Preterm Infant Body Composition: Method and Clinical Study

Method Development, Feasibility and Clinical Pilot Study of Air Displacement Plethysmography for Longitudinal Body Composition Measurements of Preterm Infants in Hospital

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A Thesis

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Abstract

Background: Inadequate nutrition during the postnatal period may be associated with adverse outcomes in later life. Tailoring nutrition to promote optimal growth requires monitoring body composition (BC). This could guide nutritional strategies to promote optimal outcomes, however preterm infant BC data is lacking.

Objectives: To introduce and assess the feasibility of air displacement plethysmography (PEA POD) as a bedside tool in the NICU; Second, to longitudinally measure preterm infant BC in hospital.

Methods: This was a longitudinal, observational study. Inclusion criteria: infants 24-37 weeks gestational age (GA); informed consent. Exclusion criteria: chromosomal/congenital abnormalities; hydrops fetalis. Preterm infant BC assessed by PEA POD; anthropometric measures were collected. Infants assessed from study inclusion to hospital discharge. For reference, 23 term infants were measured.

Results: PEA POD was a feasible bedside tool to measure preterm infant BC that was accepted by parents and free of adverse events. Longitudinal measures demonstrated preterm infants (n=65) gained fat mass (FM) and fat free mass (FFM) at differing rates, leading to an overall %FM increase in hospital. Our data suggests that, at term corrected age, preterm infants may achieve a BC similar to full term infants at birth.

Discussion: This study established PEA POD as a useful bedside clinical tool for preterm infants. Longitudinal BC changes in preterm infants using PEAPOD in hospital is described for the first time. Future application of PEA POD can expand longitudinal measures to create reference preterm infant BC data for evaluating quantity and quality of growth in relation to nutritional management.

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List of Abbreviations, Nomenclature and Symbols

AAP	American Academy of Pediatrics
ADP	Air-Displacement Plethysmography
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
DEXA	Dual Energy X-ray Absorptiometry
ELBW	Extremely Low Birth Weight (<1000g)
FM	Fat Mass
FFM	Fat Free Mass
GA	Gestational Age
g/kg/d	grams/kilograms/day
IV	Intravenous
MRI	Magnetic Resonance Imaging
N	Number of subjects or observations in sample
NICU	Neonatal Intensive Care Unit
PI	Ponderal Index

PMA	Post Menstrual Age
QC	Quality Control
SD	Standard deviation
SGA	Small for Gestational Age
VLBW	Very Low Birth Weight (<1500 g)

1.0 Introduction

1.1 Overview

Preterm birth is defined as birth of an infant less than 37 completed weeks of gestational age. The World Health Organization estimates that 15 million infants every year are born prematurely, with many experiencing disability and complications upon survival as a result of their developmental immaturity.

The number of infants born preterm has increased around the world and in Canada. The exact cause of preterm birth is unknown; however several risk factors have been associated with this event such as multiple births, infection and maternal chronic conditions such as high blood pressure and diabetes. Possible reasons for the rise in preterm birth include increased maternal age at pregnancy, increased rates of diabetes and obesity as well as greater use of infertility treatments often leading to multiple infant pregnancies.

1.2 Rationale for this work

Through the advances that have been made in neonatal medicine, the survival rate of preterm infants, especially those born less than 30 weeks of gestation has greatly increased over the past several decades. However, morbidity rates amongst this population still remain high. There is strong evidence that shows that preterm infants are at increased risk of suffering from deficits in

neurodevelopment as well as at increased risk of developing cardiovascular and metabolic disease in later life. Therefore the focus of health care providers and research now turns to improving the short and long term outcomes and quality of life of these infants. Although there are guidelines on weight gain and growth rate preterm infants should achieve, very little is known about the quality of growth these infants exhibit but also the provision of nutrition needed to promote an optimal growth trajectory and body composition has yet to be determined.

To our knowledge, no studies have been performed that accurately and longitudinally assess preterm infant body composition during hospital stay at the bedside. Understanding how these infants grow during the early postnatal period is the first step in assessing the body composition that is being achieved with current nutritional support practices. Due to a lack of appropriate techniques available to measure body composition non-invasively and accurately in infants, body composition data are lacking. In many cases preterm infants are clinically unstable and unable to be measured using traditional techniques such as dual energy X-ray absorptiometry (DEXA) or deuterium dilution, leaving a gap in knowledge of the quality of growth preterm infants display. With the introduction of the PEA POD, it is now possible to measure this vulnerable population. These data will contribute to reference data of how stable preterm infants grow during the early postnatal period, which can be used in clinical practice to guide nutritional strategies in order to promote optimal growth and development. This

would aid in the development of preventative measures that can be instituted in the early neonatal period to reduce morbidities associated with prematurity.

1.3 Research Objectives

The primary research objectives for this thesis were:

1. To introduce a new medical device, the PEA POD, into the NICU at McMaster Children's Hospital and assess its feasibility as a bedside tool to measure body composition of preterm infants
2. Longitudinally measure preterm infant body composition during the first weeks of life during hospital stay at bedside:
    i. Examine the relationship of body composition to postmenstrual age (PMA) and body weight at test using absolute values, as well as fat free mass index (FFMI) and fat mass index (FMI)
    ii. Investigate whether body mass index (BMI: weight/length^2) or ponderal index (PI: weight/length^3) is a better proxy for fat mass and fat free mass
3. Measure healthy full term infants within 72 hours of birth in order to compare preterm to term infant body composition

2.0 Literature Review

2.1 Introduction

Upon delivery, the preterm infant is faced with a drastically different environment from in utero and several factors will influence the subsequent development and health of the infant. This section reviews current knowledge of preterm infant growth, nutrition and body composition, and identifies gaps in knowledge.

2.2 Growth and Nutrition of the Fetus

An individual experiences the highest rate of growth in their lifetime while *in utero*. Intrauterine growth is slow during the first weeks but accelerates significantly later in pregnancy; between 23-27 weeks of gestation is approximately 21g/kg/d which declines to 12 g/kg/d during weeks 35-37 of gestation, where the average growth velocity from 23-37 weeks of gestation is approximately 16 g/kg/d[1]. From 25 weeks gestation through to the last trimester of pregnancy until birth, the body weight of the infant increases by 10-15% per week, leading to a 6-fold increase of body mass. During normal pregnancy, nutrients available to the infant to achieve these high growth rates are regulated by the placenta. When born prematurely, this maternal support is interrupted and the infant and healthcare providers are faced with the challenge of taking over these roles.

Fetal and postnatal infant nutrition differ significantly in mode, amount and timing of delivery. Important nutrients for the developing fetus include

glucose, amino acids, fatty acids and several micronutrients; the exact intakes of these nutrients while in utero have yet to be determined, which presents a challenge as to how to appropriately feed a preterm infant with adequate amounts of these critical nutrients[2]. Amino acid delivery rate after birth is lower than what is achieved in utero. Additionally, the fetus has a greater dependence on carbohydrates rather than fats as an energy source, whereas fat becomes the primary source of energy after birth[3].

2.3 Growth and Nutrition of the Preterm Infant

Preterm infants are at a developmental disadvantage upon birth, often requiring continued medical intervention for respiratory support, gastrointestinal and metabolic issues and are at increased risk of infection. Those born less than 35 weeks often experience difficulties with sucking and swallowing, as well as digestion and nutrient absorption due to the immaturity of their gastrointestinal tract[4]. These added barriers to receiving and processing nutrition could explain the difficulty preterm infants experience in achieving growth as recommended. Evidence exists to show that preterm infants typically display poor postnatal growth which in some cases may not be recovered before hospital discharge[5].

2.3.1 Factors Influencing Postnatal Growth

Growth is a complex interaction of genetics, hormones, nutrition and environmental factors. Maternal anthropometry, behaviours and health can influence not only birth weight of her infant, but also body composition. Parity

and maternal BMI have been shown to be associated with fat at birth[6–8]. There is a positive correlation between maternal weight gain during pregnancy and birth weight[8]. Infants born to mothers that smoke during pregnancy are smaller than infants born to non-smoking mothers and this reduction in weight was observed to be primarily due to a smaller FFM compartment[8].

2.3.2 Current Practice and Recommendations

The Canadian Pediatric Society and American Academy of Pediatrics (AAP) recommends that "growth of postnatal preterm infants in both their anthropometric indices and body composition should be the same as the normal fetus of the same gestational age"[9]. Based on this goal, recommendations of nutritional intakes have been made, but are often difficult to achieve due to clinical and metabolic instability of the infant. Current trends in nutrition favour earlier and more aggressive nutritional support to minimize the interruption of nutrients that occurs upon birth, thereby reducing the amount of initial weight loss after birth and reducing the amount of time required to regain this weight. Parenteral nutrition of glucose, amino acid and lipid solutions are provided initially, when enteral feeding is not yet possible. It is recommended that enteral feeds be introduced as soon as they can be tolerated to avoid atrophy of the gastrointestinal tract and to promote development of gut hormone release and gut motility[10]. Breast milk has been recognized as the gold standard for preterm infant nutrition because of the associated beneficial effects it has on host defence,

digestion, absorption, GI function, neurodevelopmental outcomes and improvements in maternal well being[10]. However, breast milk does not contain sufficient amounts of key nutrients to support small preterm infants (<1500g birth weight) and is fortified with additional nutrients to meet the recommended needs[11]. The optimal formula and feeding practices have yet to be determined, but it is recommended that infants receive 120 kcal/kg/d. When compared with the fetus, preterm infants receive higher rates of glucose and lipids, and significantly lower amounts of protein[12]. Carlson and Zielger found that energy and protein intakes fell short of those estimated to be needed in utero growth[12].

In addition to the recommended nutrient intakes and growth rates from the AAP[13], several different growth curves have been constructed for guiding and monitoring preterm infant growth. Intrauterine growth curves created from cross-sectional, gender-specific birth weight percentiles of infants born at varying gestational ages provide a prescriptive guide to growth of the preterm infant[14]. However, based on their construction from cross-sectional data these growth charts are not representative of actual continuous growth of the preterm infant and are therefore not a physiologically appropriate reference. Additionally, since infants lose weight after birth which they will not regain for 1-2 weeks, expecting a preterm infant to achieve a growth trajectory on par with the recommended values of a fetus in utero may be an unrealistic goal because of the morbidities associated with prematurity and this initial weight loss puts them on a different growth trajectory[1]. An alternative approach is to use reference growth curves

constructed from sequential measurements of preterm infants. The limitation of this approach is that such growth curves reflects growth impairment and effects of nutrition and care.[4] These curves would indicate what growth can be achieved using current medical intervention and nutritional support, but by no means could be used as a prescriptive guide to care. The optimal growth trajectory to which an infant should adjust to after birth is unknown; thereby limiting the definition of optimal nutritional support for the preterm infant.

Much attention has focused on improving nutritional strategies and growth of these infants, but many do not receive adequate nutrient intakes during hospital stay and do not achieve recommended reference growth. Achievement of high growth rates without imposing metabolic stress is a challenge. A common observation amongst this population is postnatal growth restriction, especially in sick infants and those born at lower gestational ages[10]. Ehrenkranz showed that VLBW preterm infants born between 24 and 29 weeks gestation do not achieve the 50th birth weight percentile of the intrauterine reference at hospital discharge and consistently do not even achieve the 10th percentile birth weight [15,16]. Poor postnatal growth is not an observation restricted to the most prematurely born infants, it was found to be a universal observation in both early and late preterm infants and appears to continue beyond infancy, and into childhood[4,17,18].

This universal postnatal growth restriction is related to the observed accumulation of nutrient deficits during the early postnatal period which are not

regained before the infants were discharged from hospital despite nutrient intakes guided by current recommendations[19]. These observations highlight the importance of providing optimal nutritional support as early as possible but may also indicate that current recommendations may still be inadequate to achieve growth rates similar to that of a fetus in utero.

2.3.3 The Dilemma

The nutritional and growth challenges that face preterm infants have become particularly relevant as there is abundant evidence to show that growth patterns during the early postnatal period may have significant effects on short and long-term development and health. Most epidemiological studies were retrospective in nature and looked at the effects of being born premature or small at birth on later health outcomes in adolescence and adulthood. Unfortunately, because of a lack of information regarding body composition, it is unclear how body composition or growth of the FM and FFM compartment influences later health outcomes of former preterm infants. Early retrospective studies showed that being born small is associated with increased BMI into adulthood[20]. Several studies have shown that poor growth, especially in head circumference is associated with motor and cognitive impairments at school age[21–23]. Poor nutrition during critical periods of brain growth has shown to result in deficit in behaviour, learning and memory. However, others have suggested that faster growth supported by early provision of amino acids, high energy intakes and breast milk

can produce positive results in growth and neurodevelopment[24–27]. A study in ELBW infants, showed that those that achieved the highest rate of growth during hospital stay displayed improved scores on the Bayley II Mental Developmental Index and Psychomotor Developmental Index tests, had fewer abnormal neurologic examinations and impairments at 18 and 22 months' corrected age[28].

Despite the positive association between rapid growth and neurodevelopmental outcomes, there is also evidence which suggests that rapid weight gain during infancy may be associated metabolic and cardiovascular disturbances in adolescence and adulthood[29]. Several studies in humans have shown that infants who exhibit catch-up growth show higher risk of experiencing a coronary event[30,31] and death from cardiovascular disease[32], hypertension[33], increased visceral adiposity[34], obesity[20,35] and increased incidences and risk of developing type II diabetes[29,35–38]. In contrast to this, preterm infants that displayed a more modest growth rate in infancy from being randomized to a lower-nutrient diet displayed reduced fasting split proinsulin (elevated levels would suggest an insulin-resistant state) when compared with those infants who were given a nutrient –enriched formula[39]. Although beneficial in the short term, rapid growth during the early postnatal period may be detrimental in the long term.

2.3.4 What is optimal?

Evidence exists that the timing of nutrient delivery may play an important role in promoting optimal outcomes. Several studies indicate that catch-up growth is a time sensitive phenomenon, and that if it does not occur during the early postnatal period, it will likely not occur at all. The first 4 weeks of life in preterm infants may represent a critical growth window. In a randomized control trial in preterm infants fed preterm formula vs. term formula, those fed preterm formula showed improved developmental outcomes at school age[24]. Further, early provision of 3g/kg/ day of amino acids (within the first 5 days of life) when compared with infants who received this protein after 5 days of life improved growth and developmental outcomes suggesting that the prime nutritional window is short[26]. It is well established that postnatal weight gain is influenced by energy intake and that it is possible to manipulate the growth trajectory of infants by altering nutrient intakes and/or feeding schedule[40] – an indication of the significant role nutrition plays in the growth of these infants. This nutritional support can be manipulated to promote an appropriate growth trajectory. Despite the attention that has been paid to nutrition and growth of preterm infants, optimizing both continues to be a challenge complicated by critical gaps in knowledge.

2.4 Evaluating Growth

Currently, in neonatal intensive care, routine serial measurements of weight, length and head circumference are used as surrogate parameters of nutritional status to adjust nutrient and fluid intakes of preterm infants while in hospital. Tracking these measurements over time and comparing them to reference growth charts provides an indication of the adequacy of growth that is being achieved. Weight measurements on an electronic scale are accurate but are sometimes complicated by attached equipment which may influence the resulting weight measurement. Head circumference is a particularly important measurement as it is used as an indication of brain growth and development. It is measured with a measuring tape while the infant is in their bed, and is therefore minimally disruptive to the infant. Length measurements can be made using either a measuring tape or length board; and provides a crude interpretation for how weight is being gained. The latter two measurements are heavily influenced by the individual performing the measurement; and if not properly trained, accurate and reproducible measurements are not possible, causing misleading growth interpretation.

Since these measurements are easily acquired, most infant growth studies have only focused on these measures. Although useful, they only provide a single dimension of growth, and do not provide details of the quality of growth of the infant. Although weight gain is the most commonly used parameter to assess

growth, perhaps there is a better parameter to track infant growth and aid in determining an optimal growth trajectory.

2.5 Measuring Body Composition

Body composition data, especially of preterm infants is useful in monitoring the quality of growth in relation to their nutritional regimen. The issue with only measuring weight is that it does not provide any information of FM or FFM development. Two infants with the same weight may have vastly different body composition. So, instead of solely monitoring weight gain, PI and BMI, which represent an index of weight independent of stature, have been used as a crude index of fatness.

The only direct method of measuring body composition is through chemical analysis of cadavers. Although a direct method, measurements may be influenced by post mortem changes that occur and affect the accuracy of the body composition measurements. There are several indirect methods available to estimate body composition, many of which are widely used and validated in the adult population. Fewer methods are suitable for the infant population, let alone the preterm infant population due to a lack of accurate techniques or the requirement of high subject compliance and specially trained personnel. Each method employs different physiological theories, operate using different assumptions and use different models of the body.

` Skinfold thickness is an anthropometric measure that uses calipers to pinch the skin at various sites of the body to estimate the subcutaneous adipose layer. This method converts the raw measures to percentage fat and is based on the assumption that subcutaneous adipose reflects a constant proportion of total body fat and that areas which are chosen for measurements represent an average thickness of fat. Measurement of skinfold thickness was found to be a useful initial rough estimate of body fat and composition in young infants despite some error[41]. Although a non-invasive and easily accessible tool, this is an unreliable method because of the potential of introducing error from a lack of valid conversion formulas and observer error[42].

Bio-electrical impedance analysis (BIA) is a non-invasive, portable and inexpensive method that uses the electrical conductivity of the body's lean mass compartment to estimate total body water and hydration status[43]. BIA lacks a standardized and validated method for infants and although algorithms have been proposed to estimate TBW, precision and accuracy of this method is still controversial. This method is complicated by the hydration changes that occur during infancy and the low fat content of infants[44].

Total body water can be determined by applying dilution kinetics of a measured dose of a stable isotope - commonly deuterium. When deuterated water is given to the subject, the concentration of the tracer is determined from the blood and urine to quantify total body water after collection of samples at

specified time points[45]. Deuterium dilution is considered to be the gold standard method of measuring body composition, however it is not practical for routine research or clinical use due to the significant time commitment, expense and complex analyses that is required[46].

Imaging techniques such as magnetic resonance imaging (MRI) and ultrasound have also been applied to measuring body composition. These techniques face the issue of translating a cross sectional slice of the body to whole body composition slice and the applicability to whole body measurement[44].

Dual energy x-ray absorptiometry (DEXA) uses 2 x-ray beams of differing energies to scan the body. Based on the differential absorption of the 2 x-rays, bone mineral content, fat and the lean mass compartments are calculated. There is minimal exposure to radiation, testing time is relatively short but requires compliance from the subject to remain motionless – which is sometimes a difficult task with infants. This method has been validated for use in the infant population and is a popular method of choice amongst infant body composition research[41,47,48].

Since its introduction in 2004, ADP (PEA POD, COSMED, USA Inc) has become a gold standard method to measure body composition of infants. The device uses whole body densitometry to determine BC. Body mass and volume are measured within the unit to calculate body density of the infant. PEA POD uses known densitometric equations to calculate FM and FFM. This system is

housed on wheels, with a warmed test chamber allowing the device to be brought to bedside, and can accommodate the preterm infant population who often cannot regulate their own body temperature. This device has been systematically tested and validated using volume phantoms, a bovine tissue phantom and full term newborn infants which were compared with a 4-compartment model[49] and deuterium dilution method[50]. More recently this system has been shown to be an accurate, reliable and precise technique to measure body composition of preterm infants[51]. This device has proven to be an acceptable reference method for measuring body composition.

2.6 Current Body Composition Data of Preterm Infants

Few data are available on preterm infant body composition due to lack of a reliable, non-invasive technique. Available data primarily describe body composition of preterm infants at the time of discharge from hospital and onwards, with an emphasis on the FM compartment of the body because it has the greatest inter-individual variation and is most sensitive to changes in nutritional status[44]. To our knowledge, there are no published studies that accurately characterize longitudinal preterm infant whole body composition during hospital stay or during the early postnatal period using ADP.

2.6.1 Body Composition before Hospital Discharge

The first studies of preterm infant body composition came from whole-body chemical carcass analysis of stillborn infants. The results of these studies

are likely not representative of body composition of the reference fetus as this method is known to underestimate body water[46] due to the losses that occur after death. Additionally, these studies did not accurately report gestational ages of the infants measured, cause of death nor the conditions under which these infants were born[3,44]. In the past, skinfold thickness measurements were also used to track fat changes, but do not provide accurate quantitative information of body composition [52,53]

It is logical to assume that infants born prematurely have low body fat content at birth considering that most fat accretion of the fetus occurs during the third trimester of pregnancy. One study measured body composition of appropriate for gestational age (AGA) preterm infants using DEXA within the first 48 hrs of life and found that %FM was constant[54] (Figure 2); and the %FM values were more similar to what is expected for a full term infant, and are therefore higher than expected for the reference fetus[55]. This may be a result of the observed tendency of DEXA to systematically overestimate the FM compartment[56,57]. Similarly, DEXA was used to measure body composition of AGA preterm and term infants within the first 2 weeks of life but the results were corrected for the overestimation of FM. Percentile curves for whole lean mass, body fat mass, %FM, bone area and bone mineral content were produced (Figure 1) [58].

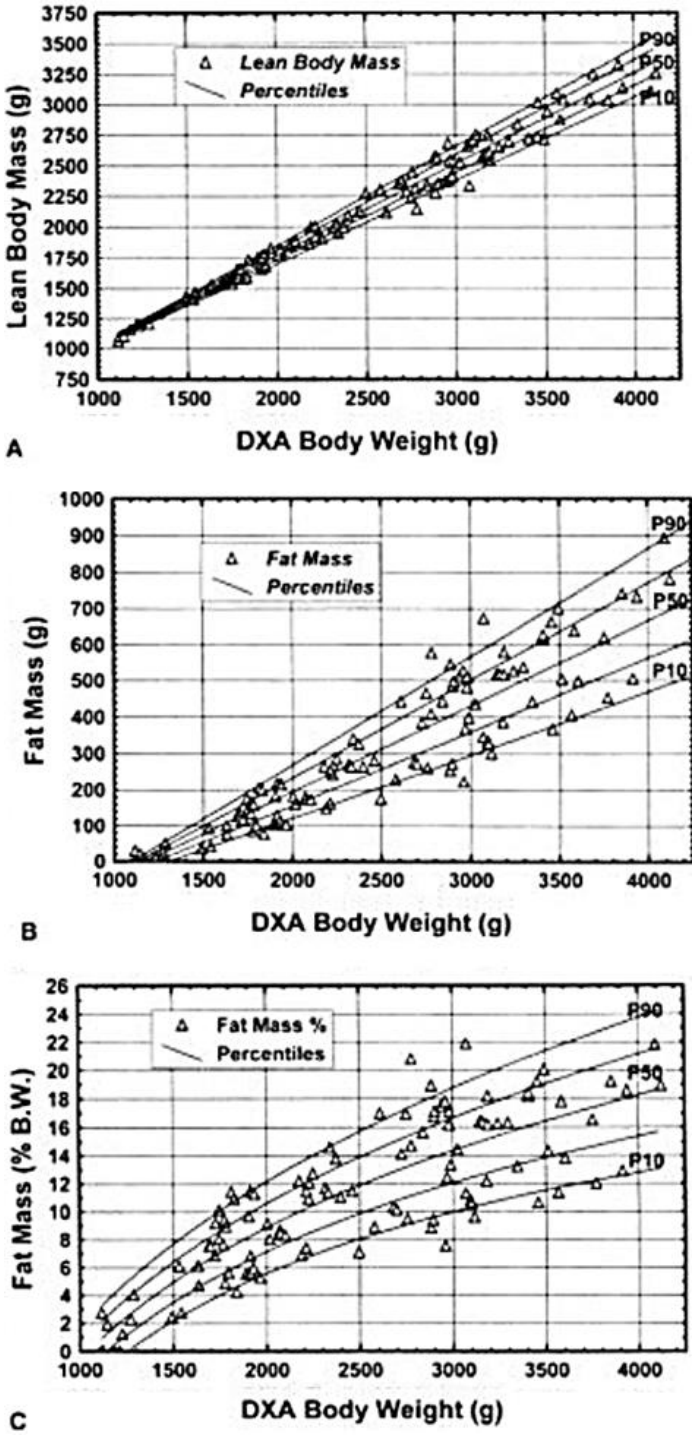

Figure 1. Individual values and percentile curves for lean mass, fat mass and %FM using DEXA of preterm and term infants by Rigo et al.

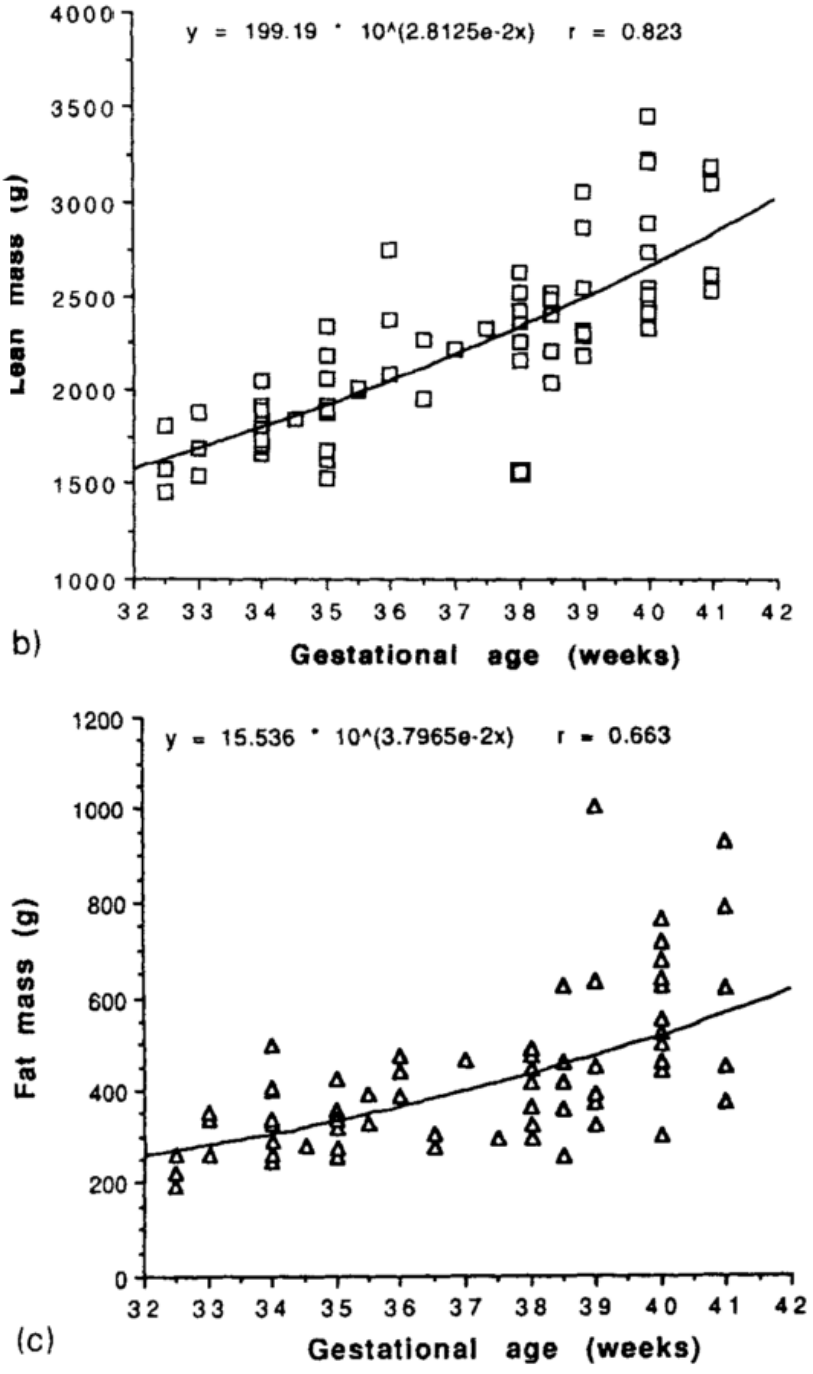

Figure 2. Correlation between GA and individual values of total lean mass and FM measured by DEXA of preterm and term infants by Lapillonne et al.

Body Composition at Hospital Discharge

Few studies have been performed at time of hospital discharge; body composition of preterm infants at this time is unclear. One study found that preterm infants had a %FM higher than in infants of similar mean body weight and of the reference infant of similar corrected age. The results from preterm infants were more comparable to the %FM of the 2 week old term reference infant[59,60].

2.6.2 Body Composition at Term Corrected Age

Preterm infants are smaller in length and weight at term corrected age, but it is unclear if their body composition similar to that of their healthy full term counterparts[61,62]. Inconsistency exists in reported %FM of preterm infants at term corrected age as either having higher %FM than healthy full term infants or no difference. A lack of consensus may be due to variable methodology used to measure body composition including isotope dilution, DEXA, MRI and ADP each with its own set of assumptions and model calculations. Additionally, there is heterogeneity among the populations included in these studies associated with different nutritional regulations and customs in various global centres and the inclusion of SGA infants who are known to have a different growth pattern than AGA preterm infants[54,63]

A recent systematic review and meta-analysis compared body composition at term corrected age of infants born preterm and those born at term[62]. The results of this analysis showed that preterm infants have a different body composition at term corrected age than newborn full term infants. These infants have significantly less total body FFM, but have a more modest reduction in amount of FM, which is to be expected because of their overall smaller stature. Interestingly, preterm infants have a greater %FM which appears be the result of an overall lesser amount of absolute FFM, rather than an increase in the FM compartment[62] (Table 1). Only studies that directly compared body composition of AGA preterm infants at term corrected age and infants born at term were included, except those that used methods of anthropometric derived conversion formulas (ie. Skinfold thickness, bioelectrical impedance analysis). To account for the differences in methodologies used in each of the studies, the differences in body composition between preterm and term populations were analyzed within each study and then the calculated differences were compared between studies. Therefore, only eight studies were included, of 388 preterm and 345 full term infants (mean PMA at test: 39.5±1.5 wks vs. 39.5±1.5 wks). Analysis of was limited by the heterogeneity in the measures of body composition, methodology, and global centres. In line with the findings of the meta-analysis, one study not included in the analysis also found that FFM was lower, and %FM was higher at term corrected age when compared with full term infants[64].

Table 1. Summary of results from meta-analysis comparing body composition of preterm infants at term corrected age and healthy full term infants at birth[62]

Outcome	No. of Studies Included in the Meta-analysis (No. of Patients)	Mean Effect of Preterm Birth on Body Composition at TEA Compared With Infants Born Full-term (95% CI)	*P*
%TBF	7 (672)	Increased in preterm infants by 3.0% (0.25%–5.88%)	.03
FM	5 (297)	Decreased in preterm infants by 50 g (10–90 g)	.02
FFM	3 (137)	Decreased in preterm infants by 460 g (270–340 g)	<.0001
Weight	7 (577)	Decreased in preterm infants by 590 g (440–750 g)	<.0001
Length	4 (383)	Decreased in preterm infants by 3.7 cm (2.8–4.6 cm)	<.0001
Head circumference	5 (450)	Decreased in preterm infants by 1 cm (0.5–1.5 cm)	<.0001

2.6.3 Body Composition within the First Year of Life

Few studies have been conducted past term corrected age to characterize longitudinal body composition growth of preterm infants. Most studies have focused on body composition changes and growth trajectories in response to dietary intervention, differing nutritional program and formula compositions. The results of studies that have been done remain controversial – there is no clear definitive consensus on the body composition changes preterm infants undergo during this time period and how they compare with those born full term.

One study showed that when corrected for age, lean mass was less than the Fomon's reference infant. However, the same study showed when corrected for weight, preterm infants displayed higher %FM which was a function of increased absolute FM[65]. A study using ADP found that at 3 to 4 months corrected age %FM and FFM was similar between the preterm and term groups of infants. Although %FM and FFM differences between 3-4 months did not reach statistical significance ($p=0.07$), the preterm infant group did have a slightly higher %FM ($27.7\pm1.7\%$ vs. $23.9\pm0.75\%$).

2.6.4 Body Composition beyond the First Year of Life

The body composition of former preterm infants in childhood and adulthood has been reported to be different when compared to those born at term. However, there is no clear consensus on body composition of former preterm infants or any related metabolic complications.

Several groups investigated former preterm using DEXA and skinfold thickness between the mean ages of 5-12 years and found that overall %FM was reduced when compared with former full term born infants of the same age[37,66,67]. Two of the studies found that this reduction in %FM was a result of less absolute FM compartment since the lean mass compartment was similar between the two groups[66,67]

In contrast, two studies found that body composition of former preterm infants do not differ significantly from those born at term between the mean ages of 2-8 and 12-18 years of age[68]. One study of former ELBW infants adolescents were overall smaller than AGA adolescents in height, weight and head circumference, lean mass, fat mass and bone mineral content, but concluded that that the relative body composition of adolescents of these groups was also similar, despite the ELBW adolescents being overall smaller in size[69].

In addition to a difference in %FM at term, some studies have found that the distribution of fat is also altered in preterm infants with a reported tendency to gain intra-abdominal (visceral) fat. The distribution and quantity of adipose tissue is a marker for morbidity risk; there is a consensus that the intra-abdominal adipose depot is damaging to health and linked to the development metabolic syndrome and cardiovascular disease in later life[70]. There is concern that the adipose pattern in infancy could carry over in adolescence and adulthood, placing preterm infants at added risk of metabolic disturbances.

Studies of former preterm infants using MRI, ^{1}H magnetic resonance spectroscopy and DEXA[60] found they had increased central and intra-abdominal fat when compared with individuals that were born at term: at time of discharge[60], term corrected age[61,71], between 4-7 years[66], at a mean age of 11.2 years[67] and between 18-27 years of age[72]. Although it is unclear whether fat deposition is result of size at birth prematurity or catch up growth, illness severity was found to be related to the magnitude of increases in visceral fat[61].

2.7 The Problem

The main issue is that information on preterm infant body composition during the early postnatal period is largely unknown, due to a lack of appropriate techniques or methods that can be applied to this population. It has been well established from the early origins of health and disease that early postnatal experiences can influence health outcomes into adolescence and into adulthood.

Despite the advances that have been made in nutrition and growth of preterm infants, both still remain highly controversial topics. Optimizing nutrition is a daunting and complicated task due to the lack of knowledge of optimal growth patterns and the clinical instability of the infants. With the overwhelming evidence that a trade-off exists between rapid catch up growth and later health outcomes, the balance to maximize neurologic outcomes without sacrificing cardiovascular and metabolic health has not yet been determined. Research therefore needs to focus on characterizing the quality of growth preterm infants

achieve with current practices and evaluate the subsequent health outcomes in order to make adjustments in the future to improve short and long term outcomes of preterm infants. It was therefore the aim of the study to first introduce and assess the feasibility of ADP as a bedside tool in the NICU and then longitudinally measure preterm infant BC in hospital.

3.0 Materials and Methods

3.1 Study Setting

This was a single-centre study; all measurements were performed at McMaster Children's Hospital, Hamilton, Ontario. Preterm infants in the NICU and Level II Nursery were measured at bedside. Healthy full term infants were measured in a designated quiet study room in the 4C Ward.

3.2 Description of Project

A longitudinal, observational study was conducted. Basic subject characteristics (gestational age, gender, birth weight, somatic classification, head circumference, length) were recorded from patient charts at bedside or from the electronic data management system, Sovera. Maternal information was also collected from patient charts stored on Sovera.

Preterm infants received parenteral and enteral feeding with expressed breast milk and/or formula and/or human milk fortifier until discharge from hospital. Infants were fed according to unit protocol.

Body composition was assessed using air displacement plethysmography (PEA POD - Infant Body Composition System, COSMED, USA; version 3.3.0) by 2 trained operators with the assistance of a nurse. Infants wore caps to flatten

hair against their head. They were assessed before feeding at specified handling times, as per hospital protocol.

3.3 Method Development

The first several weeks of study start up were used to measure preterm infants of gestational age greater than 30 weeks to establish testing procedure with stable preterm infants in the Level III NICU nursery.

3.3.1 PEA POD Door Adjustments for Testing

We received a spare inner door plate from COSMED with several rubber gaskets to which we could make adjustments. The rubber gasket is a rubber ring with an overhanging lip which acts as the seal of the volume chamber to prevent air leakage.

Design 1:

The first attempt involved cutting a small slit in the rubber gasket to allow the IV line to run through. Calibration volume tests using a known metal volume phantom and a water phantom were run to test accuracy and precision. Each volume test consisted of a calibration test, 3 volume tests and a final calibration. For every volume test the IV line was run through the slit.

Design 2:

We cut off the overhanging edge of the rubber gasket such that the rubber only filled the channel of the inner plate (see Figure 4). A foam ring with a narrow

slit in it to accommodate the IV line was placed over top of the rubber filled channel to maintain an air tight seal (see Figure 5). Calibration volume tests using a known metal volume phantom and a water bottle phantom were run to test accuracy and precision.

Figure 4. Foam ring applied over top of the rubber filled channel of the inner door plate of the PEA POD.

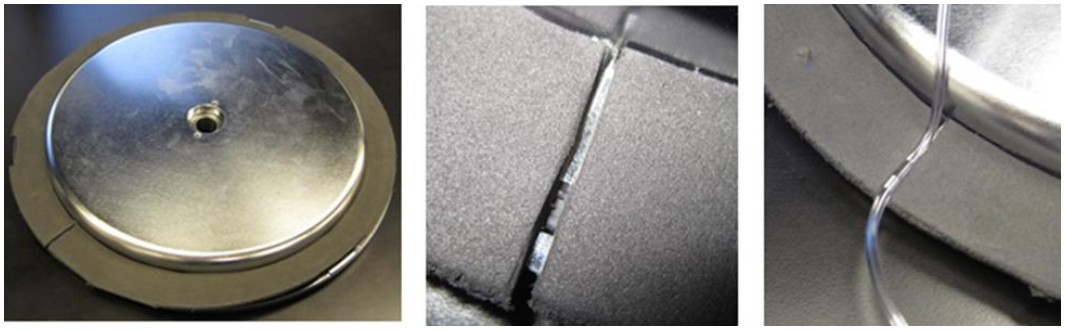

Figure 5. Complete foam ring on inner door plate, slit made in foam ring to accommodate IV line, IV line set inside foam slit.

3.4 Feasibility

In order to assess the feasibility of the PEA POD to measure preterm infants at the bedside in a clinical setting upon its introduction into the NICU at McMaster Children's Hospital, several factors were assessed: quality control

results, the number of cases of adverse events relating to study inclusion, length of time needed for quality control testing and test set up and measurement, ease of consenting and infant participation and acquisition of measurements.

3.5 Body Composition Measurements

3.5.1 PEA POD - Description of System

The PEA POD's components are housed on a movable cart, making this device mobile. It consists of 2 chambers, a reference volume chamber and a test chamber which are connected by a diaphragm that oscillates. A calibration valve connects the 2 chambers - when open, it allows the test chamber to be connected to the known reference volume which is used for calibration. Pressure transducers are connected to the two chambers. In addition to the volume chambers, the PEA POD consists of an air circulation and heating system, inclinometer and electronics box to control the diaphragm, calibration valve, pressure transducers. The PEA POD is outfitted with a computer monitor and an electronic scale on the top surface of the cart. The scale system was chosen for its stability. It has a maximum capacity of 12kg and a resolution of 0.1 g.[73,74]

Air is continuously circulated from the outside environment to the test chamber through the circulation and heating systems. The test chamber contains a slide out plastic tray where the subject is placed, and is maintained at 31ºC for subject comfort. An electromagnet system closes the chamber door during testing.

3.5.2 Operating Principles of PEA POD Device

The PEA POD uses the principle of whole body densitometry to determine body composition. Densitometry is based on calculating body density by measuring body mass and volume and using it to calculate the amount of fat and lean tissue in the body. Subject volume is measured in the test chamber by applying Boyle's and Poisson's Laws relating pressure changes to volume of air in the chamber. Density is then easily calculated from subject mass and volume and used to calculate %FM by inserting it into a standard formula according to a two compartment model.

3.5.3 Description of Testing

The PEA POD required approximately 2 hours to allow the test chamber to warm to 31ºC. Before each measurement, daily Quality Control tests consisting of scale and test chamber calibration were performed; including each time the device was switched off or re-located. The subject is first weighed on the scale. During this time the test chamber door is closed and sealed while a 2-point calibration is performed. While the test chamber is empty, the pressure changes are monitored with the calibration valve open and closed which produces a linear relationship between the inverse ratio of the pressure changes in the test and reference chamber. Once this calibration is completed, the door automatically opens so that the infant can be placed on the tray and closed into the test chamber.

A volume measurement has a duration of approximately 2.5 minutes, at which point the chamber door opens and the subject is removed.

Subjects are nude and required to wear a thin cap, provided by COSMED USA for each measurement. The surface area of clothing and hair has the potential to significantly affect volume measurements.

3.5.4 Training of personnel and data quality control

Training was provided to the study team by a COSMED, USA representative in February 2012. Further practice was performed in a lab setting without human subjects to perfect and practice the protocol of a measurement. As mentioned above quality control testing of the electronic scale and volume chamber of the PEA POD was performed before every measurement.

3.6 Study Population

Potential infants were recruited for this study by a study coordinator during both antenatal and postnatal periods. During the postnatal period, infants were screened for exclusion criteria using patient charts on Meditech, the electronic patient information management system and/or during morning rounds with physicians and nurse practitioners. During the antenatal period, infants were identified using mother's chart and gestational age data of the infant. Exclusion criteria were implemented after birth for the infants recruited antenatally. From February 2012-June 2013, 127 preterm infants were successfully consented to this study, 65 were enrolled in the study. Inclusion criteria were all infants born with a

gestational age of 24-36 weeks and having received informed and written consent from a parent or legal guardian.

Twenty-three healthy appropriate for gestational age full-term newborn infants born at McMaster Children's Hospital from January 2013-May 2013 were recruited as a reference group. These infants were measured once within the first 72 hours of life. Inclusion criteria were all infants with a gestational of 37-42 weeks and having received informed and written consent from a parent or legal guardian.

Exclusion criteria for all infants were presence of major congenital and/or chromosomal abnormalities and hydrops fetalis with clinical symptoms, severe cardiac or brain or gastrointestinal disease.

3.7 Study Measures

Body Composition – Estimates of %FM, %FFM and absolute values of FM and FFM of infants were measured using air displacement plethysmography (PEA POD, COSMED, USA). A description of testing procedures and operating principles are above.

Weight: Infant weight was measured using the electronic scale house on the PEA POD. Infant weight measured using scales in the NICU were also recorded when available.

Length: Length measurements were performed using a pediatric length board on a flat surface with the assistance of a bedside nurse.

Head circumference: Measurements were performed by a bedside nurse using a flexible measuring tape.

3.8 Time Points of Measurement

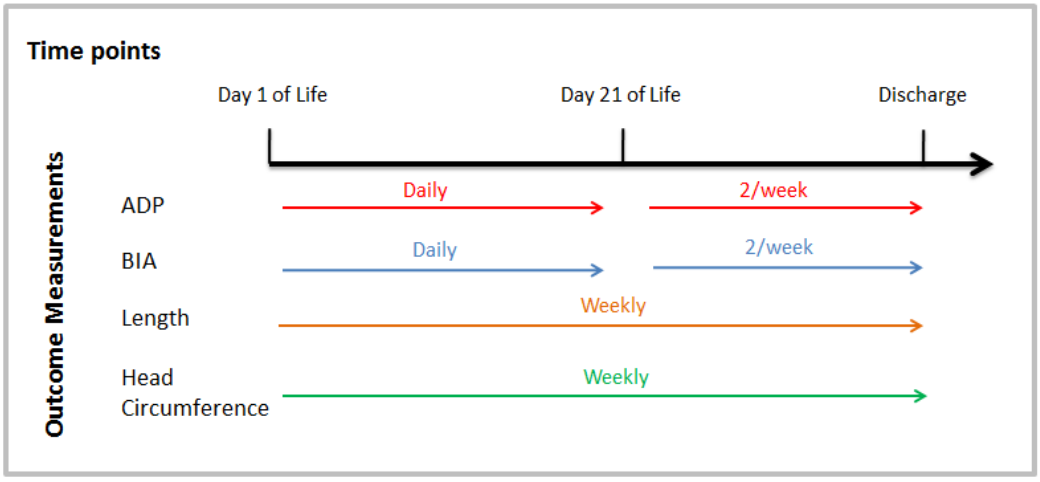

Figure 3. Time points of measurements.

3.9 Data analysis

All analyses were performed using Excel. Descriptive statistics were expressed as a mean (standard deviation) or frequency (percent). Body composition and body indices data were displayed as scatter plots and linear regression analyses were performed. Slopes of the trend lines were analyzed using Graphpad Prism 6 to compare the trajectory of each body compartment between AGA and SGA preterm infants. Term infant body composition was displayed as mean (standard deviation) in all figures due to the cross sectional nature of data

collection. FMI was calculated as ($FM/length^2$), FFMI was calculated as ($FFM/length^2$).

3.10 Ethical Considerations

This study was first presented and approved by the Neonatal Research Committee at McMaster University, then submitted to the Hamilton Integrated Research Ethics Board of McMaster University, Ontario and approved (REB# 11-499). Written and informed consent was obtained from infants' parents.

4.0 Results

4.1 Overview

This chapter discusses the method development and feasibility aspect of using PEA POD to measure preterm infants during hospital stay; then describes body composition findings of preterm and term infants from the clinical pilot study.

4.2 Study Participation

Among 127 successfully consented preterm infants who met the study inclusion criteria, 65 preterm infants were included in this study. Among 26 successfully consented full term infants, 23 were included in this study. Reasons for exclusion of already consented infants were: discharge or transfer from hospital before measurement could be obtained; infants became ill and/or were not clinically stable for measurement; and for several months, there were technical and environmental issues that interfered with successfully measuring infants.

4.3 Method Development

4.3.1 Procedure

The PEA POD is currently stored in an equipment room in the NICU and is brought to infant bedside when needed. No one on the research team was able to handle infants; therefore the assistance of the infant's bedside nurse was

required to perform a measurement. Measurements could only be performed at specified handle times of the infant to ensure that they were not additionally disrupted due to inclusion in the study.

Development of optimal measurement procedure was performed within the first weeks of the study after delivery of the PEA POD to McMaster Children's Hospital. In this case optimal is defined as a method least disruptive to the infant and neonatal nursing staff and maximal efficiency of measurement. The procedure is as follows:

1. Ensure PEA POD is turned on at least 2 hours before planned measurement in storage location. It takes approximately 1.5 hours for the PEA POD test chamber to heat to the required 30ºC.
2. Once PEA POD has warmed up, perform QC tests.
3. Confirm with bedside nurse that next handle time for infant is appropriate for PEA POD measurement.
4. Move PEA POD to infant bedside approximately 45 mins before next handle time to allow PEA POD to acclimatize to the slightly different environment and to perform QC tests and scale calibration at the new location.
5. While running QC tests, prepare PEA POD for testing: place blanket on scale, prepare items for taring volume chamber and scale (ie. ID bracelet, electrodes), get length board from storage, take out wireless pulse

monitor, and ensure infant identification information has been inputted into program.

6. At time of measurement, while nurse is undressing infant, begin PEA POD testing sequence – this step is key for timing later on. The device performs a calibration that takes approximately 2-3 mins, while this occurs the infant is weighed. After the PEA POD finishes its calibration the volume test chamber door pops open and the infant is placed inside for their volume test. If not timed properly, taking the infant out of their incubator and weighing them may take less time than the calibration test at which point the infant is naked and in room air where they may get cold and uncomfortable and cannot yet enter the warmed volume test chamber for their measurement.
7. After volume test the infant is measured using the length board (once per week).
8. PEA POD and all measurement items are cleaned at bedside before moving the PEA POD back to the clean equipment room.

The time required of the PEA POD operator for a single measurement is approximately 45 mins, beginning at time the PEA POD is moved to infant bedside to time PEA POD is moved back to storage. The actual time required for infant testing is approximately 10 minutes beginning with undressing the infant to dressing the infant back in their bed.

4.3.2 Door Adjustment Studies

It was very clear early in testing that cutting a small slit in the rubber gasket to accommodate an IV line was allowing enough air leakage from the volume chamber to produce highly varied, invalid volume results (results not shown). After a short testing period with this method we moved on to a different design.

Volume testing of the foam ring door design was performed in stages. First we performed volume and autorun QC tests using the standard metal volume phantom and a 2L water bottle phantom with the foam ring alone taped in place without use of IV line. Results showed that this ring supplied adequate coverage and seal to the volume chamber, preventing air leakage where 6/7 tests passed and consistent volumes of each phantom were measured. Table 1 in the Appendix shows full volume output and comments.

Next, a small slit was cut into the foam ring to test for air leakage using the metal volume phantom and water bottle phantom. Results when an IV line was inserted into the slit and when the slit was left empty were consistent; results showed that of 15/16 tests, the SD of measured phantoms were <0.003. 100% of tests using the metal volume phantom passed testing, measuring the expected volume of 3.0182-3.0302L. Full volume testing results are shown in Table 2 in the Appendix.

The next step was testing the PEA POD's ability to measure the metal phantom under 3 different configurations indicated in Table 2. 100% of volume tests performed using configuration 1 failed to achieve a mean volume between 3.0182-3.0302L, 3/10 tests failed to consistently measure the mean volume of the phantom producing a SD >0.003. Similarly, 100% of volume tests performed using configuration 2 failed to measure the appropriate volume of the known metal phantom and 2/10 tests failed to achieve a SD <0.003. However results from configuration 3 show that 7/9 tests measured the appropriate volume of the phantom and 100% of tests had a SD <0.003.

Table 2. List and description of 3 configurations of PEA POD testing.

Configuration Number	Calibration Configuration	Volume Measurement Configuration
1	No IV Tube through door seal or in Tray	IV Tube running through door seal and into the Tray; Volume Phantom in Tray
2	IV Tube in Tray, but not through door seal	
3	IV Tube running through door seal and into the Tray	

Additional testing using configuration 3 showed consistent and accurate results. See Table 3 in Appendix. To ensure the IV line stays in the slit in the foam, tape is applied to the line to keep it in place- if it slips out of position air leakage is introduced.

4.4 Feasibility

4.4.1 Ease of Consenting

Of the documentation available from February – September 2012 and January - April 2013, there was a 53.8% success rate of consenting an infant into the study after approaching parents. Although not quantified, documented reasons for unsuccessful consent include:

- Language barrier
- At the time, there were many other studies running in the NICU and parents were sometimes overwhelmed and not interested in being involved in more than one research study or hearing about another study
- Some parents were not interested in being involved in research at all
- Some parents felt the PEA POD measurements required too much additional infant handling

In some cases, the parents of infants who met inclusion criteria were not approached because the infant was to be discharged/transferred from hospital soon, they were too anxious or stressed to be approached and/or parents could not be located. Although not quantified, we did receive excellent feedback from families about this study. Overall, parents were supportive and interested in being involved.

4.4.2 Adverse Events

Since enrolling infants into this study in February 2012, no infant experienced an adverse event relating to inclusion in this study or PEA POD measurement. As a safety measure, infants were monitored for oxygen saturation and heart rate wirelessly for the duration of PEA POD testing. No test had to be terminated or interrupted due to oxygen desaturation, irregular heart rate or infant discomfort.

4.4.3 Quality Control (QC)

Before every measurement, the PEA POD scale is calibrated and a QC test is performed to assess environmental and device stability, as well as volume performance by measuring a known 3 L volume phantom. A resulting measure between 3.0182-3.0302L and a standard deviation less than 0.003L was required to 'pass'. The only instance when QC testing did not pass was during the period of technical problems and during a period of construction in the NICU which disturbed the environment.

4.5 Clinical Pilot Study

4.5.1 Description of Infants and Mothers

The age range at birth of infants included in this study was 27-41 completed weeks of gestation. The distribution of infants included in this study are shown in Figure 6.

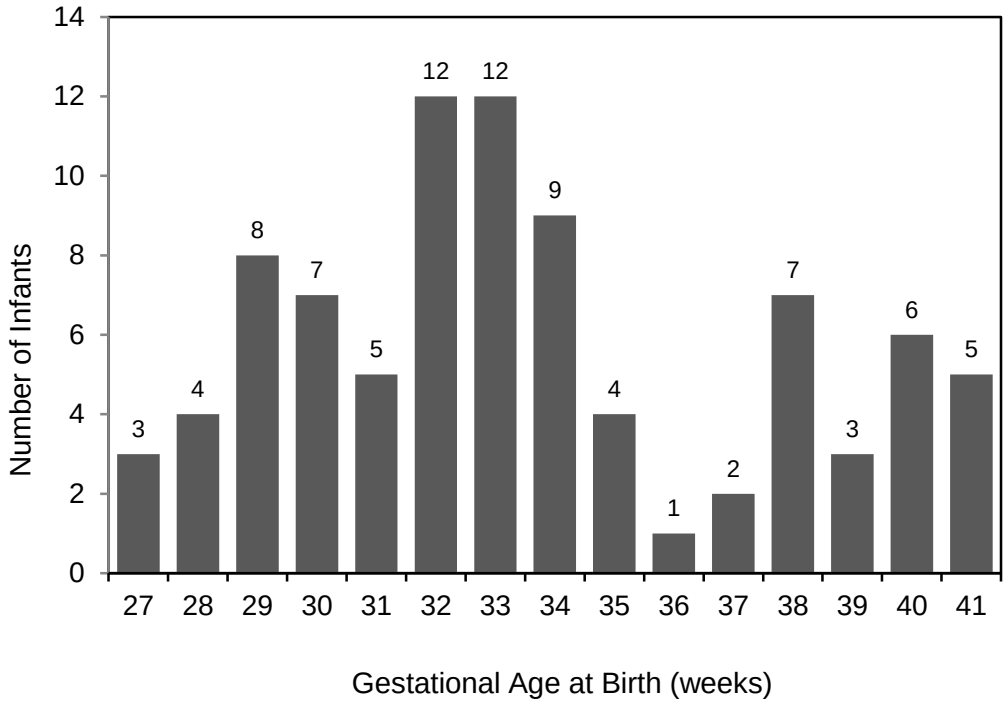

Figure 6. Distribution of preterm and term infants according to their gestational age at birth.

The range of birth weights of preterm infants was 960-2985 g, and the mean was 1753 ± 520 g. The range of birth weights of term infants was 2910-3590 g, and the mean was 3567 ± 370 g.

Table 3: Basic subject characteristics and anthropometry of preterm and full term infants at time of birth. Age, weight and head circumference expressed as mean ± SD.

	Population	At Birth
Gestational Age	Preterm	32.0 ± 2
	Term	39.5 ± 1
Males/Females	Preterm	32/33
	Term	8/15
Singletons/Twins/Triplets	Preterm	34/29/2
	Term	23/0/0
Weight (g)	All Preterm	1753 ± 520
	27 weeks	1063 ± 38
	28 weeks	1288 ± 46
	29 weeks	1204 ± 120
	30 weeks	1452 ± 220
	31 weeks	1715 ± 500
	32 weeks	1901 ± 430
	33 weeks	1935 ± 480
	34 weeks	2020 ± 280
	35 weeks	2512 ± 446
	36 weeks	2985 ± 0
	Missing	0
	All Term	3567 ± 370
	37 weeks	3135 ± 320
	38 weeks	3451 ± 320
	39 weeks	3777 ± 340
	41 weeks	3636 ± 400
	Missing	0
Head Circumference (cm)	All Preterm	29 ± 3
	27 weeks	25 ± 0

	28 weeks	27 ± 1
	29 weeks	26 ± 1
	30 weeks	28 ± 2
	31 weeks	29 ± 2
	32 weeks	30 ± 2
	33 weeks	31 ± 2
	34 weeks	30 ± 1
	35 weeks	33 ± 1
	36 weeks	33 ± 0
	Missing	2
	All Term	34 ± 1
	37 weeks	34 ± 0
	38 weeks	34 ± 1
	39 weeks	35 ± 1
	41 weeks	35 ± 1
	Missing	0
Small for Gestational Age (n)	Preterm	7
	Term	0
Large for Gestational Age (n)	Preterm	1
	Term	0

The range of maternal ages of preterm infants was 18-39 years of age and the mean age was 31.2±6 years. The largest proportion of mothers were between the ages 30-34 (Table 4). The overall prevalence of maternal smoking during pregnancy was 36.6%, including 25% who quit smoking during pregnancy and 11.6% that continued smoking during pregnancy. The prevalence of mothers with a normal pre-pregnancy BMI was 40.4%, while 21.1% had a pre-pregnancy BMI of 30 or above, classifying these women as obese.

Table 4. Distribution of age, parity and habits of mothers of preterm infants in this study.

	Frequency	%
Maternal Age (years)		
<20	3	5.7
20-24	7	13.4
25-29	12	23.1
30-34	18	34.7
>35	12	23.1
Total	52	100
Parity (n)		
1	19	36.5
2	6	11.6
3	10	19.2
4	11	21.1
5>	6	11.6
Maternal Smoking (n)		
None	31	59.6
Quit smoking in pregnancy	13	25.0
Smoked during entire pregnancy	6	11.6
Missing	2	3.8
Maternal Diabetes (n)		
Yes	6	11.6
No	43	82.7
Missing	3	5.7
Pre-pregnancy BMI (kg/m^2) (n)		
<18.5	2	3.8
18.5-24.9	21	40.4
25-29.9	4	7.8
30>	11	21.1
Missing	14	26.9

4.5.2 Body Composition of Infants

An overall increase in body weight of preterm infants was observed during the course of hospital stay (Figure 7).. Within 72 hours of life, infants born at term have an average body weight of 3387 ± 356g.

Linear trend lines were included to better visualize the general pattern of body weight at specified postmenstrual ages. The slopes of the trend lines of AGA and SGA preterm infants were not significantly different ($p=1.00$). Therefore the overall weight gain of preterm infants was 27.5g/day. SGA preterm infants gain weight at a similar but lower trajectory than AGA preterm infants, where the y-intercepts of these 2 groups of preterm infants were significantly different ($p<0.0001$). Extrapolation of the trend line shows that AGA preterm infants will achieve a similar weight to that of full term infants at birth by 41 weeks PMA (see Table 5).

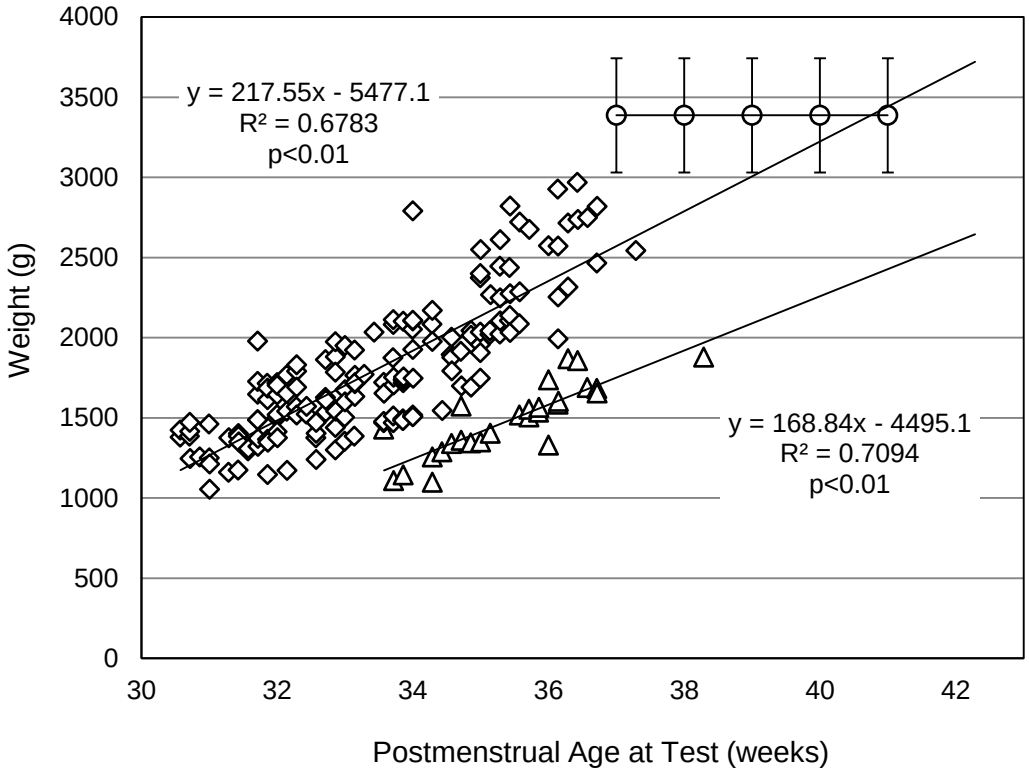

Figure 7. Body weight of AGA preterm (diamond), SGA preterm (triangle) and full term (circle) infants presented by postmenstrual age at test.

Absolute FM of preterm and term infants is positively correlated with age at test (Figure 8). The slopes of the trend lines of FM gain of AGA and SGA preterm infants were not significantly different ($p=1.00$). The y-intercept of these 2 groups of preterm infants were significantly different ($p<0.01$). Therefore, preterm infants gain 4.85 g/day of FM during hospital stay. Infants born at term and measured within 72 hours of life have a mean fat mass of 474 ± 156g. Extrapolation of the trend line shows that AGA preterm infants will achieve a FM similar to that of full term infants at birth by 40 weeks PMA (Table 5).

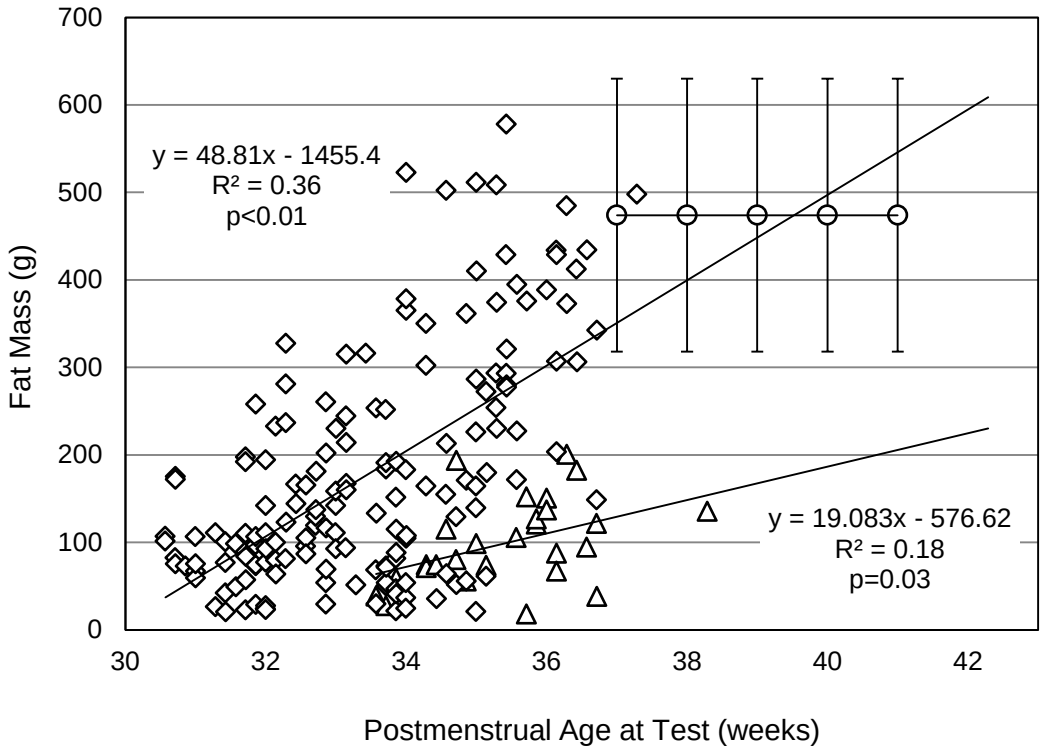

Figure 8. Absolute whole body fat mass of all observations of AGA preterm (diamond), SGA preterm (triangle) and full term (circle) infants by postmenstrual age at time of test.

The FFM compartment increases in preterm infants during hospital stay; full term infants have a mean FFM of 2913 ± 282 g within 72 hours of birth. SGA preterm infants display a FFM trajectory not significantly different ($p=1.00$) to AGA preterm infants but at a lower weight, with the y-intercepts of the trend lines being significantly different ($p<0.0001$). Preterm infants display an overall increase in FFM of 22.75 g/day. In both populations FFM is positively correlated with age at time of test. AGA preterm infants will achieve a FFM similar to that of term infants at birth by 42 weeks PMA (Table 5).

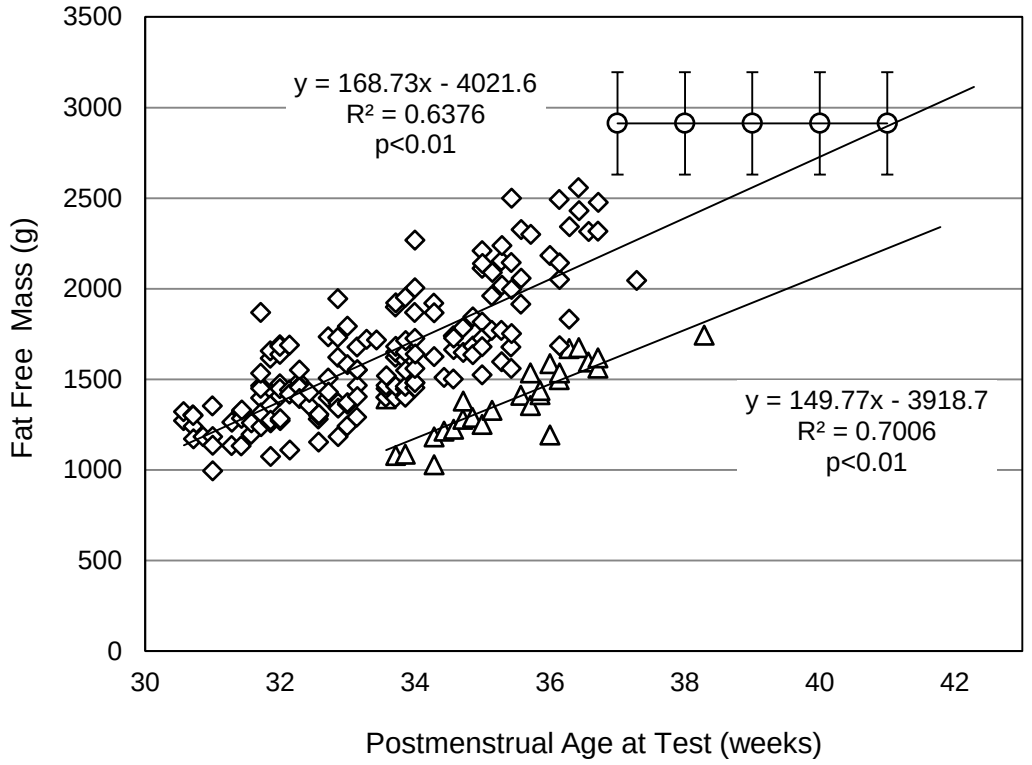

Figure 9. Absolute whole body fat free mass of AGA preterm (diamond), SGA preterm (triangle) and full term (circle) infants by postmenstrual age at time of test.

Results from % body composition analysis showed that preterm infants accrete FM and FFM at differing rates, leading to an overall increase in %FM and a subsequent decrease in %FFM during hospital stay as shown in Figures 10 and 11, respectively. SGA preterm infants display a more modest increase in %FM that is significantly different ($p<0.0001$) to that of AGA preterm infants. Within 72 hours of life, term infants have a mean %FM of 13.8 ± 4% and mean % FFM of 86.2 ± 4%. Extrapolation of the trajectory of %FM increase of AGA preterm infants show that by the time these infants reach a PMA of 37 weeks, they will have achieved a similar %FM to that of full term infants at birth.

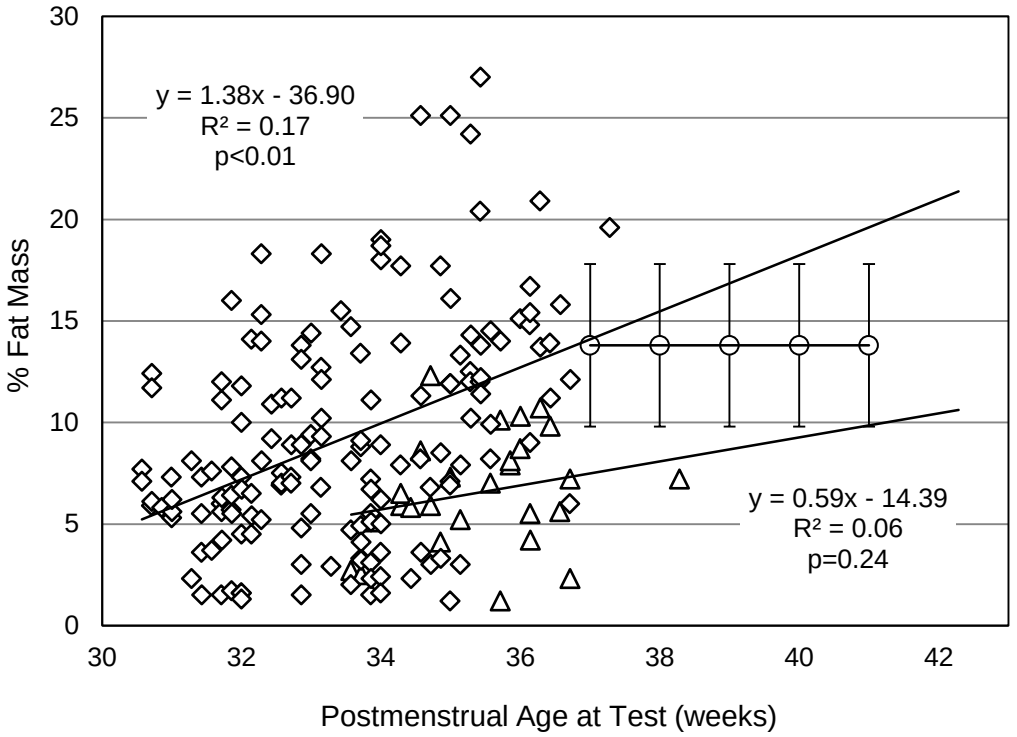

Figure 10. Total % FM of AGA preterm (diamond), SGA preterm (triangle) and full term (circle) infants by postmenstrual age at time of test.

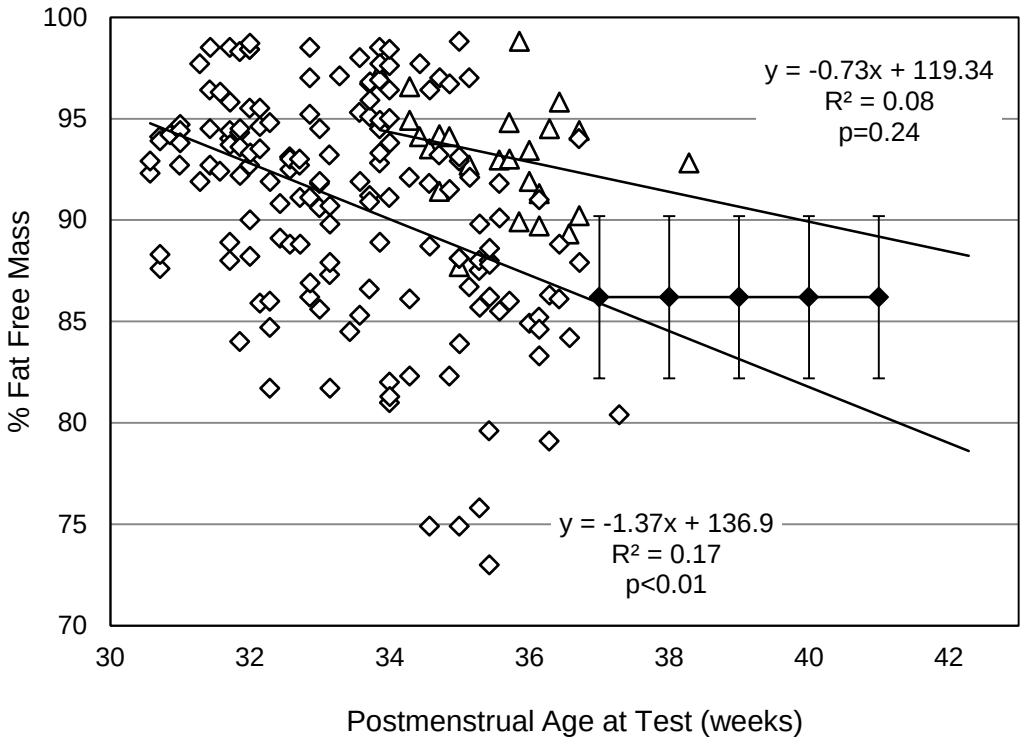

Figure 11. Total % FFM of all preterm (unfilled) and term infants (filled) by postmenstrual age at time of test.

Table 5. Extrapolated trajectory of weight, fat mass, fat free mass and %fat mass of preterm infant growth to term corrected age.

Week	Weight (g)		FFM (g)		FM (g)		%FM	
	AGA	SGA	AGA	SGA	AGA	SGA	AGA	SGA
37	2572.25	1751.98	2221.41	1622.79	350.57	129.451	14.16	7.44
38	2789.8	1920.82	2390.14	1772.56	399.38	148.534	15.54	8.03
39	3007.35	2089.66	2558.87	1922.33	448.19	167.617	16.92	8.62
40	3224.9	2258.50	2727.60	2072.10	497.0	186.70	18.30	9.21
41	3442.45	2427.34	2896.33	2221.87	545.81	205.783	19.68	9.80
42	3660.00	2596.18	3065.06	2371.64	594.62	224.866	21.06	10.39

When %FM in preterm infants are grouped by gestational age at birth, it appears that irrespective of gestational age at birth, %FM increases at a similar rate (Figure 12). SGA and LGA infants were excluded. Gestational age weeks 27, 31, 35 and 36 were also excluded due to the small sample size; each of these groups had 3 or fewer infants. Table 5 provides the regression equations of the trend lines of each gestational age group of infants depicted in Figure 12; the slopes of the lines provide an indication of the gain in %FM per day and are similar to each other with an average %FM increase of 0.45 ± 0.07% per day.

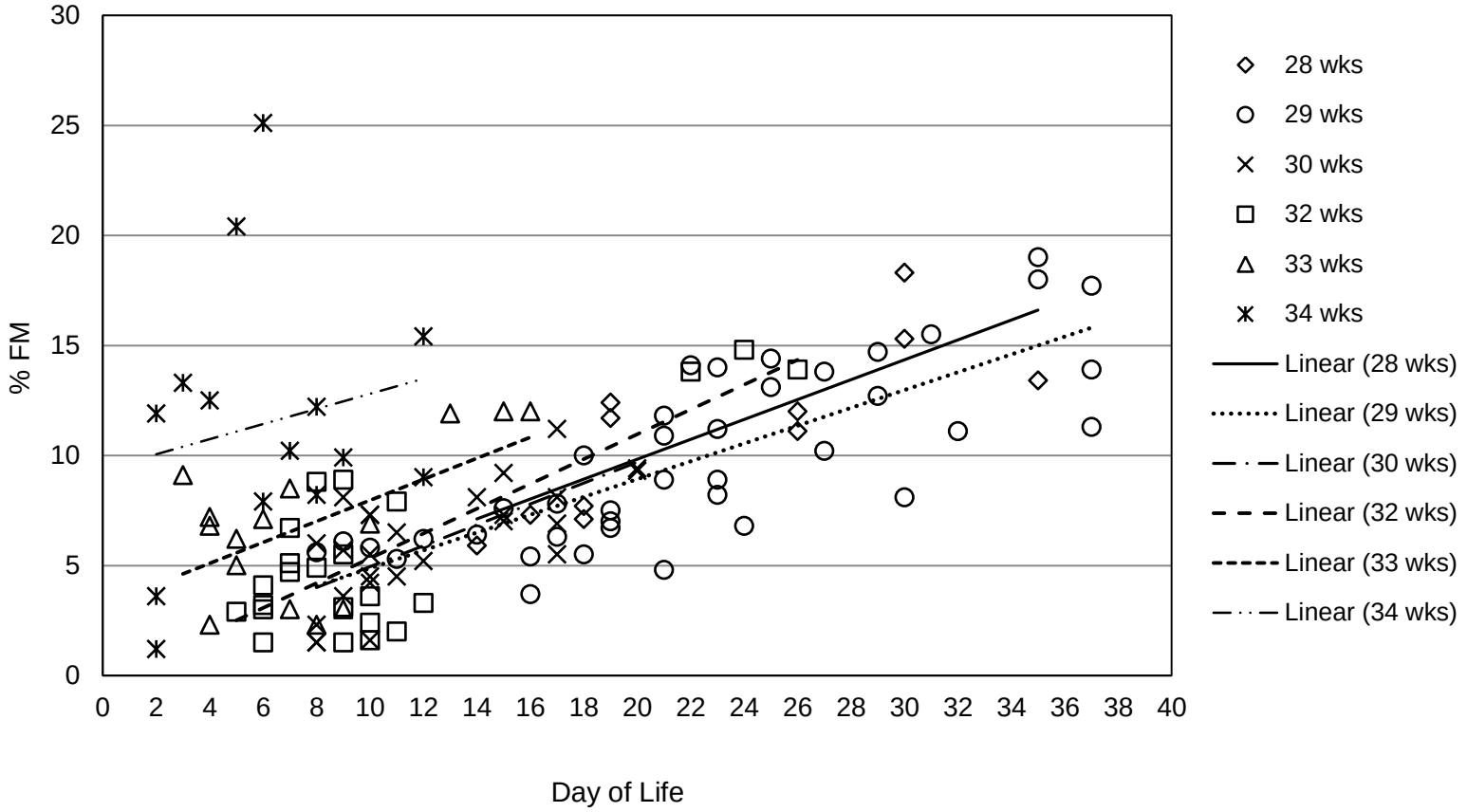

Figure 12. % FM of preterm infants grouped by their gestational age at birth and presented by day of life at time of test. SGA and LGA infants were excluded. Gestational age groups with 3 or less infants were also excluded.

Table 6. Regression equations for % FM of preterm infants grouped by gestational age at birth in Figure 12.

Gestational Age	Regression Equation and R^2 value	Change in %FM/day
28 weeks	y = 0.45x + 0.81 R^2 = 0.65	0.45
29 weeks	y = 0.40x + 0.84 R^2 = 0.62	0.40
30 weeks	y = 0.47x + 0.21 R^2 = 0.50	0.47
32 weeks	y = 0.56x - 0.30 R^2 = 0.64	0.56
33 weeks	y = 0.47x + 3.20 R^2 = 0.33	0.47
34 weeks	y = 0.34x + 9.38 R^2 = 0.03	0.34

Fat mass, FFM and %FM as a function of weight of preterm and term infants are shown in Figures 13-15. FM, FFM and % FM were positively and linearly related to body weight at time of test. Weight alone contributed 69% of the variance of FM, 97% of variance of FFM and 29% of variance to %FM.

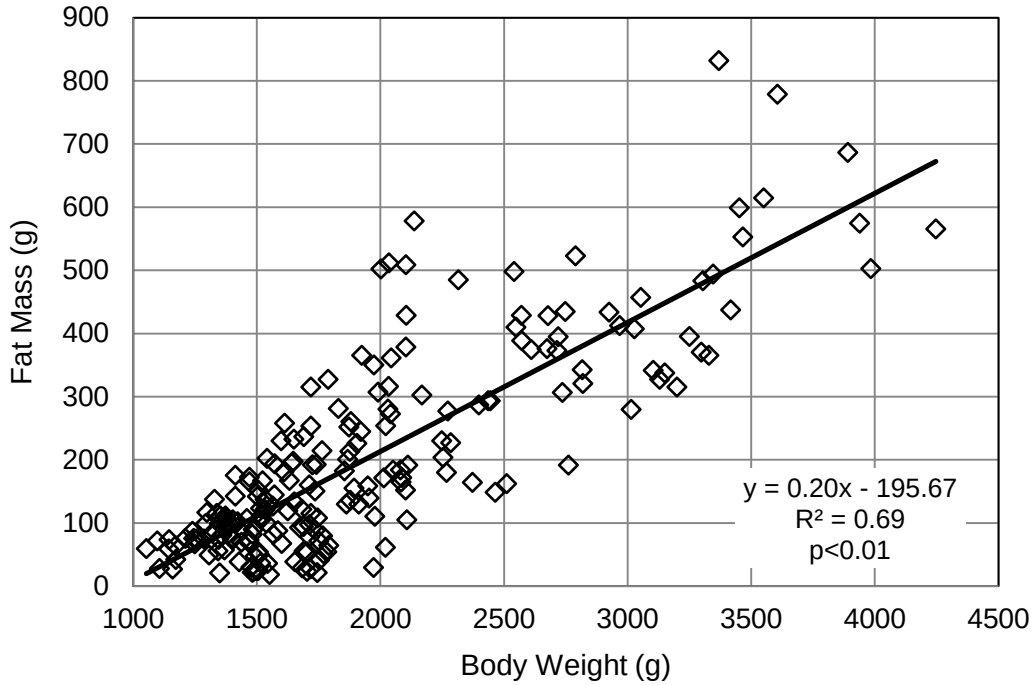

Figure 13. Individual values for whole body FM related to total body weight for 186 and 23 observations in preterm and term infants.

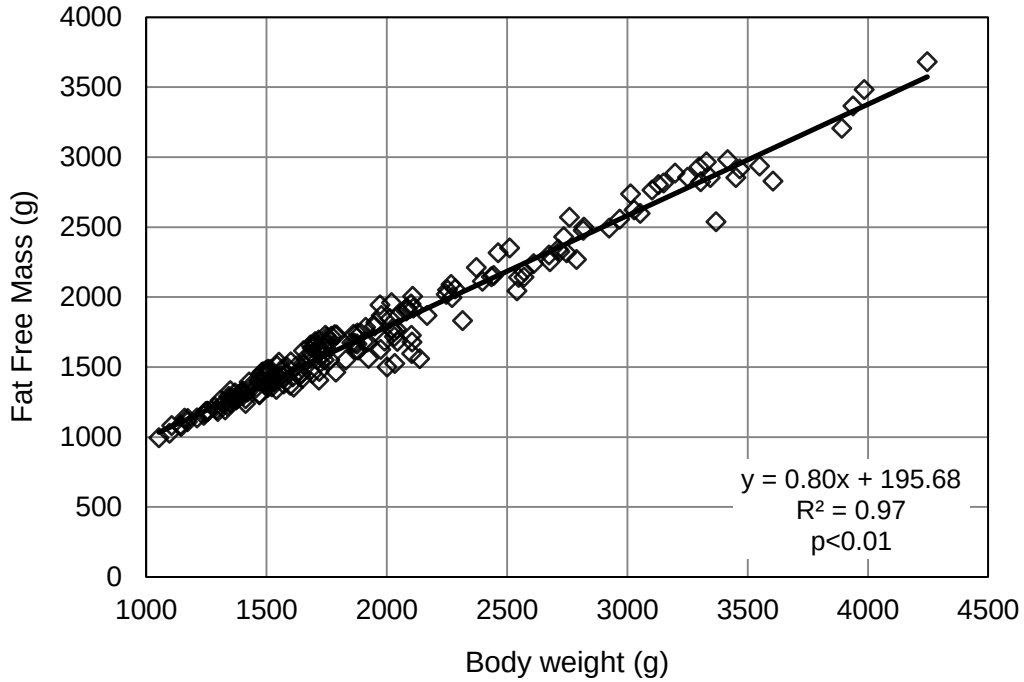

Figure 14. Individual values for whole body fat free mass related to total body weight for 186 and 23 observations in preterm and term infants, respectively.

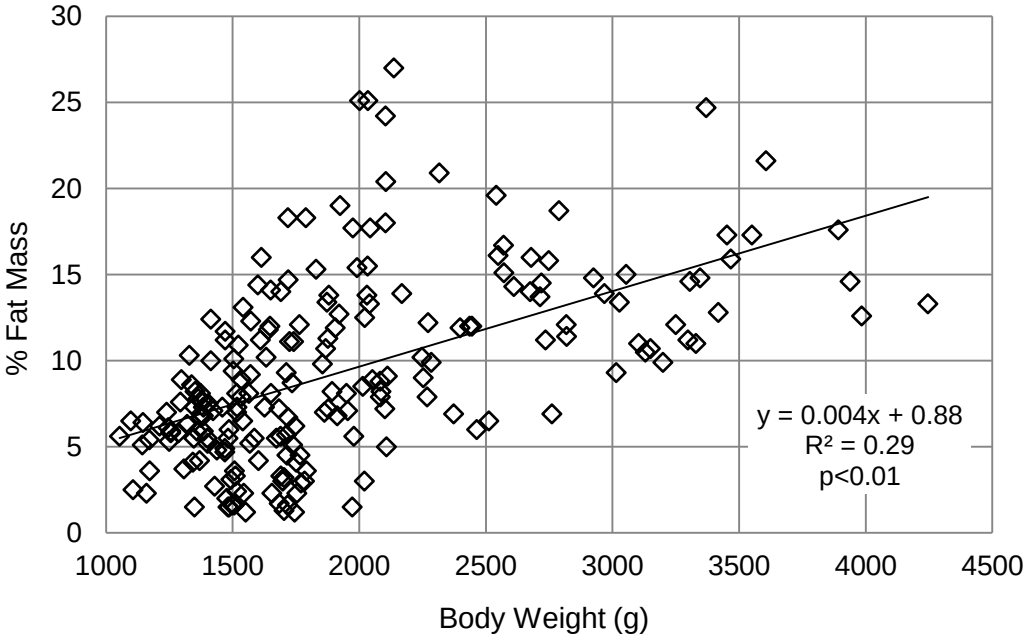

Figure 15. Individual values for %FM related to total body weight for 186 and 23 observations in preterm and term infants, respectively.

An alternative method to present body composition data is to adjust FM and FFM compartments for length to allow for independent evaluation of both compartments. Figures 16 and 17 show size adjusted development of FM and FFM compartments of the preterm infants included in this study; in both cases, FMI and FFMI were positively correlated with age at test.

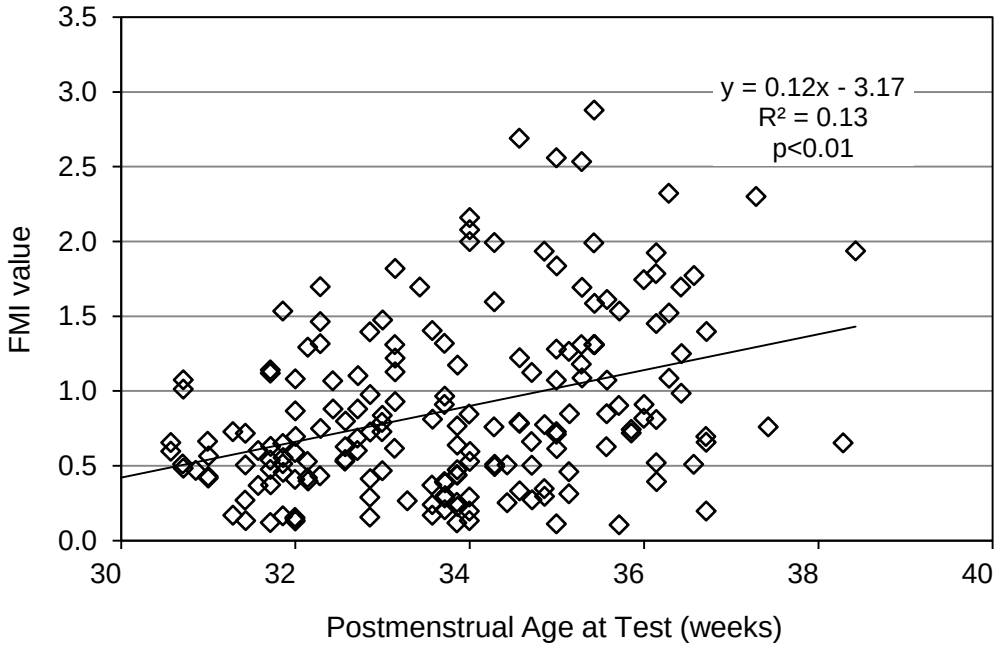

Figure 16. Fat mass index (FMI) of 186 observations preterm infants by postmenstrual age at test.

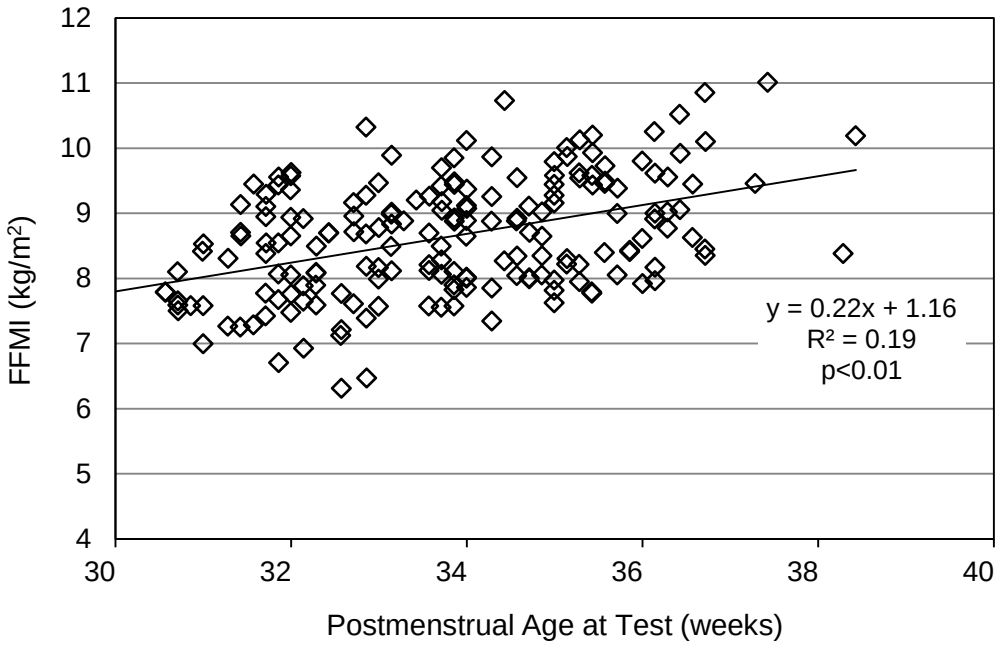

Figure 17. Fat free mass index of 186 observations of preterm infants by postmenstrual age at test.

4.6 Anthropometric Indices as a Proxy for Body Composition

Correlations between FM, %FM and BMI and PI in preterm infants are shown in Figure 18 and 19 and Table 6. The correlation coefficient for the relationship between FM and BMI was higher than that for the relationship between FM and PI (0.69 vs. 0.36). This was also the case for the relationship between % FM and BMI and PI (0.54 vs. 0.33).

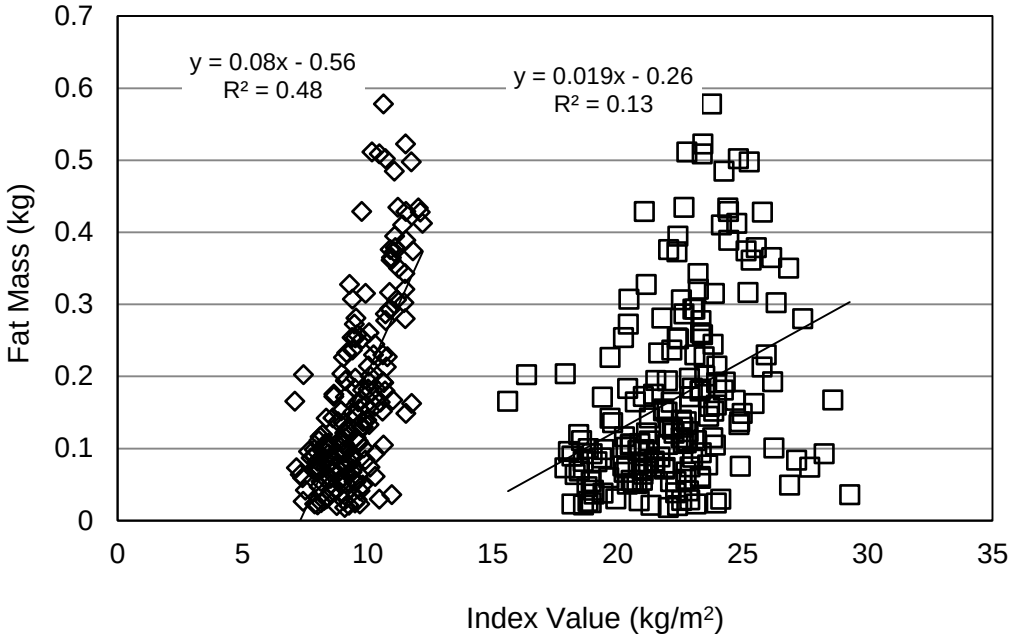

Figure 18. Correlation between whole body fat mass compartment (kg) and BMI (diamonds) and PI (squares) of 186 observations of preterm infants.

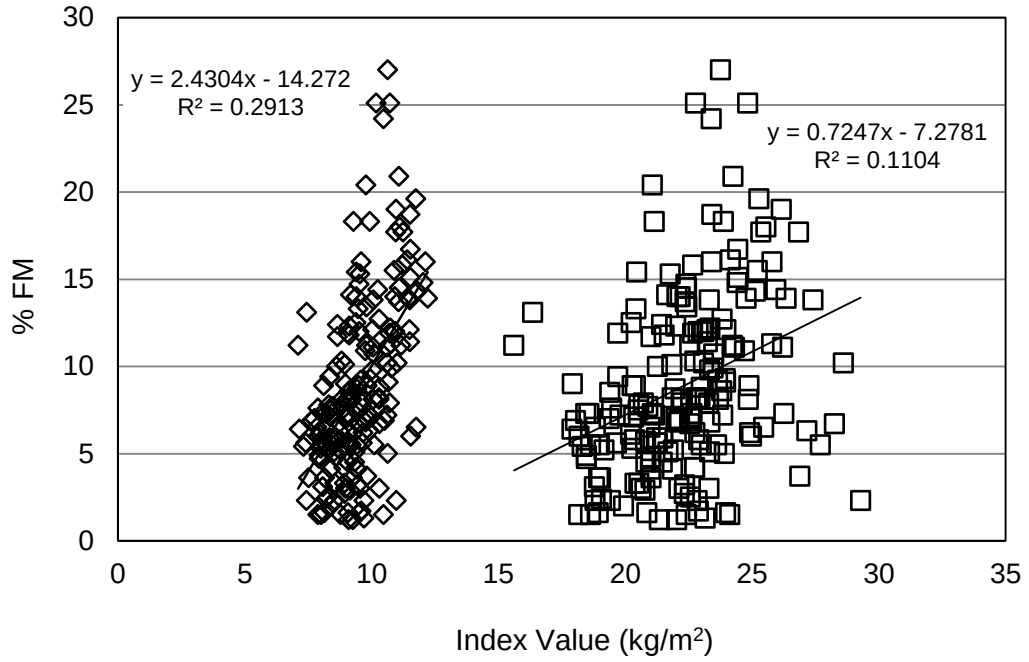

Figure 19. Correlation between %FM and BMI (diamonds) and PI (squares) of 186 observations of preterm infants.

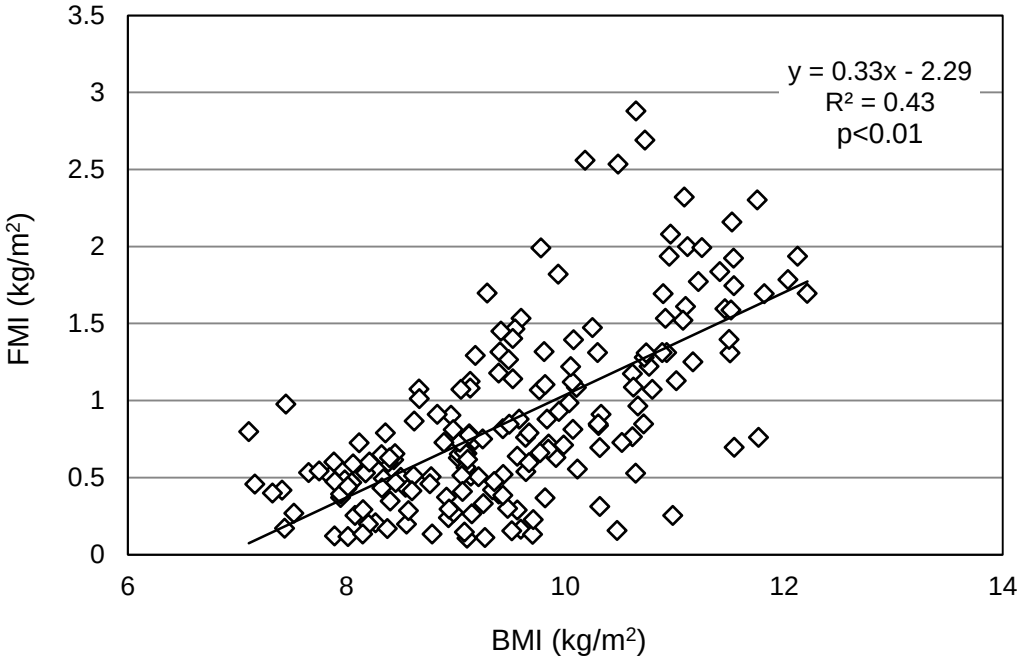

Figure 20. Correlation between FMI and BMI of 186 observations of preterm infants.

Table 7. Correlation coefficients, r square values and regression equations when % FM and FM was regressed on PI and BMI in 186 observations of preterm infants.

	Independent Variable	**R value**	**R^2 value**	**Regression equation**	**p**
% FM	BMI	0.54	0.30	% fat = 2.43(BMI) - 14.272	<0.01
	PI	0.33	0.11	% fat = 0.7247(PI) - 7.2781	<0.01
FM (g)	BMI	0.69	0.48	FM = 0.0761(BMI) - 0.5559	<0.01
	PI	0.36	0.13	FM = 0.0192(PI) - 0.2592	<0.01

5.0 Discussion

5.1 Overview

This section will begin with a discussion of the feasibility of the PEA POD and then the clinical observations in comparison with previous studies. Finally, I will present the strengths, limitations, implications of these results and possible future research that can be performed.

5.2 Method Development

This study was designed to introduce a new device, the PEA POD, into McMaster Children's Hospital and determine its feasibility as a clinical research and bedside tool. We showed that the PEA POD can be used routinely in a clinical setting to measure preterm infants at bedside. Support for this conclusion comes from the successful participation of 65 preterm infants, achieving over 180 measurements, no adverse events or parental complaints, and the precise and accurate QC test results gathered before each measurement.

Performing adjustments to the PEA POD door to accommodate one IV line were successful. Support for this conclusion comes from performing over 60 volume measurements of a known volume phantom, which produced accurate and precise results.

5.2.1 Door Adjustment Studies

The development of a method to measure infants with vascular access represents an entire population of infants that have never been measured before. Many preterm infants receive nutrition, fluids and medication intravenously during the early days of life which excludes them from eligibility for a PEA POD measurement since they need to be fully enclosed inside the volume test chamber. We were unable to lock or disconnect the IV line at any point, to avoid the risk of introducing infection to the infant. Therefore the successful results of these adjustments are an exciting next step towards gaining more knowledge of preterm infant growth.

The results from this investigation were produced in a controlled setting within a storage room without human infants involved. There is the possibility that the procedure and design of the door may need to be adjusted when real infants are being measured. It is also important to be mindful of tugging at the line as to not cause any discomfort for the infant. Another point to consider when measuring infants is the length of the IV line and how much of it will feed into the volume chamber. The additional weight and volume the lines introduce will need to be calibrated upon initiation of the infant measuring sequence. That being said, how much volume and weight of the IV line influences calibration and body composition measurement has yet to be examined.

5.3 Feasibility

Upon acquisition of the PEA POD, it was introduced and integrated into the NICU at McMaster Children's Hospital. This process required training of the research team, and introduction and education of the neonatal staff. I consider the 186 measurements in 65 preterm infants over the course of approximately 12 months obtained from this study as evidence to demonstrate that PEA POD can be used routinely at bedside in a clinical setting.

I have demonstrated that it is feasible to measure preterm infants at bedside in a clinical setting using the PEA POD at McMaster Children's Hospital; however, based on my experience and communication with other groups using the PEA POD in a similar setting, there are several adjustments that could be implemented in the future to enhance measurement efficiency and acquisition. Since members of the study team are not able to handle infants it would be beneficial to add a nurse to the study team who is trained on the device. The nurse would be responsible for handling the infant which would eliminate the need of the bedside nurses who are often overwhelmed and sometimes not willing to help perform a measurement. Although the PEA POD is housed on wheels, it is large, heavy and requires 2 individuals to move it to infant bedside. If space permitted it would be ideal to leave the PEA POD in a designated study room and bring infants to it. This procedure is done at other centres and would greatly reduce the time required for measurement.

Further evidence of the PEA POD's successful integration into the NICU and Level II nursery is its use for another study in the NICU which is running smoothly. I would expect that the PEA POD could easily be implemented in other NICUs and Level II nurseries.

One factor that influenced if the PEA POD can be used in a clinical setting was infant enrolment and parental experience. At the beginning of the study, consenting was slow. I suspect this to be mostly because of the associated learning curve of the new study coordinator to become comfortable with the study, speaking with parents and determining the best method to approach parents. The overall impression we received from parents was a positive one. We were never contacted by parents with complaints about the study or device.

Although there was a 47.6% drop out rate after successful consent, there are several possible explanations which are unique to this unit and this rate does not necessarily reflect normal circumstances. Our patient pool was mainly from the Level III NICU nursery which is where clinically unstable infants are admitted. Once these infants are stable, they are immediately transferred to a surrounding Level II nursery for the duration of time of care until they are discharged home. Two unique circumstances occurred that also interfered with measuring infants and prevented us from measuring infants we had already consented. Firstly, a technical issue with the door hinge allowed air leakage from the volume test chamber which led to a loss of approximately 8% of consented

infants. Sample size was comparable[63] or higher[64] than other published studies in older stable preterm infants using PEA POD.

5.4 Clinical Pilot Study: Comparison with Previous Studies

There is an overall increase in weight during hospital stay; FM and FFM accretion in preterm infants occurs at differing rates leading to an overall increase in %FM and subsequent decrease in %FFM during hospital stay. Indices of body size, BMI, PI, do not show good correlation of fatness in this population. FMI and FFMI are presented for preterm infants and show a positive linear association with PMA.

5.4.1 Preterm Body Composition

To our knowledge, this is the first to describe longitudinal body composition of preterm infants using PEA POD during hospitalization. In this study, preterm infants born AGA display a weight gain within the 10^{th}-50^{th} percentile of the reference birth weight growth chart from 30 to 36 weeks PMA. By comparing the slopes of the trend lines, SGA preterm infants appear to gain weight as the same rate as their AGA counterparts but on a lower weight trajectory and remain below the 10^{th} percentile of the reference birth weight chart from 33-36 weeks PMA.

Both FM and FFM compartments show a positive association with PMA. When the trend lines of the AGA and SGA preterm infants are compared it

appears that SGA preterm infants accrete FM as a slower rate than AGA preterm infants. This may be a result of it often being easier to meet the nutritional and energy requirements of larger infants, or a reflection of the effects of restricted growth in utero. In contrast, when trend lines of FFM of SGA and AGA preterm infants are compared, it appears that FFM is accreted at as similar rate. This relationship between FFM and FM accretion between SGA and AGA infants explains why we observed AGA preterm infants display greater rate of %FM increases than SGA preterm infants. In both cases FM and FFM are gained at differing rates leading to an overall increase in %FM. This observed result is to be expected as early infancy is generally associated with rapid FM accretion in all infant populations and infants are receiving high calorie diets.

%FM of each gestational age group at birth shows a positive linear relationship with day of life. When comparing the trend lines of these groups, they appear to have a very similar slope which would suggest that irrespective of gestational age at birth, these infants accrete %FM at the same rate. To our knowledge this relationship has not been documented in published literature, but demonstrates the need for additional investigation into preterm infant body composition across various populations and regions to determine if this is the usual occurrence. Preterm infants exhibit low %FM early in life which can be attributed to the fact that the third trimester of pregnancy is a period of rapid fat gain in utero which they miss.

Other than dated, unreliable chemical carcass analysis data, the only three comparable studies to the present one used DEXA to measure body composition. We found that values for FM, FFM and %FM were positively related to body weight at time of measurement. Lapillone *et al* showed that lean mass and FM increased exponentially in AGA infants between the ages of 32-41 weeks (Figure 2). Lean mass measurements were similar to the FFM compartment measured in this study (Figure 9) despite our model including the additional bone mineral content compartment. The reported FM from the Lapillone study was slightly higher than our findings (Figure 8), which may be related to the tendency of DEXA to overestimate FM and not applying the correction for it[54]. Comparison of our body composition data plotted by weight as per the Rigo *et al* study[58], to that of infants in the first two weeks of life (Figure1) demonstrate similar absolute amounts and trajectory of FM, FFM and %FM[58]. Our results show that FFM is highly linearly correlated with weight (R^2=0.97). The linear trend line of FFM tracks very closely to the calculated 50th percentile of lean body mass with the exception that our values are slightly higher to account for the inclusion of bone mineral content in this compartment, whereas with DEXA measures isolate lean mass from bone mass. Similarly, the linear trend line of our FM measurements were also very similar to the 50th percentile, except at body weights <1750g our data shows slightly higher FM values. The same is seen when comparing %FM, at the larger body weights our data are very similar to the presented 50th percentile (Figure 1), but at weights <1750g we measured higher %FM. Reasons for the

differences at smaller body weight may be a result of different methods used to measure body composition. The modelling used to create the percentiles will also produce differences from our presented raw data and trend lines. In another study[60], the linear trend line of global FM by body weight was y = -392 + 0.30x and had a similar R^2 value to our data (0.74 vs. 0.69). Despite the similarities in findings, displaying the data by body weight does not take into account the developmental stage of the infant. It may be the case that an older infant may have a different body composition to a younger infant of the same body weight. To our knowledge there are no comparable published works to the present study using the PEA POD.

Data from this study were in agreement with unpublished BC data of preterm and term infants using DEXA. In the previous study, preterm infant BC was assessed longitudinally when the infant had achieved full enteral feeds, at term, 3 months and 6 months corrected age; while term infants were assessed at term, 3 and 6 months postnatal age. The individual infants from this study tracked closely to the trajectory observed from the DEXA data (Appendix: Table 1), further establishing the usefulness of ADP for measuring preterm infant body composition.

It has been argued that body composition data should be presented such that FM and FFM compartments are adjusted for length against age. Presenting body composition data as FMI and FFMI allows independent analysis of FM and

FFM compartments[75]. This is in contrast to normalizing body compartments by weight to produce %FM and %FFM. This representation does not take into account the dependence of each compartment on each other. % body composition will not be able to distinguish between individuals with the same %FM due to having the same FFM but different FM, or the same FM but different FFM. Therefore our body composition data are represented as FMI and FFMI by age, and shows a positive relationship with PMA at test. No other published study has presented body composition of preterm infants by normalizing each compartment by length. This method of presentation of reference body composition needs further exploration.

5.4.2 Term body composition at birth

Several other studies have investigated body composition of healthy full term infants using PEA POD at time of birth. The term infants in this study had a mean %FM of 13.8% which is more similar to what has been previously reported by European groups[2,76] than with a similar American study[64]. Despite use of the same method, European groups found that %FM was 14.8%[2] and 11.9%[76], while the American study found % FM to be 18.7%[64]. It has been suggested that these differences may be a reflection of regional differences in maternal diet, behaviour and environment which are known to influence fetal growth.

5.4.3 Preterm vs. Term Body Composition

Previous studies have reported significant differences in body composition of preterm infants at term corrected age when compared with healthy full term infants at time of birth. Within our study, few preterm infants were measured at term corrected age, as they had already been discharged from our hospital. I extrapolated the linear trend line to estimate an approximate trajectory of growth to compare with the term infant population at birth.

The weight trajectory of AGA preterm infants would suggest that they will likely achieve a body weight similar to that of full term infants at term corrected age, in contrast SGA preterm infants would appear to fall below observed body weight of full term infants. Ramel *et al*[64] also found that between 40-42 weeks corrected age, weight between preterm and term infants were not different. This is in contrast to several studies that reported AGA preterm infants are smaller in weight at term corrected age[62]. These differences may be a result of differences in methods used to measure body composition or regional differences in nutrition and behaviours.

Interestingly, the %FM trajectory AGA preterm infants appear to be higher than the measured %FM of term infants, with a large cluster of individuals achieving a similar and in some cases higher %FM at 35-36 weeks PMA than term infants. Based on the trajectory of %FM, FM and FFM, I would speculate that AGA preterm infants actually display lower FFM and a similar FM

compartment to term infants at birth, rather than similar FFM compartment and higher FM compartment. SGA preterm infants appear to be on a slightly lower %FM trajectory than AGA preterm infants but appear to be on a trajectory to achieve %FM similar to term infants. These suggested findings support other studies which show that preterm infants do have a different body composition at term corrected age[64], including a recent meta-analysis which concluded that AGA preterm infants display a greater %FM as a function of reduced absolute FFM[62]. Additional studies are required to investigate this since reduced FFM gains provide an indication that organ, bone and brain growth may not be optimal.

It is a challenge to compare the results of our study with the findings of others due to the wide variation in inclusion criteria, ages of infants and methods used to measure body composition and lack of observational studies in AGA preterm infants. Some studies focus on SGA preterm infants, while others include infants based on preterm classification and mix SGA, AGA and LGA preterm infants despite differences in growth that they may introduce.

5.4.4 Anthropometric Indices of Body Size

There is increasing interest in body composition of infants, however, there are few easily accessible, accurate methods to do so. Therefore proxies of body fatness such as BMI and PI have been used in the past because of the ease of calculation. In both cases weight is adjusted for height to provide an indication of

body shape, which is thought to provide a better indication of nutritional status than weight alone.

This study provides interesting observations regarding the use of BMI and PI as a proxy for body composition. Both FM and %FM were correlated with BMI and PI, but do not present any predictive capability due to the small amount of variation accounted for by the relationship. PI is often considered a better indication of body shape than BMI for the infant population but results from this study do not support this.

It has been suggested that FMI should be compared with BMI to assess the relative agreement of how well BMI implies body composition rather than using absolute FM or %FM. Similar to Davies et al[77], FMI and BMI were correlated but only 43% of the variation was accounted for by the relationship between the two factors. These measures are poor indicators of FM in individuals and it has been suggested that they should be used as an abstract index of nutritional status not as a measure of body composition or degree of fatness. Infancy represents a time of rapid composition and length changes which may make BMI and PI a poor method for predicting FM. BMI has been used in adults under the general assumption that differences in values amongst individuals is a result of differences in the fat mass compartment. Therefore, if the changes in BMI or PI value are due to differences in length, FM and lean mass, the ability to predict fatness will be poor. It would be of great interest for clinicians to have an accurate proxy for

nutritional status on occasions where more sophisticated tools to measure body composition are not available, but due to the biological variation of the components of weight gain in infancy, this may not allow creation of a simple predictive equation using weight and length.

5.5 Strengths

This study was longitudinal with serial measures and designed to address an area of clinical research that is currently lacking. For the first time, PEA POD was used as a bedside method to measure preterm infants longitudinally during the early postnatal period. Based on its design and portability, the PEA POD provides a new opportunity to non-invasively measure small preterm infants. The longitudinal design of this study will provide a better understanding of the time course of body composition changes that occur during the early postnatal period. This study also provides the base of which future studies and analyses can be examined with a larger sample size.

5.6 Limitations

The main limitation to this study was sample size and inconsistent measurement time points. Although limited to 65 preterm infants, the sample size of this study is comparable to that of current studies in this population As we mentioned previously, the NICU at McMaster Children's Hospital admits mostly critically ill infants and once stabilized are transferred to a surrounding Level II nursery for continued care until discharge home – this significantly reduced the

number of longitudinal measurements and infants suitable for measurement. This practice may also have introduced a selection bias in our sample of infants. Infants who stayed in hospital are likely those who needed additional care and therefore may not represent our goal population of 'healthy' preterm infants. These infants will have more measurements than other infants and may influence our results by being over represented in our analysis.

Several factors that we were unable to control interfered with performing measurements. We experienced a technical issue with the PEA POD which required support from COSMED to fix. Additionally, construction in our nurseries interfered with the environmental stability needed for accurate PEA POD measurement and forced the entire Level II nursery to be re-located another floor in the hospital. Despite this, the number of preterm infants included in this study is on par and in some cases greater than studies of this population.

As a single centre study, the sample of infants measured may not be representative of the general preterm infant population. The growth and resulting body composition of these infants will be influenced by nutritional management protocol at McMaster Children's Hospital.

A data set of this nature presents the challenge of analyzing correlated data which requires specialized statistical tests to account for the correlation. This data set included repeated measures of infants which could result in the underestimation of P values and increasing the chances of observing a significant

effect. In some cases it is appropriate to remove correlations from the data set but in this case, it was not possible as it would lead to a significant loss of information[78]. Additionally, the preterm group included in this study was varied, including singletons, twins and triplets, SGA and LGA infants. Nutrition and maternal characteristics were not adjusted for.

The purpose of this pilot study was to assess feasibility and report the longitudinal, observational body composition data from preterm infants. There was no expected body composition differences expected between infants or between time points. This hindered sample size calculation to be performed for this study. Instead we used a sample size of convenience; all consented infants were included in this study. The sample size and inconsistency of measurement time points did not allow for more sophisticated statistical analyses to be performed.

5.7 Implications

This work has provided the foundation for further investigation into the quality of growth of preterm and term infants at McMaster Children's Hospital. This is the first step in developing normative body composition data of preterm infants during the early postnatal period. With additional contributions, these data may present as an additional clinical tool that can used by clinicians working with these infants for better assessment of nutritional status and quality of growth. Knowledge of longitudinal changes in body composition of preterm infants will

assist in establishing nutritional guidelines and regimens to support an appropriate body composition for optimal health outcomes. Additionally, the ability to measure body composition at bedside presents the opportunity to optimize NICU release criteria beyond achieving a specified weight. With more information on health status and of the infant before discharge, post-discharge nutrition can also be tailored to the child to promote healthy growth at home.

With the adjustments that can be made to the device to measure infants with IV lines, we would be able to measure a population that has never been investigated before. This opens the doors to measuring infants soon after birth to gain a better understanding of how preterm infants accrete FM and FFM in utero, but also how these compartments change with medical care and provision of nutrients. This would also allow the measurement of younger preterm infants that still require vascular access support.

5.8 Future Work

This work should be continued as data of this population during this time period are lacking in literature. This study will be continued, adding to the robustness of the sample size to gain better insight into the body composition changes of preterm infants during the first weeks of life. A multi-level model can then be applied to the data set to produce a model of growth of FM, FFM and %FM compartments. It would also be beneficial to find collaboration with other groups measuring the same population using PEA POD. Combining the data from

different centres would allow growth trajectories to be applied to a much broader population and reduce the bias introduced from being a single-centre study.

As mentioned above, one of the limitations of the PEA POD is that infants need to be stable off respiratory support and cannot have any vascular accesses. With the goal of measuring preterm infants early in life, this is a major obstacle to obtaining these measurements. Although we have started the process of adjusting the door to accommodate a single IV line, additional work needs to be done to accommodate additional lines since most infants will have more than one IV line. The NICU is introducing new IV lines into the unit which should be used for testing. We began initial testing of multiple IV lines but additional testing is required. Accommodating 2 lines appears promising, but 3 lines does not produce accurate, precise or reproducible results. Additional areas to explore would be use of a different foam gasket and adjustment of the door hinge to allow additional space to accommodate multiple IV lines.

Now that the PEA POD has been successfully integrated into the NICU, it is possible to expand upon the initial objective of this study. Since early postnatal growth can have significant effects on later health outcomes, future studies aimed at optimizing nutritional regimes for preterm infants should assess body composition beyond hospital stay, into childhood and beyond. It would also be of interest to relate neurodevelopmental outcomes at follow-up measurements with body composition information. Future studies with larger sample size would allow

the sub-group analysis of preterm infants by somatic classification, if they were a single or multiple gestations infant and link to maternal habits and characteristics.

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Appendix

Table 1. Volume testing of foam ring alone

Volume QC									
Vol 1	Vol 2	Vol 3	Mean Vol	Mean Pass	SD	SD pass	Mean 1	Mean2	Comment
3.0277	3.029	3.03	3.0304	FAIL	0.0037	FAIL	3.0341	3.0268	Foam ring, metal phantom (first test of day)
3.0291	3.028	3.03	3.0293	PASS	0.0015	PASS	3.0317	3.027	Foam ring, metal phantom

Autorun QC											
Vol 1	Vol 2	Vol 3	Vol 4	Vol 5	Vol 6	Mean L	SD (mL)	Min (L)	Max (L)	SD Pass	Comment
3.029	3.029	3.032	3.029	3.030	3.03	3.031	1.7	3.029	3.033	PASS	Foam ring, metal phantom
3.025	3.025	3.024	3.024	3.027	3.024	3.025	1.4	3.024	3.028	PASS	Foam ring metal phantom
2.327	2.324	2.323	2.326	2.325	2.326	2.326	1.5	2.324	2.328	PASS	Foam ring, water bottle phantom
2.318	2.320	2.322	2.323	2.321	2.325	2.322	2.3	2.319	2.325	PASS	Foam ring, water bottle phantom
2.323	2.322	2.322	2.324	2.324	2.324	2.324	0.9	2.322	2.325	PASS	Foam ring, water bottle phantom

Table 2. Volume testing with foam ring and IV line

Volume QC									
Vol 1	**Vol 2**	**Vol 3**	**Mean Vol**	**Mean Pass**	**SD**	**SD pass**	**Mean 1**	**Mean2**	**Comment**
2.3243	2.3304	2.331	2.3286	FAIL	0.0037	FAIL	2.3494	2.3077	Original door, water bottle phantom
3.0276	3.0263	3.026	3.0266	PASS	0.0008	PASS	3.0282	3.0251	Original door, metal phantom
2.323	2.322	2.3261	2.3237	FAIL	0.0021	PASS	2.3243	2.3232	Original door, water bottle phantom
2.3221	2.3208	2.3238	2.3222	FAIL	0.0015	PASS	2.3205	2.324	New door, cut foam, IV line (cut at end of knot so no extra line hanging inside chamber), water bottle phantom
2.3193	2.3179	2.32	2.3191	FAIL	0.0011	PASS	2.319	2.3192	New door, cut foam, IV line (cut at end of knot so no extra line hanging inside chamber), water bottle phantom
3.029	3.0283	3.0274	3.0283	PASS	0.0008	PASS	3.0277	3.0288	New door, cut foam, no IV or anything wedged in slit, metal phantom
3.0251	3.0277	3.0253	3.026	PASS	0.0015	PASS	3.0237	3.0284	No IV – daily QC before testing

Autorun QC											
Vol 1	**Vol 2**	**Vol 3**	**Vol 4**	**Vol 5**	**Vol 6**	**Mean (L)**	**SD (mL)**	**Min (L)**	**Max (L)**	**SD Pass**	**Comment**
2.321	2.323	2.322	2.320	2.323	2.324	2.323	1.3	2.321	2.324	PASS	Original door, water bottle phantom
2.318	2.322	2.320	2.324	2.321	2.322	2.322	2.1	2.319	2.325	PASS	Original door, water bottle

											phantom
2.315	2.316	2.318	2.318	2.318	2.318	2.318	1.2	2.316	2.319	PASS	New door, cut foam, IV line (cut at end of knot so no extra line hanging inside chamber), water bottle phantom
2.325	2.325	2.326	2.323	2.324	2.323	2.325	1.3	2.323	2.327	PASS	New door, cut foam, IV line (cut at end of knot so no extra line hanging inside chamber), water bottle phantom
2.323	2.321	2.323	2.324	2.325	2.324	2.324	1.4	2.321	2.325	PASS	New door, cut foam, IV line (cut at end of knot so no extra line hanging inside chamber), water bottle phantom
3.024	3.026	3.029	3.028	3.030	3.028	3.028	2.3	3.024	3.031	PASS	New door, cut foam, IV line (cut at end of knot so no extra line hanging inside chamber), metal phantom
3.028	3.026	3.027	3.027	3.029	3.029	3.028	1.1	3.02	3.029	PASS	New door, cut foam, IV line (long line hanging inside chamber), metal phantom
3.025	3.028	3.028	3.027	3.027	3.027	3.027	1.1	3.025	3.028	PASS	New door, cut foam, no IV or anything wedged in slit, metal phantom
3.025	3.024	3.023	3.022	3.025	3.024	3.024	1	3.023	3.026	PASS	New door, cut foam, no IV or anything wedged in slit, metal phantom
3.025	3.022	3.027	3.027	3.023	3.029	3.027	2.1	3.023	3.029	PASS	New door, cut foam, no IV or anything wedged in slit, metal phantom

Table 3. Volume testing with varying calibration procedures.

Vol1	Vol2	Vol3	Mean Vol	Mean Pass	ST DEV	SD Pass	Mean 1	Mean 2	Comment/Configuration
3.0251	3.0277	3.0253	3.026	PASS	0.0015	PASS	3.0237	3.0284	No IV – daily QC before testing
2.9696	2.974	2.9715	2.9717	FAIL	0.0022	PASS	2.9715	2.9719	1
3.0243	3.0228	3.0259	3.0243	PASS	0.0016	PASS	3.0238	3.0249	3
2.9712	2.9705	2.9712	2.971	FAIL	0.0004	PASS	2.9718	2.9701	2
2.9679	2.9657	2.9674	2.967	FAIL	0.0011	PASS	2.9692	2.9648	2
2.9664	2.971	2.9708	2.9694	FAIL	0.0026	PASS	2.9709	2.9679	1
3.0209	3.0191	3.0201	3.0201	PASS	0.0009	PASS	3.0154	3.0247	3
3.0239	3.0232	3.0237	3.0236	PASS	0.0003	PASS	3.0233	3.0239	3
2.9687	2.9698	2.9715	2.97	FAIL	0.0014	PASS	2.9701	2.9699	1
2.9759	2.9777	2.977	2.9769	FAIL	0.0009	PASS	2.9779	2.9758	1

2.9724	2.9724	2.9709	2.9719	FAIL	0.0008	PASS	2.9697	2.9741	2
2.9764	2.9766	2.9787	2.9772	FAIL	0.0013	PASS	2.9837	2.9707	2
3.0229	3.0219	3.0213	3.0221	PASS	0.0008	PASS	3.024	3.0201	3
3.0271	3.0268	3.0285	3.0275	PASS	0.0009	PASS	3.0269	3.0281	3
3.0033	3.0027	3.0002	3.0021	FAIL	0.0017	PASS	2.9786	3.0254	1
2.9783	2.9797	3.0184	2.9921	FAIL	0.0227	FAIL	2.9904	2.9938	1
2.9732	2.9741	2.9756	2.9743	FAIL	0.0012	PASS	2.976	2.9726	2
2.9748	2.9715	2.9793	2.9752	FAIL	0.0039	FAIL	2.9763	2.9742	2
3.0106	3.0069	3.0117	3.0097	FAIL	0.0025	PASS	3.0271	2.9923	3*
3.0196	3.0249	3.0209	3.0218	PASS	0.0028	PASS	3.0217	3.0219	3
3.0275	3.0277	3.028	3.0277	PASS	0.0002	PASS	3.0317	3.0238	3
3.0239	3.0233	3.0243	3.0238	PASS	0.0005	PASS	3.0244	3.0232	3
2.9971	3.0003	3.0028	3.0001	FAIL	0.0029	PASS	2.9766	3.0234	1

3.0344	2.9977	2.9961	3.0094	FAIL	0.0217	FAIL	2.9847	3.034	1
2.9955	2.9991	3.0017	2.9988	FAIL	0.0031	FAIL	2.98	3.0175	2
3.0081	3.0072	3.0076	3.0076	FAIL	0.0004	PASS	3.0234	2.9918	3*
2.9799	2.9824	2.9864	2.9829	FAIL	0.0033	FAIL	2.9829	2.9828	2
3.0222	3.026	3.0248	3.0243	PASS	0.002	PASS	3.025	3.0237	No IV – daily QC
2.9826	2.981	2.9854	2.983	FAIL	0.0022	PASS	2.9854	2.9806	2
2.9829	2.9876	2.9912	2.9872	FAIL	0.0042	FAIL	2.986	2.9885	1
2.9775	2.9809	2.9817	2.98	FAIL	0.0022	PASS	2.9798	2.9803	2
2.9859	2.987	2.987	2.9866	FAIL	0.0007	PASS	2.9848	2.9885	1
3.0256	3.0253	3.0278	3.0262	PASS	0.0014	PASS	3.0264	3.026	3

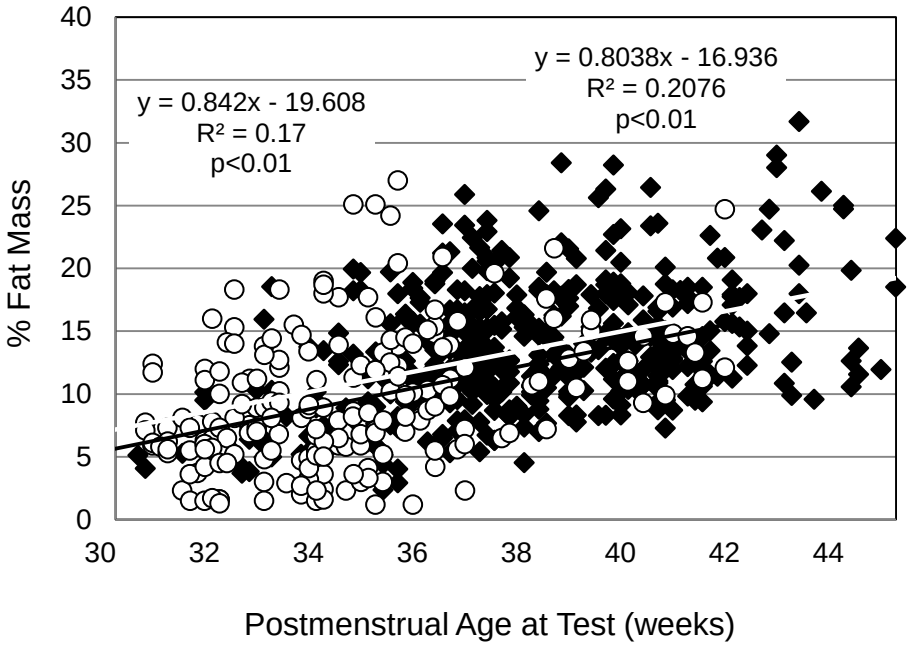

Figure 1. Percentage fat mass results of the present study (circles) and previous data set using DEXA (solid diamond) of preterm and term infants.