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An exploration of body composition in healthy early and full term infants using Air Displacement Plethysmography shortly after birth

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Abstract

Background: The Developmental Origin of Health and Disease theory suggests environmental factors during gestation are important early predictors of later disease. There is a wealth of evidence identifying an association between low and extreme birth weights and an increased risk of adverse health outcomes in later life. The importance of growth in early life led to standardised monitoring of body weight, length and head circumference at birth and throughout infancy. Evidence now suggests body composition, specifically adiposity, in early life to be a better marker of poor health outcomes in later life. Gestation is a continuum and during each week of gestation the foetus continues to accrue fat mass (FM) and fat free mass (FFM), which are not routinely measured at birth. Development of air displacement plethysmography (ADP) presents a valid and reliable technique to measure FM and FFM of infants at birth. Majority of infants are born at term gestation (37 to <42 weeks). Early term infants (37 to <39 weeks) have a higher risk of developing adverse clinical outcomes and later health issues compared to full term infants (39 to <42 weeks). It is currently unknown whether there are differences in FM and FFM between infants born early versus full term.

Aim: To investigate the FM and FFM of healthy early and full term New Zealand (NZ) infants within three days of birth.

Methods: Healthy term infants were recruited from Auckland City Hospital (ACH), NZ as part of this cross-sectional observation study. Weight, length and waist circumference were measured using standardised techniques. ADP was used to measure FM and FFM of infants. Infants were grouped into early or full term categories. Waist circumference was divided by length to give the waist to length ratio (WLR). Two indices of length-normalised body composition were calculated: a FM index (FMI) and FFM index (FFMI) derived by dividing FM and FFM values (kg) by length² (m²). Independent 2-tailed t-tests were used to compare the body composition measurements between early and full term infants and between genders.

Results: 255 healthy term infants were recruited. There were no differences in the percentage of FM and FFM between early term and full term infants (10.2±4.0% vs 11.1±4.1%, $P=0.109$ and 90.0±4.0% vs 89.0±4.1%, $P=0.110$). Full term infants had significantly higher FMI and FFMI compared to early term infants (1.44±0.6 vs 1.26±0.06, $P=0.02$ and 11.3±1.0 vs 10.8±0.96, $P<0.001$). Early term males had significantly heavier body weights ($P=0.04$), FFM (2793.1±332.9g vs 2619.7±315.4g, $P=0.003$), FFM % (90.8±3.8% vs 88.7±4.0%, $P=0.009$), FMI (1.15±0.55 vs 1.38±0.56, $P=0.039$) and lower FM % (9.2±3.8% vs

11.3±4.0%, $P=0.009$) than female early term infants. No gender differences within full term infants were noted in FM (g), FFM (g), FM %, FFM %, FMI or FFMI.

Conclusion: The results of this study suggest full term infants continue to gain FM and FFM along the same trajectories as that at early term gestation although they have greater FMI and FFMI than early term infants. While there were gender differences in body composition noted between early term infants, they were no longer apparent within the full term infants. This study identified the need to investigate the body composition changes of healthy early and full term infants at different time periods following birth. This will allow observation of factors which influence body composition in early life.

Keywords: *Early term, full term, infant, body composition, air displacement plethysmography, adiposity*

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Table of contents

Abstract	i
Acknowledgements	iii
Table of contents	iv
List of tables	vi
List of figures	vii
Abbreviations list	viii
Chapter 1: Introduction	1
1.1 Background	1
1.2 Justification	2
1.3 Purpose of the study	3
1.4 Aim and objectives	3
1.4.1 Aim of study	3
1.4.2 Objectives.....	3
1.4.3 Hypotheses.....	3
1.5 Thesis structure	4
1.6 Contribution of researchers	4
Chapter 2: Literature review	5
2.1 Definition and classification of term births	5
2.2 The rates of early and full term births	5
2.3 Causes of early term birth	6
2.4 Consequences of being born early term	7
2.5 Early life programming	8
2.6 Early adiposity and later health outcomes	8
2.7 Monitoring growth and health	9
2.8 Techniques for measuring infant body composition	11
2.8.1 Skinfold thickness.....	11
2.8.2 Doubly labelled water	11
2.8.3 Multi-compartment models.....	12
2.8.4 Bioelectrical impedance analysis	12
2.8.5 Dual energy x-ray absorptiometry	12

2.8.6 Air displacement plethysmography	13
2.9 Fat mass and fat free mass of infants.....	16
2.10 Summary	17
Chapter 3: Thesis manuscript	19
3.1 Abstract	19
3.2 Introduction	19
3.3 Materials and methods	20
3.3.1 Design and participants	20
3.3.2 Data collection	20
3.3.3 Statistical analysis	21
3.4 Results	22
3.4.1 Participant characteristics.....	22
3.4.2 Body composition with gestational age.....	22
3.4.3 Comparison of anthropometric measures of early term and full term infants	23
3.4.4 Effect of gender.....	24
3.5 Discussion	25
3.5.1 Characteristics of participants	25
3.5.2 Early term versus full term.....	26
3.5.3 Gender differences	26
3.5.4 Strengths and limitations	27
3.5.5 Conclusion.....	27
3.6 Acknowledgements	27
3.7 Conflict of interest	28
Chapter 4: Conclusion and recommendations	29
4.1 Brief overview	29
4.2 Strengths and limitations	29
4.3 Recommendations for future research	30
Reference list.....	31
Appendix A - Material used in conducting research	39
Figure A.1: Participation information sheet.....	39
Figure A.2: Information pamphlet	43
Figure A.3: Consent form	45

List of tables

Chapter 1

Table 1.1 Researchers' contributions to the study	4
---	---

Chapter 3

Table 3.1 Participant characteristics	22
Table 3.2 Comparisons of anthropometric measures between early term and full term infants	24
Table 3.3 Comparisons of anthropometric measures between male and female early term infants	24
Table 3.4 Comparisons of anthropometric measures between male and female full term infants	25

List of figures

Chapter 2

Figure 2.1 Percentage of babies, by gestation in weeks, 2006 - 2015	5
---	---

Chapter 3

Figure 3.1 Distribution of body composition measures according to gestational age	23
---	----

Appendices

Figure A.1 Participation information sheet	39
--	----

Figure A.2 Information pamphlet	43
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Figure A.3 Consent form	45
-------------------------------	----

Abbreviations list

Abbreviation	Definition
ACH	Auckland City Hospital
ADP	Air displacement plethysmography
BIA	Bioelectrical impedance analysis
BMI	Body mass index
cm	Centimeter
CS	Caesarian section
CVD	Cardiovascular disease
DLW	Doubly labeled water
DXA	Dual-energy X-ray absorptiometry
FFM	Fat free mass
FM	Fat mass
FMI	Fat mass index
FFMI	Fat free mass index
FFM %	Fat free mass percentage
FM %	Fat mass percentage
g	Gram
LGA	Large-for-gestational age
N	Number
NZ	New Zealand
SGA	Small-for-gestational age
TBW	Total body water
US	United States
WLR	Waist-to-length ratio
<	less than
>	greater than

Chapter 1: Introduction

1.1 Background

There is a wealth of evidence that growth and development in early life plays an important role in programming later health outcomes (Barker, Eriksson, Forsén, & Osmond, 2002; Carberry, Colditz, & Lingwood, 2010; Wells, Chomtho, & Fewtrell, 2007). Fetal development has been referred to as ‘plastic’ as the foetus responds to exogenous stimuli by alterations in gene expression, especially during periods of rapid tissue growth and development to sustain health (Agosti, Tandoi, Morlacchi, & Bossi, 2017; Barker et al., 2002; Barouki et al., 2012). The period from birth to two years of age has been referred to as the “critical window” due to the infant continuing to adapt to their environment (Barker et al., 2002; Wells et al., 2007). Previously, extremes in birthweight and early weight gain have been associated with later adverse health outcomes. It has been hypothesised that neonatal adiposity may be more closely related to the mechanism of programming later health than birthweight or early weight gain (Ratnasingham et al., 2017). Therefore, monitoring adiposity at birth is imperative to understand its association with later health outcomes.

In utero the foetus experiences rapid growth and accrual of fat mass during the third trimester of pregnancy and with each week of gestation, birthweight increases. Infants born early term (37 to <39 weeks gestation) are at a higher risk of adverse clinical outcomes and later health issues compared to infants born at full term (39 to <42 weeks gestation). Altered adiposity in early life has the potential to be a contributing factor to the poorer outcomes observed in early term infants (Ratnasingham et al., 2017). These concepts align with the Developmental Origin of Health and Disease theory, which proposed fetal development is plastic, in which the foetus responds to exogenous stimuli by alterations in gene expression, especially during periods of rapid tissue growth to sustain health (Agosti et al., 2017; Barker, 2007; Barouki et al., 2012). Adverse changes in programming cause and altered phenotype which limits the infants’ ability to adapt to the extrauterine environment causing disease vulnerability which is proposed to be a potential origin of non-communicable diseases (Barouki et al., 2012; Kurtoğlu et al., 2012). At birth, growth is routinely measured with anthropometric measures such as weight, length and head circumference as proxy measures of growth in utero and body composition at birth. Advanced techniques such as air displacement plethysmography (ADP) have been developed (COSMED, 2004) which assess fat mass and fat free mass in infants. These measures of body composition facilitate assessing neonatal adiposity shortly after birth.

1.2 Justification

Weight is an indicator of prenatal growth and is routinely measured to monitor postnatal growth (Ministry of Health, 2015). Epidemiological studies have associated alterations in birthweight with adverse health outcomes later in life (Barker et al., 2002). More specifically small for gestational age (SGA) births, defined by the World Health Organisation as a birth weight below the 10th percentile for gestational age (World Health Organisation, 2014), are associated with an increased risk of adverse health outcomes during infancy and in later life (Barker et al., 2002). Outcomes include moderate to severe deficits in educational achievement, attention problems and internalising behavioural problems alongside cardiovascular disease (CVD) and type two diabetes (Aarnoudse-Moens, Weisglas-Kuperus, van Goudoever & Oosterlaan, 2009; Barker, 2002; Hack et al., 2002; Singhal et al., 2003). On the other end of the spectrum, large for gestational age (LGA) births, classified as a birthweight above the 90th percentile for gestational age, are positively associated with subsequent greater body mass index (BMI) in childhood and later life (Ong, 2006; Singhal et al., 2003). Besides their differences in birthweight, both SGA and LGA births have increased risks of obesity and its associated cardiovascular and metabolic disorders. A common feature of both of these scenarios is the early accumulation of excess body fat, through excess nutrient availability in utero or rapid catch up growth immediately after birth (Ratnasingham et al., 2017). Alterations in body composition, fat mass (FM) and fat free mass (FFM), during the neonatal period may be a better marker of adverse health later in life, and more closely related to the mechanism by which maternal environment and placental adaptation mediate effects on adult health (Barker, 2002; Hull et al., 2008; Ratnasingham et al., 2017; Wells et al., 2007).

Majority of infants (90.7%) born in New Zealand are born at term gestation (37 to <42 weeks) (Ministry of Health, 2015). Birth data shows that alongside the recent decrease in the number of infants born per year in New Zealand, the proportion of early term births has increased between 2009 and 2016 (Ministry of Health, 2018). In addition to New Zealand, Australia reported similar findings from 1994 to 2009, showing an increase in those born early term and a decrease in the number of live births at full term (Nassar, Schiff, & Roberts, 2013). The increasing numbers of deliveries occurring before 39 weeks gestation is concerning (Oshiro et al., 2009; Nassar et al., 2013) as early term births are associated with an increased risk of morbidity and mortality compared to full term births (39 to <42 weeks) (Engle & Kominiarek, 2008; Fleischman, Oinuma, & Clark, 2010; Machado Jr, Passini Jr, Rosa, & Carvalho, 2014; McIntire & Leveno, 2008; Nir, Nadir, & Feldman, 2012).

Although weight is an easy non-invasive measure, it does not distinguish the compositional nature of infant growth (Carberry et al., 2010). The majority of research on body composition in early life has been conducted using methods which are impractical or of unknown accuracy (Wells et al., 2007). In

the clinical setting ADP would be a viable option for estimating FM and FFM in newborn infants. It has been shown to be a reliable technique and has been validated against methods of gold standard including multi-compartment models and doubly labelled water (DLW) (Deierlein et al., 2012; Ellis et al., 2007; Forsum, Olhager, & Törnqvist, 2016; Ma et al., 2004; Roggero et al., 2012).

Gender differences in infant body composition may predict later fat distribution and expression of risk factors and incidence of disease later in life (Fields, Krishnan, & Wisniewski, 2009). Many studies have reported term female infants having greater FM % and less FFM than age-matched male infants at one month of age (Carberry et al., 2010; Deierlein et al., 2012; Fields et al., 2009; 2011; Kirchengast, 2010; Wibaek et al., 2015). A large cohort of 743 infants by Hawkes et al. (2011) presented FM % results stratified by gestational age and sex and found female infants to have greater FM % at 38 to <40 weeks and at 40 to <42 weeks gestation, compared to males. It is important to understand the gender differences in body composition during early life to allow further research to explore the possible relationship between gender, body composition and later health outcomes (Fields et al., 2009).

1.3 Purpose of the study

The purpose of this cross sectional, observational study was to compare the body composition of healthy early term and full term infants in New Zealand using ADP. As altered fat accumulation in early life has been suggested to be a better marker of poor health outcomes later in life than adiposity and weight alone. We compared the FM and FFM between healthy early and full term infants born in New Zealand using ADP and between genders.

1.4 Aim and objectives

1.4.1 Aim of study

To investigate the fat mass and fat free mass of healthy early and full term New Zealand infants within three days of birth.

1.4.2 Objectives

1. To describe FM and FFM of term infants by week of gestational age.
2. To compare FM and FFM between infants classified as early and full term.
3. To compare the FM and FFM by gender between early term and full term births.

1.4.3 Hypotheses

H1: Early term infants will have a significantly lower FM percentage than full term infants.

H2: Male infants in both early term and full term groups will have significantly greater FM and FFM than female infants.

1.5 Thesis structure

This thesis is structured into four chapters: Chapter one, the introduction, presents the scope and justification for the study, followed by an outline of the aim and objectives. Chapter two is a review of the current literature. Chapter three, is presented as a research study manuscript prepared for submission to a peer-reviewed journal. Finally, Chapter four provides a final discussion and conclusion. Followed by the strengths and limitations of the research and the final recommendations for future research. Supplementary appendices include questionnaires and forms used in the study.

1.6 Contribution of researchers

Table 1.1 Researchers' contributions to the study

Researcher	Contributions to thesis
Annaliese Beauchamp <i>MSc Nutrition & Dietetic Student</i>	Main author of thesis; analysed data and performed statistical analysis, interpreted and discussed the results.
Dr Cath Conlon	Primary academic supervisor
A/Prof Pam von Hurst	Academic co-supervisor
Louise van Dorp, Bani Ichhpouniani, Owen Mugridge	Recruited and measured participants, collected and collated the data set.

Chapter 2: Literature review

2.1 Definition and classification of term births

Term birth includes infants born between 37 to <42 weeks (Ministry of Health, 2015; National Maternity Monitoring Group, 2014; World Health Organisation, 2012). The definition 'term birth' has been further defined to include early term (37 to <39 weeks) and full term (39 to <42 weeks) (National Maternity Monitoring Group, 2014). The recent classification of term gestation is considered to be a result of emerging evidence showing an increased risk of mortality and neonatal morbidity associated with those born early term compared to those born at full term (Engle & Kominiarek, 2008; Fleischman et al., 2010; Machado Jr et al., 2014; McIntire & Leveno, 2008; Nir et al., 2012; Parikh et al., 2014). Infants born beyond 42 weeks gestation are classified as post-term (National Maternity Monitoring Group, 2014).

2.2 The rates of early and full term births

Majority of infants are born at term with a median gestation of infants born in New Zealand (NZ) being 39 weeks (Ministry of Health, 2018). Globally, the proportion of early term deliveries have increased significantly over the past decade (Engle & Kominiarek, 2008; Ministry of Health, 2015; 2018; Nassar et al., 2013; Oshiro et al., 2009). In NZ, the proportion of infants born at 37 and 38 weeks gestation has increased in recent years despite the total number of births falling between 2006 to 2016 (Ministry of Health, 2015; 2018) (Figure 2.1).

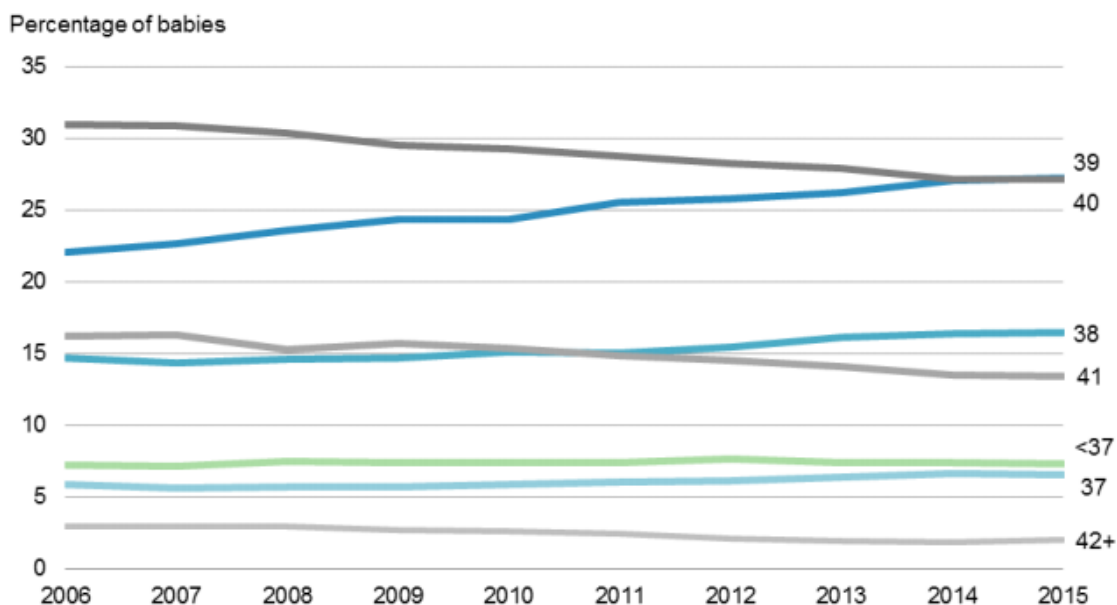


Figure 2.1 Percentage of term infants born at each week of gestation in New Zealand, 2006-2015 (Ministry of Health, 2015).

The reason for this increase in the proportion of early term births is unknown and findings are non-consistent. Studies have suggested it to be related to an increase in obstetric interventions before 39 weeks gestation; including caesarean sections (CS) or labour induction (Nassar et al., 2013; Richards et al., 2016). Spontaneous delivery has been also been reported to be the reason behind the increased early term deliveries (Barros, Clode, & Graca, 2016).

As a result of the increasing proportion of early term births in NZ, the Ministry of Health (2015) reported the proportion of infants born later to have fallen (40 weeks 31% to 26.5% and 41 weeks 16.5% to 13%) from 2006 to 2015. Similar trends have been observed in Australia (Nassar et al., 2013) and the United States (US) (Engle & Kominiarek, 2008; Oshiro et al., 2009). Recently, multiple programmes have been implemented in the US to discourage early term elective deliveries. One programme implemented in the US included the Women and Newborn Clinical Integration Program. This programme developed and implemented guidelines to discourage early term deliveries, reducing the prevalence of early term elective deliveries from 28% to less than 3% of all elective deliveries over 6 years (Oshiro et al., 2009). Reduced early term birth rates in Norway and Sweden have been attributed to the decrease in clinician-initiated obstetric interventions within the early term gestational period (Richards et al., 2016).

2.3 Causes of early term birth

Births before 39 weeks are either spontaneous, elective with no medical indication or with medical indication. Although the mechanism of which cause spontaneous births before 39 weeks are unknown, identifiable risk factors include short intervals between subsequent pregnancies, intrauterine infection, multiple pregnancies, low maternal BMI during pregnancy, psychological or social stress, smoking and pre-eclampsia (Muglia & Katz, 2010).

Women request elective caesarian deliveries with no clear medical indication for a range of personal and societal reasons. Main themes include anxiety for lack of support during labour, to avoid elements of vaginal delivery, including pain and perineal trauma, to avoid later maternal morbidity and concerns for fetal injury/death (Ecker, 2013; Wiklund, Edman, & Andolf, 2007).

Medical induction of labour is performed when the maternal and fetal risks of continuing the pregnancy outweighs the risks of early delivery (Spong et al., 2011). Risks associated with continuing the pregnancy include compromising of the foetus, uterine rupture, haemorrhage and still birth (Spong et al., 2011). Indications to induce labour before 39 weeks' gestation include maternal hypertension, diabetes mellitus, preeclampsia, placenta abruption, respiratory disease, anaemia, hormonal disease and multiple pregnancies (Brown et al., 2016; Spong et al., 2011). Although with appropriate planning and

support elective CS prior to 39 weeks gestation may be reduced, there will always be a number of infants born early term due to spontaneous delivery and medical reasons.

2.4 Consequences of being born early term

Historically, term infants have been perceived to be a homogeneous group which are not associated with an increased risk of adverse neonatal outcomes (Fleischman et al., 2010). However, emerging evidence has confirmed infants born early term are physiologically and metabolically immature (Engle & Kominiarek, 2008). Being born prior to 39 weeks increases the risk of experiencing significant neonatal complications, such as mortality and morbidity, when compared to full term births (Brown et al., 2013; Engle & Kominiarek, 2008; Fleischman et al., 2010; Machado Jr. et al., 2014; McIntire & Leveno, 2008; Nir et al., 2012; Parikh et al., 2014; Platt, 2014). Short-term adverse neonatal outcomes observed in those born before 39 weeks include neonatal respiratory morbidities, infection, neonatal intensive care unit admissions and prolonged hospital stays (Brown et al., 2013; Ghartey et al., 2012; Gill & Boyle, 2017; Sengupta et al., 2013). Moreover, early term infants have an increased risk of cardiovascular disease (CVD), type two diabetes and increased developmental delay later in life (De Boo & Harding, 2006; Morse, Zheng, Tang, & Roth, 2009; Paz et al., 2017). Gill and Boyle (2017) suggested the classifications of preterm and term gestation should not be considered as a dichotomy but as a continuum, and research suggests the risk and severity of adverse outcomes reduce with increasing gestational age (McIntire & Leveno, 2008; McLaurin et al., 2009).

The mode of delivery has a significant impact on neonatal outcomes. Being born via CS increases the risk of adverse short-term neonatal outcomes including respiratory morbidity, neonatal intensive care unit admissions and longer hospital stays in early term infants, compared to full term births and early term infants born by vaginal delivery (Adams, Gibbons, & Tudehope, 2017; Kotecha, Gallacher, & Kotecha, 2016).

The neonatal outcomes of an infant can be altered by the mode of feeding. The rates of breastfeeding are lower following a CS compared to a vaginal delivery which is concerning as early term infants are at an increased risk of being born by CS. Reasons for reduced breastfeeding following a CS include maternal postoperative pain and ongoing management of medical problems (Beake, Bick, Narracott, & Chang, 2017). Consequently, early term infants born via CS are less likely to receive the health benefits associated with breastfeeding including reduced risk of respiratory tract infection, gastrointestinal infection and type two diabetes (Beake et al., 2017).

In addition, early term infants are also likely to experience difficulties with initiating feeding. This is suggested to be a result of the immaturity of the synchronizations of sucking and swallowing (Craighead,

2012). Early term infants who are breastfed have been found to experience difficulties in sustaining breastfeeding to meet their physiologic needs, increasing the risk of dehydration and malnourishment (Craighead, 2012).

There are many adverse outcomes associated with early term births compared to full term births. It is known that early term infants who are delivered by CS are less likely to be breastfed, which increases their risk of associated adverse outcomes. Moreover, being physiologically and metabolically immature hinders their ability to initiate and sustain feeding, heightening their risk further. Early life programming has been implicated in the link between being born early and adverse health outcomes.

2.5 Early life programming

The Barker hypothesis (proposed by Epidemiologist David Barker) discovered a relationship between impaired fetal growth, such as low birth weight and premature births, and origins of CVD, type two diabetes and hypertension in later life. These findings led to the theory that environmental factors during gestation, such as under or over nutrition, are important early predictors of later disease (Wu et al., 2004). This hypothesis led to further work in early life programming; the Developmental Origin of Health and Disease theory evolved from the Barker hypothesis (De Boo & Harding, 2006). This concept proposed that fetal development is plastic, and the foetus responds to exogenous stimuli by alterations in gene expression, especially during periods of rapid tissue growth to sustain health (Agosti et al., 2017; Barker, 2007; Barouki et al., 2012). Adverse changes in programming can impair metabolic development of the foetus and lead to detrimental effects. The permanent altered phenotype limits the infants' ability to adapt to the extrauterine environment causing disease vulnerability. This phenotype change is proposed to be a potential origin of non-communicable diseases; type two diabetes, CVD, respiratory disease syndrome and neurodegenerative disorders (Barouki et al., 2012; Kurtoğlu et al., 2012). All of which has led to the suggestion of early body composition playing a role in the programming of a variety of health outcomes later in life.

2.6 Early adiposity and later health outcomes

The relationship between early adiposity and later health outcomes is a major focus of current research. Altered fat accumulation in early life has been suggested to be a better marker of poor health outcomes later in life (Ratnasingham et al., 2017). Infant groups of concern include small-for-gestational age (SGA), large-for-gestational age (LGA) and preterm infants, all of which experience rapid fat accumulation either before or immediately after birth.

Small for gestational age infants often experience intrauterine growth restriction, as a consequence of altered maternal and/or placental function, which alone is associated with poor health

outcomes including obesity, type two diabetes and CVD later in life (Barker, 2006). Such outcomes have been associated with altered neonatal body composition rather than reduced growth or low birth weight alone (Ratnasingham et al., 2017). Studies have reported SGA infants to have reduced fat accumulation in utero compared to appropriate for gestational age infants followed by rapid fat accumulation immediately after birth, also known as catch-up growth. It is well known that catch-up growth is associated with adverse health outcomes later in life (Morrison et al., 2010).

As the relationship between birth weight and poor later health outcomes has been reported as a U-shaped curve, LGA infants also have an increased risk of poor health outcomes in later life including components of the metabolic syndrome (Boney, Verma, Tucker, & Vohr, 2005; Dyck, Klomp, & Tan, 2001). This relationship may be a result of being born to overweight or obese mothers and/or mothers with gestational diabetes mellitus (Silverman et al., 1991). The relationship between early adiposity and adverse later health outcomes in LGA infants has been attributed to mothers with excess weight gain or diabetes delivering more energy to the foetus during pregnancy leading to fetal hyperinsulinemia resulting in higher weight and fat mass at birth (Sewell, Huston-Presley, Super, & Catalano, 2006).

It is well known that preterm infants are not only born with a low birth weight but experience growth restriction after birth. This inevitable growth pattern increases their risk of developing adverse outcomes during infancy and later life (Bastek et al., 2008). Despite experiencing growth restriction after birth, preterm infants have been reported to have greater absolute fat mass at term-equivalent age compared to term infants (Roggero et al., 2009). Research has suggested a relationship between adiposity accrual after birth and duration of exposure to ex utero environment and feeding (Roggero et al., 2009).

2.7 Monitoring growth and health

Routine growth monitoring is a core component of the maternal and baby care services, conducted in many countries. This begins at birth and throughout infancy. Each routine visit, at birth and throughout infancy, provides the opportunity for weight, length and head circumference measures. Such anthropometric measures are recorded with growth indicators against age and gender appropriate referenced charts e.g. World Health Organisation growth standards. This allows clinicians to track and assess infant growth, monitor nutritional adequacy of infants' dietary intake and recognise any clinical concerns such as congenital, inherited or acquired conditions (Ministry of Health, 2013; World Health Organization, 2014).

Birthweight alone is an indicator of overall growth in utero. At birth, the infant's weight is compared against reference growth charts to indicate whether weight is outside the normal range. Extremes in

birthweight are classified to identify infants at risk of adverse neonatal outcomes and health conditions in later life. Extreme birthweights include those below the 10th percentile for gestational age which are classified as SGA while birthweights above the 90th percentile for gestational age are classified as LGA. The purpose of identifying these birthweights is to monitor, prevent and manage the risk conditions throughout the lifespan (Cook, 2013). During infancy, regular weight measures are an indication of growth and development, health, nutritional intake, illness or following changes in feeding regimen. Interpretation of current weight takes the previous weight measurements, any illness or changes in nutritional intake of regimen in to account. Accurate weight measures at birth and during infancy require infants to be naked, and the use of reliable and precise scales. Inaccurate measurements falsely indicate an infant's weight and furthermore, growth and health.

Recumbent length is routinely measured to obtain information on infant growth, act as a predictive value for final adult height, and is used against other measures to assess growth. A short length in comparison to weight or gestational age may indicate underlying pathology (Engelberts et al., 2005; World Health Organization, 2014). Common measurements of length involve laying the infant flat on a length board and measuring from the crown to the heel with the movable footboard (World Health Organization, 2014). Following good practice, the average of three measurements should be recorded to the nearest millimeter (Ministry of Health, 2013; World Health Organization, 2014).

Waist circumference is an indicator of central adiposity in adults and children (Fredriks et al., 2005) and is measured by wrapping a centimeter tape around the abdomen at the umbilical-line and recording circumference to the nearest millimeter. To obtain reliable data, the average of three measurements should be recorded (World Health Organization, 2008). Normalising waist circumference for length, known as waist circumference-length ratio (WLR), eliminates age, gender and ethnic differences and is a non-invasive indice for measuring growth (Stokes et al., 2012).

The use of weight, head circumference, length, and WLR allow clinicians to assess infant growth efficiently. However, these measurements do not indicate the composition of growth, including fat mass (FM) and fat free mass (FFM). Infants of similar age, weight and length can contain different ratios of FM to FFM (Eriksson, Löf, & Forsum, 2010). Determining the amount and distribution of FM and FFM of infants in early life is important to understand, in addition to the above measures, as it may play a role in later health outcomes.

The foetus rapidly accrues the majority of its FM in the third trimester of pregnancy. Preterm babies (born before 37 weeks gestation) experience a rapid increase in FM, with a lower proportion of FFM

following birth (Johnson et al., 2012) and reach the FM % of a term infant before term corrected age (Van Dorp, 2015). Therefore, those infants born preterm accrue different amounts of FM and FFM at different rates compared to the FM and FFM stores of an infant born at term.

2.8 Techniques for measuring infant body composition

There are several techniques available to assess body composition of infants, simple anthropometric methods and complex methods. Anthropometry is a non-invasive, indirect technique which relies on predictive equations to estimate body fat. Several techniques are available, varying in complexity and relative magnitude of error. The gold standard for body composition is cadaver analysis, the only direct measure to date (Demerath & Fields, 2014). It is the most reliable method for body composition data for the human foetus. Cadaver analysis includes chemical and anatomic analysis to provide data on body components including; skin, muscle, adipose tissue, bone and organs (Toro-Ramos et al., 2015). Therefore, its use in live infants is impractical and un-ethical. Thus, majority of the available data on infant body composition is based on indirect measures.

2.8.1 Skinfold thickness

Skinfold thickness is a quick, non-invasive, inexpensive and mobile method used to measure adiposity with theoretically defined prediction equations, such as the modified Siri equation (KV, Hemalatha, Mamidi, & Balakrishna, 2016; Schmelzle & Fusch, 2002). Skin fold assessment includes multiple measurements of the subcutaneous fat layer at numerous regions of the body including, but not limited to, triceps, biceps, suprailiac and subscapular using standard skinfold calipers (Reilly, Wilson, & Durnin, 1995). There are validated paediatric equations for skinfold thickness used in infants to estimate FM, FFM and FM %, (Hoffman et al., 2012; Sen, Bose, Shaikh, & Mahalanabis, 2010). Although this method is cost effective, it is not easy to conduct in infants and has been shown to have many sources of error and therefore questionable reliability. It is a highly sensitive technique and has been reported to have a high variance within examiners and between multiple measurements (Branson et al., 1982). An additional source of error can be the choice of prediction equation, as not all are extrapolated to the newborn and early infant period (Schmelzle & Fusch, 2002). These measures are also concerned with subcutaneous fat with no reference to visceral fat or lean body mass, thus limiting results to only describing body fatness (Van der Kooy & Seidell, 1993) which has been seen to have a low correlation with body water dilution in young children (Tennefors & Forsum, 2004).

2.8.2 Doubly labelled water

The measurement of total body water (TBW) involves periodic sampling of body fluids, before and after an administration of a stable isotope solutions (Ellis et al., 2007; Traver et al., 2009; Trowbridge

et al., 1984). This process can take up to three hours (Traver et al., 2009). The samples provide a reference measure of TBW which is then incorporated in the calculation of FFM (Nielsen et al., 2011). Doubly labelled water is a time-consuming technique which requires vigorous cooperation from participants thus unlikely to be conducted routinely as part of clinical practice. The administration of isotope solutions in infants also limits its use, as spills and dripping of isotopes lead to estimation errors (Nielsen et al., 2011).

2.8.3 Multi-compartment models

Multi-compartment models reduce assumption by measuring more than two components of body mass (Demerath & Fields, 2014). Multi-compartment models differ in the number of constituents of FFM they measure, three- versus four-compartment models (Roemmich, Clark, Weltman, & Rogol, 1997). It is known that two compartment models calculate FM based on the assumption that FFM density is constant between and within subjects (Fields, Goran, & McCrory, 2002). Multi-compartment models involve many measurement techniques to measure different FFM compartments, such as DXA for bone mineral content, DLW for TBW and whole body counting to obtain total body potassium (Ellis et al., 2007). With a multi-compartment model, the constituents of FFM are specifically measured, so the calculation of FM % is no longer based off assumptions (Demerath & Fields, 2014). However, the use of multi-compartment models in infants is not always practical due to the high participant burden; it is a time-consuming process requiring serial measurements of FFM constituents using multiple techniques which is discouraged in infants (Demerath & Fields, 2014).

2.8.4 Bioelectrical impedance analysis

Bioelectrical Impedance analysis (BIA) is a method of assessing body fat in relation to lean body mass. This method is based on the known electrical properties of FFM as it determines the bioelectrical impedance as waves pass through the relaxed motionless body (Roemmich et al., 1997). BIA determines the FFM and TBW of subjects using appropriate population equations (Kyle et al., 2004). This method is widely used in the assessment of infant total body water and adult FFM and FM. BIA is a very attractive option as it is a safe, fast, non-invasive, inexpensive and portable method used to measure body composition. However, there is room for error. Placement of electrodes must be accurate otherwise small discrepancies will cause error in results (Lingwood, 2013). The reproducibility of BIA measurements is good (Lingwood, 2013). However, there is no validated predicted algorithms for BIA in infants against FFM (Lingwood et al., 2012).

2.8.5 Dual energy x-ray absorptiometry

Dual energy x-ray absorptiometry (DXA) is a whole-body scanner which operates on a single beam mode which measures the body composition and bone mineral content of an individual. It is a

validated in vivo two-compartment model used to estimate FM and FFM in adults, children and infants (Brunton, Bayley, & Atkinson, 1993; Schmelzle & Fusch, 2002). Unlike many body composition methods, images produced by DXA can distinguish muscle mass from FFM (Demerath & Fields, 2014). Some studies have found DXA to be a highly accurate and sensitive measure of FM and FFM in paediatric subjects (Chan, 1992; Rigo et al., 1998). However, this method is known to over-predict FM in low birth weight infants (Fields, Demerath, Pietrobelli, & Chandler-Laney, 2012). Although this method uses low dose x-ray, multiple measurements may have a negative impact on infants (Demerath & Fields, 2014). Moreover, the equipment is expensive and requires the individual to lay very still in a supine position to obtain a reliable reading, which can be difficult for newborns (Demerath & Fields, 2014).

2.8.6 Air displacement plethysmography

Air displacement plethysmography (ADP) is used to measure body weight and volume and determine body density. This system utilises the inverse relationship between pressure and volume outlined by relevant physical gas laws to obtain the volume of the infant's body. This method uses the principals of densitometry to determine the FM and FFM of the subject (Urlando, Dempster, & Aitkens, 2003). ADP is well tolerated by infants, even those as small as 30 weeks (Ramel, Gray, Davern, & Demerath, 2015).

The Peapod is an infant body composition system which utilises the method of ADP to determine body composition. There are two chambers inside the PeaPod: a test chamber which houses the individual, and a reference chamber. The pressure and volume of these chambers are measured when empty and recorded as the reference values (P1 and V1). Duplicate accessories, for example feeding tube, ID bracelet and or cord clamps, are placed on the scales and within the chamber when calculating reference values as these items are likely attached to the infants during the assessment. The volume of an individual is measured indirectly by measuring the increase in pressure and the volume of air displaced in the test chamber, which is equal to his or her body volume, referred to as plethysmography.

The change in air between the chambers is measured by applying physical gas laws. As stated by Boyle's law, an amount of air compressed under isothermal conditions (constant temperature) volume (V) and pressure (P) are inversely related, or:

$$P1/P2 = V2/V1$$

However, once an infant is within the chamber the temperature changes as the chamber compresses and expands adiabatically, no longer isothermal conditions (Blaney, 2008; Fields et al., 2002;

COSMED, 2004). To correct for this discrepancy, Poisson's Law is used which describes the relationship between volume and pressure of air under adiabatic conditions

$$P_1/P_2 = (V_2/V_1)^{\gamma}$$

where γ is the ratio of heat of the gas at constant pressure to that of constant volume. (COSMED, 2004; Fields et al., 2002)

Despite the reliance on adiabatic conditions, the Peapod also accounts for the smaller volume of air under isothermal conditions, such as air within the lungs and near the skin surface and within clothing. The reason for this is based on the evidence that air under isothermal conditions is 40% more compressible than adiabatic air volumes, creating a negative volume (Blaney, 2008). Therefore, only using Poisson's law would result in an artificially reduced body volume. The air within close proximity of the surface of the individual, including their hair, and within their clothing is controlled for by wearing minimal clothing and applying a tight-fitting swim cap or baby oil to compress the hair. This ensures measuring accuracy. The air surrounding the individuals surface area is corrected by the pre-determined value calculated from their height and weight using the DuBois and DuBois formula for the Peapod:

$$B.S.A. (m^2) = 0.20247 \times \text{Height}(m)^{0.725} \times \text{Weight}(kg)^{0.425} \quad (\text{Blaney, 2008})$$

or the Boyd formula for the Peapod:

$$\text{Surface Area Artifact (L)} = [1.78.27 * \text{Length (cm)}^{0.5} * \text{Weight (Kg)}^{0.4838}] * k (L/cm^2) \quad (\text{COSMED, 2004}).$$

The isothermal air within their lungs, known as thoracic gas volume (TGV) is accounted for by either an accurate prediction or direct measurement. Infant TGV is estimated using the following equation:

$$FRC (mL) = 2.36 * \text{Length (cm)}^{0.75} * \text{Weight (kg)}^{0.63}$$

Air Displacement plethysmography determines body composition using densitometry. Prior to entering the chamber, the individuals body mass (M_B) is measured, then divided by the individuals body volume (V_B). Body density (D_B) is calculated with the following calculation:

$$D_B = M_B/V_B \quad (\text{COSMED, 2004}).$$

Densitometry includes a two-compartment model to determine body composition; FM compartment (F) and FFM compartment. In this model, the FFM compartment includes protein, water minerals and glycogen while the FM compartment consists of fat only. Each compartment has different

densities which is measured by the function of the proportions and densities within each compartment. The two-compartment model is as follows; where D_F is the density of fat, D_{FFM} is the density of FFM:

$$1/D_B = F/D_F + FFM/D_{FFM} \quad (\text{COSMED, 2004}).$$

The known densities for FM and FFM are based on the most comprehensive results and are used to compute the FFM %. The density of fat does not change depending on the individual's life stage, and is equal to 0.9007 kg/L. FFM however, changes throughout the life stages and is calculated using age and gender-specific D_{FFM} values derived from multi-compartment studies (COSMED, 2004; Urlando et al., 2003). Furthermore, Rodríguez et al. (2000) measured the water fluctuations in the first six days after birth using BIA, which were included in the calculations used by the PeaPod (COSMED, 2004). Therefore, the Pea Pod accounts for fluctuations in body water known to occur within the first six days of life (COSMED, 2004). The fat % can be defined as:

$$\% \text{ Fat } [D_F D_{FFM} / D_B (D_{FFM} - D_F) - D_F / D_{FFM} - D_F] * 100\%$$

2.8.6.1 The validity and reliability of ADP

Air displacement plethysmography relies on predictive equations to calculate FM which assumes the density and proportions of the components of FFM do not differ between infants (Fields et al., 2002). Another assumption APD relies on is the hydration levels of FFM, questioning its reliability within individuals with different hydration statuses. However, despite this ADP has been found to be an accurate and validated technique for use in infants (Deierlein et al., 2012; Ellis et al., 2007; Forsum et al., 2016; Ma et al., 2004; Roggero et al., 2012; Urlando et al., 2003) against methods of gold standards including four compartment models and DLW and has minimal safety concerns (Ellis et al., 2007). This model also accommodates the majority of infant behaviour including crying and moving (Deierlein et al., 2012). Air displacement plethysmography has become more commonly used as a method to measure body composition in infants (Andersen et al., 2013; Elisabet, Elisabeth, & Caroline, 2016; Eriksson et al., 2010; McLeod et al., 2015; Ramel et al., 2015; Sengupta et al., 2013; Van Dorp, 2015).

Assessing body composition of infants is important in gaining an insight to fetal adaptation and developmental programming of subsequent health (Demerath & Fields, 2014). Many validated neonatal body composition methods are impractical for use outside of a clinical setting as they are labour intensive, time consuming and have large room for error (Deierlein et al., 2012). The availability of the Peapod has allowed researchers and health professionals to measure the body composition of infants accurately in a quick, comfortable and safe way.

2.9 Fat mass and fat free mass of infants

There is a wealth of studies which have used ADP to assess fat mass and fat free mass in healthy term infants (Andersen et al., 2013; Au et al., 2013; Carberry et al., 2010; Deierlein et al., 2012; Eriksson et al., 2010; Fields et al., 2011; Hawkes et al., 2011; Henriksson, Löf, & Forsum, 2015; Josefson, Hoffmann, & Metzger, 2013; Simon et al., 2013; Wibaek et al., 2015). There is a large variation in the timing after birth which infants were measured. A number of these studies have reported findings for infants shortly after birth (< three days) (Andersen et al., 2013; Au et al., 2013; Carberry et al., 2010; Deierlein et al., 2012; Fields et al., 2013; Josefson, et al., 2013; Simon et al., 2013; Wibaek et al., 2015). While others have included measurements within four days (Hawkes et al., 2011) and before 10 days after birth (Eriksson et al., 2010; Henriksson et al., 2015).

The sample size of many studies reporting body composition of term infants within three days of birth have small sample sizes ranging from 21 to 45 participants (Carberry et al., 2010; Josefson et al., 2013; Simon et al., 2013; Wibaek et al., 2015). While Hawkes et al. (2011) and Au et al. (2013) included large sample sizes of 698 and 599 term infants, respectively.

Majority of studies measuring the FM and FFM of term infants reported their findings by gender (Andersen et al., 2013; Carberry et al., 2010; Deierlein et al., 2012; Eriksson et al., 2010; Fields et al., 2011; Hawkes et al., 2011; Henriksson et al., 2015; Wibaek et al., 2015). While other studies reported their findings as a total group including male and female infants (Au et al., 2013; Josefson, et al., 2013; Simon et al., 2013).

Although these studies include term infants, were conducted using ADP and were all carried out within the past 10 years, comparing them is difficult. This is due to many factors. One reason for incomparability is the lack of evidence to guide a standard procedure for timing of assessment of term infants leading to large variability amongst studies.

Previous studies measuring the body composition of term infants within three days of birth report values for FM (g) ranging between 335g and 348g and FFM (g) ranging between 2898g and 2937g for total group (Josefson et al., 2013; Simon et al., 2013). However, the study sample of these studies is small with 27 to 46 participants. While those who reported their findings stratified by gender reported FM (g) values ranging between 370g and 400g for males and 400g for females and FM % values ranging between 10.7 % and 12.1 % for males and 13.2 % for females (Deierlein et al., 2012; Fields et al., 2011). While Fields et al. (2011) further reported FFM (g) values for male and female term infants, 3050g and 2630g, respectively.

Although it has been recognised that early term infants have a higher risk of adverse clinical outcomes compared to the full term infant none of the studies to date have compared FM and FFM between early and full term infants.

A large population-based cohort study measured FM and FFM of 743 preterm and term infants by gestational age and gender using ADP (Hawkes et al., 2011). Their main goal was to develop normal reference values of FM % in infants within four days of birth. Hawkes et al. (2011) revealed an upward trend in the FM % increasing with gestational age and becoming more pronounced with gestational age. Furthermore, the FM % was significantly higher in female infants compared to males in all gestational categories, which is in alignment with the findings of other studies mentioned above.

Length-normalised indices, FM index (FMI) and FFM index (FFMI), have been developed to compare the FM and FFM of infants of varying lengths (Goswami et al, 2016).

2.10 Summary

There is a wealth of evidence that growth in early life plays an important role in programming later health. Much of this evidence to date has been identified in low birth weight infants indicating that body size or body composition at birth may be an important contributing factor to later health.

Due to the importance of growth in early life we monitor simple anthropometric measures such as body weight, length and head circumference in infants. However, these measures are quite crude. With the development of ADP for infants we can now further explore body composition in early life by extending these measures to calculating FM and FFM.

The majority of infants are born at term gestation (37 to <42 weeks) however gestation is a continuum and during each week of gestation the foetus will continue to accrue both FM and FFM. Infants born early term (37 to <39 weeks) are at a higher risk of adverse clinical outcomes and later health issues compared to infants born at full term (39 to <42 weeks). Body composition in early life may be an important variable which contributes to the poorer outcomes observed in early term infants. However, it is currently unknown whether there is a significant difference in FM and FFM between infants born early versus full term.

Chapter 3: Thesis manuscript

Body composition of healthy term infants shortly after birth using Air Displacement Plethysmography: an observational study

3.1 Abstract

Background: Early term infants (37 to <39 weeks) are associated with a higher risk of adverse clinical outcomes and later health issues compared to full term infants (39 to <42 weeks). It is unknown whether early term and full term infants differ in fat mass (FM) and fat free mass (FFM) at birth. **Aim:** To investigate the fat mass and fat free mass of healthy early and full term New Zealand infants within three days of birth. **Participants:** A convenience sample of 255 healthy term infants were recruited to take part in this cross sectional observational study. **Measurements:** Anthropometric and body composition, including FM and FFM, measurements were performed using air displacement plethysmography (ADP). Infants were grouped into early and full term categories. Statistical analysis included independent 2-tailed t-test and Pearson's correlation. **Results:** There were no differences in the percentage of FM and FFM between early term and full term infants ($10.2\pm 4.0\%$ vs $11.1\pm 4.1\%$, $P=0.109$ and $90.0\pm 4.0\%$ vs $89.0\pm 4.1\%$, $P=0.110$). Full term infants had significantly higher FMI and FFMI compared to early term infants (1.44 ± 0.6 vs 1.26 ± 0.06 , $P=0.02$ and 11.3 ± 1.0 vs 10.8 ± 0.96 , $P<0.001$). Early term males were significantly heavier ($P=0.04$) and had higher FFM ($2793.1\pm 332.9\text{g}$ vs $2619.7\pm 315.4\text{g}$, $P=0.003$), FFM % ($90.8\pm 3.8\%$ vs $88.7\pm 4.0\%$, $P=0.009$), FMI (1.15 ± 0.55 vs 1.38 ± 0.56 , $P=0.039$) and lower FM % ($9.2\pm 3.8\%$ vs $11.3\pm 4.0\%$, $P=0.009$) than female early term infants. No gender differences within full term infants were noted in FM, FFM, FM % or FFM %. **Conclusion:** The results of this study suggest body composition differences exist between healthy early and full term infants, which highlights the need to track body composition changes at regular time periods following birth.

Keywords: *Early term, full term, infant, body composition, air displacement plethysmography, adiposity*

3.2 Introduction

The majority of infants (90.7%) born in New Zealand are born at term gestation (37 to <42 weeks) (Ministry of Health, 2015). Birth data shows that alongside the recent decrease in the number of infants born per year in New Zealand, the proportion of early term births has increased between 2009 and 2016 (Ministry of Health, 2018). However, the increasing numbers of deliveries occurring before 39 weeks gestation is concerning (Oshiro et al., 2009; Nassar, Schiff, & Roberts, 2013) as early term births are associated with an increased risk of adverse clinical outcomes and later health issues compared to infants born at full term (39 to <42 weeks) (Engle & Kominiarek, 2008; Fleischman, Oinuma, & Clark, 2010; Machado Jr, Passini Jr, Rosa, & Carvalho, 2014; McIntire & Leveno, 2008; Nir, Nadir, & Feldman,

2012). Furthermore, gender differences in infant body composition may predict later fat distribution and expression of risk factors and incidence of disease later in life (Fields, Krishnan, & Wisniewski, 2009).

There is a wealth of evidence that growth and development in early life plays an important role in programming later health outcomes (Barker, Eriksson, Forsén, & Osmond, 2002; Carberry, Colditz, & Lingwood, 2010; Wells, Chomtho, & Fewtrell, 2007). In utero the foetus experiences rapid growth and accrual of fat mass during the third trimester of pregnancy and with each week of gestation, birthweight increases. It has been hypothesised that neonatal adiposity may be more closely related to the mechanism of programming later health than birthweight or early weight gain (Ratnasingham et al., 2017). Therefore, monitoring adiposity at birth is imperative to understand its association with later health outcomes.

The majority of research on body composition in early life has been conducted using methods which are impractical or of unknown accuracy (Wells et al., 2007). Advancements in body composition methods such as Air Displacement Plethysmography (ADP) have made it possible to assess the body composition of infants and estimate fat mass (FM) and fat free mass (FFM) (Carberry et al., 2010). These measures of body composition facilitate assessing neonatal adiposity shortly after birth. In this cross sectional, observational study, we assessed the FM and FFM of healthy early term and full term infants in New Zealand using ADP and furthermore investigated differences between genders.

3.3 Materials and methods

3.3.1 Design and participants

This observational study recruited 255 healthy term infants (37 to <42 weeks) born at Auckland City Hospital, New Zealand, between May 2015 and September 2016. Infants were excluded from the study if they were born with any birth defects, congenital abnormalities, inability to tolerate the measuring process involved with ADP or were born preterm (<37 weeks) or post term (>42 weeks).

The study was approved by New Zealand Southern Health and Disability Ethics Committee (HDEC) (15/STH/S2). Auckland District Health Board research office provided permission for the study (Ref. No. A+6691). Informed parental written consent was obtained, and participation was voluntary.

3.3.2 Data collection

Anthropometric measurements

Infant crown to heel length was measured to the nearest 0.1 cm using the infant length board (Holtain Limited Harpenden Neonatometer). Waist circumference was measured around the widest part

of the waist with a disposable measuring tape to the nearest 0.1cm. For each parameter an average of three measurements was used. All measurements were conducted by a trained researcher.

Body composition assessment

Infant body composition was assessed using the Peapod an infant-ADP System (Life Measurement Inc., Cosmed, California, USA) within three days of birth. Infants were assessed nude, within three days of birth. The Peapod chamber and scales were calibrated daily, prior to measurements, as part of quality control. Duplicate accessories (feeding tube, ID bracelet and/or cord clamp) were used to tare the Peapod scales and calibrate the chamber. Weight was measured to the nearest 0.10 g on the Peapod integrated electronic scales. The infant was then fitted with a standard tight fitting disposable hat and laid into the chamber for two minutes, where the infant's body volume was measured. ADP determines body composition using densitometry. It measures the mass and volume of the infant, which when divided by each other (mass/volume) calculates the density and furthermore fat and fat-free mass. Using densitometric equations, FM %, FM and FFM are derived (Blaney, 2008; COSMED, 2004).

3.3.3 Statistical analysis

Infants were grouped into early term (37 to <39 weeks gestation) and full term (39 to <41 weeks gestation) (National Maternity Monitoring Group, 2014). Information on infant gestational age, gender and ethnicity were collected through self-administered questionnaires and medical records. Study population descriptive analyses are reported as frequency and (%) and mean \pm SD. Scatter plots were constructed and inspected for the relationship between gestational ages of 37 to 42 weeks gestation and body composition measures. Normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk test. Non-parametric data was tested for homogeneity of variance using the Levenes test and was treated as normal due to the large sample size (Pallant, 2005). Linear regression model and Pearson's correlation were applied to determine the strength of the relationships. Waist circumference (cm) to length (cm) ratio (WLR) was calculated by dividing waist circumference by length. Two indices of length-normalised body composition were calculated: a FM index (FMI) and FFM index (FFMI) derived by dividing FM and FFM values (kg) by length² (m²) (Goswami et al, 2016; Haisma et al., 2005). Each was expressed as kg/m² and the mean and SD are presented. Independent 2-tailed t-tests were used to compare the growth measures and body composition parameters between early and full term groups and gender. P-values <0.05 were considered significant.

3.4 Results

3.4.1 Participant characteristics

255 healthy term infants were included in this study, 145 males and 110 females. Infants were categorised by early term (n=97) and full term (n=158) birth. The participant characteristics of the total group and by early and full term birth are shown in Table 3.1.

Table 3.1 Participant Characteristics

Characteristics	Gestational age categories		
	Total group (N=255)	Early term infants (n=97)	Full term infants (n=158)
Gender, n (%)	Male	145 (57)	95 (60)
	Female	110 (43)	63 (40)
Ethnicity ^a , n (%)	European	78 (31)	49 (31)
	Māori	11 (4)	5 (3)
	Pacific	31 (12)	20 (13)
	Asian	64 (25)	42 (26)
	South Asian/Indian	31 (12)	17 (11)
	Other	4 (2)	2 (1)
	Not reported	36 (14)	23 (15)
Gestational age at birth, weeks Mean ± SD	39.3 ± 1.1	38.2 ± 0.5	40.0 ± 0.9

^a Self-reported by parent or caregiver

3.4.2 Body composition with gestational age

FM (g) increased linearly with gestational age ($R^2=0.043$, $P=0.001$). FFM (g) also increased with gestational age ($R^2=0.216$, $P<0.001$). There was no significant relationship between either FM % or FFM % and gestational age ($R^2=0.009$, $P=0.128$).

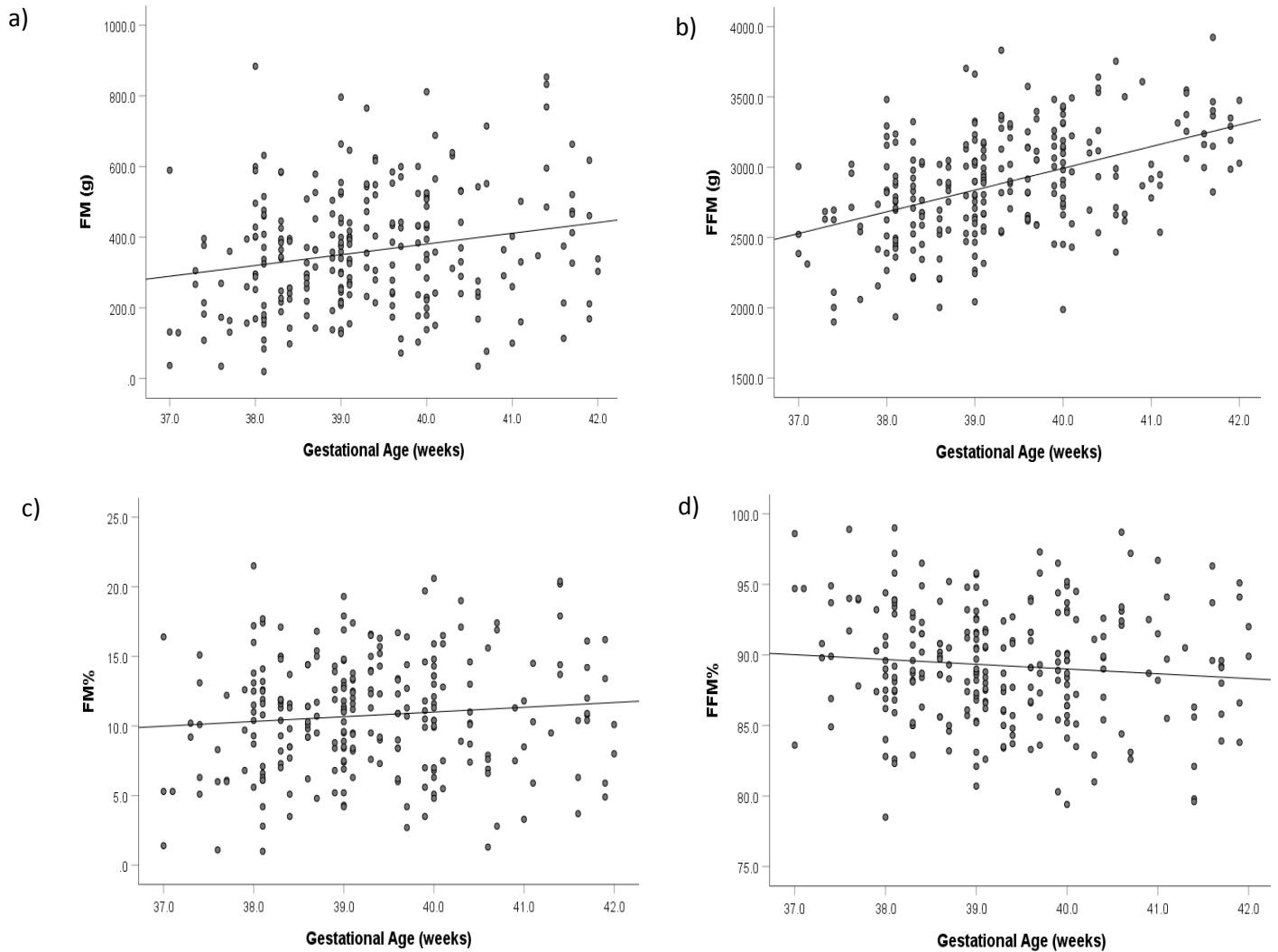


Figure 3.1 Distribution of body composition measures according to gestational age in 255 term infants. Measures a) FM; b) FFM; c) FM %; and d) %FFM. FM, fat mass; FFM, fat free mass; FM %, percentage fat mass; %FFM percentage fat free mass

3.4.3 Comparison of anthropometric measures of early term and full term infants

There was no significant difference in the postnatal age of the early versus full term infants at the time of assessment ($P=0.094$). Full term infants weighed more ($3381.3\pm 459.0\text{g}$ vs $3000.7\pm 440.2\text{g}$ $P<0.001$) had higher FM ($384.1\pm 171.3\text{g}$ vs $318.0\pm 154.0\text{g}$ $P=0.02$), FFM ($2997.2\pm 355.7\text{g}$ vs $2692.1\pm 357.0\text{g}$, $P<0.001$) and longer length ($51.4\pm 2.1\text{cm}$ vs $50.0\pm 2.3\text{cm}$, $P<0.001$) than early term infants. There were no significant differences noted in FM % or FFM % between early term and full term infants ($P=0.109$). When normalised for length, full term infants continue to have greater FMI and FFMI than early term infants ($P<0.02$) (Table 3.2).

Table 3.2 Comparison of anthropometric measures between early term and full term infants

	Gestational age categories			P*
	Total group (N=255)	Early term infants (n=97)	Full term infants (n=158)	
Postnatal age at assessment, days	1.7 ± 0.8	1.8 ± 0.8	1.6 ± 0.9	0.094
Weight, g	3236.5 ± 487.5	3000.7 ± 440.2	3381.3 ± 459.0	<0.001*
Length, cm	50.8 ± 2.3	50.0 ± 2.3	51.4 ± 2.1	<0.001*
WLR	0.64 ± 0.0	0.64 ± 0.1	0.64 ± 0.0	0.975
Fat mass, g	359.0 ± 168.0	318.0 ± 154.0	384.1 ± 171.3	0.02*
Fat-free mass, g	2888.2 ± 381.7	2692.1 ± 357.0	2997.2 ± 355.7	<0.001*
Fat mass, %	10.8 ± 4.1	10.2 ± 4.0	11.1 ± 4.1	0.109
Fat-free mass, %	89.2 ± 4.1	90.0 ± 4.0	89.0 ± 4.1	0.110
FMI, kg/m ²	1.37 ± 0.6	1.26 ± 0.56	1.44 ± 0.6	0.02*
FFMI, kg/m ²	11.13 ± 1.0	10.8 ± 0.96	11.33 ± 1.0	<0.001*

Data are Mean ± SD

P*significant difference (p<0.05) between early term and full term infants (Independent T-Test)

3.4.4 Effect of gender

Early term male infants weighed more (3089.2 ± 442.1g vs 2906.4 ± 442.6g, $P=0.04$) and were longer (50.5 ± 2.4cm vs 49.2 ± 2.1cm $P=0.007$) than early term female infants. Early term males had higher FFM (2793.1 ± 332.9g vs 2619.7 ± 315.4g, $P=0.003$) and FFM % (90.8 ± 3.8g vs 88.7 ± 4.0g, $P=0.009$) than early term females who had higher FM % (11.3 ± 4.0% vs 9.2 ± 3.8%, $P=0.009$). Female early term infants had a significantly greater FMI than male early term infants ($P=0.039$) (Table 3.3).

Table 3.3 Comparison of anthropometric measures between male and female early term infants (N=97)

	Male (n=50)	Female (n=47)	P*
Gestational age at delivery, weeks	38.2 ± 0.5	38.1 ± 0.5	0.365
Postnatal age at measurement, days	2.0 ± 1.0	2.0 ± 1.0	0.657
Weight, g	3089.2 ± 442.1	2906.4 ± 422.6	0.04*
Length, cm	50.5 ± 2.4	49.2 ± 2.1	0.007*
WLR	0.63 ± 0.1	0.65 ± 0.1	0.600
Fat mass, g	296.1 ± 148.9	340.9 ± 157.4	0.153
Fat-free mass, g	2793.1 ± 332.9	2619.7 ± 315.4	0.003*
Fat mass, %	9.2 ± 3.8	11.3 ± 4.0	0.009*
Fat-free mass, %	90.8 ± 3.8	88.7 ± 4.0	0.009*
FMI, kg/m ²	1.15 ± 0.55	1.38 ± 0.56	0.039*
FFMI, kg/m ²	11.0 ± 1.0	10.6 ± 0.88	0.107

Data are Mean ± SD

P*Significant difference (P <0.05) between males and females (Independent T-Test)

The mean postnatal age at measurement of full term male infants was significantly higher than full term female infants (2.0 ± 1.0 days vs 1.0 ± 1.0 days, $P=0.042$). Male full term infants were longer

(51.8 ± 2.0 cm vs 50.9 ± 2.2 cm, $P=0.009$) then full term female infants. Full term female infants had a greater WLR than full term male peers (0.65 ± 0.0 vs 0.63 ± 0.0 , $P=0.008$). No significant differences were noted between gender in terms of body composition and weight amongst the full term infants. No significant differences in FMI or FFMI between male and female full term infants (Table 3.4).

Table 3.4 Comparison of anthropometric measures between male and female full term infants (N=158)

	Male (n=95)	Female (n=63)	<i>P</i> *
Gestational age at delivery, weeks	39.9 ± 0.9	40.1 ± 0.8	0.052
Postnatal age at measurement, days	2.0 ± 1.0	1.0 ± 1.0	0.042*
Weight, g	3419.1 ± 444.3	3324.1 ± 478.1	0.203
Length, cm	51.8 ± 2.0	50.9 ± 2.2	0.009*
WLR	0.63 ± 0.0	0.65 ± 0.0	0.008*
Fat mass, g	388.3 ± 161.1	377.7 ± 186.6	0.706
Fat-free mass, g	3031.0 ± 361.6	2946.4 ± 343.1	0.144
Fat mass, %	11.2 ± 3.9	11.0 ± 4.4	0.771
Fat-free mass, %	88.8 ± 3.9	89.0 ± 4.4	0.758
FMI, kg/m ²	1.44 ± 0.6	1.4 ± 0.65	0.939
FFMI, kg/m ²	11.3 ± 1.0	11.38 ± 1.0	0.621

Data are Mean ± SD

*Significant difference between males and females ($P < 0.05$) (Independent T-Test)

3.5 Discussion

The primary purpose of this study was to explore the body composition of healthy term infants born in New Zealand using ADP. Although this study sample was a convenience sample and not a representation of the national population, this study provides a snapshot of infants born at term in New Zealand. Infants were categorised into early term (37 to <39 weeks) and full term (39 to <42 weeks) births and furthermore by gender.

3.5.1 Characteristics of participants

Participants were recruited from Auckland City Hospital (ACH) between May 2015 and September 2016. ACH has one of the largest maternity services in New Zealand (Auckland District Health Board, 2016). Majority of infants born in New Zealand are born at term. In 2013, 88.7% of total births at ACH were term births (Auckland District Health Board, 2013). Hence, ACH was an ideal setting for recruiting and collecting body compositional data of New Zealand born term infants.

Participants included 255 healthy term infants. The mean gestational age of this cohort was 39.3 weeks. All infants of this study had a normal birthweight with the average weight near birth being 3.23 kg which is comparable with the average birthweight of infants in New Zealand being 3.41 kg (Ministry of

Health, 2010). The study population included 97 early term infants of which 60% were male and 158 full term infants of which 50% were male.

3.5.2 Early term versus full term

Our results revealed infants born early term are significantly smaller in terms of length, absolute mass, FM and FFM than full term infants. The lower absolute FM and FFM values in early term infants was suggested to be partially caused by their significantly shorter length when compared to full term infants

There is a lack of studies assessing FMI and FFMI of term infants at birth. However the FMI and FFMI has been assessed in term infants near birth, beyond 2 weeks postpartum, as a part of longitudinal studies (Anderson, 2009; Goswami et al., 2016). Future research could benefit from conducting a longitudinal study of comparing the FMI and FFMI of early term and full term infants.

3.5.3 Gender differences

Among early term infants, weight, FFM and FFM % was higher in males than in females. Males had a higher FFM in conjunction with no differences in FM. However, female early term infants had a greater FMI than early term males. Our findings agree with Carberry et al. (2010) who assessed healthy term infants using ADP and found term males to weigh more and have a higher FFM than females. The higher proportion of FFM observed in males has been found to be due to greater proportions of lean body mass, total body water, bone mineral content and total body potassium (Butte et al., 2000). The enhanced accretion of lean body mass in males in fetal life has been assumed to be a result of the production of the male sex steroid hormone, testosterone (Hull et al., 2008). Whereas female sex hormones are relatively quiescent during perinatal development (Hull et al., 2008).

Early term female infants had a greater FM % (11.4%) than early term males (9.2%). During childhood, females as young as 5 years old, have a higher FM % in conjunction with lower lean body mass than male peers (Shaw, Crabtree, Kibirige, & Fordham, 2007; Taylor, Gold, Manning, & Goulding, 1997). Whether this difference is apparent at birth is controversial (Butte et al., 2000; Carberry et al., 2010; Eriksson et al., 2010). A large study measured the FM % of 743 healthy infants using ADP within the first four days of life and found female infants aged 38 to 39 weeks plus six days to have a significantly higher FM % than male infants (Hawkes et al., 2011). These findings agree with the current study. Copper et al. (1993) speculated that increased subcutaneous fat stores in females may be related to their better neonatal outcomes in comparison to males.

In contrast, among full term infants, no significant differences in body composition were observed. Although there was a significant difference in postnatal age between genders at measurement it should be noted that one day is unlikely to make a difference to the body composition of healthy term infants. Our findings disagree with those of Simon et al. (2013) who found full term male infants measured within 4 days of birth to have greater FFM stores and lower FM % than full term females.

3.5.4 Strengths and limitations

A key strength to the current study was measuring the FM and FFM of term infants using ADP as this method has been validated and is regarded as an accurate and reliable measure of the body composition of term infants (Ma et al., 2004). However, due to ADP being a 2-compartment method based off assumptions, it is unable to differentiate the components of FFM such as fluid, muscle and bone. The inclusion of a combination of body composition techniques (Skinfold thickness, multi-compartment models, doubly labeled water, DXA) to measure lean body mass, total body water, bone mineral content and total body potassium of the body, have been considered ideal to avoid any potential variations. However, this combination of techniques can be time consuming, potentially invasive and impractical for the use in infants (Butte et al., 2000; Demerath & Fields, 2014).

Another limitation to this study is the possible influence of hydration status of the infant at the time of measurement impacting on the accuracy of the result. The protocol for the study was to measure infants before being fed or at least one hour after feeding. While majority of the infants were measured following this protocol, this was not always possible. Measurements were organised around medical procedures and assessments which took precedence over research measurements

3.5.5 Conclusion

In summary, we provide body composition values for healthy term infants born in New Zealand using ADP. This study showed full term infants continue to accrue FM and FFM along the same trajectories as that at early term gestation although they have significantly greater FM and FFM when adjusted for length. The FM and FFM gender differences noted in this study were only apparent between early term births. The practical relevance of these findings requires further investigation in terms of measuring early and full term infants weekly after birth to track body composition changes.

3.6 Acknowledgements

We gratefully acknowledge the parents for allowing their infants to participate in this study, as well as thanking ACH for generously providing us with the space for the current research.

3.7 Conflict of interest

The authors report no conflicts of interest.

Chapter 4: Conclusion and recommendations

4.1 Brief overview

This study aimed to explore the fat mass (FM) and fat free mass (FFM) of healthy term infants born in New Zealand using air displacement plethysmography (ADP). Although the study sample is not a representative of New Zealand population it does provide a snap shot of healthy term infants born in New Zealand. Assessing 255 healthy term infants we found early term infants to be smaller in length and body weight than full term infants, with no difference in the percentage of FFM. Our hypothesis of early term infants having a lower FM percentage than full term infants was not supported by our results. Full term infants presented greater FMI and FFMI compared to early term infants. We hypothesised male infants in both early and full term groups would have a significantly greater FM than female peers. We found early term males to have a greater absolute FFM and FFM% than females, but no significant difference was seen between genders in full term infants.

4.2 Strengths and limitations

Conducting FM and FFM measurements using ADP is a strength to the current study. Although ADP relies on assumptions for FFM and its components, questioning its reliability within individuals with different hydration statuses. It has been found to be an accurate and validated technique for use in infants (Deierlein et al., 2012; Ellis et al., 2007; Forsum et al., 2016; Ma et al., 2004; Roggero et al., 2012; Urlando et al., 2003) against methods of gold standards including four compartment models and doubly labelled water and has minimal safety concerns (Ellis et al., 2007). The inclusion of a combination of body composition techniques (Skinfold thickness, multi-compartment models, doubly labeled water, DXA) to measure lean body mass, total body water, bone mineral content and total body potassium of the body, have been considered ideal to avoid any potential variations. However, this combination of techniques can be time consuming, potentially invasive and impractical for the use in infants (Butte et al., 2000; Demerath & Fields, 2014).

The time an infant was last fed and passed urine poses as a possible limitation to the current study as the hydration status of the infant at the time of measurement may impact the accuracy of the result. The protocol for the study was to measure infants before being fed or at least one hour after feeding. While majority of the infants were measured following this protocol, this was not always possible. Measurements were organised around medical procedures and assessments which took precedence over research measurements.

4.3 Recommendations for future research

1. This study identified the need to investigate the body composition (FM and FFM) changes of healthy early and full term infants at regular time periods following birth. This will allow observation of factors which influence body composition in early life, including mode of feeding.
2. Develop normative reference values for FM, FFM, FM % and FFM % of early term and full term infants at birth using a robust, ethically representative sample of New Zealand.
3. To investigate whether the mode of delivery has an impact on body composition of term infants at birth.
4. Conduct a robust longitudinal study collecting body compositional data from birth to adulthood to fully understand the impact of body composition in early life on later health outcomes.

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Appendix A - Material used in conducting research
Figure A.1: Participation information sheet

Participant Information Sheet

Early nutrition and body composition in babies

You and your baby are invited to take part in a research study looking at nutrition and growth in preterm and term babies.

Please take your time to think about and decide whether you wish to take part in the project. You are encouraged to discuss your baby's participation in the project with family / whanau.

Taking part is a completely voluntary (your choice) and if you decide you do not wish for your baby to take part, it will not affect you or your baby's current or future healthcare in any way. You may withdraw your baby's participation at any time.

Purpose of this study

How babies feed and grow is important for health. Although all babies are weighed after birth, we know very little about the amount of fat in their body relative to muscle (often referred to as body composition). Knowing more about babies' body composition will help us to understand how nutrition can support optimal growth. This study will measure the body composition of babies born both at term and preterm. This will help us to understand the relationships between preterm birth, how preterm babies are fed between birth and discharge from hospital, and body composition and how body composition of preterm babies compares with that of healthy term babies. In the future, this study may help us guide parents of preterm and term babies on the best advice for feeding and growth of their baby.

We are aiming to recruit moderate to late preterm babies (born between 32 and 36 weeks' gestation) and term babies (37 to 42 weeks' gestation). We are able to measure body composition using a technique called air displacement plethysmography in a PeaPod. This non-invasive and completely safe technique is routinely used in the care of many babies worldwide.

Project procedures

All measurements and data collection will be done while your baby is in hospital. For preterm babies we will measure their body composition weekly whilst they are in hospital. For term babies we will measure their body composition once before going home. Babies will be measured in the PeaPod (see picture below), as well as having their length, waist and

head circumference measured. Measuring your baby in the PeaPod is very quick and takes about 5 minutes.

As well as this we will collect data on how your baby has been fed and relevant medical information about your baby from their medical notes.

Data management

Each baby will be issued with a participant code that will be used for all data collection and measurements to ensure all information remains anonymous. Data will be confidential and only members of the research team will have access to it.

All data collected will only be used for the purpose of this study and will be stored securely.

You will be provided with a summary of the findings of the study when it is finished. Results will also be published in a scientific journal and may be presented at a conference to help guide future feeding practices of babies.

What is the PeaPod?

This equipment measures body composition- so it tells us how much fat your baby has. The technique measures the amount of air your baby pushes out of the machine, so it is completely safe, non-invasive and is used routinely in health services around the world.

Participant rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- *decline to answer any particular question;*
- *decline any measurements of your baby;*
- *withdraw from the study at any time;*
- *ask any questions about the study at any time during participation, and*
- *be given access to a summary of the project findings when it is concluded*

General Information

An interpreter will be provided if required.

You may have a friend, family or whanau support to help you this study and any other explanation you may require prior to deciding whether to participate or not. You,





and/or a friend, family or whānau support can accompany your baby during any measurements.

This study has received ethical approval from the Southern Health and Disability Ethics Regional Ethics Committee (15/STH/52) and locality approval from ADHB Research Review Committee (A+6691).

Project contacts

If you have any questions or concerns about the study at any stage you can contact:

Professor Frank Bloomfield

f.bloomfield@auckland.ac.nz

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021 497598

Liggins Institute
University of Auckland
Private Bag 92019

Auckland 1142

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c.conlon@massey.ac.nz

(09) 414 0800 ext 43658

021 1730428

Massey University
Private Bag 102904
North Shore City

Auckland

If you have any queries or concerns regarding your rights as a participant in this project you can contact an Independent Health and Disability Advocate. This is a free service provided under the Health & Disability Commissioner:

Telephone (NZ Wide): 0800 555 050

Free Fax (NZ Wide): 0800 2787 7678 (0800 2 SUPPORT)

Email: advocacy@hdc.org.nz

If you require Māori cultural support, talk to your whānau in the first instance. Alternatively you may contact the administrator for He Kamaka Waiora (Māori Health Team) by telephoning 09 486 8324 ext 2324

Thank you, tēnā koe, for making the time to read about, and for considering taking part in this project.

Figure A.2: Information pamphlet



What is the PeaPod?

The PeaPod measures how much fat your baby has. It works by using air and it is completely safe, non-invasive and routinely used in health services around the world.



What does it involve?

We will weigh your baby first and then measure how much body fat your baby has using the PeaPod—it's a bit like an incubator.

Your baby will only need to be inside the PeaPod for 2 minutes.

Can I stay with my baby?

Yes, you are welcome to stay with your baby.

Our research nurse will talk to you and explain what happens.

QUESTIONS YOU MAY HAVE

How long will it take?

Measuring your baby will only take 2 minutes but it takes us a few more minutes to undress and weigh them!

Will baby feel anything?

No, it is completely non-invasive. The PeaPod only uses air to measure your baby. Inside the PeaPod it's a bit like an incubator, safe and warm.

My baby is preterm is it still safe?

Yes, we have measured lots of preterm and very small babies and it is completely safe. At the moment we don't know how much fat preterm babies should have so by taking part in research you are helping us to find out.

Do I have to take part?

No, it's your choice and even if you agree to have your baby measured in the PeaPod you can stop at any time.

Who can I talk to about the PeaPod?

You can speak to the nurse looking after your baby and they can arrange for someone from the research team to come and speak with you.

Can I see the PeaPod before I decide to take part?

Yes one of our researchers will be happy to show you the PeaPod.

Will I get my baby's results?

Yes, we will fill in your baby's results below.




Your baby's results

Current weight _____g


Your baby has _____g of body fat

and _____g lean body mass.


Figure A.3: Consent form



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Te Whānau Whānau o Tōrangitiro



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TE KUNENGA KI PŪREHURŌA
UNIVERSITY OF NEW ZEALAND

Consent Form

Early nutrition and body composition in babies

English	I wish to have an interpreter	Yes	No
English	I wish to have an interpreter	Yes	No
Maori	E hiahia ana ahau ki tetahi kaiwhakamaori / kaiwhaka pakeha korero	Ae	Kao
Cook Island	Ka inangaro au i tetahi tangata uri reo	Ae	Kare
Fijian	Au gadreva me dua e vakadewa vosa vei au	lo	Sega
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu	L	Nakai
Samoaan	Ou te mana'o ia i ai se fa'amatala upu	loe	Leai
Tokelaun	Ko au e fofou ki he tino ke fakaliliu te gagana Peletania ki na gagana o na motu o te Pahefika	loe	Leai
Tongan	Oku ou fiema'u ha fakatonulea	lo	Ikai

- I have read and I understand the information sheet dated [Final version 6/3/2015] for volunteers taking part in the project called **Early nutrition and body composition in babies**. I have had the opportunity to discuss this project. I am satisfied with the answers I have been given.
- I have had the opportunity to use family / whanau support or a friend to help me ask questions and understand the project.
- I have had time to consider whether to take part in this project.
- I understand that taking part in this project is voluntary (my choice) and that I can stop taking part at any time and this will in no way affect my continuing or future health care.
- I understand that my participation in this project is confidential and that no material which could identify me will be used in any reports.
- I know who to contact if I have questions about the project in general or if I experience any ill effects resulting from my involvement in the project.
- I consent to my baby being measured using the PeaPod and other growth measurements (length, head circumference, waist circumference) being taken from my baby for the purpose of this study.
- I consent to medical information being collected from the medical records made during my baby's hospital admission.
- I consent to being contacted in the future regarding my baby's progress

I _____ (full name) hereby consent to my baby _____ (name) taking part in this study.

Signature: _____

Date: _____ Time: _____

Interpreter:

I _____ translated the project to the participant.

Signature: _____

Date: _____

Early nutrition and body composition in babies Consent Form v1.2 23/04/15
1