UNIVERSITY *of* TASMANIA

Body composition during early infancy: pre- and postnatal determinants and assessment approaches

by

Manoja Prabhashani Herath

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STATEMENTS AND DECLARATIONS

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The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Candidate

Manoja P. Herath, School of Health Sciences, University of Tasmania

Supervisors

Dr Kiran D.K. Ahuja, School of Health Sciences, University of Tasmania -- Author 1

Prof Andrew P. Hills, School of Health Sciences, University of Tasmania -- Author 2

Prof Nuala M. Byrne, School of Health Sciences, University of Tasmania -- Author 3

Dr Jeffrey M. Beckett, School of Health Sciences, University of Tasmania -- Author 4

Other Contributing Author(s)

Dr Sisitha Jayasinghe, School of Health Sciences, University of Tasmania -- Author 5

Contribution of work by co-authors for each paper:

PAPER 1: Determinants of Infant Adiposity across the First 6 Months of Life: Evidence from the Baby-bod study

Located in Chapter 4

Author contributions:

The candidate was the primary author and contributed approximately 80% of the work, including formulating research questions, data collection, statistical analysis, interpretation and writing of the manuscript. Author 1 contributed to conceptualisation/design, formulating research questions, statistical analysis, and interpretation of data. Authors 2 and 3 contributed to conceptualisation/design, funding and formulating research questions. Author 4 contributed to formulating research questions and interpretation of data. Author 5 contributed to data collection. All authors critically reviewed and revised the content.

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Located in Chapter 5

Author contributions:

The candidate was the primary author and contributed approximately 80% of the work, including conception and design, study protocol, literature search, data extraction, quality assessment, meta-analyses and writing of the manuscript. Author 1 contributed to the study protocol and data verification. Authors 2 and 3 contributed to the study protocol. Author 4 contributed to study protocol, literature search and quality assessment. All authors critically reviewed and revised the content.

We, the undersigned, endorse the above-stated contribution of work undertaken for each of the published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed:

Manoja P. Herath	Dr Kiran D.K. Ahuja	Prof Tracey Dickson
Candidate	Primary Supervisor	Associate Dean, Research Performance
School of Health Sciences	School of Health Sciences	College of Health and Medicine
University of Tasmania	University of Tasmania	University of Tasmania
Date: 10/11/2021	Date: 10 November 2021	Date: 10 November 2021

Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

The research detailed in Chapter 3 received approval from the Human Research Ethics Committee, Tasmania; approval number: H0020469.

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Signed:

Manoja P. Herath

Date: 10/11/2021

PUBLICATIONS AND PRESENTATIONS

First-Author Journal Publications

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2) **Herath MP**, Hills AP, Beckett JM, Jayasinghe S, Byrne NM, Ahuja KDK. Maternal pre- and perinatal influences on secular birthweight trends in Tasmania: A retrospective analysis of all live singleton births 2005-2018. Nutrition Society of Australia Conference. December 2021.

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1. Jayasinghe S, **Herath MP**, Beckett JM, Ahuja KDK, Byrne NM, Hills AP. Anthropometry-based prediction of body fat in infants from birth to 6 months: the Baby-bod study. *European Journal of Clinical Nutrition*. . 2021. 75(4): p. 715-723.

2. Jayasinghe S, **Herath MP**, Beckett JM, Ahuja KDK, Byrne NM, Hills AP. Exclusivity of breastfeeding and body composition: learnings from the Baby-bod study. *International Breastfeeding Journal*. 2021;16(1):41.

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- An honorarium of $1000 was received for an invited webinar based on the research findings of the study on "Determinants of infant adiposity across the first 6 months of life" at Dietitian Connection®, an online platform of nutrition professionals representing 45 countries.

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COMMON ABBREVIATIONS

ADP: Air Displacement Plethysmography

BMI: Body Mass Index

CI: Confidence Interval

DD: Deuterium Dilution

FFM: Fat-free Mass

FM: Fat Mass

FM/FFM^{p}: Log-log Index

FMI: Fat Mass Index

%FM: Percent Fat Mass

GDM: Gestational Diabetes Mellitus

GWG: Gestational Weight Gain

HBW: High Birthweight

IGDMtr: Infants Born to Mothers Treated for GDM

INGT: Infants Born to Mothers with Normal Glucose Tolerance

LBW: Low Birthweight

NGT: Normal Glucose Tolerance

nGWG: Net Gestational Weight Gain

ppBMI: Pre-pregnancy BMI

ST: Skinfold Thicknesses

TBW: Total Body Water

tGWG: Total Gestational Weight Gain

WHO: World Health Organisation

ABSTRACT

Background, research gaps and aims

Obesity has reached pandemic levels among adults worldwide and the increasing prevalence among children and adolescents is alarming. There is strong evidence that prenatal and early postnatal growth and developmental plasticity play a pivotal role in determining the risk of obesity and co-morbidities across the life span. In humans, developmental plasticity is heightened during the so-called first 1000 days (from conception to two years of age), such that a stimulus or an insult during this sensitive period may cause permanent alterations in the growth trajectory with potential for lifelong consequences. In this context, optimising growth during early life through appropriate nutrition and environmental exposures is of paramount importance.

Clinical assessment of growth in infants has long been based on anthropometric measures, with birthweight being the most widely used marker of foetal nutrition and other intrauterine exposures. Both extremities of the birthweight spectrum, i.e., low birthweight (LBW) and high birthweight (HBW), have shown associations with obesity during childhood and adulthood. Hence, a better understanding of secular trends in birthweight and associated maternal factors at the population level may help in planning preventative strategies to reduce LBW and HBW rates and improve population health. Thus far, secular trends in birthweight and associations with maternal factors have not been studied in Tasmania, the Australian state with the highest rates of obesity in children and adults.

As birthweight is only a crude indicator of the nutritional status of a newborn, body composition assessment is a valuable addition providing information of differential growth in fat mass (FM) and fat-free mass (FFM). Mounting evidence indicates that relatively greater gains in adiposity during early development is associated with later life obesity. Several pre- and postnatal risk factors for increased adiposity during infancy have been identified; however, there are inconsistencies and contradictions in findings. Differences in the age of infants, the technique used to estimate body composition, and the index used to elucidate variability of adiposity may explain some of these discrepancies. Further, *in utero* exposure to gestational diabetes mellitus (GDM) has been identified as one of the main risk factors for increased adiposity at birth. An increasing awareness of short- and long-term adverse effects of GDM to both mother and infant has led to substantial improvements in perinatal care and provision of treatments for women with GDM during recent years. However, evidence on whether treatments for GDM can normalise adiposity in newborns is still inconsistent.

Among the various body composition techniques available, air displacement plethysmography (ADP using PEA POD) is one of the most "practical" approaches for use in early infancy. ADP allows precise estimations of body composition rapidly and non-invasively. However, the PEA POD only accommodates infants up to ~6 months of age (<10 kg body weight). Therefore, alternative body composition techniques should be used in longitudinal studies that follow-up infants beyond 6 months of age. The deuterium dilution (DD) technique is another body composition approach suitable for use in infants and has good reliability and validity. It can be used in infants from birth, is comparatively inexpensive, and collected body fluids can be stored until analysed. The DD technique may be a suitable alternative when infants can no longer be accommodated in the PEA POD. Thus far, only one study has reported that body composition measurements obtained using PEA POD and DD are in agreement. Infants who participated in this study were predominantly Asian, and their ages varied from 0.4-24.4 weeks. Thus, additional research is needed to test the agreeability of the two techniques in infants of other ethnicities and in larger samples of the same age.

The four studies undertaken for this PhD thesis aimed to improve the knowledge and understanding of determinants and measures of infant body composition. The first study explored secular birthweight trends and associated maternal factors in Tasmania. The second study identified pre- and postnatal determinants of adiposity from birth to 6 months of life in a sample of healthy Tasmanian infants. The third study systematically reviewed the literature reporting different adiposity measures in newborns of mothers with GDM controlled with therapeutic interventions compared to those with normal glucose tolerance (NGT). The fourth and final study appraised the agreement of body composition measures assessed via PEA POD in relation to DD technique in 6-month-old infants.

Methods and results

Perinatal data of all live-born singletons and their mothers, linked by the Tasmanian Data Linkage Unit (n = 81700), were used to investigate the secular trends (from 2005 to 2018) in birthweight and associations with pre-pregnancy and pregnancy maternal factors in Tasmania. Over the 14 years, mean birthweight (3425 g to 3359 g) and the proportion of HBW (14.2% to 11.0%) decreased, while the proportion of LBW increased (4.8% to 6.5%). However, as of 2018, the rate of HBW (1 out of 9 babies) was still higher than the rate of LBW (1 out of 15 babies). A downward shift in gestation length distribution, increased rates of mothers with caesarean delivery, hypertensive disorders, age >35 years, and changes in ethnic demographics with an increased number of indigenous or immigrant mothers, may have contributed to this trend towards smaller babies. Although the rates of pre-pregnancy obesity and GDM (well-known risk factors of HBW) have risen, and maternal smoking (a major risk factor for LBW) has markedly

decreased over the period, the impact of these changes in mothers was not apparent in the infant birthweight trend.

A prospective longitudinal cohort study design was used to investigate associations between pre- and postnatal factors and infant adiposity measures from birth to 6 months. This study was conducted at the Launceston General Hospital, Tasmania, from September 2017 to October 2019. Information on pre- and postnatal exposure variables was obtained from mothers through an interviewer-administered questionnaire, and infant body composition was measured using PEA POD. Linear mixed-effects modelling with backward stepwise regression was used to assess longitudinal associations between pre- and postnatal factors and infant adiposity. To test whether the discrepancies in predictors of infant adiposity in the literature stemmed from using various indices to measure adiposity, a range of adiposity measures, i.e., FM, percent fat mass (%FM), fat mass index (FMI) and log-log index (FM/FFM^{P}), were used. The body composition of 322 infants was assessed within 72 hours of birth, and of those, 174 and 109 were followed up at 3 and 6 months, respectively. Positive associations were observed between gestation length and infant FM, parity and infant %FM and FMI, and pre-pregnancy body mass index (ppBMI) and infant %FM at birth. Male infant sex and formula feeding were negatively associated with all adiposity indices at 6 months. Surprisingly, maternal intake of iron supplements during pregnancy was negatively associated with infant FM, %FM and FMI at 3 months and FM/FFM^{p} at 6 months; however, this finding should be interpreted with caution as our analysis lacked information on doses and duration of prenatal supplements. These results suggest that some of the inconsistency in the literature regarding pre- and postnatal impacts on infant adiposity is potentially due to the use of different measures of adiposity in various studies.

We systematically reviewed the literature reporting FM, %FM and skinfold thickness (ST) in infants of mothers with GDM controlled with therapeutic interventions (IGDMtr) following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. In total, 25 studies were included in the systematic review, of which 17 were included in the meta-analysis. Regardless of the type of treatment (insulin, metformin, glyburide), treating GDM lowered FM in newborns compared to no treatment. A meta-analysis of all studies showed that, compared to infants exposed to normal glucose tolerance (INGT), IGDMtr had higher overall adiposity (mean difference, 95% confidence interval (CI)) as measured by FM (68.46 g, 29.91 to 107.01) and %FM (1.98%, 0.54 to 3.42). In contrast, subcutaneous adiposity measured with ST did not differ between the two infant groups. However, a meta-analysis of a subgroup of more recent studies (data collection occurred during or after 2010) showed there were no significant differences in FM, %FM and ST between INGT and IGDMtr. It is possible that more intensive

management of blood glucose levels in mothers with GDM during recent years has normalised the adiposity in their infants.

We used Bland-Altman analysis to evaluate the agreement of body composition measures (FM, %FM and FMI) assessed via PEA POD and the DD technique in a sample (n = 72) of 6-month-old infants. The differences between the two methods were not constant (FM: bias = 25.26, 95%CI = -65.92 to 116.45; %FM: 0.33; -0.93 to 1.60; FMI: 0.06; -0.15 to 0.27); however, the limits of agreement (LOA) were wide and significant proportional bias was identified with DD technique underestimating infant adiposity at lower values and overestimating infant adiposity at higher values, in comparison to PEA POD. Further analyses were performed to investigate whether the adiposity values at lower or upper extremities significantly affected the bias. When the analysis was conducted with mean values above the first quartile (n = 53), LOA was somewhat narrower (FM: -667.84 to 519.91; %FM: -9.15 to 7.96; FMI: -1.58 to 1.27), and no proportional bias was detected ($p > 0.1$ for all). Wide LOA and significant proportional bias were detected in the analysis for mean values below the third quartile (n = 53). Our results indicate that DD may be a suitable alternative method to assess body composition beyond 6 months of age in infants whose adiposity level was not at the lower end of the adiposity spectrum at 6 months of age.

Conclusions

Through this research, we aimed to address some controversies and gaps in the field of *in utero* and postnatal influences on early-life body composition. Our findings on the maternal role on infant birthweight may assist authorities to plan intervention strategies and public health awareness programs that optimise the birthweight of Tasmanian infants. As observed in the longitudinal study, some prenatal factors such as ppBMI and parity may only be associated with infant weight and adiposity at birth. Conversely, the effects of other prenatal factors, for example, maternal supplement intake, may manifest later in infancy without being evident at birth. By showing an association between supplemental iron intake during pregnancy and infant adiposity at 3 and 6 months, we have generated a new hypothesis that should be explored in future studies. Further, formula-fed infants having lower adiposity than breastfed infants at 6 months of age may appear as counter-intuitive to the well-known protective effect of breastfeeding for obesity. As the literature suggests that this association reverses after 12 months of age, an additional body composition assessment of the infants who participated in our study at 12 months would have helped to discern the effects of feeding mode (although a follow-up study that assessed the infants at 9 and 12 months was started as a part of this PhD project, it could not be completed due to the COVID-19 lockdown). Moreover, our finding of IGDMtr having higher overall adiposity but similar subcutaneous adiposity compared to INGT indicates that there may be excessive non-

subcutaneous fat accrual in IGDMtr. It has opened a new avenue for future researchers to distinguish adipose tissue distribution of IGDMtr vs INGT. Normalisation of adiposity in newborns with treatments in recent studies highlights the importance of intensive management of blood glucose levels in mothers with GDM. Finally, as FM alone (without adjustment for body size) cannot elucidate between individual variability, and %FM is considered statistically flawed as a measurement of adiposity, future research should consider conceptually and statistically robust approaches such as FMI or FM/FFM^{p} to explore factors contributing to adiposity in infants. Readers should be mindful that the determinants of infant adiposity differ between studies based on the selection of adiposity measure. Taken collectively, this thesis has generated important insights in understanding the determinants of early-life growth and the techniques of assessing the body composition of infants.

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CHAPTER 1 : GENERAL INTRODUCTION

1.1 BACKGROUND

The pandemic of overweight and obesity is one of the most significant public health challenges worldwide [1, 2]. The global prevalence of obesity has nearly tripled since 1975 [3]. In 2016, 1.9 billion (39%) of the world's adult population were overweight, and of these, 650 million (19%) were obese [3]. Further, the mean global body mass index (BMI) increased by the equivalent of 1.5 kg per person per decade, and the projections indicate that without intervention, approximately one out of five adults will be obese by 2025 [4]. Similar increases in mean BMI have been observed in children and adolescents (aged 5-19 years), and in 2016, 340 million were found with overweight or obesity [3, 5]. In 2019, 38 million children under the age of 5 years were overweight or obese [3]. These global trends in overweight and obesity are also evident in the Australian population. During 2017-18, two-thirds (67%) of adults (12.5 million) and one quarter (24.9%) of Australian children aged 2-17 years (1.2 million) were overweight or obese [6]. Noticeably, across all states and territories of Australia, Tasmania, the smallest state with a population of 0.5 million, recorded the highest proportions of overweight/obesity among both adults (70.9%) and children (28.7%) [7, 8]. These statistics reinforce the extent of the problem of overweight and obesity among both children and adults and the consequent public health challenge in Australia, and particularly in Tasmania.

Overweight and obesity are associated with several adverse health consequences. Adults with overweight or obesity are at increased risk of several life-threatening chronic conditions such as cardiovascular diseases, e.g., coronary heart disease, stroke, hypertension; insulin resistance, e.g., type 2 diabetes mellitus; certain types of cancers, e.g., colon cancer, endometrial cancer; and gall bladder disease [9]. Being overweight or obese resulted in an estimated 4 million deaths and 120 million disability-adjusted life years globally in 2015 [10]. Australian adults with overweight or obesity have also reported higher rates of chronic conditions than those of normal weight, with severity rising as BMI increases [11]. Overweight and obesity are also associated with several non-fatal health conditions, including sleep apnoea, chronic musculoskeletal problems, and infertility. Furthermore, potential psychosocial aspects of obesity, including prejudice and discrimination, body image dissatisfaction and eating disorders, cannot be underestimated [12]. Childhood obesity can adversely affect the physiological as well as mental health and well-being of children. Health consequences associated with childhood obesity include abnormal glucose metabolism, hepatic steatosis (fatty liver disease), menstrual abnormalities, sleep apnoea, asthma and orthopaedic complications. Further, social problems such as discrimination and social marginalisation can lead to negative body image, low self-confidence and low self-esteem, which

may also impact academic performance [13]. Importantly, childhood obesity and associated health risks are more likely to persist into adulthood [14, 15].

Understanding the origins of obesity is beneficial in the design and implementation of effective strategies to prevent the condition and its adverse health outcomes. Although obesity has a genetic basis, environmental influences are required for its manifestation [16]. Among the numerous obesogenic lifestyle factors, excessive food intake and insufficient physical activity are the leading causes; however, the beneficial effects of these modifiable risk factors, assessed through clinical trials, have been small or short-lived [17]. A more recent finding which offers hope for preventing the obesity epidemic is that intrauterine and postnatal environments are critical determinants of programming of later life obesity [18]. This has been explained as a consequence of developmental plasticity, where one's genotype can form different physiological or morphological states in response to influences of environmental conditions [19]. In humans, developmental plasticity is at its maximum in the first 1000 days of life, the period from conception to two years of age. As such, a stimulus or an insult occurring at this sensitive period of development may cause permanent alterations in the growth trajectory and, thereby, the lifelong health of an individual [20]. In this context, Barker et al. [21] proposed the "Foetal Origins of Adult Disease" concept, later termed the 'Developmental Origins of Health and Disease (DOHaD)' hypothesis. It was based on the results of a retrospective epidemiologic study that showed individuals with lower birthweight due to undernutrition *in utero* had a higher death rate from ischaemic heart disease. Supported by evidence from numerous subsequent epidemiological studies, there is now a strong consensus that optimal nutrition and environmental conditions are fundamental in the first 1000 days, the time of most rapid growth, for later life health [22].

For over a century, clinical assessment of infant growth has relied on anthropometric measurements, with birthweight being the most widely used marker of foetal nutrition and *in utero* exposures. Evidence suggests that both the extremities of the birthweight spectrum, LBW or HBW (also known as macrosomia), can lead to obesity and associated adverse health outcomes in later life [23-25]. LBW is defined as birthweight less than 2500 g, while weights above 4000 g are termed HBW, irrespective of gestational age [26]. Nevertheless, anthropometric indices are only a proxy of nutritional status; they are incapable of differentiating between various components of body composition, including lean mass, bone mass and FM. There may be significant variability in the body composition of infants: for example, smaller and thinner Indian babies have been found with more adiposity compared to large Caucasian babies [27]. Body composition, and in particular, relative proportions of FM and FFM (all other components of the body except fat, e.g., muscles, bones, tissues and organs), are linked with the programming of

human metabolism [28]. On this basis, the assessment of infant body composition to identify early life markers of future obesity risk has gained increased attention in recent decades.

1.2 IDENTIFYING THE RESEARCH GAPS

1.2.1 Trends in infant birthweight and associated maternal characteristics in Tasmania

As the assessment of infant body composition is not always feasible, it is also imperative to investigate how various maternal exposures affect infant birthweight. Several maternal demographic and lifestyle characteristics have been identified as significant predictors of birthweight. Notably, advanced maternal age (>35 years), stress during pregnancy, smoking and alcohol intake during pregnancy have been reported to be associated with LBW, while high ppBMI, excess gestational weight gain (GWG) and GDM are well-known contributors to HBW. The prevalence of LBW, HBW, and their risk factors vary according to the population studied, with high rates commonly found in resource-limited areas [29]. Compared to other states in Australia, Tasmania has the highest proportion of people living in low socio-economic regions [30], with a higher prevalence of LBW (7.4%) and HBW (1.7%, for this analysis HBW has been defined as birthweight of 4,500 grams or more) among live-born babies compared to Australia overall (6.7% and 1.2%, respectively) [31]. Identifying risk factors of LBW or HBW may help in planning interventions for optimising the weight of infants at birth. Birthweight trends and the relationships between maternal pre-pregnancy and pregnancy variables with LBW and HBW have not been investigated in Tasmania.

1.2.2 Discrepancies between the determinants and measures of body composition during early infancy

Several pre-and postnatal factors impact body composition during infancy, including maternal age, parity [32], ethnicity [33], maternal ppBMI [34, 35], GWG [36, 37], *in utero* exposure to GDM [38, 39], prenatal tobacco exposure [40, 41], infant birthweight [42], curtailed infant sleep [43] and infant feeding characteristics [44, 45]. Nevertheless, there are significant discrepancies in the effect of these factors on infant body composition. For example, while considerable evidence suggests that maternal obesity is associated with increased infant FM [46, 47], other studies have shown no association [48, 49]. Further, the majority of studies [50-52] have focussed on a single time-point, e.g., birth or a particular age, rather than investigating the associations across infancy. It is possible that these associations change over time; hence, assessment of body composition at a single time point may not present the full picture. A study that assessed infant body composition at birth and five months of age reported that maternal BMI was a significant predictor of FM at birth, but not at five months of age [50]. Moreover, ethnicity and geographical location are important determinants of body composition [53]. Although a few studies have

investigated the role of foetal and early postnatal environments on the body composition of Australian infants [54, 55], to the best of our knowledge, no study has examined the determinants of body composition variation across early infancy in Tasmania: the state which has the highest rate of obesity in children in Australia. Finally, the use of absolute FM values without adjusting them for body size can compromise its clinical relevance. For example, FM alone cannot elucidate inter-individual variability of fatness, nor can it rank individuals in terms of disease risk [56]. Different indices derived from FM, including %FM (FM adjusted for total body weight) [57], FMI (FM adjusted for height/length) [58], and FM/FFM^p (FM adjusted for FFM) [59], have been used as measures of infant body composition. However, their interrelationships and maternal determinants have not been investigated thoroughly.

1.2.3 Contradictions in the association between GDM and infant adiposity

Among various prenatal factors that can alter the body composition of neonates, *in utero* exposure to GDM, glucose intolerance that occurs or is first diagnosed during pregnancy [60], is considered of utmost importance. The worldwide prevalence of GDM is increasing (36% in some populations), accompanied by rising rates of overweight and obesity and increasing age among pregnant women [61]. In Australia, GDM currently affects an estimated 12-14% of pregnant women [62]. A growing body of literature has investigated the body composition of newborns of women with GDM compared to infants of women with NGT; however, findings have been inconsistent and contradictory. In spite of good glycaemic control during pregnancy, infants born to mothers with GDM have increased FM compared to infants of mothers with NGT [63]. In contrast, it has been shown that %FM in neonates was normalised when good glycaemic control was achieved by mothers with GDM [64]. Many previous studies have combined GDM with pregestational diabetes, although they are metabolically distinct disorders. A subgroup analysis of a previous systematic review and meta-analysis [65] that compared adiposity in infants born to diabetic mothers versus non-diabetic mothers reported greater adiposity in infants of GDM mothers compared to non-diabetic mothers. However, the authors did not consider whether the mothers with GDM were treated or not, and consequently, the meta-analysis included studies in which GDM mothers were treated and not treated. In addition, they did not review what therapeutic interventions were used to control GDM in the studies where GDM mothers were treated and whether the various treatment regimens had affected infant body composition differently.

1.2.4 Agreement between the infant body composition predicted by ADP (PEA POD) and DD technique

Although various methods are available for assessing body composition, their use in the paediatric population may be limited due to physiology and behaviour unique to infants. ADP and DD are two body composition techniques that have been used in infants with good reliability and validity. While the advantages of the ADP technique include non-invasiveness, very short assessment time and no radiation exposure [66], the paediatric ADP instrument (PEA POD) is limited to infants weighing less than 10 kg (approximately 6 months of age). The DD technique can be used in all ages from birth, is comparatively inexpensive and collected body fluids can be stored until analysed [67]. However, when using DD for infants, dose spillage can be common, and the procedures involved are quite time consuming (waiting period for dose equilibration in the body). Therefore, PEA POD may be more attractive than DD for the 0-6 months age group. There is a dearth of data on the suitability of DD for body composition assessment beyond 6 months of age in infants whose body composition was measured with PEA POD up to 6 months of age. Good agreement between body composition measurements obtained with PEA POD and DD at 6 months of age may imply DD is a good alternative method for longitudinal studies when infants grow beyond the capacity of the PEA POD.

1.3 STUDY SIGNIFICANCE

Environmental exposures during intrauterine and neonatal life play an important role in programming the susceptibility in later life obesity and its associated chronic diseases. Assessment of infant body composition has potential importance in early identification of future obesity risk. Although multiple pre-and postnatal factors that impact infant body composition have already been described, significant inconsistencies exist in the literature. Moreover, in the Australian context, Tasmania has the highest rates of obesity among children and adults. However, so far, no data are available on how *in utero* and postnatal factors influence early-life body composition changes in Tasmanian infants. Findings from this research contribute to the current understanding of how *in utero* and postnatal factors influence early-life body composition. They may also assist in planning interventions targeted at improving the health and well-being of the population, improving public awareness and health literacy of future parents and state-level policy changes regarding reducing health burden related to obesity. Additionally, the prospective cohort study of this research was a part of a multi-country study that aimed to develop international infant body composition reference charts. These charts will be helpful in characterising the optimal quality of growth during infancy.

1.4 RESEARCH AIMS

In an effort to address the above gaps and contradictions in the literature, this PhD thesis primarily aimed to contribute to the understanding of pre-and postnatal influences on infant growth and body composition and validate commonly used methods for assessing infant body composition. The specific aims were to:

1. Examine birthweight trends, changes in maternal characteristics, and associations between maternal characteristics and birthweight outcomes in Tasmania 2005-2018 (**secular trends study**).
2. Explore the associations between pre-and postnatal factors and different measures of body composition in Tasmanian infants from birth to 6 months (**prospective longitudinal study**).
3. Systematically review the literature reporting adiposity in newborns of mothers whose GDM was controlled with therapeutic interventions (**systematic review and meta-analysis**).
4. Evaluate the agreement of infant body composition assessed via ADP (PEA POD) and DD technique in 6-month-old infants (**body composition methods comparison study**).

1.5 THESIS ORGANISATION

This doctoral thesis is a combination of traditional thesis text (Chapters 1, 2, 3, 6, and 7) and peer-reviewed published articles (Chapters 4 and 5).

Chapter 1: The general introduction provides the rationale for the study with a brief background of the topic of interest, current situation and gaps in research, the significance of the proposed research, aims of the project and the general layout of the thesis.

Chapter 2: The literature review provides detailed background information, key themes or debates, evaluation of the relevant and important research in the field and gaps this study attempts to fill.

Chapter 3: Includes aims, methodology, results and discussion of the secular trends study that used Tasmanian Perinatal Data Collection Variables 2005-2018 linked by the Tasmanian Data Linkage Unit to examine recent trends in birthweight and maternal characteristics in Tasmania and associations between birthweight outcomes and maternal factors (research aim 1).

Chapter 4: Includes the aims, methodology, results and discussion of the prospective longitudinal study that examined associations between pre-and postnatal factors and measures of

body composition in Tasmanian infants from birth to 6 months (research aim 2). This study was also a part of a larger multi-country collaborative project ('Developing better information globally on young children's body composition') with data for the Australian arm collected at the Launceston General Hospital, Tasmania, September 2017 to October 2019.

This manuscript has been published:

Herath, M.P., et al. Determinants of Infant Adiposity across the First 6 Months of Life: Evidence from the Baby-bod study. *Journal of Clinical Medicine*, 2021. **10**(8): 1770.

A webinar conducted by the candidate on this research at Dietitian Connection® is available online: Infant adiposity webinar | Dietitian Connection

Chapter 5: Includes the aims, methodology, results and discussion of the systematic review and meta-analysis that reviewed the literature reporting adiposity in newborns of mothers with GDM controlled with therapeutic interventions (research aim 3).

This manuscript has been published:

Herath, M.P., et al. Gestational Diabetes Mellitus and Infant Adiposity at Birth: A Systematic Review and Meta-Analysis of Therapeutic Interventions. *Journal of Clinical Medicine*, 2021. **10**(4): 835.

Chapter 6: Includes the aims, methodology, results and discussion of the body composition methods comparison study that evaluated the agreement of several indices of infant body composition assessed via ADP and DD in 6-month-old Caucasian infants (research aim 4). The principles and procedures of body composition techniques used in the PhD project are described in detail.

Chapter 7: The discussion provides a summary of the key findings, how these findings interlink with the aims of the project and relate to previous research, plus limitations and potential improvements.

Chapter 8: Restates the thesis statement and highlights the key points of the overall research, the relevance and implications of the findings, and makes recommendations for future work.

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CHAPTER 2 : LITERATURE REVIEW

2.1 BACKGROUND

2.1.1 The global epidemic of obesity

The global prevalence of obesity has nearly tripled since 1975. In 2016, 1.9 billion (39%) of the world's adult population was estimated to be overweight, and of these, 650 million were deemed to be in the obese category (19%) [3]. Estimations based on data from 19.2 million adults across 186 countries from 1975 to 2014 show that the mean global BMI increased by 0·63 kg/m² per decade for men and 0·59 kg/m² per decade for women. Projections indicate that without intervention, approximately one out of five adults will be obese by 2025 [4].

Similar trends have been observed in children and adolescents. Pooled population data from 1975 to 2016 also demonstrate an increase in mean BMI in children and adolescents worldwide [5]. In 2016, 41 million children under the age of 5 years, and 340 million children and adolescents aged 5-19 years were estimated with overweight or obesity globally. In 2019, there was an estimated 38 million children under 5 years of age deemed overweight or obese [3]. In Australia, the prevalence of overweight and obesity in all age groups is rising at an alarming rate. According to national data (2014-2015), the proportion of adults classed as overweight or obese was nearly two-thirds, rising from 56 to 63% during the decade 1995-2015 [11]. In 2014-2015, 20% of children aged 2-4 years and 27% of children and adolescents aged 5-17 years deemed overweight or obese [8]. These statistics reinforce the extent of the obesity problem as a major public health challenge.

Overweight and obesity are typically defined as conditions where excess body fat accumulates and often results in adverse health outcomes [3]. BMI, calculated as body weight in kilograms divided by height in metres squared (kg/m^2), is the most commonly used indicator to classify overweight and obesity in adults (**Table 2.1**). Different classifications are used for children and adolescents because body composition fluctuates to a great extent as a child grows, along with differences pertaining to sexual maturation [3].

Age group		Indicator	Normal weight	Overweight	Obese
Adults	≥ 20 years	BMI (kg/m^2)	>18.5 to ≤ 24.9	>25.0	>30.0
Adolescents	5-19 years	BMI Z	>-2 to ≤ 1 SD	>1 to ≤2 SD	>2 SD
Children	0-5 years	WH Z	>-2 to ≤ 2 SD	>2 to ≤3 SD	>3 SD

Table 2.1 World Health Organisation (WHO) classification of body weight.

BMI: body mass index; SD: standard deviation; WH: weight-for-height; Z: z score

2.1.2 Health impacts of overweight and obesity

Overweight and obesity increase the risk of metabolic syndrome, a disorder defined as the presence of central adiposity (BMI ≥30 kg/m^2 or waist circumference higher than ethnic-specific cut-off values) with two or more of the following components: hypertriglyceridemia, hypertension, low level of high-density lipoprotein, and increased fasting plasma glucose [68]. Metabolic syndrome is a significant risk factor for several life-threatening non-communicable diseases such as cardiovascular diseases, e.g., coronary heart disease, stroke, hypertension; insulin resistance, e.g., type 2 diabetes mellitus, certain types of cancers, e.g., colon cancer, endometrial cancer; and gall bladder disease [9]. Obesity is also associated with several non-fatal health conditions, including respiratory disorders such as sleep apnoea, chronic musculoskeletal problems, infertility and skin disorders. The psychosocial aspects of obesity, for example, prejudice and discrimination, body image dissatisfaction and eating disorders, should also not be underestimated [12]. It has been reported that overweight and obesity contributed to 4 million deaths and 120 million disability-adjusted life years globally in 2015 [10], with the leading cause of death being cardiovascular disease [69]. Australian adults with overweight or obesity were reported to have higher rates of many chronic conditions compared with adults of normal weight, with severity rising with increasing BMI [11].

Maternal obesity is the most common issue encountered in obstetric practice. A BMI of 30 kg/m^2 or higher at the first antenatal consultation (within the first ten weeks of pregnancy) is considered obesity in pregnancy [70]. There has been a sharp increase in the prevalence of overweight and obesity among pregnant women globally, as well as in Australia [71]. For example, first antenatal visit BMI data of Australian women who gave birth in 2014 showed almost half with overweight and one-fifth with obesity [72]. A higher ppBMI increases the risk of pregnancy complications, including preeclampsia, GDM, thromboembolism, postpartum haemorrhage, induced labour, caesarean section, anaesthetic complications and wound infections. Infants born to mothers with obesity are at high risk of stillbirth, macrosomia, congenital anomalies, premature birth, and neonatal death [70, 73].

Childhood obesity can adversely affect the physiological as well as the mental health and wellbeing of children. Health consequences associated with childhood obesity include increased risk of cardiovascular diseases, abnormal glucose metabolism, hepatic steatosis (fatty liver disease), abnormalities in menstrual cycle, sleep apnoea, asthma and orthopaedic complications. Further, social problems such as discrimination and social marginalisation can lead to negative body image, low self-confidence and low self-esteem, which may also impact academic performance [13]. Importantly, childhood obesity and associated health risks are more likely to persist into adulthood [14, 15].

2.1.3 Origins of obesity

Obesity is a complex, multifactorial chronic disease resulting from a combination of behavioural, genetic and environmental factors.

2.1.3.1 Exposure to the obesogenic environment and individual behaviours

Body mass increases when the body remains in positive energy balance, i.e., energy intake exceeds energy expenditure, commonly over an extended period [74]. Globalization and rapid socioeconomic transitions around the world over the last few decades have created an environment that promotes high energy intake and reduces activity, referred to as an 'obesogenic environment'. Such obesogenic socioeconomic transitions include abundant availability of inexpensive, energy-dense, nutrient-poor food, increased intake of processed food and sugar-sweetened beverages, and replacement of energy-intense manual work by sedentary "desk jobs" [75, 76]. Personal behaviours identified as risk factors of obesity include eating large portions, irregular mealtimes, eating a majority of food during the night, lack of time for exercise, emotional stress, smoking, alcohol consumption [77]. However, beneficial effects of interventions targeted for correcting such obesogenic adult lifestyles have shown small benefits have often been small or short-lived [17], suggesting that the onset of the risk lies much earlier in the life span, and the interventions at a later stage of life may be too late to remedy the problem. Moreover, the fact that not all the individuals exposed to an "obesogenic environment" become obese indicates that genetic and developmental pathways are also involved in driving individuals toward increased adiposity/obesity.

2.1.3.2 Genetic predisposition to obesity

Advances in genomics have allowed researchers to identify numerous genes that contribute to determining the phenotype of the most common form of obesity [78, 79]. Since the identification of the first genetic locus (named as fat mass and obesity-associated gene) that showed an unequivocal association with adiposity in multiple populations in 2007, over 500 genetic loci have been discovered for different adiposity traits. These loci mostly have shown an association with BMI (341 loci), while some have shown an association with BMI-adjusted waist-to-hip ratio (129 loci) [80]. However, the most common 32 loci only account for less than 1.5% of the overall inter-individual variation in BMI [81], indicating that risk factors of obesity are beyond genetics.

2.1.3.3 Early metabolic programming of obesity and adiposity

2.1.3.3.1 The DOHaD hypothesis (Barker hypothesis)

In 1989, Barker and colleagues revealed that individuals with lower birthweights due to undernutrition *in utero* had a higher death rate from ischaemic heart disease [21]. This inspired

the revolutionary idea that the 'intrauterine environment influences the risk of non-communicable diseases in adulthood' [82]. While Barker's discovery was limited to the fact that LBW can lead to chronic diseases in adulthood, subsequent studies showed that the relationship between birthweight and later life disease risk is indeed U shaped; individuals with both LBW and HBW have increased risks of obesity-related diseases during adulthood [83, 84]. Afterwards, a series of comprehensive epidemiological studies supported the phenomenon that the "programming" of adult diseases might continue up to early infancy [85].

2.1.3.3.2 The life course approach

Obesity and associated non-communicable diseases do not follow the traditional medical model in which an individual is healthy until he/she contracts the disease. According to the life-course approach, the risk of metabolic diseases increases throughout life starting from the intrauterine period, although the greatest increases are observed during adulthood (Figure 2.1). This increase in risk of metabolic diseases has been explained as a consequence of the decline in plasticity where one genotype forms different physiological or morphological states in response to influences of environmental conditions [19]. In humans, plasticity is at its maximum during the first 1000 days of life, the period from conception to two years of age. During this period, most of systems and organs mature completing much of biological development [86]. Once the offspring adapts its growth trajectory in the time of foetal life and early infancy, it is relatively irreversible [20]. This is referred to as "programming", a process in which the occurrence of an insult or a stimulus at a sensitive period of development can affect the structure and physiology of cells and organs that lead to lifelong consequences [87]. Along these lines, a stimulus that results in excess accumulation of adipose tissue *in utero* or early infancy may predispose individuals to obesity in later childhood and adulthood. This suggests that early identification of markers and timely intervention in early life may help reduce obesity risk, while the benefits of late interventions may be limited [88, 89].

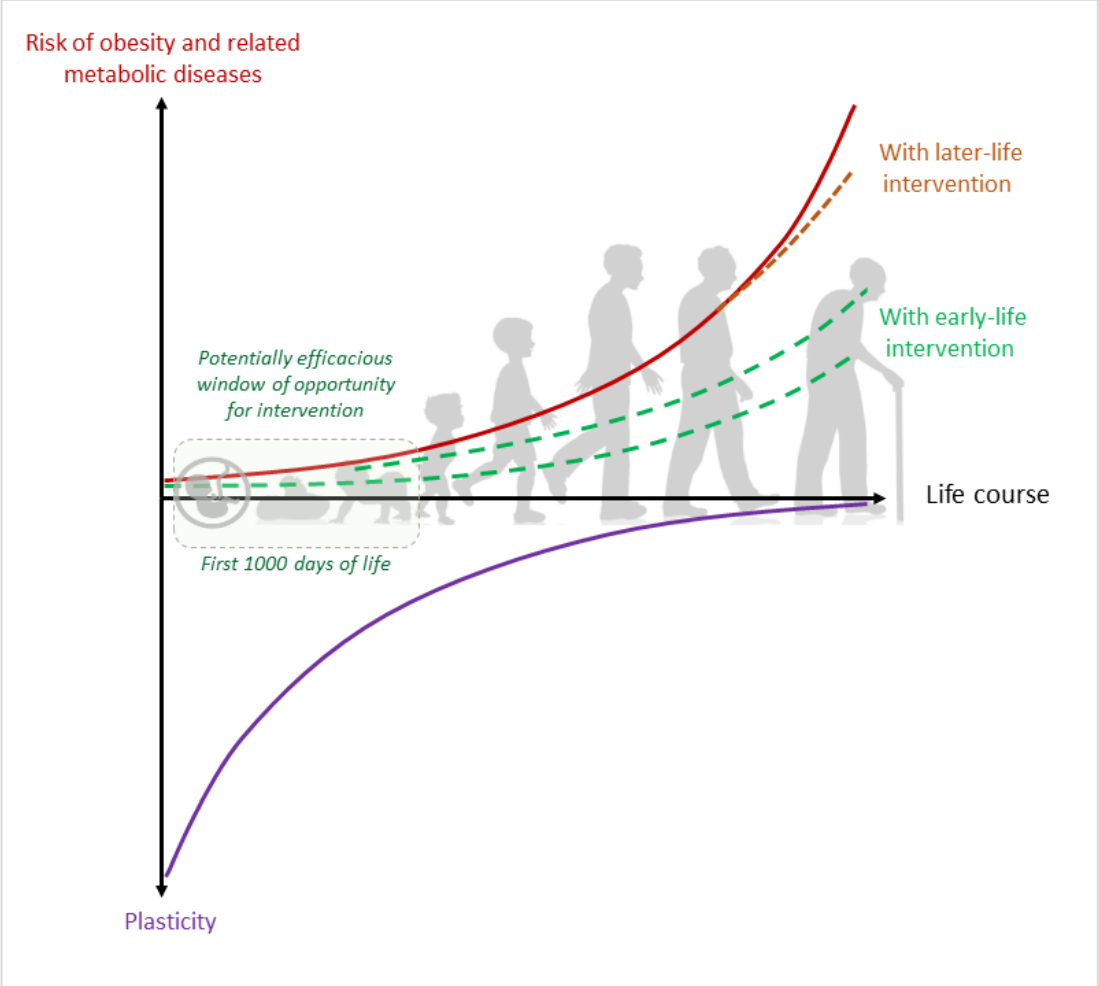

Figure 2.1 Potential benefits of early interventions for reducing the risk of obesity and related diseases in adulthood by adopting the "life course approach".

Adapted from Godfrey et al. 2010 [89] and Symonds et al. 2013 [90].

2.2 ASSESSMENT OF GROWTH AND BODY COMPOSITION DURING INFANCY

Assessment of growth is an important part of infant health surveillance [91]. For over a century, clinical assessment of infant growth relied on anthropometric measurements such as weight and length. While birthweight is the most widely used marker of foetal nutrition and *in utero* exposures [92], growth charts that take weight and length/height into account are commonly used to monitor the nutritional status of infants and children [93]. Nevertheless, these anthropometric indices are only proxies of nutritional status; they are incapable of differentiating between the components of the body. Measurement of different body compartments is referred to as body composition assessment; it can give valuable information on the human biological response to various environmental influences [67, 94]. The components that make up the body can be described in a range of ways, including the use of five interrelated levels of increasing complexity: atomic, molecular, cellular, tissue–organ, and whole-body (Figure 2.2) [95]. The molecular level, in which total body water (TBW), FM, protein and minerals are the major compartments, is particularly useful in evaluating, monitoring and management of nutrition in infants [96].

Chemical carcass analysis is the only direct method for body composition assessment [94]. The negligible amount of data obtained with the chemical analysis of cadavers present in the literature are from mostly newborns who were below the fiftieth percentile on the growth curve and cannot represent the body composition of infants with normal growth [97]. All other body composition evaluation models are either indirect or doubly indirect (relies upon another indirect model) and incorporate a number of theoretical assumptions [91, 94]. The four-compartment (4C) model that divides body compartments at the molecular level is considered the "gold standard" reference method for in vivo body composition measurement (Figure 2.3). Because 4C models are expensive and associated with a substantial burden to participants, 2-compartment (2C) models, which divide the body into FM and FFM, are commonly used [98].

Although there is a range of indirect body composition methods, their use in infants can be associated with unique challenges related to infant physiology (e.g., rapidly changing body composition) and behaviour (e.g., movement) [67]. The following sections include a brief literature review of various body composition techniques currently used in assessing body composition in infants, with more emphasis on the techniques used in the studies involved in this thesis: anthropometry, isotope dilution and ADP. An overview of the characteristics of commonly used body composition techniques in infants is outlined in Table 2.2.

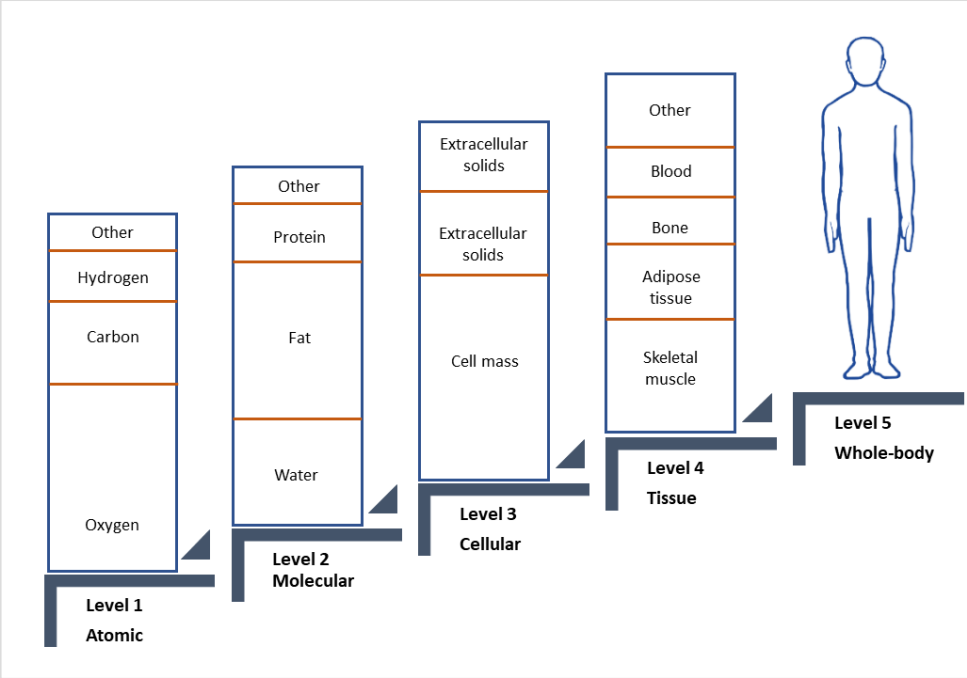

Figure 2.2 The five levels of human body composition.

Adapted from Wang et al. 1992 [95] and Guppy et al. 2012 [99]

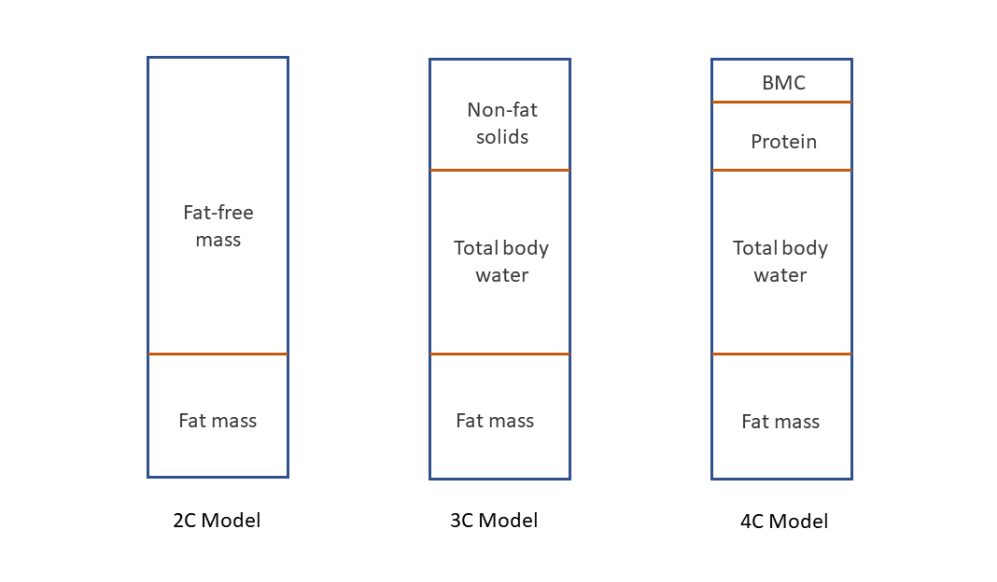

Figure 2.3 Different models for body composition assessment based on various body compartments.

Adapted from Ellis et al. 2007 [96] and Andrews et al. 2019 [100]; BMC: bone mineral content

2.2.1 Anthropometry

Anthropometric parameters such as height- and weight-based indices, body circumferences and skinfolds are extensively used as proxies of adiposity due to simplicity, low cost, portability and suitability of field use and low-resource settings [101]. BMI (weight/length^2) is widely used as a screening tool for identifying overweight and obesity in children [102]. As the association between BMI and fatness changes with age, height and sexual maturation, the BMI value of a child is often interpreted in relation to children of the same age and sex, i.e., BMI-for-age percentiles [102]. Although BMI is effective in tracking weight status in populations, a high BMI can result from increased FM or FFM, and vice versa. Therefore, BMI is considered a poor index to identify excessive adiposity at the level of an individual [103]. Ponderal index (weight/length^3) is another weight and height-based index that is less correlated with length than BMI, and therefore, a better proxy of adiposity for children and adolescents. Yet, due to a lack of reference data, the ponderal index is not as commonly used as BMI [104].

Growth references based on sex and age-adjusted percentiles and z-scores for weight, height, BMI or ponderal index are used in identifying infants deviated from normal growth patterns. They show how typical children in a population grew during a specific period; for example, reference charts by the Centers for Disease Control and Prevention (CDC) 2000 show growth patterns in children in the United States of America (USA) [105]. However, reference charts are population-specific and may not show ideal growth patterns [106]. Alternatively, WHO growth standards based on healthy breastfed infants of different ethnicities (6 countries) provide information on optimal levels for growth and development during infancy [107]. Nearly 100 countries have fully adopted WHO growth standards, while some countries (e.g., USA, China) have partially adopted them [105]. Nonetheless, a recent review by Marume et al. [108] revealed that infant growth trajectories in many countries significantly deviated from WHO growth standards and highlighted the importance of adopting regional-specific standards to increase the sensitivity of identifying children with suboptimal growth.

Body circumferences are another proxy for body composition. Waist circumference is a commonly used effective measure of abdominal adiposity [109], and it has shown associations with insulin resistance [110] and cardiovascular risk [111] in children. In addition, mid-arm circumference is suggested as a reliable adiposity measure in infants, and it correlated well with body fat estimations by ADP [112]. Moreover, indices based on circumferences such as waist-to-hip ratio and waist-to-height ratio were better predictors of obesity-related disease risk in children than BMI [113-115]. Body circumferences are considered less erroneous compared to skinfold measurements [116]. However, they cannot differentiate subcutaneous fat from visceral

fat, are prone to intra- and inter-observer variations, and interpretation can be difficult due to lack of reference data and cut-off values [117].

Skinfold thickness (ST) is a proxy for subcutaneous fat accumulation [118]. Body sites for ST measurements can be central (e.g., subscapular, abdomen, and suprailiac) or peripheral (e.g., biceps, triceps, quadriceps, and calf). For consistency, one side of the body is measured, either left or right. In children, the commonly measured ST sites are triceps, biceps, subscapular and suprailliac [119]. Single-site ST measurements have shown high correlations with total body fat in infants [120]. Normative data for ST measurements have been established for infants from different ethnic populations [121-123]. These provide an external point of comparison to aid in interpreting ST values from one site or a summation of two or more sites. Although ST measurements are fast, inexpensive, and relatively non-invasive, they may result in high intra- and inter-measurer errors in the absence of well-versed training and quality control [101]. Particularly with infants and young children, excessive movement during the assessment and variations in skin compressibility can cause measurement errors. The reliability of ST measurements in 0-4 years old children is reported to be 60-70% [91]. Other problems associated with ST measurements in infants are the cause of pain/trauma, specifically for preterm infants, and difficulty in differentiating fat-skin layer from muscle [124]. Also, ST measurements may not be appropriate for longitudinal studies because of poor within individual accuracy [125].

Various combinations of anthropometric measurements have been used to develop prediction equations for obtaining total adiposity [67, 108]. Statistical modelling of these equations requires the response variable (e.g., body composition measures such as FM, %FM) to be normally distributed, the associations between the response variable and predictor variables (e.g., simple anthropometric measures) to be linear, and the response variable to have a constant variance [91]. Previous studies have identified FM as a better outcome variable than %FM for predicting total adiposity in infants using anthropometric measurements [120, 126]. Among different single-site ST measurements, subscapular ST is found to be more predictive of FM in infants. As the subcutaneous fat layer around the limbs is uneven, triceps ST can be a poor predictor of overall adiposity [120, 127]. Developing a parsimonious prediction equation with data of a subsample and using that equation to estimate the body composition of the study sample can significantly reduce time, recourses and efforts in large epidemiological studies [91]. When applying a prediction equation to a study sample that is different to the sample it has been developed in, a cross-validation study is always recommended. Cauble et al. [128] and our group [129] cross-validated commonly used infant FM prediction equations including, Deierlein et al. [130], Catalano et al. [131], Lingwood et al. [126], and Aris et al. [127], using ADP PEA POD as the

criterion method, and determined that FM and %FM approximations by the equations had poor agreements with objective estimations derived using ADP.

2.2.2 Isotope dilution

Water is the most abundant molecule in the body; in healthy adults, ~60% of body weight and ~73% of FFM is water. In healthy term newborns, ~80-83% of FFM is water, and this declines rapidly over the first few months and steadily until ~5 years of age, reaching the proportions seen in adults. Isotope dilution, also known as hydrometry, is a method that has been used for over 60 years to estimate TBW in humans [132]. TBW derived from isotope dilution, combined with other methods such as DXA and ADP, forms the gold-standard 4C model and can also be used in 2C models [133].

Isotope dilution is based on the principle that FM is anhydrous, and TBW is distributed constantly in the FFM compartment of the body [134]. By dispensing a known amount of a tracer to the TBW pool and measuring its concentration in body water at equilibrium, the volume of TBW can be estimated by applying the dilution principle ($C_1V_1 = C_2V_2$). In the 2C model, TBW is divided by age-specific hydration factors to approximate the amount of FFM [132]. FM is then calculated by subtracting FFM from the body weight. The calculations are described in detail in Chapter 6.

Commonly used tracers are stable (non-radioactive) isotopes such as deuterium (^2H) and oxygen-18 (^{18}O). Natural water is mainly comprised of ^1H and ^{16}O and contain very little ^2H and ^{18}O. It can be made to contain larger amounts of ^2H or ^{18}O, which is referred to as labelled water. ^2H_2O is used more commonly as it is about 100 times cheaper than $^2H_2{}^{18}O$. During the testing, a dose of labelled water is given orally as a drink. After mixing with body water, the tracer is excreted from the body in urine, saliva, sweat and human milk. The dilution of the tracer in the body can be easily measured in saliva or urine; however, equilibration takes longer in urine than in saliva. Two procedures are used: 1) back extrapolation, where post-dose urine samples are collected for 7 days in infants and 14 days in adults, and 2) equilibration/plateau method, where saliva samples are collected after 2-5 hours of dose administration. The enrichment of the tracer in the samples is analysed using Isotope Ratio Mass Spectrometry (IRMS) or Fourier Transform Infrared Spectrometry (FTIR) [117, 132].

The deuterium dilution (DD) technique has shown good validity against chemical analysis in mammals [135] and 4C models in adults as well as children [136, 137]. Error rates reported are low: 1% for TBW and 0.5% for FFM [117]. Since water turnover is high in infants, the back-extrapolation approach has been recommended for infants [132, 134]; however, Salazar et al.

[138] have shown that the back-extrapolation and plateau method generate comparable results in young children.

The DD technique can be used in all ages and to assess longitudinal changes in body composition before and after an intervention. It is suitable for field settings as collected samples can be stored until analysed [132]. However, the cost is high due to the expense of stable isotopes and analytical equipment. Because of the time-consuming nature due to waiting periods between sample collections, the possibility for dose spillage and difficulty in collecting samples, the procedure may be challenging for the untrained with the paediatric population. Similar to other 2C approaches, when DD is used as part of a 2C model, the measurements may be affected by the hydration status of the participant [117, 132].

Recently, Wells et al. [139] published body composition (FMI and FFMI) reference charts for Caucasian children aged 6 weeks to 5 years based on data of TBW assessments using isotope dilution. These demonstrate that FMI rises rapidly up to 6 months, then declines almost plateauing by the age of 2 years in both males and female infants. The DD technique is widely used in a range of settings, including work by the International Atomic Energy Agency to develop body composition reference data for children (0-2 years).

In addition to body composition assessment, the DD technique is also widely used for the assessment of exclusivity of breastfeeding [140]. The DD dose-to-mother method is the only objective and non-invasive method currently available for assessing breastfeeding exclusivity in infants. In this technique, a dose of labelled water with ^{2}H is given to a lactating mother, which is dispersed in her body within a few hours and incorporated in her breastmilk, and the infant receives ^{2}H through breastfeeding. The milk intake can be estimated by sampling saliva from infant and mother for a 14 day period [141]. Additionally, the dose-to-mother technique has been used to explore the effects of the introduction of complementary foods to infants, where most of the studies show no significant reduction of breastmilk intake with the provision of complementary food items [140].

2.2.3 ADP

ADP technology originated in Germany more than 100 years ago [142]. Presently, ADP devices are manufactured by Cosmed Inc. (Concord, California, USA). There are two commercially available ADP body composition systems: BOD POD (for children from 6 years of age and adults; accommodates up to 150 kg) and PEA POD (for infants 0 to 6 months of age; accommodates up to 10 kg). The BOD POD adapted with Paediatric Option (a customised seat, modified window and calibration standards) is used in children from approximately 2–6 years of age (up to 30 kg) [143].

ADP technology involves estimating the body volume of a participant in a test chamber by detecting the change in the air pressure compared to a reference chamber with controlled air pressure [143]. The underlying principle is that when the participant is in an enclosed chamber maintained at a constant temperature, a volume of air is displaced (similar to the body volume of the participant), which results in a change in air pressure. By applying Boyle's law, the volume of the participant can be determined by measuring the change in air pressure. Once body volume is derived, with measured body mass, body density is calculated. Then, assuming a 2C model, densities of FM and FFM are assigned; the density of FM is a constant (0.9007 g/mL) and age- and gender-specific FFM density coefficients, to determine %FM [144]. The calculations are described in detail in Chapter 6.

The PEA POD software assigns FFM density values by Fomon et al. by default [145]. These values account for rapid change in density of FFM during the first few days of life due to water loss. Alternatively, FFM densities by Butte et al. can be assigned [44]. A notable disparity between these two models is that hydration factors for the neonatal period are lower in the Fomon model compared to the Butte model. Consequently, %FM estimates generated using the Fomon model may be higher than the values obtained using the Butte model. Therefore, the FFM model should be taken into consideration in longitudinal research and when comparing infant body composition of different studies [67, 146].

Since the PEA POD was introduced in 2003, several studies have investigated its accuracy and reproducibility. In a literature search between 2003 and 2017, Mazahery et al. [146] identified 12 such studies, with three studies using animal tissues, six with full-term infants and three with pre-term infants. %FM obtained from PEA POD has shown reasonable accuracy against the chemical analysis of 24 bovine tissue phantoms (weighing 1.39 to 9.95 kg to approximate infants of 0-6 months of age) [147] and 12 piglets (weighing 1.03 to 8.49 kg) [148]. In full-term infants, no bias has been reported for PEA POD estimated %FM compared to a 4C model (infant age: 2-17 weeks, weight: 2.7-7.1 kg) [149] and DD technique (infant age: 0.4-24.4 weeks, weight: 2.7 to 7.4 kg) [150]; however, 95% limits of agreement were wide in both the studies (− 6.8% to 8.1% and − 6.84% to 6.71%, respectively). In contrast, PEA POD appears to underestimate %FM compared to DXA measurements, particularly at lower body fat levels, in full-term infants aged approximately 6 months [151]. A similar modest accuracy has been reported for pre-term infants, with no bias but wide limits of agreement, against a 3C model [152] and isotope ($H_2{}^{18}O$) dilution technique [153].

Although reproducibility was poor in low %FM values in the study with piglets [148], a reasonable reproducibility (with narrow limits of agreement) has been observed in the studies that used PEA POD with term [145, 150] and pre-term [153] infants. For example, in term infants

of less than 3 months of age [145], within-day reliability was -2.0% to 1.2%, and between-day reliability was -2.2% to 1.7%. Moreover, the reproducibility appears not to be influenced by infants' behavioural states such as movements, intense crying, urination and defecation [145, 150].

Three studies [154-156] have evaluated the validity of the ADP instrument adapted for 2-6-year-old children (BOD POD with Paediatric Option), and their results are conflicting. Fields and Allison [154] compared ADP measurements in 2-6-year-old children against a 4C model and reported no bias. On the contrary, the other two studies reported significant differences between %FM derived from the Bod Pod with Paediatric Option and deuterium dilution technique in 6 to 48 months old children [155] and in 3 to 5 years old children [156]. It is important to note here that the 4C model generates more accurate results due to the use of fewer assumptions. Moreover, Fields and Allison [154] revealed that the PEA POD underestimated %FM by 7% in a subgroup of infants who cried during the volume measurement. This suggests that there may be influences of infant vocalisation in the volume chamber of PEA POD in the other two studies.

The ADP (PEA POD) system has become increasingly popular in paediatric research. As it has a very short assessment time (2-5 minutes), no radiation exposure and is non-invasive, multiple assessments can be made without concerns [146]. It allows infant behaviours such as crying or movement. In fact, among the ADP devices, PEA POD has the highest diaphragm frequency that allows for consistency and stability and takes numerous volume measurements that cancel the movement artefacts. The operating procedures are relatively simple with step-by-step instructions during calibration and testing: on average, a novice may take less than one hour to learn how to use the machine [67, 143, 157].

Nonetheless, ADP technology is not available for infants aged from 6 months to 2 years. The maximum weight that the PEA POD can accommodate is 8-10 kg, and 20-30% of infants aged 4-6 months may exceed this limit. Further, this method is not suitable for infants who require oxygen or intravenous fluids. Another limitation is that it cannot give regional estimates of body fat. Although PEA POD algorithms account for the rapid variation of FFM density during 0-6 months of age, individual variations in hydration may result in less accurate body composition results. Moreover, ADP devices are expensive and not portable, and require high-cost maintenance, and stable room conditions (temperature, pressure) for operation [67, 91, 143, 157].

As found in the systematic search by Mazahery et al. [146], 74 clinical published studies had used PEA POD to access infant body composition during the 14 years since the introduction of the device (2003-2017). In another recent review, Hamatscheck et al. [158] constructed age-specific growth charts for term and preterm infants by abstracting data from published studies (until 30 April 2019) that used ADP to measure body composition during the first 6 months of life. This compilation of 78 studies with only term infants, 19 with only preterm infants and 13 with both term and preterm infants, demonstrated that term infants had a steeper %FM development (11% to 25%, at 40- and 52-weeks postmenstrual age, respectively) compared to preterm infants (16% to 24%). FM development showed a similar pattern. In contrast, preterm infants showed a catch-up growth in FFM from 40 to 60 weeks postmenstrual age, with no significant differences (term infants: 2900 g to 5130 g; preterm infants: 2500 g to 5050 g).

2.2.4 Bioelectrical impedance analysis (BIA)

Bioelectrical impedance is a "field-friendly" body composition tool, and less prone to operator error compared to anthropometric predictions, and therefore suitable for resource-poor settings, large cohort studies and national surveys [159]. It is based on electrical characteristics of biological materials: it measures resistance (also called impedance) of body tissues to a small alternating electrical current. Bioelectrical impedance methods include bioimpedance spectroscopy (BIS), single-frequency BIA, and multi-frequency BIA [160]. Single-frequency BIA has been used in most of the studies with children because BIS and multi-frequency BIA devices became commercially available recently [161]. Typically, whole-body BIA assessment involves placement of 4 standard electrodes (two electrodes on the hand and two on the foot); however, an eight-point tactile electrode impedance has shown superior performance than four-point BIA [161]. BIA requires infants to remain still, and the inability to restrain the infants can result in significantly higher impedance values [117].

Theoretically, BIA models the body as a combination of five cylinders (conductors), the trunk, arms and legs, composed of a conductive compartment (FFM, comprising water and electrolytes) and an insulator (FM, a tissue with little to no water) [162]. Based on Ohm's law, impedance is directly proportional to the height/length and inversely proportional to the cross-sectional area of the body. Once impedance is determined, it is used in a validated prediction equation to calculate TBW, and thereby FFM and FM. In a recent systematic search, Lyons-Reid et al. [163] identified 46 published BIA equations for use in infants below 2 years of age; most of these equations (39 out of 46) have been developed for infants less than 6 months, and of those 36 have been validated.

BIA prediction equations are based on the impedance index, height^2/impedance, and by including height, it accounts for the differences in body size [164]. For this reason, these equations are population-specific or can only be used with individuals that closely resemble the size and shape of the reference population. When selecting a BIA equation, the age and ethnicity of the infants should be considered [161]. Moreover, BIA prediction equations depend on the assumption of constant tissue hydration, but hydration status rapidly changes during infancy. Consequently, despite the advantages such as quick assessment (several determinations take only a few seconds), non-invasiveness, portability and low cost, BIA is considered problematic for the paediatric population and produces no better body composition measurements than anthropometry [67, 163]. Piccoli et al. [165] have developed an alternative approach, bioelectrical impedance vector analysis (BIVA), for which prediction equations are not required, and Wells et al. [166] have proposed further adjustments to improve its accuracy. With additional work, BIVA may potentially provide a better substitution for BIA [166].

2.2.5 Dual-energy X-ray absorptiometry (DXA)

DXA is a special imaging modality that is different from normal x-ray systems as it uses a special beam filtering and near-perfect spatial registration of the two attenuations [167]. DXA is the gold-standard method for measuring bone mineral density and is widely used for quantifying total and regional fat, lean and bone mass in the body [91]. In the 4C model, DXA is used to measure bone mineral content. Another emerging use of DXA is measuring body volume, which may eliminate the need for densitometric techniques such as ADP for 4C models [168]. The underlying principle of DXA is that different tissues attenuate x-ray differently, with the lowest attenuation resulting by fat while the highest is by bone. DXA device use 2 energy levels of x-ray (high and low) to differentiate between tissues, e.g., adipose tissue and soft tissues. Differences in software, hardware and algorithms utilised in DXA devices have been found to impact the body composition estimates [169].

Although several studies have assessed the validity and reliability of DXA against criterion methods in adults and older children, only a few studies have been conducted with children less than 2 years of age, and its accuracy is not adequately confirmed in this population [170]. DXA appears to overestimate FM (up to 500 g) and %FM (by 3%-5%) in 0-6-month-old infants compared to ADP, and this is thought to be due to methodological differences between the 2 techniques. However, DXA measured FFM has been reported as similar to ADP measured FFM [158]. Moreover, DXA has shown good agreement with magnetic resonance imaging (MRI) in measuring total body FM and FFM, but it has underestimated trunk FM and FFM in 1-month old infants [171].

Hamatscheck et al. [158] identified 28 studies (14 with term infants, 9 with pre-term infants and five both) that used DXA to measure body composition in infants less than 6 months of age, published during the last 3 decades (1990-2019). This lack of studies with infants may be mainly due to the concerns of exposure to ionising radiation, although the radiation exposure of a typical DXA scan is less than 1 day's exposure (<1 micro-Sievert) to background radiation. Limitations of DXA include the high cost and requirement of radiologic certification. Moreover, some institutional review boards do not approve DXA scans for infants less than 3 months or more than 2 scans per year [91]. As movements can cause artefacts, infants need to be kept motionless by swaddling (using the same size and type of blanket for all measurements) and keeping the environment dark and quiet to make them drowsy [67]. However, these strategies may be ineffective for infants under 6 months, and they may need to be positioned on their stomachs [91]. Shepherd et al. [172] has developed a protocol for infant research that involves DXA scans, which has been shown to reduce the failure rate (nearly 21%) due to motion artefacts.

2.2.6 Magnetic resonance imaging (MRI) and quantitative magnetic resonance (QMR)

MRI is an imaging technique that can be used to estimate total and regional tissue and organ volumes. It uses the magnetic properties of the nuclei of hydrogen atoms (protons) in biological tissues (normally in water and fat). When an individual is placed on a strong magnetic field, these protons align like small magnets. Then a pulse radiofrequency field is applied for protons to absorb energy. When the radiofrequency field is turned off, the protons emit the absorbed energy which is detected by a receiver coil to produce high-resolution images. Contiguous image protocols allow estimates of tissue volumes, which are multiplied by the corresponding tissue density to calculate the tissue mass [173]. As ionising radiation is not involved in MRI, it is safe to be used in infants, but with several protection requisites. Usually, MRI scanner rooms have a low temperature; thus, infants should be kept warm by using a blanket. Also, infant-specific earplugs should be used to protect them from the noise of the scanner. To avoid artefacts due to movement, ideally, infants are scanned when sleeping after having a feed. Accordingly, MRI is more practicable for infants less than 6 months of age because it may be hard to achieve compliance with older infants [174]. Further, due to the high cost and longer image processing time, MRI is recommended when the research needs estimations of specific tissue volumes, for example, intra-abdominal adipose tissue [67, 94].

QMR is a more recent magnetic resonance model that has shown high precision and accuracy for measurements of FM, FFM and TBW [175]. It's a non-imaging technique. In QMR, the processed signal from the whole body is received at once (no spatial encoding), making it different to MRI [175, 176]. Specific QMR systems have been developed for infants weighing up to 12 kg (EchoMRI-Infant, Echo Medical Systems, Houston, TX) and children weighing 3 to

50 kg (EchoMRI-AH, Echo Medical System, Houston, TX). With mathematical adjustments for data fitting, these have produced body composition estimates comparable with whole-body chemical analysis, 4C model, ADP, DXA and DD [175-177]. As there are validated QMR systems already available for adults, this technology is a promising tool that can be used throughout the lifespan. More recently, QMR systems that can separately quantify visceral adipose tissue (VAT), brown adipose tissue (BAT) and subcutaneous adipose tissue (SAT) also have been developed [178]. Unlike MRI or DXA, QMR is faster (<3 min assessment time) and does not require infants to be motionless [177]. Even so, the devices are highly expensive (~$450,000), and standard procedures and further validations are needed to expand their use in the paediatric population [91, 178].

2.2.7 Ultrasound

Ultrasound is frequently used during pregnancy to assess foetal growth and detect congenital anomalies, but its use as a body composition technique is limited [179]. Ultrasound can measure the thickness of fat, muscle and bone tissues and is capable of providing regional compositions [180]. The underlying principle is that when an ultrasound beam is transmitted through the skin, the amount of ultrasound waves reflecting (echo) is dependent on the variations in acoustic impedance between two tissue interfaces, for example, skin-subcutaneous fat interface or muscle-bone interface. The transducer of the scan head performs a dual function of transmitting and receiving ultrasound beams. It converts the received echoes to signals where each reflected wave represents a dot, and these dots combine to form an image [180]. The scanning procedure is quick and straightforward: the gel is applied, and the transducer head is moved over the skin while taking the images. However, interpretation of the images needs expertise. Electronic callipers are used to measure the thickness of tissues of interest, and these thickness values can be used in validated equations to predict body density and total body %FM [181].

Ultrasound is safe (no ionising radiation), relatively inexpensive and portable [182]. With ultrasound, SAT and VAT thicknesses can be measured directly at various axial sections of the abdomen [179]. It also can be used to assess liver steatosis (fatty liver) [183]. Hence, ultrasound provides a feasible option when criterion methods such as MRI are not available [117]. Moreover, ultrasound provides a more accurate and reliable alternative for measuring subcutaneous fat in place of using skinfold calipers, as infant movement and skin compressibility can affect the reliability of caliper measurements [184]. Ultrasound measured VAT and SAT has shown good agreement with estimations derived by MRI [185] and BIS [176] in infants. In addition, ultrasound has shown promising results in evaluating intracellular muscle fat content in children; it may be useful for assessment of the quick and intense muscle mass loss in neonates admitted to neonatal intensive care unit (NICU) [186]. However, due to the lack of standard operating

procedures and validated age-appropriate prediction equations for calculating total body adiposity, and the necessity of technical skills for interpreting the results, use of ultrasound in infant body composition research is limited at present [117].

Table 2.2 Specifications of commonly used technologies for infant body composition assessment.

	ADP	Isotope dilution	ADP	DXA	BIA	MRI	Ultrasound
Safety	High	Medium	High	Medium	High	High	High
Cost	High	High	High	High	Low to High	Very High	Medium to High
Time involved	Low	High	Low	High	Low	Medium to High	Medium
Compliance	High	Medium	High	Medium	Medium	Medium	Medium
Operator skills	Medium	Medium	Medium	High	Low	High	Medium to High
Portability	Low	High	Low	Low	High	Low	Low
Accuracy	Medium to High	High	Medium to High	Low	Medium	Medium	Medium
Precision	High	High	High	Medium to High	High	High	High

ADP: air displacement plethysmography; BIA; bioelectrical impedance analysis; DXA: dual-energy X-ray absorptiometry; MRI: magnetic resonance imaging.
Adapted from Ward et al. 2013 [187] and Shepherd et al. 2016 [188].

2.3 DETERMINANTS OF BODY COMPOSITION DURING INFANCY

Given that excessive fat accumulation during early life can predispose to obesity [13], the identification of factors that determine body composition during infancy has gained increased attention during the past decade. Body composition, particularly relative proportions of FM and FFM, are linked with the programming of human metabolism [28]. Excess FM, low FFM, or both in infancy are risk factors for subsequent metabolic disorders [13]. Several pre-pregnancy, pregnancy and postnatal factors may contribute to excessive adiposity in early life. Since the mother provides the intrauterine environment for the developing foetus, it is expected that nutritional, endocrine, social, behavioural and environmental factors of the mother during pregnancy are reflected in the body composition of the infant at birth [189]. During the postnatal period, infant feeding practices play the most important role, providing a great opportunity to modify the effects of any adverse *in utero* exposures on adiposity of the offspring [190, 191].

2.3.1 Maternal pre-pregnancy and pregnancy factors

2.3.1.1 Pre-pregnancy weight

Maternal pre-pregnancy overweight and obesity have shown independent associations with excess adiposity in infants, increasing their susceptibility to later life obesity and an intergenerational cycle of obesity [192]. A recent systematic review demonstrated that the odds of children (age 1-18 years) being overweight/obese was 1.8 times higher (odds ratio: 1.80, 95% CI: 1.25 to 2.59) for mothers with obesity compared to mothers with normal weight before pregnancy [193]. Similarly, another systematic review showed that FM (standardised mean difference: 0.38; 95%CI: 0.30 to 0.46) and %FM (0.31%; 0.23% to 0.39%) were greater in children (age 0-6 years) born to mothers with overweight/obesity compared to children of mothers with normal weight [194]. The underlying mechanism of this relationship is not yet clearly elucidated; however, changes in maternal metabolism (increased glucose and fatty acids) is thought to play a role in permanently altering foetal metabolic programming, including hypothalamic response to leptin, and thereby appetite regulation and physiology of the pancreatic beta cells [195].

Despite the clear positive associations between maternal obesity and overweight/obesity in older children, the literature on associations between pre-pregnancy overweight/obesity and offspring adiposity during early infancy is inconsistent. The positive associations between maternal ppBMI and infant FM or %FM at birth in some studies [35, 57] were not found by others [49], even though all studies used the same technique (ADP) to assess body composition. This discrepancy may be due to differences in the statistical analysis used to test the associations (multiple linear regression with adjustments for confounding factors such as maternal weight gain during

pregnancy, age, ethnicity and infant sex vs correlation coefficient analysis which does not adjust the effect for confounders) or age of the infant. Longitudinal studies to investigate the persistence of this association are limited. Maternal ppBMI does not appear to impact infant adiposity during the postnatal period, for example, at 3-months [196], 5-months [50] or 6-months [196] of age. Additionally, sexual dimorphism in offspring body composition in response to maternal overweight/obesity has been found in a few studies. In a cross-sectional study [197], maternal obesity showed significant positive associations with FM at birth only in male infants. A longitudinal study [198] showed that, %FM was higher in girls born to obese mothers compared to girls of non-obese mothers at 9 months and 1-year of age, but the differences were not significant at 3-6 months and 1-6 years. In boys, there was no difference in %FM until the age of 4 years, but for 4-6 years, the boys of mothers with obesity had significantly higher %FM than their counterparts.

In contrast, maternal underweight, a marker of foetal undernutrition, increases the risk of LBW, small-for-gestational-age (SGA) and preterm births [199, 200]. These undernourished infants have lower adiposity at birth [201]; however, when exposed to energy-dense food environments postnatally, they experience excessive catch-up growth that promotes increased fat deposition in early childhood [88]. As shown in animal models, offspring of mothers with malnutrition have low leptin levels at birth which results in increased appetite, a mechanism to promote catch-up growth [202]. Rapid weight gain associated with catch-up growth is identified as a risk factor for later obesity [203].

2.3.1.2 GWG

Similar to ppBMI, GWG is an indicator of the nutritional status of the mother during pregnancy, with inadequate and excessive GWG reflecting undernutrition and overnutrition, respectively [204]. Among different classifications of GWG, Institute of Medicine (IOM) guidelines for total GWG (tGWG) are the most widely used. tGWG is defined as the amount of weight a pregnant woman gains between the time of conception and the onset of labour, and ~35% of it is the weight of the infant, placenta and amniotic fluid [205]. Excessive tGWG is associated with greater FM [37, 206, 207] and %FM [206, 207] in infants born to mothers who started their pregnancy with normal ppBMI, but not in infants born to mothers who had overweight or obese ppBMI. Inadequate tGWG has shown no association with infant adiposity, but the infants of mothers with inadequate tGWG have been found to be shorter than the infants of mothers who had adequate tGWG at 1 week of age [37]. Additionally, the rate of weight gain (kg/week) during different stages of pregnancy based on tGWG has been shown to impact infant body composition differently. Estampador et al. [47] examined the correlations of weight gain rate at early-, mid- and late-pregnancy, with FM at birth, and revealed that the association was only significant at

mid-pregnancy (r = 0.41, p = 0.03). However, Starling et al. [35] did not found such a variation, and weight gain rates at early-, mid-and late-pregnancy were independently positively associated with infant FM and %FM at birth.

While most studies have used tGWG as a categorial variable when assessing these associations, a few have used this as a continuous variable. For example, Nehab et al. [208] reported that tGWG (kg) was positively associated with infant FM as well as %FM at birth, after adjusting for birthweight, gender, arterial hypertension, and GDM and ppBMI. Conversely, in the study by Breij et al. [57], tGWG was not significantly associated with infant %FM after adjusting for ppBMI, gestational age, infant sex, birthweight standard deviation (SD) score and birth length SD score. These inconsistencies found at birth are also seen later in infancy. At 5 months of age, tGWG was significantly positively associated with infant FM, independent of ppBMI, ethnicity, FM at birth, breastfeeding exclusivity, and rapid infant growth [50], but it was not significantly associated with FM or %FM at 3 and 6 months of age when the effects were corrected for ppBMI, maternal age, height, parity, smoking during pregnancy, ethnicity, education level, infant sex and infant age at the assessment [196].

As mentioned previously, tGWG includes the weight of the infant, placenta and amniotic fluid; thus, it may not accurately reflect the actual weight gain of the mother. On the contrary, net gestational weight gain (nGWG) calculated as the difference between maternal weight measured after delivery and prior to pregnancy, reflects the actual weight gain of the mother. Therefore, conceptually it may be a better indicator of maternal nutritional status. Heude et al. [209] measured nGWG to calculate nGWG rate (nGWG in grams divided by gestational age in weeks) and categorised it into: "Low", <74 g/week; "Normal", 74-282 g/week; "Medium-high", 282-389 g/week; and "High", >389 g/week, to investigate its relationship with adverse pregnancy outcomes. So far, no previous study has explored the associations of nGWG with infant adiposity.

2.3.1.3 GDM

GDM is defined as glucose intolerance first diagnosed in pregnancy [210]. A growing body of literature has investigated the body composition of newborn infants of women with GDM compared to infants of women with NGT; however, their findings have been inconsistent and contradictory. Independent of birthweight, infants of mothers with GDM have increased FM compared to infants of mothers with NGT [211, 212], a result not observed by some researchers [64]. The basic biological mechanism is that excess blood glucose in hyperglycaemic mothers is transferred to the foetus during pregnancy, and in response to elevated blood glucose levels, the infant body secretes increased amounts of insulin, which results in higher fat deposits [213]. Interestingly, another study found that GDM was the main predictor of infant adiposity in boys

but it was not a significant predictor of adiposity in girls [55]. In a systematic review and meta-analysis [65] of observational studies that compared adiposity in infants born to diabetic (all types of diabetes) and non-diabetic mothers, a subgroup analysis showed a significantly greater FM (62 g; 29 to 94; p = 0.0002) and %FM (1.7%; 0.7% to 2.8%; p = 0.002) in infants of mothers with GDM compared to infants of mothers with NGT. However, there was high heterogeneity (I^2 = 97%) between the studies included in this meta-analysis, and importantly, in some studies, the blood glucose levels of GDM mothers were not controlled with therapeutic interventions.

The level of glycaemic control and various therapeutic interventions may affect infant body composition differently. In a randomised controlled trial that investigated whether treatment for GDM can normalise infant adiposity at birth, Landon et al. [214] found that mean FM in infants of mothers who received diet therapy (*n* = 427) and insulin, if required (*n* = 36), for controlling GDM, was significantly lower than that of the infants (*n* = 473) whose mothers received usual prenatal care (427 ± 198 g vs 464 ± 222 g, p = 0.003). An observational study of 599 term babies (67 exposed to GDM) showed that neonatal %FM did not vary by the GDM status of mothers, potentially due to good maternal glycaemic control [64]. Conversely, other groups have reported that FM increased in newborn infants of women with and without GDM, despite attempts to control glycaemia throughout the pregnancy [63, 215]. To ascertain the impact of glycaemic control using treatments for GDM regarding infant adiposity, we conducted a systematic review and meta-analysis [216], which showed significant differences in FM and %FM between infants of mothers treated for GDM and infants of mothers with NGT only existed in 'pre-2010' studies. There was no significant difference in FM and %FM in the two infant groups in 'post-2010' studies, which may be attributed to more intensive management of hyperglycaemia in the 'post-2010' period. The systematic review is presented in Chapter 3 in this thesis.

2.3.1.4 Smoking

Several meta-analyses have shown strong associations between maternal smoking during pregnancy and child overweight or obesity [217-220]. Smoking during pregnancy, either active or passive, is a well-recognised risk factor for foetal growth restriction characterised by LBW or SGA [40, 221-223]. Infants with LBW or SGA often have rapid catch-up growth during infancy, which may be a contributing factor for obesity in children exposed to smoke in utero [224]. The direct toxic effects of products of cigarette smoking, such as nicotine and carbon monoxide, and confounding effects of poor maternal nutrition in smoking mothers, have been suggested as the potential mechanisms by which smoking reduces birthweight of infants born to mothers who smoke during pregnancy [225-227]. Nicotine has a vasoconstrictive effect that results in impaired blood flow from the placenta to the foetus [225]. Carbon monoxide displaces oxygen in

haemoglobin in foetal arterial blood, causing hypoxia in the developing foetus that leads to reduced energy availability for growth [226]. The negative effect of smoking on foetal growth may also be due to the increased energy expenditure and lower caloric intake during pregnancy, resulting in maternal undernutrition or inadequate GWG [227-230]. However, others have shown no differences in ppBMI or GWG between smoking and non-smoking maternal groups [40, 231].

Although prenatal smoking is a well-known predictor of foetal growth restriction, few studies have investigated its impact on the body composition of infants, and their findings are contradictory. While some studies showed that maternal smoking was associated with lower FFM, but not FM [40, 232], others reported that women who smoked during pregnancy gave birth to newborns with lower FFM as well as lower FM compared to those who never smoked [233, 234]. The effect of maternal prenatal smoking may be mediated by infant feeding mode. At 1 month of age, formula-fed infants of smoking mothers have higher percent FFM (%FFM) and lower %FM compared to those of non-smoking mothers. However, no significant differences were found in %FM and %FFM of infants of smoking mothers vs infants of non-smoking mothers from 2 months to 1 year of age, independent of the feeding mode [235]. In another study, FFM of the infants of mothers who smoked prenatally was greater (154.7 g; 0.5 g to 309.0 g; $p = 0.049$) than their counterparts at around 5 months of age when the effects were adjusted for breastfeeding exclusivity, but the increase in FM was not significant, indicating a catch-up growth only in lean tissue [234]. Further, Harrod et al. [236] investigated whether the association between prenatal smoking and infant body composition is dependent on the quantity and timing of smoking after adjustments for significant confounders: ppBMI, GWG, gestational and chronological age, offspring sex, gravidity, maternal age, ethnicity, educational status, household income, and physical activity. They showed that every additional packet of cigarettes consumed significantly decreased newborn FFM (-2.1 g; -2.9 g to -1.3 g; $p < 0.001$) and FM (-0.7 g; -1.1 g to -0.3 g; $p < .001$), and cessation of smoking before late pregnancy resulted in no difference in FFM and FM in the two newborn groups. This comparable body composition measures between neonates of mothers who stopped smoking late pregnancy and neonates who were never exposed to smoking could be because the growth of FM and FFM in the foetus largely occurs during the third trimester.

2.3.1.5 Intake of micronutrient supplements

During pregnancy, the requirements for essential vitamins and minerals (collectively known as micronutrients), increases markedly to support the cellular and metabolic functions in the growing foetus and placenta [237]. For certain nutrients, e.g., iron, folate, vitamin B6 and iodine, the increase in the requirement is even higher than that of energy [238]. Numerous studies have shown that several micronutrient deficiencies are associated with pregnancy complications

including folate, iron, vitamin D, vitamin B_{12}, zinc, iodine, and selenium, commonly provided in prenatal supplements [237]. However, although the IOM [239] and the National Health and Medical Research Council (NHMRC) of Australia [240] recommend that women increase their daily intake of most micronutrients, the WHO recommends supplemental intake of only folic acid and iron [241] to prevent maternal anaemia, LBW, preterm delivery, neural tube defects, cleft lip and palate and congenital heart defects.

Folic acid is the synthetic form of vitamin B_9, which is converted to folate in the body. Folate is essential for methylation reactions through the one-carbon cycle and its deficiency in critical growth periods results in congenital malformations, particularly neural tube defects [242]. De-Regil et al. [243] reviewed data of 5 trials (7391 women) and identified that supplemental folic acid during preconception, on its own or in combination with other micronutrients, prevented neural tube defects. Folic acid supplementation is recommended for all pregnant women in Australia, and recommended daily doses are: at least 400 µg for a minimum 1 month before pregnancy, 600 µg for a minimum 3 months during pregnancy [240].

Iron promotes the production of haemoglobin: the carrier of oxygen from lungs to tissues, and is involved in DNA synthesis [244]. A systematic review by Figueiredo et al. [245] that synthesised data of 71 studies (916,990 pregnant women in total), showed that maternal anaemia as a consequence of iron deficiency is a key risk factor of intrauterine growth restriction and LBW (adjusted odds ratio: 1.23, 1.06 to 1.43). The WHO recommendation is daily supplementation with 30 mg to 60 mg of elemental iron during pregnancy; however, some governing bodies recommend iron supplementation only for women with anaemia (after assessing haemoglobin level at the first antenatal visit and around 28th week of gestation), not for all pregnant women [246].

Apart from the benefits shown for single-nutrient supplements such as iron and folic acid, there is promising evidence for multiple micronutrient supplementations in pregnant women as a strategy for preventing LBW and SGA births and preterm births [247]. Although the evidence base for the benefits of prenatal supplements is from developing countries, the use of prenatal micronutrient supplements continues to increase in pregnant women in developed countries [248]. Concurrent use of better-quality nutrient-rich diets and prenatal micronutrient supplements may result in the total intake of certain micronutrients exceeding the daily recommendations [242]. Although the harmful effects of micronutrient deficiencies are well-studied, little attention has been paid to the impact of excess micronutrient intake during pregnancy. Even less is known regarding the long-term effects of antenatal supplements, particularly on offspring body composition at birth and beyond. Animal studies indicate that excessive intake of methyl vitamins like folate or vitamin B_6 during gestation can affect food intake regulation and alter the

metabolism of the offspring, promoting fat deposition and weight gain [242], but evidence in human trials is scarce. Dougan et al. [249] explored data of 29160 mother-daughter pairs and found no relationship between prenatal vitamin intake and obesity in childhood (ages 5 and 10 years) and adulthood (>18 years), despite limitations such as recall bias and fatness determined using a figure rating scale, BMI and waist circumference. Moreover, FM in newborns whose mothers consumed the recommended 400 µg of folic acid daily was not significantly different from those who did not [233]. However, in the study by Sauder et al. [250], intake of multivitamins was not associated with infant FM or FFM at birth, or rates of change in FM and FFM during the first 5 months of life, but it was inversely associated with rate of change in %FM after adjusting for potential confounders including breastfeeding. This finding suggests there may be persist effects of prenatal micronutrient intake on offspring adiposity beyond birth.

2.3.1.7 Diet

Maternal diet during the prenatal period has shown significant associations with foetal growth. Apart from adequate micronutrient intake, macronutrient balance, diet quality, dietary patterns and meal timings are important aspects of maternal diet [251]. The intake of carbohydrates was positively associated with BMI peak, i.e., an initial increase in BMI during early infancy that peaks at 6-12 months of age [252]. The intake of sugar rather than starch largely contributed to this effect, with each additional 25 g of sugar intake by mothers (n = 910) associated with a higher infant pre-peak velocity (0.02/month; 0.01/month to 0.03/month) and a higher BMI peak (0.07; 0.01, 0.13). The authors concluded that high consumption of food items with added sugar during pregnancy might result in greater adiposity in infancy. In another analysis by the same group of investigators [253], the association between maternal macronutrient intake and abdominal adiposity of a sub-sample of the infant cohort (n = 379) who underwent MRI scans, was explored. A maternal diet higher in protein and lower in carbohydrate or fat during 26-28 weeks of gestation was associated with a lower abdominal adiposity in the neonates (age 2 weeks).

To investigate the effect of maternal diet quality on infant body composition, several studies have used the Healthy Eating Index 2010 (HEI-2010): a validated diet quality scoring tool developed by the United States Department of Agriculture [254]. Using this tool, Shapiro et al. [255] examined maternal diet quality at 8-24 weeks of gestation (median = 17 weeks) and 24-32 weeks of gestation (median = 27 weeks, n = 1079). They found that a lower HEI-2010 score (poor diet quality) was significantly associated with higher infant FM (0.74 g; 1.49 g to 40.0 g; $p < 0.05$) %FM (0.58%; 0.07% to 1.1%, $p < 0.05$), after adjusting for ppBMI, physical activity, maternal age, smoking, energy intake, preeclampsia, hypertension, infant sex, and gestational age. Similarly, Tahir et al. [256] showed an inverse association between maternal diet quality and infant FM and %FM. However, Gonzalez-Nahm et al. [257] found no significant associations

between HEI-2010 measured maternal diet quality and infant overall and abdominal adiposity at 6 and 12 months of age and after adjusting for maternal ppBMI, race, education, age, smoking, parity, calorie intake, weeks of breastfeeding and infant age at measurement.

Dietary pattern assessment accounts for complex food consumption behaviours and incorporates interactions between nutrients. Higher intake of fruit and vegetables and lower intake of fast-food during gestation (maternal dietary intake information collected at 26-28 weeks of gestation) was associated with lower adiposity (measured with ST) during 18-54 weeks of infant age [258]. Moreover, a dietary pattern characterised by the intake of eggs, potatoes and other starchy vegetables, non-whole grains, and a low intake of dairy, dark-green vegetables, whole grains, and soy was associated with greater newborn %FM [259]. Interestingly, maternal night-fasting interval (determined as the longest fasting interval between calorie-containing food or beverage taken from 7.00 pm to 7.00 am) in the late second trimester of pregnancy, was positively associated with neonatal adiposity [260].

2.3.1.8 Physical activity level

Pregnant women are encouraged to engage in moderate-intensity physical activity for 150 minutes per week; however, studies show that only 1 in 7 pregnant mothers meet this requirement [261]. Exercising during pregnancy has a number of benefits to the mother, including prevention of excessive weight gain, reducing the risk of GDM, hypertension, caesarean deliveries and postpartum depression [262]. In a recent systematic review and meta-analysis of 135 studies (166094 women), Davenport et al. [263] reported a 39% decrease in the odds of having a HBW infant for mothers who exercised during pregnancy compared to those who did not, without any increase in odds of LBW, SGA, preterm birth or intrauterine growth restriction.

The effect of maternal physical activity during pregnancy on infant body composition can be modulated by the intensity, type, frequency, duration of physical activity, and time period of the pregnancy [233, 264-266]. Bisson et al. [264] measured maternal physical activity during early-pregnancy (at 17 weeks of gestation) and late pregnancy (at 36 weeks of gestation) showed that performing vigorous physical activity during early pregnancy was associated with lower %FM in newborns ($2.3 \pm 0.8\%$, $p = 0.003$) while moderate physical activity during late pregnancy was associated with increased FFM (2.0 ± 0.8 g, $p = 0.012$). These effects did not change after the analyses were adjusted for infant sex, gestational age at delivery, maternal ethnicity, ppBMI, smoking habits prior to pregnancy, parity, daily energy intake and energy expenditure spent in sports and exercise prior to pregnancy. Similarly, FM was lower in the neonates whose mothers performed moderate-intensity exercise (exercise that that did not result in heavier breathing) during the 15th week of gestation (recalled by mothers) than those of mothers who never exercised [233]. In contrast, in the study by Mudd et al. [266], moderate physical activity at any

trimester was not associated with infant adiposity at 2 weeks. However, vigorous physical activity during the third trimester was associated with a lower FM change from birth to 4 years ($\beta = 0.006$, $p = 0.025$). Similar findings were reported by Harrod et al. [265]; total energy expenditure at early- and mid-pregnancy was not associated with neonatal adiposity, but newborns of mothers who had a higher level of total energy expenditure during late pregnancy had lower FM (41.1 g; $p = 0.03$).

2.3.1.9 Socio-demographic factors: age, parity and ethnicity

Average maternal age in pregnancy is increasing worldwide; in some cases, women delay their pregnancy until after 40 years of age due to educational, occupational or economic reasons [267]. Maternal age greater than 35 years at pregnancy has been identified as a risk factor for adverse pregnancy outcomes that can affect the growth of the foetus. For example, the incidence of GDM is higher in older pregnant women compared to their younger counterparts, independent of obesity and ethnicity. Pancreatic beta-cell function and insulin sensitivity decline with age making women more susceptible to glucose intolerance [268]. Moreover, older women are reported to have an increased risk of SGA births due to insufficient placental perfusion or transplacental flux of nutrients [269] and stillbirths relative to mechanisms involving the exposure of gametes to intensified oxidative stress [270]. On the other hand, teenage mothers also are at high risk of delivering preterm or LBW infants [271]. This has been explained using the concept of foeto-maternal competition for nutrients. In adolescent mothers who are still growing, leptin surges in the third trimester increase the use of energy for the growth of the mother, restricting the growth of the foetus [272].

Maternal age has shown a positive correlation with birthweight; however, this association was no longer significant when the effect was adjusted for parity (number of previous viable pregnancies) [273]. Similarly, in the studies [32, 54] that explored predictors of neonatal adiposity, when maternal age and parity were included as two independent predictors in the models, only parity showed a significant correlation with infant FM and %FM. Catalano et al. [32] examined the correlation between several maternal factors and body composition of 186 newborns and reported that the highest variation in infant FM was explained by parity ($R^2 = 0.08$) followed by other variables such as gestational age, pre-pregnancy weight, GWG, and neonatal sex. In another comparatively larger study ($n = 599$), Au et al. [54] reported that infants born to primiparous or multiparous women have higher %FM compared to infants born to nulliparous women. It has been speculated that there can be a cumulative effect of pregnancy on women's metabolism that affects foetal growth. Evidence that the risk of type 2 diabetes increases with increasing parity has been used to support the former notion. Conversely, Sauder et al. [50] showed that maternal age or gravidity (number of times a woman has been pregnant) was not a

significant predictor of FM or %FM at 5 months of age. Their findings partially agreed with the outcomes of the study by Breij et al. [196], where maternal age was not a significant predictor at 3 and 6 months while a significant positive correlation of parity with %FM was found at 3 months, but not at 6 months.

Ethnic background is identified as another key factor that contributes to variations in infant body composition. Sletner et al. [33] compared anthropometric parameters (abdominal circumference and ponderal index) of infants born in Asia, Middle-East, Africa and South/Central America (n = 282) versus western Europe (n = 229), to reveal that the former group of infants were characterised with a 'thin fat phenotype'. Similarly, South Asian infants (n = 30) were found with lower FFM and higher FM compared to White European infants (n = 30) in the study by Stanfield et al. [274]. This was also the first study to show the early manifestation of body composition phenotype with elevated total and central adiposity characteristics of Asian adults compared to those of European ancestry [275, 276]. Additionally, Paley et al. [277] showed that newborns of African-American, Asian and Hispanic background have greater truncal adiposity (measured with subscapular ST) compared to those of Caucasian descent; however, significant differences in FM was found only in boys. Conversely, Singh et al. [278] reported, there was no difference in FM estimated from anthropometric measurements between African-American (n = 104) and Caucasian (n = 274) infants. However, there is evidence that racial differences in body composition found at birth may carry forward during early infancy. For example, compared to non-Hispanic White infants, Hispanic infants had higher FM and %FM while infants of Black origin had lower FM and %FM at 5 months of age [50].

2.3.2 Infant and postnatal factors

2.3.2.1 Infant sex

Sex differences in body composition have been recognised throughout life, with females having greater total adipose tissue and less lean mass/FFM, than males. These differences are primarily attributed to sex steroid hormones (testosterone and estrogen) and intensify after puberty. However, sexual dimorphism in body composition has been observed well before puberty and tracked back to childhood and even to birth. Testosterone enhances the synthesis of protein and thereby increases FFM [279]. Testosterone production by the testes in male infants starts during foetal life, and this was assumed to be the reason for sex differences in body composition at birth, found in some studies [44, 280, 281]; however, others [282, 283] have reported no differences at birth. Additionally, the sex differences at birth were found to be dependent on gestational age; among full-term infants, females had higher FM than males (11.1 ± 3.7 vs 9.0 ± 3.3 %; p = 0.047), but the difference was not significant amongst preterm infants [284]. The concentration of testosterone in male infants increases considerably (near to concentrations found in adult men) during the early postnatal time, peaking around 6-8 weeks of age and culminating by 4-5 months of age [279]. This testosterone surge in the "mini-puberty" period of infancy is shown to be associated with linear growth velocity from birth to 6 months of age (n = 18570), with the length of male infants increasing 2-4 cm per year more than female infants, and such differences in linear growth velocity have not been found after 6 months of life [285]. A definitive effect of "mini-puberty" on infant body composition has not been discovered; however, Davis et al. demonstrated that, at 5 months of age, male infants had greater FFM and lesser %FM, compared to female infants- changes expected from an elevated testosterone level [286].

2.3.2.2 Infant feeding practices

Infant feeding mode is a crucial determinant of postnatal growth and body composition. Breast milk is considered as the optimal source of nutrition for infants because it confers a number of benefits to the infant, such as transfer of immunity from mother (mainly via colostrum), improved cognitive development, and establishment of a healthy intestinal microbiome [287-289]. Further, breastfeeding is known to protect against later life obesity and several diseases, including diabetes, asthma and inflammatory bowel disease [290-292]. The WHO recommends that infants should be exclusively breastfed for at least up to 6 months [293]; however, globally, less than 40% of infants are exclusively breastfed during the first 6 months of life, even though the maternal medical conditions that prevent breastfeeding are not common [294].

Growth trajectories of infants differ between breastfed and formula-fed infants, with the latter having 400-600 g more weight by 1 year of age [295]. A meta-analysis by Gale et al. [296]

revealed that, compared to breastfed infants, formula-fed infants had lower FM at 3, 4 and 6 months, but this trend reversed by 12 months. Additionally, FFM was higher in formula-fed infants throughout the first year of life than their counterparts [296]. The authors explained that the higher level of leptin (appetite-regulating hormone) found in breast-fed infants during <4 months of age but not later in infancy could be one reason for this difference. Further, formula milk contains more protein than breast milk, resulting in greater accretion of lean mass/FFM. This higher adiposity in breastfed infants during pre-weaning age (~6 months) has been explained as an evolutionary adaptation to store energy to support the infant during the unstable weaning period. The reversal of this trend by 12 months, with formula-fed infants having more FM, suggests that their metabolism may have been programmed to catch up with the FM deficit. It is possible that this programming persists through childhood to adulthood, making formula-fed individuals end up with obesity [296]. On the contrary, several contemporary studies [297-299] have demonstrated no difference in FM or %FM between breastfed and formula-fed infants up to 7 months of age. They argued that the findings of the previous systematic review [296] might be due to publication bias and the inclusion of small studies with large effect sizes. In spite of this, greater FFM (~200-300 g during 4-6 months of age) in formula-fed infants has been consistent in the literature, signifying excess gain of lean mass rather than adipose tissue may be on the causal pathway of early life formula-feeding and later life obesity.

The introduction of complementary foods to infants is recommended from 6 months onwards [293]. The association between age at which solids were introduced and infants BMI is found to be U-shaped, with the introduction of solids at 5-6 months being optimal for a normal BMI at one year of age [300]. Early (<4 months) or late (>7 months) introduction of solids has been associated with an increased likelihood of childhood overweight/obesity [301]. Even though the timing of complementary foods may impact overall growth, the limited studies on its effect on body composition parameters (%FM, %FFM) have failed to identify any association at 5 months [50] or 3 years of age [302].

2.3.2.3 Infant sleep

Curtailed sleep is identified as one of the modifiable risk factors for childhood obesity [303]. A previous meta-analysis of 19 cross-sectional studies published before 2008 (30002 participants) showed an association between shorter sleep duration and overweight/obesity during childhood but failed to reveal its causal link [304]. Later, several meta-analyses of longitudinal studies [305-309] identified that short sleep increases the risk of later overweight/obesity during early childhood, mid-childhood, and adolescence. Further, sleep patterns and chronotypes have been linked to obesity risk [310]. Research on the mechanisms underlying the sleep-obesity association have mostly focussed on changes in hormones such as leptin and ghrelin that result in increased

appetite [311]. However, these biological pathways are understudied in children and the existing limited research shows conflicting results [312, 313]. More recent studies suggest that behavioural pathways play a more important role in moderating weight gain related to sleep restriction in children. Such eating behaviours include increased energy intake, late-night eating, emotional eating, and eating in response to external cues [310]. Nevertheless, very little is known about sleep pattern-associated behavioural changes in infancy and the risk of obesity. Moreover, the previous meta-analyses that investigated the effect of sleep duration on risk of obesity [304-309] have used BMI as a proxy for body composition. A recent systematic review [314] concluded that there is not enough evidence for an association between short sleep duration during early infancy (<2 years) and increased %FM, FMI or ST in later childhood. Hence, current understanding on the role of sleep patterns during very early childhood on risk of excessive adiposity remains inconclusive.

2.4 CONCLUSION

Maternal and postnatal exposures during early life may mediate the programming of obesity and its related diseases in later life. There has been a growing interest in infant body composition assessment as a useful research and diagnostic tool for early identification of future obesity risk. Several pre- and postnatal factors may alter the body composition during infancy; however, significant variations exist in the literature and studies that have investigated these relationships are mostly cross-sectional. Moreover, of the technologies available for quantifying body composition in children, ADP is preferred by researchers; however, it has not been validated in infants aged 6 months to 2 years. Hence, a research gap exists regarding the comparability of measurements obtained using ADP with other alternative body composition methods suitable for this age group.

2.5 SETTING OF THE PHD STUDY

According to currently available data (2017-2018), across all states and territories in Australia, Tasmania reports the highest combined prevalence of overweight and obesity among adults (70.9%) and children 2-17 years (28.7%) [6]. These alarming figures indicate that Tasmania needs urgent action to reverse current obesity trends, and interventions should be started from early life (prenatal period or infancy) when the programming of subsequent health occurs.

Prior to any intervention, it is important to objectively evaluate the current health status and recent trends of the targeted population, through observational studies. So far, few studies [315-317] have investigated the effect of maternal factors on the growth/health of Tasmanian infants, and these are limited to birth [315-317], the Aboriginal population [315] or crude measurements such as weight and skinfolds [315-317]. Therefore, exploring the current growth patterns in Tasmanian infants with longitudinal assessments of body composition and an examination of how maternal and postnatal factors contribute to observed trends, would benefit future health policy decisions.

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CHAPTER 3 : BIRTHWEIGHT TRENDS AND ASSOCIATED MATERNAL CHARACTERISTICS IN TASMANIA

3.1 ABSTRACT

Background: Birthweight is the most widely used index to predict infant mortality and morbidity and is a crucial determinant of adult diseases. Both LBW and HBW have been associated with obesity during childhood and adulthood. Tasmania records the highest rates of obesity among children and adults in the country. However, secular trends in birthweight and association with maternal factors have not been explored.

Aim(s): To determine recent trends in birthweight and maternal characteristics in Tasmania and associations between birthweight outcomes and maternal factors.

Methods: In this retrospective cohort study, perinatal data (2005-2018) of live-born singletons, and their mothers linked by the Tasmanian Data Linkage Unit, were analysed. The main outcome measures were mean birthweight, LBW (birthweight <2500 g), HBW (birthweight >4000 g), and their association with maternal factors explored using regression analysis.

Results: Data of 81700 babies (51.3% male) were included, an average of 5836 births per year. Over the 14 years, mean birthweight (3425 g to 3359 g) and the proportion of HBW decreased (14.2% to 11.0%), while the proportion of LBW increased (4.8% to 6.5%). A downward shift in gestation length distribution, along with increased rates of mothers with caesarean delivery, hypertensive disorders, age >35 years, indigenous or non-Caucasian ethnicity, and assisted conception, contributed to this trend. Rising rates of pre-pregnancy obesity and GDM, factors known to increase birthweight, and the marked reduction in smoking, a key factor that reduces birthweight, did not explain the observed trend.

Conclusion(s): Birthweight in Tasmania declined between 2005 and 2018, along with increases in LBW and decreases in HBW. Decreases in gestation length and changes in maternal characteristics over the period partly explained the observed trend in infant birthweight. Future studies should consider other maternal factors potentially contributing to these trends, including GWG and glycaemic control in diabetic mothers.

3.2 INTRODUCTION

Birthweight is the most widely used index to determine infant mortality and morbidity, childhood developmental problems and also predict health and disease in adulthood [92]. According to the WHO, infants weighing less than 2500 g at birth are with LBW, and those who weigh over 4000 g have HBW, irrespective of their gestational age [26]. LBW infants are at 40 times higher risk of mortality than infants with normal birthweight (NBW) and may have cognitive deficits, motor delays, cerebral palsy, and other behavioural and psychological problems [318, 319]. HBW, on the other hand, may result in shoulder dystocia, brachial plexus injury, neonatal hyperbilirubinaemia and hypoglycaemia, and postpartum haemorrhage [320, 321]. In addition, individuals born with LBW and HBW are at high risk of obesity during later life [23-25]. Although the precise mechanisms for these associations are yet to be elucidated, the role of elevated leptin during the critical periods of growth on the risk of later life obesity has been suggested. Infants born with HBW have high leptin levels at birth, while in contrast, infants with LBW display high leptin levels during "catch-up" growth [24].

Several maternal demographic, health, nutrition and lifestyle factors during the prenatal period have been related to infant birthweight. Maternal smoking, alcohol consumption, low socioeconomic status, hypertensive disorders, and advanced maternal age (>35 years) are known causes of LBW [322], whereas high ppBMI, excess GWG and GDM are mainly associated with HBW [323]. As most of these factors are modifiable, investigating their associations at the population level is useful to inform public health measures to prevent LBW and HBW.

Birthweight outcomes vary according to the population studied [29]. During 1980-2000, the mean birthweight and proportions of HBW increased in many developed countries [324, 325], including some parts of Australia [326, 327], and this was attributed to the high prevalence of pre-pregnancy obesity and GDM in mothers. Data from the United States in the early 2000s suggested a reversal of the previous upward trend, mainly due to obstetric intervention mediated reductions in gestational length [328]. However, in the Australian context, one recent study [323] from the Northern Territory reported that birthweight is still on an upward trajectory.

Of all the states in Australia, Tasmania, the smallest state with a population of ~0.5 million, records the highest rates of overweight/obesity in children and adults and people living in low socioeconomic areas, both important determinants of birthweight [7, 30]. To the best of our knowledge, no study has investigated the trends in birthweight and associated maternal factors in Tasmania. In this study, we explored: 1) birthweight trends in live singleton infants in Tasmania from 2005 to 2018, 2) changes in maternal characteristics over this period, and 3) associations of maternal characteristics with birthweight outcomes. A better understanding of

recent trends in birthweight and associated maternal factors will inform health care policy setting and preventative health strategies to improve population health.

3.3 METHODS

3.3.1 Data source and study cohort

The Tasmanian Perinatal Data Collection, managed by the Department of Health, consists of demographic and health information on mothers and infants across the state. All singleton live births from 1st January 2005 to 31st December 2018, with at least 20 weeks of gestational age and weighing 400-6000 g at birth, were used in the current analysis.

3.3.2 Exposures

The continuous exposure variables (also modified as categorical for logistic regression) were: maternal age, the difference between mother's date of birth and date of delivery (<18, 18-35 or >35 years); parity, number of previous pregnancies that have resulted in a live birth/stillbirth, including the current pregnancy (primiparous vs multiparous); maternal ppBMI (underweight: <18.5, normal: 18.5-25, overweight: 25-30, and obese: >30 kg m^{-2}); socioeconomic status, based on the area of residence of the mother using the Index of Relative Advantage and Disadvantage (IRSAD) decile rankings within the state (1-3: low, 4-7: medium, or 8-10: high); gestation length (<37 weeks: preterm vs ≥37 weeks: term). Categorical exposure variables were: indigenous status: non-indigenous vs indigenous (Aboriginal or Torres Strait Islander); mother's country of birth: Australia vs overseas-born; diabetes status: non-diabetic, pre-existing diabetes (type 1 and type 2) or GDM; hypertensive disorders: none, hypertension or pre-eclampsia; smoking/alcohol consumption/drug use during pregnancy: no vs yes; type of conception: spontaneous vs assisted; mode of delivery: vaginal, emergency caesarean or elective caesarean; and infant sex: female vs male.

3.3.3 Outcomes

Infants' first weight obtained after birth was the primary outcome variable. Mean birthweight and proportion of LBW (<2500 g), NBW (2500 to 4000 g) and HBW (>4000 g) was calculated for each year.

3.3.4 Statistical analysis

Descriptive statistics included mean (standard deviation) for normal continuous variables, median (interquartile range) for skewed continuous variables, and count (percentage) for categorical variables. Birthweight z-scores based on infant sex and gestational age were

calculated using the INTERGROWTH-21st International Newborn Size at Birth Standards application [329]. Normal distribution of the birthweight and gestation length spectrums in 2005 and 2018 were compared, and associations between maternal prenatal variables and mean birthweight were explored using multiple linear regression. The relationship of maternal exposure variables to the two abnormal birthweight categories (LBW and HBW) was investigated with multinominal logistic regression, using NBW as the reference group. Separate models were built using the same explanatory variables in two data sets, i.e., 2005-2011 (Model 1) and 2012-2018 (Model 2). These models were adjusted for infant sex. Model 3 was set as Model 2 further adjusted for ppBMI. Outcomes of Model 3 were used to identify maternal determinants of birthweight outcomes as it contains effects adjusted for ppBMI. All analyses were conducted using R statistical software (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria) [330].

3.3.5 Missing data

Of the total 81700 infants, the proportions with missing data for the variables considered in all the models were: maternal age (2.4%), indigenous status (1.3%), country-born (0.3%), parity (0.2%), IRSAD ranking (0.5%), diabetes status (2.3%), hypertensive disorders (1.1%), smoking (2.8%), alcohol consumption (3.2%), illegal drug use (1.6%), type of conception (1.1%), and these were excluded from the analysis.

3.3.6 Ethics

The study was approved by the Human Research Ethics Committee, Tasmania (Approval No. H0020469).

3.4 RESULTS

3.4.1 Study cohort

From 2005 to 2018, 84338 deliveries were recorded, and data of live singletons (n = 81700, 51.3% male) were included in the analysis (Figure 3.1). The number of live singleton births per year ranged between 5385 (in 2018) and 6182 (in 2008).

3.4.2 Trends in birthweight

From 2005 to 2018, mean birthweight decreased by 66 g (3425 g to 3359 g, $p < 0.001$). Specifically, mean birthweight fluctuated between 2005-2009 and markedly declined after 2010 (Figure 3.2). Based on the INTERGROWTH-21st newborn size standards, the mean birthweight z-scores of Tasmanian infants ranged from 0.520 (in 2015) to 0.607 (in 2009). These positive z-scores indicated that, on average, the birthweights of Tasmanian infants were greater compared to the reference infant population (Figure 3.3). Moreover, there was a disproportionate downward shift in birthweight distributions between 2005 and 2018 (Figure 3.4). The quantiles at 0.1, 0.25, 0.5, 0.75, and 0.90 decreased by 85 g, 54 g, 30 g, 62 g and 84 g, respectively, with greater drops at both ends of the spectrum. The percentage of LBW infants increased from 4.8% to 6.5%, attributable to the increase (2.8% to 4.5%) in the percentage of preterm deliveries with LBW. The percentage of HBW infants decreased from 14.2% to 11.0%, with a reduction (14.2% to 10.9%) in term deliveries with HBW (Figure 3.5).

3.4.3 Trends in maternal characteristics

Pronounced decreases were observed in the percentages of pregnant women who smoked (27.4% to 16.6%), consumed alcohol (18.2% to 1.9%) and used illegal drugs (4.0% to 1.9%; Figure 3.6). The proportions of pregnant women with low socioeconomic status (30.4% to 28.6%) and age <18 years (4.1% to 1.6%) also decreased. In contrast, the percentage of women diagnosed with GDM (1.7% in 2005 to 12.3% in 2018) and pre-pregnancy overweight/obesity (40.5% in 2013 to 48.8% in 2018) rapidly increased. Increases were also observed in the proportion of women with hypertensive disorders (5.9% to 6.4%), assisted conception (2.1% to 5.7%), age >35 (12.1% to 14.4%) and mean maternal age (28.5 to 29.4 years). The ethnic composition of women also changed; overseas-born mothers more than doubled (6.1% vs 13.4%), with a 1.5-fold increase in indigenous mothers (3.7% to 5.7%). The proportion of mothers who underwent caesarean section rose (26.2% to 33.8%), with increases in both emergency (12.6% to 17.4%) and elective (13.6% to 16.4%) caesareans. In addition, a downward shift in the bimodal gestation length spectrum was also observed (Figure 3.7). In 2005, the main peak appeared just above 40 weeks with a

lower peak around 38.5 weeks. In contrast, in 2018, two peaks were at around 39 and 38 weeks, though there was no clear separation.

3.4.4 Associations between birthweight outcomes and maternal characteristics

3.4.4.1 Mean birthweight

When effect sizes were sorted by descending order, smoking (effect size: -221 g; 95% CI: -233 to -208 g), pre-eclampsia (-139 g; -171 to -107 g), illegal drug use (-99.2 g; -129 to -69.3 g), non-Caucasian ethnicity (-92.7 g; -107 to -78.0 g), alcohol consumption (-58.4 g; -82.3 to -34.5g), hypertension (-36.9 g; -60.3 to -13.5 g), indigenous ethnicity (-29.3 g; -49.6 to -9.06 g), and maternal age (-2.29 g; -3.22 to -1.36 g) were negatively associated with mean birthweight (Figure 3.8). In contrast, pre-existing diabetes (311 g; 260 to 363 g), gestation length (197 g; 194 to 199 g), elective caesarean (89.6 g; 76.3 to 103 g), parity (46.2 g; 41.9 to 50.5 g), GDM (19.3 g; 2.45 to 36.1 g), and ppBMI (10.3 g; 9.59 to 11.1 g) were positively associated with mean birthweight. The models only explained part of the variation in birthweight (R^2 for Model 1, 2, and 3 of linear regression were 0.41, 0.49 and 0.50, respectively).

3.4.4.2 LBW and HBW

Factors associated with LBW, were, in descending order of the strength of association, pre-eclampsia (odds ratio [OR]: 2.99; 95% CI: 2.96 to 3.02), smoking (OR: 2.87; 2.59 to 3.18), underweight ppBMI (OR: 1.75; 1.73 to 1.77), using illegal drugs (OR: 1.73; 1.69 to 1.76), emergency caesarean (OR: 1.72; 1.53 to 1.94), low socioeconomic status (OR: 1.47; 1.37 to 1.57), overseas-born (OR: 1.42; 1.37 to 1.47), elective caesarean (OR: 1.38; 1.31 to 1.45), middle socioeconomic status (OR: 1.33; 1.23 to 1.44), hypertension (OR: 1.25; 1.24 to 1.26), indigenous-ethnicity (OR: 1.12; 1.10 to 1.13), assisted conception (OR: 1.08; 1.07 to 1.10) and age >35 years (OR: 1.06; 1.02, 1.09) (Figure 3.9). Conversely, multiparity (OR: 1.82; 1.68 to 1.97), obese ppBMI (OR: 1.79; 1.65 to 1.94), pre-existing diabetes (OR: 1.71; 1.70 to 1.71), emergency caesarean (OR: 1.64; 1.49 to 1.80), overweight ppBMI (OR: 1.45; 1.34 to 1.58), and age <18 years (OR: 1.24; 1.23 to 1.25) increased the probability of delivering an infant with HBW. Emergency caesarean section was associated with both LBW and HBW. Remarkably, GDM was not a risk factor for HBW, but infants exposed to GDM had a slightly higher chance of having LBW (OR: 1.03; 1.01 to 1.05). These factors partially explained the variation in the likelihood of having an infant with LBW or HBW (R^2 for Model 1, 2, and 3 of logistic regression were 0.37, 0.44, and 0.54, respectively).

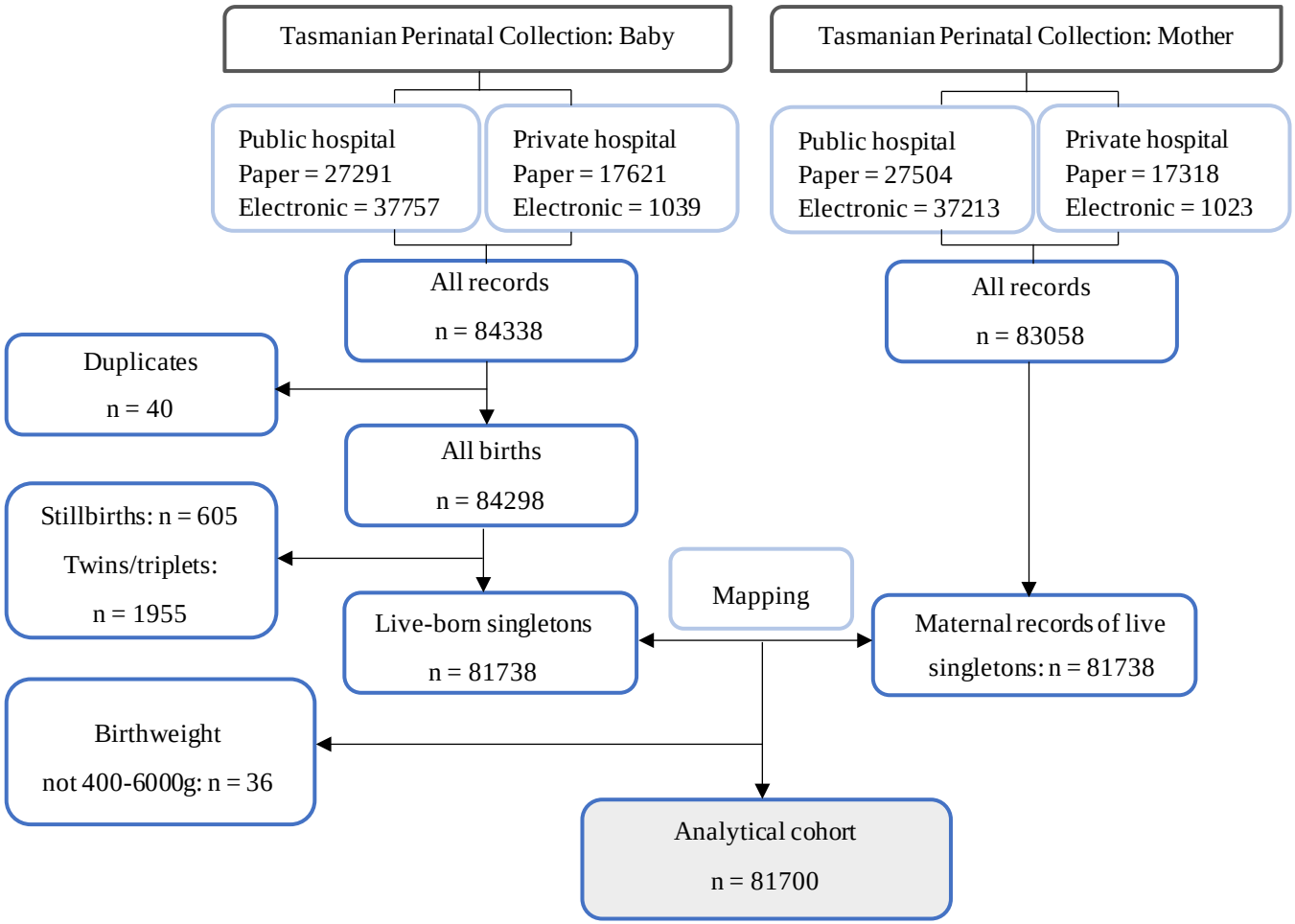

Figure 3.1 Selection of the analytical cohort from Tasmanian Perinatal Data Collection 2005-2018.

Data recorded in the Tasmanian Perinatal Collection are 2 types: 'Paper': data were collected in paper forms and transcribed later in the electronic perinatal database system; 'Electronic': data were directly entered in the electronic perinatal database system.

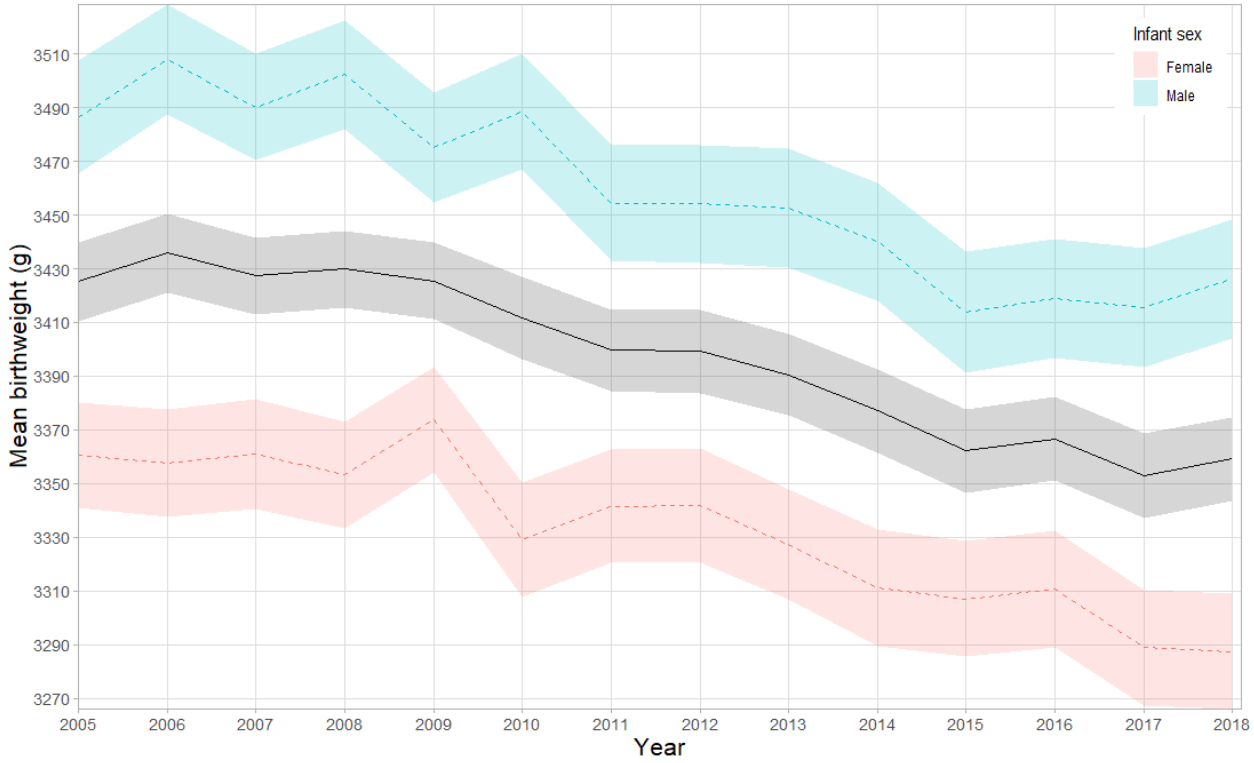

Figure 3.2 Mean birthweight for live-born singletons in Tasmania, 2005-2018.

Middle solid line represents the weight of all infants and dashed lines show the weight of male and female infants; shaded area shows 95% Confidence Interval.

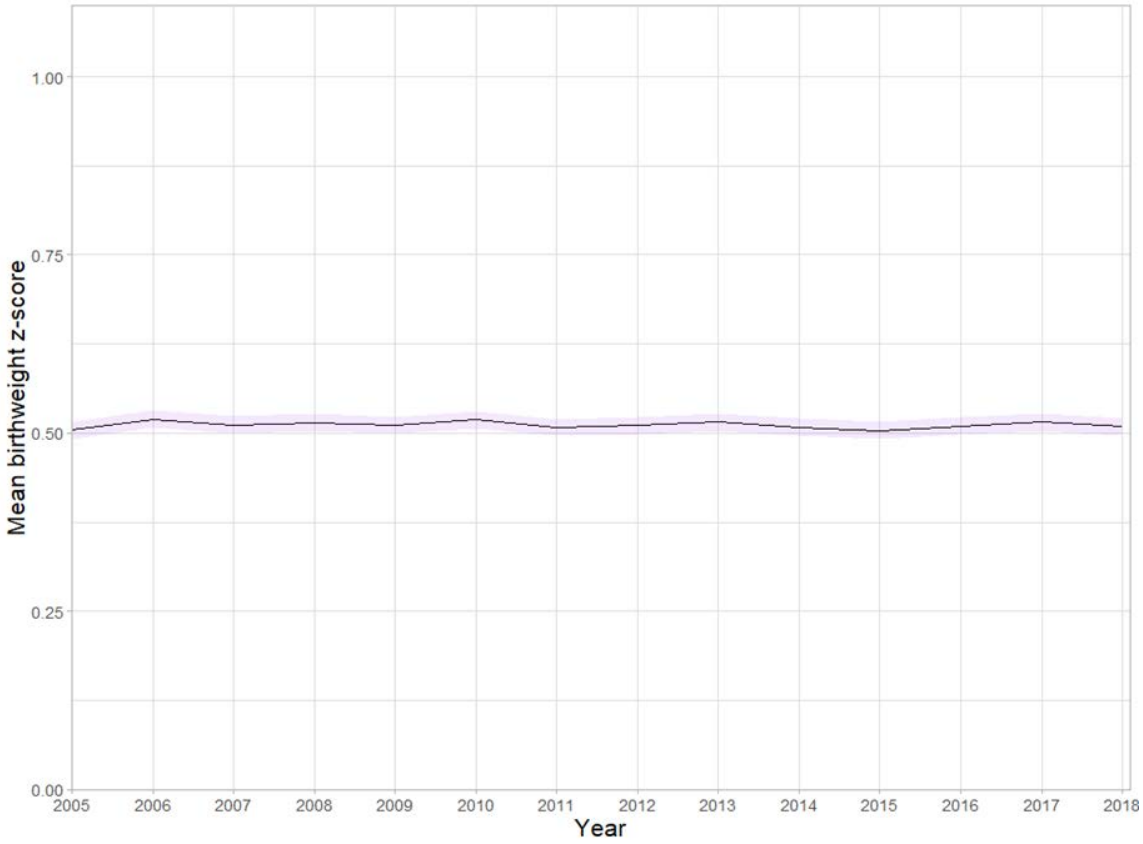

Figure 3.3 Mean birthweight z-scores for live-born singletons in Tasmania, 2005-2018.

Middle solid line represents the mean of birthweight z-scores adjusted for infant sex and gestational age; Purple shaded area shows 95% Confidence Interval.

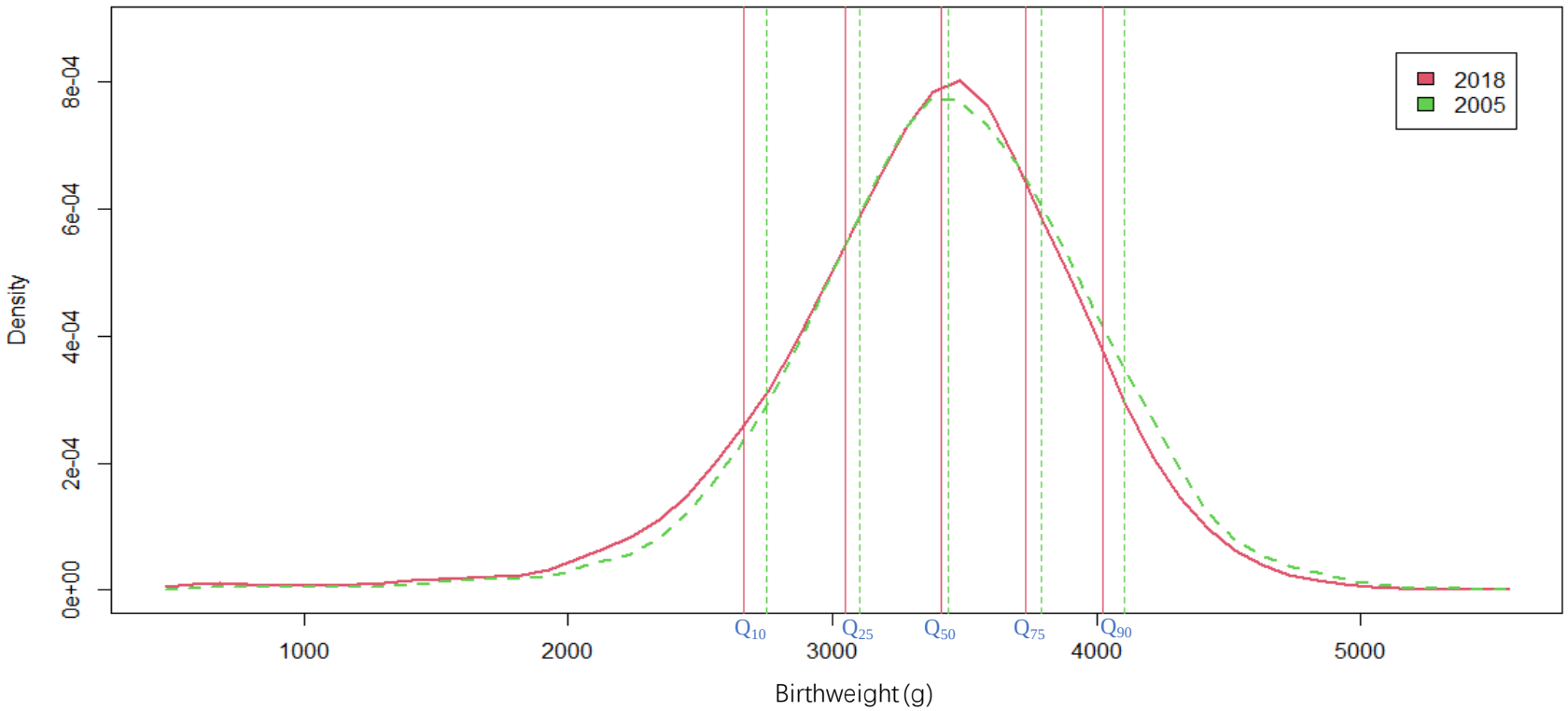

Figure 3.4 Birthweight distribution in live singletons in Tasmania, 2005 and 2018.

Curves show Kernel Density Estimation by year, where the area under the curve is 1, and the probability of a value being between two values of the x-axis is the area under the curve between those two points. Straight lines show quantiles at 0.10 (Q_{10}), 0.25 (Q_{25}), 0.50 (Q_{50}), 0.75 (Q_{75}) and 0.90 (Q_{90}).

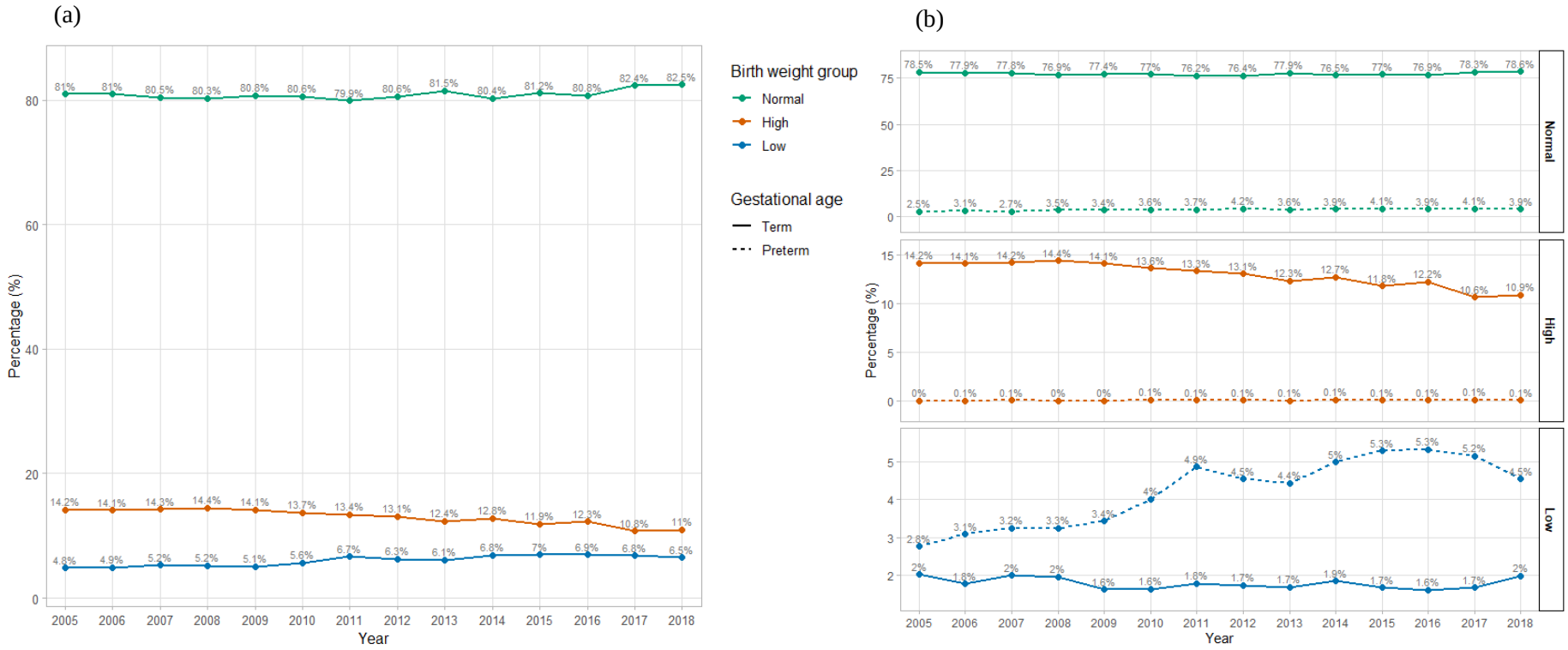

Figure 3.5 Prevalence of NBW, LBW and HBW in live-born singletons in Tasmania 2005-2018.

All infants (a); infants grouped by gestational age (b).

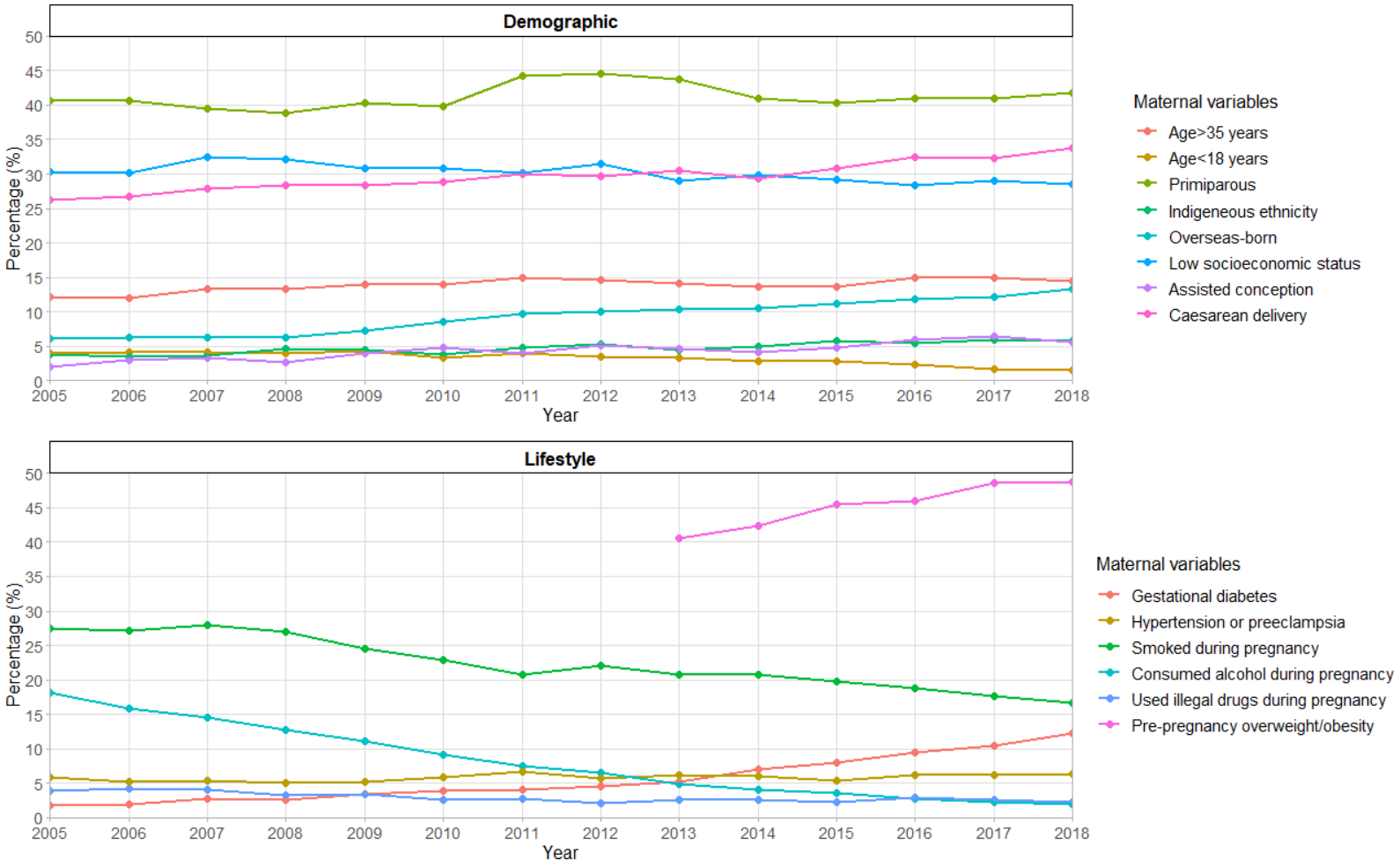

Figure 3.6 Prevalence of risk factors of LBW and HBW in mothers of live singleton infants born in Tasmania, 2005-2018.

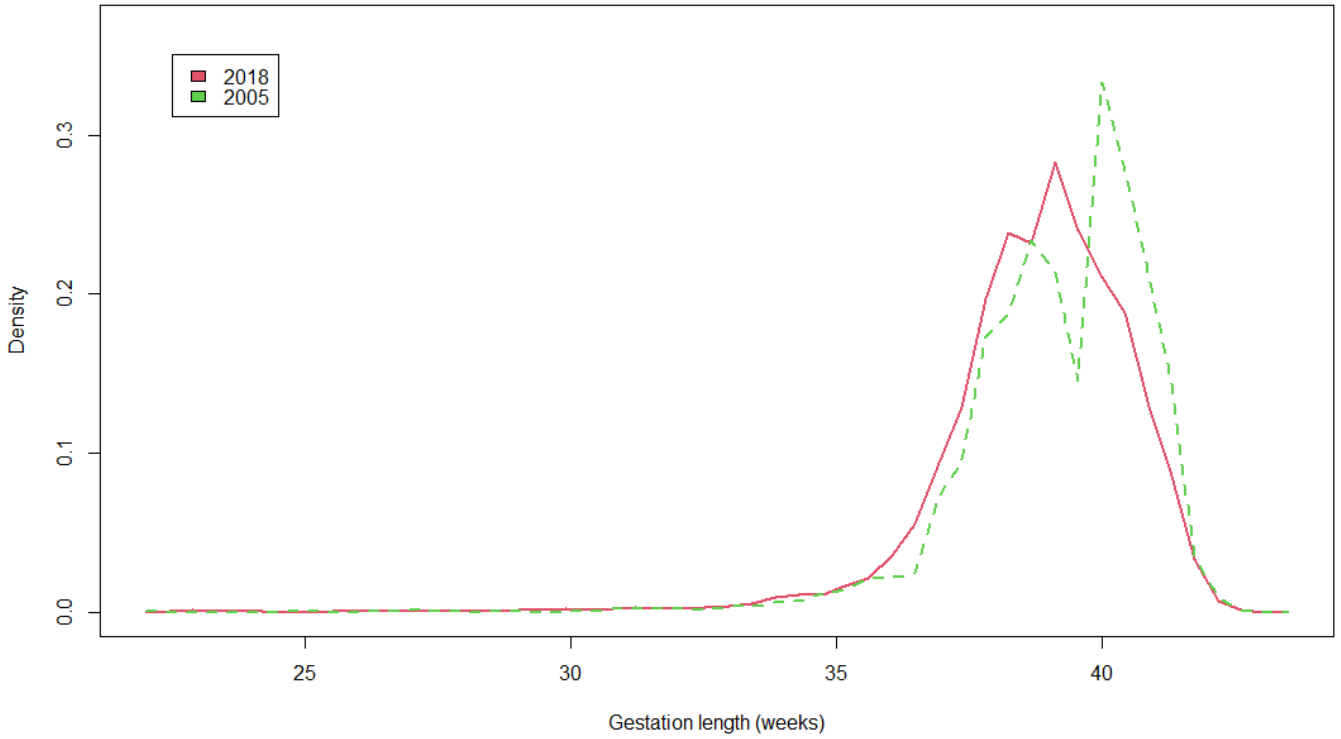

Figure 3.7 Gestation length distribution in live singletons in Tasmania, 2005 and 2018.

Curves show Kernel Density Estimation by year, where the area under the curve is 1, and the probability of a value being between two values of the x-axis is the area under the curve between those two points.

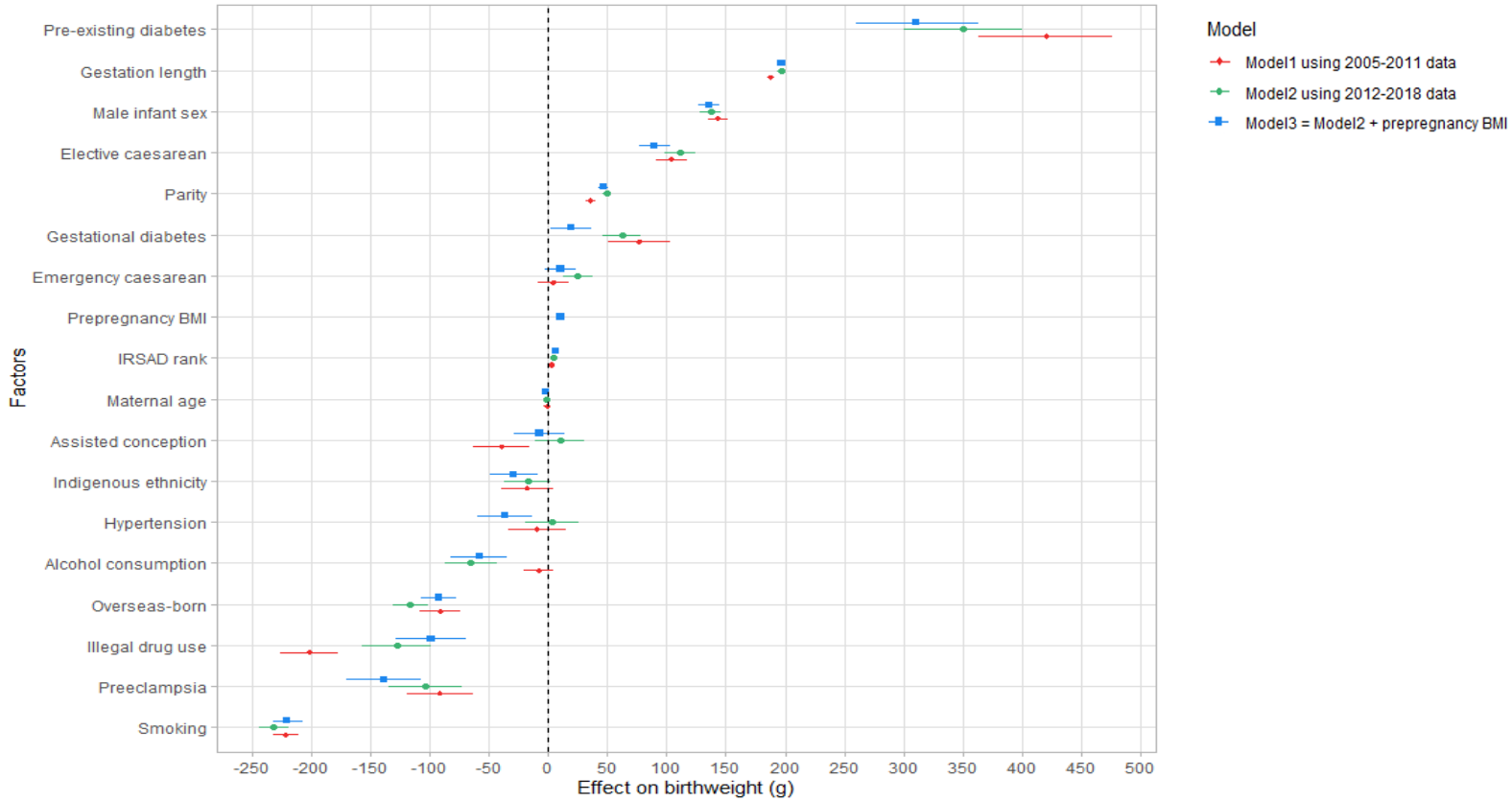

Figure 3.8 Effects of maternal factors on infant birthweight.

Estimates were calculated using linear regression modelling for birthweight in grams; whiskers show 95% CI; the broken vertical line is the line of no effect; BMI: body mass index; IRSAD: Index of Relative Socioeconomic Advantage and Disadvantage; Models are adjusted for year of birth.

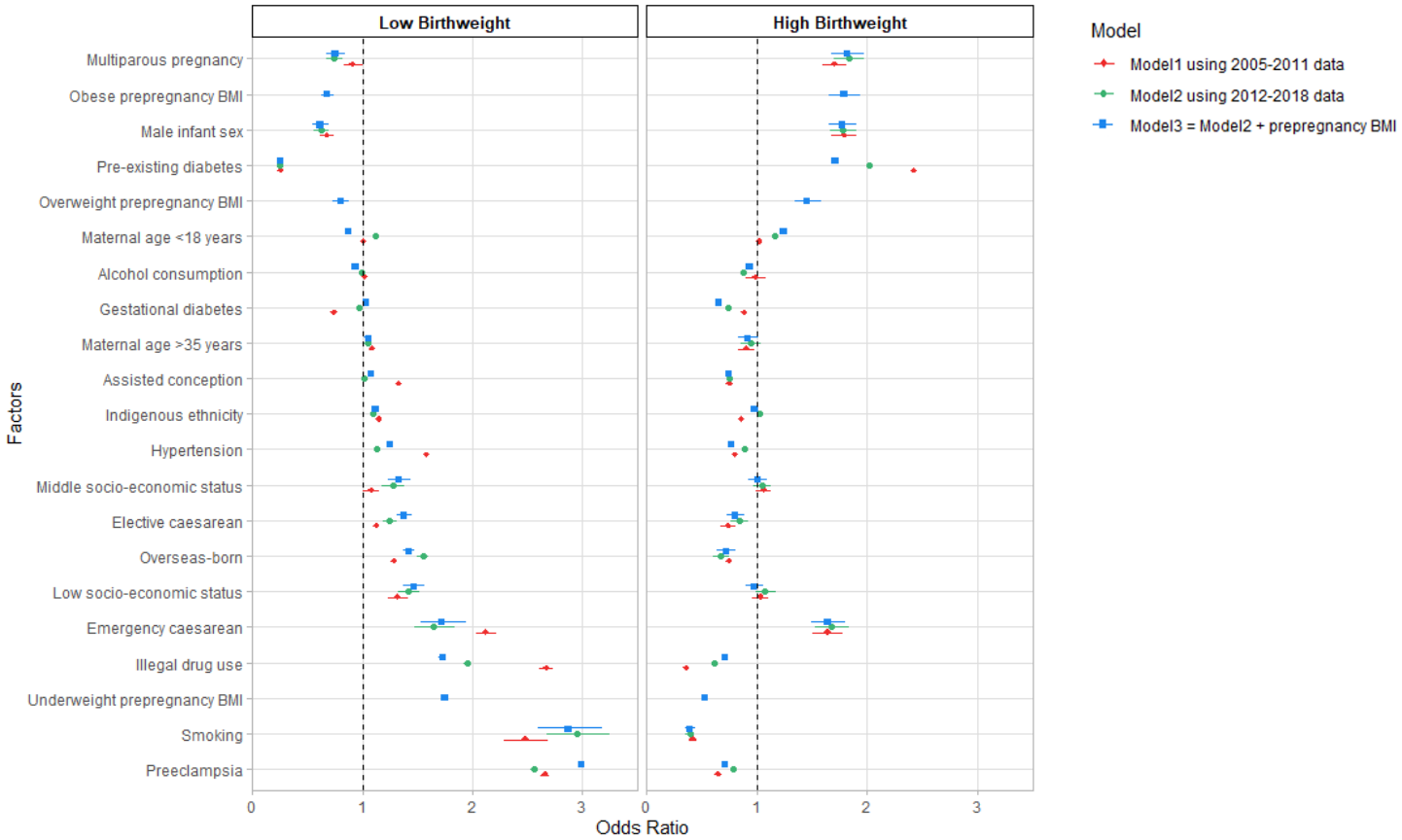

Figure 3.9 Effects of maternal factors on the odds ratio of LBW and HBW.

Estimates were calculated using multinominal regression modelling for birthweight categorised as low (<2500 g) and high (>4000 g); whiskers show 95% CI; the broken vertical line is the line of no effect (odds ratio = 1); BMI: body mass index; Models are adjusted for gestation length (<37 weeks vs ≥37 weeks) and year of birth; Odds ratio (95%CI) for gestation length: <37 weeks in Models 1, 2 and 3 were 38.1 (35.4 to 41.0), 50.7 (45.3 to 56.7) and 50.3 (44.7 to 56.6), respectively, and not shown in the figure.

3.5 DISCUSSION

From 2005 to 2018, the mean birthweight of live-born singletons in Tasmania decreased by 66 g (3425 g to 3359 g). This trend was accompanied by a decrease in the proportion of HBW (14.2% to 11.0%) and an increase in the proportion of LBW (4.8% to 6.5%) infants. Increases in the prevalence of hypertensive disorders, caesarean delivery, maternal age >35 years, assisted conception, indigenous and overseas-born mothers, along with a downward shift in gestation length distribution, contributed to this trend. Interestingly, the rise in factors known to increase birthweight, e.g., pre-pregnancy obesity and GDM, and marked reductions in factors that decrease birthweight, e.g., smoking and alcohol consumption, did not prevent the trend towards smaller babies.

More recent studies have noted a reversal in the upward birthweight trend observed in many countries during the mid to late 20th century. In a study in Queensland [326], birthweight increased steadily (1.9 g per year) from 1988 to 2000, and from 2001 to 2005, there was a slight decrease. Comparing the Australian Institute of Health and Welfare reports 2005 [331] and 2018 [332], the decline in birthweight from the early 2000s seems to be Australia-wide, except for the Northern Territory (3246 g to 3265 g). The findings of our study are also in line with those from the United States where birthweight decreased by 27 g (3410 g to 3383 g) in term singletons between 2000-2008 [333] and by 68 g (3315 g to 3247 g) in first-birth singletons between 1990-2013 [328]. These small reductions in mean birthweight may seem insignificant, especially because it is still within and towards the mid-point of the normal birthweight range (2500 g to 4000 g). However, given the strong evidence for early life growth and later-life health [82, 89, 334], these trends may have long term consequences for the health of a population. A study by Imai et al. showed that the mean birthweight of women born in Iceland dropped by 94 g (3732 g to 3638 g) from 1925 to 1934 due to the impact of the 'Great Depression' and those who were with lighter birthweight had increased risk of obesity and hyperglycaemia during adulthood [335].

The rising rates of pre-pregnancy obesity and GDM, and substantial reductions in maternal smoking, alcohol consumption and illegal drug use observed in the current study, are mirrored in other developed settings. For example, in the United States, the prevalence of pre-pregnancy obesity increased by 11.1% (26.1% to 29.0%) in only 3 years (2016 to 2019) [336], whereas the prevalence of smoking decreased from 9.2% in 2010 to 6.9% in 2017 [337] Among Australian states, Tasmania in 2018 had the highest rate of pre-pregnancy obesity (26.4%) and hypertensive disorders (8.1%) and the second-highest rate of maternal smoking (17.2% vs 24.9% in Northern Territory). The age-standardised incidence of GDM (for all live births 2016-17) in Australia was 15%, with both the Australian Capital Territory and the Northern Territory having the highest incidence (17%), and Tasmania the second-lowest with 13.5% [338]. The elevated rates in risk

factors for HBW and LBW highlight the need for better health education in women of childbearing age in Tasmania.

The upward trend of birthweight found in previous studies [323, 324, 326, 327] was due to rising rates of GDM, high ppBMI and excessive GWG, and lower rates of maternal smoking. GDM, high ppBMI and excessive GWG are conditions in which the developing foetus is exposed to overnutrition (excess amounts of glucose and/or fatty acids), and in response, the foetus secretes increased amounts of insulin, which results in higher fat deposits and HBW [195, 213]. On the other hand, maternal smoking may cause reduced birthweight due to direct toxic effects of products of cigarette smoking such as nicotine and carbon monoxide, and confounding effects of poor maternal nutrition in smoking mothers [225-227]. Treatment for GDM, such as dietary intervention and insulin therapy, can significantly reduce mean birthweight and HBW in infants compared to no treatment [214]. Further, while high ppBMI and excessive GWG increase birthweight, the effect of GWG is higher than ppBMI [339]. The fact that Tasmanian infants did not show an upward trend in birthweight, despite increasing rates of GDM and pre-pregnancy obesity suggests that the effects of both conditions may have been controlled to some extent. The drop in mean birthweight was greater from 2010 since the collection of ppBMI data started in Tasmanian Hospitals and screening mothers for GDM increased. We hypothesise that these changes in obstetric care may have led to increased awareness of the detrimental effects of GDM and pre-pregnancy obesity and facilitated diet and lifestyle changes and medication use (for GDM), leading to better control of blood glucose levels and GWG. This may, in turn, have averted excessive growth in some infants and contributed to the downward trend in birthweight.

Several maternal factors may have contributed to the downward trend in birthweight, including increased caesarean deliveries and induced labour [328]. Notably, emergency caesarean delivery was associated with both HBW and LBW, indicating both infant groups were delivered before full gestation, and this explains why there were greater falls in birthweight at lower and upper quantiles. Elective caesareans performed upon maternal request also increased the risk of LBW, highlighting the importance of avoiding elective procedures when there are no obstetric indications. Major reasons cited for maternal preference for caesarean-section over vaginal delivery include fear of childbirth and negative previous birth experiences, thus may be resolved through counselling approaches [340, 341]. Further, increases in the rates of mothers with gestational hypertension and age >35 years were two other contributing factors. Hypertension may restrict foetal growth by reducing uteroplacental blood flow [342], and giving birth after the age of 35 years is associated with pre-eclampsia and preterm delivery [343]. Further, upsurges in the incidence of GDM in Tasmanian mothers also slightly increased the risk of LBW in infants as GDM is a known risk factor for premature birth [344]. Moreover, the ethnic composition of

Tasmanian mothers has changed, with greater numbers of Asian [345] and indigenous [323] mothers, who generally have babies with lower birthweight than White mothers. Finally, different processes used in assisted reproductive technology may increase the risk of LBW; for example, frozen embryo transfer has resulted in a lower risk of LBW compared to fresh embryo transfer [346].

Given that Tasmania currently reports the highest rates of child obesity across Australia, an investigation of recent birthweight trends in Tasmania provides a unique opportunity to understand any contribution from drifts in early growth patterns during recent years to elevated obesity trends. The use of population-wide data minimises selection bias and enhances generalisability of the study findings to the Tasmanian population. As missing data in the data set were very low (<3.2% in all selected variables), the bias introduced by the exclusion of missing data is expected to be small. A missing data rate of 5% or less in a data set has been considered inconsequential [347]. Moreover, we could account for the effect of ppBMI on birthweight trends from 2012-2018, which is not the case in many previous studies [323, 326, 327]. We used the WHO recommended method for classifying infants at risk. Categorisation of infants based on birthweight for age: SGA, AGA or LGA [348] was beyond the scope of this study; however, we have accounted for gestational age in our model constructions. Besides, others have shown that whether born as SGA or AGA, infants with very LBW have complications postnatally [349]. Other limitations of our study are the retrospective nature and lack of data on two important confounding factors, namely, GWG and glycaemic control in GDM mothers. Furthermore, as data on most maternal factors were collected using an interview-based approach, self-reporting bias and social desirability bias may have affected responses. For example, smoking may have been underreported by the mothers because of the inclination to provide socially acceptable responses [350]. However, declines in the rates of undesirable maternal behaviours such as smoking and alcohol consumption have been observed throughout Australia during the study period [331, 332].

Although it is imperative to investigate how birthweight is affected by various maternal exposures, birthweight is only a proxy of an infant's nutritional status; it is incapable of differentiating between various components of body composition, including lean mass, bone mass and FM. Body composition may significantly vary among infants and be linked with the programming of human metabolism [28]. Excess FM accumulation during foetal life has been found to increase the risk of obesity in later childhood and adulthood [13]. In this context, exploring how maternal factors affect infant body composition has gained increased attention in recent years [35, 208, 351]. This has been facilitated by the availability of technology to estimate infant body composition noninvasively, accurately and rapidly, such as ADP [66]. Future studies

would benefit from examining recent trends in infant body composition measurements and their association to maternal exposures.

3.6 CONCLUSION

Birthweight in Tasmania reduced significantly between 2005 and 2018, with a 23% decrease in the rate of HBW and a 35% increase in the rate of LBW. We identified several contributing factors to this decline in birthweight, including some deterioration of the health of pregnant women indicated by increases in the prevalence of hypertensive disorders and GDM, changes in the obstetric practices signified by an increased number of caesarean sections, and assimilation of mothers of different ethnic backgrounds. Further, mothers who smoked, used illegal drugs, were diagnosed with hypertension or pre-eclampsia, were underweight before pregnancy, or of low socio-economic status had a high risk of delivering a baby with LBW. In contrast, the risk of having a HBW baby was higher for women who were overweight/obese before pregnancy and diagnosed with pre-existing diabetes. These findings may assist authorities to understand the underlying causes of the current downward birthweight trend, and plan interventions and increase health care for women who are at increased risk of having LBW and HBW babies. In addition, as our models could only explain approximately 50% of the variation in birthweight outcomes, further investigations are required. Since birthweight is a crude indicator of infant health, gathering objective information on body composition in a representative sample of newborns would provide valuable additional information alongside anthropometric growth data.

The next chapter includes an appreciation of body composition and related pre- and postnatal factors in a select Tasmanian infant sample.

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CHAPTER 4 : DETERMINANTS OF INFANT WEIGHT AND ADIPOSITY ACROSS THE FIRST 6 MONTHS OF LIFE

4.1 ABSTRACT

Background: Excess adiposity in infancy may predispose individuals to obesity later in life. The literature on determinants of adiposity in infants is equivocal.

Aim(s): To investigate pre-pregnancy, pre- and postnatal determinants of infant weight different adiposity indices in, i.e., FM, %FM, FMI and FM/FFM^{p}, from birth to 6 months.

Results: Three hundred and twenty-two, 174 and 109 infants were assessed at birth and 3 and 6 months, respectively. Body composition was measured utilising ADP, and linear mixed-effects regression was used for statistical analysis. Positive associations were observed between gestation length and infant weight and FM, maternal self-reported ppBMI and infant %FM, and parity and infant %FM and FMI at birth. Surprisingly, maternal intake of iron supplements during pregnancy was associated with infant FM, %FM and FMI at 3 months and FM/FFM^{p} at 6 months. Male infants were heavier at all time points and had lower adiposity (evidenced in all indices) at 6 months than female infants. Formula feeding was negatively associated with all adiposity indices at 6 months.

Conclusion(s): Pre-pregnancy and pregnancy factors influence adiposity during early life, and these impacts may not be found when using infant weight as a proxy for adiposity. Any unfavourable impacts of body composition during foetal life may be modulated postnatally via infant feeding practices. Moreover, as these associations are dependent on the adiposity indices used, it is crucial that researchers use conceptually and statistically robust approaches such as FM/FFM^{p}.

4.2 INTRODUCTION

The prevalence of obesity and comorbidities are rising at alarming rates across all age groups worldwide [69]. Growth and development in early life play a pivotal role in determining the risk of obesity over the life course [17]. Programming of the growth trajectory in humans occurs during the first 1000 days of life (from conception to 2 years of age), when developmental plasticity is at its maximum [86]. Across this critical period, insults or inappropriate stimuli can result in metabolic and structural alterations in cells, organs and systems that may be irreversible [87]. For example, excess accumulation of adipose tissue in foetal life or early infancy may predispose individuals to obesity in later childhood and adulthood [13].

Several pre-pregnancy, pre- and postnatal factors have been identified as predictors of adiposity in infants, including maternal pre-pregnancy body mass index (ppBMI), education, low socioeconomic status, smoking and infant feeding mode [50, 57, 352]. Nevertheless, the literature is equivocal on the impacts of some of the factors, and other factors have not been adequately studied. For example, considerable evidence suggests that maternal obesity during conception is associated with increased FM [46, 47], but this relationship was not evident in other studies [48, 49]. Similarly, the positive association between exposure to GDM *in utero* and FM in newborns reported in some studies [39, 63, 353] was not found in others [64, 354, 355]. Additionally, although the use of vitamin and mineral supplementation is common in pregnancy, evidence on how these supplements affect the growth and body composition of infants is scarce [250]. Finally, maternal factors and their effects on infant adiposity have more commonly been studied at a single timepoint, in most cases at birth [51, 52]. As associations between maternal factors and adiposity at birth have been reported to change over the first 5 months of life [50], studying the combined effect of a range of pre-pregnancy, pre- and postnatal factors throughout infancy could provide a better understanding of the modulation of adiposity accretion during this critical period.

Accurate estimation of adiposity in infants was challenging until the development of the ADP PEA POD system, a rapid and non-invasive two-compartment technique with excellent reliability and validity [150]. Weight is used as a proxy for adiposity in infants when resources are limited for assessing body composition. The expression of the outcomes of the two-compartment model, i.e., FM and FFM, as absolute values without adjusting for body size, can compromise its clinical relevance. For example, FM alone cannot elucidate interindividual variability of fatness, nor can it rank individuals in terms of disease risk [56]. Nonetheless, the absolute values of FM have been used as a measure of adiposity in infants [233]. Additionally, different indices derived from FM, including %FM (FM adjusted for total body weight) [57], FMI (FM adjusted for height/length) [58], and FM/FFM^{p} (FM adjusted for FFM) [59], have been used, but their

interrelationships and, whether their determinants are identical, have not been investigated thoroughly.

Identifying the links between pre-pregnancy and prenatal factors and neonatal adiposity could provide valuable insights for optimising maternal health to promote better cardiometabolic outcomes later in life for their offspring. Following up on these associations across infancy, with adjustments for key confounding postnatal factors, may advance the understanding of how long these prenatal influences can last and how any undesirable impacts can be ameliorated postnatally. Moreover, comprehension of associations between these factors and various adiposity indices may inform the selection of appropriate measure(s) for future research. In this longitudinal cohort study, we investigated associations between pre-pregnancy, pre- and postnatal factors and infant weight and different measures of adiposity from birth to 6 months.

4.3 METHODS

4.3.1 Participant recruitment

The Baby-bod study is the Australian arm of a multi-country collaborative project exploring body composition in healthy infants across the first two years of life. Participant recruitment and all assessments in the Baby-bod study were conducted at the Launceston General Hospital, Tasmania, Australia, from September 2017 to October 2019. Inclusion criteria were mothers with a singleton term pregnancy (gestation length at birth between 37^{+0} and 41^{+6} weeks), ≥18 years of age and able to speak and understand English. Exclusion criteria were newborns with congenital anomalies or admitted to the neonatal intensive care unit, mother's inability to negotiate the informed consent process, and difficult birthing experience as judged by a clinician. All eligible mothers were approached within 72 hours of delivery and provided with participant information sheets. Informed written consent was obtained from the mothers (also from the fathers, if present) who agreed to participate in the first assessment. All research procedures and protocols were approved by the Human Research Ethics Committee (Tasmania, reference: H0016117).

4.3.2 Assessment of exposure variables

Maternal demographic information (age, ethnicity, highest education, occupational status, parity), lifestyle characteristics (smoking, routine intake of iron and folic acid throughout pregnancy), diagnosis of GDM, and pre-pregnancy weight were obtained through an interviewer-administered screening questionnaire. Infant feeding pattern in the month prior to each follow-up visit (3 and 6 months) was obtained using a food frequency questionnaire. All questionnaires used in this study were designed for the multi-country project and used at all the study sites.

4.3.3 Anthropometric and body composition assessment of infants

Infant weight, length, head circumference and body composition were recorded at birth and 3 and 6 months after. Nude weight was obtained using a Seca 374 digital baby scale (Seca, Hamburg, Germany) to the nearest 5 g up to 7.5 kg and the nearest 10 g beyond. Crown-to-heel length was measured using a Seca 417 infantometer (Seca, Hamburg, Germany) to the last completed millimetre. A flexible steel tape (Cescorf, Porto Alegre, Brazil) was used to assess head circumference to the last completed millimetre. All measurements were taken in duplicate by two trained research assistants and averaged. When the duplicate readings were out of the study's tolerance ranges, i.e., weight: 50.0 g, length: 7.0 mm, and head circumference: 5 mm, the assessment was repeated, and only measurements within the tolerance were averaged.

Body composition in infants was assessed using the ADP PEA POD system (COSMED USA Inc., Concord, CA, USA; software version 3.5.0) according to the manufacturer's guidelines. PEA POD, a two-compartment approach, uses the gas laws of Boyle and Poisson, and principles of whole-body densitometry. Body density was used to estimate FM, assuming that the density of FM was constant and assigning age and gender-specific FFM density values determined by Fomon et al. [356]. The physical design and the operating procedures of the PEA POD have been described in detail elsewhere [150].

4.3.4 Calculation of infant adiposity indices

FMI was calculated as FM (kg)/length $(m)^2$. %FM was calculated as FM (kg) *100%/ body weight (kg). For calculating FM/FFM^p, first FM and FFM were natural log transformed. Next, log FM was regressed on log FFM to find distinct regression coefficients for each timepoint. These coefficients were used for p to calculate FM/FFM^p, and the derived values were multiplied by 1000 to enhance the readability [59].

4.3.5 Maternal anthropometry

Postdelivery weight was measured at enrolment (within 72 hours of delivery) using a Seca 876 flat scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a Seca 264 digital stationary stadiometer (Seca, Hamburg, Germany) to the nearest 0.1 cm at enrolment or the first follow-up visit in cases when the mother was unable to stand after the delivery. ppBMI was calculated as the mother's self-reported pre-pregnancy weight (kg) divided by height squared (m^2). Maternal net gestational weight gain (nGWG) was calculated as the difference between the mother's postdelivery weight (kg) and self-reported pre-pregnancy weight (kg).

4.3.6 Statistical analysis

Continuous predictors were: maternal age (years), parity, ppBMI (kg/m^2) and nGWG (kg). Categorical predictors were: diagnosis of GDM (yes vs no (reference group)), routine intake of supplemental iron (yes vs no (reference group)) and folic acid (yes vs no (reference group)), smoking (0–3 days (reference group) vs 4–7 days per week) during pregnancy, highest education (up to high school vs university/professional training (reference group)), occupational status (unemployed vs employed (reference group)), infant sex (male vs female (reference group)) and infant feeding mode (exclusive breastfeeding (reference group), partial breastfeeding (both breastmilk and formula milk), and formula feeding). Longitudinal associations of predictor variables and infant weight and adiposity indices were examined with linear mixed-effects (LME) models using backward stepwise regression. LME models take the correlation between repeated measures on the same individual into account and allow for missing data in the outcome measure, assuming that it was missing at random [357]. Moreover, LME modelling requires the presence of all the predictor variables at all the timepoints considered. Consistent with the multicounty study, we collected data on infant feeding only at 3 and 6 months. Thus, separate models were developed considering all 3 timepoints, i.e., birth, 3 months and 6 months (Model 1), and considering only 3 months and 6 months (Model 2). Model 1 included all pre-pregnancy and pregnancy predictor variables considered. Model 2 included all variables in Model 1 plus infant feeding mode and respective adiposity measure at birth. A p-value <0.2 was considered the cut-off inclusion of a predictor variable in the model. Infant sex and gestation length were included in the models independent of their p values. The interactions between maternal factors and infant age at the assessment, i.e., birth (reference level), 3 months and 6 months, were explored to understand whether the associations of maternal prenatal factors changed with infant age. Variance inflation factors (VIFs) were computed to confirm there was no multicollinearity between exposure variables (all <2.0). Residual plots of each model were examined to confirm the assumptions of normality, linearity and homoscedasticity. Cook's distance influence statistics were computed to identify unduly influential observations in the model construction, and the values flagged as being influential were checked for genuineness. All statistical analyses were conducted using R Project for Statistical Computing (version 3.5.3) in R Studio (version 1.1.463, Vienna, Austria) [330]. Statistical significance level was two-tailed and set at $p < 0.05$.

4.4 RESULTS

4.4.1 Participants

Of the 1375 mothers approached, 322 mothers (44.5% primiparous) agreed to participate in the study. Their newborns were assessed at birth, and 191 (58.6%) of them at-tended the 3-month follow-up, and 183 (56.1%) attended the 6-month follow-up (Figure 4.1). ADP PEA POD measurements were available for 174 infants at 3 months and 109 infants at 6 months. Reasons cited for lack of follow-up included lack of time and moving to another area. Seventy-four of the infants who participated in the 6-month follow-up did not have PEA POD measurements due to following reasons: parents chose the alternative body composition technique offered in the study (DD technique, discussed in Chapter 6), parents decided to have only the anthropometric measurements, and finally, some PEA POD measurements failed because of excessive crying by infants when inside the test chamber which triggered an alarm. None of the infants had a body weight >10 kg (the maximum capacity of the PEA POD). Infants with complete data for all variables of interest were included in the regression analyses. Maternal and infant characteristics were similar for the group who commenced the study and those who were included in the final analyses (Table 4.1).

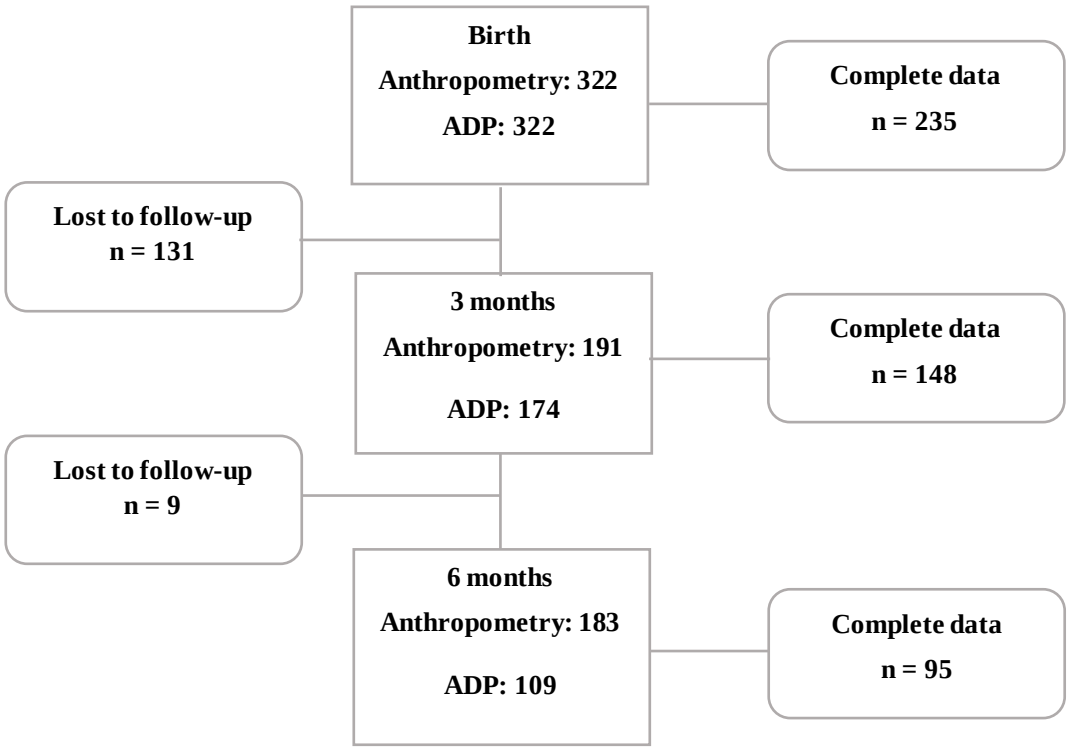


Figure 4.1 The flow of the participants of the Baby-bod study.

ADP: Air Displacement Plethysmography. Anthropometric measurements include weight, length, head circumference of infants. Complete data indicates the number of infants included in the analysis after removing the infants with missing data for maternal variables considered.

Table 4.1 Characteristics of the full-cohort and analytical cohort.

Characteristic	Full cohort	Analytical cohort			
	Birth N = 322	Birth N = 235	3 months N = 148	6 months N = 95	p-value
Infant sex[1]					>0.9
Female	167 (51.9%)	127 (54.0%)	79 (53.4%)	52 (54.7%)	
Male	155 (48.1%)	108 (46.0%)	69 (46.6%)	43 (45.3%)	
Gestation length (weeks)[2]	39.50 (1.14)	39.53 (1.15)	39.61 (1.14)	39.75 (1.16)	0.3
Maternal ethnicity[1]					0.8
Caucasian	294 (91.6%)	217 (92.3%)	139 (93.9%)	89 (93.7%)	
Other	27 (8.4%)	18 (7.7%)	9 (6.1%)	6 (6.3%)	
Unknown	1				
Maternal age[2]	29.87 (5.21)	29.84 (5.26)	30.42 (5.00)	30.63 (4.56)	0.3
Parity[1]					0.9
Primiparous	143 (44.4%)	107 (45.5%)	71 (48.0%)	44 (46.3%)	
Multiparous	179 (55.6%)	128 (54.5%)	77 (52.0%)	51 (53.7%)	
Maternal prenatal BMI (kg/m^2)[3]	25.20 (22.00, 29.70)	25.10 (22.30, 29.80)	25.30 (22.40, 28.85)	25.30 (22.15, 28.40)	0.7
Unknown	25				
Supplemental iron intake during pregnancy[1]					
Yes	232 (79.5%)	180 (76.6%)	112 (75.7%)	73 (76.8%)	>0.9
No	60 (20.5%)	55 (23.4%)	36 (24.3%)	22 (23.2%)	
Unknown	30				
Supplemental folic acid intake during pregnancy[1]					
Yes	177 (64.1%)	141 (60.0%)	93 (62.8%)	60 (63.2%)	0.8
No	99 (30.7%)	94 (40.0%)	55 (37.2%)	35 (36.8%)	
Unknown	46				

[1] n (%); [2]Mean (SD);[3]Median (IQR); significance tests: Pearson's Chi-squared test for categorical variables; Kruskal-Wallis rank-sum test for continuous variables; BMI: body mass index

4.4.2 Baseline characteristics of mothers

The age of mothers at delivery ranged from 18 to 48 years, with a mean ± SD of 29.9 ± 5.2 years. Mothers were predominantly Caucasian (91.3%), and most routinely consumed supplemental iron (79.5%) and supplemental folic acid (64.1%) throughout pregnancy (Table 4.2). Of the 297 mothers who had records of ppBMI, more than half were overweight, and nearly 1 in 10 mothers had been diagnosed with GDM. There were no significant differences in maternal sociodemographic characteristics (age, ethnicity, highest education, occupational status, parity, height, and pre-pregnancy weight) between the full (n = 322) and analytical cohorts (n = 235) (Table 4.1).

Table 4.2 Baseline characteristics of mothers.

Variable (n = 322)	**Missing Values**	**n (%)/Mean (± SD)/ Median (25th and 75th Percentiles)**
Age (years) *	0	29.9 (5.2)
Ethnicity	1	
Caucasian		294 (91.3)
Other		27 (8.4)
Highest education	0	
University/professional training		235 (73.0)
Up to high school		87 (27.0)
Occupational status	0	
Employed		283 (87.9)
Unemployed		39 (12.1)
Parity	1	
Primiparous		143 (44.5)
Multiparous		178 (55.5)
ppBMI (kg/m^2) **	25	25.2 (22.0, 29.7)
Pre-pregnancy weight status	25	
Non-overweight (BMI < 25)		142 (47.8)
Overweight (BMI ≥ 25)		155 (52.2)
nGWG **	8	8.5 (4.2, 12.4)
GDM	0	
No		290 (90.1)
Yes		32 (9.9)
Smoking during pregnancy	0	
0–3 days per week		304 (94.4)
4–7 days per week		18 (5.6)
Intake of supplemental iron	30	
Yes		232 (79.5)
No		60 (20.5)
Intake of supplemental folic acid	46	
Yes		177 (64.1)
No		99 (35.9)
Gestation length (weeks) *	0	39.5 (1.1)

Numbers represent count (%) for categorical variables and mean (SD) *, or median (25th and 75th percentiles) ** for continuous variables; SD: standard deviation; BMI: body mass index; nGWG: net gestational weight gain.

4.4.3 Infant characteristics from birth to 6 months

The weight of infants increased by 80% from birth to 3 months (3283 ± 449.3 g vs 5931 ± 801.3 g), in contrast to a 30% increase from 3 to 6 months (7632 ± 946.2 g at 6 months) (Table 4.3). As expected, there were significant changes in all adiposity measures; greater increases from birth to 3 months and relatively smaller increases from 3 to 6 months (all $P < 0.001$). On average, FM increased by 3-fold from birth to 3 months (353.6 ± 161.0 vs 1412.3 ± 397.8), but the increase was smaller from 3 to 6 months (1859 ± 412.4 g at 6 months). %FM more than doubled during the first 3 months (10.5% ± 3.8% vs 23.7% ± 4.6%); however, the increase was not as pronounced during the subsequent 3-month period (25.4% ± 4.5% at 6 months). A similar trend was observed in FMI (birth: 1.4 ± 0.6; 3 months: 3.9 ± 1.1; 6 months: 4.4 ± 1.0). Conversely, FM/FFM^p displayed dramatic changes corresponding to fluctuations in FM and FFM in 0–6-month-old infants. FM/FFMp increased by approximately 6-fold during the first 3 months (36.0 ± 14.4 at birth vs 251.5 ± 62.9 at 3 months), and by 5-fold during the next 3 months (1519 ± 336.1 at 6 months). Infant feeding mode changed substantially from 3 months (68.9% exclusively breastfed) to 6 months (16.9% exclusively breastfed).

Table 4.3 Characteristics of infants at birth, 3 months and 6 months.

Variable	Birth		3 Months		6 Months	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Age (days)	317	1.8 (1.1)	191	88.3 (7.9)	181	180.3 (8.4)
Sex*	322		191		182	
Female		167 (51.9)		100 (52.4)		93 (51.1)
Male		155 (48.1)		91 (47.6)		89 (48.9)
Weight (g)	317	3283 (449.3)	191	5931 (801.3)	181	7632 (946.2)
Length (cm)	316	49.5 (2.1)	191	59.8 (2.2)	182	66.1 (2.5)
Head circumference (cm)	317	34.4 (1.2)	191	40.2 (1.2)	180	43.1 (1.3)
FM (g)	314	353.6 (161.0)	174	1412.3 (397.8)	109	1859 (412.4)
FFM (g)	314	2933 (346.7)	174	4473 (477.0)	109	5421 (557.1)
%FM	314	10.5 (3.8)	174	23.7 (4.6)	109	25.4 (4.5)
%FFM	314	89.5 (3.8)	174	76.3 (4.6)	109	74.6 (4.5)
FMI (kg/m^2)	314	1.4 (0.6)	174	3.9 (1.1)	109	4.4 (1.0)
FFMI (kg/m^2)	314	12.0 (0.9)	174	12.5 (0.9)	109	12.6 (0.8)
FM/FFM^p	314	36.0 (14.4)	174	251.5 (62.9)	109	1519 (336.1)

Numbers represent mean (SD) for continuous variables and count (%) for categorical variables *; SD: standard deviation; n: number of infants for each variable of interest; FM: fat mass; FFM: fat-free mass; %FM: percent fat mass; %FFM: percent fat-free mass; FMI: fat mass index; FFMI: fat-free mass index; p: relevant regression coefficient (described in detail in methods).

4.4.4 Correlations between infant weight and adiposity indices

Weight was highly correlated with all adiposity indices: FM (r = 0.96), FMI (r = 0.89), %FM (r = 0.87) and FM/FFM^{p} (r = 0.79). FM, %FM and FMI were highly correlated with each other (r > 0.80), while their correlation with FM/FFM^{p} was moderate (r = 0.65–0.78).

4.3.5 Associations between pre- and postnatal factors and infant weight and adiposity indices

4.4.5.1 Weight (g)

Infants' birthweight increased by 174.26 g (95%CI: 114.67 to 233.87 g) with each one week increase in gestation length. Male infants were heavier than female infants at birth (181.58 g; 44.02 to 319.34 g), and at 3 months (421.68 g; 208.63 to 632.36). The increase in weight was not significantly different between the 2 sexes from 3 to 6 months, after adjusting for infant feeding mode.

4.4.5.2 FM (g)

Gestation length was positively associated with FM at birth (34.07 g; 0.64 to 67.48, Table 4.4), but this effect was not evident at 3 and 6 months (Table 4.5). The increase in FM was significantly smaller from birth to 3 months (−205.30 g; −352.30 to −58.36, Table 4.4) and from birth to 6 months (−273.72 g; −446.49 to −100.95, Table 4.4) in infants born to mothers who had supplemental iron during pregnancy compared to the infants of mothers who did not consume supplemental iron, and these effects did not significantly change even after adjusting the effects for the infant feeding mode (Table 4.5). Growth in FM from 3 to 6 months was lower in male infants compared to female infants (−73.14 g; −305.70 to −40.67, Table 4.5), and in formula-fed infants compared to exclusively breastfed infants (−248.27 g; −470.16 to −25.62, Table 4.5).

4.4.5.2 %FM

Maternal ppBMI (0.08%; 0.005 to 0.016) and parity (0.72%; 0.21 to 1.24, Table 4.4) were positively associated with %FM at birth, and these effects did not last at 3 or 6 months. The association between iron intake and %FM was not significant at birth; however, in infants born to mothers who consumed iron supplements, the increase in %FM was significantly lower at 3 months (−2.47%; − 4.24 to −0.70, Table 4.4) and 6 months (−2.95%; −5.06 to −0.83, Table 4.4) compared to infants of mothers who did not take iron supplements, and similar effects were found after accounting for the feeding mode (Table 4.5). Increases in %FM from 3 to 6 months were lesser in male infants compared to female infants (−2.14%; −3.75 to −0.54, Table 4.5), and in formula-fed infants compared to exclusively breastfed infants (−3.84%; −6.47 to −1.21, Table 4.5).

4.4.5.3 FMI

Parity was the only significant predictor of FMI at birth (0.12 kgm^{-2}; 0.02 to 0.22, Table 4.4). Compared to infants of mothers who did not take iron supplements, the increase in FMI was significantly lower in infants born to mothers who had supplemental iron during pregnancy from birth to 3 months (−0.48 kgm^{-2}; −0.82 to −0.13, Table 4.4) and from birth to 6 months (−0.53 kgm^{-2}; −0.95 to −0.12, Table 4.4), which did not significantly change after adjusting for the infant feeding mode. Elevations in FMI from 3 to 6 months were smaller in male infants compared to female infants (−0.47 kgm^{-2}; −0.78 to −0.16, Table 4.5), and in formula-fed infants compared to exclusively breastfed infants (−0.81 kgm^{-2}; −1.33 to −0.29, Table 4.5).

4.4.5.4 FM/FFM^p

None of the potential predictors considered in our analysis was associated with FM/FFM^p at birth. The increase in FM/FFM^p from birth to 6 months was lower in infants born to mothers who had iron supplements (−216.22; −310.57 to −121.89, Table 4.4) and higher in those of mothers who had folic acid supplements (112.53; 31.03 to 194.05, Table 4.4); however, only the effect of iron was significant when adjusted for the effect of infant feeding. Similar to all other indices, increases in FM/FFM^p from 3 to 6 months were also lower in male infants (−119.08; −225.99 to −12.80, Table 4.5) and formula-fed infants (−162.75; −322.41 to −4.23, Table 4.5).

Table 4.4 Longitudinal associations between pre-pregnancy and prenatal factors and indices of infant adiposity from birth to 6 months.

Parameter	Weight (g)		FM (g)		%FM		FMI		FM/FFM^p	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
ppBMI (kg/m^2)	NS								NS	
Association at birth			2.31	(−3.20 to 7.83)	**0.08**	**(0.005 to 0.16)**	0.01	(−0.002 to 0.03)		
Change from birth to 3 months			4.95	(−3.14 to 13.03)	0.02	(−0.09 to 0.14)	0.01	(−0.02 to 0.03)		
Change from birth to 6 months			−1.46	(−11.45 to 8.53)	0.09	(−0.23 to 0.06)	−0.01	(−0.04 to 0.02)		
nGWG (kg)	NS		NS				NS		NS	
Association at birth					0.04	(−0.02 to 0.05)				
Change from birth to 3 months					0.004	(−0.04 to 0.05)				
Change from birth to 6 months					−0.002	(−0.06 to 0.05)				
GDM: yes	NS		NS		NS				NS	
Association at birth							−0.17	(−0.52 to 0.18)		
Change from birth to 3 months							−0.27	(−0.80 to 0.27)		
Change from birth to 6 months							0.1	(−0.47 to 0.68)		
Intake of supplemental iron: yes	NS									
Association at birth			17.01	(−85.27 to 119.34)	0.42	(−0.80 to 1.64)	0.07	(−0.17 to 0.31)	2.45	(−49.26 to 54.16)
Change from birth to 3 months			**−205.34**	**(−352.30 to −58.36)**	**−2.47**	**(−4.24 to −0.70)**	**−0.48**	**(−0.82 to −0.13)**	**−36.53**	**(−118.00 to 44.86)**
Change from birth to 6 months			**−273.72**	**(−446.49 to −100.95)**	**−2.95**	**(−5.06 to −0.83)**	**−0.53**	**(−0.95 to −0.12)**	**−216.22**	**(−310.57 to −121.89)**
Intake of supplemental folic acid: yes	NS				NS		NS			
Association at birth			−4.54	(−92.84 to 83.81)					−0.88	(−45.42 to 43.67)
Change from birth to 3 months			101.77	(−27.38 to 230.93)					12.01	(−59.24 to 83.38)
Change from birth to 6 months			142.11	(−6.84 to 291.07)					**112.53**	**(31.03 to 194.05)**
Parity	NS									
Association at birth			29.72	(−8.90 to 68.33)	**0.72**	**(0.21 to 1.24)**	**0.12**	**(0.02 to 0.22)**	2.57	(−16.94 to 22.08)
Change from birth to 3 months			−20.8	(−79.28 to 37.69)	−0.51	(−1.31 to 0.30)	−0.12	(−0.28 to 0.05)	0.62	(−59.24 to 83.38)
Change from birth to 6 months			22.62	(−42.67 to 37.69)	−0.09	(−1.00 to 0.80)	0.01	(−0.17 to 0.19)	**46.87**	**(31.03 to 194.05)**
Highest education: up to high school										
Association at birth	−100.50	(−258.63 to 57.72)	NS		NS		NS		NS	
Change from birth to 3 months	−145.38	(−368.38 to 77.62)								
Change from birth to 6 months	24.04	(−238.58 to 286.65)								
Gestation length (weeks)										
Association at birth	**174.26**	**(114.67 to 233.87)**	**34.07**	**(0.64 to 67.48)**	0.44	(−0.01 to 0.89)	0.08	(−0.01 to 0.18)	−0.52	(−17.38 to 16.34)
Change from birth to 3 months	−33.07	(−110.56 to 44.40)	−13.53	(−62.01 to 34.93)	−0.69	(−1.36 to −0.02)	−0.13	(−0.27 to 0.002)	−6.03	(−32.70 to 20.61)
Change from birth to 6 months	−20.70	(−111.18 to 69.86)	5.96	(−50.74 to 34.93)	−0.38	(−1.18 to 0.41)	−0.05	(−0.21 to 0.10)	**34.33**	**(3.48 to 65.18)**
Infant sex: male										
Association at birth	**181.58**	**(44.02 to 319.34)**	−6.14	(−82.30 to 70.11)	−0.71	(−1.73 to 0.32)	−0.10	(−0.30 to 0.11)	−5.69	(−44.23 to 32.85)
Change from birth to 3 months	**350.88**	**(171.00 to 530.76)**	39.57	(−69.50 to 148.64)	−0.66	(−2.17 to 0.86)	−0.04	(−0.33 to 0.26)	−19.82	(−80.11 to 40.51)
Change from birth to 6 months	**262.12**	**(49.61 to 474.61)**	**−145.16**	**(−273.16 to −17.15)**	**−2.74**	**(−4.50 to−0.97)**	**−0.52**	**(−0.87 to −0.17)**	**−141.1**	**(−211.12 to −71.11)**

Estimates of the predictors were obtained from Model 1 of stepwise mixed-effects linear regression conducted with backward elimination (at $p > 0.2$) separately for each outcome measure. Gestation length and infant sex were included in all the models; FM: fat mass; FFM: fat-free mass; %FM: percent fat mass; %FFM: percent fat-free mass; FMI: fat mass index; ppBMI: pre-pregnancy body mass index; nGWG: net gestational weight gain. Reference groups for categorical variables include GDM: no, antenatal iron: no, antenatal folic acid: no, infant sex: female, highest education: university/ professional training. *NS* indicates that the predictor was removed from the model during backward elimination. Other exposure variables and covariates considered and removed due to statistical nonsignificance and not shown in the table include maternal age, antenatal smoking, antenatal and interaction between ppBMI and nGWG. Interaction effects at 3 and 6 months show the change in the association from birth to 3 months and change in the association from birth to 6 months, respectively. Bold values denote statistical significance at $p < 0.05$.

Table 4.5 Longitudinal associations between pre-pregnancy, pre- and postnatal factors and indices of infant adiposity from 3 to 6 months.

Parameter	Weight (g)		FM (g)		%FM		FMI		FM/FFM[p]	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
ppBMI (kg/m^2)	NS								NS	
Association at 3 months			4.22	(−5.46 to 13.95)	0.12	(−0.01 to 0.24)	0.01	(−0.01 to 0.04)		
Change from 3 to 6 months			−3.99	(−14.80 to 6.81)	−0.09	(−2.33 to 0.05)	−0.01	(−0.03 to 0.02)		
nGWG (kg)	NS		NS						NS	
Association at 3 months					0.05	(−0.01 to 0.11)				
Change from 3 to 6 months					−0.04	(−0.09 to 0.02)				
GDM: yes	NS		NS		NS				NS	
Association at 3 months							−0.38	(−0.97 to 0.20)		
Change from 3 to 6 months							0.19	(−0.33 to 0.71)		
Intake of supplemental iron: yes	NS									
Association at 3 months			**−200.29**	**(−370.56 to −29.53)**	**−2.16**	**(−3.80 to −0.53)**	**−0.44**	**(−0.82 to −0.06)**	−41.61	(−133.64 to 50.43)
Change from 3 to 6 months			−79.16	(−257.60 to 99.28)	−0.38	(−2.25 to 1.50)	0.01	(−0.36 to 0.36)	**−187.87**	**(−329.58 to −47.13)**
Intake of supplemental folic acid: yes	NS				NS		NS			
Association at 3 months			69.53	(−80.18 to 218.01)					9.65	(−71.74 to 91.02)
Change from 3 to 6 months			57.62	(−100.83 to 216.8)					94.56	(−29.44 to 218.09)
Parity	NS									
Association at 3 months			−5.37	(−77.22 to 66.42)	0.24	(−0.54 to 1.02)	−0.01	(−0.20 to 0.18)	−2.42	(−40.54to 35.69)
Change from 3 to 6 months			37.64	(−30.34 to 105.66)	0.39	(−0.42 to 1.21)	0.10	(−0.06 to 0.26)	51.28	(−3.86 to 106.43)
Highest education: up to high school										
Association at 3 months	−213.35	(−479.43 to 55.26)	NS		NS		NS		NS	
Change from 3 to 6 months	164.21	(−54.03 to 382.73)								
Gestation length (weeks)										
Association at 3 months	47.68	(−51.62 to 145.79)	20.40	(−37.62 to 78.55)	−0.31	(−0.96 to 0.33)	−0.02	(−0.18 to 0.13)	−1.13	(−32.41 to 30.16)
Change from 3 to 6 months	−28.50	(−102.75 to 45.89)	−13.50	(−71.91 to 45.07)	0.11	(−0.61 to 0.83)	−0.03	(−0.17 to 0.11)	38.23	(−8.20 to 84.88)
Infant sex: male										
Association at 3 months	**421.68**	**(208.63 to 632.36)**	31.78	(−97.63 to 160.13)	−1.22	(−2.66 to 0.20)	−0.11	(−0.44 to 0.22)	−16.08	(−86.54 to 54.35)
Change from 3 to 6 months	−70.99	(−246.60 to 104.62)	**−173.14**	**(−305.70 to −40.67)**	**−2.14**	**(−3.75 to −0.54)**	**−0.47**	**(−0.78 to −0.16)**	**−119.08**	**(−225.99 to −12.80)**
Feeding mode: mixed−feeding										
Association at 3 months	−178.01	(−415.49 to 50.01)	−142.69	(−313.19 to 27.45)	−1.27	(−3.24 to 0.70)	−0.37	(−0.78 to 0.04)	−20.77	(−128.22 to 86.63)
Change from 3 to 6 months	60.90	(−246.42 to 368.73)	78.70	(−152.69 to 311.11)	0.53	(−2.14 to 3.22)	0.24	(−0.30 to 0.78)	−56.75	(−213.94 to 99.41)
Feeding mode: formula feeding										
Association at 3 months	159.60	(−88.92 to 398.62)	97.99	(−66.17 to 259.38)	1.54	(−2.90 to 3.38)	0.42	(−0.02 to 0.82)	1.36	(−92.08 to 94.81)
Change from 3 to 6 months	−110.43	(−405.09 to 184.33)	**−248.27**	**(−470.16 to -25.62)**	**−3.84**	**(−6.47 to −1.21)**	**−0.81**	**(−1.33 to −0.29)**	**−162.75**	**(−322.41 to −4.23)**

Estimates of the predictors were obtained from Model 2 of stepwise mixed-effects linear regression conducted with backward elimination (at $p > 0.2$) separately for each outcome measure. Gestation length and infant sex were included in all the models despite their P values, and effects are adjusted for respective adiposity measure at birth; FM: fat mass; FFM: fat-free mass; %FM: percent fat mass; %FFM: percent fat-free mass; FMI: fat mass index; ppBMI: pre-pregnancy body mass index; nGWG: net gestational weight gain. Reference groups for categorical variables include GDM: no, antenatal iron: no, antenatal folic acid: no, infant sex: female, feeding mode: exclusive breastfeeding. *NS* indicates that the predictor was removed from the model during backward elimination. Other exposure variables and covariates considered and removed due to statistical nonsignificance and not shown in the table include maternal age, antenatal smoking, antenatal and interaction between ppBMI and nGWG. Interaction effects at 3 and 6 months show the change in the association from birth to 3 months and change in the association from birth to 6 months, respectively. Bold values denote statistical significance at $p < 0.05$.

4.5 DISCUSSION

In this longitudinal cohort study, we explored associations between pre-pregnancy, pre- and postnatal factors and infant weight and adiposity measures (FM, %FM, FMI and FM/FFM^p), across 0–6 months of age. Of the various predictors considered, only gestation length and infant sex were related to infant weight. This highlights the importance of gathering body composition information to understand the impact of maternal and postnatal factors on infant growth and development. We identified positive associations between gestation length and infant FM, maternal self-reported ppBMI and infant %FM, and parity and infant %FM and FMI, at birth. Surprisingly, maternal intake of iron supplements during pregnancy was negatively associated with infant FM, %FM and FMI at 3 months, and FM/FFM^p at 6 months. Male infant sex and formula feeding were negatively associated with all adiposity indices at 6 months. Our findings imply that, pre-pregnancy and pregnancy factors influence body composition during early life, and any unfavourable impacts may be modulated during the postnatal period, particularly via infant feeding practices. Moreover, the associations we observed were dependent on the adiposity measure used. Therefore, it is critical that researchers understand the strengths and limitations of different adiposity indices and use conceptually and statistically robust approaches such as FM/FFM^p.

Gestation length at delivery has been reported as the strongest predictor of birth measurements in many studies. Positive associations of gestation length with infant weight and FM found in the current study is in accordance with the earlier findings [50, 54, 358, 359]. Moreover, we observed a positive effect of parity on infant %FM and FMI at birth. Increases in infant fatness in successive pregnancies have been explained as a function of changes in the mother's metabolism as a cumulative effect of advancing age and prior pregnancies [360].

Maternal ppBMI is an indicator of maternal nutrition status during conception. The increases in newborn weight and adiposity with increasing ppBMI has been explained by the fact that high blood glucose levels in mothers with excess weight triggers the production of insulin that in turn increases lipogenesis and excessive fat deposition in the foetus [361]. Though it was not evident in our study, others [320, 362] have shown that infant birthweight increases with increasing ppBMI. Our finding of the impact of ppBMI on infant %FM was consistent with those who reported a positive association between ppBMI and %FM at birth [35, 57] and others who did not find significant associations at 3 months [196], 5 months [50] and 6 months [196]. However, the positive association between ppBMI and FM/FFM^p at birth, reported by Abreu et al. [59], was not evident in our study. Conversely, some researchers have shown that ppBMI is not associated with FM or %FM, even in newborns [208].

Between 3 and 6 months, to our surprise, we found a negative association between maternal supplemental iron intake during pregnancy and infant adiposity measures, which did not significantly change even after the models were adjusted for infant feeding mode. This result may be because mothers who took iron supplements were generally more aware of health issues in pregnancy and thus led a healthier lifestyle which promoted leanness in their infants. Physiologically, it is also possible that high iron stores in infants born to mothers who took iron supplements promoted the production of red blood cells, myoglobin, and muscle growth [363], leading to the relative increase in FFM and reductions in adiposity. Further, we observed that the increase in FM/FM^{p} from birth to 6 months was significantly larger in infants born to mothers who consumed folic acid supplements during pregnancy, but this relationship was no longer significant after adjusting for the feeding mode. Dahly et al. [233] have also shown that FM was not different in newborns whose mothers met the recommended daily allowance of folate (400 µg dietary folate equivalents) vs those of mothers who did not. Nonetheless, since our data were limited to whether mothers consumed supplemental iron/folic acid during pregnancy or not, our results should be interpreted with caution. We also acknowledge that due to our low recruitment rate (23.4% from the mothers approached) and dependence on self-reported data, the prevalence of supplement intake observed in the study group may not be representative of all pregnant women in Tasmania. Future research should consider validated data on the dosage and length of supplement intake during pregnancy on infant adiposity at birth and long-term.

Other predictors of adiposity increase from 3 to 6 months of age were infant sex and infant feeding mode. Prior studies have noted higher adiposity levels in female infants in contrast to higher FFM in male infants [50, 52, 54, 196]. Some have explained this as a result of a testosterone surge, referred to as “mini-puberty”, during early infancy (2-5 months) in male infants, which promotes the growth in FFM, reducing relative FM [279]. However, others [44, 280, 281, 284, 286] have shown that sexual dimorphism in adiposity starts from birth based on testosterone production by testes in male infants starting during foetal life. Furthermore, compared to infants who were exclusively breastfed, formula-fed infants had a significantly lower increase in adiposity (evident in all indices) from 3 to 6 months. Our result is consistent with the findings of a systematic review of 15 studies that compared the body composition of breastfed vs formula-fed infants [296]. The authors reported that compared to breastfed infants, formula-fed infants had lower FM and %FM at 3–4 months, as well as at 6 months, and this could be due to higher leptin levels, characteristic of breastfed infants. Another possibility would be that formula milk contains more energy and protein compared to breastmilk, which may promote growth in FFM, thereby resulting in lower levels of relative fatness [364]. However, in our infants, this difference in adiposity attributed to the feeding mode was not evident at 3 months. The reason could be that we analysed infants’ feeding mode with a 1-month feed recall, and it may have not

accurately depicted the infants' diet in the period of 0–3 months. On the other hand, the reason may be that more time was needed to reflect the changes due to differences in feeding mode in infants' body composition. Nevertheless, despite the increased adiposity found in breastfed infants compared to formula-fed infants during 0–6 months of age, a large body of evidence suggests breastfeeding has a protective effect against obesity and adiposity later in life [365, 366].

Previous studies have identified excessive GWG during pregnancy based on the weight gained between conception and the onset of labour, following the recommendations of the Institute of Medicine. This includes the weight of the infant, placenta and amniotic fluid, which account for ~35% of the GWG; therefore, it may not accurately reflect the actual weight gain of the mother [367]. In our analysis, we used nGWG (weight calculated as the difference between pre-pregnancy and post labour weight) to assess the association between true weight gain of the mother and measures of infant adiposity and did not find any association. Except for one study [209] that investigated the association between nGWG and risk of large-for-gestational-age birth, nGWG has not been considered as a predictor of infant adiposity in any previous studies, and it is, therefore, difficult to compare our results. If nGWG is adopted in future research, it may help to identify more accurate associations specific to the real weight gain of the mother. Moreover, GDM was not a significant predictor of adiposity in our infants; however, we acknowledge the low number of mothers with GDM in our study precludes a robust conclusion. In our systematic review and meta-analysis [216] of studies comparing adiposity in infants born to mothers with GDM and mothers with NGT, we have shown that, despite treatments for GDM, infants exposed to GDM *in utero* had higher total body adiposity than the infants born to mothers with NGT (Chapter 5). Infants of mothers who smoked during pregnancy are distinguished by lower FFM [40, 359], which might affect measures of %FM or FM/FFM^{p}. Nonetheless, we did not observe a significant relationship between maternal smoking and infant adiposity, potentially due to the nature of the approach taken by the multi-country study to collect information. The smoking status of mothers was recorded as a dichotomous variable as "smoked 0–3 days" or "smoked 4–7 days"; hence, (light) smokers were included in the same group as non-smokers, and we were unable to further separate the non-smokers from light smokers. Further, our maternal cohort was predominantly Caucasian, employed and had university education/professional training. Consequently, maternal ethnicity, occupation status, and education were not significant predictors of infant adiposity.

Although adiposity is widely expressed with absolute values of FM, normalising FM for size is fundamental to understand the relative fatness of individuals [56]. Most commonly, FM is normalised for overall body weight, i.e., %FM, but this approach cannot effectively distinguish fatness between individuals as %FM is affected by changes in FM as well as FFM [368]. FMI is

recommended as a more appropriate approach that allows independent evaluation of FM relative to body size (height) [56]. In addition, recent research has demonstrated that FMI is a more reliable index than %FM when assessing neonatal adiposity [369]. In contrast, some studies [59, 368] have suggested that an appropriate index should adjust the originator of the risk (FM) for a variable that is bearing the risk (FFM), thus recommending the index of FM/FFM^{p}; however, to the best to our knowledge, only one study [59] used FM/FFM^{p} to identify maternal predictors of infant adiposity at birth. Our data demonstrate that FM/FFM^{p} is highly sensitive to rapid changes in adiposity during this critical period of growth, with drastic increases from birth (~36) to 6 months (~1500). Further research is required to test its reliability and validity in longitudinal studies.

To our knowledge, this is the first study that concurrently explores the determinants of different indices of adiposity in early infancy. Other strengths of our study are the use of a validated and reliable technique to evaluate infant body composition, prospective longitudinal study design and satisfactory sample size compared with similar studies. Nonetheless, the inclusion of mostly healthy mother-infant dyads may have introduced selection bias to our study limitting the generalisability of our findings. Moreover, participant loss-to-follow-up may have introduced attrition bias. The reasons for loss-to-follow-up were moving away from the research location, work or childcare responsibilities, or other commitments. Despite the PEA POD being the most "practical" body composition assessment technique for infants of 1–10 kg body weight, potential sources of measurement error include hydration status, body moisture, temperature, and body hair [146]. Another limitation of our study is that the maternal variables, including pre-pregnancy weight, were self-reported by mothers in an interview-based approach and can be subjected to recall inaccuracies and social desirability bias. Particularly, self-reported ppBMI is used in many studies as a crude measure of maternal adiposity as obtaining objective maternal body composition measurements before conception and throughout pregnancy would be extremely difficult in practice, although it would be ideal. We also concede that the associations we report on prenatal supplements have been analysed without considering the variations in doses; therefore, the results should be translated with caution. Further, we acknowledge there are other potential predictors of neonatal body composition that we have not adjusted for in our results. These may include prenatal factors such as maternal dietary intake [257], use of other micronutrient supplements (e.g., vitamin D [370], iodine [371]) or combined nutritional supplements during pregnancy [247], maternal physical activity level [266], and postnatal factors such as infant milk feeding patterns (e.g., variations in volume and frequency [372]) and infants' exposure to micronutrients (e.g., iron supplementation is recommended for breastfed infants since the concentration of iron in breastmilk is very low and declines with time; in contrast, formula-fed infants may get iron from iron-fortified formula [363]).

4.6 CONCLUSIONS

Using infant weight as a proxy for adiposity may hinder understanding the influences of pre- and postnatal factors on infant growth and development. In our study, gestation length, parity, and ppBMI were significant predictors of adiposity in newborns, and male infant sex, intake of supplemental iron during pregnancy, and mode of feeding significantly contributed to variations in adiposity of 3–6-month-old infants. However, these associations were dependent on the adiposity index used. Our results highlight the importance of optimal maternal health and lifestyle during pre-pregnancy and pregnancy periods in determining adiposity in early life. Our findings also suggest that any negative prenatal impacts on neonatal adiposity may be ameliorated during the postnatal period, potentially with infant feeding practices. Additionally, it is critical that researchers understand the strengths and limitations of respective approaches when choosing a measure of adiposity to investigate its relationship with potential predictor variables. On the understanding that FM cannot identify relative fatness of individuals and %FM is statistically flawed, future research should use more conceptually and statistically robust adiposity measures. FM/FFM^{p} can account for changes in both FM and FFM; therefore, we suggest that it may be a better index for tracking variations in relative fatness in infants and identifying relationships with maternal factors. Further, longitudinal studies beyond early infancy are required to inform long-standing links between pre-pregnancy, pre- and postnatal factors and offspring growth trajectory.

Although we did not observe an association, exposure to GDM is a key determinant of newborn adiposity. It is not clear in the literature whether this effect lasts even after treatment for GDM. On the other hand, PEA POD (the device used to measure infant adiposity in the prospective study) is limited to infants less than 6 months old; hence, other options should be explored for further follow-ups. The next two studies (Chapter 5 and 6) address these two gaps, respectively.

CHAPTER 5 : GDM CONTROLLED WITH THERAPEUTIC INTERVENTIONS AND ADIPOSITY IN NEWBORNS

5.1 ABSTRACT

Background: Individuals exposed to untreated GDM *in utero* have an elevated risk of obesity and type 2 diabetes in adulthood. Increased adiposity during early infancy is a plausible mediator of this increased risk of later-life metabolic disorders. The evidence for whether good glycaemic control in mothers with GDM can normalise infant adiposity is inconsistent, and the effect of different treatments for GDM on neonatal adiposity is understudied.

Aim(s): To systematically review studies reporting FM, %FM and ST at birth in infants of mothers with GDM controlled with therapeutic interventions.

Methods: MEDLINE, Embase, CINHAL, PubMed and Web of Science databases were searched. Inclusion criteria were infants' age < 1-month, and availability of information on therapeutic interventions used to control GDM and at least one infant adiposity measure. The quality of the studies was assessed using the Evidence Project risk of bias tool. For data synthesis, included studies were categorised according to comparison groups: (1) infants born to mothers treated for GDM (IGDMtr) vs untreated for GDM; (2) infants born to mothers treated with different therapies for GDM and (3) IGDMtr vs infants exposed to normal glucose tolerance (INGT).

Results: In total, 25 studies were included, and of them, only 15 reported the level of glycaemic control in mothers. Treating GDM lowered FM in newborns compared to no treatment. There was no difference in FM and ST as per the type of treatment (insulin, metformin, glyburide). As evidenced in the meta-analysis of 17 studies (published 1980-2020), IGDMtr had higher overall adiposity (mean difference, 95% CI) measured with FM (68.46 g, 29.91 to 107.01) and %FM (1.98%, 0.54 to 3.42) but similar subcutaneous adiposity measured with ST, compared to INGT. This suggests that IGDMtr may be characterised by an excessive non-subcutaneous fat accretion. Subgroup analysis showed that significant differences in FM and %FM between IGDMtr and INGT only existed in 'pre-2010′ studies, while there was no significant difference in FM and %FM in IGDMtr compared to their counterparts in 'post-2010′ studies. This may be attributed to more intensive management of hyperglycaemia that has been adopted in the 'post-2010′ period.

Conclusion(s): Intensive glycaemic control in GDM may normalise the adiposity in infants at birth. Future studies should report the treatments and level of glycaemic control in GDM mothers

throughout the pregnancy to enable robust conclusions. Additionally, any differences in the adipose tissue distribution of IGDMtr compared to INGT would benefit from further research.

5.2 INTRODUCTION

The prevalence of GDM is rising globally, affecting up to 38% of pregnancies in some populations [373]. As well as causing complications during pregnancy and delivery including macrosomia, shoulder dystocia and preterm birth, exposure to GDM *in utero* places offspring at an increased risk of obesity and type 2 diabetes in later life [374, 375]. The mechanisms associated with this increased risk of obesity and type 2 diabetes are not well understood; however, increased adiposity during foetal growth has been suggested as a potential mediator [376]. The Pedersen hypothesis [377] suggests that, as glucose freely crosses the placenta, maternal hyperglycaemia in diabetic pregnancies leads to foetal hyperinsulinaemia, causing accelerated foetal uptake of glucose (foetal glucose steal phenomenon) and deposition of excess foetal adipose tissue [378]. The impact of GDM on adipose tissue growth in the foetus can be identified with adiposity measures at birth, for example, FM, %FM and ST [212].

Diagnosis and management of GDM continue to be controversial. The earlier definition of GDM, i.e., "any degree of glucose intolerance that occurs or is first diagnosed during pregnancy" [379], was used for many years and enabled a uniform approach to the detection of GDM. However, the classification of women with unrecognized overt diabetes as GDM and providing treatments accordingly may not be effective because risks associated with type 1 and type 2 diabetes are greater than GDM [380]. In the latest clinical practice recommendations by the American Diabetes Association [210], GDM is defined as "glucose intolerance first diagnosed during the second or third trimester of pregnancy in women without overt diabetes prior to pregnancy, which resolves postnatally", and this involves risk-based screening for type 2 diabetes or prediabetes at their initial prenatal visit. Nonetheless, different criteria are currently being used worldwide to diagnose GDM. A landmark change in these diagnostic thresholds occurred when the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study [381] demonstrated a positive linear association between increasing levels of plasma glucose and adverse pregnancy outcomes and subsequently, lowered thresholds for screening GDM. These new diagnostic thresholds (fasting plasma glucose 5.1–6.9 mmol/L, 1-h plasma glucose $\geqslant$10.0 mmol/L or 2-h 8.5–11.0 mmol/L) were promulgated by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) in 2010 and by the WHO in 2013, and this enabled detection of more GDM cases [382].

Awareness of the adverse outcomes associated with GDM has been a driver for substantial improvements in perinatal care for pregnant women with GDM in recent years [383]. The first-

line treatment for GDM involves lifestyle changes, e.g., modified diet and increased physical activity, and nearly two-thirds of women can achieve glycaemic targets with this approach [384]. When blood glucose levels are not adequately controlled with modified lifestyle alone, supplementary pharmacological treatments such as metformin, glyburide or insulin are added to the therapeutic regimen [385]. Glycaemic control in GDM women using modified dietary interventions alone has resulted in lower birthweights and less macrosomia [386], despite the high heterogeneity in diet observed among different populations [387]. Similarly, using pharmaceutical interventions along with or without lifestyle changes has resulted in reduced risk of macrosomia [388] and has prevented GDM-associated adverse health conditions in neonates [389]. Nevertheless, the effect of GDM treatments on neonatal adiposity is understudied, and the evidence for whether good glycaemic control in GDM can normalise foetal adiposity is contradictory [55, 63, 64]. To ascertain the impact of glycaemic control in GDM on infant adiposity at birth, we systematically reviewed studies reporting adiposity in newborns of mothers with GDM controlled with therapeutic interventions.

5.3 METHODS

This work was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [390]. The protocol is registered in PROSPERO (CRD42020175338).

5.3.1 Search strategy

Electronic searches were conducted in three stages with the assistance of a Research Librarian at the University of Tasmania, Australia. First, a limited search was undertaken in Medline and Scopus, using search terms: “gestational diabetes”, “body composition” and “infants”. The title, abstract and index terms of the retrieved articles were scanned to build a keyword list. In the second step, a broader search was conducted (March 2020), using the identified terms in MEDLINE in Ovid, Embase, CINHAL, PubMed and Web of Science databases, limiting the results to studies published in “English” language, “human” species and “infants” age group. The search strategy for MEDLINE is shown in Figure 5.1, and a similar approach was used in other databases. Finally, we manually scanned the reference lists of included articles, relevant reviews, and citations to identify any additional studies. Hand searches were not conducted for any specific journal, and we did not trace any grey literature.

Step	Searches
1	(gestation* or pregnan* or maternal or mother*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
2	(diabet* or hyperglyc?mi* or glyc?mi* or glucose intolerance or impaired glucose tolerance or insulin resistance).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
3	(infan* or neonat* or child* or newborn* or offspring* or birth).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
4	("body composition" or fat or adiposity).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
5	("air-displacement plethysmography" or "isotope dilution" or "deuterium dilution" or "bioelectrical impedance" or "total body electrical conductivity" or "dual-energy x-ray absorptiometry" or "photon-counting computed tomography" or "photon absorptiometry" or "total body potassium" or "magnetic resonance imaging" or "skinfold" or "skin fold" or anthropometry).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
6	1 and 2 and 3 and 4 and 5
7	limit 6 to (english language and humans and "all infant (birth to 23 months)")

Figure 5.1 Search strategy in Medline via Ovid.

5.3.2 Eligibility criteria

We included all study types reporting adiposity in infants exposed to GDM. Inclusion criteria were: (1) data collected at birth or <1-month infants' age; (2) availability of infant adiposity measure(s); i.e., FM, %FM or ST; and (3) availability of information regarding what therapeutic measures were undertaken to control GDM. Exclusion criteria were (1) examination of maternal glycaemia as a continuous variable; (2) assessment of only foetal measurements (e.g., ultrasound scans); (3) merging of data for GDM exposed infants with pregestational diabetes-exposed infants; (4) full report of the study not published in English; and (5) review articles, protocol papers and conference abstracts. When there were multiple publications from the same sample of study participants, we only included the paper that presented the most appropriate data for the purpose of this review.

5.3.3 Study selection

The results emanating from database searches were imported into the Covidence software® [391]. After removing duplicates, the search outputs were independently reviewed at the title and abstract level by M.P.H. and K.D.K.A/J.M.B. to find potentially eligible articles. These articles were screened at the full-text level by the same reviewers to determine the eligibility of the papers for data extraction.

5.3.4 Quality assessment

The methodological quality of the selected studies was assessed by two reviewers (M.P.H. and J.M.B) using the Evidence Project risk of bias tool. This tool is appropriate for assessing study rigour for both randomised and non-randomised intervention studies. The Evidence Project risk of bias tool includes eight items: (1) cohort, (2) control or comparison group, (3) pre-post intervention data, (4) random assignment of participants to the intervention, (5) random selection of participants for assessment, (6) follow-up rate of 80% or more, (7) comparison groups equivalent on sociodemographics, and (8) comparison groups equivalent at baseline on outcome measures [392]. For criterion 7, we considered infant sex and ethnicity as the relevant sociodemographic characteristics. If authors reported that study arms were equivalent on only one sociodemographic variable, we considered the meeting of the criterion as "Partial". Additionally, if the study arms were not equivalent on at least one sociodemographic variable, we considered that the criterion was not met. Any disagreements between the two reviewers regarding the inclusion of studies and quality assessment were resolved by discussion and consensus.

5.3.5 Data extraction

A pre-designed data collection form was used to extract information from each paper. This information included: (1) study characteristics (author, year, design, time period of data collection, state/country of the study, inclusion and exclusion criteria), (2) study groups (sample size, male%), (3) method(s) (GDM screening/diagnostic criteria, treatments to control GDM, target blood glucose level, degree of glycaemic control, body composition measurement technique) and (4) outcomes (FM, %FM, ST).

5.3.6 Data analysis

For the purpose of data synthesis, the included studies were categorised according to comparison groups: (1) infants born to mothers treated for GDM (IGDMtr) vs untreated for GDM; (2) infants born to mothers treated with different therapies for GDM and, (3) IGDMtr vs INGT. When blood glucose levels of GDM mothers were controlled with any form of therapeutic intervention (including lifestyle modification and/or pharmaceutical interventions), they were considered as 'treated', and usual antenatal care without any specific treatment for GDM was considered as 'untreated'. When an adequate number of studies were available, meta-analyses were performed with the inverse variance statistical method and random effects analysis model (RevMan version: 5.4.0) [393]. Mean difference at a 95% CI was used to combine the results. Forest plots were used to demonstrate the outcomes. Heterogeneity between the studies in meta-analyses was determined with a Chi^2 test on the Q statistic (variance of the observed effect sizes in the meta-analysis), Tau^2 (between-study variance of the true effect sizes) and I^2 (proportion of the observed variation in the effect size due to differences in the true underlying effect sizes, as opposed to sampling error). An alpha level <0.05 was considered statistically significant. Potential sources of heterogeneity, i.e., level of glycaemic control in GDM mothers, any advances in the effectiveness of treatments for GDM in 'recent' years (defined as study data collection occurred during or after 2010: referred as post-2010) compared to 'pre-2010′ (defined as study data collection occurred before 2010), GDM diagnosis criteria and body composition assessment technique, were investigated with subgroup analyses. Sensitivity testing was performed with 'leave-one-out' testing.

5.4 RESULTS

5.4.1 Study selection

Of the 1072 references identified through database searching, 19 matched inclusion-exclusion criteria (Figure 5.2). An additional six papers were identified through a review of reference lists, relevant reviews, and forward citations. In total, 25 studies [55, 63, 64, 212, 214, 355, 394-412] were included in the systematic review, of which 17 [55, 63, 64, 212, 394-396, 398, 400, 403-410] were included in the meta-analysis.

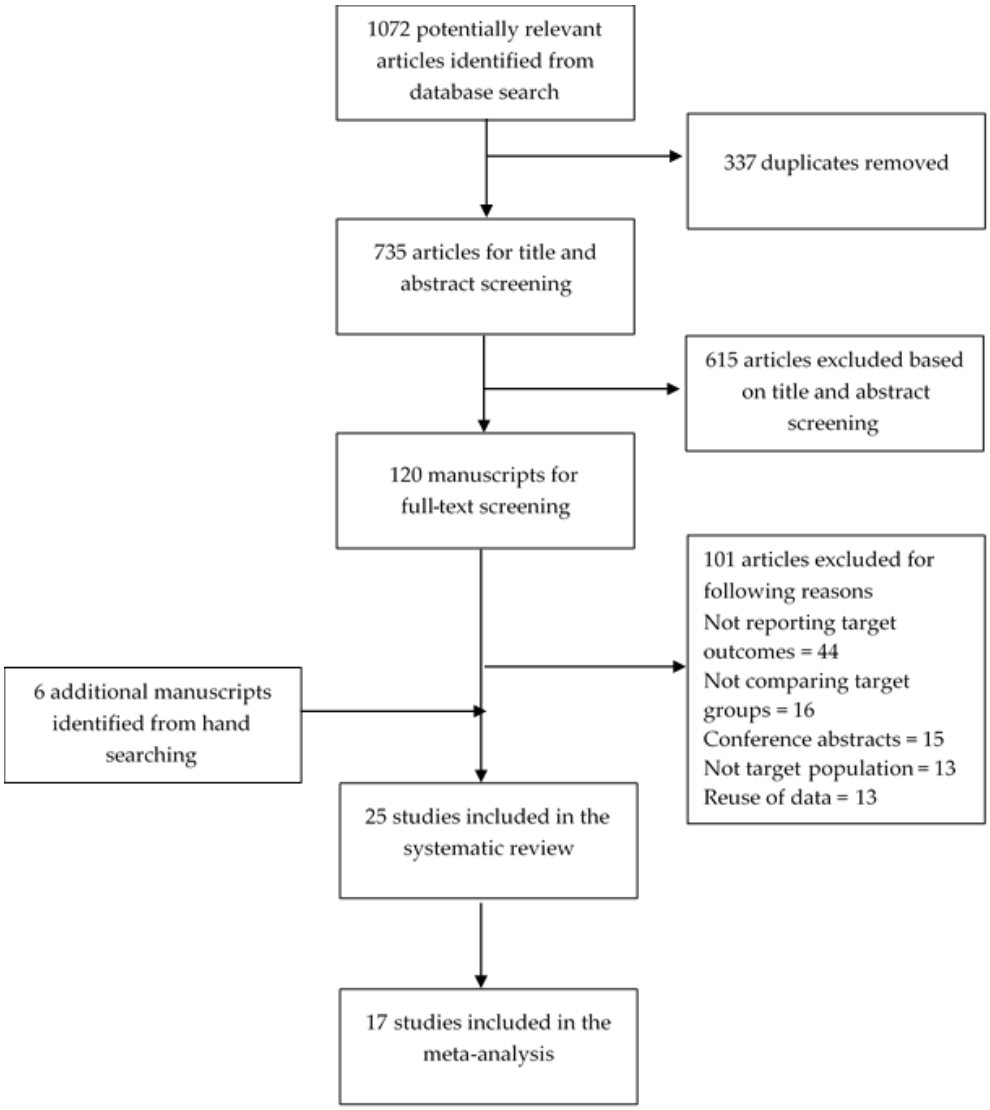


Figure 5.2 Flow of information through the different phases of the review.

5.4.2 Description of the studies

The selected references included three randomised clinical trials [214, 401, 402], 15 cohort studies [55, 212, 394, 396-398, 403, 404, 406-412], 3 case-control studies [63, 400, 405] and 4 cross-sectional studies [64, 355, 395, 399]. The included studies were published between 1980 and 2020, and from 12 different countries, i.e., the United States [212, 214, 355, 395, 396, 398, 402], Australia [55, 64, 410], New Zealand [397, 400], Australia and New Zealand [401], Germany [403, 404, 406], Sweden [63, 409], China [399], France [408], Italy [394], Malaysia [412], Spain [405], the United Kingdom [411] and Turkey [407]. Sample sizes varied from 25 to 1000 (Table 5.1).

Eleven guidelines developed between 1964 and 2014 were used for screening and diagnosing GDM by 22 studies [55, 64, 212, 214, 355, 394-399, 401-411] (Table 5.2). The remaining three studies [63, 400, 412] used center-specific criteria for screening and diagnosing GDM. From the 25 studies, 24 used an oral glucose tolerance test (OGTT) to diagnose GDM, and the other [394] used White's classification based on the age of onset and duration of diabetes. Commonly used criteria included Carpenter and Coustan (1982), Australasian Diabetes in Pregnancy Society (ADIPS, 1998), and the International Association of Diabetes and Pregnancy Study Groups (IADPSG, 2011). Seven of the guidelines utilised a screening oral glucose challenge test (OGCT) prior to an OGTT, while other guidelines used only a diagnostic OGTT. Only six of the guidelines tested plasma glucose 3-hours post OGCT. The cut-offs for fasting, 1-hour, 2-hour and 3-hour blood glucose ranged between 5.0-7.0 mmol/L, 9.2-11.0 mmol/L, 8.0-9.1 mmol/L and 6.9-8.0 mmol/L, respectively. Seven GDM criteria required two or more abnormal values, while three guidelines required only one abnormal value, for the diagnosis of GDM.

Fasting and 2-hour post-prandial plasma glucose targets for treated-GDM mothers differed between studies as follows; 5.0 mmol/L and 6.7 mmol/L [408, 412], 5.3 mmol/L and 7.8 mmol/L [405], 5.3 mmol/L and 6.7 mmol/L [214, 402], 5.5 mmol/L and 6.5 mmol/L [400], 5.5 mmol/L and 6.7 mmol/L [212, 396], and 5.5 mmol/L and 7.0 mmol/L [55, 64, 397, 401]. Two studies [63, 407] used HbA1c between 3.5–5.3% as the mean blood glucose target.

Table 5.1 Characteristics and findings of the studies.

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method/ST Measurements	Findings
(1) Treated GDM vs no treatment for GDM							
Landon, 2009 **Randomised trial** **2002–2007** **Bethesda, MD, USA [214]**	Control = 473 Treatment = 485	At 24th and 30th weeks using 4th International workshop conference criteria	Diet therapy (*n* = 427) and insulin (*n* = 36)	Targeted for fasting glucose <5.3 mmol/L or 2-hour post-prandial glucose, <6.7 mmol/L Good glycaemic control achieved	Birth	FM was calculated as proposed by Catalano et al., 1995. Flank skinfold (data not given)	FM: Lower in treatment group (427 ± 198 vs 464 ± 222, p = 0.003)
(2) Different treatment regimens for GDM							
(a) Studies that measured only skinfolds							
Simmons, 1997 **1991–1992** **Middlemore Hospital and National Women's Hospital, Auckland, New Zealand [397]**	All GDM Non-insulin = 11 (46%) Insulin = 9 (33%)	At 28–32 weeks gestation, using modified O'Sullivan criteria	All women received dietary therapy	Targeted fasting glucose >5.5 mmol/L and/or 2-hour post-prandial glucose >7.0 mmol/L	<24 h	Subscapular	ST: Not significantly different subscapular 5.4 (4.8–7.0) vs 6.8 (5.0–7.9)
Rowan, 2008 **randomised, open-label trial** **10 New Zealand and Australian urban obstetrical hospitals [401]**	All GDM Metformin = 363 Insulin = 370	According to the criteria of the Australasian Diabetes in Pregnancy Society (ADIPS)	Metformin = 363 Insulin = 370	Aimed for the capillary glucose levels recommended by the ADIPS (after an overnight fast, <5.5 mmol/L; 2-hour post-prandial level, <7.0 mmol/L	<48 h	Triceps and subscapular	ST: Metformin group not significantly different from insulin group triceps (5.2 ± 1.6 vs 5.1 ± 1.2, p = 0.30) subscapular (5.2 ± 1.5 vs 5.2 ± 1.3, p = 0.60)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
(b) Studies that measured body composition							
Catalano, 2003 **Prospective cohort** **1990 -2000** **Pregnancy Diabetes Clinic in Cleveland** **Ohio, USA [212]**	NGT = 220 (54%) GDM = 195 (51%)	National Diabetes Data Group criteria	Diet only = 128 Diet + insulin = 67	Targeted fasting glucose >5.5 mmol/L and/or 2-hour post-prandial glucose >6.7 mmol/L; Women maintained glucose values within the target range with diet and exercise (66%), plus insulin (34%)	<72 h	TOBEC	FM: Higher in diet + insulin group (492 ± 215 vs 407 ± 196, p = 0.006) %FM: Higher in diet + insulin group (13.6 ± 4.6 vs 11.7 ± 4.5, p = 0.007)
Lain, 2009 **Randomised clinical trial** **2002–2005** **Magee-Women's Hospital, Pittsburgh, Pennsylvania [402]**	Insulin = 41 (55.3%) Glyburide = 41 (58.5%)	Carpenter and Coustan criteria. Participants with a glucose level of >7.5 mmol/L had a 3-hour 100-g OGTT	Insulin = 41 Glyburide = 41	Targeted fasting glucose >5.5 mmol/L and/or 2-hour post-prandial glucose >6.7 mmol/L. Post-prandial dinner glucose was increased in the glyburide group.	<36 h	TOBEC Triceps, subscapular, suprailiac, and anterior thigh ST (individual and sum given)	FM: Insulin group not significantly different from glyburide group (370 ± 167 vs 473 ± 278, p = 0.06) %FM: Insulin group not significantly different from glyburide group (11.2 ± 4.2 vs 12.8 ± 5.7, p = 0.18) ST: Insulin group not significantly different from glyburide group triceps (3.9 ± 0.7 vs 3.9 ± 0.9, p = 0.89), subscapular (4.1 ± 1.0 vs 4.5 ± 1.3, p = 0.10), suprailiac (2.1 ± 0.6 vs 2.1 ± 0.6, p = 0.85) and thigh (5.1 ± 1.2 vs 5.4 ± 1.7, p = 0.28)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
(3) Treated GDM vs NGT				‡ *IGDMtr compared to INGT*			
(a) Studies that measured only skinfolds							
Stevenson, 1991 **Cross-sectional** **USA [395]**	AGA NGT = 20 LGA NGT = 20 AGA GDM = 13	O'Sullivan and Mahan criteria	Dietary control	'Well-managed GDM'	<72 h	Triceps	Not significantly different triceps compared to AGA NGT group (5.0 ± 1.1 vs 4.3 ± 0.8, p > 0.05) and LGA NGT group (5.0 ± 1.1 vs 6.2 ± 2.0, p = 0.058)
Vohr, 1995 **Prospective longitudinal cohort** **1991–1993** **Women and Infants' hospital, Rhode Island [396]**	AGA NGT = 69 AGA GDM =62 LGA GDM = 57 LGA NGT = 74	Carpenter and Coustan criteria	Diet only = 385 Diet + insulin = 34 Diet includes 45–50% carbohydrates, 25% protein, and 25% fat.	Targeted fasting glucose >5.5 mmol/L and/or 2-hour post-prandial glucose >6.7 mmol/L. The management team worked with all mothers to maintain BGL targets	20 ± 12 h	Triceps, subscapular, abdominal, suprailiac, and medial calf ST	<u>AGA GDM vs AGA NGT</u> Not significantly different triceps (3.5 ± 0.9 vs 3.6 ± 0.8), subscapular (3.9 ± 1.0 vs 3.9 ± 0.9), abdominal (3.5 ± 1.0 vs 3.7 ± 0.9), suprailiac (3.4 ± 0.9 vs 3.6 ± 1.0) and medial calf (4.8 ± 1.1 vs 5.1 ± 1.1) <u>LGA GDM vs LGA NGT</u> Not significantly different subscapular (5.5 ± 1.5 vs 5.3 ± 1.3), suprailiac (4.9 ± 1.1 vs 4.5 ± 1.1) and medial calf (6.7 ± 1.3 vs 6.3 ± 1.1) significantly higher triceps (4.7 ± 1.0 vs 4.5 ± 1.0) and abdominal (5.3 ± 1.4 vs 4.9 ± 1.2) <u>LGA GDM vs AGA GDM</u> Significantly higher subscapular (5.5 ± 1.5 vs 3.9 ± 1.0), Abdominal (5.3 ± 1.4 vs 3.5 ± 1.0), suprailiac (4.9 ± 1.1 vs 3.4 ± 0.9) and medial calf (6.7 ± 1.3 vs 4.8 ± 1.1) Not significantly different triceps (4.7 ± 1.0 vs 3.5 ± 0.9)
Ng, 2004 **Cross-sectional** **Prince of Wales Hospital Hong Kong [399]**	NGT = 40 (50%) GDM = 42 (45.5%)	ADIPS criteria (1998)	Low-energy diet (1800 kcal/d)	Not reported	< 24 h	Triceps and subscapular	ST: Not significantly different triceps (4.8(4.2–5.1) vs 4.7(4.1–5.5)) and subscapular (4.8(4.3–5.3) vs 4.8(4.1–5.3), p > 0.05)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
Westage, 2006 **case-control** **1999–2001** **Middlemore Hospital, South Auckland** **New Zealand [400]**	NGT = 95 GDM = 138	Local criteria for diagnosis of GDM fasting glucose ≥5.5 mmol/Land/or a 2-h value after a 75 g glucose load ≥9.0 mmol/l	Insulin, usually as lispro insulin up to three times daily along with Humulin N if target fasting glucose exceeded two occasions or post-prandial readings were consistently high.	Target fasting glucose <5.5 mmol/L and post-prandial readings <6.5 mmol/l.	<24 h	Triceps and scapular	ST: Significantly higher triceps (5.0 ± 1.2 vs 4.4 ± 1.0) and scapular (5.6 ± 1.6 vs 4.4 ± 1.0)
Kara, 2017 **Cohort** **Ataturk University, Medical Hospital, Erzurum, Turkey [407]**	NGT = 20 GDM = 15 groups were matched for gestational age and sex	At 24-28 gestational week using WHO criteria	All were treated with dietary intervention, physical activity recommendation, and lifestyle management. All of them (diabetic) have used insulin therapy.	While the mean HbA1c level of mothers with GDM was 5.9 ± 1.7%, that of the controls was 5.2 ± 0.33%; there was no significant difference. Therefore, mothers with GDM were well controlled.	Birth	Triceps, Biceps, subscapular	ST: Significantly higher triceps (3.9 ± 0.7 vs 3.3 ± 1.1, p = 0.009) and subscapular (3.8 ± 0.8 vs 3.4 ± 1.2, p = 0.04) Not significantly different biceps (2.8 ± 0.6 vs 2.6 ± 0.9, p = 0.32)
Mitanchez, 2017 **prospective cohort exposure-matched cohort** **2010–2013** **Paris, France [408]**	Lean NGT = 164 Lean GDM = 41 Obese NGT = 120 Obese GDM = 90	Fasting blood glucose (FBG) in the first trimester for women with BMI ≥30 kg/m2, and a 75 g OGTT between 24–28 weeks regardless of maternal BMI. At 32 weeks 75 g OGTT, International Association of Diabetes and Pregnancy Study Groups (IADPSG) Criteria.	The first-line treatment was dietary intervention with a standard 1800 kcal daily meal plan divided into three meals and snacks. Insulin treatment after two weeks of failed dietary therapy.	Target fasting glucose <5.0 mmol/L and post-prandial level <6.7 mmol/L.	<72 h	Triceps, biceps, suprailiac and subscapular	ST: <u>Normal weight group</u> Not significantly different sum of ST (triceps, biceps, subscapular, suprailiac) (18.6 ± 3.7 vs 17.8 ± 3.1, p > 0.05) <u>Obese group</u> Not significantly different sum of ST (19.9 ± 44 vs 19.0 ± 3.5, p > 0.05)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
Prentice, 2019 **Prospective cohort** **2001–2009 and 2011–2013** **Rosie Maternity Hospital, Cambridge, UK [411]** **(additional data provided by authors)**	Earlier GDM = 98 (53%) Recent GDM = 122 (54%) Recent NGT = 876 (52%)	At around 28 weeks using IADPSG criteria	"Earlier" GDM was mostly treated with diet and lifestyle modification, with or without insulin. 19% of the 'earlier' GDM group were not diagnosed and did not receive any treatment. "Recent" all GDM women received standardised dietary and lifestyle advice and metformin and/or insulin if required.	Not reported	< 8 days	Triceps, subscapular, flank, quadriceps	ST: Earlier GDM Not significantly different sum of ST (triceps, subscapular, flank, quadriceps) (26.0 ± 6.3 vs 24.6 ± 6.0) Significantly higher skinfold SDS (0.31 ± 0.85 vs 0.03 ± 0.86) Recent GDM Significantly lower sum of ST (20.0 ± 3.6 vs 24.6 ± 6.0) Significantly lower skinfold SDS (−0.41 ± 0.61 vs 0.03 ± 0.86)
Buhling, 2012 **Prospective cohort** **2005–2006** **Hamburg, Germany [404]** **(additional data provided by authors)**	NGT = 142 GDM = 30	GDM was defined according to the clinic's guidelines, O'Sullivan criteria.	Treated with diet or diet + insulin.	Not reported	< 72 h	Left anterior iliac spine, at the lower angle of the left scapula, at the middle of the femur, above the left quadriceps femoris and at the middle of the left triceps, midway between acromion and olecranon	ST: Not significantly different all 4 sites triceps, 4.6 ± 0.9 vs 4.8 ± 1.5, p = 0.67 scapular, 4.3 ± 1.41 vs 4.1 ± 0.97, p = 0.54 iliac, 4.4 ± 1.3 vs 4.2 ± 1.0, p = 0.45 femur, 5.2 ± 1.8 vs4.7 ± 1.4, p = 0.72
(b) Studies that measured body composition						**'Dauncy et al. equation [413]'**	
Enzi, 1980 **Cohort** **Italy [394]**	NGT = 17 GDM = 17	White's classification, class A (abnormal glucose tolerance that reverted to normal postpartum)	Low-carbohydrate diet	Not reported	Birth	FM and FM% calculated by Dauncy et al. equation Sum of subscapular, subcostal, tricipital, and crural ST	FM: Not significantly different (553 ± 49 vs 386 ± 22) %FM: Significantly higher (17.1 ± 1.7 vs 12.2 ± 0.5) ST: Significantly higher sum of ST (23.0 ± 1.4 vs 17.8 ± 0.7)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
Naf, 2012 **Prospective case-control** **Joan XXIII University Hospital, Tarragona, Spain [405]**	NGT = 130 (46.1%) GDM = 84 (53.2%)	National Diabetes Data Group criteria were used to define GDM before 30 weeks	Diet = 48 Diet + insulin = 29	Target fasting glucose values <5.3 mmol/L and or 1-hour post-prandial values <7.8 mmol/L. GDM women had higher levels of fasting glucose 4.5 ± 0.4 vs 4.8 ± 0.6 mmol/L	<48 h	FM by Dauncy et al. equation. Triceps, biceps, subscapular, and flank ST (data not given)	FM: Not significantly different (291 ± 131 vs 318 ± 133, p = 0.198)
							'Weststrate and Deurenberg equation [118]'
Ubel, 2014 **Cohort** **Abteilung für Geburtshilfe und Perinatalmedizin der Frauenklinik, Klinikum rechts der Isar, Technische Universität München Munich, Germany [406]**	Lean NGT = 15 (46.7%) Obese NGT = 13 (61.5%) Obese GDM = 16 (81.3%)	Hyperglycaemia and Pregnancy Outcome (HAPO) criteria	Diet = 7 Insulin treated = 9	fasting BGL at 3rd trimester did not significantly differ between the groups and was <5.1 mmol/L	1 week	FM by the equations of Weststrate and Deurenberg Sum of Biceps,triceps, subscapular, suprailiac	FM: Significantly higher compared to lean NGT (694 ± 117, vs 583 ± 139, p < 0.05); Not significantly different compared to obese NGT (694 ± 117, vs 660 ± 114, p > 0.05) ST: Significantly higher compared to lean NGT (21.6 ± 2.4 vs 18.9 ± 3.1) Not significantly different compared to obese NGT (21.6 ± 2.4 vs 20.3 ± 2.6)
							'Catalano et al. equation [131]'
Aman, 2011 **Case-control** **Örebro University Hospital, Sweden [63]**	NGT= 28 GDM = 10	2-hour capillary whole-blood glucose concentration above 11 mmol/l, following a 75 g OGTT after 24th week of pregnancy	Dietary adjustments and multiple pre-meal insulin injections.	Daily blood glucose target, HbA1c 3.5–5.3% Glycaemic control was fairly good, with mean HbA1c values below the upper reference limit for healthy from the 24th to the 36th week of gestation.	< 2 days	FM by Catalano et al., equation. Triceps, subscapular and abdomen flank ST	FM: Significantly higher (700 ± 200 vs 500 ± 200, p < 0.01) %FM: Significantly higher (17.0 ± 3.2 vs 13.5 ± 3.5, p < 0.01) ST: Significantly higher in triceps (6.6 ± 1.7 vs 5.3 ± 1.1, p < 0.05) and subscapular (6.0 ± 2.1 vs 4.8 ± 1.1, p < 0.05) Not significantly different in abdominal flank (5.1 ± 1.5 vs 3.9 ± 1.0, p > 0.05)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
Schaefer-Graf,2011 **Cohort** **2007–2008** **Vivantes Medical Center, Berlin, Germany [403]**	NGT = 190 (48.4%) GDM = 150 (44.0%)	American Diabetes Association criteria for measurements in venous plasma. With respect to lower glucose concentrations in capillary compared with venous blood, the threshold for fasting glucose was modified into 5.0 mmol/L, while post challenge capillary glucose levels correspond with those in venous blood.	Dietary instruction and performed self-monitoring of BGL. Insulin therapy given before 36 weeks gestation based on BGL and/or foetal abdominal circumference (AC).	fasting <5.0 mmol/L or 2-h postprandial < 6.7 mmol/L or when AC > 75th percentile fasting <5.0 mmol/L or 2-h postprandial <11.1 mmol/L 'Well-controlled' Maternal serum glucose levels did not differ between control subjects and women with GDM	<48 h	FM by Catalano et al., equation.	FM: Significantly higher (433 ± 14 vs 381 ± 13, p < 0.01)
Maple-Brown, 2019 **Longitudinal cohort study** **2011–2017** **Northern Territory, Australia [410]**	Indigenous NGT = 117 Indigenous GDM/DIP = 278 Non-indigenous NGT = 118 Non-indigenous GDM/DIP* = 461	GDM were diagnosed by either the ADIPS guidelines or a universal 75 gm OGTT and revised glucose cut points as recommended by the WHO. DIP, was defined as diabetes first identified in pregnancy, but with glucose or HbA1c values higher glucose than GDM), and identified from medical records	Diet only or Metformin only or Insulin only or Metformin and insulin	Not reported	<72 h	FM by Catalano et al. equation.	FM: Not significantly different (11.3 ± 4.2 vs 11.5 ± 3.7, p = 0.65) <u>Non-indigenous</u> Significantly lower (10.2 ± 3.7 vs 11.5 ± 3.5, p = 0.0006)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
Samsuddin, 2020 **Prospective cohort** **2014–2017** **Tertiary antenatal clinic, Kuala Lumpur, Malaysia [412]**	Obese NGT = 94 Non-obese NGT = 268 GDM = 145 BMI categories (Asian) Normal:18.5–22.9 kg/m^2; Overweight: 23–27.4 kg/m^2; Obese: ≥27.5 kg/m^2	FPG ≥ 5.1 mmol/L and/or 2-hour glucose ≥7.8 mmol/L after a 75 g OGTT (based on the study center's definition and the Malaysian 2015 Clinical Practice Guideline	Nutrition therapy. If >30% of the self-monitoring of blood glucose values is beyond target despite compliance with medical nutrition therapy, insulin therapy is initiated	The glycaemic targets for GDM in the study center: fasting 3.5 – 5.1 mmol/L, pre-meals 4.0 -5.8 mmol/L, 2-hours post-prandial 4.0 - 6.7mmol/L. Well-treated GDM mothers (pre-delivery HbA1c 5.3%)	<24 h	FM by Catalano et al. equation. Sum of flank, triceps, subscapular ST	FM: Not significantly different compared to non-obese NGT (909 ± 113 vs 924 ± 149, p > 0.05) Significantly lower compared to obese NGT (909 ± 113 vs 973 ± 149, p < 0.05) ST: Significantly lower sum of ST (flank, triceps, subscapular) compared to obese NGT (14.2 ± 3.0 vs 16.1 ± 5.3, p < 0.05) Not significantly different compared to non-obese NGT (14.2 ± 3.0 vs 14.4 ± 2.8, p > 0.05)
						'TOBEC'	
Okereke, 2001 **Cohort** **1998–2000** **Metro Health Medical Center, Cleveland, USA [398]**	NGT = 44 (58.8%) GDM = 34 (59.1%)	Carpenter and Coustan criteria	Diet = 23 Diet + insulin = 11	Not reported	<48 h	TOBEC paediatric model HP-2	FM: Significantly higher (480 ± 210 vs 360 ± 150, p = 0.01) %FM: Significantly higher (13.2 ± 4.3 vs 10.5 ± 3.8, p = 0.01)
Catalano, 2003 **Prospective cohort** **1990 -2000** **Pregnancy Diabetes Clinic in Cleveland** **Ohio, USA [212]**	NGT = 220 (54%) GDM = 195 (51%)	At 26 to 28 weeks using National Diabetes Data Group criteria	Diet only = 128 Diet + insulin = 67	Targeted fasting glucose >5.5 mmol/L and/or 2-hour post-prandial glucose >6.7 mmol/L. Women maintained glucose values within the target range with diet and exercise (66%), plus insulin (34%).	<72 h	TOBEC Triceps and subscapular, flank, thigh, abdominal ST	FM: Significantly higher (436 ± 206 vs 362 ± 198, p = 0.0002) %FM: Significantly higher (12.4 ± 4.6 vs 10.4 ± 4.6, p = 0.0001) ST: Significantly higher at all 5 sites triceps (4.7 ± 1.1 vs 4.2 ± 1.3, p = 0.0001) subscapular (5.4 ± 1.4 vs 4.6 ± 1.2, p = 0.0001) flank (4.2 ± 1.2 vs 3.8 ± 1.0, p = 0.0001) thigh (6.0 ± 1.4 vs 5.4 ± 1.5, p = 0.0001) abdominal wall (3.5 ± 0.9 vs 3.0 ± 0.8, p = 0.0001)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
						'ADP (PEA POD)'	
Brumbaugh,2013 **Cross-sectional** **University of Colorado Hospital or Denver Health. Colorado, USA [355]**	Normal NGT = 13 (53.8%) Obese/GDM = 12 (66.7%) Both groups matched for ethnicity	At 24–28 weeks using Carpenter and Coustan criteria	2 were diet control, 10 required insulin or glyburide.	Not reported	1–3 weeks	ADP (PEA POD) Sum of triceps and subscapular ST	%FM: Not significantly different 14.7 ± 3.0 vs 13.1 ± 5.0, p = 0.36 ST: Significantly higher sum of ST (11.7 ± 1.3 vs 9.9 ± 2.0, p = 0.01
Lingwood, 2011 **Prospective cohort** **2009–2010[a]** **Royal Brisbane and Women's Hospital Queensland, Australia [55] (additional data provided by authors)**	NGT = 77 (53%) GDM = 84 (50%)	ADIPS criteria	Dietary and physical activity advise. Insulin treatment was begun if more than two glucose measurements exceeded the target range in 1 week.	Target BGLs were set according to current ADIPS guidelines: 5.5 mmol/L or lower fasting, and 7.0 mmol/L or lower 2-h post-prandial. 80% met both current fasting and post-prandial ADIPS targets. 75% met the lower targets of the American Diabetes Association (5.3 and 6.7 mmol/L)	<6 days	ADP (PEA POD)	FM: Significantly higher (413 ± 192 vs 350 ± 162, p = 0.003) %FM: Significantly higher (12.1 ± 4.3 vs 10.1 ± 4.1, p = 0.003)

First Author, Year, Study Design, Time of Data Collection, Location	Study Groups *n* (Males%)	GDM Identification/ Definition	Treatment(s)	Target Blood Glucose Levels (BGLs) and Level of Glycaemic Control	Infants' Age	Infants' Body Composition Assessment Method	Findings
Au, 2013 **Cross-sectional** **September-October 2010** **Royal Prince Alfred Hospital** **Sydney, Australia [64]**	NGT = 532 (53%) GDM = 67 (42%)	ADIPS criteria.	Dietary and physical activity advice. Insulin therapy was commenced when glycaemic targets could not be met.	Good glycaemic control was achieved in 90% of women meeting both fasting and post-prandial ADIPS targets	<48 h	ADP (PEA POD)	%FM: Not significantly different 7.9 ± 4.5 vs 9.3 ± 4.3, p = 0.018
Andersson-Hall, 2018 **Longitudinal cohort** **2009–2018** **6 antenatal health units and Sahlgrenska University Hospital** **Gothenburg, Sweden [409]**	Normal weight group 83 (50.6%) Obese group 26 (65.4%) GDM group 26 (38.5%)	All pregnant women had non-fasting blood glucose measured regularly throughout pregnancy, and women with an elevated non-fasting glucose (> 8 mmol/l) underwent OGTT. GDM mothers were identified based on the European Association for the Study of Diabetes criteria, at 27 ± 7 gestational weeks.	All 26 received diet and lifestyle advice, 4 received insulin.	Not reported	4–10 days	ADP (PEA POD)	FM: Normal weight group Significantly different (640 ± 200 vs 500 ± 230, p = 0.0034) Obese group Not significantly different sum of ST (640 ± 200 vs 580 ± 170, p = 0.29) %FM: Normal weight group Significantly different (16.44 ± 4.68 vs 13.5 ± 4.6, p = 0.0036) Obese group Not significantly different sum of ST (16.44 ± 4.68 vs 15.23 ± 3.86, p = 0.26)

Studies are grouped according to the type of the outcome, and within these groups, the studies are sub-grouped according to the body composition technique used. %FM: percent fat mass; ADP: air displacement plethysmography; AGA: appropriate for gestational age; BMI: body mass index (kg/m^2); h: hours; FM: fat mass (g); GDM: gestational diabetes mellitus; IGDMtr: infants exposed to treated GDM; INGT: infants exposed to normal glucose tolerance; LGA: large for gestational age; NGT: normal glucose tolerance; OGTT: oral glucose tolerance test; ST: skinfold thickness (mm); TOBEC: total body electrical conductivity; *DIP: diabetes in pregnancy (defined as diabetes first identified in pregnancy, but meeting glucose or HbA1c values diagnostic of overt diabetes outside pregnancy); ‡ body composition data for 'treated GDM vs. NGT' subgroup are presented as IGDMtr vs. INGT.3.2.1. GDM screening criteria and target blood glucose concentrations.

Table 5.2 Diagnostic criteria for GDM used by the studies included in the review.

Criteria name	Year	Screening oral glucose challenge test (OGCT)	Diagnostic oral glucose tolerance test (OGTT)	Studies in the review

		Dose	1h mmol/L	Fasting mmol/L	Dose	1h mmol/L	2h mmol/L	3h mmol/L	Abnormal values	
O' Sullivan and Mahan **[414]**	1964	50 g fasting	140	5.0	100 g	9.2	8.1	6.9	2 or more	[395]
White's Classification **[415]**	1978	Pregnant women are categorised according to duration and age of onset of diabetes								[394]
US National Diabetes Data Group **[416]**	1979	50 g fasting	7.8	5.8	100 g	10.5	9.1	8.0	2 or more	[212, 405]
Carpenter and Coustan **[417]**	1982	50 g fasting	7.2	5.3	100 g	10.0	8.6	7.8	2 or more	[355, 396, 398, 402]
Modified O'Sullivan Criteria by Court et al. **[418]**	1985	50 g fasting	7.8	5.8	100 g	10.0	8.9	7.8	2 or more	[397]
European Association for the Study of Diabetes (EASD) **[419]**	1991	_	_	7.0	75 g	11.0	9.0	_	Either the 0-h or 1-h concentration should meet or exceed stated values in addition to a 2-h value	[409]
Australasian Diabetes In Pregnancy Society (ADIPS) **[420]**	1998	50 g or 75 g non-fasting	50 g: 7.8 or 75 g: 8.0	5.5	75 g	_	8.0 or 9.0 *	_	1 or more	[55, 64, 399, 401]
4th International workshop conference **[60]**	1998	50 g fasting	7.2 or 7.8	5.3	100 g or 75 g	10.0	8.6	7.8 (3 hr value only for 100 g test)	2 or more	[214]
American Diabetes Association (ADA) **[421]**	2004	50 g fasting	7.2 or 7.8	5.3	100 g or 75 g	10.0	8.6	7.8 (3 hr value only for 100 g test)	2 or more	[403, 404]
International Association of Diabetes and Pregnancy Study Groups (IADPSG) **[422]**	2010	_	_	5.1	75 g	10.0	8.5	_	1 or more	[406, 408, 410, 411]
World Health Organisation (WHO) **[423]**	2014	_	_	5.1–6.9	75 g	10.0	8.5–11.0	_	1 or more	[407]

Guidelines are ordered according to the year of the publication. All values are for venous plasma glucose, except for O'Sullivan and Mahan criteria, which are venous whole blood glucose. IADPSG criteria are based on the Hyperglycaemia and Pregnancy Outcome (HAPO) study. OGCT: oral glucose challenge test; OGTT: oral glucose tolerance test.

5.4.3 Adiposity Assessment techniques used in the studies

Anthropometric and/or body composition information was available in 13 studies, including ADP [55, 64, 355, 409], total body electrical conductivity (TOBEC) [212, 398, 402], or anthropometric equations proposed by Catalano et al. [63, 214, 403, 410, 412], Dauncy et al. [394, 405], and Weststrate and Deurenberg [406]. The most commonly assessed individual ST sites were triceps and subscapular, and four studies [406, 408, 411, 412] presented the sum of ST at different sites (data of individual sites were not available).

5.4.4 Quality assessment

Of the eight criteria listed in the Evidence Project risk of bias tool, two criteria, "(3) pre-post intervention data" and "(8) comparison groups equivalent at baseline on outcome measures", were not applicable for the studies selected for this review (Table 5.3). All selected studies used non-probability sampling strategies (convenience or self-selected sampling); thus, the criterion "random selection of participants for assessment" was not met by any of them. All studies met the "control or comparison group" criterion. Nineteen studies [55, 212, 214, 394, 396-398, 401-412] met the criterion "cohort", and except for 1 study [397], all others met the criterion of "follow-up rate of 80% or more". Only the three randomised control trials (RCT) [214, 401, 402] included in the review met the criterion of "(4) random assignment of participants to the intervention". Results of the assessment of the criterion "(7) comparison groups equivalent on sociodemographics" varied across the studies, with the following outcomes: "Equivalent" [355, 397, 400, 402], "Partially Equivalent" [212, 214, 398, 399, 401, 403-405, 407, 409, 411], "Not Equivalent" [64, 406, 412], and "Not Reported" [55, 63, 394-396, 408, 410].

Table 5.3 Quality assessment of the studies included in the review, using the Evidence Project risk of bias tool.

First Author	Year	Evidence Project risk of bias tool items									
		(1) Cohort	(2) Control or Comparison Group	(3) Pre/Post Intervention Data	(4) Random Assignment of Participants to the Intervention	(5) Random Selection of Participants for Assessment	(6) Follow-up Rate of 80% or More [a]		(7) Comparison Groups Equivalent on Sociodemographic [b]		(8) Comparison Groups Equivalent at Baseline on Disclosure
		Judgement	Judgement	Judgement	Judgement	Judgement	Judgement	Follow-up rate	Judgement	Comment	Judgement
Enzi	1980	Yes	Yes	No	NA	No	Yes	87.5%	NR		NA
Stevenson	1991	No	Yes	No	NA	No	NA		NR		NA
Vohr	1995	Yes	Yes	No	NA	No	Yes	100%	NR		NA
Simmons	1997	Yes	Yes	No	NA	No	No	57%	Yes	Ethnicity and sex not significantly different	NA
Okereke	2001	Yes	Yes	No	NA	No	Yes	100%	Partial	Sex not significantly different, ethnicity significantly different	NA
Catalano	2003	Yes	Yes	No	NA	No	Yes	100%	Partial	Ethnicity significantly different, sex not significantly different	NA
Ng	2004	No	Yes	No	NA	No	NA		Partial	Sex not significantly different	NA
Westgate	2006	No	Yes	No	NA	No	NA		Yes	Sex and ethnicity not significantly different	NA
Rowan	2008	Yes	Yes	No	Yes	No	Yes	97.6%	Partial	Ethnicity not significantly different	NA
Lain	2009	Yes	Yes	No	Yes	No	Yes	82.8%	Yes		NA
Landon	2009	Yes	Yes	No	Yes	No	Yes	93.9%	Partial	Ethnicity not significantly different	NA
Aman	2011	No	Yes	No	NA	No	NA		NR		NA
Lingwood	2011	Yes	Yes	No	NA	No	Yes	100%	NR		NA
Naf	2011	Yes	Yes	No	NA	No	Yes	100%	Partial	Sex not significantly different	NA
Schaefer-Graf	2011	Yes	Yes	No	NA	No	Yes	100%	Partial	Sex not significantly different	NA

First Author	Year	Evidence Project risk of bias tool items									
		(1) Cohort	(2) Control or Comparison Group	(3) Pre/Post Intervention Data	(4) Random Assignment of Participants to the Intervention	(5) Random Selection of Participants for Assessment	(6) Follow-up Rate of 80% or More [a]		(7) Comparison Groups Equivalent on Sociodemographic [b]		(8) Comparison Groups Equivalent at Baseline on Disclosure
		Judgement	Judgement	Judgement	Judgement	Judgement	Judgement	Follow-up rate	Judgement	Comment	Judgement
Au	2012	No	Yes	No	NA	No	NA		No	Significant difference in maternal ethnicity	NA
Buhling	2012	Yes	Yes	No	NA	No	Yes	100%	Partial	Ethnicity not significantly different	NA
Brumbaugh	2013	No	Yes	No	NA	No	NA		Yes	Sex and ethnicity not significantly different	NA
Ubel	2014	Yes	Yes	No	NA	No	Yes	100%	No	Sex significantly different	NA
Mitanchez	2017	yes	Yes	No	NA	No	Yes	90.3%	NR		NA
Kara	2017	Yes	Yes	No	NA	No	Yes	100%	Partial	Sex not significantly different	NA
Andersson-Hall	2018	Yes	Yes	No	NA	No	Yes	83%	Partial	Sex not significantly different	NA
Maple-Brown	2019	Yes	Yes	No	NA	No	Yes	100%	NR		NA
Prentice	2019	Yes	Yes	No	NA	No	NR		Partial	Sex not significantly different	NA
Samsuddin	2020	Yes	Yes	No	NA	No	Yes	100%	No	Ethnicity significantly different	NA

NA: not applicable; NR: not reported. Studies are ordered according to the year of the publication. [a] Follow-up rate was calculated as the number of participants at the final assessment*100 divided by the number of participants at the first assessment, as stated in the paper. [b] Infant sex and ethnicity were considered as sociodemographic characteristics. If the authors have only reported that the study arms are equivalent on one of the sociodemographic characteristics, it was indicated as "Partial", while if the study arms were not equivalent at least on one of the socio-demographics, it was decided that the criterion was not met ("No").

5.4.5 Effects of treatments for GDM on infant adiposity

5.4.5.1 Treated GDM vs no Treatment for GDM

One RCT [214] investigated whether treatment for GDM normalised infant adiposity at birth. In this study of 958 GDM women (485 treated vs 473 no treatment), mean FM in infants of GDM mothers who received the treatment of diet therapy (n = 427) and insulin, if required (n = 36), was significantly lower than that of control infants whose mothers received usual prenatal care (427 ± 198 g vs 464 ± 222 g, p = 0.003).

5.4.5.2 Different Treatment Regimens for GDM

Two studies [212, 397] compared the effects of treating GDM with lifestyle modification alone vs lifestyle modification plus insulin, on infant birth measurements. A small study [397] with a sample size of 20, found no significant differences in mean subscapular ST, between GDM exposed infants whose mothers were treated with 'diet alone' and 'diet with insulin'. A comparatively larger study [212], with a sample size of 195, revealed that compared to 'diet and exercise only', infants whose mothers were treated with 'diet, exercise and insulin' had higher FM (492 ± 215 g vs 407 ± 196 g, p = 0.006) and %BF (13.6% ± 4.6% vs 11.7 ± 4.5%, p = 0.007). These effects persisted even after adjusting for gestational age, maternal pregravid weight and parity. Two RCTs [401, 402] investigated the difference in adiposity in infants of GDM mothers, who were treated with pharmacological treatments for GDM. Rowan et al. [401] compared treating GDM women with metformin (with supplemental insulin, if required, n = 363) to treatment with insulin alone (n = 370) and reported that triceps (5.2 ± 1.6 vs 5.1 ± 1.2, p = 0.30) and subscapular (5.2 ± 1.5 vs 5.2 ± 1.3, p = 0.60) ST (mm) were not significantly different between the groups. Lain et al. [402] compared insulin (n = 41) with glyburide (n = 41), and found no significant differences in mean triceps ST (3.9 ± 0.7 vs 3.9 ± 0.9, p = 0.89), subscapular ST (4.1 ± 1.0 vs 4.5 ± 1.3, p = 0.10), suprailiac ST (2.1 ± 0.6 vs 2.1 ± 0.6, p = 0.85), thigh ST (5.1 ± 1.2 vs 5.4 ± 1.7, p = 0.28), FM (370 ± 167 vs 473 ± 278, p = 0.06) or %FM (11.2 ± 4.2 vs 12.8 ± 5.7, p = 0.18). None of the studies compared 'lifestyle modification alone' with 'pharmaceutical interventions'.

5.4.5.3 IGDMtr vs INGT

5.4.5.3.1 FM

Ten studies [55, 63, 212, 394, 398, 403, 405, 406, 409, 412] reported the effect of treated GDM compared to NGT on infant FM. Overall, IGDMtr had significantly higher FM (mean difference, 95% CI: 68.46 g, 29.91 to 107.01) than INGT (Figure 5.3).

5.4.5.3.2 %FM

Nine studies [55, 63, 64, 212, 355, 394, 398, 409, 410] investigated the effect of treated GDM compared to NGT on infant %FM. In the pooled result, %FM (1.98%, 0.54 to 3.42) in IGDMtr was significantly higher than INGT (Figure 5.4).

5.4.5.3.3 ST

The number of studies that reported ST at individual skinfold sites were as follows: triceps = 8 [63, 212, 395, 396, 400, 404, 407, 411]; subscapular = 7 [63, 212, 396, 400, 404, 407, 411]; flank = 3 [63, 212, 411]; and abdominal = 2 [212, 396]. None of the comparisons of skinfold sites were significantly different between IGDMtr and INGT infants in the pooled results; triceps: 0.14 mm, −0.35 to 0.63 (Figure 5.5A); subscapular: 0.44 mm, −0.15 to 1.02 (Figure 5.5B); flank: 0.04 mm, −1.35 to 1.44 (Figure 5.5C) and abdominal: 0.33 mm, −0.06 to 0.72 (Figure 5.5D). Several other ST sites, i.e., biceps [407], quadriceps [411], suprailiac [396], iliac [404], femur [404], thigh [212] and calf [396], were reported in single studies, and therefore, a meta-analysis could not be performed.

Four studies compared IGDMtr vs INGT using sum of ST at different body sites, therefore they were not included in the meta-analysis. Of those, two reported that the sum of ST at 'triceps and subscapular'[355] and 'subscapular, subcostal, tricipital and crural' [394] was significantly higher in IGDMtr. Another study [412] reported that the sum of ST at 'flank, triceps and subscapular' was not significantly different between IGDMtr and INGT. A study [399] in which data were not normally distributed presented median and interquartile range and reported that triceps (4.8 mm (4.2–5.1) vs 4.7 mm (4.1–5.5)) and subscapular (4.8 mm (4.3–5.3) vs 4.8 mm (4.1–5.3)) ST were not significantly different between the two infant groups.

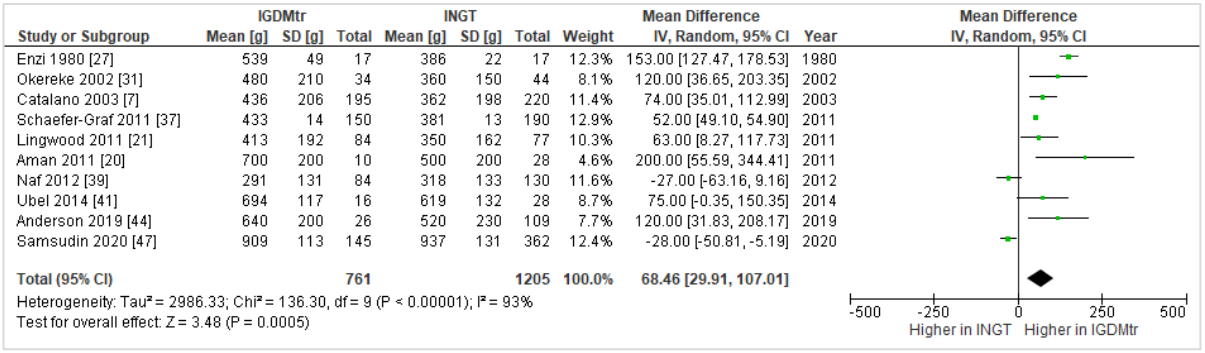


Figure 5.3 Forest plot comparing FM in IGDMtr and INGT.

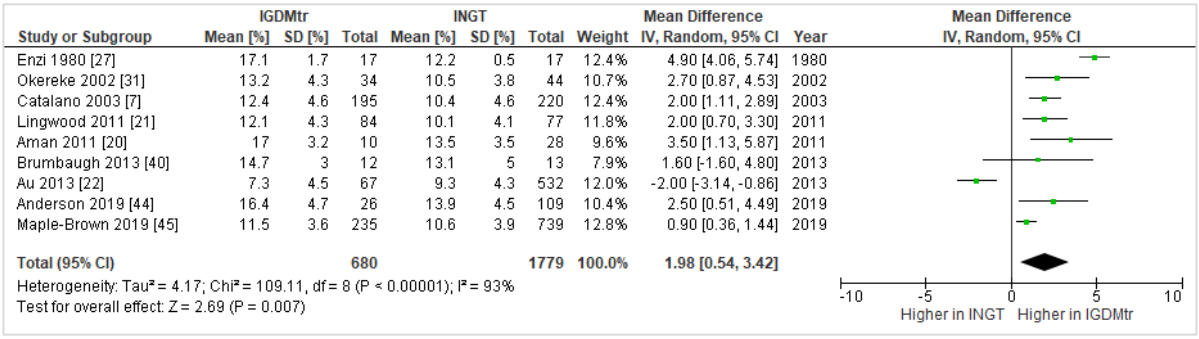
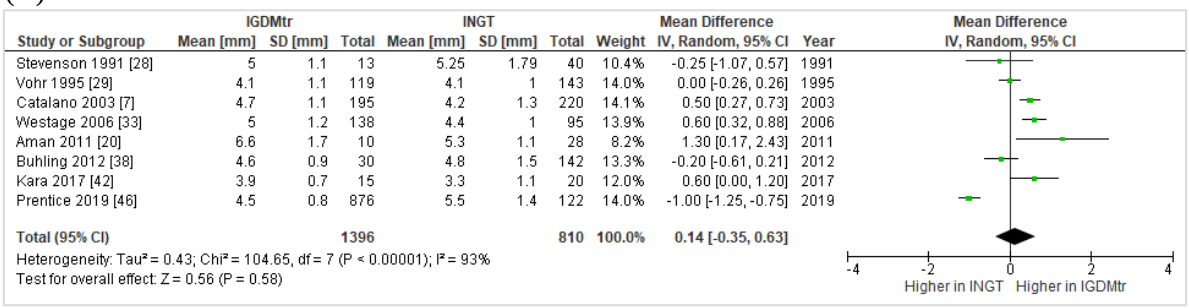
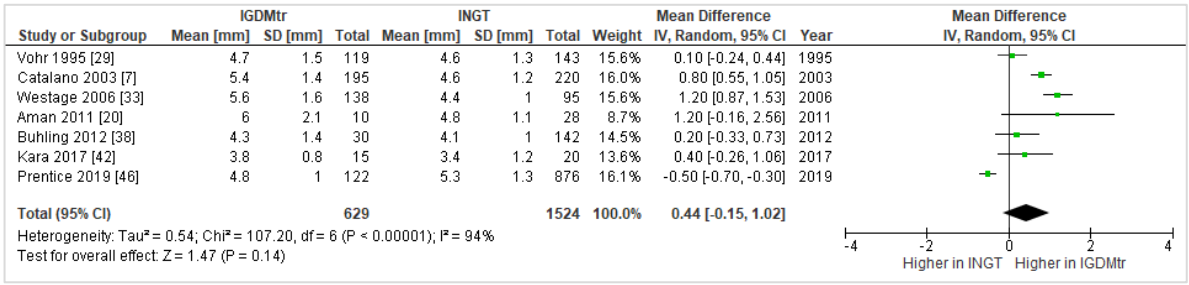


Figure 5.4 Forest plot comparing %FM in IGDMtr and INGT.

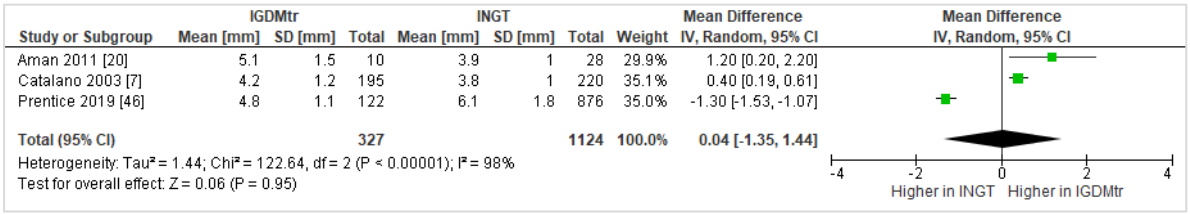
(A)



(B)



(C)



(D)

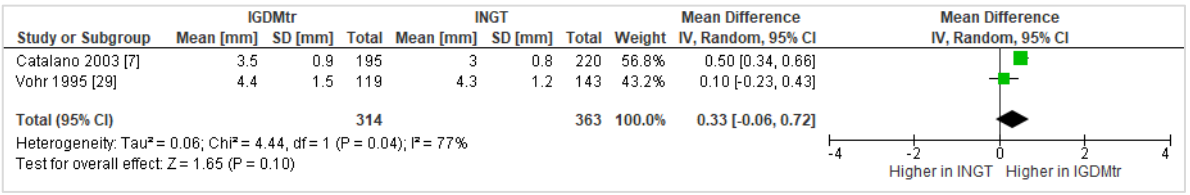


Figure 5.5 Forest plots comparing skinfold thickness at triceps (a), subscapular (b), flank (c) and abdomen (d) in IGDMtr and INGT.

5.4.5.4 Heterogeneity between the Studies that Compared adiposity in IGDMtr vs INGT

A high proportion of the observed heterogeneity in all the meta-analyses (as indicated by an I^2 statistic >90%) was due to underlying between-study differences [424]. We considered whether the GDM mothers achieved good glycaemic control with the treatments as one of the potential sources of heterogeneity. However, the information on the level of glycaemic control in GDM mothers was not reported in 40% of studies [355, 394, 397-400, 404, 408, 410, 411]. Therefore, the studies in which the authors stated that the mothers achieved good glycaemic control were separated from other studies, to see if the achievement of good glycaemic control mediated the relationship between GDM and infant adiposity. The test for subgroup differences indicated that there was no statistically significant subgroup effect of studies indicating GDM mothers achieving good glycaemic control on infant FM ($p = 0.76$), %FM ($p = 0.15$), triceps ($p = 0.34$) and subscapular ST ($p = 0.73$).

The test for subgroup differences in 'pre-2010′ vs 'post-2010′ studies showed a statistically significant subgroup effect on FM ($p = 0.03$, Figure 5.6) and %FM ($p = 0.02$, Figure 5.7). There was no significant difference in FM and %FM between IGDMtr and INGT in 'post-2010′ studies, whereas, in 'pre-2010′ studies, FM and %FM were significantly higher in IGDMtr compared to their counterparts. Further, subgroup analyses by infant body composition assessment technique were performed for infant FM and %FM. There was no significant effect ($p = 0.28$) of body composition technique on infant FM (Figure 5.8). Subgroup difference in %FM was significant ($p < 0.00001$); however, the number of studies and participants who contributed to subgroups were considerably different (Figure 5.9). %FM measured with ADP (0.93%, −1.61 to 3.47) or the Catalano et al. equation (1.93%, −0.56 to 4.43) did not significantly differ between IGDMtr and INGT. %FM measured by TOBEC (2.13%, 1.34 to 2.93) or the Dauncy et al. equation (4.90%, 4.06 to 5.74) was higher in IGDMtr. Leave-one-out sensitivity analysis demonstrated that removing the studies that had used the Catalano equation or TOBEC changed the overall effect for %FM to statistical non-significance. Of note, from the four studies that used ADP [55, 64, 355, 409], three [55, 64, 409] affirmed good glycaemic control in mothers. Sensitivity analysis performed after removing the study [355] with no data on glycaemic control did not change the pooled result for the ADP subgroup. Moreover, leave-one-GDM-criteria-out sensitivity testing for FM and %FM did not show significant changes in the pooled effects. Specifically, the sensitivity analysis for White's classification, which is different from other criteria that use an OGTT, did not significantly change the overall results for infant FM and %FM (Figure 5.10).

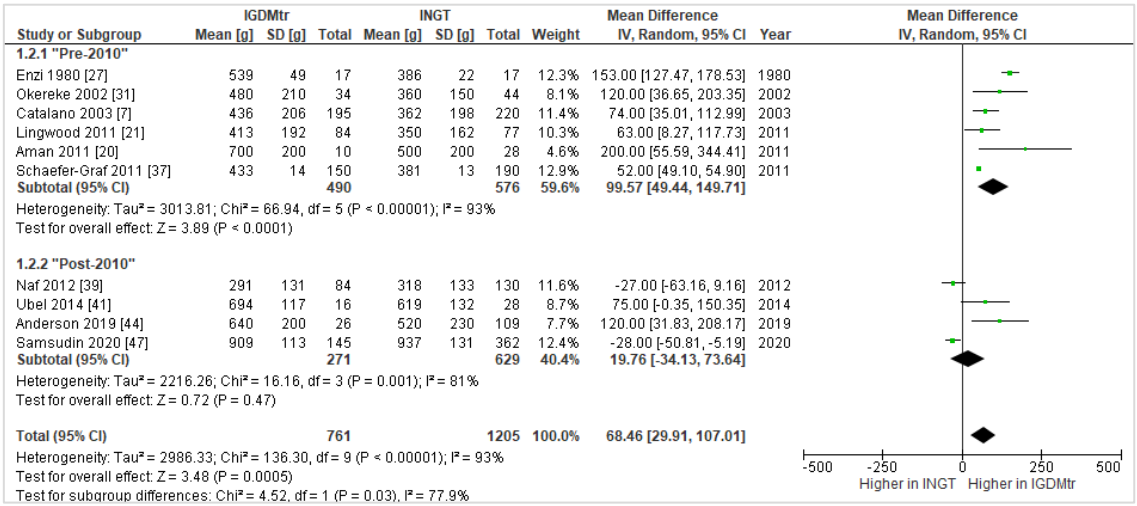


Figure 5.6 Forest plot comparing FM in IGDMtr and INGT by subgroup analysis of time of the study; 'pre-2010' vs 'post-2010'.

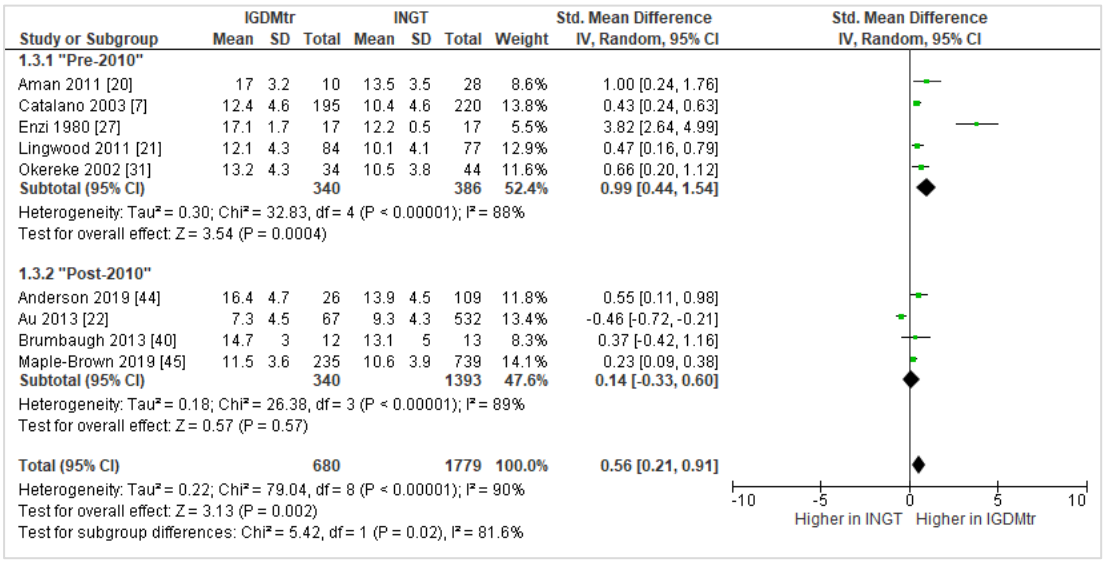


Figure 5.7 Forest plot comparing %FM in IGDMtr and INGT by subgroup analysis of time of the study; 'pre-2010' vs 'post-2010'.

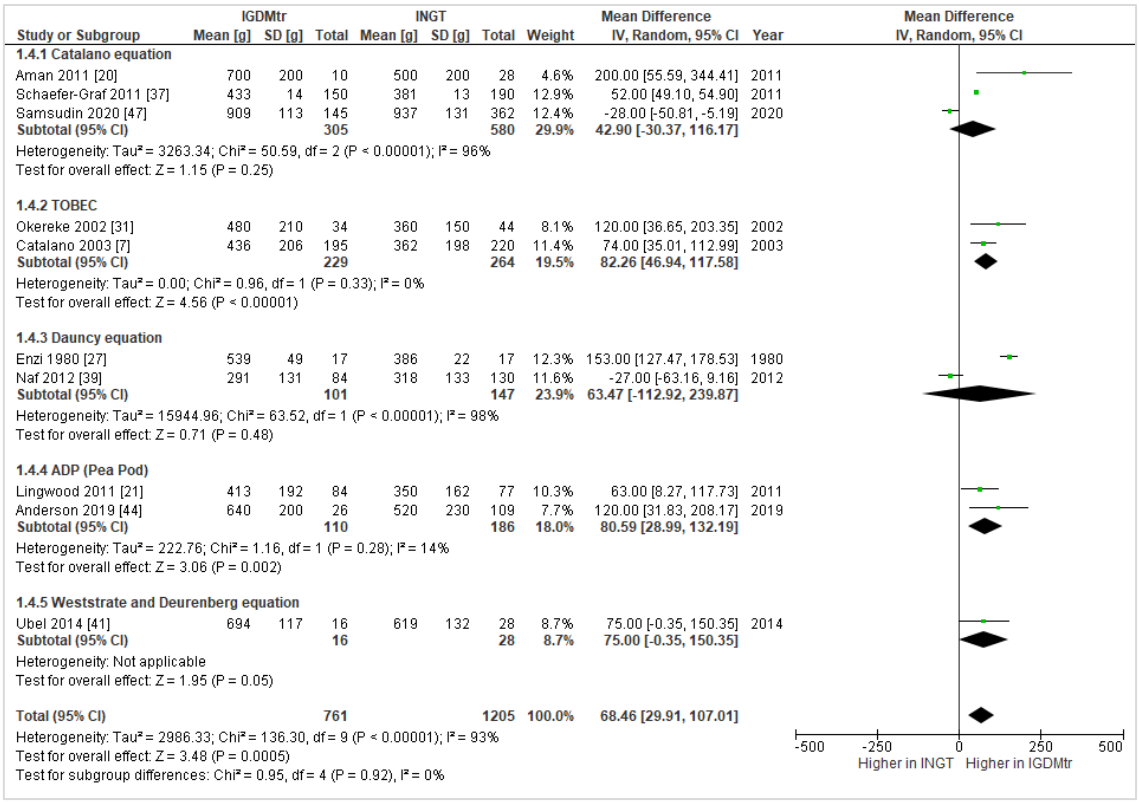


Figure 5.8 Forest plot comparing FM in IGDMtr and INGT by subgroup analysis of infant body composition assessment technique.

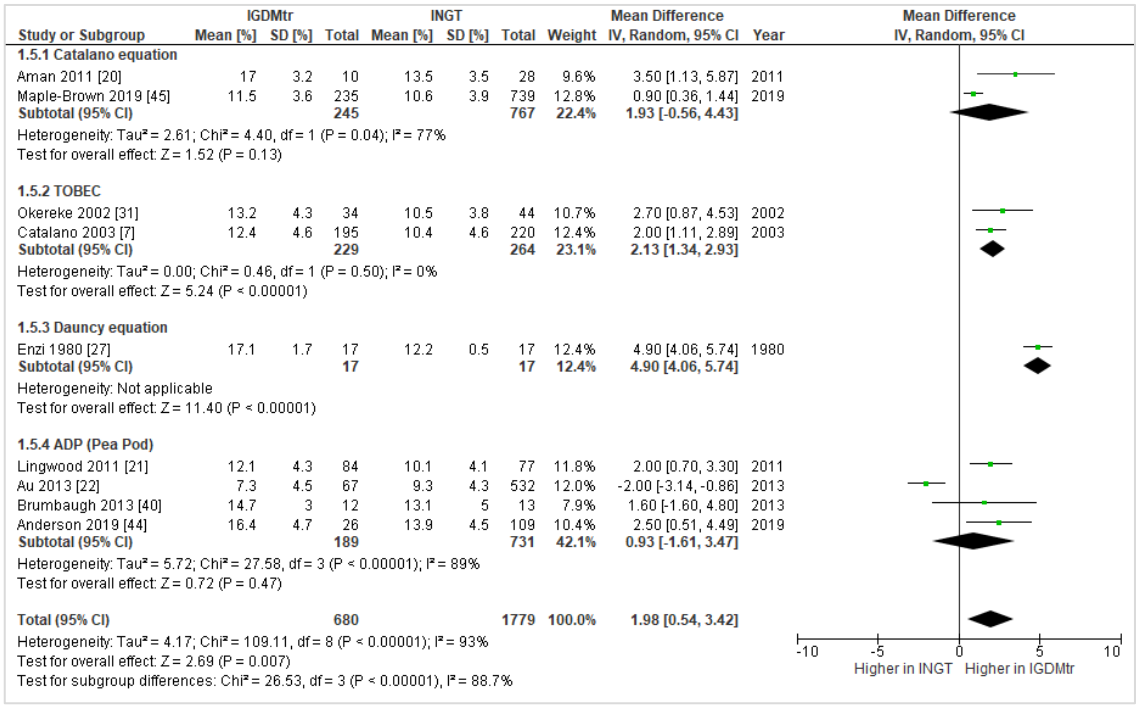
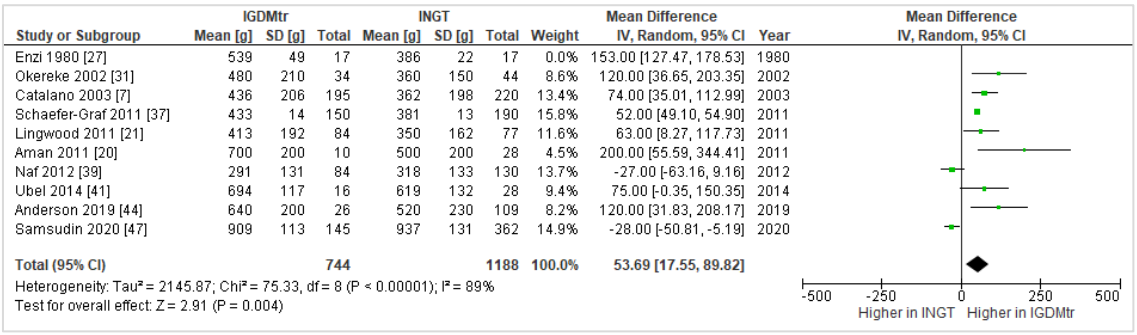


Figure 5.9 Forest plot comparing percent %FM in IGDMtr and INGT by subgroup analysis of infant body composition assessment technique.

(A)



(B)

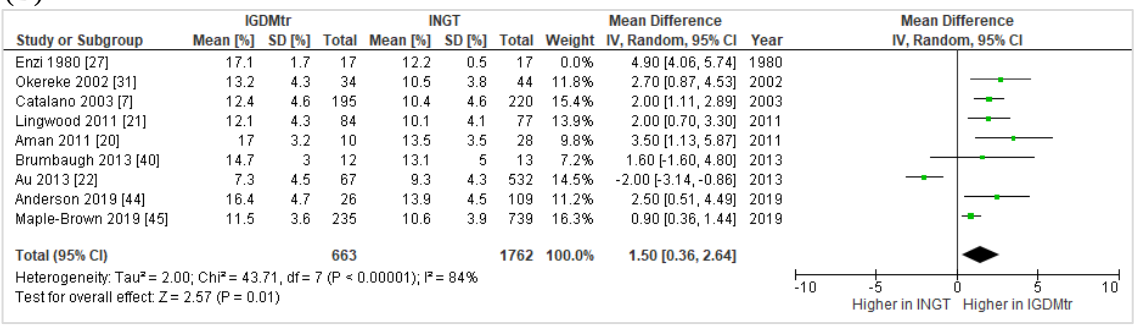


Figure 5.10 Forest plot comparing FM (a) and %FM (b) in IGDMtr and INGT excluding the effect of the study by Enzi et al. (1980).

5.5 DISCUSSION

We performed a systematic review and a meta-analysis of published studies (irrespective of the study designs) reporting adiposity in infants exposed to GDM controlled with therapeutic interventions. Treatment for GDM lowered newborn adiposity compared to no treatment, and there were no significant differences in adiposity in IGDMtr according to the mode of therapy; however, the evidence was insufficient due to the low number of available studies. The pooled result of all the studies (published between 1980-2020) included in the meta-analysis showed that IGDMtr had higher FM and %FM compared to INGT, and there was no significant difference in subcutaneous adiposity as measured by ST. However, a subgroup analysis indicated that the significant differences in overall adiposity between IGDMtr and INGT existed only in 'pre-2010' studies and there were no significant differences between the two infant groups in 'post-2010' studies.

Accelerated fat deposition in the foetus of GDM women can be reduced by strict glycaemic control [214]. Most women with GDM can control blood glucose with lifestyle changes such as diet modification, and increased physical activity; however, approximately one-third of women may require additional pharmacological treatments [384]. Oral diabetic medication is widely accepted by pregnant women in contrast to insulin because of easier storage, administration and lower cost [425], but unlike insulin, both metformin and glyburide cross the placenta [426]. Additionally, meta-analyses of risks and benefits of using insulin, metformin and glyburide in GDM women requiring drug treatment have shown that glyburide is inferior to both insulin and metformin, resulting in higher birthweights and increased risk of macrosomia, while metformin is associated with more preterm births than insulin [427]. On the other hand, insulin can bind to its specific receptor (in the placenta) to activate its signalling pathways; thus, insulin treatment still may have effects on placental and foetal growth [428]. One of the studies included in our review [212] reported that treatment with insulin in addition to lifestyle modification significantly increased the FM and %FM in IGDMtr as opposed to lifestyle intervention alone; however, the authors speculated that there might have been a confounding effect of other maternal factors associated with increased infant adiposity, as the former group of mothers (i.e., those who received insulin in addition to lifestyle changes) were characterised with higher pre-pregnancy weight and parity than their counterparts. Moreover, metformin and glyburide can impact foetal growth in opposite ways [429, 430]. Glyburide controls maternal hyperglycaemia by stimulating insulin production. When glyburide is transported to the foetus through the placenta, it may also increase insulin secretion by the foetal pancreas that results in foetal overgrowth [429]. On the other hand, metformin inhibits glucose and amino acid transportation from the mother to the developing foetus through the placenta [430], which may cause foetal undergrowth. Despite this,

the findings of the two RCTs included in our review [401, 402] suggested that the effects of metformin, glyburide or insulin on infant adiposity were not significantly different; nonetheless, more studies are required for definitive conclusions. As reported in two recent systematic reviews, although there are no significant differences in body composition at birth [431], children exposed to metformin *in utero* show accelerated postnatal growth, compared to those exposed to insulin [432]. Therefore, tracking body composition trajectory of children exposed to pharmacological interventions *in utero* should be a research priority.

Our meta-analysis shows that treatments for GDM normalise newborns' subcutaneous fat measured by ST, but not overall adiposity measured by FM and %FM. These findings suggest that the phenotype of the IGDMtr may be distinguished with increased non-subcutaneous adiposity. Increased intra-abdominal adiposity is associated with several metabolic disorders, while superficial subcutaneous adiposity may exert a protective effect [433]. Furthermore, exposure to excess fuels in the gestational environment may lead to increased hepatic fat deposition in the foetus, which possibly plays a role in the development of non-alcoholic liver disease in children [434]. On the other hand, the accuracy of ST measurements is dependent on the skills of the measurer, and the adiposity prediction equations with ST are highly specific to the infant population that the data were derived from [184]. Thus, differentiating adipose tissue compartments with more reliable objective techniques and assessing hepatic fat deposition in IGDMtr and INGT at birth is important to identify these differences and any effects of GDM treatments. Comparing different adiposity compartments was beyond the scope of the current review, and such studies are very limited. Two small studies [355, 435] reported that there were no significant differences in %FM, subcutaneous fat (cm^3) and intra-abdominal fat/length (cm^2) at 1–3 weeks [355], and in total adipose tissue (cm^3), subcutaneous adipose tissue (cm^3), internal abdominal adipose tissue (cm^3) at 1–2 weeks [435] in IGDMtr and INGT infants. Intriguingly, one study reported a significant increase in intrahepatocellular lipid content in IGDMtr compared to INGT, while the other did not detect such a difference. However, glycaemic control was not described in the former study, whereas ~80% of mothers in the latter study had good glycaemic control with a mean third-trimester HbA1c level of 5.3%.

There were no significant differences in FM or %FM in IGDMtr and INGT in studies 'post-2010′ or when newborn %FM was measured with ADP. These findings may be attributed to more intensive management of hyperglycaemia in the 'post-2010′ period. Following the HAPO study findings, the IADPSG proposing new diagnostic criteria in 2010, attempts have been made around the world to improve GDM diagnosis and management. Moreover, our findings highlight the importance of using more accurate and reliable objective infant body composition techniques such as ADP.

The high degree of between-study heterogeneity may have arisen from the use of a wide variety of GDM diagnostic criteria, differences in the severity of hyperglycaemia and level of glycaemic control, and confounding effects of maternal obesity, ethnicity, gestational weight gain, smoking, gestational age, infants' sex and age at the investigation. Future studies should adopt universal criteria for the diagnosis of GDM, use reliable body composition assessment techniques such as ADP, and report the treatments and level of glycaemic control in GDM mothers throughout the pregnancy to enable robust conclusions on the association between GDM and newborn adiposity.

To our knowledge, the current review is the first to simultaneously evaluate studies reporting adiposity in newborns exposed to treated GDM vs no treatment, different treatment regimens for GDM, and treated GDM vs NGT. Adiposity in infants exposed to GDM compared to NGT has been investigated in a subgroup analysis of a previous systematic review [65] that examined the literature focused on the effect of all types of maternal diabetes. The authors found higher FM, %FM, triceps ST and subscapular ST in GDM-exposed infants compared to NGT; however, in some of the studies included in their meta-analysis (e.g., HAPO Study [381]), mothers were not treated. Other strengths of our study include the search of the literature in five major databases, investigation of differences in ST sites such as abdominal and flank regions in addition to commonly reported triceps and subscapular measures, and investigation of potential sources of heterogeneity via subgroup and sensitivity analyses. Limitations of our study were that we only included studies published in English, excluded studies in which GDM status was self-reported by mothers where no information was reported on the use of treatments for glycaemic control, and considered only the most common measures of adiposity, i.e., FM, %FM and ST, for comparison purposes.

5.6 CONCLUSIONS

Irrespective of the therapeutic strategy, treatment for GDM appears to reduce excess adiposity characteristic for newborns exposed to untreated GDM, but the evidence is limited. Due to the potential effects of oral hypoglycaemic medications on foetal growth, further studies on the impact of different GDM therapies on newborn adiposity are also warranted. Despite the significant heterogeneity found between the studies, our meta-analysis of studies published 1980-2020 revealed higher overall adiposity (as measured with FM and %FM) but similar subcutaneous adiposity (as measured with ST) in IGDMtr compared to INGT, suggesting that higher adiposity in IGDMtr may be due to excess non-subcutaneous (e.g., visceral) fat accrual. Future studies should distinguish adipose tissue distribution of IGDMtr and INGT with sufficient power to confirm these differences. However, we did not observe differences in FM and %FM in studies in the last decade, indicating that glycaemic control in GDM mothers in these studies have normalised adiposity in infants at birth. This may be attributed to more intensive management of hyperglycaemia that has been adopted in the 'post-2010' period. This underscores the importance of rigorous control of hyperglycaemia in mothers with GDM through healthy maternal lifestyle choices or pharmacological interventions when suitable. Future studies should report the level of glycaemic control in mothers treated for GDM to enable robust conclusions.

CHAPTER 6 : A COMPARISON OF BODY COMPOSITION MEASURED WITH ADP (PEA POD) AND DD TECHNIQUE IN 6-MONTH-OLD INFANTS

6.1 ABSTRACT

Background: An appreciation of infant body composition is helpful to understanding the 'quality' of growth in early life and may have implications for predisposition to later overweight and obesity. ADP (using PEA POD) allows rapid, non-invasive and precise assessment of body composition in infants; however, it only accommodates infants up to ~6 months of age (or 8-10 kg body weight).

Aims(s): We evaluated the comparability of body composition assessed via ADP (PEA POD) and DD technique in 6-month-old infants.

Methods: FM, %FM and FMI obtained using PEA POD, and DD technique in 72 infants were compared using Bland-Altman analysis.

Results: No significant constant bias was found between the two methods (FM: bias = 25.26, 95%CI = -65.92 to 116.45; %FM: 0.33; -0.93 to 1.60; FMI: 0.06; -0.15 to 0.27); however, the limits of agreement (LOA) were wide and significant proportional bias was identified with DD technique underestimating infant fatness at lower mean values. For the mean values above the first quartile, LOA was somewhat narrow (FM: -667.84 to 519.91; %FM: -9.15 to 7.96; FMI: -1.58 to 1.27), and no significant proportional bias was detected ($p > 0.1$ for all).

Conclusions: DD technique may be a suitable alternative body composition method when infants cannot be accommodated in the PEA POD but only for those who are not at the lower end of the adiposity spectrum at 6 months of age.

6.2 INTRODUCTION

Assessment of infant body composition has become an increasingly important area of research due to the association between early growth and subsequent obesity and metabolic diseases [17, 436, 437]. Body composition can be assessed using a range of approaches and models, with the most common methods considering body weight as comprising various components. The four-component (4C) model that divides the body into FM, TBW, bone mineral, and protein, is considered as the gold standard reference method, while the most commonly used indirect methods divide the body into 2-compartments (2C): FM and FFM [98]. The use of various body composition techniques in paediatric populations is associated with practical challenges due to the distinctive physiological and behavioural characteristics of infants and children. For example, keeping an infant motionless for DXA can be challenging, whereas dose spillage can be a common problem when using DD technique in neonates [67]. ADP (PEA POD), assuming a 2C model, may be the only "practical" tool available at present for body composition assessment during early infancy [143]. The technique has several advantages over other approaches such as ease of use, very short assessment time, non-invasiveness, not affected by infants' behavioural state (e.g. movement, crying, urination) and good precision [66].

The major limitation of PEA POD is that it only accommodates infants up to 6 months of age (8-10 kg of body weight). The adult ADP system (BOD POD) adapted with a paediatric option has demonstrated good validity with the 4C model in children 2-6 years of age [154]. This leaves a gap in the use of ADP technology from 6 months to 2 years of age. Hence, for longitudinal research from birth to childhood, an alternative technique is required. To our knowledge, only a few studies [149-153] have compared body composition obtained with PEA POD to other techniques in infants. In these studies, PEA POD measurements have shown good agreement with body composition assessed using a 4C model [149] and DD [150], but not with DXA [151] in full-term infants. In preterm infants, ADP has shown good agreement with the 3-compartment (3C) model [152] and isotope dilution ($H_2{}^{18}O$) [153].

Despite being the ideal, the 4C model requires measurements of different body components; thus, it is expensive, and the associated participant burden is high. Anthropometry based prediction of body fat is simple, inexpensive and suitable for field settings; however, a poor agreement has been observed with PEA POD measurements in 0 to 6-month-old infants [128, 129]. DD may be a suitable alternative, as it can be used in all ages starting from birth and has several advantages, including safety, suitability for field use, and collected samples can be stored for long periods [117].

To date, only one study [150] has compared body composition in full-term infants measured using PEA POD and DD technique. This study compared %FM obtained using the two methods in 53 predominantly Asian infants aged 0.4-24.4 weeks. As ethnicity and postnatal age are significant predictors of infant body composition [50, 438], it may be worth testing the agreement of the two techniques in infants of other ethnicities and in larger samples of the same age. Moreover, %FM, although it is convenient as an index of fatness, is statistically biased as it can be affected by the changes in FM as well as FFM in the body [368]. FMI, normalises body fatness to body size (length in infants) and is suggested as a better index for understanding changes in adiposity over time [56]. In this study, we aimed to appraise the agreement of FM, %FM and FMI assessed via PEA POD in relation to the DD technique in predominantly Caucasian 6-month-old infants. We argue that if the body composition measurements of PEA POD and DD are comparable at 6 months, DD may be a suitable alternative to assess longitudinal changes in body composition when infant size precludes the use of PEA POD.

6.3 METHODS

6.3.1 Participants

The Baby-bod study is a prospective longitudinal cohort study conducted in the Launceston General Hospital as a part of a multi-country study that aimed to develop body composition standards [351, 439]. Inclusion criteria for this study were: mothers above 18 years of age at the time of the delivery and able to speak and understand English, a term (gestational age at birth between 37^{+0} and 41^{+6} weeks) pregnancy, and a singleton birth. Exclusion criteria were: infants with congenital birth defects or admitted to the neonatal intensive care unit, mothers with significant morbidity or incapability to negotiate the informed consent process. Infants recruited to the Baby-bod study participated in the present study at their 6-month follow-up visit. Procedures associated with body composition assessment using the ADP PEA POD and DD techniques were explained and informed written consent was obtained from the parents.

6.3.2 Protocol

The Human Research Ethics Committee (Tasmania, reference: H0016117) approved all research procedures. Infant body composition assessment using PEA POD and sample collection for DD took place in the Launceston General Hospital, Tasmania, Australia (September 2017 to October 2019). Sample analyses associated with the DD technique were conducted at the School of Health Sciences, University of Tasmania.

6.3.3 Principles

6.3.3.1 PEA POD

ADP uses gas laws that describe the relationship between pressure (P) and volume (V). According to Boyle's law, the pressure of a given mass of gas is inversely proportional to its volume at isothermal conditions.

Equation 1 $$\frac{P_1}{P_2} = \frac{V_2}{V_1}$$

Poisson's law describes that, under adiabatic conditions, the temperature of the air does not remain constant as its volume changes, and therefore, the above relationship should be modified as below.

Equation 2 $$\left(\frac{P_1}{P_2}\right) = \left(\frac{V_2}{V_1}\right)^{\gamma}$$

Here, γ is the ratio of specific heat of the gas at constant pressure to that at constant volume, and its value for air is approximately 1.4. This relationship allows the derivation of an unknown volume (V_{test}) by directly measuring the ratio of pressures in a test chamber (P_{test}) and reference chamber (P_{ref}).

Equation 3 $$V_{test} = \left(\frac{P_{ref}}{P_{test}}\right) V_{ref}$$

The PEA POD technology adapts the above equation to evaluate the body volume (V_B) of the participant, where m and b are computed in a calibration performed in the device at the beginning of each test.

Equation 4 $$V_B = m(V_{test}) + b$$

Once V_B is calculated, the PEA POD uses principles of whole-body densitometry to evaluate body density (D_B), with body mass (M_B) measured at the beginning of the test.

Equation 5 $$D_B = \frac{M_B}{V_B}$$

As the density of any material is a function of the proportions and densities of its components, whole-body density (D_B) can be defined as a function of densities of FM (D_{FM}) and FFM (D_{FFM}).

Equation 6 $$\frac{1}{D_B} = \frac{\mathrm{FM}}{\mathrm{D_{FM}}} + \frac{\mathrm{FFM}}{\mathrm{D_{FFM}}}$$

Equation 6 can be rearranged as below to express %FM.

Equation 7 $$\%\mathrm{FM} = \left[\frac{\mathrm{D_{FM}}\,\mathrm{D_{FFM}}}{\mathrm{D}_B(\mathrm{D_{FFM}}-\mathrm{D_{FM}})} - \frac{\mathrm{D_{FM}}}{(\mathrm{D_{FFM}}-\mathrm{D_{FM}})}\right] * 100\%$$

Therefore, %FM can be calculated when D_B is measured, and appropriate values are assigned for D_{FM} and D_{FFM}. The density of FM is equal to 0.9007 kg/L and considered to be constant throughout life. Age- and sex-specific estimates for D_{FFM} derived using the Fomon model [356] are used in PEA POD software.

Once %FM is determined, FM and FFM are calculated using the equations given below.

Equation 8 $$FM = \frac{(\%FM)M_B}{100\%}$$

Equation 9 $$FFM = M_B - FM$$

6.3.3.2 DD technique

Water is the largest component of the body, and it is found almost exclusively within the FFM. TBW includes both intracellular fluid and extracellular fluid and accounts for approximately 70-75% of newborn body weight and 40-60% of adult body weight. In isotope dilution, a tracer of known concentration (C_1) and volume (V_1) is administered into the TBW pool. The tracer is allowed to mix with the pool, and when it reaches equilibrium, the pool is sampled (e.g., urine, saliva, plasma). The concentration (C_2) of the tracer in the sample is measured. The volume of distribution (V_2), also known as dilution space, can be calculated as,

Equation 1 $$V_2 = \frac{C_1 V_2}{C_2}$$

Deuterium (^2H) is a stable (non-radioactive) isotope of hydrogen. It is naturally present in the TBW pool in small concentrations, usually close to 0.015 atom % ^2H. Deuterium oxide (^2H_2O, also referred to as D_2O), can be used as a tracer to measure TBW.

The dilution space is slightly larger than TBW due to the non-aqueous exchange of the isotope. For ^2H, it is 1.041 times that of TBW.

Equation 2 $$TBW = \frac{V_2}{1.014}$$

As TBW is solely contained in FFM, the proportion of water within FFM is referred to as "hydration of FFM". When TBW is estimated, an estimation of FFM can be derived using Equation 3.

Equation 3 $$FFM = \frac{TBW}{Hydration\ factor}$$

The hydration factor of infants and children continues to change with age, as during growth, muscle mass increases, thus hydration of FFM decreases. Hydration factors are available from Fomon et al. [356] for infants. Once FFM is estimated, and body weight is known, FM is calculated as,

Equation 4 FM = Body weight - FFM

6.3.3.2.1 Measurement of D_2O in saliva samples using FTIR spectroscopy

The FTIR instrument is designed to measure the intensity of the O-D peak. A full and detailed description of the FTIR principle and procedure is available elsewhere [132].

FTIR spectroscopy can be used to measure the increased concentration of deuterium above the amount naturally present, i.e., 'the enrichment', expressed as the concentration of deuterium in parts per million (ppm) by weight (mg/kg). When infrared radiation is passed through a sample, some of it is absorbed by the sample, and some of it is transmitted. Absorbance in the mid-infrared range is due to molecular vibrations of bonds between atoms of a molecule. The energy of these vibrations depends on the masses of the atoms of which the bond is made. Because of the extra neutron present in the nucleus, deuterium (^2H) is roughly twice the mass of the common stable isotope of hydrogen, protium (^1H). The substitution of deuterium for protium shifts the energy to a lower level.

Fourier transform techniques are used to convert raw absorbance data into an absorbance spectrum over a broad wavenumber range. Absorbance peak positions, commonly expressed in terms of wavenumber (cm^{-1}), frequency (THz), or wavelength (μm), are used to distinguish between the components of a sample. The peak due to D_2O (D–O bond) occurs at 2504 cm^{-1} (75.07 THz or 3.994 μm).

Beer-Lambert law: for a parallel beam of monochromatic radiation passing through a homogeneous solution, the amount of radiation absorbed (A) is proportional to the product of the concentration (c) and pathlength (l); is used to estimate the concentration of D_2O in the sample from the measured intensity of O-D peak.

Equation 5 $A \alpha c l$

$A = \varepsilon c l$

$c = \frac{A}{\varepsilon l}$ where ε is known as the extinction coefficient.

For D–O, the extinction coefficient of 7150 M^{-1} m^{-1}, and for quantitation, a cell thickness (path length) of 10^{-4} m (100 μm) is used.

6.3.4 Procedure

Infants were measured using the two techniques on the same day, PEA POD followed by the DD technique.

6.3.4.1 PEA POD

ADP measurements were conducted using the PEA POD infant body composition assessment system (COSMED USA, Inc., Concord, CA, USA; software version 3.5.0, Figure 6.1A). The physical design and the operating procedures of the PEA POD have been described in detail elsewhere [144, 150]. In brief, quality control procedures were conducted each day before testing infants, using calibration weight and volume phantom provided by the manufacturer to evaluate the stability and performance of the system. Prior to testing, crown to heel length of the infant was measured using an infantometer (Seca, Hamburg, Germany) to the last completed millimetre and entered into the system. The infant's hair was flattened against the head, either by applying baby oil with a cotton swab or placing a head cap on infants with curly/thick hair (to reduce the amount of air behaving isothermally). The default models provided by the manufacturer, i.e., density model: Fomon [356]; body surface area model: Boyd [440]; thoracic gas volume model: Stocks [441], were used. A disposable pad was placed on the scale tray, and the scale was tared. The unclothed infant was placed at the centre of the scale, and body mass was measured (Figure 6.1B). Mass measurement was repeated on occasions when excessive infant movement resulted in an error. Next, the unclothed infant was placed in the test chamber for two minutes for volume measurement (Figure 6.1C). At the end of the testing sequence, body composition values were computed by the software.

Figure 6.1 Analysis of infant body composition using ADP (PEA POD).

A: PEA POD infant body composition system used in the Baby-bod study; B: weight measurement on the electronic scale; C: volume measurement in the test chamber.

6.3.4.2 DD technique

6.3.4.2.1 Sample collection and dosing

Upon completion of PEA POD testing, two samples of saliva were collected from the infant (pre-dose sample), who had fasted for 20-30 min. A sterile cotton ball held with sterile plastic forceps was moved inside the mouth of the infant to soak saliva (Figure 6.2A). Once the cotton ball was soddened, it was placed in a 20 mL disposable syringe, and the plunger was depressed until the saliva sample was dispensed into two labelled 1 mL- cryovials. At least 0.5 mL of saliva was extracted into each vial, and if not, the procedure was repeated with a new cotton ball. One gram of undiluted D_2O (99.8 atom % D_2O) was administered to each infant using a 1 mL oral syringe (Figure 6.2B). The exact weight of the administered dose was measured to the nearest 0.001 g as the difference between the dose syringe weight before and after dose administration using an analytical balance reserved for the purpose. If any spillage occurred during dosing, the procedure was stopped, and the participant was asked to come back again within 3 weeks. Saliva samples were collected at 2.5 hours (post-dose sample I) and 3 hours (post-dose sample II) after the dose administration, in the same way described above. Infants were allowed to be fed during the equilibration period; however, mothers were asked to stop feeding at least 15 min before each saliva sampling. The samples at each time-point (pre-dose, post-dose 1, post-dose 2) were kept in small zip-lock bags to prevent cross-contamination, and samples of a single participant were placed in a larger zip-lock bag. The samples were transported to the School of Health Sciences, University of Tasmania, on ice and were stored at -80^0 C until analysed.

6.3.4.2.2 Sample analysis

On the day of analysis, samples were thawed, swirled to mix, and centrifuged at 1000 g, 4^0 C for 10 minutes to allow any solids to precipitate. D_2O concentration in each saliva sample was determined in duplicate using the Agilent 4500 FTIR portable spectroscopy instrument (Agilent Technologies, Inc., USA.) [141]. The calibration of the instrument was checked daily at the beginning of each batch of samples using 0 mg/kg and 1000 mg/kg standards. A 30 µL aliquot of sample was pipetted onto the optical window of the instrument for measurement. Air background was measured each time before measuring any standard/sample. The optical windows were cleaned after reading each standard/sample using ethanol and cotton swabs. If the coefficient of variation of duplicate measurements were not less than 1%, or if there was doubt about any measurement such as air bubbles in the optical path (as detected in the real-time spectrum), another replicate was measured.

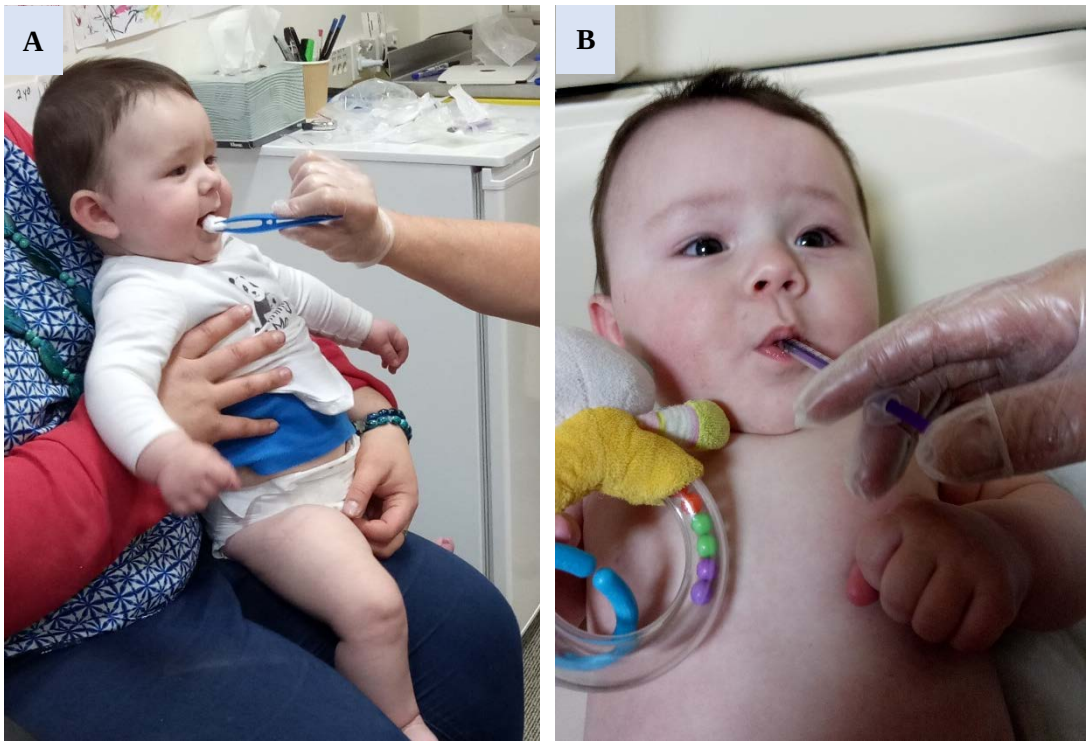


Figure 6.2 Analysis of infant body composition using DD technique.

A: collecting saliva from an infant using cotton ball and plastic forceps; B: dose administration to the infant using a dose syringe.

6.3.5 Statistical analysis

All analyses were performed using R Project for Statistical Computing (version 3.5.3) in R Studio (version 1.1.463, Vienna, Austria) [330]. Descriptive variables are expressed as mean (± standard deviation) otherwise specified. Three variables that are commonly used to express infant adiposity, i.e., absolute FM (in grams), %FM (FM divided by total body mass, multiplied by 100), and FMI (FM in kilograms divided by the length in metres squared), derived from PEA POD and DD technique were compared. Paired-sample t-test was used to detect differences between the variables obtained by PEA POD vs DD technique. The normality of differences between the two measurements was checked using a graphical approach (histograms). The degree of agreement between PEA POD and DD technique was assessed with Bland-Altman analysis. Constant bias (mean difference) was calculated as the average of the differences between the paired data, and the significance of bias was determined by assessing whether the line of equality was within the 95%CI of the mean difference. Limits of agreement (LOA) expected were defined as a priori based on comparison of PEA POD measurements with 4C reference model [149]. Possible error in the mean difference and of the LOA due to a sampling error was described with 95% CI. Proportional bias was detected by drawing a regression line of the differences in Bland-Altman plots. If significant proportional bias was found, further analyses were performed to investigate whether the values towards the end of each spectrum (lower or upper) significantly affected the bias. This was conducted by including 1) the values above the first quartile (above Q1 value, i.e., removing the lowest 25% of the values) and 2) values below the third quartile (below Q3 value, i.e., removing the highest 25% of values) of the mean of each adiposity measure in Bland–Altman analysis. Additional analyses: a) Pearson correlation coefficient analysis that quantifies the strength of the linear relationship between pairs of variables, b) linear regression to find the best line that predicts one variable from the other one, and c) linear regression to examine whether the differences in variables derived by the two techniques were a function of body mass, length, postnatal age, body volume, body density and percent TBW (%TBW), are given in supplementary files. All hypothesis tests were two-sided. $p < 0.05$ was considered statistically significant.

6.4 RESULTS

6.4.1 Infants

Of the 113 infants with PEA POD measurements and 133 with DD measurements, 72 had measurements with both the techniques (Figure 6.3). Even though we tried to convince parents to allow infants to be tested using both approaches, the final number tested with both methods differed due to the following reasons. Some parents only consented to the PEA POD measurement because they could not commit the time needed for both techniques. A few parents only consented to the DD technique as their child cried intensely inside the PEA POD at the time of previous assessment. Moreover, some PEA POD assessments were unsuccessful due to excessive crying by infants inside the test chamber that triggered an alarm. Finally, in the DD technique, a few saliva samples generated invalid results due to low or high enrichments. The infants were predominantly Caucasian, and the mean age was 5.82 months. Male infants (52.8%) slightly outnumbered female infants (47.8%) (Table 6.1).

6.4.2 Correlation and regression analysis

Medium to high positive correlations ($r > 0.55$) existed between the pairs of adiposity variables (Figure 6.4). The regression lines between pairs did not significantly deviate from 1; however, correlations between the two methods were stronger for FMI than %FM. Slopes of FM, %FM and FMI were 0.91, 0.79 and 0.91, respectively (Figure 6.5). The mean ± SD of all selected adiposity measures derived by PEA POD (FM in grams: 1,842.21 ± 410.33; %FM: 25.24 ± 4.43; FMI: 4.30 ± 0.93) did not significantly differ ($p > 0.05$ for all) from those derived by DD technique (FM: 1,816.94 ± 539.89; %FM:24.91 ± 6.37; FMI:4.24 ± 1.23).

6.4.3 Bland-Altman analysis

No constant bias was observed between the two methods in Bland-Altman analysis with all data included (FM: bias = 25.26, 95%CI = -65.92 to 116.45; %FM: 0.33; -0.93 to 1.60; FMI: 0.06; -0.15 to 0.27), as well as with using values above Q1 (FM: -73.96, -157.48 to 9.55; %FM: -0.59, -1.79 to 0.61; FMI: -0.16, 0.36 to 0.04) or values below Q3 (FM: 63.82, -48.09 to 175.74; %FM: 0.85, -0.72 to 2.42; FMI: 0.14, -0.13 to 0.40), for each adiposity measure (Table 6.2, Figure 6.7). The agreement intervals were wide, and significant proportional bias was detected in the analysis with all values and values below Q3. Negative trends of differences were evident in all the plots. In comparison to PEA POD, DD technique underestimated adiposity at lower values and overestimated adiposity at higher values. Nevertheless, with mean values above Q1, LOAs were narrower, and no significant proportional bias was detected; there was a trend where DD technique overestimated infant fatness, with the difference becoming slightly higher with increasing mean values (but $p > 0.1$ for all). Moreover, the differences in FM, %FM and FMI

between the two methods were not significantly associated ($p > 0.4$ and $R^2 < 0.01$, for all) with the body weight (Figure 6.8), length (Figure 6.9) and body volume (Figure 6.10) of the infants. However, mean differences were significantly associated with %TBW, with PEA POD underestimating fatness in infants with low %TBW and overestimating it at high %TBW (Figure 6.11).

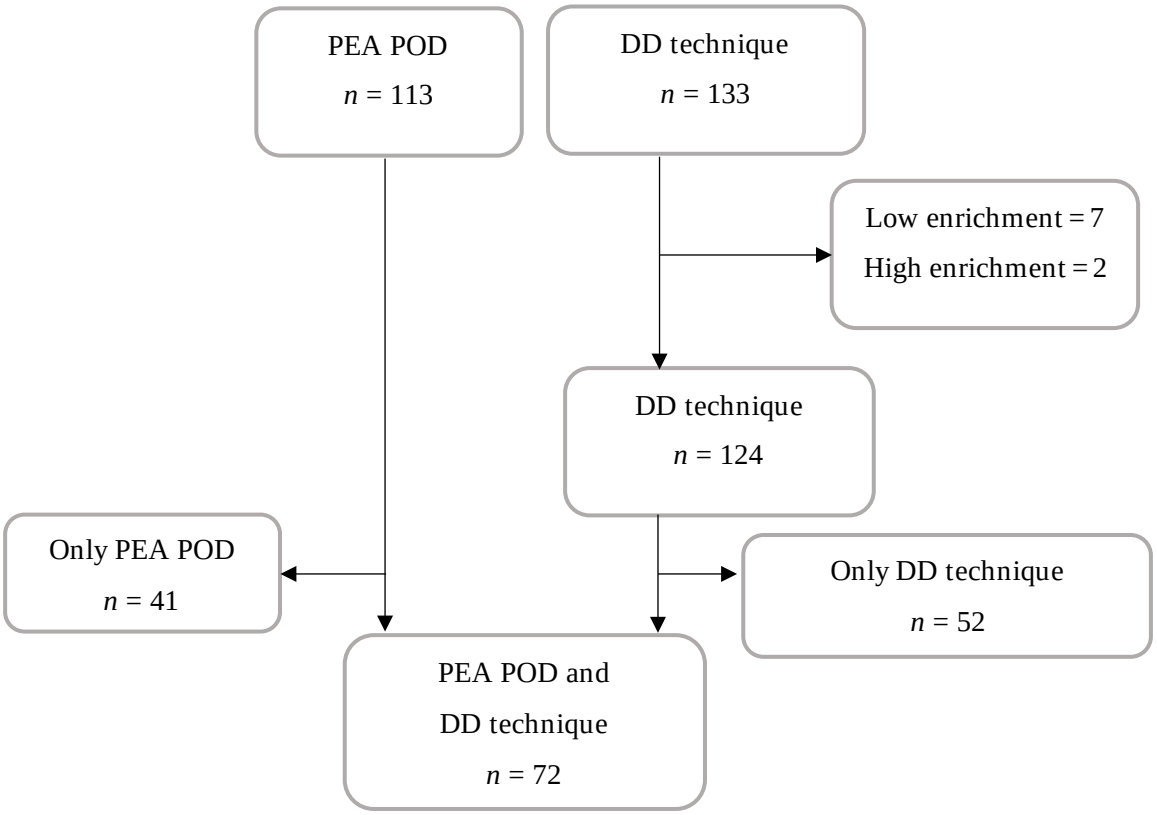


Figure 6.3 Number of infants tested with ADP (PEA POD) and DD technique.

Table 6.1 Infant characteristics and adiposity variables at 6 months.

Characteristic	Mean (SD)	Median (IQR)
Age (months)	5.82 (0.29)	5.80 (5.70, 6.00)
Infant sex*		
Female	34 (47.2%)	
Male	38 (52.8%)	
Ethnicity*		
Caucasian	67 (93.1%)	
Other	5 (6.9%)	
Gestational age (weeks)	39.69 (1.10)	39.79 (39.11, 40.43)
Weight (g)	7,256.62 (691.70)	7,153.50 (6,740.50, 7,700.00)
Length (cm)	65.49 (2.17)	65.65 (63.70, 67.03)
Head circumference (cm)	42.78 (1.25)	42.75 (41.99, 43.56)
Body mass index (kg/m^2)	16.90 (1.14)	16.79 (16.18, 17.71)
ADP (PEA POD)		
Body volume (L)	7.10 (0.76)	7.06 (6.62, 7.58)
Body density (kg/L)	1.02 (0.02)	1.02 (1.01, 1.02)
FM_PP (g)	1,842.21 (410.33)	1,761.05 (1,591.00, 2,046.80)
%FM_PP	25.24 (4.43)	24.90 (22.48, 28.23)
FMI_PP	4.30 (0.93)	4.16 (3.65, 4.91)
DD technique		
Total body water (g)	4,328.99 (489.58)	4,283.50 (4,028.75, 4,655.75)
Total body water (%)	59.76 (5.07)	59.57 (55.70, 62.32)
FM_DD (g)	1,816.94 (539.89)	1,733.00 (1,507.25, 2,231.75)
%FM_DD	24.91 (6.37)	25.11 (21.66, 30.01)
FMI_DD	4.24 (1.23)	4.20 (3.51, 5.23)
Averages of two methods		
FM (g)	1,829.58 (438.50)	1,747.57 (1,554.00, 2,098.90)
%FM	25.07 (4.78)	24.55 (22.03, 28.33)
FMI	4.27 (0.99)	4.17 (3.53, 5.02)

*Values for infant sex and ethnicity are given as count (percentage); SD: standard deviation; IQR: interquartile range; DD: deuterium dilution; PP: PEA POD; FM: fat mass; %FM: percent fat mass; FMI: fat mass index.

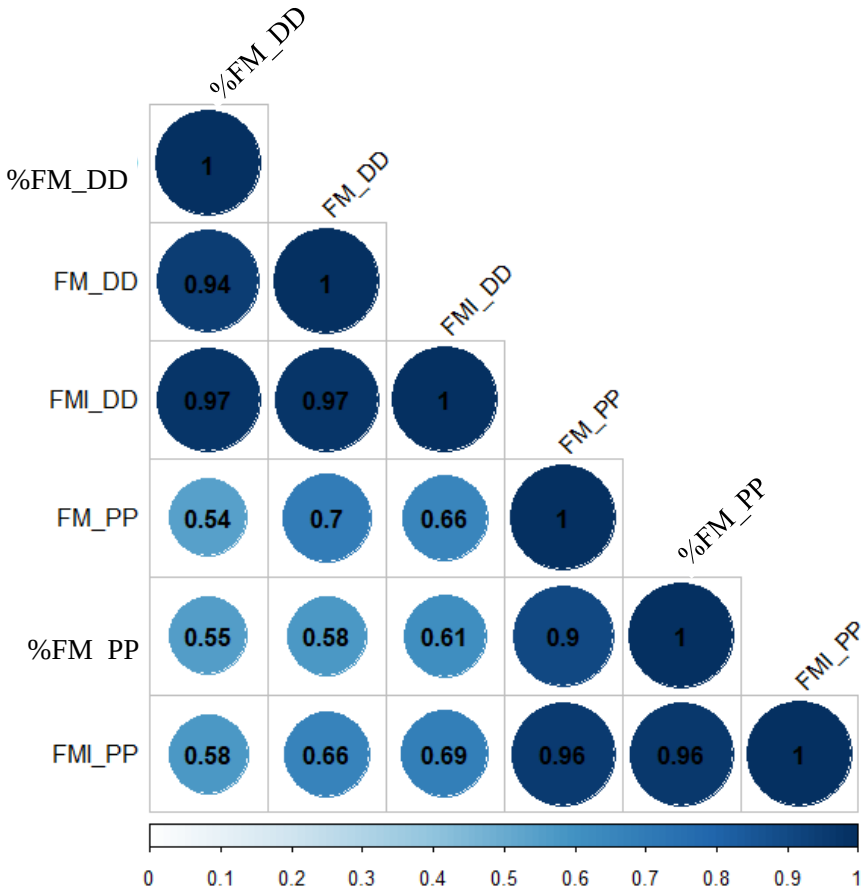


Figure 6.4 Correlation matrix for adiposity variables derived by ADP (PEA POD) and DD technique in 6 months old infants.

Method: "Pearson"; DD: deuterium dilution; PP: PEA POD; FM: fat mass; %FM: percent fat mass; FMI: fat mass index; $p < 0.0001$ for all correlations.

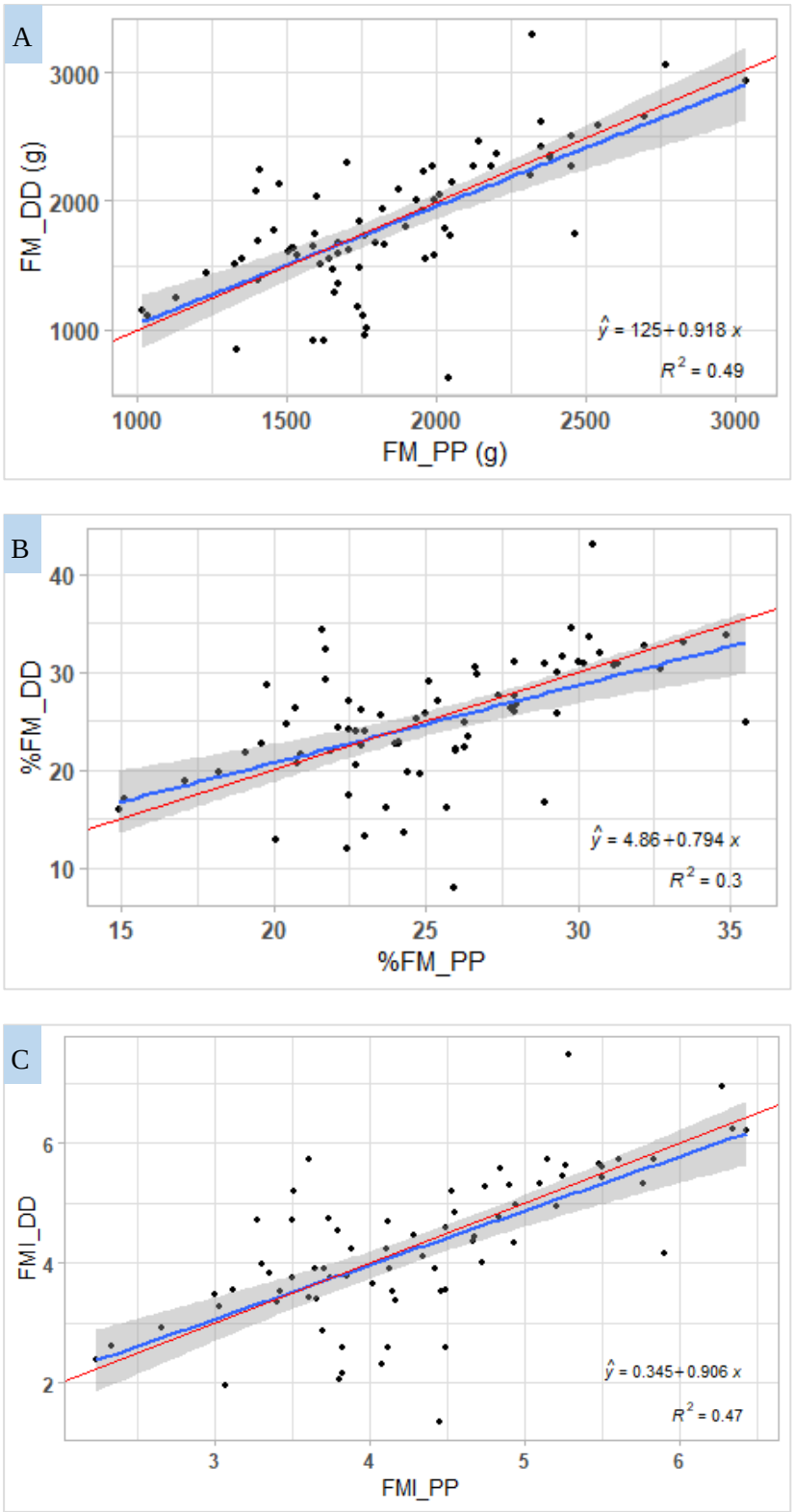


Figure 6.5 Linear regressions of adiposity values derived by ADP (PEA POD) on DD technique.

FM: fat mass (A); %FM: percent fat mass (B) FMI: fat mass index (C); DD: deuterium dilution; PP: PEA POD; regression line is given in 'blue' and line of identity (Y=X) is given in red in each plot; Shaded areas show 95%CI for regression line; Regression equations and coefficient of determination (R^2) are shown in each plot; p <0.001 for all.

Table 6.2 Bland-Altman statistics of selected adiposity measures derived by ADP (PEA POD) compared to DD technique.

	Constant bias (95%CI)	Lower LOA (95%CI)	Upper LOA (95%CI)	Slope	p-value for slope
All data (n = 72)					
FM	25.26 (-65.92, 116.45)	-735.31 (-891.87, -578.75)	785.83 (629.27, 942.39)	-0.32	0.002
%FM	0.33 (-0.93, 1.60)	-10.22 (-12.40, -8.05)	10.90 (8.72, 13.07)	-0.46	<0.001
FMI	0.06 (-0.15, 0.27)	-1.70 (-2.07, -1.34)	1.82 (1.46, 2.19)	-0.33	0.002
Mean values > Q1 (n = 53)					
FM	-73.96 (-157.48, 9.55)	-667.84 (-811.47, -524.201)	519.91 (376.28, 663.54)	-0.18	0.130
%FM	-0.59 (-1.79, 0.61)	-9.15 (-11.21, -7.08)	7.96 (5.89, 10.03)	-0.13	0.482
FMI	-0.16 (-0.36, 0.04)	-1.58 (-1.92, 1.23)	1.27 (0.92, 1.61)	-0.10	0.434
Mean values < Q3 (n = 53)					
FM	63.82 (-48.09, 175.74)	-732.01 (-924.48, -539.53)	859.66 (667.18, 1052.13)	-0.71	0.001
%FM	0.85 (-0.72, 2.42)	-10.30 (-12.99, -7.60)	11.99 (9.30, 14.69)	-0.89	<0.001
FMI	0.14 (-0.13, 0.40)	-1.72 (-2.17, -1.27)	1.99 (1.55, 2.45)	-0.68	<0.001

FM: fat mass; %FM: percent fat mass; FMI: fat mass index

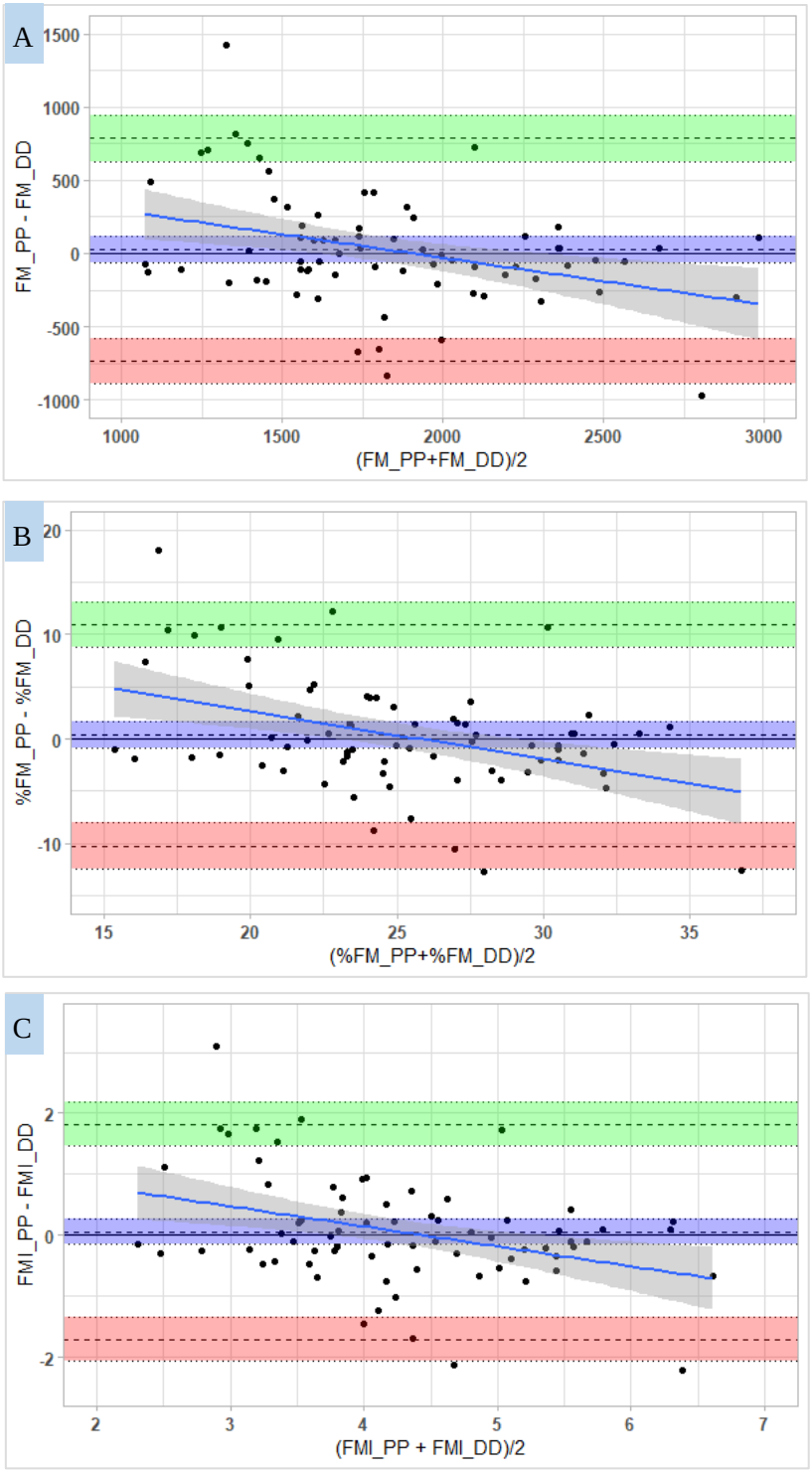


Figure 6.6 Agreement between adiposity measures derived by ADP (PEA POD) and DD technique using all data.

n = 72; FM: fat mass (A); %FM: percent fat mass (B) FMI: fat mass index (C); DD: deuterium dilution; PP: PEA POD; Y axis show difference between the methods and X axis show mean of the two methods for respective adiposity measure. The solid line represents the mean differences between the 2 methods (bias), and the dashed lines are the limits of agreement (±2 SD from the mean difference diagonal line (blue colour) represents proportional bias line); shaded areas around solid and dashed lines show 95%CI.

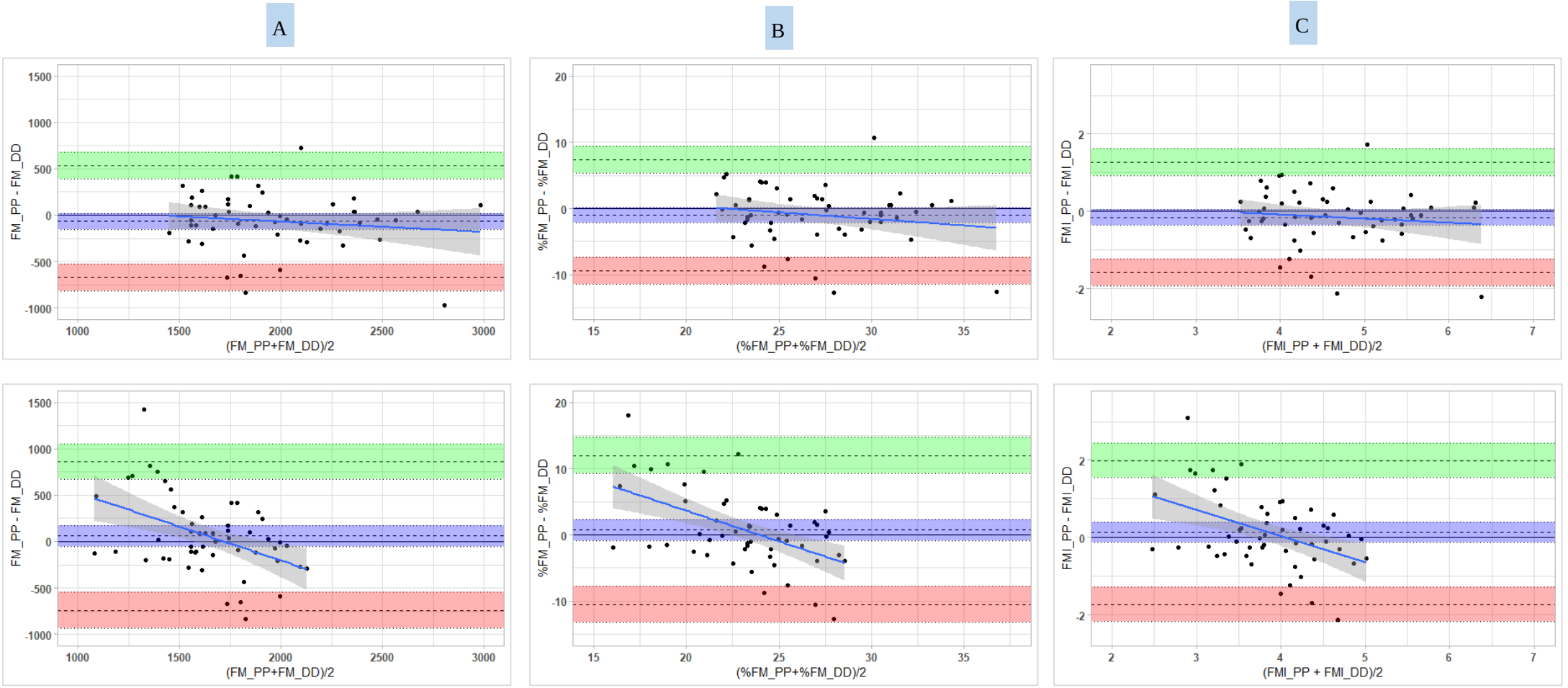


Figure 6.7 Agreement between adiposity measures derived by ADP (PEA POD) and DD technique using selected data.

Top row shows analysis with values >first quartile and bottom row shows analysis with values <third quartile; FM: fat mass (A); %FM: percent fat mass (B) FMI: fat mass index (C); DD: deuterium dilution; PP: PEA POD; Y axis show difference between the methods and X axis show mean of the two methods for respective adiposity measure. The solid line represents the mean differences between the 2 methods (bias), and the dashed lines are the limits of agreement (± 2 SD from the mean difference diagonal line (blue colour) represents proportional bias line); shaded areas around solid and dashed lines show 95%CI.

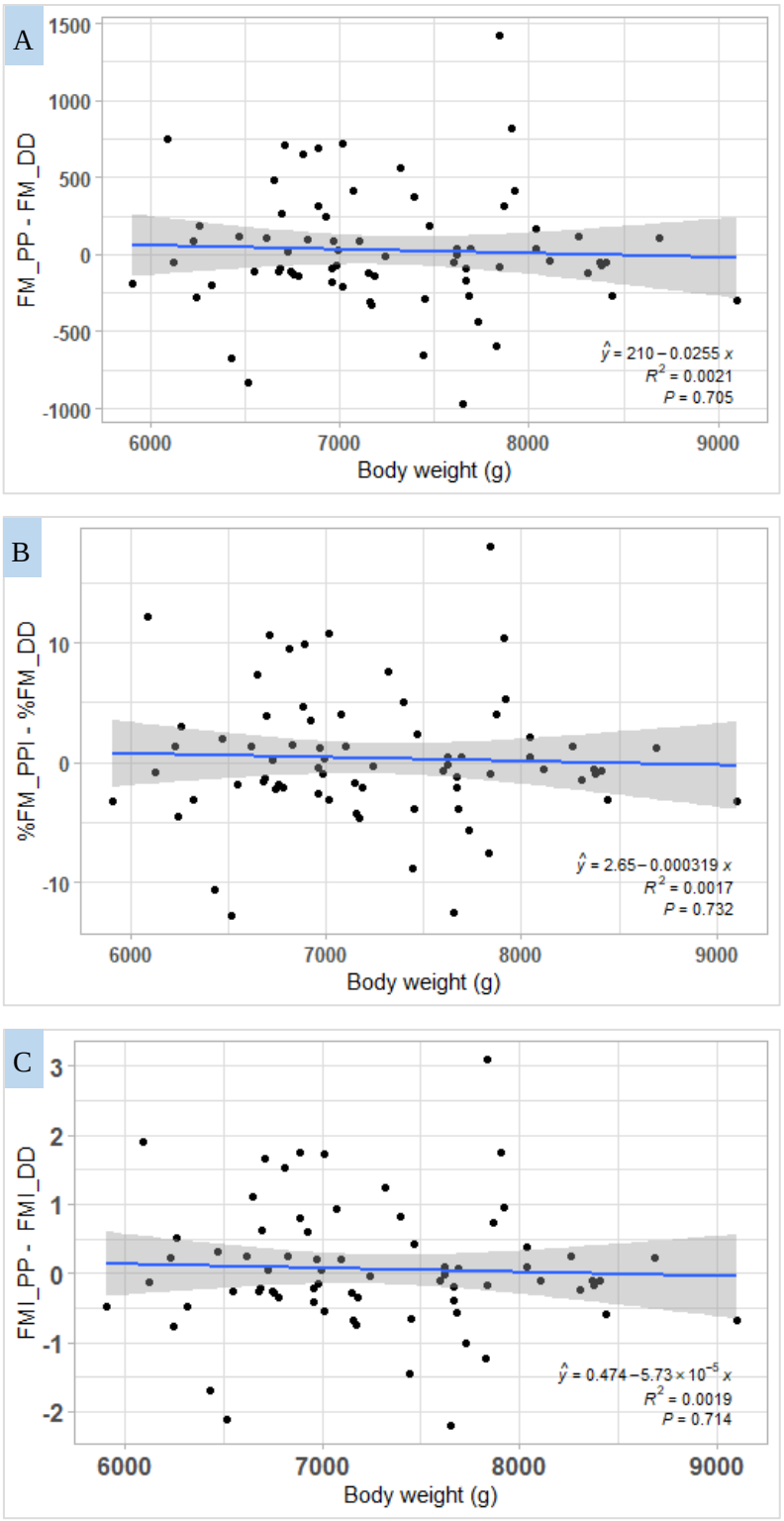


Figure 6.8 Linear regressions of differences in adiposity measures on infants' body weight.

FM: fat mass (A); %FM: percent fat mass (B) FMI: fat mass index (C); DD: deuterium dilution; PP: PEA POD; regression line is given in 'blue' and shaded areas show 95%CI for regression line; Regression equations, coefficient of determination (R2) and p-value are shown in each plot.

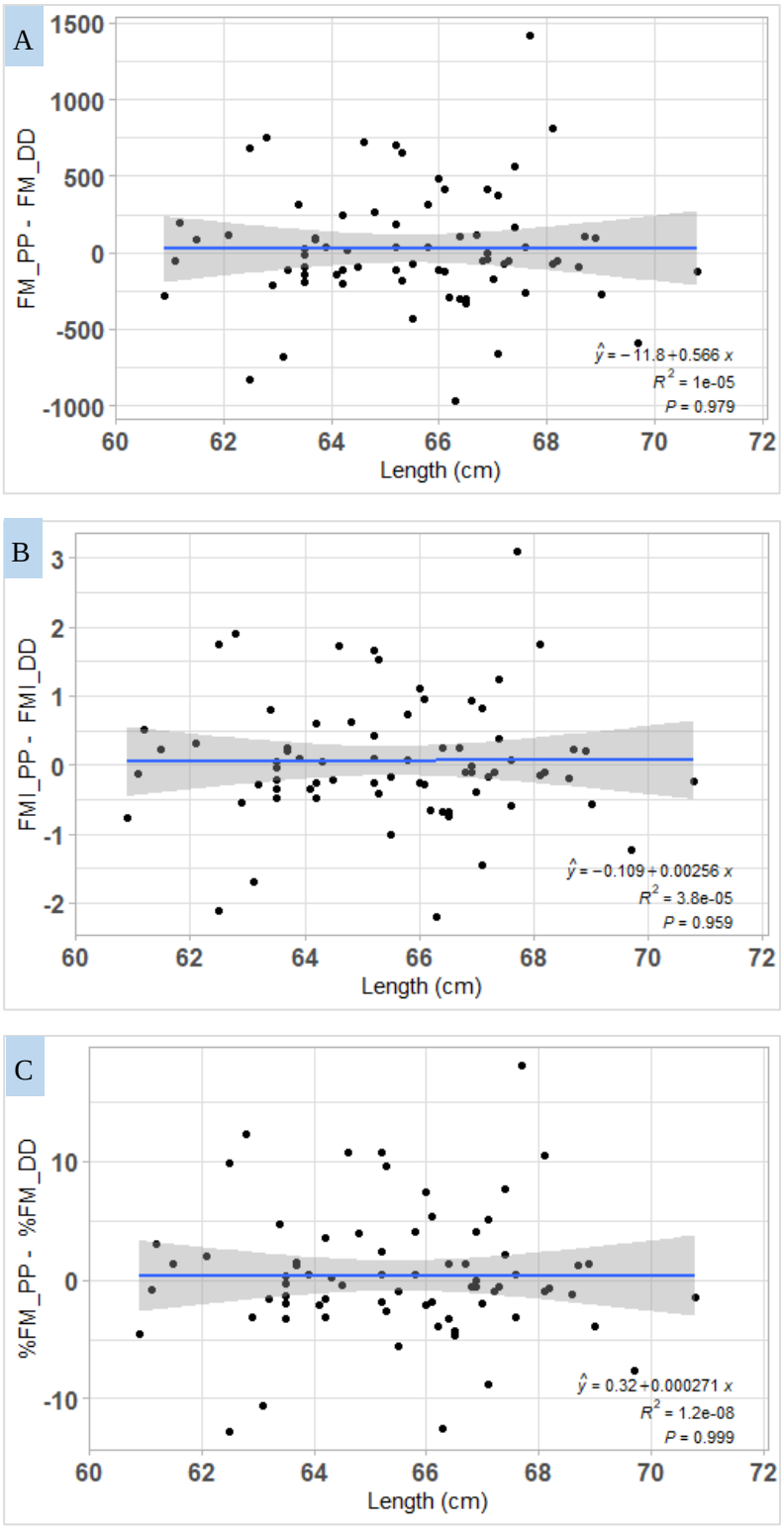


Figure 6.9 Linear regressions of differences in adiposity measures on infants' length.

FM: fat mass (A); %FM: percent fat mass (B) FMI: fat mass index (C); DD: deuterium dilution; PP: PEA POD; regression line is given in 'blue' and shaded areas show 95%CI for regression line; Regression equations, coefficient of determination (R2) and p-value are shown in each plot.

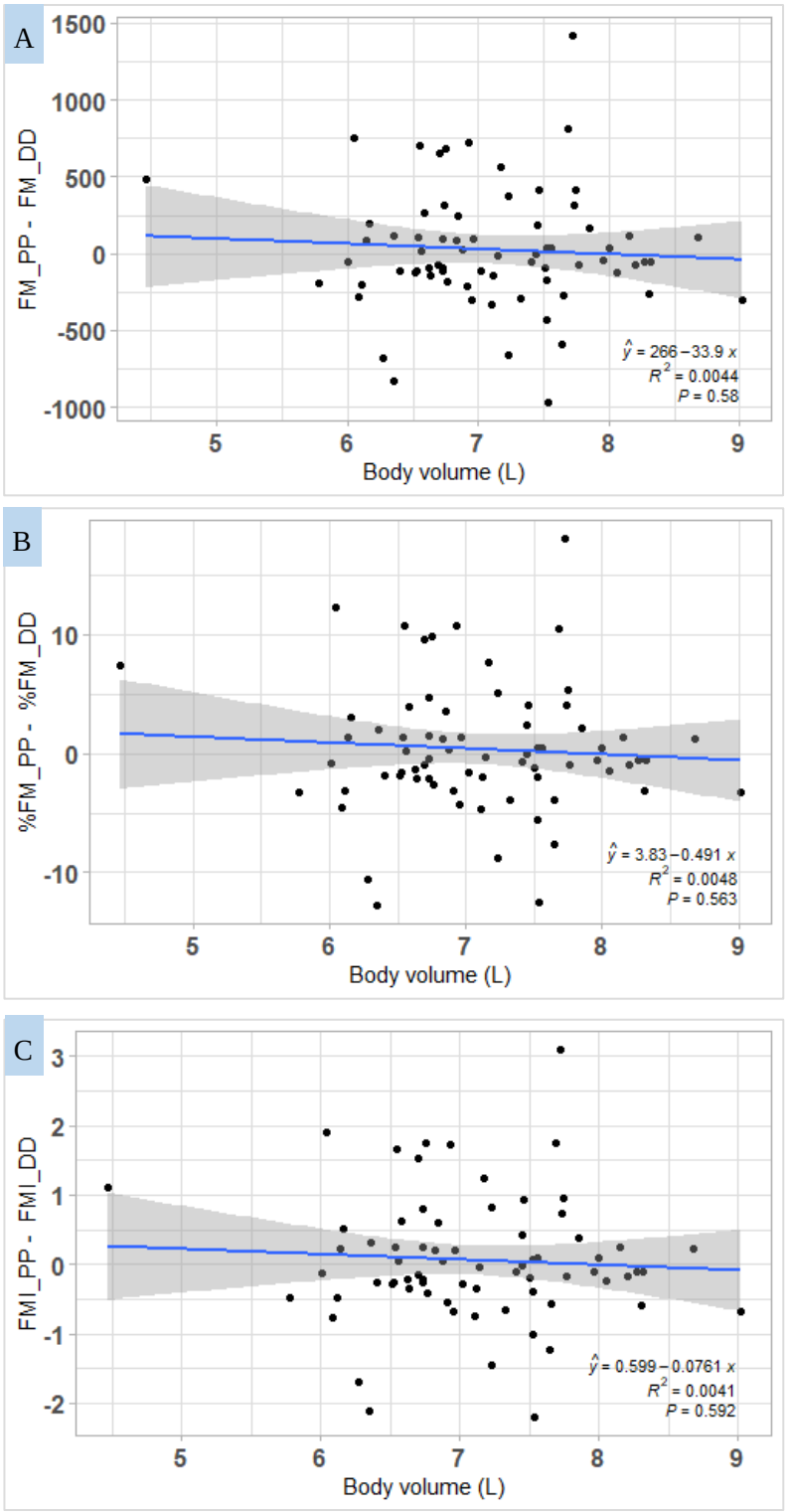


Figure 6.10 Linear regressions of differences in adiposity measures on infants' body volume.

FM: fat mass (A); %FM: percent fat mass (B) FMI: fat mass index (C); DD: deuterium dilution; PP: PEA POD; regression line is given in 'blue' and shaded areas show 95%CI for regression line; Regression equations, coefficient of determination (R^2) and p-value are shown in each plot.

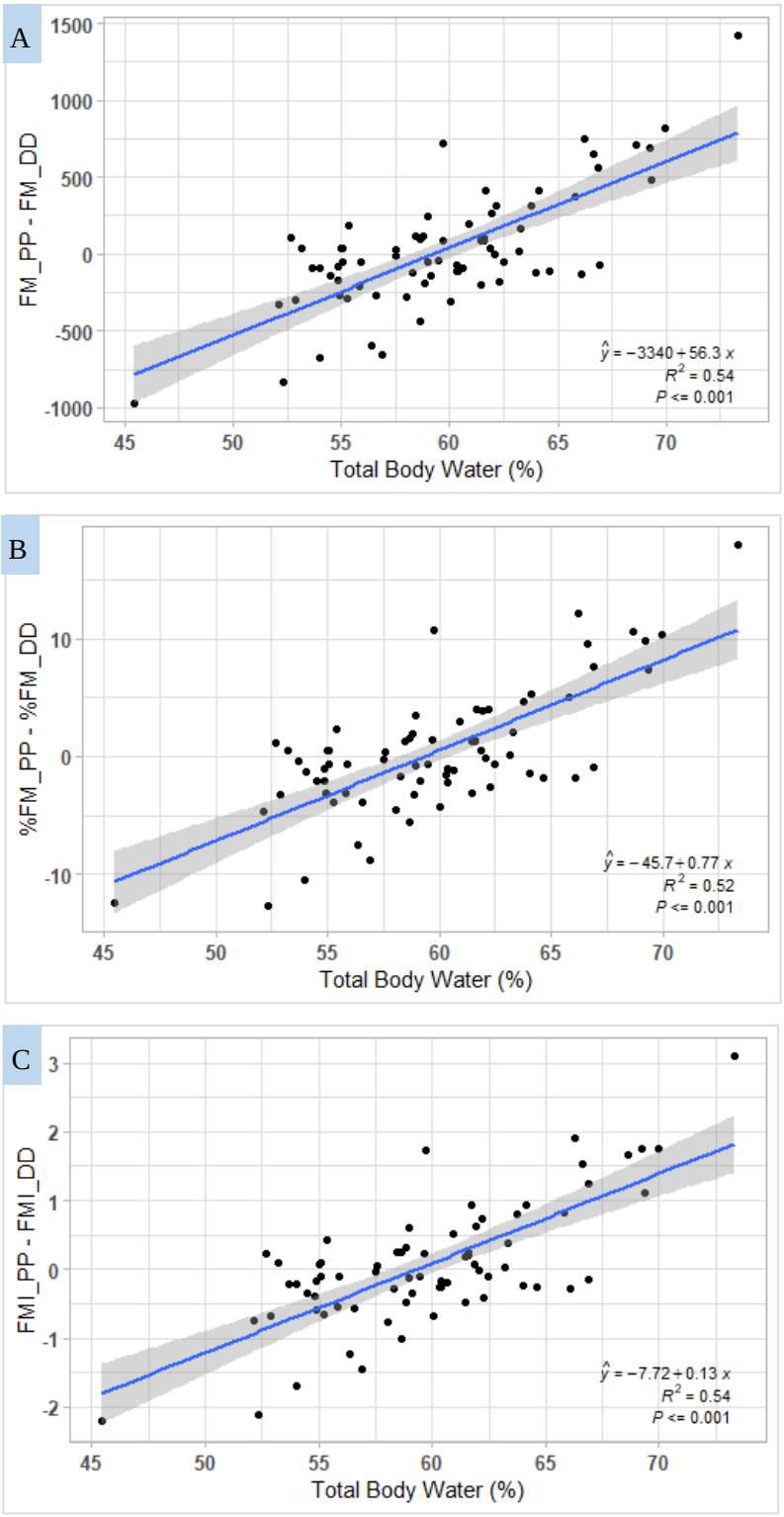


Figure 6.11 Linear regressions of differences in adiposity measures on infant's total body water (%).

FM: fat mass (A); %FM: percent fat mass (B) FMI: fat mass index (C); DD: deuterium dilution; PP: PEA POD; regression line is given in 'blue' and shaded areas show 95%CI for regression line; Regression equations, coefficient of determination (R^2) and p-value are shown in each plot; $p < 0.01$ for all measures; the regression outputs did not significantly change after removing outliers.

6.5 DISCUSSION

In this study, we investigated the agreement of 3 adiposity measures (FM, %FM and FMI) obtained using ADP (PEA POD) and the DD technique in predominantly Caucasian infants aged 5.82 ± 0.29 months and weighing 7.3 ± 6.9 kg. Means for %FM in girls and boys in our study were 25.8 ± 4.94 % and 24.4 ± 4.60 %, respectively, representing lower average body fat levels compared to reference data by Butte et al. (32.0 ± 4.4 for girls and 29.1 ± 4.7 for boys at 6 months of age) [44]. Our results show no constant bias between the 2 methods, in other words, the values derived from one method are not consistently greater or lesser than the other method by a constant amount. But there was significant proportional bias; the difference in values resulting from the two methods decreased with increasing average values. Specifically, compared to PEA POD, DD technique had a tendency to underestimate infant fatness at lower adiposity values (e.g., %FM < ~22%). These results suggest that a change in body composition assessment method from PEA POD to DD technique in longitudinal studies may only be suitable for infants who are not at the lower end of the body fat spectrum for their age. The difference in each adiposity measure between PEA POD and DD technique was not dependent on infant weight, length, postnatal age, volume and density. However, a significant association existed between the differences in adiposity measures and %TBW. At lower %TBW levels, PEA POD tended to underestimate infant fatness, while at higher %TBW values, it had a propensity to overestimate infant fatness.

Our results differ somewhat from the study by Ma et al. [150] that reported an excellent agreement between %FM measured with PEA POD and DD technique. In accordance with Ma et al. [150], the mean difference in %FM in our study did not significantly differ from zero. However, although infant %FM ranged from 9-37% in their study, they did not observe any proportional bias, as we did for mean %FM < ~22%. Moreover, their LOA for %FM (-6.84%, 6.71%) was narrower than ours (-10.22%, 10.90%). There could be several potential contributors to these discrepancies. Firstly, our cohort is different, based on infant age (5.82 ± 0.29 months vs 5.8 ± 6.0 weeks, respectively) and ethnicity (Caucasian vs Asian). The infants in the present study are older, and this could have contributed to increased inter-individual variation. Asian infants are characterised with more FM and less FFM than white Caucasians [274, 442], and these ethnic differences may have an impact on the measurements. Secondly, as shown in Figure 6.11, significant variations in %TBW of infants who participated in this study may have influenced the results; principles used in PEA POD are based on assumptions of body density, ignoring the inter-individual variation in the constituents of FFM.

Ellis et al. [149] compared %FM in full-term infants (age 2-17 weeks) derived by PEA POD against a 4-compartment model and reported that there was no significant bias; however, in their

study, LOA was wider (− 6.8% to 8.1%) than the study by Ma et al. [150] but narrower than ours. Fields et al. [151] compared FM and %FM using PEA POD and DXA in term-born infants at 6 months of age and reported that estimates of FM (2,284 ± 449 vs 1,921 ± 492 g; $P < 0.001$) and %FM (31.1 ± 3.6% vs 26.7 ± 4.7%; $P < 0.001$) by DXA were significantly greater than PEA POD. They showed that the difference in %FM reduced with increasing mean %FM values, and a similar outcome was evident in our study where significant differences existed at low %FM levels. However, we did not detect any relationship between body weight and infant fatness, as observed by Fields et al. [151], who reported that significant differences in FM and %FM occurred when infant weight was less than 7 kg.

A 4C model that independently assesses the constituents of the FFM is considered the gold standard for body composition assessment. PEA POD and DD technique are 2C models based on several assumptions [67]; thus, they may not provide the most accurate estimations of body composition [443]. Fields et al. [444] compared the accuracy of FM assessed by ADP (BOD POD), DD technique, DXA, and hydrostatic weighing against a 4C model and concluded that ADP prevailed over the other methods. They also highlighted that DD technique might be associated with the highest error as it assumes a constant hydration status, but individuals may have significant differences in hydration status. Our results support this finding, as we observed a significant variation in %TBW in 6-month-old girls and boys, resulting in a wider range of values for adiposity measured with DD technique compared to PEA POD. Other plausible reasons for reduced accuracy of the DD technique may include dosing issues, although we took all precautions to ensure that infants consumed the doses correctly. Insensible water loss during the equilibration period may be another issue; we assumed that the loss of deuterium in urine and sweat is minimal and can be ignored.

PEA POD software assigns values for the density of FFM based on the age and sex of the infants; however, the density of FFM can also vary according to hydration status. Additionally, to account for differences in compressibility of air in the thoracic cavity and near the skin surface, the PEA POD software makes corrections in the measured body volume by predicting thoracic gas volume and surface area artefacts [144]. These predictions may introduce errors at the individual level. Moreover, hair, body moisture and temperature have been shown to significantly affect %FM measurements by ADP, with increases in body temperature and moisture resulting in underestimation of body fatness [445, 446]. In our study, significant differences between PEA POD and DD technique existed at low mean values of body fat. This is consistent with previous reports that showed PEA POD produced measurement errors at lower body fat levels; PEA POD even yielded negative %FM values for infants that are not physiologically plausible [146, 152].

Also, the precision of ADP measurements has been reported as being lower at low body volumes [447]. On the whole, PEA POD and DD technique may have inherent potential sources of errors, but they are still valuable tools to track body composition during the postnatal period, as 4C models are expensive and impractical to use in the paediatric population.

One of the strengths of our study is the reasonable sample size compared to similar studies [149-151]. Importantly, participating infants were all approximately the same age (6 months), limiting the effect age-related variations in body composition may have on comparisons. In addition, this is the marginal age where PEA POD measures may become limited due to infant size, and the DD technique may be considered as an alternative assessment option, making this an ideal age group to make such a comparison. Moreover, this is the first study to compare PEA POD and DD technique measurements in term Caucasian infants at 6 months of age and compared agreement between three common adiposity measures, FM, %FM and FMI. One of the limitations of our study was the assumption that any changes in the TBW in infants due to milk intake and urination during the equilibration period is minimal and, therefore, can be ignored. It is recommended that participants fast during the equilibration period, or the volume of liquids consumed during this period is subtracted from the calculated TBW [132]. Furthermore, due to the practical challenges of working with infants and for reducing participant burden, we did not perform repeat PEA POD assessments, which may have helped improve accuracy and allow a determination of precision.

6.6 CONCLUSION

In conclusion, PEA POD and DD technique are in good agreement in the majority of 6-month-old infants. Therefore, DD technique may be a suitable alternative method to assess body composition when the PEA POD can no longer accommodate infants. Additional research is required to understand the reasons for the significant inter-method differences observed at low body fat levels. Additionally, correlations of measurements between PEA POD and DD technique were stronger for FMI than %FM. Given that FM and %FM are statistically flawed in expressing body fatness, we encourage future researchers to use FMI in tracing paediatric body composition. Future studies should also explore alternative body composition techniques suitable for infants with lower body fat levels until reliable ADP equipment to accommodate children 6-24 months of age is developed.

CHAPTER 7 : GENERAL DISCUSSION

Higher adiposity during critical periods of development, such as the first 1000 days of life, is likely to increase the risk of obesity and associated metabolic diseases in adulthood [28]. Research efforts to identify pre- and postnatal risk factors for increased adiposity during early life have mostly used proxies for adiposity, such as birthweight, BMI and skinfolds, and findings from the relatively small number of studies that assessed body composition are contradictory [39, 46-49, 63, 64, 353-355]. Moreover, most of the evidence has emerged from retrospective and cross-sectional studies [41, 50, 57, 208, 448], and there is a paucity of prospective longitudinal studies.

The primary aim of this thesis was to understand pre-and postnatal influences on infant body composition and to validate adiposity measurement approaches. This research is unique as for the first time, body composition and associated maternal factors have been investigated in infants born in Tasmania, the state with the highest rates of child and adult obesity in Australia. Two different approaches were used: 1) a study that explored birthweight trends over the years 2005 to 2018 and associated maternal factors in Tasmanian infants; and 2) a prospective longitudinal cohort study that investigated the body composition of infants born at the Launceston General Hospital, one of the three main public hospitals in Tasmania, and associated pre- and postnatal factors, from birth to 6 months. In the latter study, associations were explored using various adiposity indices, another distinctive objective not previously researched. Additionally, this was the first study to measure body composition in a large sample of 6-month-old Caucasian infants using ADP (PEA POD) and the DD technique - two of the preferred body composition techniques in pediatric research. Finally, quantification of overall differences in adiposity between infants of mothers treated for GDM and mothers with NGT through a meta-analysis is a further unique aspect of this research.

Birthweight is commonly used as a proxy for adiposity in newborns when resources are limited for assessing body composition. In comparison to infants born with NBW, infants born with LBW or foetal growth restriction have lower FM at birth, whereas those born with HBW have higher FM at birth. Since birthweight is largely influenced by maternal pre-pregnancy and pregnancy factors [449], exploring trends in birthweight and associated maternal factors can provide valuable insights into current obesity trends in a population. To identify birthweight trends and maternal determinants in Tasmania, we used the perinatal data collection managed by the Department of Health, Tasmania, Australia. Analysis of data of all live singleton births from 2005 to 2018 (referred to as secular trends study hereon) revealed that the mean birthweight (3425

g to 3359 g) and the proportion of HBW (14.2% to 11.0%) decreased over the 14-year period, while the proportion of LBW (4.8% to 6.5%) increased (Chapter 3). The findings of our study are consistent with recent studies from the United States where birthweight decreased by 27 g (3410 g to 3383 g) in term singletons over the 8 years from 2000 to 2008 [333] and by 68 g in first-birth singletons over the 23 year period from 1990 to 2013 [328]. Reductions in gestational length attributable to obstetric interventions such as caesarean delivery and induced labour have been suggested as a major reason for this downward birthweight trend [328]. Results from our study show that between 2005 to 2018, the gestational age distribution of live singleton births in Tasmania has shifted towards the left. In 2005, the main peak appeared just above 40 weeks whereas, in 2018, it was at around 39 weeks. This change was in accordance with the increase (26.2% to 33.8%) in caesarean deliveries.

Two other factors that may have contributed to the fall of birthweight are increased rates of mothers with hypertensive disorders and advanced maternal age (>35 years). Foetal growth restriction may occur due to reduced uteroplacental blood flow in hypertensive mothers [342] and insufficient placental perfusion or transplacental flux of nutrients in older mothers [269]. In addition, changes in ethnic composition from primarily Caucasian to increasing numbers of Indigenous mothers and non-Caucasian mothers over the years may have contributed to the current trend. Generally, Asian [345] and Indigenous [323] infants have lower birthweights than White Caucasian infants.

An upsurge in the prevalence of maternal obesity and GDM were the most cited causes for increases in mean birthweight and HBW during 1980-2000 in many developed countries [324, 325], including parts of Australia [326, 327]. As evidenced in the secular trends study, the rates of pre-pregnancy overweight/obesity (40.5% in 2013 to 48.8% in 2018) and GDM (1.7% in 2005 to 12.3% in 2018) have increased strikingly during the study period, but infant birthweight has decreased rather than increased. This indicates that adverse impacts of maternal obesity and GDM on foetal growth may have been controlled, potentially through improved obstetric care. Specifically, infants' birthweight has started to drop decline from 2010 onwards. In Australia, there have been substantial improvements in maternal and child healthcare services in the past decade, specifically following the 2010 National Maternity Services Plan [450, 451]. As a consequence, there might have been better control of GWG in mothers with overweight/obesity and blood glucose levels in mothers with GDM, which averted increases in birthweight in their infants. However, since the current Tasmanian perinatal data collection does not contain specific information on GWG and glycaemic control in GDM mothers, further analyses on these factors were not possible. Moreover, the fact that our statistical models explained only ~50% of the

variation in birthweight also reinforces the need for further investigations on these and other maternal factors (e.g., maternal diet, supplement intake) not explored in our study.

Since birthweight is only a crude indicator of adiposity at birth, gathering objective measurements of body composition during early infancy and exploring their relationship to pre- and postnatal factors would provide valuable information on differential growth in body compartments under various exposures. In our prospective longitudinal study, we investigated associations between various pre-pregnancy, pregnancy and postnatal factors and adiposity measured with PEA POD in a sample of healthy Tasmanian infants from birth to 6 months (Chapter 4). During the period of participant recruitment (September 2017 to March 2019), we approached 1375 mothers, and 317 mother-infant dyads volunteered for the initial assessment (within 3 days of birth). The mean weight of infants was 3283 g, lower than the mean birthweight of all live-born Tasmanian infants reported in the secular trends study in 2018 (3359 g). This substantial difference (76 g) in mean birthweight between the two studies may be due to differences in the time birthweight was measured. Birthweight data in the secular trends study was measured soon after birth, whereas in our prospective longitudinal study, infant age at time of measurement varied from 0-3 days. Physiological weight loss due to fluid reduction (water loss) during the first few days of life is common in infants [452]. Moreover, the proportions of HBW (4.1%) and LBW (5.4%) among the infants who participated in the prospective longitudinal study were lower than the state-wide rates in 2018 for all live births in the secular trends study (11.0% and 6.5%, respectively). This could be due to the strict inclusion criteria used as our study is a part of a multi-country project that aims to develop body composition reference charts for healthy infants. We excluded newborns with congenital anomalies or who were admitted to the NICU. Infants with LBW or HBW are often admitted to the NICU for special medical care for conditions such as respiratory distress syndrome, irregular body temperature and hypoglycaemia.

Of the various predictors considered in the prospective longitudinal study (Chapter 4), only gestation length and infant sex were associated with infant weight at birth. Birthweight increased by 174 g per every one-week increase in gestation length, and male newborns were 181 g heavier than female infants. Results of the secular trends study showed that for each additional week of gestation, birthweight increased by 197 g, with male infants 136 g heavier than female infants at birth. These differences in the estimates were expected due to two reasons. First, the secular trends study included all singleton live births, whereas the prospective longitudinal study was limited to term-born singleton live infants. Second, the predictor variables included in the model construction differed between the two studies and were dependent on the availability of data. For

example, data on maternal weight gain during pregnancy was not available in the secular trends study, while net gestational weight gain (nGWG) data was available in the prospective longitudinal study.

In the prospective longitudinal study, we investigated associations between various pre- and postnatal factors and adiposity in infants from birth to 6 months. These relationships were examined with a range of adiposity indices, i.e., FM, %FM, FMI and FM/FFM^p as used in other similar studies. Absolute FM and %FM are the most common adiposity indices utilised in paediatric research; however, concerns have been raised regarding the conceptual and statistical validity of both measures. Absolute values of FM cannot elucidate between-individual variability. On the other hand, %FM, which is FM adjusted for body weight, is statistically flawed because body weight is a combination of FM and FFM, and therefore includes FM in both numerator and denominator. Although not commonly used, FMI (FM adjusted for height) and FM/FFM^p (FM adjusted for FFM) have been presented as more appropriate measures to assess the risk imposed by body fatness [368]. We found that the determinants of these various adiposity measures may not be the same. For example, gestation length was a predictor of FM but not any other indices. Similarly, ppBMI was a predictor of %FM, not any other indices. Our findings suggest that the use of different adiposity indices could be one reason for the inconsistency in the literature regarding determinants of infant adiposity.

Previous research has emphasised that increased FM in infants born to mothers with high ppBMI is a plausible mediator of the intergenerational cycle of obesity [46, 47]. Maternal ppBMI, as well as GWG, are commonly used markers of maternal nutrition during gestation. Mothers with higher ppBMI or excessive GWG have higher levels of circulating glucose, free fatty acids and triglycerides, which cross the placenta and reach the foetus stimulating foetal insulin secretion and thereby promoting adipogenesis [361]. However, their relationship with infant FM was not evident in all studies [48, 49, 57]. Similarly, the relationship of ppBMI and GWG with infant %FM has also been contradictory: some reported that infant %FM was positively associated with both ppBMI and GWG [54], and others showed that this relationship was limited to either ppBMI [57] or GWG [208]. In our study, ppBMI was only related to infant %FM at birth. We found no association between GWG and infant adiposity, and this could be due to the method we used to calculate GWG. In contrast to commonly used GWG (the difference between pre-pregnancy weight and weight near to onset of labour), we used nGWG (the difference between pre-pregnancy weight and post-labour weight). As GWG includes the weight of the infant, placenta and amniotic fluid, we believe nGWG accurately reflects the actual weight gain of the mother, and we recommend adopting nGWG along with GWG in future research.

Micronutrient requirements increase during pregnancy, and to meet this growing demand, vitamin and mineral supplements are often recommended for pregnant women. However, concurrent intake of good-quality nutrient-rich diets and micronutrient supplements, common in women in developed countries, may result in total intakes of certain micronutrients exceeding the daily recommendations [242]. For example, the Australian recommendation for the intake of iron during pregnancy is 27 mg per day, and this can be achieved with most prenatal supplements [240]. Iron supplementation demonstrates a U-shaped risk curve, i.e., both inadequate and excessive iron levels during pregnancy can result in adverse effects on birthweight outcomes [453, 454]. We found negative associations between supplemental iron intake during pregnancy and all measures of adiposity at 3 and 6 months. The fact that there was no difference in body composition at birth between infants of mothers who took iron supplements and those who did not, indicates that both the groups of infants received iron adequate iron for foetal growth. The women who did not take iron supplements may have maintained iron levels adequate for foetal growth through their normal diet. However, the infants of the mothers who took iron supplements may have received excessive amounts of iron which was deposited during foetal growth, and this stored iron may have promoted greater production of red blood cells, myoglobin, and muscle growth during the postnatal period [363], leading to the relative increase in FFM and reductions in adiposity. Alternatively, associations observed between iron supplementation and infant body composition could be due to mothers who use supplements being more aware of health issues in pregnancy and leading healthier lifestyles consistent with infant leanness. Nonetheless, to the best of our knowledge, no previous study has investigated associations between iron supplementation and infant adiposity measures; thus, our results are novel and worthy of further investigation.

Our findings regarding the effects of maternal micronutrient supplements have little practical significance and must be interpreted with caution. Since all the questionnaires used in the prospective longitudinal study were pre-designed and used across all sites involved in the multi-country project, we did not collect additional data. For example, we only asked mothers if they took supplements throughout pregnancy or not, and we had no record of doses and period of intake. Further investigations on the longer-term effects of prenatal vitamin and mineral supplementation on infant adiposity are warranted.

Infant feeding practices are a key determinant of postnatal body composition. The WHO recommends exclusive breastfeeding up to 6 months of age and continuing breastfeeding with complementary foods for 2 years or more for optimal growth and health [455]. In our study, formula-fed infants had lower adiposity than breastfed infants at 6 months, potentially due to a

higher protein level in formula-milk compared to breastmilk and the promotion of growth in FFM [456]. This association may appear to be counterintuitive given the well-known protective effect of breastfeeding for later life obesity but has been reported in several other similar studies. A systematic review [296] of 15 such studies highlighted higher adiposity in breastfed infants compared to formula infants around 6 months of age. The authors explained this finding as an evolutionary adaptation as at approximately 6 months of age many infants start weaning; having enough energy stored by this time may be a mechanism to prepare the infant for the unstable weaning period. Moreover, by the age of 12 months, this association seems to reverse with formula-fed infants having high adiposity as well as rapid weight gain; both are risk factors of future obesity. As our study was limited to 6 months, we could not observe these associations. Although we started to follow-up infants who participated in the prospective longitudinal study when they were 9 and 12 months old, with the view to this being an additional study within the PhD program, it had to be stopped due to the COVID-19 lockdown in 2020.

The prevalence of GDM has increased globally as a consequence of rising rates of maternal pre-pregnancy overweight/obesity and advancing maternal age [457]. While some have demonstrated an increase in birthweight and adiposity at birth in infants exposed to GDM *in utero* [55, 63], others have failed to identify such an association [64, 458]. In the secular trends study (Chapter 3), we have shown that exposure to GDM was positively associated with birthweight of Tasmanian infants. However, in the prospective longitudinal study (Chapter 4), it was not a significant determinant of infant adiposity from birth to 6 months. One reason for this may be that the number of infants born to mothers with GDM in the cohort study was too low to detect an association. Participation in our prospective longitudinal study was voluntary, and we adopted strict inclusion/exclusion criteria such as the omission of infants admitted to NICU after birth. Correspondingly, the rate of GDM in the prospective longitudinal study (9.9%) was lower than the rate found in the secular trends study (12.3% in 2018), and the rate of GDM in the state (13.5% for all live births 2016-17) [338]. GDM increases the risk of caesarean delivery or operative vaginal delivery in mothers. The neonates of mothers with GDM are prone to adverse birth outcomes, including hypoglycaemia, shoulder dystocia, respiratory distress and birth traumas [459] and often need to be admitted to the NICU after birth. During the study period, approximately 7-8% of newborns at the Launceston General Hospital were admitted to the NICU soon after birth.

The contradictory findings in the literature regarding the impact of GDM on neonatal adiposity may have arisen from differences in blood glucose control using various treatment strategies in mothers with GDM. As identified by the HAPO study [381], the association between maternal

glycaemia and infant adiposity is a continuum, where infant adiposity increases with increasing maternal glucose concentrations. When GDM is well-controlled, infants seem to have normal birthweight or body composition [64, 214]. It is possible that the mothers with GDM who participated in our prospective longitudinal study had adequately controlled blood glucose levels, making GDM a non-significant determinant of infant body composition.

In our systematic review (Chapter 5), we appraised literature reporting adiposity in newborns born to mothers treated for GDM. Limited evidence showed that treating GDM lowered FM in newborns compared to no treatment, regardless of the type of treatment (insulin, metformin, glyburide). A meta-analysis of adiposity in infants of mothers who were treated for GDM compared to infants of mothers with NGT, showed that the former group had higher overall adiposity (FM and %FM), but there was no significant difference in subcutaneous adiposity (ST). This finding indicated that, despite treatment to control blood glucose levels in GDM mothers, their infants might be distinguished by a non-subcutaneous fat accrual (e.g., visceral fat) leading to increased total body adiposity. Studies comparing different adiposity compartments in infants are limited, with small sample sizes and conflicting findings [355, 435]. Hence, further research is warranted to identify early changes in different body compartments in the offspring of mothers with GDM.

Notably, we found no significant differences in FM and %FM between the two infant groups in more recent studies (post-2010). This could be due to strict management of blood glucose levels with improved perinatal care for mothers with GDM and increasing awareness of short- and long-term health outcomes of the disease for both mother and infant. However, 60% of the studies included in this meta-analysis did not report the level of glycaemic control in GDM mothers. Future studies should obtain reliable information on glycaemic levels throughout pregnancy to evaluate the 'true' effect of GDM following treatment.

To derive adiposity estimates from birth to 6 months in our prospective longitudinal study, we used the ADP PEA POD system. PEA POD is often the preferred technique during infancy because it is non-invasive, rapid, has been validated against the gold standard 4C method, and accommodates different infant behaviours (e.g., movement, crying) [66]. The major limitation of the PEA POD is that it only accommodates infants up to 6 months of age (approximately 8-10 kg of body weight). A Paediatric Option with appropriate hardware (custom-designed seat and paediatric calibration cylinder) and software has been developed for the BOD POD (adult ADP system) to assess body composition in children aged 2-6 years, and has been validated against a 4C model in children in this age range [154]. However, to date, body composition estimates obtained from ADP with Paediatric Option in children from 6 months to 2 years of age have been

not validated against a 4C model. We hypothesised that DD is a suitable alternative technique for infants who can no longer be accommodated in the PEA POD. Only one previous study of predominantly Asian infants [150] has reported that body composition measurements in term-born infants (aged from 0.4 to 24.4 weeks) obtained by PEA POD did not significantly differ from DD. However, in our evaluation of the agreement of body composition assessed via PEA POD and DD in 6-month-old predominantly Caucasian infants, we identified significant proportional bias with DD technique underestimating infant fatness compared to PEA POD at lower adiposity values. In an additional analysis using mean values above the first quartile, such proportional bias was not observed. Results suggest that DD might be a suitable technique to assess longitudinal body composition after 6 months of age in infants who are not at the lower end of the adiposity spectrum at 6 months. Further research is warranted to better understand the reasons for this difference.

CHAPTER 8 : SUMMARY AND CONCLUSIONS

The main findings of studies comprising this thesis (and visually depicted in Figure 8.1), have important implications for clinical practice and future research on infant growth and body composition. We have shown that the mean birthweight and proportion of HBW infants decreased while the proportion of LBW infants increased in Tasmania from 2005 to 2018. However, as of 2018, the rate of HBW (1 out of 9 babies) was still higher than the rate of LBW (1 out of 15 babies). Women who had hypertensive disorders, smoked, used illegal drugs, consumed alcohol during pregnancy or were aged >35 years, had a higher likelihood of giving birth to infants with LBW. Compared to Caucasian mothers, the risk of having a LBW baby was higher for Indigenous and non-Caucasian mothers. Women with overweight/obese ppBMI and pre-existing diabetes had a higher risk of having HBW infants. Although GDM was not a risk factor for HBW in our study, infants of mothers with GDM had higher birthweight than infants born to mothers with NGT. These findings will be helpful in determining strategies to improve public awareness and health literacy of future parents and state-level policy changes. Importantly, since the rate of pre-pregnancy overweight/obesity has markedly risen over the years, there is an urgent need for health authorities to take further action to raise the awareness of women to maintain a healthy weight during pre-pregnancy. Also, recording maternal weight at the start (e.g., pre-pregnancy weight, weight at first antenatal visit) and the end of pregnancy (e.g., onset of labour, post-delivery), and blood glucose level in mothers treated for GDM as a part of routine clinical practice, may assist to better understand the impact of GWG and glycaemic control in GDM pregnancies on foetal growth.

For the first time, we assessed body composition in Tasmanian infants from birth to 6 months and explored pre- and postnatal determinants of selected adiposity measures across infancy. We showed that both pre- and postnatal factors influence adiposity from birth to 6 months of age, indicating any unfavourable influences during the intrauterine period may be ameliorated until this point, during the postnatal period. The finding that high maternal ppBMI can increase adiposity in newborns provides further endorsement of the importance of mothers maintaining a healthy weight during pre-conception for healthy growth in infants. However, given that BMI can introduce bias in predicting effects related to obesity, as it does not take age, sex, muscle mass, bone structure or distribution of body fat into consideration, the use of objective methodological approaches such as ADP to assess body composition in mothers should be encouraged. Our analysis of postnatal infant feeding practices demonstrated that formula feeding during the first 6 months may alter the normal adipose tissue development trajectory in infants, highlighting the importance of exclusive breastfeeding up to 6 months for normal growth patterns

during early infancy. We also generated a hypothesis regarding the impact of micronutrient supplement intake during pregnancy on postnatal body composition of the offspring, which should be tested with adequate information regarding dose and duration of different supplements. Another important finding of this study was that associations between maternal factors and infant adiposity depended on the measure of adiposity used. This explains some of the inconsistency in the current literature on maternal impacts of infant adiposity and reinforces the need to use conceptually and statistically robust approaches such as FMI or FM/FFM^{p} along with commonly used indices such as FM and %FM in research. It also informs readers to be mindful of the fact that the determinants may differ between studies based on which adiposity index has been used.

By systematically reviewing the literature that reported effects of GDM controlled with therapeutic interventions on infant adiposity at birth, we found that treatments for GDM lowered newborn adiposity compared to no treatment. There were no significant differences in adiposity in infants of mothers who were treated for GDM according to the mode of therapy. Infants of mothers who were treated for GDM had higher total body adiposity compared to infants of mothers with NGT, but there was no significant difference in subcutaneous adiposity, suggesting that increased adiposity accumulation infants of mothers who were treated for GDM may be non-subcutaneous (e.g., brown fat or visceral fat). This opens a new avenue for future researchers to distinguish adipose tissue distribution in infants of mothers who were treated for GDM vs infants of mothers with NGT. A meta-analysis of recent studies (studies conducted after 2010) suggested that treatments for GDM can normalise adiposity in infants, and we assume this might be due to increased prenatal care for mothers with GDM in recent years. We noted that nearly 60% of the studies included in our systematic review had not reported the level of glycaemic control in mothers treated for GDM; hence, future studies should obtain reliable information on glycaemic levels throughout pregnancy to evaluate the 'true' effect of GDM after treatments.

Furthermore, some of the discrepancies of determinants of early life body composition may have arisen due to differences in confounder adjustments between the studies. Future studies should adjust the estimates of exposure effects for potential covariates (e.g., the association between GDM and infant adiposity is likely to be confounded by maternal BMI, but only a few studies have included maternal BMI as a confounder in regression analysis). The exposures that have been scarcely researched, and hence should be considered in future studies are maternal intake of prenatal supplements such as iron, folic acid and vitamin D, and supplements/vitamins for infants. It is also important to understand how far into the life course the influences of early life exposures last. Therefore, longitudinal studies investigating such associations from infancy to childhood and adolescence, where risks of early-life exposures may manifest, are warranted.

Finally, evidence from our methods comparison study showed that body composition data obtained using the PEA POD are comparable with the DD technique in 6-month-old infants whose adiposity levels were not at the lower end of the spectrum. We observed similar results using different adiposity measures such as FM, %FM, and FMI, suggesting that, regardless of the adiposity index, these two techniques can be used in 6-month-old infants whose adiposity levels are not too low. Additional research is required to understand why PEA POD and DD are not in good agreement in infants at low body fat levels.

Overall, the findings of the studies detailed in this thesis advance previous knowledge on determinants and measures of accumulation of adiposity in early life and provide important insights for future infant body composition research. Given that compositional changes present during infancy may persist to childhood and beyond, making individuals susceptible to obesity and associated diseases, our research findings contribute to the body of knowledge on the importance of early identification of changes in body composition as a way of monitoring potential risk of progression to overweight and obesity.

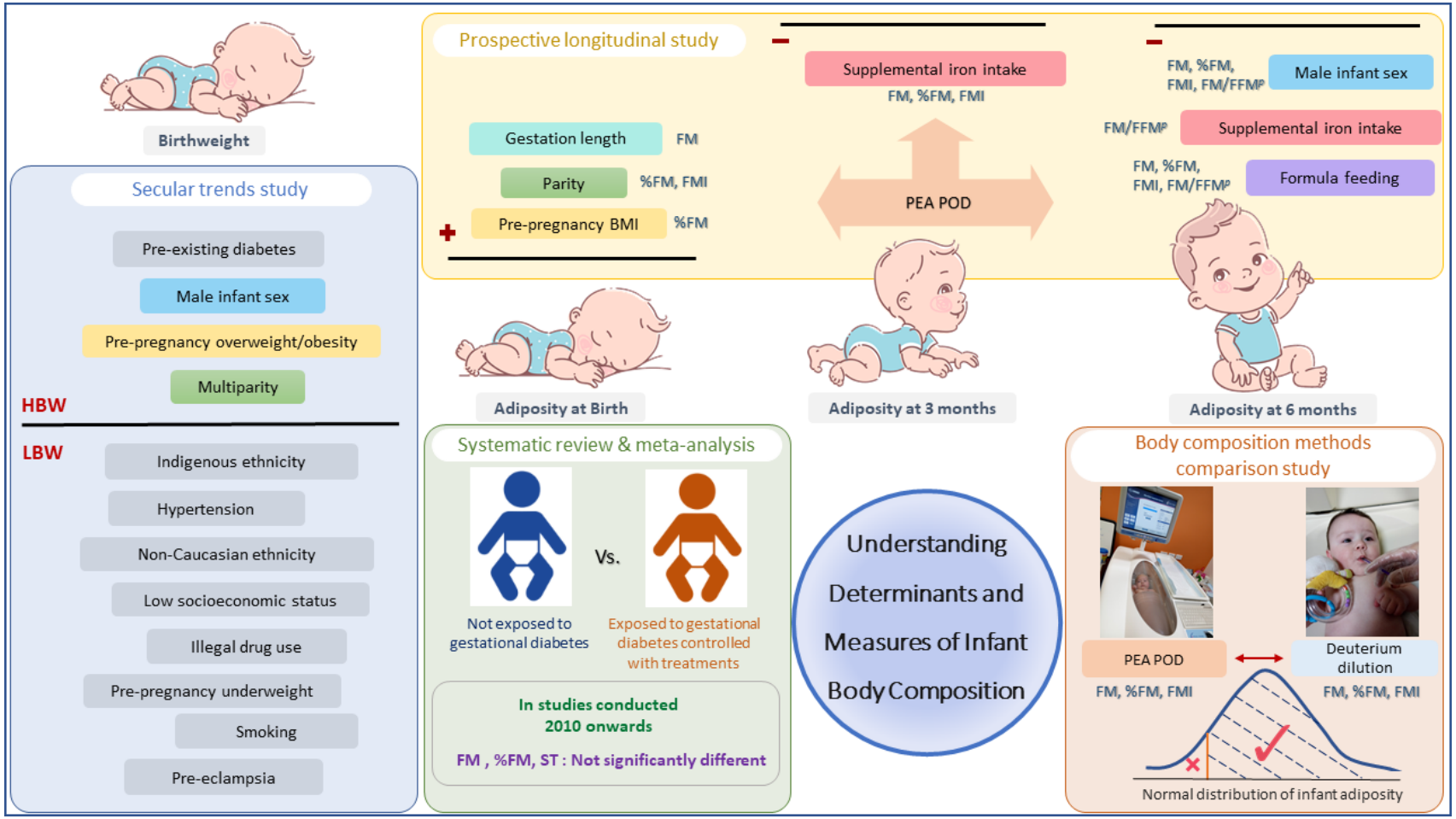


Figure 8.1 A visual abstract of main findings.

HBW: high birthweight; LBW: low birthweight; FM: fat mass; %FM: percent FM; FMI: fat mass; FM/FFM^{p}: log-log index; ST: skinfold thickness.

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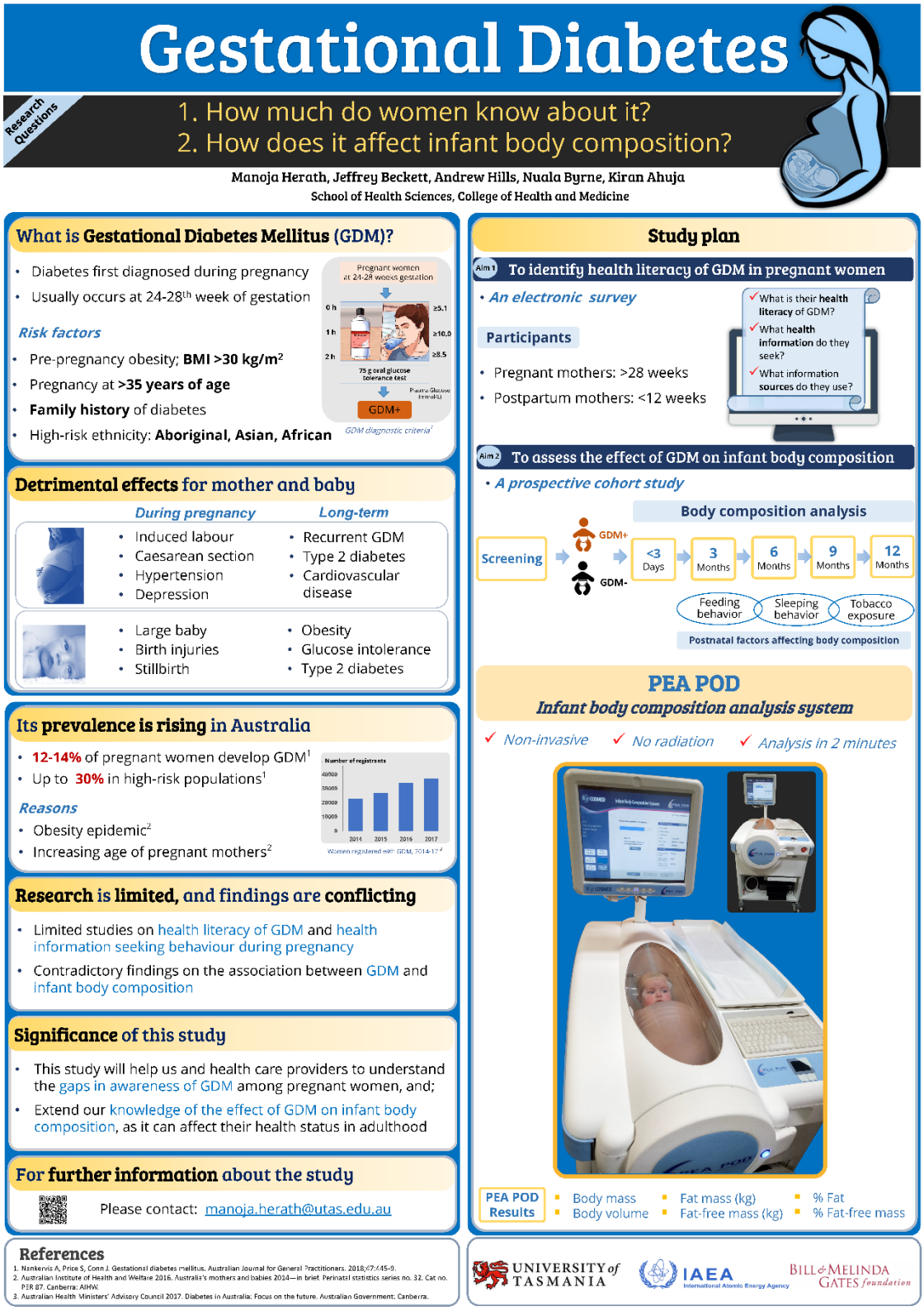
APPENDICES

Appendix 1: Copy of poster for Annual Graduate Research Conference, University of Tasmania (2018)



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Appendix 2: Certificate for Nestle Emerging Researcher Award 2021