDS values are based on UK 1990 growth reference.



Similar to weight trajectories, height gain patterns among controls also had crossing point at 12 months (Figure 8.4) since the secretors were shorter at birth but gained more height during infancy and ended up being taller at 24 months, but none of these cross-sectional comparisons reached significance. Meanwhile, there was barely any difference in height gain between secretor and non-secretor among SGA infants. Among OGDM, the secretors showed a trend towards more gain in height in early infancy (0-3 months) whereas the non-secretor showing more upward trend

between 3-12 months. The secretor OGDM were always taller than their non-secretor counterparts at all time points.

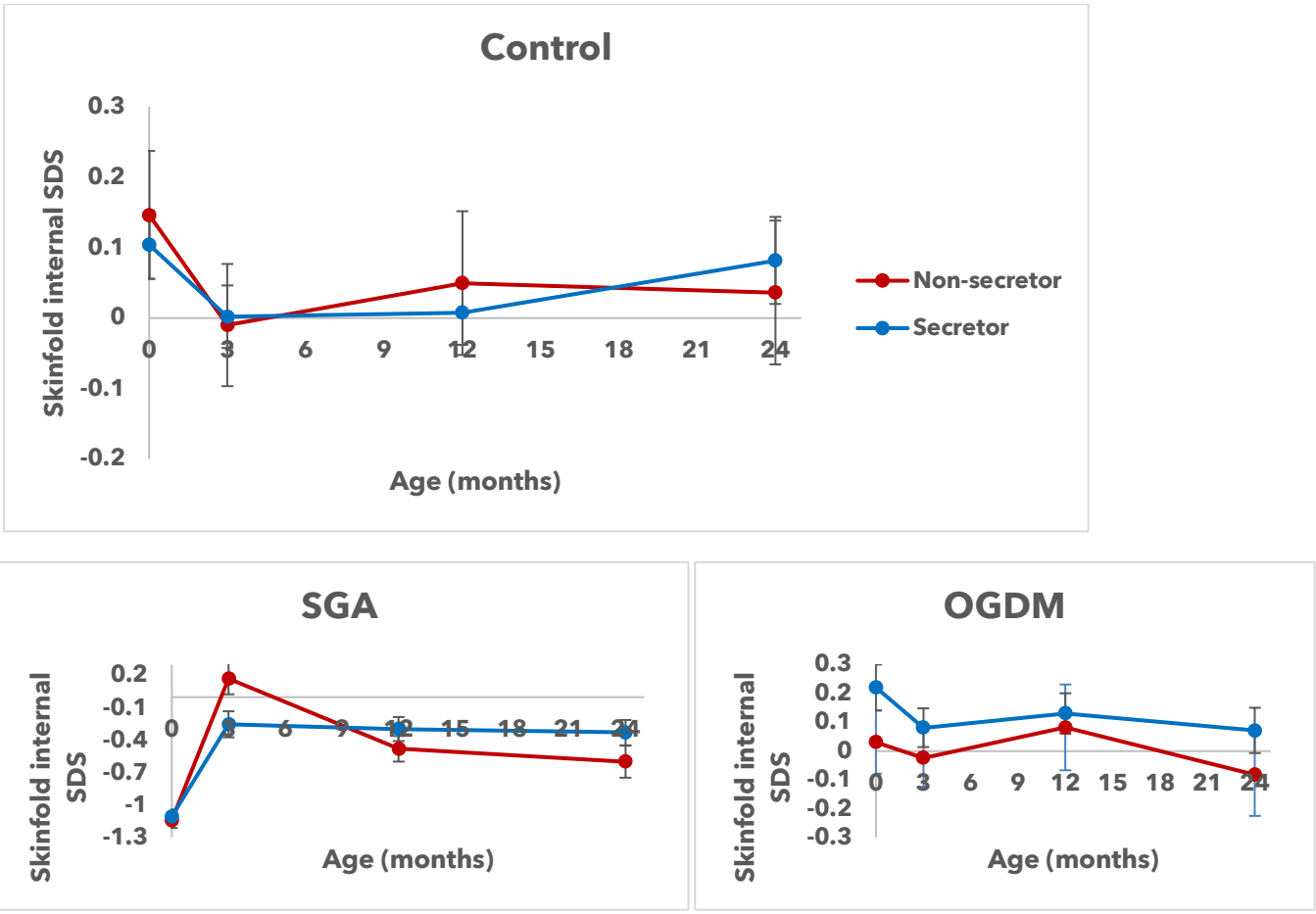
Figure 8.4 Infancy height trajectories between secretors and non-secretors
Values are mean±SEM. All SDS values are based on UK 1990 growth reference.



In skinfold trajectories, while there was little difference between secretors versus non-secretors among both controls and OGDM (Figure 8.5), SGA non-secretors caught-up more than the secretors, resulting in significantly greater adiposity at 3 months (*p=0.03*). The SGA non-secretors then showed significantly less adiposity gains compared to the secretors, especially between 3-12 months (*p=0.005*). An

identical pattern was also seen in BMI trajectories of SGA secretors versus non-secretors (*p* for 3-12 months BMI gain=*0.016*, Appendix 7).

Figure 8.5 Infancy skinfold trajectories between secretors and non-secretors
Values are mean±SEM. All SDS values are based on UK 1990 growth reference.



In the CBGS-BF (an ongoing CBGS cohort involving control infants only), infant morbidities were recorded carefully by mothers and then reported to the research team during each visit. Interestingly, the rate of infection and/or antibiotic exposure was slightly higher among the secretors in the first 3 months but slightly lower from

3-12 months compared to the non-secretor infants (Table 8.1). However, statistical significance was not reached, possibly due to lack of power and imbalanced classification (lower than expected number of non-secretor infants).

Table 8.1 Number of infants with infection/antibiotic exposure based on their *FUT2* secretor status

Values are N(%)

Time period	Infant FUT2 status		
	Secretor	**Non-secretor**	***p***
0-6 weeks	10 out of 74 (14%)	1 out of 12 (8%)	*0.618*
6 weeks-3 months	15 out of 74 (20%)	1 out of 12 (8%)	*0.324*
3-6 months	11 out of 74 (15%)	3 out of 12 (25%)	*0.378*
6-12 months	18 out of 71 (25%)	4 out of 12 (33%)	*0.562*

8.3.1.2 In relation to hormonal and lipid biomarkers

As described in Chapter 6, capillary IGF-1 and C-peptide concentrations were measured in the 3 infant groups at 12 months of age, although the number of samples from non-secretors were small, especially for C-peptide measurement (Table 8.2).

Table 8.2 Number of subjects in each infant groups with IGF-1 and C-peptide results at 12 months based on their secretor status

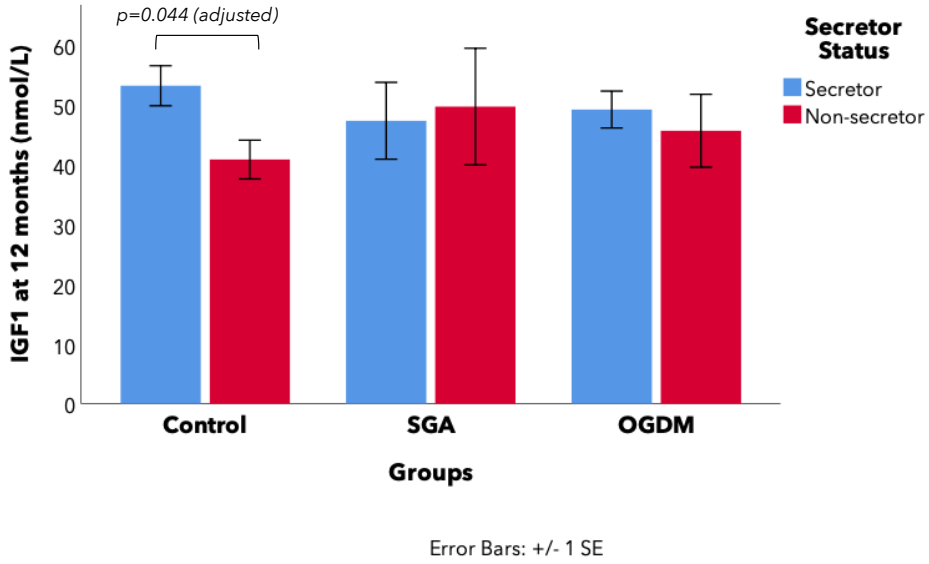
	Controls	**SGA**	**OGDM**
IGF-1			
Secretors	64	15	53
Non-secretors	26	6	13
C-peptide			
Secretors	26	15	13
Non-secretors	6	5	7

IGF-1 levels at 12 months were different between secretor and non-secretor only among controls, with higher level among secretors (mean±SD=50.9±24.8 vs 43.4±19.1, respectively, Figure 8.6). This difference persisted after adjustment for infant sex and age at 12 month-visit.

From a previous CBGS study among controls only, apart from 12 months, capillary IGF-1 levels were also measured at other time points: 3, 18, and 24 months. At 3 months, secretor controls (N=80) also had higher IGF-1 level at 3 months compared to non-secretors (N=30), *p=0.007*. However, this significant difference was confounded by 0-3 months infant feeding history (with more breastfed infants among non-secretors), and was attenuated after adjustment for infant sex, gestational age, age at visit, and infant feeding history (*p=0.056*, Appendix 8). No difference was observed at 18 and 24 months.

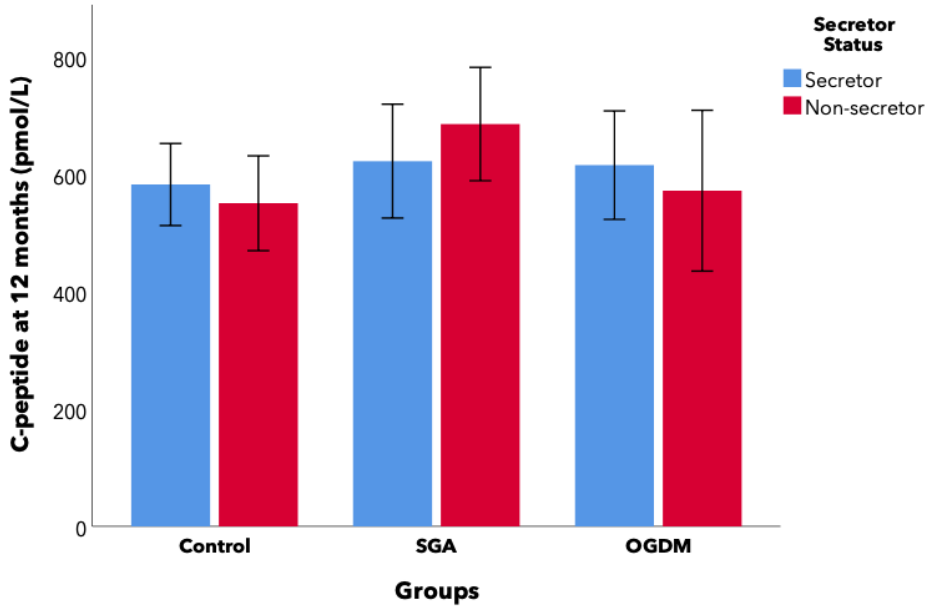
There was no difference in IGF-1 level at 12 months between secretors and non-secretors among infants born SGA and OGDM, although numbers were small.

Figure 8.6 IGF-1 level at 12 months between secretors and non-secretors in each infant group



With respect to capillary C-peptide level, there was no significant difference between secretors and non-secretors in any of the 3 infant groups (Figure 8.7).

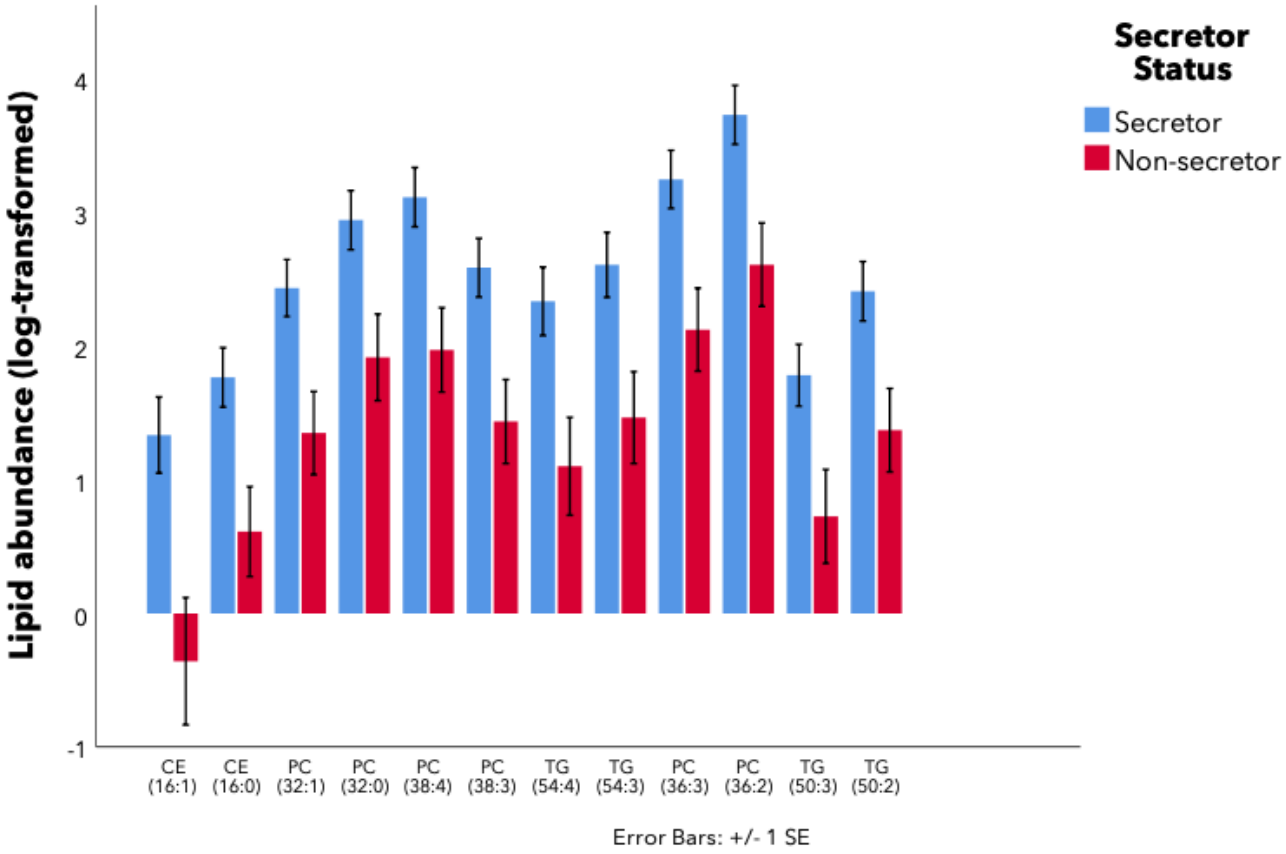
Figure 8.7 C-peptide level at 12 months between secretors and non-secretors in each infant group



Lipidomic analysis by infant secretor status was only possible among controls due to lack of SGA and OGDM subjects with both genotyping and lipidomic data. As illustrated in Figure 8.8, there were significantly higher abundance of several infant capillary lipid species among secretor controls (N=69) compared to the non-secretor counterparts (N=21) measured at 3 months.

Figure 8.8 Comparison of lipid abundance between secretors versus non-secretors among control infants

Representatives of lipid species are shown in the graph. All *p values* <0.05



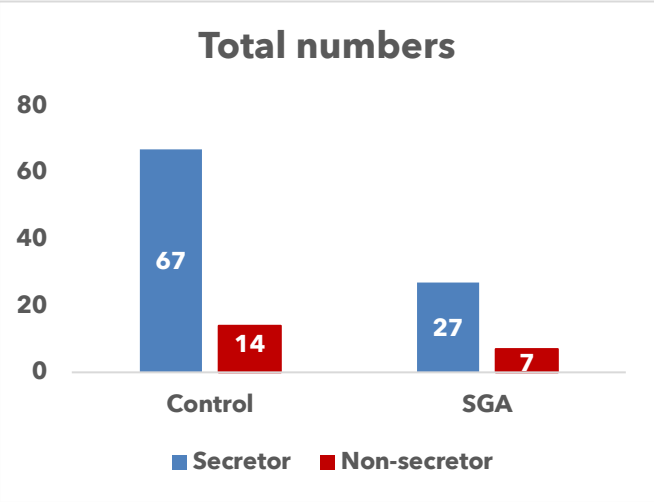
8.3.2 Maternal FUT2 secretor status

8.3.2.1 In relation to infant growth and adiposity

The maternal FUT2 genotypes were available only for subsets of CBGS controls and SGA. From the total of 115 mothers, 83% of control mothers were secretors and 79% of mothers of SGA infants (Figure 8.9).

Figure 8.9 Prevalence of FUT2 secretor and non-secretor among CBGS mothers

Total N=115



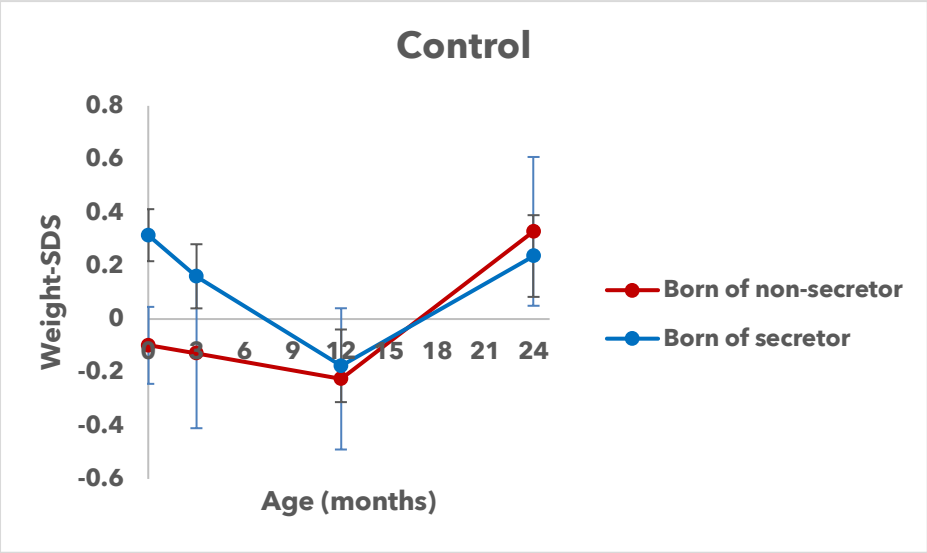
Group	Non-secretors (%)	Secretors (%)	*p (against controls)*
Control	17.3	82.7	
SGA	20.6	79.4	*0.676*
All	18.3	81.7	

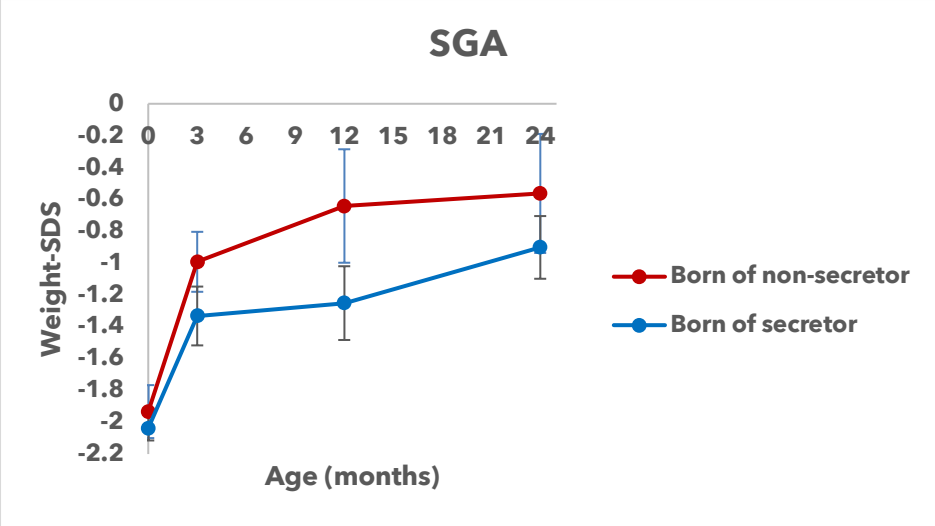
Regarding infancy weight, control infants born of secretor mothers were slightly heavier at birth than infants of non-secretor mothers (*p=0.064*, unadjusted). By contrast, SGA infants born of secretor mothers had lower weight gain to 24 months than SGA infants of non-secretor mothers (Figure 8.10).

Similar patterns were observed for infant height and adiposity (BMI and skinfolds) gains: 1) control infants born of secretor mothers were longer and more adipose at

birth than control infants of non-secretor mothers; 2) SGA infants of secretor mothers appeared to gain less in length and adiposity throughout infancy, especially between 0-3 months (Appendix 9-11). However, numbers were small and none of these differences was statistically significant.

Figure 8.10 Infancy weight trajectories according to maternal *FUT2* secretor status
Values are mean±SEM. All SDS values are based on UK 1990 growth reference.





As mentioned previously, only control infants (from CBGS-BF cohort) had available data on morbidities during infancy. In this group, the incidence of infection and/or exposure to antibiotics at all time points was comparable, e.g. 18% at 6 weeks among infants born to secretor mothers vs 22% of infants of non-secretors , *p=0.754*.

8.3.2.2 In relation to human milk oligosaccharides

This analysis was conducted only among control mothers in the CBGS-BF, who exclusively breastfed their infants from birth to 6 weeks. As shown in Figure 8.11, there was a clear difference in HMO profile between secretor (total N=39) and non-secretor mothers (total N=5). Secretors' milk was abundant in 2FL, while non-secretors produced little to none of this HMO species. LNNT was also produced at a higher level in the milk of secretor mothers. On the contrary, 3-FL was in much higher in the milk of non-secretors compared to secretors.

Over time, there was a general decreasing trend in 2FL among secretors, and decreasing trends in LNT, LNnT, and 6SL among both groups. Conversely, increasing trends were observed for 3FL and 3SL in both groups.

The associations between HMO intake and infant growth and adiposity outcomes were then explored. The intake of each HMO species at 6 weeks was calculated by multiplying its concentration (g/L) by BM intake volume (L/day, details in Section 8.3.2). Of 7 HMO species included in the analyses, 4 of them: 2-FL (only present in secretors), 3-FL, 3SL, and 6SL, showed significant positive correlations with growth and adiposity parameters at 6 weeks and 3 months (Table 8.3).

From Table 8.3, 2-FL and 6SL intake appeared to have the strongest associations to infant weight and adiposity outcomes at 6 weeks and 3 months. However, total BM intake seemed to affect these associations since the significance disappeared after adjusting for this factor. Only 6SL correlations with weight at 6 weeks as well as weight and subcutaneous adiposity at 3 months remained significant even after adjustment for total BM intake. No associations found between HMO intake and infant growth parameters beyond 3 months.

Figure 8.11 Distinct profiles of human milk oligosaccharides (HMO) between secretors and non-secretors

2-FL=2-fucosyllactose, 3-FL=3-fucosyllactose, LNFP I=lacto-N-fucopentaose I, LNT=lacto-N-tetraose, LNnT= lacto-N-neotetraose, 3SL=3′-sialyllactose, 6SL=6′-sialyllactose

y axis = HMO concentration (g/L), x axis = time point (weeks)

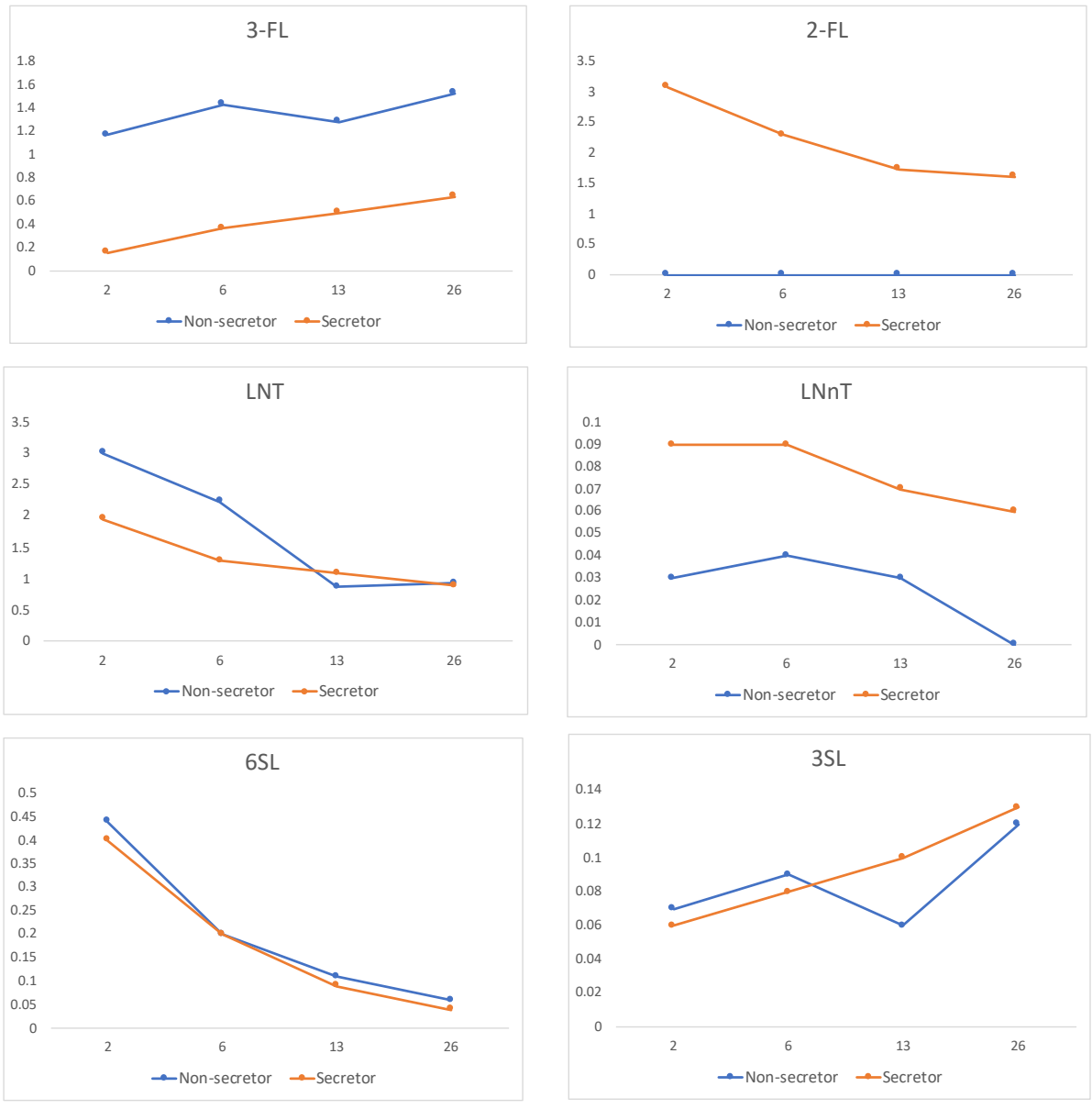


Table 8.3 The associations between HMO intake and early infancy growth parameters

All infants are from control group

Total N in 2FL model=35, 3FL=40, 6SL=40

Weight, length, and BMI SDS are based on UK 1990 growth reference. SFT SDS are internally-derived, adjusted for infant sex, gestational age, and age at visit.

2FL models only involved infants born of secretor mothers

p values are adjusted for infant sex, gestational age, and postnatal age at measurement. 3FL and 6SL models were additionally adjusted for maternal *FUT2* secretor status. Significant *p* values are written in bold.

*remained significant even after adjusting for BM intake volume (kg/day)

2-FL=2-fucosyllactose, 3-FL=3-fucosyllactose, 3SL=3'-sialyllactose, 6SL=6'-sialyllactose, HC=head circumference, WC=waist circumference

Outcomes: Growth parameters	**Predictors: HMO intake at 6 weeks (g/day)**							
	2-FL		**3-FL**		**3SL**		**6SL**	
	R	***p***	**R**	***p***	**R**	***p***	**R**	***p***
6 weeks								
Weight SDS	0.44	***0.008***	0.31	*0.052*	0.32	***0.05***	0.57*	***<0.0001***
Length SDS	0.4	***0.016***	0.13	*0.413*	0.25	*0.129*	0.43	***0.005***
HC SDS	0.38	***0.037***	0.32	*0.069*	0.24	*0.18*	0.58*	***<0.0001***
BMI SDS	0.31	*0.071*	0.34	***0.028***	0.26	*0.108*	0.47	***0.002***
Mean SFT SDS	0.4	***0.016***	0.3	*0.056*	0.37	***0.02***	0.48	***0.001***
WC	0.41	***0.013***	0.15	*0.36*	0.28	*0.08*	0.41	***0.009***
3 months								
Weight SDS	0.37	***0.029***	0.24	*0.138*	0.22	*0.176*	0.56*	***<0.0001***
Length SDS	0.38	***0.024***	0.06	*0.725*	0.28	*0.093*	0.38	***0.014***
HC SDS	0.35	*0.061*	0.3	*0.087*	0.15	*0.426*	0.51*	***0.002***
BMI SDS	0.25	*0.149*	0.31	***0.048***	0.12	*0.49*	0.53*	***<0.0001***
Mean SFT SDS	0.18	*0.297*	0.28	*0.079*	0.4	***0.012***	0.45	***0.003***
WC	0.2	*0.247*	0.31	*0.055*	0.26	*0.11*	0.37	***0.017***

Figure 8.12 Correlation between HMO intake and infant weight at 6 weeks
SDS are based on UK 1990 growth reference

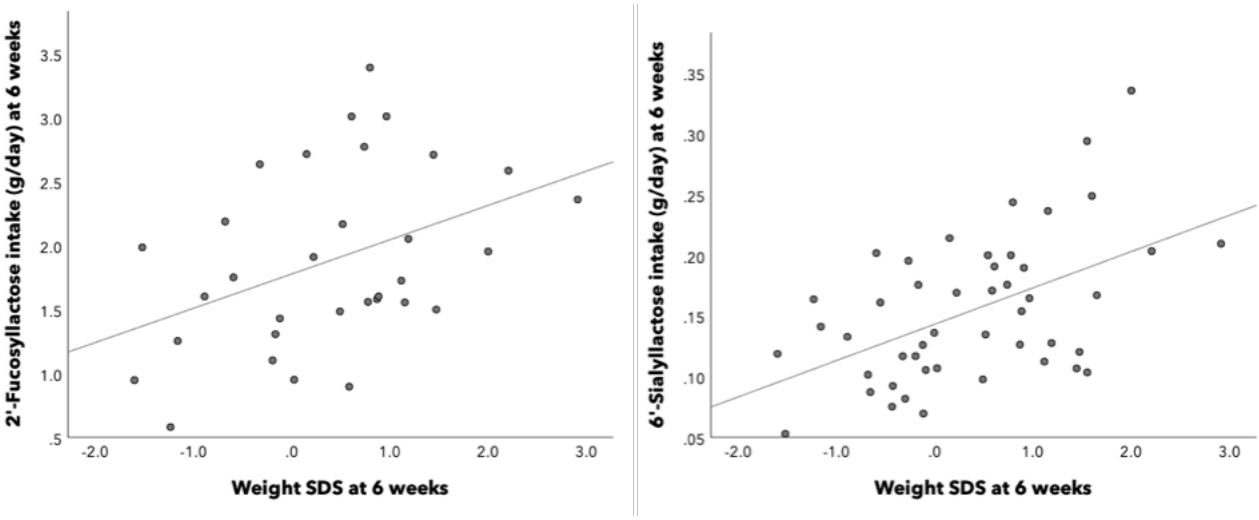
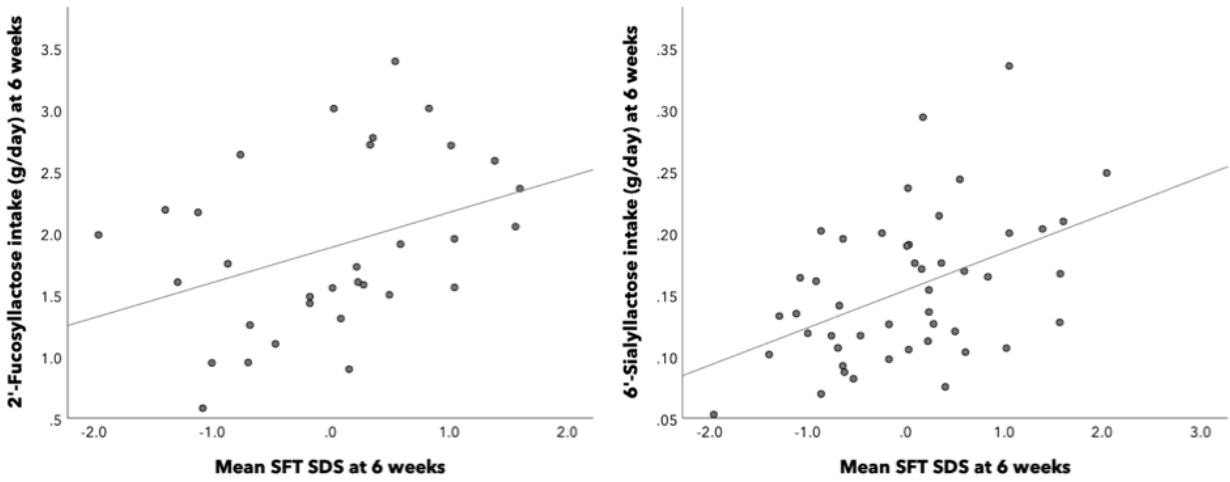


Figure 8.13 Correlation between HMO intake and infant subcutaneous adiposity at 6 weeks
SDS are internally-derived, adjusted for infant sex, gestational age, and age at visit



8.4 Discussion

This study explored the influence of maternal and infant *FUT2* genotypes on early growth and adiposity among infants with different *in utero* exposures.

Compared to controls, SGA and OGDM had a higher percentage of non-secretors, especially OGDM. This may become another clue relating *FUT2* inactivity with T1D, following the established higher T1D susceptibility among non-secretor individuals[213,312]. From the literature, Smyth *et al* (2011) linked *FUT2* non-secretor allele with higher T1D risk while also providing resistance to Norovirus infection[213]. This was supported by a recent study (2019) involving 1831 infants that reported higher respiratory and gastrointestinal infections among *FUT2* negative infants between 6-24 months[210]. The comparison of infection rate during infancy according to the infant's secretor status did not reach significance in the CBGS.

Similar to that, maternal *FUT2* positivity in the CBGS also did not correlate with infection risk during infancy. This was in contrast to a 2019 study involving *FUT2* and *FUT3* genotyping in several Asia, Africa, and Latin America countries. In this study, infants born to secretor mothers had a higher risk of any type of diarrhoea (hazard ratio 1.38, confidence interval 1.15-1.66)[311].

With respect to growth and adiposity, there was no significant difference observed between secretors and non-secretors among control infants and OGDM, despite the higher IGF-1 level at 12 months and overall lipid abundance measured at 3 months among secretor controls. Conversely, *FUT2* positivity affected the adiposity trajectories of SGA, especially between 3-12 months. In the first 3 months, all infants caught-up in weight and adiposity as the whole SGA group (Chapter 4), but those with inactive *FUT2* showed more pronounced gains, especially in subcutaneous adiposity. They then caught down between 3-12 months while the secretors gained

adiposity more stably during the period. It is also intriguing to hypothesise that *FUT2* inactivity may accentuate the typical growth trend in this group of infants.

From the perspective of maternal *FUT2* genotypes, there was also no statistically significant differences in growth between infants born of secretor or non-secretor mothers in both controls and SGA. This finding supports a study by Sprenger *et al*[316] that found no effect of maternal secretor status and the growth of their infants until 4 months of age. However, several apparent differences, although not significant, still need further longitudinal investigation on a larger cohort, especially heavier at birth among secretor controls and less weight and height catch-up among secretor SGA, compared to the secretors in their corresponding infant groups.

FUT2 enzyme defines HMO production, in quantity and in quality, with lower abundant and less diverse HMO produced by non-secretor mothers[216]. In this study, in concordance with the literature[216,321], the non-secretors produced negligible to none 2-FL, much lower LNnT, and higher amount of 3-FL compared to the secretor counterparts. Therefore, 2-FL analyses only involved individuals with active *FUT2*.

Out of 7 HMO species involved in the analysis, the intake of 4 of them showed a strong positive association with infant's growth and adiposity: 2-FL and 3-FL as neutral and fucosylated while 3'SL and 6'SL are sialylated and acidic HMO. From all of them, 6SL appeared to have the strongest associations with all growth and adiposity parameters.

A recently published large study in Malawi examined the relations between HMO contents and infant's growth and development[322]. Involving 659 subjects, they

failed to present any associations between fucosylated HMO with growth, unlike 2-FL and 3-FL in this study. In contrast, 3SL and 6SL correlated with HC gain between 6-12 months in that Malawian study, but in different directions, positive for 3SL but negative for 3SL. In the CBGS, these 2 sialylated HMO, especially 6SL, were both positively associated with all parameters at much earlier time points: 6 weeks and 3 months.

Sialylated oligosaccharides contain sialic acid (SA) residues that provide plenty of benefits to infant's health and growth, especially during neonatal period. These include immune maturation, protection to pathogenic agents, and optimal gut function. Moreover, since SA is an essential component of brain gangliosides, SA is important during cognitive development[323].

In this study, similar to the other research on *FUT2*, low power is a common problem when comparing secretors versus non-secretors. This is caused by an imbalance in classification since the proportion of non-secretors is at least 3 times lower than secretors. Due to being underpowered, it was therefore not always possible to present models that are fully adjusted for covariates. A similar study in a much larger cohort is then encouraged, involving SGA, OGDM, and control infants, to replicate and confirm these findings. In addition, microbiota in breast milk and infant gut would help in completing the missing link between maternal *FUT2* status and infant's growth outcomes.

8.5 Conclusions

From the preliminary results presented in this chapter, both maternal and infant *FUT2* secretor status may have impact on infancy growth, adiposity, and overall health, presumably via HMO production (maternal) and host resistance to infections or putative gut microbiota changes (infant). More thorough and longitudinal investigations in a larger population are needed to confirm the findings of this study.

Chapter 9 Discussion

This chapter summarises each work of this thesis by describing how it has or has not addressed the hypothesis (Table 1.6), reflecting on its limitations, and generating questions for further research. Besides, strengths and limitations of the study in general are also displayed, followed by future plan and direction of the study (CBGS).

9.1 Results and implications of each study

9.1.1 Growth trends in offspring of mothers with GDM (Chapter 3)

Hypothesis/research question:

Changes in GDM diagnosis criteria and treatment modalities in the last decade would have impacted growth outcomes among offspring of mothers with GDM.

Methods:

The hypothesis was tested by comparing growth and adiposity outcomes between 2 groups of offspring of mothers with GDM born in non-overlapping years

Key findings:

While the 'earlier' OGDM (born in 2001-2009) still exhibited the classical features of OGDM (heavier and of more adipose at birth, followed by catch-down weight gain

until 12 months of age), 'recent' OGDM (born in 2011-2013) displayed normalisation of birth weight and reduced adiposity at birth, followed by increased weight and adiposity gains in the first 3 months. Despite those gains, the 'recent' OGDM group had persistently lower subcutaneous until 24 months. Although unexpected, these findings actually provided compelling insights to the earlier studies reporting normalisation of neonatal body composition among OGDM by Au[240] and Logan *et al*[179].

Furthermore, since the 'recent' OGDM was born of more obese mothers with a higher level of hyperglycaemia during pregnancy compared to the 'earlier' OGDM, defined by the same IADPSG criteria, the differences observed between these 2 groups were unlikely to be caused by the inclusion of milder GDM cases. Rather, given the non-overlapping period of mothers recruited and treated for GDM in the 2 populations, it can be hypothesised that the 'recent' OGDM outcomes might relate to the more intensive GDM management in this group, including tighter glycaemic control and the use of metformin that can cross the placenta. Although there were no detailed GDM medication data on the 'earlier' group, the use of metformin during that period was almost non-existent. However, this requires more investigations to explore the interplay between this environmental factor and genetic predisposition affecting OGDM, as well as the long-term consequences of this birth size normalisation and reduced adiposity during infancy.

Limitations:

1. Detailed medication history was only available to 'recent' OGDM, but not among 'earlier' OGDM, and it has limited the analysis on how maternal GDM treatment associates with the offspring's growth outcomes.

2. Detailed antenatal information (especially pregnancy weight gain) and glucose control during pregnancy among GDM mothers were not available to all women in the study.
3. Detailed infant body composition assessment, for example by MRI or ADP, was not conducted in the study and offspring's body composition parameters were only reflected from skinfold thickness measurement and adiposity indices, such as BMI and PI. As sustained reduced adiposity was one of the distinctive growth features among 'recent' OGDM, a more accurate estimation of adiposity will be of benefit.

Implications:

The normalisation of birth weight among 'recent' OGDM, presumably due to more stringent GDM management, is likely to be advantageous at birth as it could reduce the risk of perinatal complications related to macrosomia, e.g. prematurity, shoulder dystocia, Caesarean delivery, hypoglycaemia, and jaundice. However, the potential longer-term metabolic implications still need to be explored, especially with regard to its distinct postnatal growth trajectories: rapid weight gain immediately after birth (0-3 months) and continuing reduced adiposity (at least until 24 months).

Further questions to investigate:

1. Will reduced adiposity at birth observed among 'recent' OGDM encourage subsequent 'catch-up' growth and increased metabolic risk in later life?
2. Will the trend of more stringent GDM management lead to more SGA deliveries in the future?

3. How to find the trade-off of GDM diagnostic criteria and treatment modalities that best reduce maternal and infant morbidities during ante- and early postnatal periods, but also possess the least future metabolic risk for the infants?

9.1.2 Investigating early life physical and biochemical similarities between SGA and OGDM, in separate comparison to controls (Chapter 4, 5, and 6)

Hypothesis/research question:

As SGA (typically, although not always, reflecting insufficient placental nutritional transport) and OGDM (representing *in utero* hyperglycaemia and hyperinsulinemia), possess similar adulthood risks despite having opposite *in utero* conditions, there should be metabolic commonalities shared during early life. The similarities (or differences) between infant groups could be influenced by antenatal/maternal factors and early postnatal exposures (growth and early feeding).

Methods:

The research question was addressed by comparing physical and biochemical markers between SGA and OGDM, separately against controls. Physical parameters included weight, height, subcutaneous and abdominal adiposity, while biochemical analytes consisted of IGF-1, C-peptide, and lipidomics. OGDM refers to 'recent' OGDM born in 2011-2013 from CBGS2.

Key findings:

All the comparisons between SGA and 'recent' OGDM against controls discovered in this thesis are summarised in Table 9.1.

Table 9.1 Significant differences between SGA/OGDM versus controls

[a]'Recent' OGDM
[b]significant between SGA and controls (*p=0.045*) and approached significance between OGDM and controls (*p=0.056*)
[c]similar results but only significant among OGDM

	Compared to controls		
	SGA and 'Recent' OGDM	**SGA only**	**'Recent' OGDM[a] only**
Maternal factors	• More diverse ethnicities • More deprived household (higher IMD) • More primiparous (only significant among SGA) • Shorter • Higher smoking rate during pregnancy	More primiparous	• More obese (higher pre-pregnancy BMI) • Higher pregnancy OGTT
Birth characteristics	• Delivered at earlier GA • Higher C-section rate	Higher rate of twin pregnancy	
Growth and adiposity	• Higher weight and adiposity gains between 0-3 months (especially among SGA) • Reduced subcutaneous adiposity between 3-24 months • Lower visceral abdominal fat	• Smaller and shorter at all time points • Lower waist circumference at all time points • Higher catch-up rate in weight, height, and skinfold gains from 0-12 months	• Reduced statural gain from 0-3 months • Reduced weight, height, and skinfold gains from 3-12 months • Increased weight and height gains from 12-24 months

	thickness at all time points	• Higher catch-down rate in weight and height from 0-12 months
Hormones	Lower capillary IGF-1 level at 12 months[b], affected by infant sex, feeding type, and infancy growth pattern	Positive association between capillary IGF-1 level at 12 months with growth parameters at 24 months
Lipidomics	• Distinct separation against controls in multivariate models reflecting lipid abundance • Generally lower abundant in glycerolipids, sphingolipids, and glycerophospholipids, but higher in sterol lipids	

Growth and adiposity parameters

While 'recent' OGDM did not display classical birth anthropometry and postnatal growth features (Chapter 3), the contemporaneously-recruited SGA infants performed comparably to the literature[21,139] with typical rapid postnatal catch-up in weight and adiposity. These excessive gains were clearly seen during the first 3 months of life occurred not only among those with birth weight lower than -2 SDS, but also among those between -1.5 to -2 SDS. Although catch-up growth among SGA is not a novel finding, the contribution of more rapid fat mass development in only 3 months postnatally among this group of infants was still interesting to

observe, which could indicate altered metabolism and a thrifty phenotype caused by inadequate nutrition during fetal period[21,23,24].

The similar growth outcomes observed in SGA and 'recent' OGDM included rapid weight and adiposity gains immediately after birth (between 0-3 months), reduced subcutaneous adiposity between 3-24 months, and lower medial/visceral abdominal fat thickness (reflecting fat between internal organs) at all time points (Table 9.1).

Hormonal and lipidomic outcomes

From this study, both SGA and OGDM had lower capillary IGF-1 level measured at 12 months compared to controls (Table 9.1). In all groups, infant sex and feeding type had a significant impact on IGF-1 level, with female and/or mixed/formula-fed infants had a higher level, similar to reports in the literature[200,260]. Interestingly, the associations between IGF-1 and catch-up growth pattern were clearer among controls and OGDM, but not with SGA. In both controls and OGDM, IGF-1 levels at 12 months were positively associated with and could predict growth parameters at 24 months, which was in concordance with the previous findings[264,265].

Meanwhile, C-peptide level at 12 months did not seem to differ across these infant groups of different *in utero* exposures. However, the positive correlation between this hormone and visceral abdominal fat thickness is novel and deserves further studies.

Unbiased lipidomics can be useful to recognise potential lipid biomarkers among infant groups at risks, in comparison to a control group, using small samples in dried

blood spots. In this study, a distinct separation was observed between SGA and controls as well as between OGDM and controls, but not between SGA and OGDM. Moreover, these 2 groups of infants with a history of *in utero* nutritional insults had similar patterns of lipid abundance against controls, persisting from 3 to 12 months: a generally lower abundance of glycerolipids, sphingolipids, and glycerophospholipids, but higher of sterol lipids, possibly influenced by PPARα[278]. However, interpretation of these results as the aetiology or the implication of being SGA/OGDM would need more sets of mechanistic studies. In addition, research to validate and confirm the correlations found between those lipid groups (especially glycerolipids) with growth gains in SGA and OGDM in this study are also of importance.

Using lipid ratios to represent 3 key enzymes in lipid metabolism, SCD1, FADS1, and FADS2, SCD1 activity appeared as the most promising proxy that could predict subsequent weight and adiposity gains, especially among the control infants. This is in concordance with previous studies on animals and humans that have linked SCD1 activity to obesity and metabolic risks[283,287]. Validation is still needed, especially among SGA and OGDM where the results were more variable, and should be complemented with the measurement of enzymatic activity.

Limitations:

1. Adiposity assessment in this study only involved subcutaneous depot derived from skinfold thickness measurement and abdominal compartment from ultrasound estimates. Detailed infant body composition assessment that includes total and regional fat- and fat-free mass can be advantageous, especially when conducted in the first 3 months of life.

2. The IGF-1 and C-peptide results from this study need further confirmation in a larger cohort involving SGA and OGDM with hormones being measured at the same time with stricter sampling and processing protocols.
3. Hormone measurement being analysed in this study was only from 1 timepoint at 12 months and therefore cannot observe the change of IGF-1/C-peptide during infancy/childhood.
4. The hormonal study did not include other hormones that could influence infant growth, e.g. IGF-2, adiponectin, leptin, cortisol, etc.
5. The evaluation of how much postnatal feeding (compared to *in utero* conditions) affecting distinctive lipidomic features between SGA or OGDM separately against controls cannot be effectively performed since the proportion of formula-feeders among controls was too small.
6. Complementary feeding was not included as a covariate in the regression models. Although this information was recorded (as food diary) at 6, 12, and 24 months of age, the nutritional analysis had not been completed at the time of writing this thesis.

Implications:

Any similar characteristics between SGA and OGDM observed in this study, both physical and biochemical, could implicate metabolic derangements that may sustain from early life (childhood) to later life (adulthood).

With regard to growth, the first 3 months of life deserves further investigation with more frequent and detailed measurements (i.e. at 2, 4, 6, 8 weeks of age) since during this period, both SGA and OGDM gained weight and adiposity rapidly. More

studies should also be conducted to demonstrate how this period could affect later metabolic risk. If the effect is substantial, this period should be considered as a critical window of opportunity during this period where growth is highly modifiable, infant feeding/nutrition is relatively easier to control, and the metabolic impact is long-lasting.

Besides, persisting lower visceral abdominal fat thickness observed in both groups at all time points deserves further study, especially with regard to its implications to later health and metabolic risks.

The hormonal study in this thesis could not sufficiently address the question of how similar/different IGF-1 and C-peptide levels between infants at risk (SGA/OGDM) and controls due to technical issues. Besides, other hormones with biologically plausible effects on growth also need to be measured, including IGF-2, leptin, adiponectin, insulin, and cortisol. However, this work could confirm that IGF-1 and C-peptide seemed to relate with growth gains not only among controls, but also among SGA and OGDM.

Although lipid metabolism is complex and dynamic, several lipid species that distinguished SGA and OGDM from controls could serve as candidate early life biomarkers. Further mechanistic studies are needed to investigate 1) if those lipids were causing or resulting from being born SGA/OGDM deserves further investigations, 2) the extent of early nutrition affecting those distinguishing lipids, 3) the best time point to capture these potential biomarkers.

As key enzymes in lipid metabolism, SCD1, FADS1, and FADS2 activities could potentially predict subsequent growth and adiposity gains during infancy among

controls. The lack of associations between the lipid ratios (acting as proxies to those enzymes) among SGA and OGDM need to be explored further.

Further questions to investigate:

1. How would subgroups of SGA (symmetrical vs asymmetrical, with versus without indicators of placental insufficiency, with versus without pre-eclampsia or other maternal hypertensive disorders, with or without chromosomal abnormalities, etc) differ in growth pattern and adiposity outcomes during infancy, as well as in the biochemical and lipidomic profiles?
2. As growth and adiposity outcomes between the 'recent' and 'earlier' OGDM were of significant difference, comparing biochemical and lipidomic properties between these 2 OGDM groups would be of importance.
3. How could complementary feeding and weaning period influence, confound, or mediate the commonalities as well as the differences between SGA and OGDM compared to controls?

9.1.3 Breast milk and *FUT2* exploratory study

Aims:

1. To investigate how much exclusive breastfeeding duration could influence immediate and subsequent infant growth and adiposity development
2. To explore the associations between both breast milk macronutrient *concentrations* and *intake* with infant growth and adiposity

3. To examine the associations between both maternal (via HMO) and infant *FUT2* polymorphisms and infant growth, adiposity, and overall health

Methods:

The concentrations of lactose, protein, triglycerides (representing breast milk macronutrients), and 7 HMO species were measured from hindmilk samples at 6 weeks of age. Breast milk intake volume was estimated via dose-to-the mother deuterium oxide turnover technique using maternal and infant urines collected between 4-6 weeks of age. The value was used as the multiplication factor to assess each breast milk macronutrient and HMO intake. *FUT2* secretor status was analysed from both maternal and infant saliva.

Key findings and implications:

From this study, exclusive breastfeeding could protect against early childhood adiposity and this effect was dose-dependent. However, it was highly confounded by the introduction of mixed-feeding and BM intake volume. There was also evidence for 'reverse causality' during early infancy: poor initial growth gains leading to formula introduction and consequently greater weight and adiposity gains in later infancy.

In agreement with the existing literature[204,302], BM lactose and protein intake were positively associated with later growth gains. However, the lack of associations between BM fat intake and growth was unexpected since the previous studies have

consistently reported inverse associations between BM fat and subsequent infant adiposity[304,305].

Confirming the CBGS previous publication[293], butyrate content, but *not* intake, was negatively associated with infant's growth and adiposity parameters. It is then intriguing to hypothesise that the association between BM butyrate and infant growth is either mediated or confounded by intake volume due to 2 reasons: 1) butyrate concentration was negatively correlated with BM intake volume, and 2) butyrate has been evidenced to reduce appetite and food intake in mice[301].

The influence of infant and maternal *FUT2* status on infant growth was explored in this study. Both SGA and OGDM had a higher proportion of non-secretors although it was only significant among OGDM. SGA infants with inactive *FUT2* also gained more weight and adiposity between 3-12 months compared to the secretor SGA. Among controls, *FUT2* secretor status may associate with a higher abundance of lipids measured at 3 months and higher IGF-1 level at 12 months.

No significant associations found between both maternal and infant *FUT2* status with infection and antibiotic consumption during infancy. However, this could be due to limited variation to observe, since this cohort consisted of healthy infants born of healthy mothers without any significant pregnancy complications. There were interesting positive associations between 4 out of 7 HMO species being investigated in the study, especially 6 sialyllactose (6SL), with infant growth and adiposity outcomes.

Although preliminary, the results from the *FUT2* analyses provided more clues into inherent factors influencing growth and need to be replicated in larger infant cohorts.

Limitations:

1. Although the application of strict criteria had resulted in a clean and well-designed cohort, the results of the study were often not significant due to too low samples.
2. Breast milk study was only conducted among controls, but not SGA and OGDM.
3. Maternal *FUT2* genotyping analysis was only available to SGA and controls, but not OGDM.

Further questions to investigate:

1. Breast milk intake volume negatively related to protein and butyrate concentrations. Could it reflect that infants have an innate ability to limit or regulate the intake of breast milk based on its nutritional content, e.g. consume less if the milk contains too much protein or butyrate, and vice versa?
2. Is there any cut-off for optimal breastfeeding duration for best possible infant growth and adiposity outcomes (e.g. the benefits could only be obtained if the infant is breastfed for *at least* "x" months/weeks) or is it completely a dose-dependent effect (i.e. the longer the duration, the more benefit obtained)?
3. Since FUT2 enzyme defines the synthesis and secretion of soluble blood antigens in other body fluids and intestinal mucosa, could *FUT2* polymorphisms affect nutritional absorption in the gut and digestive system in general?

4. The influence of HMO in modulating infant gut microbiota and how it would affect infant growth, adiposity and general health: is it more about the variety of HMO or is there any species with remarkable effects than the others?

9.2 Strengths and limitations of the study in general

All subjects were part of the Cambridge Baby Growth Study which were recruited from a single maternity unit and can be a representative population of the East of England, and thus, where relevant, all growth parameters were converted into SDS using the UK 1990 growth reference. The main anthropometric measures in this study included weight, length, skinfold thickness to reflect subcutaneous adiposity, and abdominal adiposity consisting of waist circumference and abdominal fat thickness. As most study participants were of White/British/Caucasian origin and with the use of the UK 1990 growth reference, the results of this study should be applicable to British and European/Caucasian populations.

The main strengths of this study originated from the design of CBGS as a longitudinal birth cohort with wide-ranging data and biological samples collection during the first 2 years of life. The longitudinal aspect of the study had made it possible to examine infant's growth as a continuous variable and to classify them into catch-up, catch-down, or no-change, and see how they relate to subsequent growth or biochemical measures.

This study also employed more detailed anthropometric measurements apart from weight and height, such as skinfold and abdominal fat thicknesses, which could

provide more insights on body composition and fat depots. Additional visits at 2 and 6 weeks among controls had also obtained a closer and valuable look into early infancy period. This would be interesting to do similar observational studies across SGA infants and OGDM.

The comprehensive perinatal questionnaire (Appendix 1) allowed the statistical analyses performed in this thesis to be adjusted for demographic factors and potential covariates. In addition, the recently-recruited mother-infant pairs in the CBGS-BF were strictly controlled and thus excluding several factors that can potentially confound the main outcomes being observed, especially delivery method (all infants were vaginally born) and early feeding history (all infants were solely breastfed in the first 6 weeks).

The CBGS has also benefited from a team of dedicated paediatric research nurses who have been in the study across all cohorts, especially with regard to minimising measurement bias, keeping the families in the study, and collecting complete sets of biological samples. The least invasive method possible, heel prick, has also substantially helped to collect blood samples in a longitudinal infant cohort like this.

Another strength of this study is the long-established multiple collaborations with several research groups, in and outside the UK. This enables the optimal use of biological samples collected in the study for hormonal, lipidomic, and breast milk studies, especially when the precious samples were in small amount, e.g. dried blood spots. The measurement of breast milk intake volume using the dose-to-the-mother deuterium-oxide turnover technique was also helpful to augment the literature with more direct data demonstrating the relationship between breast milk nutritional content, consumed by infants, and their growth outcomes.

However, there are several limitations of this study to be acknowledged. First and foremost, since all parts of this thesis were categorised as observational studies, all results could only imply associations, *not* causalities. As the golden rule for any observational studies, any associations observed between a predictor/exposure with an outcome could potentially be due to chance, bias, or caused/affected by the covariates[324]. However, it is also important to note that observational study design is feasible, applicable, and more ethically suitable to conduct in paediatric populations.

This study was also not immune to missing data due to missing visits, which is a common problem in the nature of longitudinal studies. Where possible, if allowed by sufficient power and number of samples, it can be accounted for in the mixed models. Additionally, since ultrasound abdominal fat thickness was not measured at birth, it was not possible to observe the extent of abdominal fat gain during the most crucial time point, immediately in the first 3 months after birth.

Another limitation is the breast milk and maternal *FUT2* studies were not conducted uniformly across all 3 groups of infants. It would be then interesting to continue these studies and confirm the findings in more SGA and OGDM subjects. There was little paternal data available, which could have been useful and interesting to analyse, especially with the recent findings linking paternal body size and early childhood growth[70,74].

9.3 Future work of CBGS

With regard to the cohort work, the visits and biological samples collection of SGA infants, OGDM, and the newly-recruited controls in the CBGS-BF are still ongoing, especially for 36 months, following the CBGS study protocol. All infants from all groups in the CBGS have also been invited to return for a single visit called the Cambridge Baby Growth Outcome Study (CBGOS) when aged between 5-11 years old. The main endpoints of the CBGOS include body composition measurement using DXA and the assessment of insulin secretion and sensitivity via oral glucose tolerance test (OGTT). Blood pressure and heart rate are also recorded as other cardiovascular risk parameters. In addition, pubertal staging is also recorded by asking the participants to complete puberty-related questionnaires with or without their parent's help.

There has also been an ongoing microbiome study in the CBGS-BF, on mother's milk and infant's gut (stool). This analysis will provide substantial insight into the role of breast milk and its constituents on early microbiota acquisition, as well as the link between infant's gut microbiota and infancy growth/adiposity.

To expand the applications of this study into wider populations, there have been some collaborations conducted with other infant cohorts, such as the Hormonal and Epigenetic Regulators of Growth (HERO-G) in The Gambia and the Soweto Baby Growth Study in Soweto, South Africa. These collaborative projects would include growth comparisons, hormone measurements, and lipid profiling analysis.

References

1. Barker DJP. The fetal and infant origins of adult disease. *BMJ*. 1990;301:1111.
2. Rose G. Familial patterns in ischaemic heart disease. *Brit J Prev Soc Med*. 1964;18:75-80. doi:10.1136/jech.18.2.75
3. Barker DJP, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet*. 1986;1(8489):1077-1081. doi:10.1016/S0140-6736(86)91340-1
4. Barker D, Osmond C, Winter P, Margetts B. Weight in infancy and death from ischaemic heart disease. *Lancet*. 1989;2(8663):577-580. doi:10.31729/jnma.1018
5. Barker DJ., Godfrey K., Gluckman P., Harding J., Owens J., Robinson J. Fetal nutrition and cardiovascular disease in adult life. *Lancet*. 1993;341(8850):938-941. doi:10.1016/0140-6736(93)91224-A
6. WHO. *Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013-2020.*; 2013. doi:978 92 4 1506236
7. WHO. Obesity and overweight. http://www.who.int/mediacentre/factsheets/fs311/en/. Published 2020. Accessed December 22, 2020.
8. Nguyen DMD, El-Serag HB. The epidemiology of obesity. *Gastroenterol Clin North Am*. 2010;39(1):1-7. doi:10.1016/j.gtc.2009.12.014.The
9. WHO. Childhood overweight and obesity. http://www.who.int/dietphysicalactivity/childhood/en/. Published 2017. Accessed June 30, 2017.
10. Health and Social Care Information Centre. *Statistics on Obesity, Physical Activity and Diet: England, 2016.*; 2016. doi:978-1-84636-274-3
11. Kahn S, Hull R, Utzschneider K. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444:840-846. doi:10.1038/nature0548
12. Cornier MA, Marshall JA, Hill JO, Maahs DM, Eckel RH. Prevention of overweight/obesity as a strategy to optimize cardiovascular health. *Circulation*. 2011;124(7):840-850.

doi:10.1161/CIRCULATIONAHA.110.968461

13. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, Obesity, and Mortality from Cancer in a Prospectively Studied Cohort of U.S. Adults Eugenia. *N Engl J Med*. 2003:977-985.
14. Adams K, Schatzkin A, Harris T, et al. Overweight, Obesity, and Mortality in a Large Prospective Cohort of Persons 50 to 71 Years Old. *NEJM*. 2006;355(8):763-778. doi:10.1056/NEJMoa1505949
15. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*. 2018;14(2):88-98. doi:10.1038/nrendo.2017.151
16. Diabetes UK. *Facts and Stats*.; 2016. doi:10.1007/s11245-009-9073-4
17. Hall KD, Kahan S. Maintenance of Lost Weight and Long-Term Management of Obesity. *Med Clin North Am*. 2018;102(1):183-197. doi:10.1016/j.mcna.2017.08.012
18. Montesi L, Ghoch M El, Brodosi L, Calugi S, Marchesini G, Grave RD. Long-term weight loss maintenance for obesity: A multidisciplinary approach. *Diabetes, Metab Syndr Obes Targets Ther*. 2016;9:37-46. doi:10.2147/DMSO.S89836
19. Stettler N. Nature and strength of epidemiological evidence for origins of childhood and adulthood obesity in the first year of life. *Int J Obes*. 2007;31(7):1035-1043. doi:10.1038/sj.ijo.0803659
20. Druet C, Stettler N, Sharp S, et al. Prediction of childhood obesity by infancy weight gain: An individual-level meta-analysis. *Paediatr Perinat Epidemiol*. 2012;26(1):19-26. doi:10.1111/j.1365-3016.2011.01213.x
21. Cho WK, Suh B-K. Catch-up growth and catch-up fat in children born small for gestational age. *Korean J Pediatr*. 2016;59(1):1-7. doi:10.3345/kjp.2016.59.1.1
22. Dunger DB, Salgin B, Ong KK. Early childhood contributions to insulin resistance. In: Zeitler PS, Nadeau KJ, eds. *Insulin Resistance: Childhood Precursors and Adult Disease*. Humana Press; 2008:81-92.
23. Ong KK, Ahmed ML, Emmett PM, Preece M. A., Dunger, D. B, the ALSPAC Study Team. Association Between Postnatal Catch-Up Growth and Obesity in Childhood: Prospective Cohort Study. *BMJ*. 2000;320:967-971. doi:10.1177/000922800003901210
24. Ibáñez L, Ong K, Dunger DB, De Zegher F. Early development of adiposity and insulin

resistance after catch-up weight gain in small-for-gestational-age children. *J Clin Endocrinol Metab*. 2006;91(6):2153-2158. doi:10.1210/jc.2005-2778

25. Touger L, Looker HC, Krakoff J, Lindsay RS, Cook V, Knowler WC. Early growth in offspring of diabetic mothers. *Diabetes Care*. 2005;28(3):585-589. doi:10.2337/diacare.28.3.585
26. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol Rev*. 2014;94(Table 1):1027-1076. doi:10.1152/physrev.00029.2013
27. Mensah G, Wei G, Sorlie P, et al. Decline in Cardiovascular Mortality: Possible Causes and Implications. *Circ Res*. 2017;120(2):366-380. doi:10.1161/CIRCRESAHA.116.309115. Decline
28. Barker DJP, Osmond C. Diet and coronary heart disease in England and Wales during and after the second world war. *J Epidemiol Community Health*. 1986;40(1):37-44. doi:10.1136/jech.40.1.37
29. Payne RA. Cardiovascular risk. *Br J Clin Pharmacol*. 2012;74(3):396-410. doi:10.1111/j.1365-2125.2012.04219.x
30. Committee on preventing the global epidemic of cardiovascular disease: meeting the challenges in developing countries. *Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health*. (Fuster V, Kelly BB, eds.). Washington: The National Academies Press; 2010. http://www.nap.
31. Forsdahl A. Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? *Br J Prev Soc Med*. 1977;31(2):91-95. doi:10.1136/jech.31.2.91
32. Notkola V, Punsar S, Karvonen MJ, Haapakoski J. Socio-economic conditions in childhood and mortality and morbidity caused by coronary heart disease in adulthood in rural Finland. *Soc Sci Med*. 1985;21(5):517-523. doi:10.1016/0277-9536(85)90035-8
33. Buck C, Simpson H. Infant diarrhoea and subsequent mortality from heart disease and cancer. *J Epidemiol Community Health*. 1982;36(1):27-30. doi:10.1136/jech.36.1.27
34. Marmot M, Shipley M, Rose G. Inequalities in death - specific explanations of a general pattern? *Lancet*. 1984;May 5:1003-1006.

35. Hinkle L. Coronary heart disease and sudden death in actively employed American men. *Bull N Y Acad Med*. 1973;49(6):467-474.
36. McCay C. Is longevity compatible with optimum growth? *Science (80-)*. 1933;77:410-411.
37. McCance RA. Food, growth, and time. *Lancet*. 1962;2:671-676.
38. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ*. 1991;303(6809):1019-1022. doi:10.2337/dc10-1141
39. Hales CN, Barker DJP. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Int J Epidemiol*. 2013;42(5):1215-1222. doi:10.1093/ije/dyt133
40. Tian G, Guo C, Li Q, et al. Birth weight and risk of type 2 diabetes: A dose-response meta-analysis of cohort studies. *Diabetes Metab Res Rev*. 2019;35(e3144):1-7. doi:10.1002/dmrr.3144
41. Mu M, Wang SF, Sheng J, et al. Birth weight and subsequent blood pressure: A meta-analysis. *Arch Cardiovasc Dis*. 2012;105(2):99-113. doi:10.1016/j.acvd.2011.10.006
42. da Silveira VMF, Horta BL. Birth weight and metabolic syndrome in adults: Meta-analysis. *Rev Saude Publica*. 2008;42(1):10-18. doi:10.1590/S0034-89102008000100002
43. Wang SF, Shu L, Sheng J, et al. Birth weight and risk of coronary heart disease in adults: a meta-analysis of prospective cohort studies. *J Dev Orig Health Dis*. 2014;5(6):408-419. doi:10.1017/S2040174414000440
44. Risnes KR, Vatten LJ, Baker JL, et al. Birthweight and mortality in adulthood: A systematic review and meta-analysis. *Int J Epidemiol*. 2011;40(3):647-661. doi:10.1093/ije/dyq267
45. Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: A meta-analysis. *Am J Epidemiol*. 2007;165(8):849-857. doi:10.1093/aje/kwk071
46. Knop MR, Geng TT, Gorny AW, et al. Birth weight and risk of type 2 diabetes mellitus, cardiovascular disease, and hypertension in adults: A meta-analysis of 7 646 267 participants from 135 studies. *J Am Hear Assoc*. 2018;7(23):1-13. doi:10.1161/JAHA.118.008870
47. Baker JL, Olsen LW, Sørensen TIA. Weight at birth and all-cause mortality in adulthood. *Epidemiology*. 2008;19(2):197-203.
48. Eriksson JG, Forsén T, Tuomilehto J, Osmond C, Barker DJP. Early adiposity rebound in

childhood and risk of Type 2 diabetes in adult life. *Diabetologia*. 2003;46(2):190-194. doi:10.1007/s00125-002-1012-5

49. Ravelli G, Stein Z, Susser M. Obesity in young men after famine exposure in utero and early infancy. *NEJM*. 1976;295(7):349-353. doi:10.1249/00005768-199407000-00008
50. Ong KK, Elmlinger M, Jones R, et al. Growth hormone binding protein levels in children are associated with birth weight, postnatal weight gain, and insulin secretion. *Metabolism*. 2007;56(10):1412-1417. doi:10.1016/j.metabol.2007.06.004
51. Milovanovic I, Njuieyon F, Deghmoun S, Chevenne D, Levy-Marchal C, Beltrand J. SGA children with moderate catch-up growth are showing the impaired insulin secretion at the age of 4. *PLoS One*. 2014;9(6). doi:10.1371/journal.pone.0100337
52. Stroescu R, Micle I, Marginean O, et al. Is small for gestational age status associated with an increased risk of atherogenesis? *Maedica (Buchar)*. 2013;8(4):315-320. http://www.ncbi.nlm.nih.gov/pubmed/24790660.
53. Kramer MS, Martin RM, Bogdanovich N, Vilchuk K, Dahhou M, Oken E. Is restricted fetal growth associated with later adiposity? Observational analysis of a randomized trial. *Am J Clin Nutr*. 2014;100(1):176-181. doi:10.3945/ajcn.113.079590.1
54. Olaiya MT, Wedekind LE, Hanson RL, et al. Birthweight and early-onset type 2 diabetes in American Indians: differential effects in adolescents and young adults and additive effects of genotype, BMI and maternal diabetes. *Diabetologia*. 2019;62(9):1628-1637. doi:10.1007/s00125-019-4899-9.Birthweight
55. Lucas A. Programming by early nutrition in man. *Ciba Found Symp*. 1991;156:38-50, discussion 50-5.
56. Hanson MA, Gluckman PD. Developmental origins of health and disease - Global public health implications. *Best Pract Res Clin Obstet Gynaecol*. 2015;29(1):24-31. doi:10.1016/j.bpobgyn.2014.06.007
57. Lawlor DA, Smith GD, O'Callaghan M, et al. Epidemiologic evidence for the fetal overnutrition hypothesis: Findings from the Mater-University study of pregnancy and its outcomes. *Am J Epidemiol*. 2007;165(4):418-424. doi:10.1093/aje/kwk030
58. Painter RC, Osmond C, Gluckman P, Hanson M, Phillips DIW, Roseboom TJ.

Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. *BJOG An Int J Obstet Gynaecol*. 2008;115(10):1243-1249. doi:10.1111/j.1471-0528.2008.01822.x

59. Neel J V. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genet*. 1962;14:353-362.
60. Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low bir thweight with diabetes and vascular disease. *Lancet*. 1999;353:1789-1792. doi:10.1016/S0140-6736(98)07546-1
61. Adair LS. Child and adolescent obesity: Epidemiology and developmental perspectives. *Physiol Behav*. 2008;94(1):8-16. doi:10.1016/j.physbeh.2007.11.016
62. Wrottesley S V., Lamper C, Pisa PT. Review of the importance of nutrition during the first 1000 days: Maternal nutritional status and its associations with fetal growth and birth, neonatal and infant outcomes among African women. *J Dev Orig Heal Dis*. 2015;7(2):144-162. doi:10.1017/S2040174415001439
63. Fewtrell M, Michaelsen KF, van der Beek E, van Elburg R, eds. *Growth in Early Life: Growth Trajectory and Assessment, Influencing Factors and Impact of Early Nutrition*. Milton, Queensland, Australia: John Wiley & Sons; 2016.
64. Mook-Kanamori DO, Durmuş B, Sovio U, et al. Fetal and infant growth and the risk of obesity during early childhood: The generation R study. *Eur J Endocrinol*. 2011;165(4):623-630. doi:10.1530/EJE-11-0067
65. Burton GJ, Jauniaux E. What is the placenta? *Am J Obs Gynecol*. 2015;213(4):S6.e1-S6.e4. doi:10.1016/j.ajog.2015.07.050
66. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr*. 2013;13(59):1-13. doi:10.1186/1471-2431-13-59
67. Toro-Ramos T, Paley C, Pi-Sunyer FX, Gallagher D. Body composition during fetal development and infancy through the age of 5 years. *Eur J Clin Nutr*. 2015;69(12):1279-1289. doi:10.1038/ejcn.2015.117
68. Carlsen EM, Renault KM, Nørgaard K, et al. Newborn regional body composition is influenced by maternal obesity, gestational weight gain and the birthweight standard score. *Acta Paediatr*. 2014;103(9):939-945. doi:10.1111/apa.12713

69. Modi N, Thomas EL, Harrington TAM, Uthaya S, Doré CJ, Bell JD. Determinants of adiposity during preweaning postnatal growth in appropriately grown and growth-restricted term infants. *Pediatr Res*. 2006;60(3):345-348. doi:10.1203/01.pdr.0000232732.93000.52
70. Pomeroy E, Wells JCK, Cole TJ, O'Callaghan M, Stock JT. Relationships of maternal and paternal anthropometry with neonatal body size, proportions and adiposity in an Australian cohort. *Am J Phys Anthr*. 2015;156(4):625-636. doi:10.1002/ajpa.22680
71. Kozuki N, Katz J, Lee ACC, et al. Short Maternal Stature Increases Risk of Small- for-Gestational-Age and Preterm Births in Low- and Middle-Income Countries : Individual Participant Data Meta-Analysis and Population Attributable Fraction. *J Nutr*. 2015;145:2542-2550. doi:10.3945/jn.115.216374.preterm
72. Lewis RM, Demmelmair H, Gaillard R, et al. The placental exposome: Placental determinants of fetal adiposity and postnatal body composition. *Ann Nutr Metab*. 2013;63(3):208-215. doi:10.1159/000355222
73. Voerman E, Santos S, Golab BP, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PLoS Med*. 2019;16(2):1-22. doi:10.1371/journal.pmed.1002744
74. Linabery AM, Nahhas RW, Johnson W, et al. Stronger influence of maternal than paternal obesity on infant and early childhood BMI: The Fels longitunal study. *Pediatr Obes*. 2013;8(3):159-169. doi:10.1038/jid.2014.371
75. Victora CG, Villar J, Barros FC, et al. Anthropometric characterization of impaired fetal growth risk factors for and prognosis of newborns with stunting orwasting. *JAMA Pediatr*. 2015;169(7):1-10. doi:10.1001/jamapediatrics.2015.1431
76. Kozuki N, Lee AC, Silveira MF, et al. The associations of birth intervals with small-for-gestational-age, preterm, and neonatal and infant mortality: A meta-analysis. *BMC Public Health*. 2013;13(SUPPL.3):S3. doi:10.1186/1471-2458-13-S3-S3
77. Kozuki N, Lee AC, Silveira MF, et al. The associations of parity and maternal age with small-for-gestational-age, preterm, and neonatal and infant mortality: A meta-analysis. *BMC Public Health*. 2013;13(SUPPL.3):S2. doi:10.1186/1471-2458-13-S3-S2
78. Sferruzzi-Perri AN, Vaughan OR, Forhead AJ, Fowden AL. Hormonal and nutritional drivers

of intrauterine growth. *Curr Opin Clin Nutr Metab Care*. 2013;16(3):298-309. doi:10.1097/MCO.0b013e32835e3643

79. Grieger JA, Grzeskowiak LE, Clifton VL. Preconception Dietary Patterns in Human Pregnancies Are Associated with Preterm Delivery. *J Nutr*. 2014;144(7):1075-1080. doi:10.3945/jn.114.190686
80. Belkacemi L, Nelson DM, Desai M, Ross MG. Maternal Undernutrition Influences Placental-Fetal Development. *Biol Reprod*. 2010;83(3):325-331. doi:10.1095/biolreprod.110.084517
81. Group THSCR. Hyperglycemia and Adverse Pregnancy Outcomes. *NEJM*. 2008;358:1991-2002. doi:10.1056/NEJMoa1208410
82. Graves E, Hill DJ, Evers S, et al. The impact of abnormal glucose tolerance and obesity on fetal growth. *J Diabetes Res*. 2015;2015(847674):1-10. doi:10.1155/2015/847674
83. Kamana K, Shakya S, Zhang H. Gestational diabetes mellitus and macrosomia: A literature review. *Ann Nutr Metab*. 2015;66(suppl2):14-20. doi:10.1159/000371628
84. Grieger JA, Clifton VL. A review of the impact of dietary intakes in human pregnancy on infant birthweight. *Nutrients*. 2015;7(1):153-178. doi:10.3390/nu7010153
85. Horan MK, McGowan CA, Gibney ER, Donnelly JM, McAuliffe FM. The association between maternal dietary micronutrient intake and neonatal anthropometry - Secondary analysis from the ROLO study. *Nutr J*. 2015;14(105):1-11. doi:10.1186/s12937-015-0095-z
86. Hanson MA, Bardsley A, De-Regil LM, et al. The International Federation of Gynecology and Obstetrics (FIGO) recommendations on adolescent, preconception, and maternal nutrition: "Think Nutrition First" #. *Int J Gynaecol Obs*. 2015;131 Suppl:S213-53. doi:10.1016/s0020-7292(15)30034-5
87. Horan MK, McGowan CA, Gibney ER, Donnelly JM, McAuliffe FM. Maternal low glycaemic index diet, fat intake and postprandial glucose influences neonatal adiposity - Secondary analysis from the ROLO study. *Nutr J*. 2014;13(78):1-12. doi:10.1186/1475-2891-13-78
88. Jiménez-Chillarón JC, Díaz R, Martínez D, et al. The role of nutrition on epigenetic modifications and their implications on health. *Biochimie*. 2012;94(11):2242-2263. doi:10.1016/j.biochi.2012.06.012
89. Desclée de Maredsous C, Oozeer R, Barbillon P, et al. High-Protein Exposure during Gestation or Lactation or after Weaning Has a Period-Specific Signature on Rat Pup Weight,

Adiposity, Food Intake, and Glucose Homeostasis up to 6 Weeks of Age. *J Nutr*. 2016;146(1):21-29. doi:10.3945/jn.115.216465

90. Ferraro ZM, Gaudet L, Adamo KB. The potential impact of physical activity during pregnancy on maternal and neonatal outcomes. *Obs Gynecol Surv*. 2012;67(2):99-110.
91. Barakat R, Perales M. Resistance exercise in pregnancy and outcome. *Clin Obstet Gynecol*. 2016;59(3):591-599. doi:10.1097/GRF.0000000000000213
92. Beeson JH, Blackmore HL, Carr SK, et al. Maternal exercise intervention in obese pregnancy improves the cardiovascular health of the adult male offspring. *Mol Metab*. 2018;16(June):35-44. doi:10.1016/j.molmet.2018.06.009
93. Fernandez-Twinn DS, Gascoin G, Musial B, et al. Exercise rescues obese mothers' insulin sensitivity, placental hypoxia and male offspring insulin sensitivity. *Sci Rep*. 2017;7(March):1-11. doi:10.1038/srep44650
94. Banderali G, Martelli A, Landi M, et al. Short and long term health effects of parental tobacco smoking during pregnancy and lactation: A descriptive review. *J Transl Med*. 2015;13(327):1-7. doi:10.1186/s12967-015-0690-y
95. Behnke M, Smith VC. Prenatal substance abuse: Short- and long-term effects on the exposed fetus. *Pediatrics*. 2013;131(3):e1009-24. doi:10.1542/peds.2012-3931
96. Huo W, Xia W, Wan Y, et al. Maternal urinary bisphenol A levels and infant low birth weight: A nested case-control study of the Health Baby Cohort in China. *Env Int*. 2015;85:96-103. doi:10.1016/j.envint.2015.09.005
97. Andersen GS, Girma T, Wells JCK, Kastel P, Michaelsen KF, Friis H. Fat and fat-free mass at birth: Air Displacement plethysmography measurements on 350 ethiopian newborns. *Pediatr Res*. 2011;70(5):501-506. doi:10.1203/PDR.0b013e31822d7470
98. Au CP, Raynes-Greenow CH, Turner RM, Carberry AE, Jeffery H. Fetal and maternal factors associated with neonatal adiposity as measured by air displacement plethysmography: A large cross-sectional study. *Early Hum Dev*. 2013;89(10):839-843. doi:10.1016/j.earlhumdev.2013.07.028
99. Paviotti G, De Cunto A, Travan L, Bua J, Cont G, Demarini S. Longitudinal growth and body composition of twins versus singletons in the first month of life. *Sci World J*.

2013;2013(108189):1-3. doi:10.1155/2013/108189

100. Sudfeld CR, McCoy DC, Danaei G, et al. Linear growth and child development in low- and middle-income countries: A meta-analysis. *Pediatrics*. 2015;135(5):e1266-75. doi:10.1542/peds.2014-3111
101. Monteiro POA, Victora CG. Rapid growth in infancy and childhood and obesity in later life - A systematic review. *Obes Rev*. 2005;6(2):143-154. doi:10.1111/j.1467-789X.2005.00183.x
102. Michaelsen KF. WHO Growth standards-should they be implemented as national standards? *J Pediatr Gastroenterol Nutr*. 2010;51(Suppl. 3):151-152. doi:10.1097/MPG.0b013e3181f1ba10
103. Macdonald PD, Ross SRM, Grant L, Young D. Neonatal weight loss in breast and formula fed infants. *Arch Dis Child Fetal Neonatal Ed*. 2003;88(6):F472-76. doi:10.1136/fn.88.6.f472
104. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatr*. 2006;95(SUPPL. 450):76-85. doi:10.1080/08035320500495548
105. Wen X, Kleinman K, Gillman MW, Rifas-Shiman SL, Taveras EM. Childhood body mass index trajectories: Modeling, characterizing, pairwise correlations and socio-demographic predictors of trajectory characteristics. *BMC Med Res Methodol*. 2012;12(1):1-13. doi:10.1186/1471-2288-12-38
106. Fomon SJ, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr*. 1982;35(5 Suppl.):1169-1175. doi:10.1093/ajcn/35.5.1169
107. Soriguer Escofet FJC, Esteva De Antonio I, Tinahones FJ, Pareja A. Adipose tissue fatty acids and size and number of fat cells from birth to 9 years of age - A cross-sectional study in 96 boys. *Metabolism*. 1996;45(11):1395-1401. doi:10.1016/s0026-0495(96)90121-3
108. Murray PG, Clayton PE. Endocrine control of growth. *Am J Med Genet Part C Semin Med Genet*. 2013;163C(2):76-85. doi:10.1002/ajmg.c.31357
109. Küpers LK, L'Abée C, Bocca G, Stolk RP, Sauer PJJ, Corpeleijn E. Determinants of weight gain during the first two years of life-the GECKO drenthe birth cohort. *PLoS One*. 2015;10(7):1-15. doi:10.1371/journal.pone.0133326
110. Chiavaroli V, Derraik JGB, Hofman PL, Cutfield WS. Born Large for Gestational Age: Bigger

Is Not Always Better. *J Pediatr*. 2016;170:307-311. doi:10.1016/j.jpeds.2015.11.043

111. Bove I, Campoy C, Uauy R, Miranda T, Cerruti F. Trends in early growth indices in the first 24 months of life in Uruguay over the past decade. *J Heal Popul Nutr*. 2014;32(4):600-607. doi:10.3329/jhpn.v32i4.3016
112. Ong KK, Preece MA, Emmett PM, Ahmed ML, Dunger DB, for the ALSPAC Study Team. Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast-feeding: longitudinal birth cohort study and analysis. *Pediatr Res*. 2002;52(6):863-867. doi:10.1203/01.pdr.0000036602.81878.6d
113. Gale C, Logan KM, Jeffries S, et al. Sexual dimorphism in relation to adipose tissue and intrahepatocellular lipid deposition in early infancy. *Int J Obes*. 2015;39(4):629-632. doi:10.1038/ijo.2015.4
114. Buyken AE, Karaolis-Danckert N, Remer T, Bolzenius K, Landsberg B, Kroke A. Effects of breastfeeding on trajectories of body fat and BMI throughout childhood. *Obesity*. 2008;16(2):389-395. doi:10.1038/oby.2007.57
115. Cheng TS, Loy SL, Cheung YB, et al. Sexually dimorphic response to feeding mode in the growth of infants. *Am J Clin Nutr*. 2016;103(2):398-405. doi:10.3945/ajcn.115.115493
116. World Health Organization. Breastfeeding. https://www.who.int/maternal_child_adolescent/topics/child/nutrition/breastfeeding/en/. Accessed August 29, 2019.
117. Jensen SM, Ritz C, Ejlerskov KT, Mølgaard C, Michaelsen KF. Infant BMI peak, breastfeeding, and body composition at age 3 y. *Am J Clin Nutr*. 2015;101(2):319-325. doi:10.3945/ajcn.114.092957
118. Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B. Growth of breast-fed and formula-fed infants from 0 to 18 months: The DARLING study. *Pediatrics*. 1992;89(6):1035-1041.
119. Gale C, Logan KM, Santhakumaran S, Parkinson JRC, Hyde MJ, Modi N. Effect of breastfeeding compared with formula feeding on infant body composition: A systematic review and meta-analysis. *Am J Clin Nutr*. 2012;95(3):656-669. doi:10.3945/ajcn.111.027284

120. Johnson L, Van Jaarsveld CHM, Llewellyn CH, Cole TJ, Wardle J. Associations between infant feeding and the size, tempo and velocity of infant weight gain: SITAR analysis of the Gemini twin birth cohort. *Int J Obes*. 2014;38(7):980-987. doi:10.1038/ijo.2014.61

121. Bergmann KE, Bergmann RL, Von Kries R, et al. Early determinants of childhood overweight and adiposity in a birth cohort study: Role of breast-feeding. *Int J Obes*. 2003;27(2):162-172. doi:10.1038/sj.ijo.802200

122. Ejlerskov KT, Christensen LB, Ritz C, Jensen SM, Molgaard C, Michaelsen KF. The impact of early growth patterns and infant feeding on body composition at 3 years of age. *Br J Nutr*. 2015;114(2):316-327. doi:10.1017/S0007114515001427

123. Regnault N, Botton J, Forhan A, et al. Determinants of early ponderal and statural growth in full-term infants. *Am J Clin Nutr*. 2010;92:594-602. doi:10.3945/ajcn.2010.29292.

124. WHO Multicentre Growth Reference Study Group. Assessment of differences in linear growth among populations in the WHO Multicentre Growth Reference Study. *Acta Paediatr*. 2006;Suppl 450:56-65. doi:10.1080/08035320500495514

125. Villar J, Papageorghiou AT, Pang R, et al. Monitoring human growth and development: A continuum from the womb to the classroom. *Am J Obs Gynecol*. 2015;213(4):494-499. doi:10.1016/j.ajog.2015.07.002

126. Currie J, Moretti E. Biology as destiny? Short- and long-run determinants of intergenerational transmission of birth weight. *J Labor Econ*. 2007;25(2):231-263. doi:10.1086/511377

127. Poore KR, Hollis LJ, Murray RJS, et al. Differential pathways to adult metabolic dysfunction following poor nutrition at two critical developmental periods in sheep. *PLoS One*. 2014;9(3). doi:10.1371/journal.pone.0090994

128. Friedman JE. Obesity and gestational diabetes mellitus pathways for programming inmouse,Monkey, and man-where do we go next? The 2014 norbert freinkel award lecture. *Diabetes Care*. 2015;38(8):1402-1411. doi:10.2337/dc15-0628

129. Remacle C, Bieswal F, Bol V, Reusens B. Developmental programming of adult obesity and cardiovascular disease in rodents by maternal nutrition imbalance. *Am J Clin Nutr*. 2011;94:1846-1852. doi:10.3945/ajcn.110.001651.1

130. Reusens B, Theys N, Dumortier O, Goosse K, Remacle C. Maternal malnutrition programs

the endocrine pancreas in progeny. *Am J Clin Nutr*. 2011;94(6):1824-1829. doi:10.3945/ajcn.110.000729

131. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev*. 2005;85(2):571-633. doi:10.1152/physrev.00053.2003
132. Oliveira AC, Andreotti S, Chimin P, et al. Neonatal streptozotocin-induced diabetes in mothers promotes metabolic programming of adipose tissue in male rat offspring. *Life Sci*. 2015;136(1):151-156. doi:10.1016/j.lfs.2015.06.024
133. Capobianco E, Fornes D, Linenberg I, Powell TL, Jansson T, Jawerbaum A. A novel rat model of gestational diabetes induced by intrauterine programming is associated with alterations in placental signaling and fetal overgrowth. *Mol Cell Endocrinol*. 2016;422:221-232. doi:10.1016/j.mce.2015.12.020
134. Finken MJJ, Van Der Steen M, Smeets CCJ, et al. Children born small for gestational age: differential diagnosis, molecular genetic evaluation, and implications. *Endocr Rev*. 2018;39(6):851-894. doi:10.1210/er.2018-00083
135. Lapillonne A, Bronsky J, Campoy C, et al. Feeding the late and moderately preterm infant: a position paper of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *JPGN*. 2019;69(2):259-270. doi:10.1097/mpg.0000000000002397
136. Zeve D, Regelmann MO, Holzman IR, Rapaport R. Small at birth, but how small? The definition of SGA revisited. *Horm Res Paediatr*. 2016;86(5):357-360. doi:10.1159/000449275
137. Schlaudecker EP, Munoz FM, Bardají A, et al. Small for gestational age: Case definition & guidelines for data collection, analysis, and presentation of maternal immunisation safety data. *Vaccine*. 2017;35(48):6518-6528. doi:10.1016/j.vaccine.2017.01.040
138. Lee ACC, Kozuki N, Cousens S, et al. Estimates of burden and consequences of infants born small for gestational age in low and middle income countries with INTERGROWTH-21 st standard: Analysis of CHERG datasets. *BMJ*. 2017;358:1-11. doi:10.1136/bmj.j3677
139. Castanys-Muñoz E, Kennedy K, Castañeda-Gutiérrez E, et al. Systematic review indicates

postnatal growth in term infants born small-for-gestational-age being associated with later neurocognitive and metabolic outcomes. *Acta Paediatr*. 2017;106(8):1230-1238. doi:10.1111/apa.13868

140. Balasuriya CND, Stunes AK, Mosti MP, et al. Metabolic outcomes in adults born preterm with very low birthweight or small for gestational age at term: A cohort study. *J Clin Endocrinol Metab*. 2018;103(12):4437-4446. doi:10.1210/jc.2018-00464
141. Lei X, Chen Y, Ye J, Ouyang F, Jiang F, Zhang J. The optimal postnatal growth trajectory for term small for gestational age babies: A prospective cohort study. *J Pediatr*. 2015;166(1):54-58. doi:10.1016/j.jpeds.2014.09.025
142. Fattal-Valevski A, Toledano-Alhadef H, Leitner Y, Geva R, Eshel R, Harel S. Growth patterns in children with intrauterine growth retardation and their correlation to neurocognitive development. *J Child Neurol*. 2009;24(7):846-851. doi:10.1177/0883073808331082
143. Biosca M, Rodríguez G, Ventura P, et al. Central adiposity in children born small and large for gestational age. *Nutr Hosp*. 2011;26(5):971-976. doi:10.3305/nh.2011.26.5.5162
144. Rodriguez-Lopez M, Osorio L, Acosta-Rojas R, et al. Influence of breastfeeding and postnatal nutrition on cardiovascular remodeling induced by fetal growth restriction. *Pediatr Res*. 2016;79:100-106. doi:10.1038/pr.2015.182
145. Okada T, Takahashi S, Nagano N, Yoshikawa K, Usukura Y, Hosono S. Early postnatal alteration of body composition in preterm and small-for-gestational-age infants: implications of catch-up fat. *Pediatr Res*. 2015;77(1):136-142. doi:10.1038/pr.2014.164
146. Roth CL, Sathyanarayana S. Mechanisms affecting neuroendocrine and epigenetic regulation of body weight and onset of puberty: potential implications in the child born small for gestational age (SGA). *Rev Endocr Metab*. 2012;13:129-140. doi:10.1007/s11154-012-9212-x
147. Lumey L, Stein A, Susser E. Prenatal famine and adult health. *Annu Rev Public Heal*. 2011;32:1-26. doi:10.1146/annurev-publhealth-031210-101230.
148. Ekelund U, Neovius M, Linné Y, Brage S, Wareham NJ, Rössner S. Associations between physical activity and fat mass in adolescents: The Stockholm Weight Development Study. *Am J Clin Nutr*. 2005;81(2):355-360. doi:81/2/355 [pii]
149. Ong K, Petry C, Emmett P, et al. Insulin sensitivity and secretion in normal children related to

size at birth , postnatal growth , and plasma insulin-like growth factor-I levels. *Diabetologia*. 2004;47:1064-1070. doi:10.1007/s00125-004-1405-8

150. Dunger DB, Salgin B, Ong KK. Session 7: Early nutrition and later health; Early developmental pathways of obesity and diabetes risk - Symposium on "Nutrition in early life: New horizons in a new century." *Proc Nutr Soc*. 2007;66(3):451-457. doi:10.1017/S0029665107005721
151. Morley R, Fewtrell MS, Abbott RA, et al. Neurodevelopment in children born small for gestational age: A randomized trial of nutrient-enriched versus standard formula and comparison with a reference breastfed group. *Pediatrics*. 2004;113:515-521.
152. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. Consensus Statement: Management of the child born small for gestational age through to Adulthood: A Consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *J Clin Endocrinol Metab*. 2017;92:804-810. doi:10.1210/jc.2006-2017
153. Singhal A, Kennedy K, Lanigan J, et al. Nutrition in infancy and long-term risk of obesity: evidence from 2 randomized controlled trials. *Am J Clin Nutr*. 2010;92:1133-1144. doi:10.3945/ajcn.2010.29302.
154. Lanigan J, Singhal A. Early nutrition and long-term health: A practical approach. *Proc Nutr Soc*. 2009;68(4):422-429. doi:10.1017/S002966510999019X
155. Setji T, Brown A, Feinglos M. Gestational diabetes mellitus. *Clin Diabetes*. 2005;23(1):17-24. doi:10.15537/smj.2015.4.10307
156. Keely E, Barbour L. *Management of Diabetes in Pregnancy*. (De Groot L, Chrousos G, Dungan K, Al. E, eds.). Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. https://www.ncbi.nlm.nih.gov/books/NBK279010/.
157. Group NDD. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes*. 1979;28(December):1039-1057.
158. Lee AJ, Hiscock RJ, Wein P, Walker SP, Permezel M. Gestational diabetes mellitus: Clinical predictors and long-term risk of developing type 2 Diabetes - A retrospective cohort study using survival analysis. *Diabetes Care*. 2007;30(4):878-883. doi:10.2337/dc06-1816

159. Catalano PM, Ehrenberg HM. The short- and long-term implications of maternal obesity on the mother and her offspring. *BJOG*. 2006;113(10):1126-1133. doi:10.1111/j.1471-0528.2006.00989.x

160. Hadden DR, Hillebrand B. The first recorded case of diabetic pregnancy (Bennewitz HG, 1824, University of Berlin). *Diabetologia*. 1989;32(8):625. doi:10.1007/BF00285339

161. Hoet J, Lukens F. Carbohydrate metabolism during pregnancy. *Diabetes*. 1954;3(1):1-12. doi:10.1016/B978-0-7216-9654-6.50050-3

162. Noctor E, Dunne FP. Type 2 diabetes after gestational diabetes: the influence of changing diagnostic criteria. *World J Diabetes*. 2015;6(2):234-244. doi:10.4239/wjd.v6.i2.234

163. Catalano PM, McIntyre HD, Cruickshank JK, et al. The hyperglycemia and adverse pregnancy outcome study: Associations of GDM and obesity with pregnancy outcomes. *Diabetes Care*. 2012;35(4):780-786. doi:10.2337/dc11-1790

164. Kim SY, England JL, Sharma JA, Njoroge T. Gestational diabetes mellitus and risk of childhood overweight and obesity in offspring: A systematic review. *Exp Diabetes Res*. 2011;2011. doi:10.1155/2011/541308

165. Johns EC, Denison FC, Norman JE, Reynolds RM. Gestational diabetes mellitus: mechanisms, treatment, and complications. *Trends Endocrinol Metab*. 2018;29(11):743-754. doi:10.1016/j.tem.2018.09.004

166. American Diabetes Association. Gestational Diabetes Mellitus. *Diabetes Care*. 2003;26(Supplement 1):S103-5. doi:10.1016/j.ecl.2019.05.001

167. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2011;34(Suppl 1):S62-9. doi:10.2337/dc11-S062

168. International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care*. 2010;33(3):676-682. doi:10.2337/dc09-1848

169. O'Sullivan J, Mahan C. Criteria for the oral glucose tolerance test in pregnancy. *Diabetes*. 1964;13:278-285.

170. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. *Am J Obstet Gynecol*. 1982;144(7):768-773. doi:10.1016/0002-9378(82)90349-0

171. Weiss PAM, Haeusler M, Kainer F, Purstner P, Haas J. Toward universal criteria for gestational diabetes: Relationships between seventy-five and one hundred gram glucose loads and between capillary and venous glucose concentrations. *Am J Obstet Gynecol*. 1998;178(4):830-835. doi:10.1016/S0002-9378(98)70500-9

172. Coustan D, Lowe L, Metzger B, Dyer A. The HAPO Study: Paving The Way For New Diagnostic Criteria For GDM. *Am J Obs Gynecol*. 2010;202(6):654.e1-654.e6. doi:10.1016/j.ajog.2010.04.006.The

173. Han S, Crowther CA, Middleton P. Interventions for pregnant women with hyperglycaemia not meeting gestational diabetes and type 2 diabetes diagnostic criteria (Review). *Cochrane Collab*. 2012;(1). doi:10.1002/14651858.CD009037.pub2.www.cochranelibrary.com

174. Duran A, Śaenz S, Torreĵon MJ, et al. Introduction of IADPSG criteria for the screening and diagnosis of gestational diabetes mellitus results in improved pregnancy outcomes at a lower cost in a large cohort of pregnant women: The St. Carlos gestational diabetes study. *Diabetes Care*. 2014;37(9):2442-2450. doi:10.2337/dc14-0179

175. Long H, Cundy T. Establishing consensus in the diagnosis of gestational diabetes following HAPO: Where do we stand? *Curr Diab Rep*. 2013;13(1):43-50. doi:10.1007/s11892-012-0330-3

176. Bhupathiraju SN, Hu FB. Epidemiology of obesity and diabetes and their cardiovascular complications. *Circ Res*. 2016;118(11):1723-1735. doi:10.1161/CIRCRESAHA.115.306825

177. Diabetes UK. Facts and figures. https://www.diabetes.org.uk/professionals/position-statements-reports/statistics. Published 2018. Accessed January 30, 2020.

178. NICE guideline. Diabetes in pregnancy: management from preconception to the postnatal period. https://www.nice.org.uk/guidance/ng3/chapter/Introduction. Published 2015. Accessed January 8, 2020.

179. Logan KM, Emsley RJ, Jeffries S, et al. Development of early adiposity in infants of mothers with gestational diabetes mellitus. *Diabetes Care*. 2016;39(6):1045-1051. doi:10.2337/dc16-0030

180. Farrar D, Simmonds M, Griffin S, et al. The identification and treatment of women with

hyperglycaemia in pregnancy: An analysis of individual participant data, systematic reviews, meta-analyses and an economic evaluation. *Health Technol Assess (Rockv)*. 2016;20(86):1-382. doi:10.3310/hta20860

181. Zhang C, Rawal S, Chong YS. Risk factors for gestational diabetes: is prevention possible? *Diabetologia*. 2016;59(7):1385-1390. doi:10.1007/s00125-016-3979-3
182. Zhu Y, Zhang C. Prevalence of Gestational Diabetes and Risk of Progression to Type 2 Diabetes: a Global Perspective. *Curr Diab Rep*. 2016;16(1):1-11. doi:10.1007/s11892-015-0699-x
183. Lowe WL, Scholtens DM, Sandler V, Hayes MG. Genetics of gestational diabetes mellitus and maternal metabolism. *Curr Diab Rep*. 2016;16(2):1-10. doi:10.1007/s11892-015-0709-z
184. Chu S, Callaghan W, Kim Y, et al. Maternal obesity and risk of gestational diabetes mellitus. *Diabetes Care*. 2007;30(8):2070-2076. doi:10.2337/dc06-2559a.The
185. Kim SY, England L, Wilson HG, Bish C, Satten GA, Dietz P. Percentage of gestational diabetes mellitus attributable to overweight and obesity. *Am J Public Heal*. 2010;100(6):1047-1052. doi:10.2105/AJPH.2009.172890
186. Hedderson M, Gunderson E, Ferrara A. Gestational weight gain and risk of gestational diabetes mellitus. *Obs Gynecol*. 2010;115(3):597-604. doi:10.1038/jid.2014.371
187. Bao W, Michels KB, Tobias DK, et al. Parental smoking during pregnancy and the risk of gestational diabetes in the daughter. *Int J Epidemiol*. 2016;45(1):160-169. doi:10.1093/ije/dyv334
188. Zhang C, Ning Y. Effect of dietary and lifestyle factors on the risk of gestational diabetes: review of epidemiologic evidence. *Am J Clin Nutr*. 2011;94(6):1975-1979. doi:10.3945/ajcn.110.001032.1
189. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet*. 2009;373(9677):1773-1779. doi:10.1016/S0140-6736(09)60731-5
190. Schwartz N, Nachum Z, Green MS. The prevalence of gestational diabetes mellitus recurrence - effect of ethnicity and parity: a metaanalysis. *Am J Obstet Gynecol*. 2015;213(3):310-317. doi:10.1016/j.ajog.2015.03.011
191. Raiten DJ, Namasté S, Brabin B, et al. Executive summary - Biomarkers of nutrition for

development: Building a consensus. *Am J Clin Nutr*. 2011;94(suppl)(2):633S-650S. doi:10.3945/ajcn.110.008227

192. Strimbu K, Tavel J a. What are biomarkers? *Curr Opin HIV AIDS*. 2011;5(6):463-466. doi:10.1097/COH.0b013e32833ed177.What
193. Prentice PM, Olga L, Petry CJ, et al. Reduced size at birth and persisting reductions in adiposity in recent, compared with earlier, cohorts of infants born to mothers with gestational diabetes mellitus. *Diabetologia*. 2019;62(11):1977-1987. doi:10.1007/s00125-019-4970-6
194. Furse S, Snowden SG, Olga L, et al. Evidence from 3-month-old infants shows that a combination of postnatal feeding and exposures in utero shape lipid metabolism. *Sci Rep*. 2019;9(1):1-10. doi:10.1038/s41598-019-50693-0
195. Olga L, van Diepen JA, Bobeldijk-pastorova I, et al. Lipid ratios representing SCD1, FADS1, and FADS2 activities as candidate biomarkers of early growth and adiposity. *EBioMedicine*. 2021;63(103198):1-12. doi:10.1016/j.ebiom.2020.103198
196. Davis DR, Apley J, Fill G, Grimaldi C. Diet and retarded growth. *Br Med J*. 1978;1(6112):539-542. doi:10.1136/bmj.1.6112.539
197. Dekhtyar S, Wang HX, Scott K, Goodman A, Koupil I, Herlitz A. Associations of head circumference at birth with early-life school performance and later-life occupational prestige. *Longit Life Course Stud*. 2015;6(1):26-42. doi:10.14301/llcs.v6i1.308
198. De Lucia Rolfe E, Modi N, Uthaya S, et al. Ultrasound estimates of visceral and subcutaneous-abdominal adipose tissues in infancy. *J Obes*. 2013;2013. doi:10.1155/2013/951954
199. Yackobovitch-Gavan M, Gat-Yablonski G, Phillip M. Nutritional biomarkers for growth outcomes: Perspective of the endocrinologist. *World Rev Nutr Diet*. 2013;106:19-25. doi:10.1159/000342562
200. Ong KK, Langkamp M, Ranke MB, et al. Insulin-like growth factor I concentrations in infancy predict differential gains in body length and adiposity: The Cambridge Baby Growth Study. *Am J Clin Nutr*. 2009;90(1):156-161. doi:10.3945/ajcn.2008.27408
201. Regnault N, Botton J, Heude B, et al. Higher cord C-peptide concentrations are associated

with slower growth rate in the 1st year of life in girls but not in boys. *Diabetes*. 2011;60(8):2152-2159. doi:10.2337/db10-1189

202. Prentice P, Koulman A, Matthews L, Acerini CL, Ong KK, Dunger DB. Lipidomic analyses, breast- and formula-feeding, and growth in infants. *J Pediatr*. 2015;166(2):276-281.e6. doi:10.1016/j.jpeds.2014.10.021
203. Acharjee A, Prentice P, Acerini C, et al. The translation of lipid profiles to nutritional biomarkers in the study of infant metabolism. *Metabolomics*. 2017;13(3):1-9. doi:10.1007/s11306-017-1166-2
204. Prentice P, Ong KK, Schoemaker MH, et al. Breast milk nutrient content and infancy growth. *Acta Paediatr*. 2016;105(6):641-647. doi:10.1111/apa.13362
205. Ballard, O. Morrow A. Human Milk Composition: Nutrients and Bioactive Factors. *Pediatr Clin North Am*. 2013;60(1):49-74. doi:doi:10.1016/j.pcl.2012.10.002.
206. Czosnykowska-Łukacka M, Królak-Olejnik B, Orczyk-Pawiłowicz M. Breast Milk Macronutrient Components in Prolonged Lactation. *Nutrients*. 2018;10(1893):1-15. doi:10.3390/nu10121893
207. Turpin W, Bedrani L, Espin-Garcia O, et al. FUT2 genotype and secretory status are not associated with fecal microbial composition and inferred function in healthy subjects. *Gut Microbes*. 2018;9(4):357-368. doi:10.1080/19490976.2018.1445956
208. Ferrer-Admetlla A, Sikora M, Laayouni H, et al. A natural history of FUT2 polymorphism in humans. *Mol Biol Evol*. 2009;26(9):1993-2003. doi:10.1093/molbev/msp108
209. Payne DC, Currier RL, Staat MA, et al. Epidemiologic association between FUT2 secretor status and severe rotavirus gastroenteritis in children in the United States. *JAMA Pediatr*. 2015;169(11):1040-1045. doi:10.1001/jamapediatrics.2015.2002
210. Barton SJ, Murray R, Lillycrop KA, et al. FUT2 Genetic Variants and Reported Respiratory and Gastrointestinal Illnesses During Infancy. *J Infect Dis*. 2019;219:836-843. doi:10.1093/infdis/jiy582
211. Azad MB, Wade KH, Timpson NJ. FUT2 secretor genotype and susceptibility to infections and chronic conditions in the ALSPAC cohort. *Wellcome Open Res*. 2018;3(0):65. doi:10.12688/wellcomeopenres.14636.2
212. McGovern DPB, Jones MR, Taylor KD, et al. Fucosyltransferase 2 (FUT2) non-secretor status

is associated with Crohn's disease. *Hum Mol Genet*. 2010;19(17):3468-3476. doi:10.1093/hmg/ddq248

213. Smyth DJ, Cooper JD, Howson JMM, et al. FUT2 nonsecretor status links type 1 diabetes susceptibility and resistance to infection. *Diabetes*. 2011;60(11):3081-3084. doi:10.2337/db11-0638
214. Kumar H, Wacklin P, Nakphaichit M, et al. Secretor status is strongly associated with microbial alterations observed during pregnancy. *PLoS One*. 2015;10(7):1-10. doi:10.1371/journal.pone.0134623
215. Cabrera-Rubio R, Kunz C, Rudloff S, et al. Association of Maternal Secretor Status and Human Milk Oligosaccharides with Milk Microbiota: An Observational Pilot Study. *J Pediatr Gastroenterol Nutr*. 2019;68(2):256-263. doi:10.1097/MPG.0000000000002216
216. Azad MB, Robertson B, Atakora F, et al. Human Milk Oligosaccharide Concentrations Are Associated with Multiple Fixed and Modifiable Maternal Characteristics, Environmental Factors, and Feeding Practices. *J Nutr*. 2018;148(11):1733-1742. doi:10.1093/jn/nxy175
217. Prentice P, Acerini CL, Eleftheriou A, Hughes IA, Ong KK, Dunger DB. Cohort profile: The Cambridge Baby Growth Study (CBGS). *Int J Epidemiol*. 2016;45(1):35-35g. doi:10.1093/ije/dyv318
218. Freeman J V., Cole TJ, Chinn S, Jones PRM, White EM, Preece MA. Cross sectional stature and weight reference curves for the UK, 1990. *Arch Dis Child*. 1995;73(1):17-24. doi:10.1136/adc.73.1.17
219. Chomtho S, Wells JCK, Williams JE, Davies PSW, Lucas A, Fewtrell MS. Infant growth and later body composition: Evidence from the 4-component model. *Am J Clin Nutr*. 2008;87(6):1776-1784. doi:10.1093/ajcn/87.6.1776
220. Diet, Anthropometry and Physical Activity (DAPA) Measurement Toolkit. https://dapa-toolkit.mrc.ac.uk/.
221. Freedman DS, Horlick M, Berenson GS. A comparison of the Slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular. *Am J Clin Nutr*. 2013;98(3):1417-1424. doi:10.3945/ajcn.113.065961.1
222. Aris IM, Soh SE, Tint MT, et al. Body fat in Singaporean infants: Development of body fat

prediction equations in Asian newborns. *Eur J Clin Nutr*. 2013;67(9):922-927. doi:10.1038/ejcn.2013.69

223. Borga M, West J, Bell JD, et al. Advanced body composition assessment: From body mass index to body composition profiling. *J Investig Med*. 2018;66(5):887-895. doi:10.1136/jim-2018-000722
224. Fields DA, Gilchrist JM, Catalano PM, Gianní ML, Roggero PM, Mosca F. Longitudinal body composition data in exclusively breast-fed infants: A multicenter study. *Obesity*. 2011;19(9):1887-1891. doi:10.1038/oby.2011.11
225. Pan H, Cole T. LMSgrowth, a Microsoft Excel add-in to access growth references based on the LMS method. 2012. http://www.healthforallchildren.co.uk/.
226. de Lauzon-Guillain B, Wijndaele K, Clark M, et al. Breastfeeding and infant temperament at age three months. *PLoS One*. 2012;7(1). doi:10.1371/journal.pone.0029326
227. Koulman A, Prentice P, Wong MCY, et al. The development and validation of a fast and robust dried blood spot based lipid profiling method to study infant metabolism. *Metabolomics*. 2014:1-8. doi:10.1007/s11306-014-0628-z
228. Dumas J. Procedes de l'analyse organique. *Ann Chim Phys*. 1831;47:198-205.
229. Southgate D. The relationship between food composition and available energy. Joint FAO/WHO/UNU Expert Consultation on Energy and Protein Requirements. doi:10.1017/CBO9781107415324.004
230. Wells JCK, Jonsdottir OH, Hibberd PL, et al. Randomized controlled trial of 4 compared with 6 mo of exclusive breastfeeding in Iceland: Differences in breast-milk intake by stable-isotope probe. *Am J Clin Nutr*. 2012;96(1):73-79. doi:10.3945/ajcn.111.030403
231. Haisma H, Coward WA, Albernaz E, et al. Breast milk and energy intake in exclusively, predominantly, and partially breast-fed infants. *Eur J Clin Nutr*. 2003;57(12):1633-1642. doi:10.1038/sj.ejcn.1601735
232. A. Lawlor D, Fraser A, Lindsay RS, et al. Association of existing diabetes, gestational diabetes and glycosuria in pregnancy with macrosomia and offspring body mass index, waist and fat mass in later childhood: Findings from a prospective pregnancy cohort. *Diabetologia*. 2010;53(1):89-97. doi:10.1007/s00125-009-1560-z
233. Catalano PM, Thomas A, Huston-Presley L, Amini SB. Increased fetal adiposity: A very

sensitive marker of abnormal in utero development. *Am J Obstet Gynecol*. 2003;189(6):1698-1704. doi:10.1016/S0002-9378(03)00828-7

234. Hay WW. Care of the infant of the diabetic mother. *Curr Diab Rep*. 2012;12(1):4-15. doi:10.1007/s11892-011-0243-6
235. Philipps LH, Santhakumaran S, Gale C, et al. The diabetic pregnancy and offspring BMI in childhood: A systematic review and meta-analysis. *Diabetologia*. 2011;54(8):1957-1966. doi:10.1007/s00125-011-2180-y
236. Aceti A, Santhakumaran S, Logan KM, et al. The diabetic pregnancy and offspring blood pressure in childhood: A systematic review and meta-Analysis. *Diabetologia*. 2012;55(11):3114-3127. doi:10.1007/s00125-012-2689-8
237. Metzger B, Lowe L, Dyer A, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*. 2008;358(19):1991-2002. doi:10.1056/NEJMoa1110039
238. Crowther C, Hiller J, Moss J, McPhee A, Jeffries W, Robinson J. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med*. 2005;352(24):2477-2486. doi:10.1056/NEJMp1002530
239. Landon MB, Spong CY, Thom E, et al. A multicenter, randomized trial of treatment for mild gestational diabetes. *N Engl J Med*. 2009;361(14):1339-1348. doi:10.1056/NEJMoa0902430
240. Au CP, Raynes-Greenow CH, Turner RM, Carberry AE, Jeffery HE. Body composition is normal in term infants born to mothers with well-controlled gestational diabetes mellitus. *Diabetes Care*. 2013;36(3):562-564. doi:10.2337/dc12-1557
241. Hoffman L, Nolan C, Wilson J, Oats J, Simmons D. Gestational diabetes mellitus -- management guidelines The Australasian Diabetes in Pregnancy Society. *MJA*. 1998;169(20 July):93-97.
242. World Health Organization Department of Noncommunicable Disease Surveillance Geneva. *Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications.*; 1999. doi:WHO/NCD/NCS/99.2
243. Rubin D. *Multiple Imputation for Nonresponse in Surveys*. Hoboken, New Jersey: John Wiley & Sons; 2008.

244. McElduff A. Comment on: Au et al. Body composition is normal in term infants born to mothers with well-controlled gestational diabetes mellitus. Diabetes Care 2013;36:562-564. *Diabetes Care*. 2013;36(9):2013. doi:10.2337/dc13-0834

245. Logan KM, Gale C, Hyde MJ, Santhakumaran S, Modi N. Diabetes in pregnancy and infant adiposity: systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal Ed*. 2017;102(1):F65-F72. doi:10.1136/archdischild-2015-309750

246. Langer O, Levy J, Brustman L, Anyaegbunam A, Merkatz R, Divon M. Glycemic control in gestational diabetes mellitus--how tight is tight enough: small for gestational age versus large for gestational age? *Am J Obstet Gynecol*. 1989;161:646-653.

247. Simmons D, Nema J, Parton C, et al. The treatment of booking gestational diabetes mellitus (TOBOGM) pilot randomised controlled trial. *BMC Pregnancy Childbirth*. 2018;18(151):1-8. doi:10.1186/s12884-018-1809-y

248. Lowe WL, Lowe LP, Kuang A, et al. Maternal glucose levels during pregnancy and childhood adiposity in the Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study. *Diabetologia*. 2019;42(3):372-380. doi:10.1007/s00125-018-4809-6

249. Rowan JA, Rush EC, Plank LD, et al. Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition and metabolic outcomes at 7-9 years of age. *BMJ open diabetes Res care*. 2018;6(1):e000456. doi:10.1136/bmjdrc-2017-000456

250. Hjorth-Hansen A, Salvesen Ø, Hanem LGE, et al. Fetal growth and birth anthropometrics in metformin-exposed offspring born to mothers with PCOS. *J Clin Endocrinol Metab*. 2018;103(2):740-747. doi:10.1210/jc.2017-01191

251. Ong K, Loos R. Rapid infancy weight gain and subsequent obesity: Systematic reviews and hopeful suggestions. *Acta Paediatr*. 2006;95(8):904-908. doi:10.1080/08035250600719754

252. Van Hulst A, Barnett TA, Paradis G, Roy-Gagnon MH, Gomez-Lopez L, Henderson M. Birth weight, postnatal weight gain, and childhood adiposity in relation to lipid profile and blood pressure during early adolescence. *J Am Heart Assoc*. 2017;6(8). doi:10.1161/JAHA.117.006302

253. De Lucia Rolfe E, Sleigh A, Finucane FM, et al. Ultrasound measurements of visceral and subcutaneous abdominal thickness to predict abdominal adiposity among older men and women. *Obesity*. 2010;18(3):625-631. doi:10.1038/oby.2009.309

254. Armellini F, Zamboni M, Robbi R, et al. Total and intra-abdominal fat measurements by ultrasound and computerized tomography. *Int J Obes Relat Metab Disord J Int Assoc Study Obes*. 1993;17(4):209-214.

255. Friedrich N, Thuesen B, Jrøgensen T, et al. The association between IGF-I and insulin resistance: A general population study in Danish adults. *Diabetes Care*. 2012;35(4):768-773. doi:10.2337/dc11-1833

256. Hawkes C, Grimberg A. Measuring growth hormone and insulin-like growth factor-I in infants: what is normal? *Pediatr Endocrinol Rev*. 2013;11(2):126-146.

257. Pang AL-Y, Chan W-Y. Molecular Basis of Diseases of the Endocrine System. In: *Essential Concepts in Molecular Pathology*. Elsevier Inc.; 2010:291-292. doi:https://doi.org/10.1016/C2009-0-01712-7

258. Jing Z, Hou X, Wang Y, et al. Association between insulin-like growth factor-1 and cardiovascular disease risk: Evidence from a meta-analysis. *Int J Cardiol*. 2015;198:1-5. doi:10.1016/j.ijcard.2015.06.114

259. Yosten GLC, Maric-Bilkan C, Luppi P, Wahren J. Physiological effects and therapeutic potential of proinsulin C-peptide. *Am J Physiol Endocrinol Metab*. 2014;307(11):E955-68. doi:10.1152/ajpendo.00130.2014

260. Chellakooty M, Juul A, Boisen KA, et al. A prospective study of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-3 in 942 healthy infants: Associations with birth weight, gender, growth velocity, and breastfeeding. *J Clin Endocrinol Metab*. 2006;91(3):820-826. doi:10.1210/jc.2005-0950

261. Koletzko B, Demmelmair H, Grote V, Totzauer M. Optimized protein intakes in term infants support physiological growth and promote long-term health. *Semin Perinatol*. 2019;43(7):1-8. doi:10.1053/j.semperi.2019.06.001

262. Ginsburg BE, Lindblad BS, Persson B, Zetterström R. Plasma Valine and Urinary C-peptide in Infants. The Effect of Substituting Breast-Feeding with Formula or Formula with Human Milk. *Acta Pædiatr Scand*. 1985;74(4):615-616. doi:10.1111/j.1651-2227.1985.tb11044.x

263. Inostroza J, Haschke F, Steenhout P, Grathwohl D, Nelson SE, Ziegler EE. Low-protein formula slows weight gain in infants of overweight mothers. *JPGN*. 2014;59(1):70-77.

doi:10.1097/MPG.0000000000000349

264. Rogers I, Metcalfe C, Gunnell D, Emmett P, Dunger D, Holly J. Insulin-like growth factor-I and growth in height, leg length, and trunk length between ages 5 and 10 years. *J Clin Endocrinol Metab*. 2006;91(7):2514-2519. doi:10.1210/jc.2006-0388
265. Hoppe C, Mølgaard C, Thomsen BL, Juul A, Michaelsen KF. Protein intake at 9 mo of age is associated with body size but not with body fat in 10-y-old Danish children. *Am J Clin Nutr*. 2004;79(3):494-501. doi:10.1093/ajcn/79.3.494
266. Fahy E, Subramaniam S, Brown HA, et al. A comprehensive classification system for lipids. *J Lipid Res*. 2005;46(5):839-861. doi:10.1194/jlr.E400004-JLR200
267. Westerhuis JA, Hoefsloot HCJ, Smit S, et al. Assessment of PLSDA cross validation. *Metabolomics*. 2008;4(1):81-89. doi:10.1007/s11306-007-0099-6
268. Chong J, Soufan O, Li C, et al. MetaboAnalyst 4.0: Towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res*. 2018;46(W1):W486-94. doi:10.1093/nar/gky310
269. Jin WY, Lin SL, Hou RL, et al. Associations between maternal lipid profile and pregnancy complications and perinatal outcomes: A population-based study from China. *BMC Pregnancy Childbirth*. 2016;16(1):1-9. doi:10.1186/s12884-016-0852-9
270. Morillon AC, Leite DFB, Yakkundi S, et al. Glycerophospholipid and detoxification pathways associated with small for gestation age pathophysiology: discovery metabolomics analysis in the SCOPE cohort. *Metabolomics*. 2021;17(1):1-11. doi:10.1007/s11306-020-01740-9
271. Herrera E, Ortega-Senovilla H. Disturbances in lipid metabolism in diabetic pregnancy - Are these the cause of the problem? *Best Pract Res Clin Endocrinol Metab*. 2010;24(4):515-525. doi:10.1016/j.beem.2010.05.006
272. Gueuvoghlanian-Silva BY, Cordeiro FB, Lobo TF, et al. Lipid fingerprinting in mild versus severe forms of Gestational Diabetes Mellitus. *PLoS One*. 2015;10(12):1-13. doi:10.1371/journal.pone.0144027
273. Chen Q, Francis E, Hu G, Chen L. Metabolomic profiling of women with gestational diabetes mellitus and their offspring: Review of metabolomics studies. *J Diabetes Complications*. 2018;32(5):512-523. doi:10.1016/j.jdiacomp.2018.01.007
274. Tyagi S, Gupta P, Saini A, Kaushal C, Sharma S. The peroxisome proliferator-activated

receptor: A family of nuclear receptors role in various diseases. *J Adv Pharm Technol Res*. 2011;2(4):236-240. doi:10.4103/2231-4040.90879

275. Tain YL, Hsu CN, Chan JYH. PPARs link early life nutritional insults to later programmed hypertension and metabolic syndrome. *Int J Mol Sci*. 2016;17(20):1-11. doi:10.3390/ijms17010020
276. Rees WD, McNeil CJ, Maloney CA. The roles of PPARs in the fetal origins of metabolic health and disease. *PPAR Res*. 2008;2008. doi:10.1155/2008/459030
277. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary Protein Restriction of Pregnant Rats Induces and Folic Acid Supplementation Prevents Epigenetic Modification of Hepatic Gene Expression in the Offspring. *J Nutr*. 2005;135(6):1382-1386. doi:10.1093/jn/135.6.1382
278. Kersten S. Integrated physiology and systems biology of PPARα. *Mol Metab*. 2014;3(4):354-371. doi:10.1016/j.molmet.2014.02.002
279. Al-Hasani H, Joost HG. Nutrition-/diet-induced changes in gene expression in white adipose tissue. *Best Pract Res Clin Endocrinol Metab*. 2005;19(4):589-603. doi:10.1016/j.beem.2005.07.005
280. Martin CR, Ling PR, Blackburn GL. Review of infant feeding: Key features of breast milk and infant formula. *Nutrients*. 2016;8(5):1-11. doi:10.3390/nu8050279
281. Popkin BM, Adair LiS, Ng SW. NOW AND THEN: The Global Nutrition Transition: The Pandemic of Obesity in Developing Countries. *Nutr Rev*. 2012;70(1):3-21. doi:10.1111/j.1753-4887.2011.00456.x.
282. Fahy E, Cotter D, Sud M, Subramaniam S. Lipid classification, structures and tools. *Biochim Biophys Acta*. 2011;1811(11):637-647. doi:10.1007/s10439-011-0452-9.Engineering
283. Warensjö E, Risérus U, Vessby B. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. *Diabetologia*. 2005;48(10):1999-2005. doi:10.1007/s00125-005-1897-x
284. Andersen KR, Harslof LBS, Schnurr TM, et al. A study of associations between early DHA status and fatty acid desaturase (FADS) SNP and developmental outcomes in children of obese mothers. *Br J Nutr*. 2017;117(2):278-286. doi:10.1017/S0007114516004645

285. Wolters M, Dering C, Siani A, et al. The role of a FADS1 polymorphism in the association of fatty acid blood levels, BMI and blood pressure in young children—Analyses based on path models. *PLoS One*. 2017;12(7):1-17. doi:10.1371/journal.pone.0181485

286. Wolters M, Schlenz H, Börnhorst C, et al. Desaturase activity is associated with weight status and metabolic risk markers in young children. *J Clin Endocrinol Metab*. 2015;100(10):3760-3769. doi:10.1210/jc.2015-2693

287. Vinknes KJ, Elshorbagy AK, Nurk E, et al. Plasma stearoyl-CoA desaturase indices: Association with lifestyle, diet, and body composition. *Obesity*. 2013;21(3):294-302. doi:10.1002/oby.20011

288. Czumaj A, Śledziński T. Biological role of unsaturated fatty acid desaturases in health and disease. *Nutrients*. 2020;12(2). doi:10.3390/nu12020356

289. Rakhshandehroo M, Knoch B, Müller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. *PPAR Res*. 2010;2010. doi:10.1155/2010/612089

290. Zwamborn RA, Slieker RC, Mulder PC, et al. Prolonged high-fat diet induces gradual and fat depot-specific DNA methylation changes in adult mice. *Sci Rep*. 2017;7(43261):1-9.

291. Warensjö E, Rosell M, Hellenius ML, Vessby B, De Faire U, Risérus U. Associations between estimated fatty acid desaturase activities in serum lipids and adipose tissue in humans: Links to obesity and insulin resistance. *Lipids Health Dis*. 2009;8:6-11. doi:10.1186/1476-511X-8-37

292. Ziegler EE. Growth of Breast-Fed and Formula-Fed Infants. *Nestlé Nutr Work Ser Pediatr Progr*. 2006;58:51-63.

293. Prentice PM, Schoemaker MH, Vervoort J, et al. Human Milk Short-Chain Fatty Acid Composition is Associated with Adiposity Outcomes in Infants. *J Nutr*. 2019;149(5):716-722. doi:10.1093/jn/nxy320

294. UNICEF UK, The Baby Friendly Initiative. Breastfeeding in the UK. doi:10.1108/nfs.1999.01799fab.006

295. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res*. 2013;54(9):2325-2340. doi:10.1194/jlr.R036012

296. Arnoldussen IAC, Wiesmann M, Pelgrim CE, et al. Butyrate restores HFD-induced

adaptations in brain function and metabolism in mid-adult obese mice. *Int J Obes*. 2017;41(6):935-944. doi:10.1038/ijo.2017.52

297. Pannaraj PS, Li F, Cerini C, et al. Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA Pediatr*. 2017;90095:1-8. doi:10.1001/jamapediatrics.2017.0378
298. Lin H V., Frassetto A, Kowalik EJ, et al. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One*. 2012;7(4):1-9. doi:10.1371/journal.pone.0035240
299. Brahe LK, Astrup A, Larsen LH. Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases? *Obes Rev*. 2013;14(12):950-959. doi:10.1111/obr.12068
300. Corfe BM, Harden CJ, Bull M, Garaiova I. The multifactorial interplay of diet, the microbiome and appetite control: current knowledge and future challenges. *Proc Nutr Soc*. 2015;74(3):235-244. doi:10.1017/S0029665114001670
301. Li Z, Yi CX, Katiraei S, et al. Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut*. 2018;67(7):1269-1279. doi:10.1136/gutjnl-2017-314050
302. Butte NF, Wong WW, Hopkinson JM, O'Brian Smith E, Ellis KJ. Infant feeding mode affects early growth and body composition. *Pediatrics*. 2000;106(6):1355-1366. doi:10.1542/peds.106.6.1355
303. Nommsen L, Lovelady C, MJ H, Lönnerdal B, Dewey K. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING Study. *Am J Clin Nutr*. 1991;53(2):457-465.
304. Kratz M, Baars T, Guyenet S. The relationship between high-fat dairy consumption and obesity, cardiovascular, and metabolic disease. *Eur J Nutr*. 2013;52(1):1-24. doi:10.1007/s00394-012-0418-1
305. Rolland-Cachera MF, Maillot M, Deheeger M, Souberbielle JC, Péneau S, Hercberg S. Association of nutrition in early life with body fat and serum leptin at adult age. *Int J Obes*. 2013;37(8):1116-1122. doi:10.1038/ijo.2012.185
306. Koda Y, Tachida H, Pang H, et al. Contrasting Patterns of Polymorphisms at the ABO-

Secretor Gene (FUT2) and. *Genetics*. 2001;158:747-756.

307. Ikehara Y, Yasutomi H, Kitamura T, et al. Polymorphisms of two Fucosyltransferase genes (Lewis and Secretor genes) involving type I Lewis antigens are associated with the presence of anti-Helicobacter pylori IgG antibody. *Cancer Epidemiol Biomarkers Prev*. 2001;10(9):971-977.
308. Hazra A, Kraft P, Selhub J, et al. Common variants of FUT2 are associated with plasma vitamin B 12 levels NIH Public Access Author Manuscript. *Nat Genet*. 2008;40(10):1160-1162. doi:10.1038/ng.210.Common
309. Kindberg E, Hejdeman B, Bratt G, et al. A nonsense mutation (428G→A) in the fucosyltransferase FUT2 gene affects the progression of HIV-1 infection. *AIDS*. 2006;20(5):685-689. doi:10.1097/01.aids.0000216368.23325.bc
310. Thorven M, Grahn A, Hedlund K-O, et al. A Homozygous Nonsense Mutation (428G→A) in the Human Secretor (FUT2) Gene Provides Resistance to Symptomatic Norovirus (GGII) Infections. *J Virol*. 2005;79(24):15351-15355. doi:10.1128/jvi.79.24.15351-15355.2005
311. Colston JM, Francois R, Pisanic N, et al. Effects of Child and Maternal Histo-Blood Group Antigen Status on Symptomatic and Asymptomatic Enteric Infections in Early Childhood. *J Infect Dis*. 2019;220(1):151-162. doi:10.1093/infdis/jiz072
312. Barrett JC, Clayton D, Concannon P, et al. Genome-wide association study and meta-analysis finds over 40 loci affect risk of type 1 diabetes. *Nat Genet*. 2009;41(6):703-707. doi:10.1038/ng.381.
313. Tong M, McHardy I, Ruegger P, et al. Reprograming of gut microbiome energy metabolism by the FUT2 Crohn's disease risk polymorphism. *ISME J*. 2014;8(11):2193-2206. doi:10.1038/ismej.2014.64
314. Wacklin P, Tuimala J, Nikkilä J, et al. Faecal microbiota composition in adults is associated with the FUT2 gene determining the secretor status. *PLoS One*. 2014;9(4):e94863. doi:10.1371/journal.pone.0094863
315. Korpela K, Salonen A, Hickman B, et al. Fucosylated oligosaccharides in mother's milk alleviate the effects of caesarean birth on infant gut microbiota. *Sci Rep*. 2018;8(1):1-7. doi:10.1038/s41598-018-32037-6
316. Sprenger N, Lee LY, De Castro CA, Steenhout P, Thakkar SK. Longitudinal change of

selected human milk oligosaccharides and association to infants' growth, an observatory, single center, longitudinal cohort study. *PLoS One*. 2017;12(2):1-15. doi:10.1371/journal.pone.0171814

317. Smith-Brown P, Morrison M, Krause L, Davies PSW. Mothers secretor status affects development of childrens microbiota composition and function: A pilot study. *PLoS One*. 2016;11(9):1-13. doi:10.1371/journal.pone.0161211
318. Sprenger N, Binia A, Austin S. Human Milk Oligosaccharides: Factors Affecting Their Composition and Their Physiological Significance. *Nestle Nutr Inst Workshop Ser*. 2019;90:43-56. doi:10.1159/000490292
319. Lewis ZT, Totten SM, Smilowitz JT, et al. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. *Microbiome*. 2015;3(1):15-17. doi:10.1186/s40168-015-0071-z
320. Schneider M, Al-Shareffi E, Haltiwanger RS. Biological functions of fucose in mammals. *Glycobiology*. 2017;27(7):601-618. doi:10.1093/glycob/cwx034
321. McGuire M, Meehan C, McGuire M, et al. What's normal? Oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically. *Am J Clin Nutr*. 2017;105:1085-1100. doi:10.3945/ajcn.116.139980.Am
322. Jorgensen JM, Young R, Ashorn P, et al. Associations of human milk oligosaccharides and bioactive proteins with infant growth and development among Malawian mother-infant dyads. *Am J Clin Nutr*. 2021;113:209-220. doi:10.1093/ajcn/nqaa272
323. ten Bruggencate SJ, Bovee-Oudenhoven IM, Feitsma AL, van Hoffen E, Schoterman MH. Functional role and mechanisms of sialyllactose and other sialylated milk oligosaccharides. *Nutr Rev*. 2014;72(6):377-389. doi:10.1111/nure.12106
324. Maki K, Slavin J, Rains T, Kris-Etherton P. Limitations of observational evidence: Implications for evidence-based dietary recommendations. *Adv Nutr*. 2014;5(3):7-15. doi:10.3945/an.114.005777

Appendices

Appendix 1 CBGS questionnaires

Appendix 1a Prenatal questionnaire

AFFIX MOTHER'S BARCODE

Identifying information:

Please enter answers here

Mother's - initials	
Mother's - date of birth	__ / ____ / _____
Mother's - study number	
Expected date of delivery of baby	__ / ____ / _____
Date of completing this questionnaire	__ / ____ / _____

AFFIX MOTHER'S BARCODE

A. GENERAL QUESTIONS about the mother (A1-7)

A1. What is your marital status? 1. Married 2. Cohabiting 3. Single	D1
A2. How tall are you (feet or cm)? *Be as precise as possible.*	A6
A3. What was your weight *before* pregnancy (stone or kg)?	A8
A4. What is your current weight (stone or kg)?	A9
A5. How old were you when you had your first period (years)?	C1

A6. What was your own birth weight?

Please complete one of the following lines	
Birth weight in pounds and ounces	lbs ozs
OR Birth weight in kilograms	kgs
Or Birth weight not known (please tick)	☐

A7. When were you born?

*Please tick **ONE** option from the following*	
Very Pre-term (early) Before 33 weeks	
Pre-term (early) Between weeks 33 and 36	
At term Between weeks 36 and 42	
Post term (late) After 42 weeks	
Information not known	

AFFIX MOTHER'S BARCODE

B. CURRENT AND PREVIOUS PREGNANCIES (B1-9)

B1. Did you take any medication while you *tried to become pregnant* ? 1. Yes *(please enter details below)* 2. No			B27
Name of Medicine	Condition treated	Dose per day	No. of days
E.g. Paracetamol	*E.g. Muscle aches*	*E.g. 500 mg x 3*	*E.g. 3 days*
B28	B29	B30	B31
B32	B33	B34	B35
B36	B37	B38	B39
B40	B41	B42	B43

B2. Have you taken any medicine during this pregnancy? 1. Yes *(please enter details below)* 2. No				B91
Name of Medicine	Condition treated	Total Dose per day	No. of days	Gestational Week(s)
E.g. Paracetamol	*E.g. Muscle aches*	*E.g. 1500 mg*	*E.g. 3*	*14*
B92	B93	B94	B95	B96
B97	B98	B99	B100	B101
B102	B103	B104	B105	B106
B107	B108	B109	B110	B111

B3. Have you taken any antibiotics in the last year? 1. Yes *(please enter details below)* 2. No				
Name of Antibiotic (or unknown)	Condition treated	Total Dose per day	No. of days	Date
E.g. Augmentin	*E.g. Ear infection*	*E.g. 250 mg*	*E.g. 5*	*E.g 20/10/2010*

AFFIX MOTHER'S BARCODE

B4. Have you been pregnant before? 1. Yes *(please enter details below)* 2. No							B121
Year	Length of pregnancy (weeks)	Sex of child	Birth weight (grams)	Any Disease?	Miscarriage /Abortion	Stillbirth	Ectopic Pregnancy?
E.g. 2005	*E.g. 5*	*E.g. NA*	*E.g. NA*	*E.g. NK*	*E.g. ✓*	*E.g. NA*	*E.g. No*
B122-130							
B131-139							
B140-148							
B149-157							
B158-166							

B5. How long did it take for you to become pregnant? (since trying to be pregnant / since stopping contraception) B17

0. Don't know OR Pregnancy was unplanned
1. Less than 2 months
2. 2-4 months
3. 5-6 months
4. 7-9 months
5. 10-12 months
6. 1-2 years
7. More than 2 years

B6. Did you use any *hormonal* contraception (pill, implant or hormonal intrauterine device (IUD)) **before you knew you were pregnant?** B12
(i.e. during the first weeks of this pregnancy)

1. Yes
2. No

B7. Have either you or the father been treated for infertility in connection with this pregnancy? B19

1. Yes

AFFIX MOTHER'S BARCODE

2. No *(go to Section C)*	
B8. If yes, which treatment did you or the father receive? *Please tick the appropriate box. You may choose several options and tick the appropriate box(es).* 1. Hormonal treatment *(go to question 8)* ..... 2. Insemination..... 3. IVF..... 4. Operation..... 5. Other.....	B20-24 ☐ ☐ ☐ ☐ ☐

B9. If you were treated by insemination / IVF / ICSI, was the semen: 1. from your husband ..... 2. from a donor.....	B25 ☐

B10. If you were treated by insemination / IVF / ICSI, was the egg: 1. your own..... 2. from a donor.....	B26 ☐

AFFIX MOTHER'S BARCODE

C. MOTHER'S LIFESTYLE (C1-21)

C1. Have you taken any vitamins or dietary supplements during this pregnancy? E23

1. Yes *(please add details below)*
2. No

In the column "Frequency" please choose from the following three possibilities:
1) daily, 2) more than once a week, 3) less than once a week.

	Name of product	Frequency	Gestational Week(s)
E.g.	*Vitamin C*	*daily*	*12 to 20*
Folic Acid	E30	E31	E32
Other Single Vitamins	E24	E25	E26
Other Multivitamins	E27	E28	E29
Iron	E33	E34	E35
Calcium	E36	E37	E38
Fish oil (Cod Liver Oil)	E39	E40	E41
Other E42	E43	E44	E45
Other E46	E47	E48	E49

C2. How often have you eaten or drunk of the following items on average *during this pregnancy*? *Please answer for each single item and use "0" for those that you have not used at all.* E68-75

1. Coffee (cups per day) .....
2. Tea (cups per day) .....
3. Cocoa (cups per day) .....
4. Coke (litres per week) .....
5. Beer (cans or bottles per **week**) .....
6. Wine (glasses per **week**) .....
7. Strong alcohol /Spirits (liqueur glasses per **week**).....
8. Chocolate bars (grams per week).....
   e.g. 1 Mars bar = 60 grams

AFFIX MOTHER'S BARCODE

C3. How many glasses of tap water do you drink daily? 1. None 2. 1-3 glasses / day 3. more than 3 glasses /day	E50 ☐
C4. How often do you eat eggs? 1. Several times per week 2. Several times per month 3. Less than once a month 4. Never	☐
C5. How often do you use canned foods? 1. Several times per week 2. Several times per month 3. Less than once a month 4. Never	E64 ☐
C6. How often do you eat freshwater fish (not seafood) as main course? 1. Several times per week 2. Several times per month 3. Less than once a month 4. Never	E65 ☐

C7. Are you vegetarian? 1. Yes 2. No (go to question C9)	E51 ☐
C8. If you are vegetarian, which foods do you avoid? 1. I don't eat fish or meat..... 2. I don't eat eggs and milk.....	E52-53 ☐ ☐

C9. Do you ever eat organic food ? 1. Yes 2. No (go to question C11)	E55 ☐
C10. If yes, how often ? 1. Daily / several times per week 2. 1-4 times per month 3. Very seldom / Never	E56 ☐

C11. Do you use plastic containers in the microwave for preparing and heating meals? 1. Yes 2. No (go to question C13)	E62 ☐
C12. If yes, how often ? 1. Several times per week 2. Several times per month 3. Less than once a month 4. Never	E63 ☐

AFFIX MOTHER'S BARCODE

C13. Have you smoked at all during this pregnancy? *Please also include the first few weeks of this pregnancy* 1. Yes 2. No (go to question C17)	E99
C14. If Yes, how many do you smoke <u>now *per day*</u>? 1. Cigarettes. (no per day)..... 2. Other. (please state what?).....	E104-107 ☐ ☐
C15. Did you stop smoking *after* you had become pregnant? 1. Yes 2. No *(go to question C17)* 3. Partially / No, but I reduced it.	E110 ☐
C16. If yes, in which week of pregnancy did you stop?	E111 ☐

C17. Have you used nicotine plasters /chewing gums etc. in this pregnancy? 1. Yes 2. No *(go to question C20)*	E112 ☐
C18. If yes, which product did you use? 1. Chewing gum with nicotine 2. Plaster with nicotine 3. Spray with nicotine	E113 ☐
C19. If yes, during which week(s) of pregnancy did you use it? *Write the week in the box.* 1. week 0-13 2. week 14-26 3. week 27-42 4. during the whole pregnancy	E114 ☐

C20. Are you exposed to passive smoking? e.g. does your partner smoke? 1. Yes 2. No *(go to section D)*	E115 ☐
C21. If yes, how many hours per day on average? 1. Less than 1 hour 2. 1 to 2 hours 3. More than 2 hours	E116 ☐

AFFIX MOTHER'S BARCODE

D. SPECIFIC HEALTH (D1-2)

D1. Have you, your partner, or other children had any of the following illnesses?
Please tick YES if you (mother), the father or any other children have these conditions
The research nurse can help you with this section if you have any difficulties.

		Mother	Father	Any Child
KIDNEY / BLADDER				
		Tick if YES		
Lack of one kidney	C140-143			
More than 2 kidneys	C144-147			
Cystic kidney(s)	C148-151			
Malformation of kidney (write name if possible)	C156-159			
Narrowing of ureter or urethra	C160-163			
Kidney stone(s)	C164-167			
Urinary tract infections in childhood	C168-171			
Bed wetting after 6 yrs of age	C172-175			
GONADS				
Hypospadias	C176-178			
Epispadias	C179-181			
Wrong opening of urethra	C182-184			
Undescended testis (one or both sides)	C201-203			

D2. Other health conditions in the mother

Please tick if you have ever suffered from any of the following conditions:	
Childhood-onset Diabetes (onset < 16 years old)	
Adult-onset Diabetes	
Gestational Diabetes (pregnancy-only)	
Hypertension	
Depression	
Anorexia nervosa	
Bulimia	

AFFIX MOTHER'S BARCODE

E. OCCUPATION AND EDUCATION of the mother (E1-11)

The following questions refer to your current main job, or (if you are not working now) to your last main job. Please tick one box only per question.

(National Statistics SEC self-coded method)

E1. Employee or self employed
Do (did) you work as an employee or are (were) you self-employed?

	Please tick one box
Employee	
Self-employed with employees	
Self-employed / freelance without employees (*go to question **E4***)	
Never been employed (*go to question **E7***)	

E2. Number of employees
For employees: indicate below how many people work (worked) for your employer at the place where you work (worked) For self-employed: how many people do (did) you employ?

	Please tick one box
1 to 24 people	
25 or more people	

E3. Supervisory status
Do (did) you supervise any other employees
A supervisor or foreman is responsible for overseeing the work of other employees on a day to day basis.

Please tick one box	Yes	No

AFFIX MOTHER'S BARCODE

E4. Occupation type
Please tick one box which **best** describes the sort of work you do. If you are not working now, please tick a box to show what you did in your last job.

	Please tick one box	Office use
Modern professional occupations such as: teacher – nurse – social worker - welfare officer – artist – police officer(sergeant or above) – musician – software designer		1
Clerical and intermediate occupations such as: office clerk – secretary – personal assistant – clerical worker – call centre agent – nursing auxiliary – nursery nurse		2
Senior managers or administrators (usually responsible for planning, organising and co-ordinating work and for finance)		3
Technical and craft occupations such as: motor mechanic – fitter – inspector – plumber – printer – tool maker – electrician – gardener –train driver		4
Semi-routine manual and service occupations such as: postal worker – machine operative – security guard – caretaker – farm worker – catering assistant – receptionist – sales assistant		5
Routine manual and service occupations such as: HGV driver – van driver – cleaner – porter – packer – sewing machinist – messenger – labourer – waiter/waitress – bar staff		6
Middle or junior managers such as: office managers – retail manager – bank manager – restaurant manager – warehouse manager – publican		7
Traditional professional occupations such as: accountant – solicitor – medical practitioner – scientist – civil/mechanical engineer		8

E5. Are you / were you, to your knowledge, exposed to chemicals at your work? 1. Yes 2. No *(go to question E7)*	D15 ☐
E6. If yes, which chemicals? *Write name, type or what the chemicals are used for.* _____	D16

AFFIX MOTHER'S BARCODE

F. FURTHER QUESTIONS ABOUT THE FATHER of your child (F1-9)

F1. How old is he (in years)?	A3
F2. How tall is he (feet or cm)? *Be as precise as possible.*	
F3. What is his current weight (stone or kg)?	

The following questions refer to his current main job, or (if you are not working now) to his last main job. Please tick one box only per question.

F4. Employee or self employed
Do (did) he work as an employee or is (was) he self-employed?

	Please tick one box
Employee	
Self-employed with employees	
Self-employed / freelance without employees	
Never been employed	

F5. Number of employees
For employees: indicate below how many people work (worked) for his employer at the place where he works (worked)
For self-employed: indicate below how many people he employs (employed)

	Please tick one box
1 to 24 people	
25 or more people	

F6. Supervisory status
Does (did) he supervise any other employees
A supervisor or foreman is responsible for overseeing the work of other employees on a day to day basis.

Please tick one box	Yes	No

AFFIX MOTHER'S BARCODE

F7. Occupation type
Please tick one box which **best** describes the sort of work he does. If he is not working now, please tick a box to show what he did in his last job.

	Please tick one box	Office use
Modern professional occupations such as: teacher – nurse – social worker - welfare officer – artist – police officer(sergeant or above) – musician – software designer		1
Clerical and intermediate occupations such as: office clerk – secretary – personal assistant – clerical worker – call centre agent – nursing auxiliary – nursery nurse		2
Senior managers or administrators (usually responsible for planning, organising and co-ordinating work and for finance)		3
Technical and craft occupations such as: motor mechanic – fitter – inspector – plumber – printer – tool maker – electrician – gardener –train driver		4
Semi-routine manual and service occupations such as: postal worker – machine operative – security guard – caretaker – farm worker – catering assistant – receptionist – sales assistant		5
Routine manual and service occupations such as: HGV driver – van driver – cleaner – porter – packer – sewing machinist – messenger – labourer – waiter/waitress – bar staff		6
Middle or junior managers such as: office managers – retail manager – bank manager – restaurant manager – warehouse manager – publican		7
Traditional professional occupations such as: accountant – solicitor – medical practitioner – scientist – civil/mechanical engineer		8

F8. Is / was he – to his knowledge – exposed to chemicals at his work? 1. Yes 2. No	☐ D15
F9. If yes, which chemicals? *Write name, type or what the chemicals are used for.* _____	D16

AFFIX MOTHER'S BARCODE

G: ETHNIC ORIGIN of Mother and Father

Tick one box only for each person

Mother (**M**)	Father (**F**)

2001 census ethnicity classification

White		**M**	**F**
A	British		
B	Irish		
C	Any other White background		

Mixed			
D	White & Black Caribbean		
E	White and Black African		
F	White and Asian		
G	Any other Mixed background		

Asian or Asian British			
H	Indian		
J	Pakistani		
K	Bangladeshi		
L	Any other Asian background		

Black or Black British		**M**	**F**
M	Caribbean		
N	African		
P	Any other Black background		

Other ethnic category			
R	Chinese		
S	Any other ethnic category		

Not stated			
Z	Not stated		

Appendix 1b Infant feeding practice questionnaire

The Cambridge Baby Growth Study

Infant Diet questionnaire

UNIVERSITY OF CAMBRIDGE

AFFIX

CHILD BARCODE

Date _____

Visit – 3 months

1. What milk does your baby currently have? (Please tick all that apply)

Breast milk ☐

Expressed breast milk ☐

Formula milk: infant ☐ "follow-on" ☐ other ☐

If you are giving your baby formula, please write the brand(s) you use:

_____

2. When feeding your baby, do you… (Please circle one)

Feed on demand Follow a routine Do a combination

3. If your baby has water, how much water does your baby have?

_____ mls/oz (please specify) per day

4. Does your baby have any drink other than breast/formula milk or water/anything added to the milk?

☐ Yes ☐ No

If yes, please give details _____

5. Does your baby have any solid/semi soft foods?

☐ Yes ☐ No

If yes, how many times a day? _____

If yes, at what age did this start? _____

Please answer questions 6-8 if your baby has breast milk:

6. In a typical 24 hour period, how many breast feeds does your baby have? _____

7. How many minutes does a typical breast feed last? _____

8. If your baby has expressed breast milk, how much milk does your baby have per feed?

_____ mls/oz (please specify) No. of feeds per day _____

The Cambridge Baby Growth Study

Infant Diet questionnaire

UNIVERSITY OF CAMBRIDGE

Please answer questions 9-11 if your baby has formula milk:

9. How much formula milk does your baby drink per feed? (please exclude any left over milk) _____ mls/oz (please specify) No. of feeds per day _____

10. When you make milk feeds are the scoops usually? (please circle)

a) rounded flat ready to use formula

b) loosely packed tightly packed

11. What do you add to the bottle first? (please circle)

powder first water first

12. Do you use 1 scoop formula to 1 ounce/30mls of water?

☐ Yes ☐ No

If not, how do you make the feeds? _____

13. When did you stop breastfeeding exclusively (if applicable)?

Child's age: _____Weeks _____Days

14. When did you stop breastfeeding completely (if applicable)?

Child's age: _____Weeks _____Days

15. Do you give your baby or yourself any pre- or probiotics?

If yes, please give details of any supplements _____

And/or dietary intake e.g. types of yoghurt/drink

_____

_____

16. Have you or your baby had any antibiotics or anti-fungal treatment since your last study visit?

☐ Yes ☐ No

If yes, please give details _____

17. Have you or your baby had any steroids since your last study visit?

☐ Yes ☐ No

If yes, please give details _____

THANK YOU FOR YOUR HELP

Heading 1

Appendix 1c Infant eating behaviour questionnaire

Cambridge University Hospitals NHS
NHS Foundation Trust

UNIVERSITY OF CAMBRIDGE

AFFIX CHILD BARCODE

Please complete just before 3 month visit

The Cambridge Baby Growth Study

Infant Eating Behaviour Questionnaire

These questions are about your baby's appetite over its first three months of life. We are specifically interested in the period when your baby was fed milk only, i.e. no solid foods or pre-prepared baby food yet.

D1. How would you rate your baby's appetite in its first three months?

Poor	OK	Good	Very Good	Excellent
☐	☐	☐	☐	☐

How would you describe your baby`s feeding style at a typical daytime feed in its first three months?

		Never	Rarely	Sometimes	Often	Always
D2.	My baby sucked vigorously	☐	☐	☐	☐	☐
D3.	My baby sucked steadily and rhythmically	☐	☐	☐	☐	☐
D4.	My baby seemed contented while feeding	☐	☐	☐	☐	☐
D5.	My baby frequently wanted more milk than I provided	☐	☐	☐	☐	☐
D6.	My baby loved milk	☐	☐	☐	☐	☐

These are some more questions about how your baby feeds. Again thinking back to the first three months, please choose which box is most appropriate for your baby

		Never	Rarely	Sometimes	Often	Always
D7.	My baby had a big appetite	☐	☐	☐	☐	☐
D8.	My baby finished feeding quickly	☐	☐	☐	☐	☐

Infant Eating Behaviour Questionnaire, Cambridge Baby Growth Study

		Never	Rarely	Sometimes	Often	Always
D9.	**My baby became distressed while feeding**	☐	☐	☐	☐	☐
D10.	**My baby got full up easily**	☐	☐	☐	☐	☐
D11.	**If allowed to, my baby would take too much milk**	☐	☐	☐	☐	☐
D12.	**My baby took more than 30 minutes to finish feeding**	☐	☐	☐	☐	☐
D13.	**My baby got full before taking all the milk I thought he/she should have had**	☐	☐	☐	☐	☐
D14.	**My baby fed slowly**	☐	☐	☐	☐	☐
D15.	**Even when my baby had just eaten well he/she was happy to feed again if offered**	☐	☐	☐	☐	☐
D16.	**My baby found it difficult to manage a complete feed**	☐	☐	☐	☐	☐
D17.	**My baby was always demanding a feed**	☐	☐	☐	☐	☐
D18.	**My baby sucked more and more slowly during the course of a feed**	☐	☐	☐	☐	☐
D19.	**If given the chance, my baby would always be feeding**	☐	☐	☐	☐	☐
D20.	**My baby enjoyed feeding time**	☐	☐	☐	☐	☐
D21.	**My baby could easily take a feed within 30 minutes of the last one**	☐	☐	☐	☐	☐

Please return this questionnaire to:
The Cambridge Baby Growth Study
Department of Paediatrics, Addenbrooke's Hospital, Box 116, Cambridge CB2 0QQ
If you have any questions please contact us on:
Tel. 01223 336 888
Or Email: babygrowthstudy@medschl.cam.ac.uk

Appendix 1c Infant food diary

How to fill in the diary

Understanding how food and drink influence growth is an important part of the Baby Growth study. Thank you very much for helping us by filling in these food diaries.

Please could you record everything that your child has by mouth for 3 days. Please start each day's record when you get up in the morning and fill in everything your child eats and drinks for a 24-hour period until the same time the next day. The days do not need to be one after the other. If any day is likely to be very difficult or unusual choose another day. It is very important that you do not change what your child normally eats and drinks just because you are keeping this record.

Try to fill in the food and drink given as you go through the day, as this is much easier and more accurate than trying to remember at the end of the day. We have included examples to show how we would like you to record the food and drink given.

When recording the food given please include the brand name (if known), portion size (using feeding jar size, cup or spoon size, weights from labels), any additions to the food (oils, butter, sugar/sweeteners, sauces, salt, pepper etc) and cooking methods (fried, grilled, micro-waved, roasted). It helps a great deal if you bring along labels from any unusual foods you give your child when returning your completed food diary. If someone else looks after your child for some of the time it would be most helpful if they could fill in the food given in the parts of the day when your child is with them.

Please bring the completed diaries with you when you come to Addenbrooke's or Ely Hospital for your 24 month Cambridge Baby Growth Study check. There will be someone to talk to about the diaries at this visit. If you would like some more time to fill in the diaries we can give you a stamped addressed envelope to send back the diaries. Please also bring any special food labels with you as this really helps us to analyse accurately what you have recorded in the diary.

Many thanks

V1 08/02/11

General questions about your child's food. Please add as much detail as you need

1. What type of milk does your child have? If using more than one sort of milk please tik more than one box and add more detail if you would like.

   a. Breast milk: ☐

   b. Formula: infant ☐ follow on ☐ soya ☐

   c. Other formula: ☐

   If formula, which brand do you use? _____

   d. Cows milk: whole milk ☐ semi-skimmed milk ☐ skimmed milk ☐

   e. Soya milk: ☐ other milk ☐

   Please give details of any other milk given? _____

2. If using formula please describe how you make the feed. Are scoops usually?

   flat ☐ rounded ☐ ready to use formula ☐

3. For 1 scoop of milk powder how much water do you add? Fluid ounces ____ or Millilitres ____

4. If you use formula milk powder to make up your child's feeds do you put the water or the powder in the bottle first? water first ☐ powder first ☐

2pm to 5pm							
5pm to 8pm		Day 3					
8pm to 10pm							
10pm to 6am							

What sort of bottle or cup did your baby use? Please write the number in the correct box.

Plastic trainer cup with lid ☐ Plastic bottle ☐
Plastic cup without lid ☐ China cup or mug ☐
Glass ☐ Other ☐

Drinks Day 3
The milk feeds and drinks your child has, including water, in this 24 hours can be recorded here.

Please find the appropriate time slot and then record the time your child has a drink.
Note the type of fruit juice or squash etc.
Please also include any vitamins or medicines on this sheet.

Time slot	When	Full description and/or brand of drink.	Did you dilute with water? yes/no	How much concentrate did you use?	Did you add sug-ar? Number of tea-	Breast milk-minutes child fed	How much did your child drink?
6am to 9am							
9am to 12 noon		Drinks					
12 noon to 2pm							

We hope that by answering these questions you won't have to keep repeating these details on the daily food questionnaire.

1. What type of margarine or butter do you usually use for your baby? *(If not used at all please go to question 2).* Please give the full name and brand from the packet of the type used most often. _____

2. What type of bread does your child eat most often?
   white ☐ brown ☐ whole meal ☐
   granary ☐ high fibre white ☐ other ☐
   Please give full description _____
   We will assume that you used the same bread and butter or margarine throughout the day. Please indicate on the food diary pages if a different bread, butter or margarine is used.

3. What sort of loaf do you usually use? Please tick all that apply.
   small loaf ☐ medium loaf ☐ large loaf ☐
   sliced (thin) ☐ sliced (medium) ☐ sliced (thick ☐

4. Do you avoid giving your child any of the following foods? yes ☐ no ☐
   Please tick all that apply

a, poultry	b, fish	c, beef	d, other red meat	e. eggs
f, cheese	g, milk	h, butter	I, nuts	j, wheat/gluten

5. Is your child on any kind of special diet? yes ☐ no ☐ _____

Below is an example of how we would like you to record what your child eats.

Date 12th May 2011

Day of the Week Thursday

Each day is divided into time intervals from before breakfast to the evening meal and throughout the night. Please find the appropriate time interval and write in what your child had to eat.

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
6am to 9am	7.30	Kitchen No TV High chair	Mother sister	11/2 Weetabix with 100mls of milk and 1 tbs raisins	Left 2 tsp Weetabix ate all raisins

Food Day 3.

1. As far as you know was all the food and drink taken by your baby during this 24 hours recorded? yes ☐ no ☐ not sure ☐

2. Was the food and drink for this 24 hours fairly typical for your baby?
   yes ☐ no ☐
   If no please describe how it differed from normal and if your baby was unwell during this 24 hours
   _____
   _____

3. Has anyone else looked after your baby today?
   yes ☐ no ☐
   Please record any parts of this 24-hour period when someone else looked after your baby

Start time (e.g. 9.30am)	Return time (e.g. 5.00pm)
8.45am	5.00pm

Food Day 3 continued.

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
5pm to 8pm					
8pm to 10pm					
10pm to 6am					
9am to 12 noon	10.30	Nursery No TV At table	Nursery nurse	Chopped fruit at nursery, strawberries, unpeeled apples, bananas. Long breadstick	Ate whole nursery portion. Ate half
12 noon to 2pm	12.00	Nursery No TV At table	Nursery nurse	Shepherd's Pie with peas Apple pie and custard (All food prepared and cooked at the nursery)	Ate half of a nursery portion Ate all
2pm to 5pm	3.15	Nursery No TV At table	Nursery nurse	Half a pitta bread cut into fingers with 1 tbs houmus	Ate all

First food example continued

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
5pm to 8pm	6.15	Kitchen No TV High chair	Mum sister	Fish finger, grilled, with 1 small boiled potato, 2 small broccoli florets and 2 tbs sweetcorn (frozen) Petits Filous Fromage Frais 60g Strawberry flavour	Left 1 tbs sweetcorn Ate 2
8pm to 10pm					
10pm to 6am					

What sort of plates does your child usually use: plastic ✓ china ☐ other ☐

What sort of cutlery does your child usually use: plastic ✓ metal ☐

9am to 12 noon					
12 noon to 2pm				Day 3	
2pm to 5pm					

Food Day 3

Date **Day of the Week**

When recording your child's food please give as much detail as possible about the foods used, the cooking method and any added sauces. Also please include the brand name and flavour of other foods.
For take away food or eating out please note the name of the café / restaurant.
Please write how much your child ate, excluding any leftover food, in the 'Amount eaten' column.
Don't forget to include any sweets, biscuits, crisps, fruit and spreads like marmite.

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
6am to 9am				Food	

First food example.

1. As far as you know was all the food and drink taken by your baby during this 24 hours recorded? yes ✓ no ☐ not sure ☐

2. Was the food and drink for this 24 hours fairly typical for your baby? yes ✓ no ☐
   If no please describe how it differed from normal and if your baby was unwell during this 24 hours

3. Has anyone else looked after your baby today? yes ✓ no ☐
   Please record any parts of this 24-hour period when someone else looked after your baby

Start time (e.g. 9.30am)	Return time (e.g. 5.00pm)
8.45am	5.00pm

Below is a second example of how we would like you to record what your child eats.

Date 15th May 2011

Day of the Week Sunday

Each day is divided into time intervals from before breakfast to the evening meal and throughout the night. Please find the appropriate time interval and write in what your child had to eat.

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
6am to 9am	7.45	Kitchen No TV At table	Dad	Cheerios 100g with 75mls whole milk Toast with strawberry jam	Ate half Ate all including the crusts
2pm to 5pm					
5pm to 8pm					
8pm to 10pm					
10pm to 6am					

Day 2

What sort of bottle or cup did your baby use?

Please write the number in the correct box.

Plastic trainer cup with lid	☐	Plastic bottle	☐
Plastic cup without lid	☐	China cup or mug	☐
Glass	☐	Other	☐

Drinks Day 2
Please record milk feeds, other drinks and water here.

Please find the appropriate time slot and then record the time your child has a drink.
Note the type of fruit juice or brand of squash etc.
Please also include any vitamins or medicines on this sheet.

Time slot	When	Full description and/or brand of drink.	Did you dilute with water?	How much concentrate did you use?	Did you add sugar?	Breast milk- minutes child fed	How much did your child drink?
6am to 9am							
9am to 12 noon							
12 noon to 2pm							

Drinks

9am to 12 noon	10.45	Grand-parent's house, living room, No TV	Mum, Dad, sister and Grand-parents	Haribo Starmix	10 sweets
12 noon to 2pm	1.30	Grand-parent's dining room. No TV. At table	Mum, Dad, sister and Grand-parents	1 slice chicken and 2 small roast potatoes roasted in sun-flower oil. 1/2 small carrot and 1 tbs peas boiled. 2 tbs gravy made with juices from meat and chicken Bisto gravy granules Tesco Finest chocolate ice cream	Left half of the carrot and half the peas Ate 3 tbs
2pm to 5pm	3.30	Grand-parent's house, living room, TV on		Victoria sponge made with butter and eggs strawberry jam no icing	Ate a small adult sized slice

Second food example continued

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
5pm to 8pm		Kitchen No TV At table	Mum Dad and sister	Cheese sandwich made with 2 slices of bread and medium cheddar cheese Seedless grapes, 1 inch cubes of honeydew melon	Just left 1/2 the crusts Ate 10 grapes 3 cubes melon
8pm to 10pm					
10pm to 6am					

Food Day 2.

1. As far as you know was all the food and drink taken by your baby during this 24 hours recorded? yes ☐ no ☐ not sure ☐

2. Was the food and drink for this 24 hours fairly typical for your baby?
   yes ☐ no ☐
   If no please describe how it differed from normal and if your baby was unwell during this 24 hours
   _____
   _____

3. Has anyone else looked after your baby today?
   yes ☐ no ☐
   Please record any parts of this 24-hour period when someone else looked after your baby

Start time (e.g. 9.30am)	Return time (e.g. 5.00pm)
8.45am	5.00pm

Food Day 2 continued.

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
5pm to 8pm					
8pm to 10pm				Food	
10pm to 6am					

Second food example

1. As far as you know was all the food and drink taken by your baby during this 24 hours recorded? yes ☑ no ☐ not sure ☐

2. Was the food and drink for this 24 hours fairly typical for your baby?
   yes ☑ no ☐
   If no please describe how it differed from normal and if your baby was unwell during this 24 hours
   Mainly normal but a few more sweet things than usual as with grandparents

3. Has anyone else looked after your baby today?
   yes ☐ no ☑
   Please record any parts of this 24-hour period when someone else looked after your baby

Start time (e.g. 9.30am)	Return time (e.g. 5.00pm)
8.45am	5.00pm

Food Day 1

Date **Day of the Week**

_____ _____

When recording your child's food please give as much detail as possible about the foods used, the cooking method and any added sauces. Also please include the brand name and flavour of other foods.
For take away food or eating out please note the name of the café / restaurant.

Please write how much your child ate, excluding any leftover food, in the 'Amount eaten' column.

Don't forget to include any sweets, biscuits, crisps, fruit and spreads like marmite.

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
6am to 9am				Food	
9am to 12 noon					
12 noon to 2pm				Day 2	
2pm to 5pm					

Food Day 2

Date **Day of the Week**

_____ _____

When recording your child's food please give as much detail as possible about the foods used, the cooking method and any added sauces. Also please include the brand name and flavour of other foods.
For take away food or eating out please note the name of the café / restaurant.

Please write how much your child ate, excluding any leftover food, in the 'Amount eaten' column.

Don't forget to include any sweets, biscuits, crisps, fruit and spreads like marmite.

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
6am to 9am				Food	
9am to 12 noon					
12 noon to 2pm				Day 1	
2pm to 5pm					

Food Day 1 continued.

Time slot	When	Where TV on? At table?	With whom	Food description and preparation.	Amount eaten
5pm to 8pm					
8pm to 10pm					
10pm to 6am					

What sort of plates does your child usually use: plastic ☐ china ☐ other ☐

What sort of cutlery does your child usually use: plastic ☐ metal ☐

2pm to 5pm							
5pm to 8pm							
8pm to 10pm							
10pm to 6am							

What sort of bottle or cup did your baby use? Please write the number in the correct box.

Plastic trainer cup with lid ☐ Plastic bottle ☐
Plastic cup without lid ☐ China cup or mug ☐
Glass ☐ Other ☐

Drinks Day 1
Please record milk feeds, other drinks and water here.

Please find the appropriate time slot and then record the time your child has a drink.
Note the type of fruit juice or brand of squash etc.
Please also include any vitamins or medicines on this sheet.
What sort of water does your child usually drink? Tap water ☐ Filtered water ☐ Bottled water ☐

If bottled water which type and brand? _____

To help us to decide on the correct amount of fluids for drinks, please fill your child's cup with water to the usual level, then empty the water into a measuring jug and record here.

Fluid ounces (fl.oz) _____ or Millilitres (ml) _____

Time slot	When	Full description and/or brand of drink.	Did you dilute with water?	How much concentrate did you use?	Did you add sug-ar?	Breast milk-minutes child fed	How much did your child drink?
6am to 9am							
9am to 12 noon							
12 noon to 2pm							

Food Day 1.

1. As far as you know was all the food and drink taken by your baby during this 24 hours recorded? yes ☐ no ☐ not sure ☐

2. Was the food and drink for this 24 hours fairly typical for your baby? yes ☐ no ☐
If no please describe how it differed from normal and if your baby was unwell during this 24 hours
_____
_____

3. Has anyone else looked after your baby today? yes ☐ no ☐
Please record any parts of this 24-hour period when someone else looked after your baby

Start time (e.g. 9.30am)	Return time (e.g. 5.00pm)
8.45am	5.00pm

Below is an example of how we would like you to record what your baby drinks.

Please find the appropriate time slot and then record the time your baby has a drink.
Note the type of fruit juice or brand of squash etc.

Time slot	When	Full description and/or brand of drink.	Did you dilute with water? yes/ no	How much milk powder did you use per whole cup or bottle?	How much concen-trate did you use per whole cup or bottle?	Did you add sugar? Number of tea-spoons per whole cup or bottle?	Breast milk- minutes baby fed	How much did your baby drink?
6am to 9am	7.30	Whole milk						5fl oz
9am to 12 noon	10.30	Pure apple juice	yes		100mls juice 100mls water			150mls
12 noon to 2pm	12.15	Tap water	yes					100mls
2pm to 5pm	3.00	Sainsbury's no added sugar high juice orange squash	yes		10mls in 200mls water			120mls
5pm to 8pm	6.15 7.30	Tap water Whole milk	yes					50mls 200mls
8pm to 10pm								
10pm to 6am								

What sort of bottle or cup did your baby use?
Please write the number in the correct box.

Plastic trainer cup with lid	2	Plastic bottle	2
Plastic cup without lid		China cup or mug	
Glass		Other	

Appendix 2 Anthropometric measurements in the CBGS

Measurement	Age of visit in each study			
	CBGS1	**CBGS2**	**CBGS-BF**	**CBGOS (single visit)**
Weight	0, 3, 12, 18, 24 months	0, 3, 6, 12, 24, 36 months	0, 0.5 (2w), 1.5 (6w), 3, 6, 12, 24, 36	v
Length/height	0, 3, 12, 18, 24 months	0, 3, 6, 12, 24, 36 months	0, 0.5 (2w), 1.5 (6w), 3, 6, 12, 24, 36	v
HC	0, 3, 12, 18, 24 months	0, 3, 6, 12, 24, 36 months	0, 0.5 (2w), 1.5 (6w), 3, 6, 12, 24, 36	v
WC	3 and 12 months	0, 3, 6, 12, 24, 36 months	0, 0.5 (2w), 1.5 (6w), 3, 6, 12, 24, 36	v
Skinfolds thicknesses	0, 3, 12, 18, 24 months	0, 3, 6, 12, 24, 36 months	0, 0.5 (2w), 1.5 (6w), 3, 6, 12, 24, 36	v
	Taken from 4 sites: triceps, subscapular, flank, quadriceps			Taken from 6 sites: triceps, biceps, subscapular, suprailiac, flank, quadriceps
Abdominal ultrasound	3 and 12 months	0, 3, 6, 12, 24, 36 months	1.5 (6w), 3, 6, 12, 24, 36	v
DXA scan	NA	NA	NA	v
ADP-Pea Pod	NA	NA	6 weeks and 3 months	NA

Appendix 3 Maternal demographics and birth characteristics of subsets of infant groups involved in hormonal measurements (Chapter 5)

	Control (Total N=305)	**SGA (Total N=64)**	**OGDM (Total N=50)**
Maternal demographics			
Age at birth (years)	33.35 ± 4.5	33.61 ± 4.71	34.82 ± 4.75
Caucasian ethnicity	97.2%	88.5%**	86%**
Primiparous pregnancy	46.2%	73.4%**	50%
Height (cm)	166.46 ± 7.17	162.52 ± 7.74**	164.13 ± 9.35
Pre-pregnancy BMI (kg/m^2)	24.13 ± 4.4	23.99 ± 4.61	26.06 ± 6.15
Smoking during pregnancy	1.3%	19.4%**	4.7%
Birth characteristics			
Gestational age (weeks)	40.13 ± 1.14	40.05 ± 1.63	38.89 ± 0.96**
Caesarean delivery	27.5%	41.7%*	39.1%
Male infant sex	50.8%	46.9%	60%
Weight (kg)	3.57 ± 0.431	2.521 ± 0.319**	3.308 ± 0.482**
Weight SDS[a]	0.13 ± 0.82	-2.14 ± 0.39**	0.1 ± 1.11
Length (cm)	50.8 ± 2.0	47.78 ± 2.37**	49.96 ± 1.91
Length SDS[a]	-0.07 ± 0.84	-1.7 ± 0.84**	-0.12 ± 1.02
BMI (kg/m^2)	13.92 ± 1.22	10.97 ± 0.86**	13.21 ± 1.39**
BMI SDS[a]	0.52 ± 0.96	-2.23 ± 0.86**	0.17 ± 1.17
Head circumference (cm)	34.96 ± 1.28	33.12 ± 1.35**	34.61 ± 1.14
Head circumference SDS[a]	-0.1 ± 0.93	-1.63 ± 0.7**	0.03 ± 0.94
Mean skinfolds SDS[a]	0.13 ± 0.91	-1.01 ± 0.5**	-0.15 ± 0.57
Infant feeding history (% of exclusively breastfed by 3 months)	41.2%	35.5%	43.5%

Values are mean ± SD, or %

[a]SDS, standard deviation score (for weight and length are calculated using the UK 1990 reference, for skinfolds using internal references)

SGA, small for gestational age

*p<0.05 vs. control group, **p<0.005 vs. control group

Weight-, length-, and mean skinfolds-SDS values are adjusted for gestational age, sex and postnatal age at measurement

Appendix 4 Maternal demographics and birth characteristics of subsets of infant groups involved in the lipidomic analyses (Chapter 6)

	Control (Total N=99)	**SGA (Total N = 99)**	**OGDM (Total N= 99)**
Maternal demographics			
Age at birth (years)	33.08±4.30	33.31±4.85	34.54±4.61
Caucasian ethnicity	97.3%	88.6%*	82.4%**
Primiparous pregnancy	38.7%	61.5%**	53.1%*
Height (cm)	167.56±6.59	163.06±5.93**	163.38±7.89**
Pre-pregnancy BMI (kg/m^2)	22.43±2.93	23.85±4.71	27.15±6.83**
Age at menarche	13.22±1.49	12.86±1.54	12.74±1.58
Birth characteristics			
Sex (% male)	41.4%	54.5%	40.4%
Gestational age (weeks)	40.32±1.09	39.58±1.69**	38.89±0.94**
Weight (kg)	3.645±0.452	2.446±0.292**	3.322±0.434**
Weight SDS	0.23±0.87	-2.13±0.41**	0.20±1.04
Length (cm)	51.08±1.90	47.57±2.14**	50.18±1.76**
Length SDS	-0.18±0.88	-1.66±0.83	0.09±1.05
Head circumference (cm)	35.31±1.26	32.82±1.24**	34.70±1.11**
Head circumference SDS	-0.08±1.08	-1.72±0.85**	0.20±1.05
BMI SDS	0.31±0.86	-2.51±0.99**	-0.21±1.07**
Waist circumference (cm)	33.40±2.32	28.65±2.02**	31.86±1.95**
Triceps SFT (mm)	5.18±1.05	3.60±0.61**	4.55±0.81**
Subscapular SFT (mm)	5.40±1.20	3.64±0.67**	4.83±0.89**
Flank SFT (mm)	5.66±1.17	3.89±0.92**	4.83±1.07**
Quadriceps SFT (mm)	7.20±1.49	4.45±0.86**	5.91±1.20**
Sum SFT (mm)	23.44±4.30	15.58±2.64**	20.11±3.37**

Values are mean ± SD, or %

*p<0.05 vs. control group; **p<0.005 vs. control group

SDS values are based on UK 1990 data reference, adjusted for gender, gestational age (birth and 3 months data only), and postnatal age at measurement.

SFT=skinfold thickness

Appendix 5 Cross-sectional growth data of subsets of infant groups involved in the lipidomic analyses (Chapter 6)

	Control (Total N=99)	**SGA (Total N = 99)**	**OGDM (Total N= 99)**
3 months			
Feeding nutrition (% exclusively breastfed at 3 months)	75.8%	66.2%	56.8%*
Weight SDS	0.21±1.29	-1.43±1.09**	0.29±1.33
Length SDS	0.22±1.11	-1.51±1.08**	0.12±1.28
Head circumference SDS	0.04±1.23	-1.21±1.25**	0.06±1.19
Waist circumference (cm)	40.85±2.56	38.09±2.45**	40.64±2.67
Sum skinfolds thicknesses (mm)	40.55±6.91	38.66±6.99	41.50±6.38
0-3 months weight gain SDS	-0.07±0.93	0.71±1.01**	0.10±1.14
0-3 months height gain SDS	0.36±0.72	0.13±0.77	0.04±0.76*
Catch-up weight gain[+]	21.4%	44.3%**	30.9%
Catch-up height gain[+]	30.5%	24%	19.6%
6 months			
Weight SDS	0.09±1.38	-1.32±1.16**	0.00±1.04
Length SDS	0.13±1.23	-1.26±1.20**	0.07±1.59
Head circumference SDS	0.04±1.42	-1.14±1.48**	-0.13±1.46
Waist circumference (cm)	42.91±2.93	40.63±2.43**	42.57±3.18
Sum skinfolds thicknesses (mm)	43.94±7.02	42.21±6.75	44.10±7.21
12 months			
Weight SDS	0.20±1.09	-1.13±1.16**	0.05±1.43
Length SDS	0.27±1.01	-0.86±1.25**	0.26±1.40
Head circumference SDS	0.00±1.37	-1.45±1.60**	-0.35±1.71
Waist circumference (cm)	44.55±5.75	43.10±2.43	44.92±3.16
Sum skinfolds thicknesses (mm)	41.05±7.59	39.10±6.13	39.85±7.60
3-12 months weight gain SDS	-0.30±0.92	0.28±0.88**	-0.31±0.83
3-12 months height gain SDS	-0.19±0.66	0.65±0.77**	0.02±0.63

SDS values are based on UK 1990 data reference, adjusted for sex, GA (birth and 3 months only), and postnatal age at measurement

[+]Catch-up is defined as a gain in SD score for weight or height greater than 0.67 SD between 0-3 months.

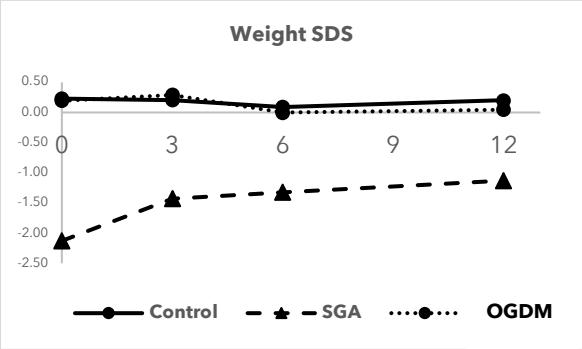
Values are mean ± SD, or %

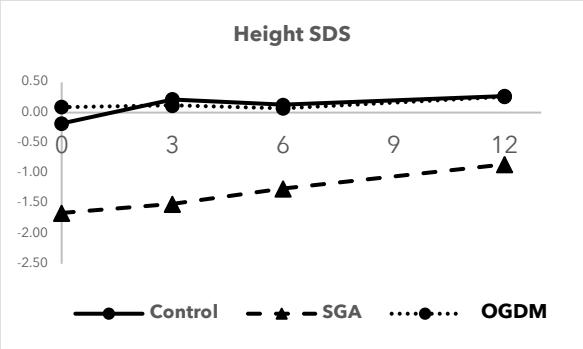
*p<0.05 vs. control group

**p<0.005 vs. control group

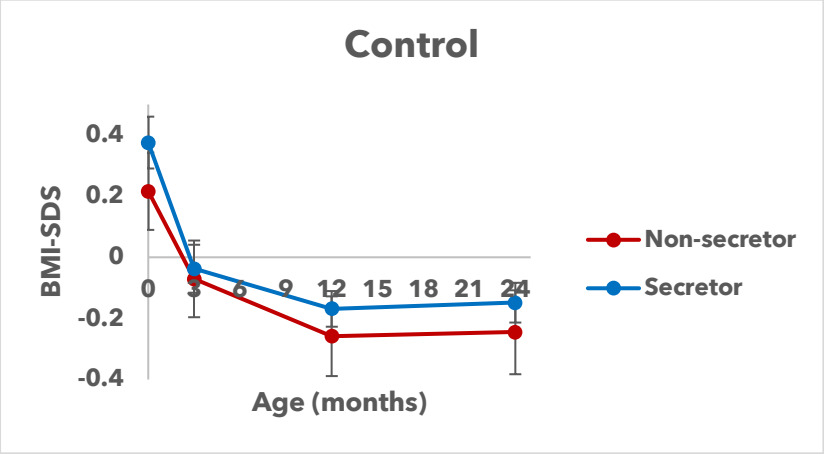
Appendix 6 Infancy weight and length trajectories of subsets of infant groups involved in the lipidomic analyses (Chapter 6)

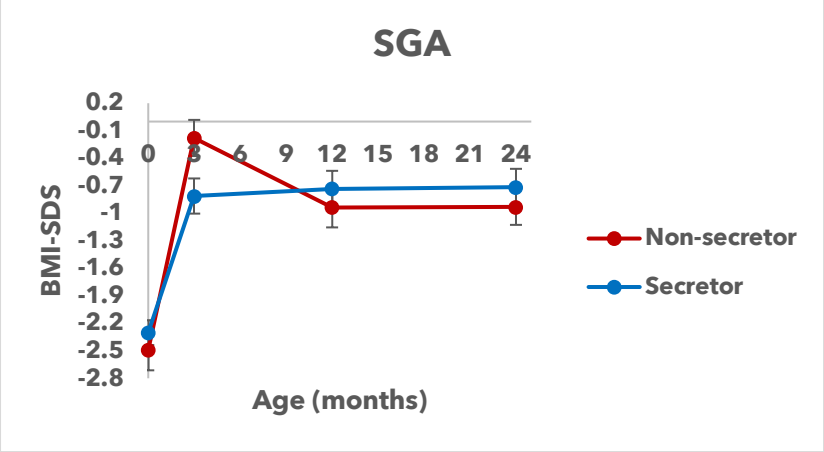
SDS values are based on UK 1990 growth reference

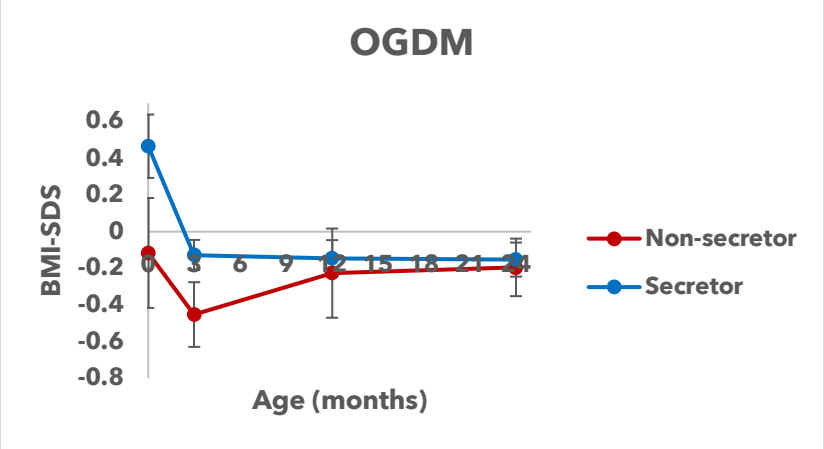




Appendix 7 BMI trajectories between *FUT2* secretor and non-secretor infants (Chapter 8)
Values are mean±SEM
SDS values are based on UK 1990 growth reference

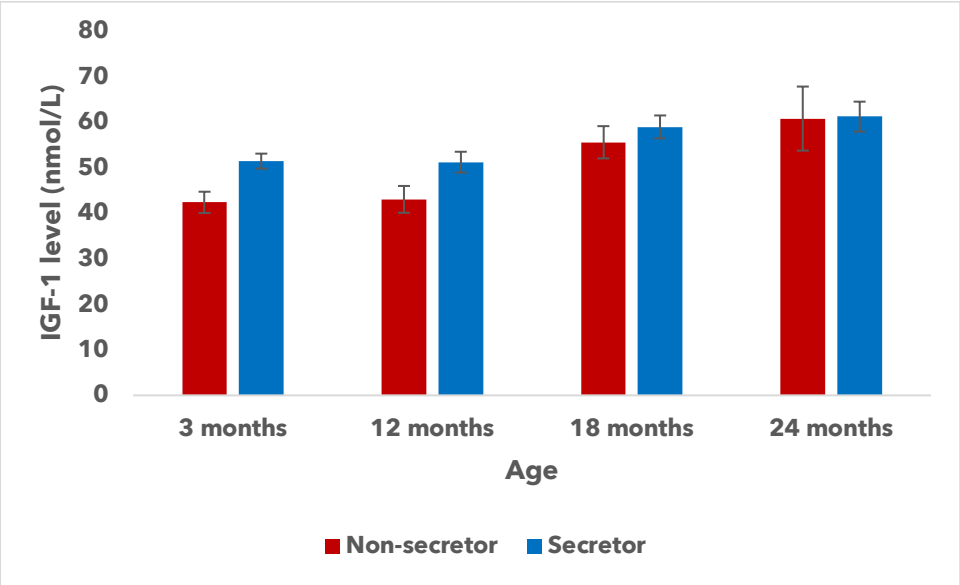






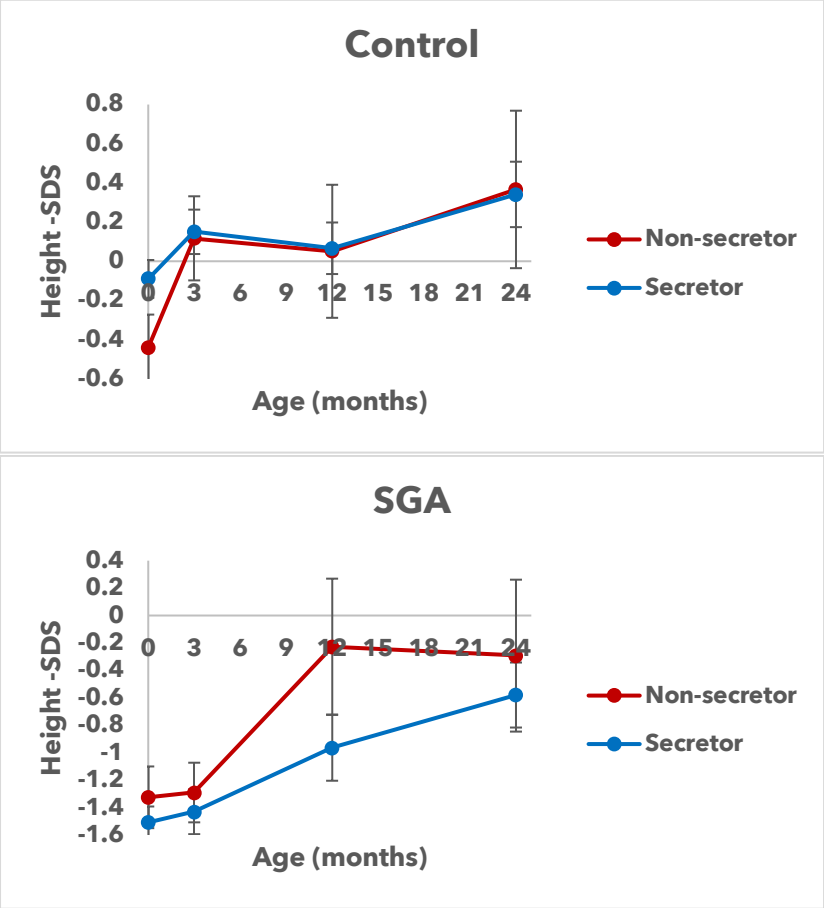
Appendix 8 Capillary IGF-1 level between secretor and non-secretor controls (Chapter 8)

Values are mean±SEM
At 3 months, *p=0.007* (unadjusted) and *0.056* (adjusted)
At 12 months, *p= 0.044* (adjusted)



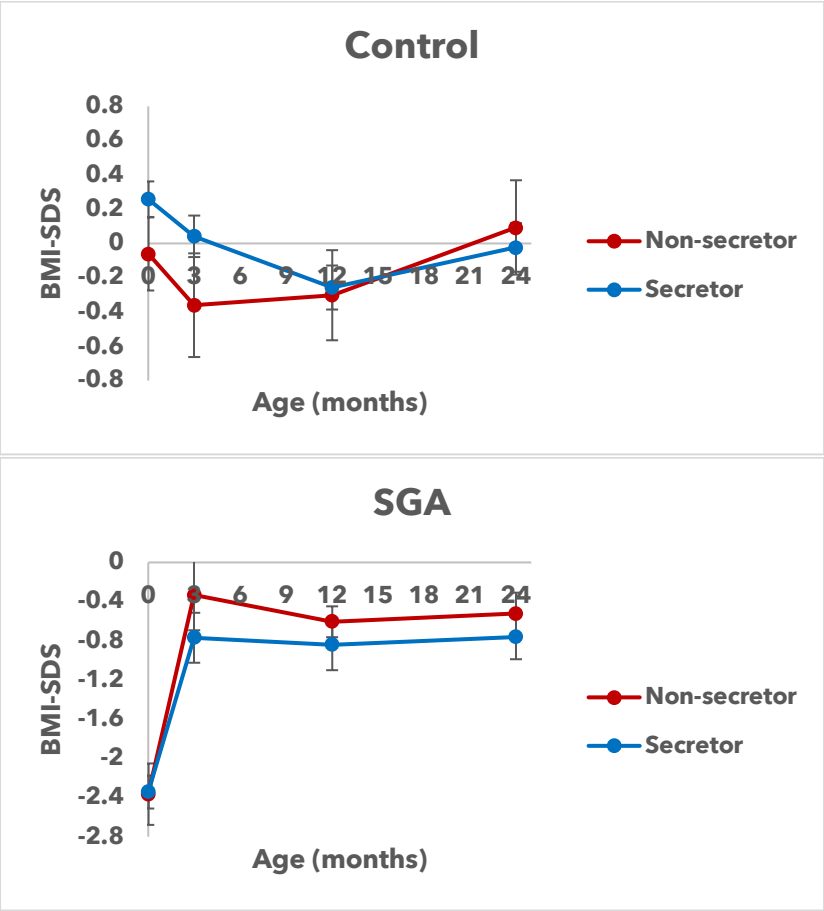
Appendix 9 Height trajectories among control and SGA infants born based on their maternal *FUT* 2 secretor status (Chapter 8)

Values are mean±SEM
SDS values are based on UK 1990 growth reference



Appendix 10 BMI trajectories among control and SGA infants born based on their maternal *FUT* 2 secretor status (Chapter 8)

Values are mean±SEM
SDS values are based on UK 1990 growth reference



Appendix 11 Skinfolds trajectories among control and SGA infants born based on their maternal *FUT* 2 secretor status (Chapter 8)

Values are mean±SEM
SDS values are internally-derived, adjusted for infant sex, gestational age (0-3 months only), and infant's age at visit