Body Composition Assessment of Premature Infants in the Neonatal Intensive Care Unit

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ABSTRACT

Premature infants experience growth alterations that place them at risk for adverse metabolic and neurodevelopmental outcomes. Monitoring the quality of weight gain through body composition assessment in the neonatal intensive care unit (NICU) may help clinicians gauge the response to nutritional provision and guide future interventions that promote adequate growth and neurodevelopment while reducing the risk for obesity and metabolic disease.

While length and weight are regularly tracked during an infant's NICU stay, these measurements do not adequately represent total body adiposity shortly after birth. Thus, a method of body composition which is non-invasive, portable, and able to be frequently utilized in both critically ill and medically stable infants is desirable. Unfortunately, many current methods of body composition are invasive, expensive, involve ionizing radiation, or are unsuitable for repeated measurement in a medically fragile infant. Thus, this dissertation project explores methods to monitor body composition in premature infants in the NICU setting with a focus on ultrasound.

The first study explored the ability of weight for length indices of the body to serve as proxies for adiposity in preterm infants. Indices examined include weight for length (W/L), body mass index (BMI), and ponderal index (PI). Each index was examined for its ability to predict fat mass (FM), fat-free mass (FFM), and percent body fat (%BF). None of the indices adequately reflected adiposity in preterm infants, indicating that assessing body composition in preterm infants requires more than weight and length measurements, and other methods of bedside assessment should be pursued.

The second study examined the ability of ultrasound to assess body composition in premature infants in the NICU setting. Ultrasound images of the biceps, abdomen, and quadriceps were obtained for assessment of adipose and muscle thickness and were compared with body composition measurements (FFM, FM, %BF) taken using air displacement plethysmography (ADP). While ultrasound measurements of biceps and quadriceps muscle thickness correlated with total FFM, ultrasound measurements were not included in final models for predicting FFM. Biceps, abdomen, and quadriceps

adipose thickness correlated with total FM and %BF, but only biceps adipose was selected in the final model predicting %BF. The sum of ultrasound adipose thickness measures was selected for the final model predicting %BF. However, all models had low predictive ability due to low proportion of variance explained (R^2) and/or high prediction error (root mean square error, RMSE).

While the study conducted here does not support the use of ultrasound measurements of adipose and muscle thickness of the biceps, abdomen, or quadriceps alone to predict body composition in preterm infants, exploration of additional sites or cross-sectional area may improve predictive ability. Additionally, ultrasound measurements may have some value as a prognostic tool for other clinical outcomes, such as neurodevelopment or readiness for NICU discharge. Regardless, this work highlights the need for clinical body composition methods appropriate for premature infants to help monitor for disease risk and assist in the refinement of current nutrition practices in the NICU.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ..... **i**

ABSTRACT ..... **ii**

LIST OF TABLES ..... **vi**

LIST OF FIGURES ..... **viii**

LIST OF ABBREVIATIONS ..... **ix**

CHAPTER 1: INTRODUCTION **1**

CHAPTER 2: LITERATURE REVIEW – CLINICAL APPLICATION OF BODY COMPOSITION METHODS IN PREMATURE INFANTS **4**

CHAPTER SYNOPSIS 5

INTRODUCTION 6

Challenges to assessing body composition in the NICU 8

Air displacement plethysmography (ADP) 9

Bioelectrical impedance analysis (BIA) 10

Skinfold Measurements 12

Ultrasound 14

DISCUSSION 15

CHAPTER 3: ANTHROPOMETRIC MEASURES DO NOT ADEQUATELY REFLECT ADIPOSITY IN PRETERM INFANTS FROM 30-63 WEEKS CORRECTED GESTATIONAL AGE **22**

CHAPTER SYNOPSIS 23

INTRODUCTION 24

METHODS 25

Statistical Analysis 26

RESULTS 27

DISCUSSION 38

ACKNOWLEDGEMENTS 40

CHAPTER 4: ULTRASOUND MEASUREMENTS OF MUSCLE AND ADIPOSE TISSUE THICKNESS ARE NOT SURROGATES FOR WHOLE BODY COMPOSITION IN PREMATURE INFANTS **42**

CHAPTER SYNOPSIS 43

INTRODUCTION 44

METHODS 45

Statistical Analysis 47

RESULTS 48
Participant Characteristics 48
Reliability 49
US measurements of muscle thickness and whole body FFM 50
US measurements of adipose tissue thickness and whole body FM and %BF 50
Sensitivity Analysis 51
DISCUSSION 52
ACKNOWLEDGEMENTS 55
CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS 62
CONCLUSIONS 63
FUTURE DIRECTIONS 64
Reliability of ultrasound measurements and longitudinal measurements 64
Ability of ultrasound measurements of muscle thickness to predict neurodevelopmental outcomes 64
Ability of ultrasound measurements to predict metabolic outcomes and corrected age at discharge from the NICU 64
Preliminary Data 65
REFERENCES 69

LIST OF TABLES

CHAPTER 2: LITERATURE REVIEW – CLINICAL APPLICATION OF BODY COMPOSITION METHODS IN PREMATURE INFANTS 4

Table 2.1: Precision and Accuracy of Body Composition Methods in Infants 17

Table 2.2: Evaluation of Body Composition Methods for Bedside Assessment of Preterm Infants 21

CHAPTER 3: ANTHROPOMETRIC MEASURES DO NOT ADEQUATELY REFLECT ADIPOSITY IN PRETERM INFANTS FROM 30-63 WEEKS CORRECTED GESTATIONAL AGE 22

Table 3.1: Infant descriptive characteristics and body composition by corrected age at measurement 30

Table 3.2: Pearson's correlation coefficients for predictors and outcomes in 260 preterm infants by corrected gestational age at measurement 31

Table 3.3: R^2 and root MSE for prediction of body composition by weight/length indices in preterm infants by corrected gestational age 32

Table 3.4: Relationship between predictors and FMI and FFMI in preterm infants by CGA group 35

Table 3.5: Relationship between W/L indices and %BF by GA at birth and sex in 260 preterm infants <32 weeks vs ≥ 32 weeks GA at birth 36

Table 3.6: Relationship between W/L indices and %BF by gestational age and sex in term infants versus preterm infants at term-corrected age 37

CHAPTER 4: ULTRASOUND MEASUREMENTS OF MUSCLE AND ADIPOSE TISSUE THICKNESS ARE NOT SURROGATES FOR WHOLE BODY COMPOSITION IN PREMATURE INFANTS 42

Table 4.1: Descriptive characteristics of preterm infants (n=63) 49

Table 4.2: Intra-rater reliability of ultrasound measurements 49

Table 4.3: Pearson's correlation coefficients for US measurements of muscle thickness and whole body FFM 50

Table 4.4: Pearson's correlation coefficients for US 51

Table 4.5: Association of individual US measurements of adipose tissue thickness with FM and %BF in 63 preterm infants measured at mean corrected age of 35 weeks 56

Table 4.6: Association of individual US measurements of muscle thickness with FFM in 63 preterm infants measured at mean corrected age of 35 weeks 57

Table 4.7: Stepwise regression analysis for prediction of FFM from US measurements of muscle thickness and USM and weight, length, and GA in 63 preterm infants at mean corrected age of 35 weeks 58

Table 4.8: Stepwise regression analysis for prediction of FM from US measurements of adipose tissue thickness and USM, weight, length, and GA in 63 preterm infants at mean corrected age of 35 weeks 59

Table 4.9: Stepwise regression analysis for prediction of %BF from US measurements of adipose tissue thickness and USA and GA in 63 preterm infants at mean corrected age of 35 weeks 60

Supplemental Table 4.1: Sensitivity Analysis: stepwise regression analysis for prediction of FM and %BF from the sum of US measurements of adipose tissue thickness, CGA, and length in 63 preterm infants at mean corrected age of 35 weeks 61

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS 62

Table 5.1: Stepwise regression analysis for prediction of corrected age at discharge from US measurements of muscle thickness and USM, GA, weight, and length in 47 preterm infants at mean corrected age of 35 weeks 67

Table 5.2: Stepwise regression analysis for prediction of corrected age at discharge from US measurements of adipose thickness and GA, CGA, and weight in 47 preterm infants at mean corrected age of 35 weeks 68

REFERENCES 69

LIST OF FIGURES

CHAPTER 3: ANTHROPOMETRIC MEASURES DO NOT ADEQUATELY REFLECT ADIPOSITY IN PRETERM INFANTS FROM 30-63 WEEKS CORRECTED GESTATIONAL AGE 22

Figure 3.1 Scatterplot of the relationship between BMI and %BF in 74 preterm infants measured between 28-33 weeks corrected gestational age. 33

Figure 3.2: Scatterplot of the relationship between BMI and %BF in 175 preterm infants measured between 34-36 weeks corrected gestational age. 33

Figure 3.3 Scatterplot of the relationship between BMI and %BF in 113 preterm infants measured between 37-42 weeks corrected gestational age. 34

Figure 3.4 Scatterplot of the relationship between WL and %BF in 111 preterm infants measured between 43-63 weeks corrected gestational age. 34

CHAPTER 4: ULTRASOUND MEASUREMENTS OF MUSCLE AND ADIPOSE TISSUE THICKNESS ARE NOT SURROGATES FOR WHOLE BODY COMPOSITION IN PREMATURE INFANTS 42

Figure 4.1: Ultrasound images of the biceps, abdomen, and quadriceps 47

LIST OF ABBREVIATIONS

%BF: percent body fat

AA: abdominal adipose

ADP: air displacement plethysmography

AIC: Akaike information criterion

AICC: Akaike information criterion corrected

ASPEN: American Society for Parenteral and Enteral Nutrition

BA: biceps adipose

BIS: bioimpedance Spectroscopy

BMI: body mass index

CGA: corrected gestational age

CI: confidence interval

CV%: coefficient of variation (percent)

DXA: dual-energy x-ray absorptiometry

ECW: extracellular water

FM: fat mass

FFM: fat-free mass

GA: gestational age at birth

ICC: intraclass correlation coefficient

ICW: intracellular water

IR: impedance ratio

LOA: limits of agreement

MF-BIA: multifrequency bioimpedance analysis

MRI: magnetic resonance imaging

NICU: neonatal intensive care unit

QA: quadriceps adipose

RMSE: root mean square error

SD: standard deviation

SF-BIA: single-frequency bioimpedance analysis

SFT: skinfold thickness

TEM: technical error of measurement

US: ultrasound

USA: sum of ultrasound adipose measurements

USM: sum of ultrasound muscle measurements

CHAPTER 1: INTRODUCTION

In 2017, approximately 10% of babies born in the Unites States were born prematurely, and in 2016, 17% of infant deaths were due to prematurity and low birthweight.[1] Prematurity places infants at risk for a myriad of growth, metabolic, and neurodevelopmental problems. This may be due, in part, to growth alternations that premature infants exhibit, including increased adiposity, decreased fat-free mass (FFM) gains, and decreased linear growth for the first 2 years of life in comparison to term infants. Weight and length are currently used to evaluate growth and adequacy of nutritional provision in the neonatal intensive care unit (NICU), but growing evidence suggests that monitoring weight quality is valuable in prevention of adverse metabolic and neurodevelopmental outcomes. Weight quality can be monitored by assessing body composition or gains in the amount of FFM and fat mass (FM). Unfortunately, very few body composition methods are easily used in the NICU setting.

The goal of nutritional provision in the NICU is to promote growth similar to that of term infants.[2] However, aggressive nutrition may promote increased gains in FM, placing infants at risk for metabolic consequences. Individualized nutritional recommendations or the benefits of “early aggressive nutrition” are debated amongst clinicians, and it is known that composition of diet can affect body composition in premature infants.

This dissertation explores the state of body composition analysis in premature infants in the NICU, beginning with a literature review of the available clinical body composition methods for premature infants in Chapter 2 and continuing with an assessment of the ability of weight for length indices to serve as proxies for body composition from 30-63 weeks corrected age in Chapter 3. Chapter 4 contains results from a study examining the use of ultrasound to assess body composition in the NICU.

In Chapter 5, conclusions from this work and future directions are presented along with preliminary data from continuing studies.

CHAPTER 2: LITERATURE REVIEW – CLINICAL APPLICATION OF BODY COMPOSITION METHODS IN PREMATURE INFANTS

***** This chapter has been submitted and is under review for publication at the *Journal of Parenteral and Enteral Nutrition*

CHAPTER SYNOPSIS

Currently, body composition assessment is not part of the routine clinical evaluation of premature infants, a population at risk for obesity and metabolic disease. Instead, measures of weight and length are used to assess growth in the neonatal intensive care unit but are known to be poor predictors of adiposity shortly after birth. Monitoring whole body composition (total body fat mass and fat-free mass) in preterm infants is essential for optimizing nutrition and promoting growth and neurodevelopment while preventing adverse metabolic outcomes. Therefore, a method which allows clinicians to track whole body composition during hospitalization is desirable. While body composition methods such as magnetic resonance imaging, stable isotope dilution, and dual energy x-ray absorptiometry have been examined in infants, they are invasive, expensive, involve exposure to radiation, and/or are unsuitable for repeated measurements in a medically fragile population. Several body composition methods with potential for clinical use at the bedside have been explored in premature infants, including air displacement plethysmography, bioimpedance, skinfold measurements, and ultrasound. In this review, we examine each method and evaluate feasibility for incorporation into clinical care. While these methods show promise for use in premature infants, further research is needed before they can be recommended for routine body composition measurement in the clinical setting.

INTRODUCTION

Altered growth in premature infants

Premature infants exhibit growth irregularities that may place them at increased risk for obesity and metabolic disease such as decreased fat-free mass (FFM) gains during infancy, increased overall adiposity compared to full term infants at term corrected age, and decreased linear growth for the first two years of life.[3–5] They also undergo an initial period of significant growth restriction followed by a period of "catch-up growth" or accelerated weight gain, resulting in a greater proportion of fat mass (FM) to FFM.[3,5–7] These growth abnormalities are concerning due to correlation with adverse metabolic and neurodevelopmental outcomes later in life.[8,9] Currently, clinicians use anthropometric measurements such as weight and length to track growth and the adequacy of nutritional support, but these measurements do not adequately represent FM in premature infants, who have greater adiposity than their term counterparts.[3,10,11] Thus, monitoring both quantity and *quality* of weight gain via body composition (BC) assessment may help clinicians to better understand and prevent adverse long term outcomes while simultaneously optimizing growth and neurodevelopment.[12]

The case for whole body composition

Clinicians are interested in whole BC assessment to monitor growth response to nutritional intake. A difference in prescribed and actual nutritional intake can negatively impact growth.[5,13–16]Although recommended goals for nutrition exist,[2,16] actual intake in the neonatal intensive care unit (NICU) varies according to mode of nutritional delivery (enteral versus parenteral) and use of human milk or infant formula. Monitoring whole BC may allow for comparison of macronutrient and micronutrient provision to optimize nutrition for preterm infants.[17–19] Furthermore, tracking BC may help to better define the relationship between early nutrition, nutrient accretion, and growth.[5]

Monitoring whole BC may also assist in assessing metabolic and neurodevelopmental risk. Multiple studies have linked early catch-up growth to later adverse metabolic outcomes such as type 2 diabetes, obesity, and cardiovascular disease.[6,20–23] Previously, we found that higher gains in FM during a premature infant's hospitalization are positively associated with blood pressure at 4 years of age.[9] Additionally, we discovered that FFM is a better predictor of neurodevelopmental outcomes than weight, and higher rates of weight gain and FFM in the NICU are associated with improved cognition and faster speed of processing in infancy and at 4 years of age.[8,9] While early catch-up growth in length, FFM, and weight during the first several months of life has been shown to improve neurodevelopmental outcomes of premature infants in many studies, gains in FM have not shown the same benefit.[6,8,22,24]

Evaluation of body composition methods for premature infants

Validation of a new BC method typically occurs by comparison with a reference method, an established technique that produces accurate and reliable estimations of BC. Because cadaver analysis is the only way to directly measure BC, every other method relies on assumptions and is subject to error.[25,26] The four-component model of BC, which divides body mass into FM and FFM components of total body water (TBW), bone, and protein is considered the most valid reference model because it includes independently measured components that are estimated by simpler models.[27,28] The four-component model is only feasible in the research setting because it requires multiple tests and infant cooperation.[29] Consequently, evaluation of newer infant BC methods often involves comparison with two- or three-component models that have been validated against three- or four-component models. The two-component model of BC divides the body into FM and FFM and assumes a constant density of FM (0.9007

g/mL) and specific age and sex-specific densities of FFM.[30] The three-component model divides the body into FM and further divides FFM into TBW and dry FFM.[31–33]

Challenges to assessing body composition in the NICU

Assessment of BC in premature infants is difficult because of the known variability in FFM composition during growth.[34,35] An infant's TBW decreases while bone mineral content increases with increasing postmenstrual age. Compounded with varying fluid status from the intravenous provision of fluids while in the NICU, two-compartment models that do not directly measure TBW may be inaccurate in critically ill infants. Fomon and Butte developed age and sex-specific normative values for TBW and other FFM components which may improve the predictive ability of BC methods that rely on this data, but these values were derived from term infants.[34–36] Similarly, methods that rely upon prediction equations to estimate an infant's thoracic volume are based upon reference data from healthy term infants and may not be accurate for preterm infants with lung disease.[37] Conducting BC assessment in the NICU setting is also challenging due to varying medical stability of infants and the need to coordinate measurements around nursing care.

While magnetic resonance imaging (MRI),[29] isotope dilution techniques, quantitative MRI, and dual energy x-ray absorptiometry (DXA)[29,38–40] have been studied in premature infants, these techniques are not suitable for repeated clinical use because they involve exposure to ionizing radiation, are expensive, require medical stability, and/or are not feasible outside the research setting. These methods have been extensively reviewed elsewhere[12,29,41] and will not be discussed in our review. The BC methods of air displacement plethysmography, bioimpedance, skinfold measurements, and ultrasound show promise for repeated clinical use in premature infants. Here, we examine each method and its potential for clinical application.

Air displacement plethysmography (ADP)

Only one device, the Pea Pod (Cosmed, Inc., California), is currently available to conduct ADP measurements in preterm infants. The Pea Pod determines percent body fat (%BF) through body measurements, gas laws, and Fomon's[34] or Butte's[35] age and sex specific densities for FFM as described elsewhere.[42–44] ADP estimates of BC rely on the Pea Pod's algorithms to account for hydration status in infancy[37,45] and estimation of functional residual capacity estimates to account for thoracic volume. The validity of measurements also relies upon accuracy of length measurements.

ADP exhibits excellent accuracy when evaluated against direct analysis of bovine phantoms[46] and superior reliability over repeated measurements[43] and has been validated against the four-compartment model in term infants.[47] Two studies to assess accuracy and precision have been conducted in premature infants (**Table 1**), but the effects of covariates such as weight, length, and sex on regression models were not examined in either study. Forsum showed moderate accuracy in %BF using ADP compared to a three-component method.[37,45] No bias was detected. However, ADP overestimated low FFM densities and underestimated high FFM densities.[45] The other study by Roggero compared BC via ADP to that obtained via stable isotope dilution studies and found good reliability, precision, and accuracy for %BF measured via ADP and $H_2{}^{18}O$ dilution. No bias was detected as %BF increased.[19]

Whether ADP can provide useful information in the acute care setting is partially dependent upon its ability to detect clinically relevant changes through longitudinal measurements of BC. In our previous work, we found that ADP is sensitive enough to detect a 30-45 gram change in FFM (~3% change) in measurements taken one week apart.[8] ADP can also detect differences in %BF of premature infants over a three to four week period.[48]

ADP is attractive for use in the clinical setting because it is non-invasive, reliable, and relatively easy for clinicians to use. Measurements of ADP are unaffected by crying and moderate movement, making it an appropriate tool for use in infants.[29,43,47] Another advantage of this method is the potential for use of ADP-based BC reference charts to monitor growth.[49,50] Tracking premature infants on these charts while monitoring clinical outcomes may help to determine if current nutritional and growth standards are appropriate.

The cost of the Pea Pod may preclude routine use in the NICU, and it is best suited for healthy infants with clinically stable respiratory status, weight between 1 and 10 kg, and independence from a central line.[44] These criteria prevent use of ADP in infants who are critically ill, such as premature neonates on ventilators and those receiving parenteral nutrition.

Bioelectrical impedance analysis (BIA)

BIA measures BC indirectly through the resistive properties of an electrical current in the body with the assumption that lean tissue produces low resistance because of its high fluid content, while adipose tissue and bone yield a greater level of resistance as a result of low fluid content.[51,52] Bioimpedance data can be collected using single-frequency (SF), multi-frequency (MF), or spectroscopy devices (BIS).[53] From bioimpedance data, TBW, extracellular and intracellular fluid (ECF, ICF), FM, FFM, and other lean tissue compartments can be estimated using mathematical modeling and prediction equations, depending on the approach.[51]

SF-BIA and MF-BIA rely on assumptions of body shape, uniform conduction of the electric current, and use of population-specific equations that are dependent on factors such as age, gender, and ethnicity, in order to generate BC or volume estimates.[53,54] Use of SF-BIA and MF-BIA, which rely on stable hydration status, is

challenging in the neonatal population.[51] TBW relative to total body mass changes throughout infancy, beginning at around 80.6% at birth and decreasing to 78.8% (females) and 79% (males) at one year of age, which has implications for the measurement of specific tissue, specifically FFM.[34] TBW may also depend on weight for gestational age, with small for gestational age infants having a greater body water content than appropriate for gestational age infants.[55] Therefore, the hydration constants for FFM used to estimate TBW in conjunction with SF- and MF-BIA equations may not be appropriate for use in a growing infant.

BIS devices, which measure impedance over a wide range of frequencies, are more promising for use because they do not rely on population-specific prediction equations.[56] However, the use of curvilinear modeling and mixture theory-based algorithms to determine TBW by spectroscopy requires inclusion of shape and density constants and resistivity coefficients, which represent the resistivity of extra- and intracellular fluid compartments to an electric current.[56,57] As a result of substantial variation in resistivity among infants, preterm infants require unique coefficients for accurate assessment of TBW, but they may only be suitable for population level estimates of TBW.[56,58,59] Infant movement and proximity of measurements to feeding times have also been shown to impact spectroscopy data, specifically extracellular resistance measurements.[60]

Multiple studies have concluded that SF-BIA data minimally improves prediction equations for whole BC beyond weight, length, and sex and that SF-BIA data is not more advantageous than weight for determination of FFM in preterm and term infants.[59,61] Nevertheless, additional research into bioimpedance approaches for BC assessment is warranted because of advantages such as suitability for non-invasive serial measurements. BIA may be used in critically ill infants despite requiring central lines,

respiratory support, and continuous monitoring of vitals.[62] However, if infants have not achieved fluid balance, SF- and MF-BIA estimates of BC may be inaccurate. BIS devices may be more appropriate for use in critically ill preterm infants because they employ spectral data modeling to determine ECF and ICF and thus, are not dependent on the assumption of fluid balance. However, several constants used in standard BIS algorithms are based on adult data and may result in errors in whole BC estimates when applied to infants.

Skinfold Measurements

Measurements of skinfold thickness (SFT) have been proposed to estimate BC in infants (**Table 2.1**). Multiple measurements taken at various body sites using calipers are then associated with whole body FM using prediction equations.[63] While these measurements are inexpensive, noninvasive, and do not involve exposure to radiation, they may not reflect whole body fat in older infants because they assess only subcutaneous adipose tissue.[29,64] Furthermore, the use of calipers is controversial in medically fragile preterm infants, and repeated, accurate measurements may be difficult to obtain because of an infant's small body size and sensitive skin.[65] SFT measurements can be influenced by fluid status[65], type of caliper used,[66,67] and amount of time the caliper is applied to the skin.[68,69]

A validated prediction equation is needed for clinicians to use SFT to determine BC. Several have been proposed, but most are derived from models developed in other clinical populations and may not be appropriate for use in premature infants.[63,70,71] Many existing prediction equations also require SFT measurements at multiple sites, which may be difficult to obtain in preterm infants. Of note, SFT measurements likely have lower intra- and inter-rater reliability than the other methods reviewed here, unless conducted by a single trained individual.[66]

A well-known model developed in preterm and term infants by Dauncey includes subscapular and triceps skinfold measurements and nine body measurements and has good agreement with published data from cadaver analysis.[69] Although not validated in preterm infants, some consider it the best method for accurate determination of BC in this population despite its complexity.[65]

Other attempts to develop and validate prediction equations using SFT have been met with mixed success,[64,72,73] likely due to the variety in model development and validation populations and the BC methods against which SFT measurements are compared. While some equations demonstrate good correlation with whole BC measured using ADP or DXA,[63] others have yielded insufficient correlation for calculation of individual BC and unacceptable levels of systematic error.

Four prediction equations for estimating whole body FM in term infants using SFT were recently developed by Deierlein, Catalano, Lingwood, and Aris.[59,74–76] While all equations include covariates such as weight, length, and gender, it is unknown whether the covariates alone can predict body composition. When compared to ADP-measured whole body FM obtained at birth, all equations except for the Catalano model yield significantly different FM. Bias was noted for the Catalano and Lingwood equations, indicating that they overestimated FM at lower FM values and underestimated FM at greater FM values. At 3 months of age, none of the equations accurately predict whole body FM in comparison to ADP-derived measurements. Bias was noted for all equations, with the Deierlein equation overestimating FM at all values and the other three equations overestimating FM at lower FM values and underestimating FM at higher FM values.[77]

While more research is needed to develop and validate prediction equations for use of SFT to assess BC in premature infants, this method may be most appropriate for

the global health care setting because of low cost and accessibility. Measurements may be affected by factors such as race and sex, making it important to develop and validate population-specific equations.[78,79]

Ultrasound

Ultrasound is a developing method for assessment of whole BC in infants and employs high-frequency sound waves to produce an image of the tissue of interest. Images are analyzed for either adipose tissue and muscle thickness (or cross-sectional area), which is then correlated with whole body FM or FFM generated by established BC methods.[80–82] Ultrasound is not routinely utilized for assessing BC in any clinical population, but several studies have shown a correlation between adipose and muscle measurements obtained using ultrasound and established BC methods in adults and adolescents.[83–88] A few of these studies have also generated prediction equations for whole body FM or FFM using ultrasound measurements.[89–92]

Ultrasound assessment of BC has been minimally explored in infants. Only cross-sectional area has been correlated with whole BC; the cross-sectional area of calf adipose tissue correlates with whole body FM (r = 0.67) measured via DXA in premature infants.[93] However, ultrasound has yet to be validated against reference methods appropriate for premature infants.

Ultrasound, a non-invasive tool that involves no ionizing radiation, is readily available in the clinical setting and can be quickly performed at the bedside in critically ill infants. Ultrasound has good reliability in adult populations[94] but appears to have variable reliability in premature infants, although data are limited.[95] The pressure at which the ultrasound transducer is applied affects the measurement of tissues and therefore, the precision of measurements.[82,96] Care must be taken to use consistent compression during measurement, though no universal method has been developed for

this purpose. The consistent identification of measurement sites may be difficult in a rapidly growing infant, and inconsistencies may impair comparison of longitudinal measurements. Refinement of ultrasound techniques, including development of a standardized protocol for use in premature infants, would greatly assist in addressing these issues. If able to accurately estimate FM, ultrasound would represent the only clinical BC assessment method with the potential to assess a patient from birth through adulthood.

DISCUSSION

The inclusion of BC assessment in clinical evaluation has the potential to provide a better understanding of growth in premature infants. All methods discussed (**Table 2.2**) show promise for clinical use and should be further explored. However, none are four-component models, and the limitations of each must be considered when choosing the best method for use in the clinical setting. ADP, which has been validated against the four-component model in term infants and compared with the three-component model in preterm infants, is currently the most accurate and reliable method for assessing body composition in preterm infants. ADP, however, requires a costly device, and infants are not eligible for measurement unless they are medically stable. BIA and SFT have been compared with the two-component model but need refinement for routine use in the clinical setting. Whole BC via ultrasound has not been validated against two- or three-component models, but we felt it warranted discussion due to its potential use in critically ill infants.

Moving forward, the first step in identifying a clinically relevant, bedside BC modality in preterm infants is the evaluation of one-time measurements. This must be followed by an assessment of the method's ability to detect longitudinal changes in whole BC, which is important to provide meaningful information for routine clinical

monitoring. Once a bedside modality is proven effective for longitudinal measures, regular assessment of BC in response to nutritional provision can be conducted. Routine monitoring may facilitate the development of new nutritional recommendations for healthy and critically ill premature infants that promote gains in FFM and reduce adiposity. Achieving such BC changes could ultimately decrease the risk for childhood obesity and metabolic disease later in life while simultaneously optimizing neurodevelopmental outcomes.

Table 2.1: Precision and Accuracy of Body Composition Methods in Infants

Method	Model Comparisons	Precision	Accuracy	Conclusion
ADP	**4-component model** isotope dilution, total body potassium counting, DXA Ellis[47]: 49 term infants	7.9% CV; mean bias 0.4%BF for repeated ADP tests (non-significant)	*%BF* Mean bias 0.6 ± 3.7% (non-significant); LOA:-6.8 and 8.1%	%BF via ADP slightly but not significantly higher than 4C model
	3-component model isotope dilution; Siri's equation Forsum[45]: 14 premature infants	Not done	*%BF* Mean bias 1.00 ± 2.91% (non-significant); LOA: -6.8 and 4.8%	%BF via ADP slightly but not significantly higher than 3C model
	2-component model isotope dilution Roggero[19]: 57 preterm infants (precision); 10 preterm infants (accuracy)	Mean bias 0.15%BF for repeated ADP tests (non-significant)	*%BF* Mean bias 0.32 ± 1.57%; LOA: -3.4% and 2.76%	%BF via ADP slightly but not significantly lower than 3C model
BIS	**2- component model** ADP *Lingwood[59]: 72 term infants at birth; 54 term infants at 6 weeks	Not done	*%BF* Mean bias 1.2-1.9%; LOA: -9.5 to 10.5% *%FFM* Birth: Mean bias 1.5% 6 weeks: Mean bias 2.6% LOA 200-600 grams (6-13% error)	BIA did not improve prediction of %BF or %FFM.
BIA-SF	**2- component model** ADP *Tint[97]: 57 term infants at birth; 46 at 2 weeks	Not done	*%FFM* Birth: Mean bias 0.7%; LOA: 7.9 and -5.8% 2 weeks: mean bias 0.3%; LOA: -7.1% and 6.4%	Inclusion of BIA only slightly improved prediction of %FFM, but BIA has limited use in predicting FFM in first weeks of life

SFT	DXA *Dung[61]: 51 male, 67 female preterm neonates	Not done	*%FFM* Males: mean bias 0.10%; 95% CI: -1.2 and 1.42% Females: mean bias -0.81%; 95% CI: -2.4 and 0.84%	Weight outperformed BIA as predictor of FFM
	2- component model <u>ADP</u> *Aris[76]: 262 term neonates	Triceps SFT: Male ICC: 0.994 Female ICC: 0.997 Subscapular SFT: Boys ICC: 0.996 Girls ICC: 0.997	*FM (kg)* Subscapular SFT: R^2=0.811; mean bias 0.003 kg (p>0.05); LOA: -0.25 and 0.26 kg	Prediction equation explained 81.1% of variance; potential use in Asian neonates
	*Cauble[77]: 95 term infants at birth; 65 term infants at 3 months; tested Deierlein, Catalano, Lingwood, and Aris prediction equations	ICC: 0.83 to 0.96; TEM: 0.23 to 0.34	*FM (kg)* Birth: Deierlein: Mean bias 0.114 kg; LOA: -0.010 and 0.328 kg Catalano: Mean bias -0.012 kg; LOA: -0.240 and 0.215 kg Lingwood: Mean bias -0.045 kg; LOA: -0.272 and 0.183 kg Aris: Mean bias -0.034 kg; LOA: -0.245 and 0.176 kg 3 months: Deierlein: Mean bias 3.325 kg; LOA: 1.789 and -4.862 kg Catalano: Mean bias -0.271 kg; LOA: -0.871 and 0.328 kg Lingwood: Mean bias -0.286 kg; LOA: -0.871 and 0.299 kg Aris: Mean bias -0.230 kg; LOA: -0.824 and 0.363 kg	Poor precision and accuracy for all prediction equations. FM via prediction equations had poor agreement with ADP measures of FM.

SFT	Daly-Wolfe[98]: 28 preterm infants; 28 term infants	All measurements within 3 mm	*%BF* Midarm circumference (MAC) and suprailiac SFT: R^2 =0.49 (preterm); 0.41 (term); p< 0.001 MAC: R^2=0.604 (preterm); p=0.008 Mid-thigh circumference: R^2 = 0.63; p<0.001 (term)	MAC and suprailiac SFT may be accurate and reliable methods to measure adiposity in preterm and term infants.
	Deierlein[74]: 128 term infants	All SFT measurements within 2 mm	*FM (kg)* Sum of triceps, subscapular, thigh SFT: R^2 = 0.81; p<0.0001; RMSE =0.08 kg	Prediction equation including sum of SFT explained 81% of variance in FM.
	Lingwood[59]: 72 term infants at birth; 65 term infants at 6 weeks	Not done	*%BF* Triceps and subscapular SFT yielded large mean bias at birth and 6 weeks Underestimation of %BF by 2.4-8.9%	SFT did not improve prediction of %BF at any age
	<u>Total body electrical conductivity (TOBEC)</u> *Catalano[75]: 194 term neonates; compared new model to Dauncey's model and TOBEC-estimated FM	CV% = <7% for SFT	*FM (g)* Catalano: (weight, length, flank SFT): R^2 = 0.84; p=0.0001 Dauncey model: R^2 = 0.54; p=0.0001	No significant difference (p=0.11) between FM via Catalano model and TOBEC in infants at birth.

SFT				
	DXA *Schmelzle[63]: 104 term and preterm infants at 0, 2, and 4 months	96% of intraindividual variation was within 5% range of variation	*FM (g)* Sum of triceps, biceps, suprailiac, and subscapular SFT: Mean bias: ≤500 g: 75 g 500-1000 g: 170 g 1001-2000: 300 g >2000 g: 370 g R^2 = 0.95; p<0.001	SFT can be used to obtain rough estimate of FM in infants but should be paired with another body composition method to improve accuracy.
	Isotope dilution *Sen[99]: 46 term infants between 6-24 months	Not done	*%BF* Biceps, triceps SFT: Mean bias -0.93%; 95%CL: -2.03, 3.89 (females) Biceps, suprailiac SFT Mean bias 1.14%; 95% CL: -0.26, 2.54 (males)	Relatively wide LOA observed; Sen prediction equation may be useful for group-level comparisons of %BF in infants
	Sheng[100]: 16 preterm infants at birth	Not done	*FM (g)* Subscapular SFT: r=0.78 *BF%* Subscapular SFT: r=0.61	Subscapular SFT performed best for assessment of FM
US	**2- component model** DXA Ahmad[93]: 102 preterm and term infants	Not done	*FM (g)* Calf cross-sectional area r=0.82 *Lean Mass (g)* r=0.81	US measurements of calf cross-sectional area may be useful in obtaining information about FM in premature infants.

Table 1. Summary of studies on the precision and accuracy of body composition methods. %BF, percent body fat, %CV, percent coefficient of variation; 4C, four-component model; 3C, three-component model; ADP, air displacement plethysmography; BIA-SF, single-frequency bioimpedance analysis; BIS, bioimpedance spectroscopy; CI, 95% confidence interval; CL, 95% confidence limits; DXA, dual energy x-ray absorptiometry; FM, fat mass; ICC, intraclass correlation coefficient; LOA, Bland Altman 95% Limits of Agreement; MAC, midarm circumference; SFT, skin fold thickness, TEM, technical error of measurement
*validation study

Table 2.2: Evaluation of Body Composition Methods for Bedside Assessment of Preterm Infants

Method	Validated in Preterm Infants?	Advantages	Disadvantages	Suggested Use
ADP	Yes	• Non-invasive • No radiation • Unaffected by movement/crying	• 1-10 kg weight limit • Infants must be on room air • No central line • Expensive • Not for critically ill infants	• Medically stable infants • NICU or outpatient clinic settings
BIA	No	• Non-invasive • No radiation • Can be performed at bedside • Appropriate for critically ill infants (BIS)	• Requires population specific information • May require stable hydration status • Sensitive to infant movement • May be affected by feeds	• Medically stable infants with balanced hydration status (BIS) • NICU or outpatient clinic settings
SFT	No	• Minimally invasive • No radiation • Can be performed at bedside • Inexpensive • Appropriate for critically ill infants	• No consensus protocol • Influenced by fluid status, caliper type, length of measurement • Low reliability with multiple observers	• Medically stable infants • Outpatient clinic setting • Global healthcare setting
US	No	• Non-invasive • No radiation • Can be performed at bedside • Appropriate for critically ill infants • Available at most hospitals	• No validated protocol • Affected by tissue compression • Consistent measurements may be difficult	• Critically ill or medically stable infants • NICU or outpatient clinic settings • Potential for use in the global healthcare setting

Table 2. Advantages and disadvantages of body composition methods used to assess preterm infants. ADP, air displacement plethysmography; BIA, bioimpedance analysis; BIS, bioimpedance spectroscopy; NICU, neonatal intensive care unit; SFT, skin fold thickness; US, ultrasound

CHAPTER 3: ANTHROPOMETRIC MEASURES DO NOT ADEQUATELY REFLECT ADIPOSITY IN PRETERM INFANTS FROM 30-63 WEEKS CORRECTED GESTATIONAL AGE

CHAPTER SYNOPSIS

Background: Growth in premature infants is currently assessed through measures of weight and length, but weight/length indices such as body mass index are known to be poor surrogates for adiposity shortly after birth. Whether these indices can predict body composition later in infancy is unknown. Should they prove to be adequate surrogates for body composition, weight/length indices could be used to assess weight quality in premature infants, potentially improving growth and guiding nutritional provision.

Objective: To determine the ability of weight/length indices to predict adiposity in premature infants from 28-63 weeks corrected gestational age.

Methods: Data from 260 preterm infants and 95 term infants was compiled from three studies conducted at the University of Minnesota's Neonatal Intensive Care Unit from 2010-2019. Whole body fat mass and fat-free mass and percent body fat were obtained using air displacement plethysmography beginning when infants were medically stable and continuing through 63 weeks corrected gestational age. Weight, length, weight/length, body mass index, and ponderal index were assessed for their ability to predict fat mass, fat-free mass, and percent body fat in four age groups. Fat mass and fat-free mass index were examined as secondary outcomes. The predictive ability of weight/length indices was also compared in preterm infants < 32 and $\geq$ 32 to 36 + 6 weeks gestational age at birth. Finally, predictive ability of indices was compared in preterm infants at term-equivalent age and term infants. The best proxy for whole body composition was determined by selection of the linear regression model with the highest variance explained (R^2) and lowest root mean square error (RMSE).

Results: BMI was the best predictor of percent body fat for the first three age groups but resulted in poorly fitting models with low variance explained (R^2 = 0.34-0.35) and high prediction errors (RMSE = 3.21-4.01). Weight for length was the best predictor of %BF for the oldest age group, but also exhibited low variance explained (R^2 = 0.13) and high prediction error (RMSE = 4.41). Weight was the strongest predictor of fat-free mass across all age groups (R^2 = 0.81-0.94; RMSE = 0.06-0.30).

Conclusions: None of the weight/length indices examined accurately represented adiposity in preterm infants. Weight/length indices cannot be used to assess adiposity in premature infants through 63 weeks corrected gestational age.

INTRODUCTION

Clinicians are interested in the quality of weight premature infants gain in the neonatal intensive care unit (NICU) because of the relationship between body composition and metabolic and neurodevelopmental outcomes. Weight quality can be assessed by monitoring changes in fat mass (FM) and fat-free mass (FFM) as infants grow. In comparison with term infants, premature infants exhibit decreased FFM gains, decreased linear growth, and increased relative adiposity at term corrected age.[3–6] They also experience a period of rapid growth or "catch-up growth", which may increase their risk for later insulin resistance, obesity, and cardiovascular disease.[22,101–103] While greater FM gains after NICU discharge until four months of age are associated with increased blood pressure at four years of age,[9] gains in FFM (but not FM) are associated with improved neurodevelopmental outcomes.[24,104]

The American Academy of Pediatrics recommends nutritional goals aimed at helping preterm infants achieve growth equal to that of term infants of the same corrected age.[105] Better nutrition support is associated with improved growth and neurodevelopmental outcomes in multiple studies. [8,106–109] However, assessing an infant's response to nutrition support is currently limited by the available growth metrics of weight and length. Despite the known relationship between early body composition and later outcomes, body composition is not routinely measured in the NICU because of the limitations of currently available tools. Many methods are expensive, invasive, or unsuitable for repeated measurements in a medically fragile population. The use of simple weight for length (W/L) indices to assess body composition would provide a fast and non-invasive way to assess a premature infant's quality of weight gain in response to nutritional provision in the NICU, regardless of medical stability. Due to growth irregularities, assessing proportionality of growth in preterm infants is also desirable. [110]

Ponderal index (PI) and body mass index (BMI) have been proposed as proxies for infant adiposity or percent body fat (%BF). In healthy term infants, neither PI or BMI can accurately predict %BF.[111] In preterm infants, BMI is a better predictor of proportionality than PI, and gender-specific BMI curves for preterm infants have been created and validated.[110,112]

Previously, we examined W/L indices (weight, length, W/L, BMI, PI) of preterm infants shortly after birth (within 72 hours) and found that none of the indices examined accurately reflected %BF.[10] Whether BMI is predictive of adiposity in preterm infants later in infancy is unknown. Thus, in this study, we aimed to determine if W/L indices are predictive of FFM, FM, or %BF in preterm infants at various timepoints after the first week of life, including 28 to 33 weeks corrected gestational age (CGA), 34 to 36 weeks CGA, 37 to 42 weeks CGA, and 50 to 63 weeks CGA.

METHODS

Data from 355 infants (preterm = 260 infants; term = 95 infants) recruited from the University of Minnesota's Neonatal Intensive Care Unit between 2010-2019 were included in this study. Study inclusion criteria included birth between < 32 to 36 + 6 weeks gestational age (GA), medical and respiratory stability, and independence from central lines at time of measurement. Infants with medical conditions (other than prematurity) known to affect growth or adiposity were excluded. All measurements were taken ≥ 7 days after birth.

The Pea Pod (Cosmed, Ltd, Concord, California) was used to evaluate body composition by air displacement plethysmography (ADP). The Pea Pod's operating procedures have been described elsewhere.[43–46] Briefly, a recumbent length board was used to measure each infant's length to the nearest 0.1 cm. Naked weight to the nearest

0.0001 kg was obtained using the Pea Pod's electronic scale followed by an approximately 2-minute body volume measurement in the device's test chamber. The body volume measurement, the known density of fat (0.9007 g/mL), and Fomon's[34] or Butte's[35] age and sex specific densities for FFM were then used by the Pea Pod's software to calculate %BF. Weight and length z-scores were calculated using the Fenton[113] growth charts for preterm infants until 50 weeks CGA, while the World Health Organization (WHO) growth charts[114] were used for preterm infants ≥ 50 weeks CGA and for term infants. All measurements were taken by a consistent study team.

Statistical Analysis

For preterm infants, anthropometric and body composition measurements were divided into four groups based upon the CGA at which they were obtained: ≤ 33 weeks corrected CGA (group one), 34 to 36 weeks CGA (group two), 37 to 42 weeks CGA (group 3), and 43 to 63 weeks CGA (group four). Since measurements were taken longitudinally during an infant's NICU stay, some infants had measurements in all CGA groups and others had multiple measurements within the same CGA group. If multiple measurements from an infant were present in the same CGA group, the measurement taken closest to the midpoint of the CGA group was selected.

The relationship between W/L indices and primary outcomes of FFM, FM, and %BF and secondary outcomes of FFM/L^2 (FFMI) and FM/L^2 (FMI) were examined by CGA group using linear regression analysis. Weight, length, W/L, BMI (W/L^2), and PI (W/L^3) were assessed as predictors. To ascertain whether gestational age at birth affected the prediction of our outcomes, we further compared the predictive ability of W/L indices in preterm infants born < 32 weeks and ≥ 32 to 36 + 6 weeks gestational age. Finally, to examine differences in predictive ability between preterm infants at term-

equivalent age and term infants, we compared W/L indices in the last two CGA groups combined. Random effects models were used to account for the correlation between repeated measurements. The best proxy for whole body composition was the linear regression model with the highest variance explained (R^2) and lowest root mean square error (RMSE). To account for the number of models examined, p-values are two sided with $p < .00025$ considered statistically significant. SAS 9.4 (SAS Institute, Cary, North Carolina) was used to perform statistical analyses.

RESULTS

Characteristics of infant participants are described by CGA group in Table 3.1. Infants in the youngest CGA group had mean GA of 29.25 weeks, were 41% male, and 16% white, while preterm infants in the oldest CGA group had mean GA of 28.81 weeks, were 53% male, and 6% white. Infants in the youngest CGA group had the lowest mean %BF (8%), while preterm infants in the oldest CGA group had the highest mean %BF (22.9%).

Table 3.2 shows the relationship between the predictors of weight, length, W/L, BMI, PI and the outcomes of FFM, FM, and %BF by CGA group. All W/L indices in each of the four CGA groups were significantly correlated with FM except for PI in CGA group four. BMI was most strongly correlated with FM in CGA groups one and two, while W/L was most strongly correlated with FM in CGA groups three and four. Weight, W/L, and BMI were significantly correlated with %BF in all CGA groups, but length exhibited significant correlation only for CGA group one, and PI was significantly correlated only in CGA groups one, two, and three. BMI was most strongly correlated with %BF *(r* = 0.58) in groups one through three, while W/L was most strongly correlated with %BF in group four *(r* = 0.43). Weight, length, W/L, and BMI were significantly correlated with FFM

across all CGA groups, but PI was only significantly correlated with FM in group one. Weight was most strongly correlated with FFM across all CGA groups.

The R^2 and RMSE of models examined in linear regression analyses are shown according to CGA group in Table 3.3. BMI was the best predictor of %BF for CGA groups one, two, and three (**Figure 3.1, 3.2, and 3.3**), while W/L was the best predictor of %BF for the oldest CGA group (**Figure 3.4**). Large prediction errors were observed for W/L indices and %BF across all CGA groups. BMI was the best predictor of FM for the first two CGA groups, while W/L was the best predictor for CGA group three, and weight was the best predictor for the oldest CGA group. Weight was the best predictor of FFM across all four CGA groups.

For our secondary analyses, BMI was a moderate predictor of FMI for the first three CGA groups, while all W/L indices were weak predictors of FMI for the oldest CGA group (**Table 3.4**). BMI was a strong predictor of FFMI for all CGA groups but generally decreased as preterm infants grew older.

The ability of W/L indices to predict %BF in preterm infants < 32 weeks GA at birth and ≥ 32-36 + 6 weeks GA at birth is shown in Table 3.5. All W/L indices were significant predictors of %BF ($p < .0001$). For all W/L indices except for PI, GA at birth (< 32 weeks or ≥ 32 weeks) was a significant predictor of %BF. A significant interaction between GA and W/L indices was present for all indices except for PI. Sex was not a significant predictor of %BF.

The relationship between W/L indices and %BF for preterm infants at term-equivalent age versus term infants is shown in Table 3.6. All W/L indices were significant predictors of %BF. For all W/L indices, term status was a significant predictor of %BF,

while sex was not a significant predictor. A significant interaction between term status and W/L indices was present for all models.

Table 3.1: Infant descriptive characteristics and body composition by corrected age at measurement

Variable[a]	CGA Group 1 (n=74)	CGA Group 2 (n=175)	CGA Group 3		CGA Group 4	
			Preterm (n=113)	Term (n=70)	Preterm (n=111)	Term (n=95)
Characteristics						
GA at birth (weeks)	29.25 ± 1.78	30.09 ± 2.79	28.74 ± 2.89	39.3 ± 0.78	28.81 ± 3.01	39.76 ± 1.04
Birthweight (g)	1238 ± 258.4	1434 ± 498.0	1185 ± 471.4	3466 ± 435.7	1207 ± 0.48	3543 ± 0.46
Birthweight z-score[b]	0.03 ± 0.68	0.02 ± 0.67	-0.10 ± 0.84	0.33 ± 0.87[c]	-0.06 ± 0.87	0.49 ± 0.91[c]
Weight/length (kg/m)	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
BMI (kg/m2)	9.37 ± 1.09	11.36 ± 1.08	13.37 ± 1.37	13.71 ± 1.01	16.81 ± 1.86	15.68 ± 1.69
PI (kg/m3)	23.37 ± 1.96	26.04 ± 2.55	28.55 ± 2.90	26.10 ± 1.98	27.80 ± 4.01	26.46 ± 2.24
Sex (male)	30 (40.54%)	89 (50.86%)	62 (54.87%)	35 (50.00%)	59 (53.15%)	47 (49.47%)
Race (white)	12 (16.22%)	64 (37.14%)	32 (28.32%)	57 (81.43%)	7 (6.31%)	78 (82.11%)
Body Composition						
CGA (weeks)	32.27 ± 1.20	35.15 ± 0.80	38.85 ± 1.45	41.74 ± 0.79	57.04 ± 3.45	50.21 ± 4.07
Body Mass (kg)	1.52 ± 0.32	2.17 ± 0.32	2.97 ± 0.54	3.80 ± 0.38	6.24 ± 1.03	5.58 ± 1.14
Length (cm)	40.03 ± 2.08	43.67 ± 2.13	46.94 ± 2.96	52.59 ± 1.58	60.82 ± 4.25	59.27 ± 3.80
Body fat (%)	8.03 ± 3.93	12.45 ± 4.71	18.23 ± 4.91	14.57 ± 3.90	22.87 ± 4.87	22.12 ± 6.78
Fat mass (kg)	0.13 ± 0.09	0.28 ± 0.13	0.55 ± 0.20	0.56 ± 0.18	1.45 ± 0.46	1.29 ± 0.59
Fat-free mass (kg)	1.39 ± 0.26	1.90 ± 0.26	2.42 ± 0.41	3.24 ± 0.30	4.77 ± 0.70	4.28 ± 0.64

[a]Continuous variables expressed as mean ± SD or *n* (%)

[b]Fenton z-score; [c]WHO z-score

BMI, body mass index; CGA, corrected gestational age; GA, gestational age at birth; PI, ponderal index

Group 1: 28-33 weeks CGA; Group 2: 34-36 weeks CGA; Group 3: 37-42 weeks CGA; Group 4: 43-65 weeks CGA

Table 3.2: Pearson's correlation coefficients for predictors and outcomes in 260 preterm infants by corrected gestational age at measurement

Predictor	Fat mass	Body fat (%)	Fat-free mass
CGA Group 1 (n=74)			
Weight (kg)	*0.753	*0.536	*0.972
Length (cm)	*0.580	*0.394	*0.913
Weight/length (kg/m)	*0.774	*0.562	*0.954
BMI (kg/m^2)	*0.782	*0.585	*0.888
PI (kg/m^3)	*0.688	*0.541	*0.637
CGA Group 2 (n=175)			
Weight (kg)	*0.608	*0.355	*0.924
Length (cm)	*0.208	-0.029	*0.842
Weight/length (kg/m)	*0.700	*0.478	*0.830
BMI (kg/m^2)	*0.729	*0.585	*0.575
PI (kg/m^3)	*0.602	*0.580	0.148
CGA Group 3 (n=113)			
Weight (kg)	*0.756	*0.402	*0.947
Length (cm)	*0.480	0.134	*0.872
Weight/length (kg/m)	*0.812	*0.497	*0.877
BMI (kg/m^2)	*0.776	*0.581	*0.618
PI (kg/m^3)	*0.485	*0.504	0.090
CGA Group 4 (n=111)			
Weight (kg)	*0.759	*0.412	*0.902
Length (cm)	*0.538	*0.204	*0.812
Weight/length (kg/m)	*0.723	*0.434	*0.781
BMI (kg/m^2)	*0.470	*0.360	*0.362
PI (kg/m^3)	0.080	0.177	-0.157

*indicates significance at $p < .05$

BMI, body mass index; CGA, corrected gestational age; PI, ponderal index; RMSE, root mean square error

Table 3.3: R^2 and root MSE for prediction of body composition by weight/length indices in preterm infants by corrected gestational age

	Body fat (%)		Fat mass		Fat-free mass	
Predictor	R^2	RMSE	R^2	RMSE	R^2	RMSE
CGA Group 1 (n=74)						
Weight (kg)	0.288	3.338	0.567	0.058	0.944	0.063
Length (cm)	0.155	3.636	0.336	0.072	0.834	0.108
Weight/length (kg/m)	0.316	3.271	0.599	0.056	0.911	0.079
BMI (kg/m^2)	0.342	3.209	0.612	0.055	0.788	0.123
PI (kg/m^3)	0.293	3.325	0.473	0.064	0.406	0.205
CGA Group 2 (n=175)						
Weight (kg)	0.126	4.419	0.369	0.101	0.854	0.100
Length (cm)	0.001	4.726	0.043	0.124	0.709	0.141
Weight/length (kg/m)	0.229	4.151	0.489	0.090	0.689	0.146
BMI (kg/m^2)	0.346	3.824	0.534	0.086	0.327	0.214
PI (kg/m^3)	0.339	3.843	0.364	0.101	0.021	0.259
CGA Group 3 (n=113)						
Weight (kg)	0.161	4.513	0.572	0.132	0.897	0.132
Length (cm)	0.018	4.884	0.223	0.178	0.761	0.202
Weight/length (kg/m)	0.248	4.275	0.656	0.118	0.769	0.198
BMI (kg/m^2)	0.337	4.012	0.598	0.128	0.381	0.325
PI (kg/m^3)	0.254	4.257	0.228	0.177	0.008	0.411
CGA Group 4 (n=111)						
Weight (kg)	0.170	4.460	0.577	0.300	0.813	0.305
Length (cm)	0.042	4.793	0.290	0.389	0.659	0.412
Weight/length (kg/m)	0.189	4.410	0.522	0.319	0.610	0.441
BMI (kg/m^2)	0.129	4.569	0.221	0.407	0.131	0.658
PI (kg/m^3)	0.031	4.819	0.007	0.460	0.025	0.697

BMI, body mass index; CGA, corrected gestational age; PI, ponderal index; RMSE, root mean square error

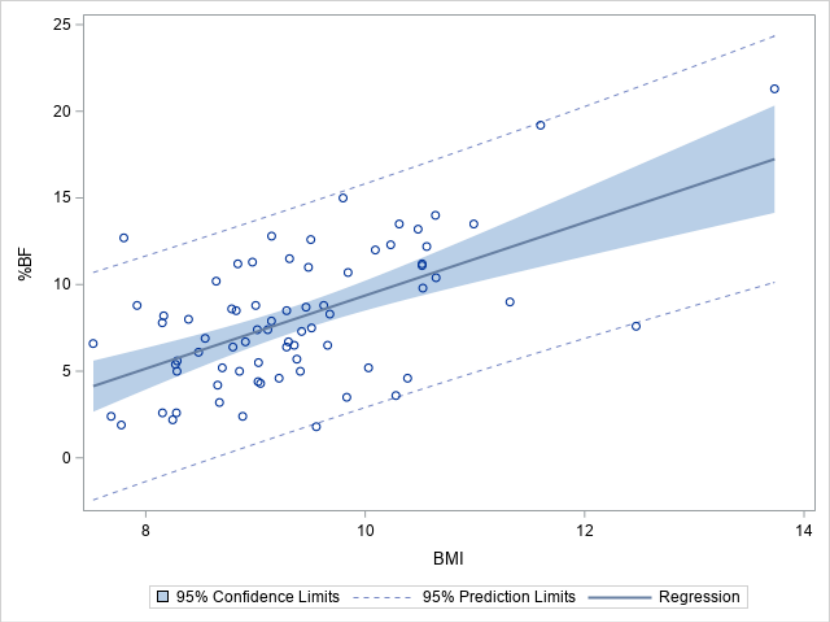

Figure 3.1 Scatterplot of the relationship between BMI and %BF in 74 preterm infants measured between 28-33 weeks corrected gestational age.

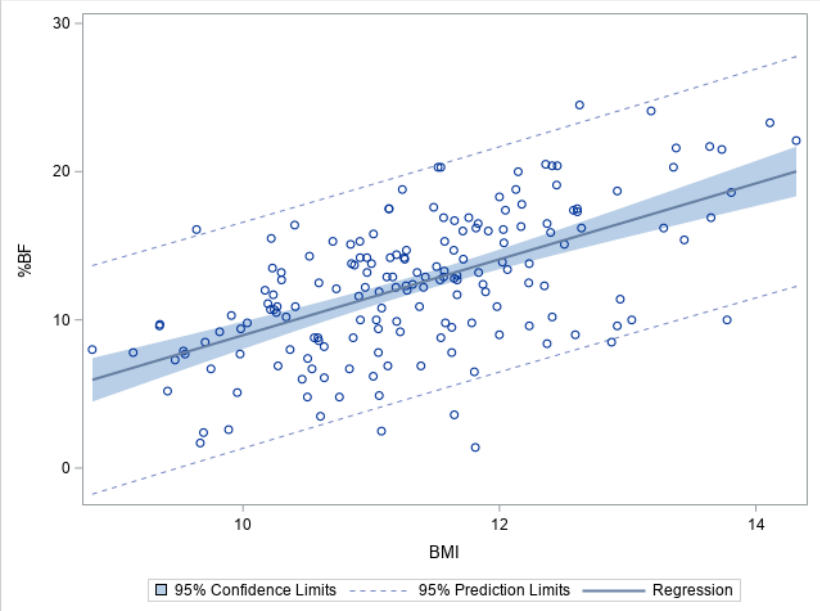

Figure 3.2: Scatterplot of the relationship between BMI and %BF in 175 preterm infants measured between 34-36 weeks corrected gestational age.

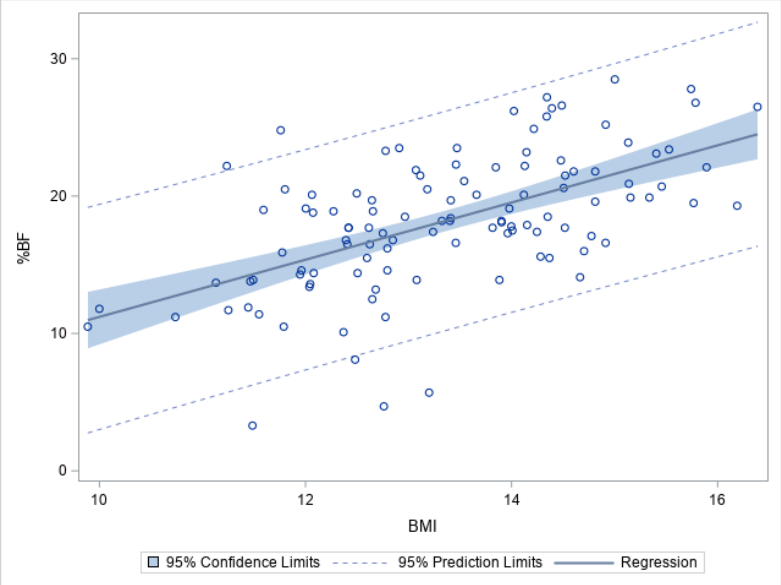

Figure 3.3 Scatterplot of the relationship between BMI and %BF in 113 preterm infants measured between 37-42 weeks corrected gestational age.

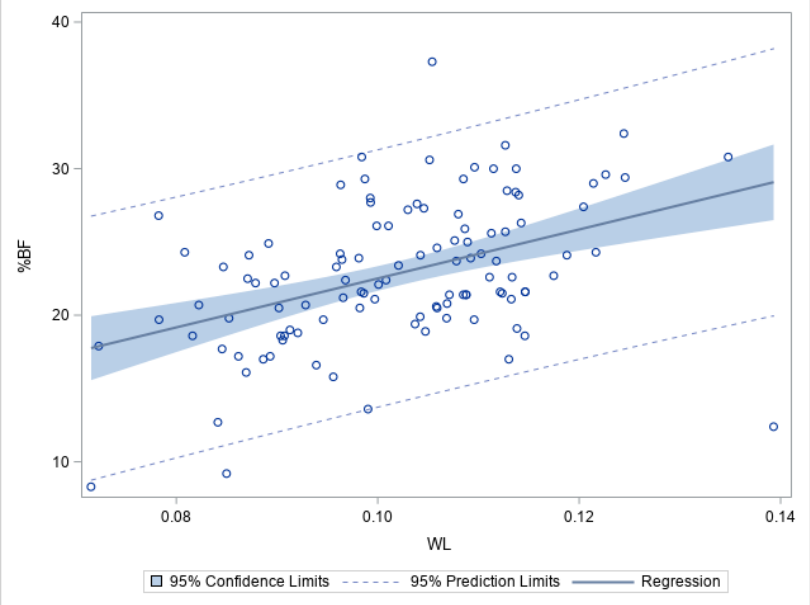

Figure 3.4 Scatterplot of the relationship between WL and %BF in 111 preterm infants measured between 43-63 weeks corrected gestational age.

Table 3.4: Relationship between predictors and FMI and FFMI in preterm infants by CGA group

	FMI		FFMI	
Predictor	R^2	RMSE	R^2	RMSE
CGA Group 1 (n=74)				
Weight/length (kg/m)	0.492	0.000034	0.804	0.000037
BMI (kg/m^2)	0.530	0.000032	0.830	0.000035
PI (kg/m^3)	0.456	0.000035	0.656	0.000049
CGA Group 2 (n=175)				
Weight/length (kg/m)	0.365	0.000051	0.606	0.000048
BMI (kg/m^2)	0.511	0.000045	0.659	0.000045
PI (kg/m^3)	0.470	0.000047	0.442	0.000057
CGA Group 3 (n=113)				
Weight/length (kg/m)	0.439	0.000062	0.567	0.000061
BMI (kg/m^2)	0.585	0.000053	0.663	0.000054
PI (kg/m^3)	0.430	0.000063	0.411	0.000071
CGA Group 4 (n=111)				
Weight/length (kg/m)	0.373	0.000084	0.308	0.000096
BMI (kg/m^2)	0.366	0.000084	0.568	0.000076
PI (kg/m^3)	0.163	0.000097	0.479	0.000083

CGA, corrected gestational age; FMI, fat mass index ($FM/length^2$); FFMI, fat-free mass index ($FFM/length^2$); RMSE, root mean square error

Table 3.5: Relationship between W/L indices and %BF by GA at birth and sex in 260 preterm infants <32 weeks vs ≥ 32 weeks GA at birth

	Body fat (%)				
Predictor	Estimate ± SE	95% CL	P-value	AICC	Df
Weight (kg)	3.30 ± 0.29	2.74, 3.86	<.0001	2764.0	467
Intercept	2.32 ± 1.06	0.24, 4.40	0.0287		467
GA	6.58 ± 1.10	4.42, 8.74	<.0001		467
Sex	-1.07 ± 0.83	-2.70, 0.56	0.1983		464
GA*Weight	-1.02 ± 0.30	-1.61, -0.43	0.0007		465
Sex*Weight	0.35 ± 0.21	-0.07, 0.76	0.1000		339
Length (cm)	0.80 ± 0.07	0.65, 0.95	<.0001	2821.4	466
Intercept	-26.4 ± 3.76	-33.7, -19.0	<.0001		467
GA	18.3 ± 3.81	10.8, 25.8	<.0001		467
Sex	-2.22 ± 2.47	-7.08, 2.65	0.3706		364
GA*Length	-0.29 ± 0.08	-0.44, -0.14	0.0002		467
Sex*Length	0.05 ± 0.05	-0.05, 0.14	0.3731		345
Weight/length (kg/m)	2.65 ± 0.21	2.24, 3.06	<.0001	2701.5	465
Intercept	-3.59 ± 1.38	-6.31, -0.87	0.0098		467
GA	8.05 ± 1.44	5.23, 10.88	<.0001		467
Sex	-1.17 ± 1.04	-3.21, 0.87	0.2613		426
GA*Weight/length	-0.81 ± 0.22	-1.24, -0.38	0.0002		467
sex*Weight/length	0.21 ± 0.15	-0.08, 0.51	0.1492		341
BMI (kg/m^2)	2.39 ± 0.18	2.04, 2.73	<.0001	2637.9	454
Intercept	-16.7 ± 2.21	-21.0, -12.3	<.0001		460
GA	10.7 ± 2.31	6.15, 15.2	<.0001		464
Sex	-0.89 ± 1.56	-3.96, 2.19	0.5706		384
GA*BMI	-0.69 ± 0.18	-1.05, -0.32	0.0002		462
sex*BMI	0.09 ± 0.12	-0.14, 0.33	0.4227		348
PI (kg/m^3)	1.37 ± 0.25	0.88, 1.86	<.0001	2989.7	433
Intercept	-21.5 ± 6.26	-33.8, -9.21	0.0006		438
GA	6.73 ± 6.50	-6.05, 19.5	0.3011		433
Sex	1.33 ± 4.21	-6.95, 9.61	0.7530		467
GA*PI	-0.22 ± 0.26	-0.72, 0.28	0.3909		431
Sex*PI	-0.06 ± 0.16	-0.37, 0.25	0.6897		467

AIC, Akaike information criterion, corrected; BMI, body mass index; GA, gestational age at birth; PI, ponderal index

< 32 wks: 191 infants, 386 measurements; ≥32 wks: 70 infants, 87 measurements

Table 3.6: Relationship between W/L indices and %BF by gestational age and sex in term infants versus preterm infants at term-corrected age

	Body fat (%)				
Predictor	Estimate ± SE	95% CL	P-value	AICC	Df
Weight (kg)	4.48 ± 0.26	3.97, 5.00	<.0001	2223.6	170
Intercept	-3.50 ± 1.33	-6.10, -0.88	0.0090		204
Term Status (TS)	16.4 ± 1.40	13.6, 19.1	<.0001		224
Sex	0.88 ± 1.27	-1.62, 3.37	0.4887		294
TS*weight	-2.97 ± 0.28	-3.52, -2.42	<.0001		187
Sex*weight	0.13 ± 0.25	-0.37, 0.63	0.6154		247
Length (cm)	1.15 ± 0.08	1.00, 1.31	<.0001	2325.7	184
Intercept	-46.7 ± 4.56	-55.7, -37.7	<.0001		185
TS	49.5 ± 4.66	40.3, 58.7	<.0001		190
Sex	2.88 ± 3.74	-4.48, 10.2	0.4413		275
TS*Length	-0.83 ± 0.08	-1.00, -0.67	<.0001		189
Sex*Length	-0.03 ± 0.07	-0.16, 0.11	0.6774		272
Weight/length (kg/m)	3.69 ± 0.21	3.28, 4.10	<.0001	2199.6	175
Intercept	-13.0 ± 1.79	-16.5, -9.45	<.0001		189
TS	22.0 ± 1.92	18.2, 25.8	<.0001		213
Sex	0.40 ± 1.74	-3.02, 3.83	0.8169		281
TS*weight/length	-2.38 ± 0.22	-2.82, -1.94	<.0001		197
Sex*weight/length	0.13 ± 0.20	-0.27, 0.53	0.5314		259
BMI (kg/m^2)	3.29 ± 0.20	2.91, 3.68	<.0001	2207.4	231
Intercept	-30.7 ± 2.94	-36.4, -24.9	<.0001		235
TS	30.3 ± 3.23	23.9, 36.6	<.0001		278
Sex	0.34 ± 2.99	-5.54, 6.23	0.9092		341
TS*BMI	-1.95 ± 0.21	-2.37, -1.53	<.0001		273
Sex*BMI	0.07 ± 0.20	-0.32, 0.46	0.7358		334
PI (kg/m^3)	1.65 ± 0.22	1.22, 2.08	<.0001	2242.3	383
Intercept	-24.9 ± 5.81	-36.4, -13.5	<.0001		383
TS	31.3 ± 6.19	19.2, 43.5	<.0001		383
Sex	7.38 ± 4.99	-2.43, 17.2	0.1400		383
TS*PI	-1.16 ± 0.23	-1.61, -0.70	<.0001		383
Sex*PI	-0.24 ± 0.18	-0.60, 0.11	0.1803		383

AIC, Akaike information criterion, corrected; BMI, body mass index; PI, ponderal index; TS, term status (1=term; 2=preterm); 163 infants, 224 measurements; term: 95 infants; 165 measurements

DISCUSSION

Weight for length indices consist of non-invasive anthropometric measures that are already obtained in the NICU on a routine basis. If reflective of adiposity, use of these indices would be a relatively simple process, potentially allowing clinicians to track the quality of weight gain throughout an infant's NICU stay. Monitoring weight quality via body composition in tandem with nutrition intervention may improve the overall ability of clinicians to optimize nutrition, promote neurodevelopment, and reduce the risk of obesity and metabolic disease.

While BMI was strongly correlated with FM and moderately correlated with %BF for all CGA groups except for group four, it was a poor predictor of %BF and FM for all CGA groups due to low R^2 values and large prediction errors. Of the W/L indices examined, BMI was the best predictor of %BF until CGA 43-65 weeks, where W/L was the best predictor of %BF. W/L indices of BMI and W/L had only moderate ability to predict an infant's whole body FM. These indices similarly predicted FM until preterm infants were of term-corrected age, when W/L (37-42 weeks CGA) or weight (43-63 weeks CGA) became better predictors.

Some have argued that use of FFMI and FMI are more useful than FFM and FM for comparison of adiposity in individuals because they are normalized for body size. Most of our predictors (W/L, BMI, PI) were calculated using length in the denominator, so we examined their ability to predict two of our outcomes on the same general scale. FFMI and FMI are essentially length-normalized indices of BMI, so it was not surprising that BMI was the best predictor of both outcomes. However, because of its ability to predict both outcomes, an elevated BMI could represent either increased FM or FFM.

Our results align with the results of our previous study and other similar studies, which found that BMI was a poor predictor for %BF and had large prediction errors.[10,111,115] A study by Roy et al. found that high BMI z-scores in early infancy (for term infants) had a greater association with later childhood obesity than W/L z-scores, indicating that although BMI is not an adequate surrogate for adiposity, it may be worth monitoring in children younger than 2 years old.[116] Furthermore, BMI measurements were more consistent than W/L measurements over time. In contrast, a study by Aris et al. did not find a difference between the ability of BMI and W/L to predict cardiometabolic outcomes in early adolescence, although adiposity was assessed using bioimpedance, which may have underestimated FM.[117]

In the clinical setting, W/L is used to assess the appropriateness of infant weight gain until age two, but BMI may be a better indication of adiposity until a preterm infant reaches 43 weeks corrected age. However, the WHO W/L growth charts do not account for infant age, which may be problematic for preterm infants, who have a greater amount of FM (and greater %BF) than their peers at term-corrected age but may be shorter in length. Unfortunately, prediction errors may prevent use of BMI to provide estimates of %BF at the individual level. Additionally, in infants and children, BMI can represent a large variation in %BF.[118]

BMI was a strong predictor for FFM between 28-33 weeks CGA, but the effect diminished with increasing age as FM and %BF increased. Weight and length were consistently good predictors of FFM across all CGA groups, which is not surprising for the younger CGA groups given that the majority of an infant's weight consists of FFM. The predictive ability of these indices decreased as preterm infants grew older, which is expected as their %BF increased.

We found that GA at birth (< 32 weeks or ≥ 32 weeks) was a significant predictor for %BF in models containing W/L indices, GA, and sex with significant interaction between GA and W/L indices (except for PI). This indicates that the predictive ability of W/L indices is affected by early versus late preterm status and should be considered as a covariate in future analyses. We also found a significant interaction between term status and W/L indices when comparing predictive ability in term infants versus preterm infants at term-corrected age. This indicates that the ability of W/L indices to predict %BF is different for term infants than in preterm infants at term-corrected age.

Our study was limited by the fact that a significant proportion of infants did not have race reported. Thus, we are unable to comment on the applicability of our results to other populations. Our study also contained twins and triplets whose measurements may have been correlated. We examined only single predictors in our study, but inclusion of additional covariates such as CGA and sex may have improved the predictive ability of our models.

To our knowledge, this is the first study to examine the relationship between W/L indices and body composition outcomes in preterm infants ≥ one week through 63 weeks CGA. We have confirmed that thus far, we do not have a way to measure adiposity in preterm infants, and weight and BMI are not adequate surrogates for adiposity. Our study results emphasize the importance of continued exploration of bedside body composition methods to help assess disease risk and improve neurodevelopmental outcomes in preterm infants.

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CHAPTER 4: ULTRASOUND MEASUREMENTS OF MUSCLE AND ADIPOSE TISSUE THICKNESS ARE NOT SURROGATES FOR WHOLE BODY COMPOSITION IN PREMATURE INFANTS

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CHAPTER SYNOPSIS

Background and Objectives: Premature infants are at risk for adverse metabolic and neurodevelopmental outcomes due to growth alterations in early infancy. Monitoring body composition by tracking gains in fat mass and fat-free mass may assist clinicians in preventing obesity and metabolic disease while promoting optimal growth and development. A prospective, observational study was conducted to determine the ability of ultrasound measurements of muscle and adipose tissue thickness to predict whole body composition, including fat-free mass, fat mass, and percent body fat.

Methods: Sixty-eight (n=68) healthy premature infants (mean gestational age at birth = 32 weeks) were recruited from the Neonatal Intensive Care Unit at the University of Minnesota. Anthropometric measurements, air displacement plethysmography, and ultrasound measurements of the biceps, abdomen, and quadriceps were conducted when infants were medically stable. Intra-rater reliability of ultrasound measurements was evaluated using percent coefficient of variation and intraclass correlation coefficient. The relationship between ultrasound measurements and body composition was assessed using Pearson's correlation coefficient and linear regression analysis.

Results: Ultrasound measurements of muscle and adipose tissue showed good to excellent intra-rater reliability (ICC = 0.87-0.98; %CV = 4.8-13.5%). Biceps (r = 0.56) and quadriceps muscle (r = 0.51) thickness were moderately but significantly correlated with fat-free mass ($p < .0001$). Adipose tissue thickness of the biceps (r = 0.43; r = 0.32), abdomen (r = 0.43; r = 0.34), and quadriceps (r = 0.40; r = 0.28) were weakly but significantly correlated with fat mass ($p < .01$) and percent body fat ($p < .05$). In stepwise linear regression analysis, biceps adipose and the sum of adipose thickness measurements were significant predictors of %BF, but prediction models had low R^2 and high RMSE. US measurements of muscle thickness were not predictive of whole body FFM.

Conclusions: Ultrasound measurements of biceps, abdomen, and quadriceps muscle and adipose tissue thickness are not adequate surrogates for whole body composition in preterm infants. Exploration of alternate measurement sites may improve predictive ability.

INTRODUCTION

Premature infants are at risk for obesity and metabolic disease because of altered growth, including increased adiposity, decreased fat-free mass (FFM) gains, and decreased linear growth for the first two years of life.[3–6] Catch up growth or a rapid period of growth in early infancy may also increase their risk for later insulin resistance, obesity, and cardiovascular disease.[22,101–103] Our previous research found that higher gains in fat mass (FM) from discharge until 4 months of age are positively associated with blood pressure at 4 years of age.[9] Additionally, we discovered that FFM is a better predictor of neurodevelopmental outcomes than weight, and gains in FFM (but not FM) are related to improved neurodevelopmental outcomes.[6,8,22,24] Little is known about the long-term consequences of growth alterations premature infants experience in the neonatal intensive care unit (NICU), but monitoring changes in body composition by tracking gains in FM and FFM may help assess their risk for later adverse metabolic and neurodevelopmental outcomes.

Currently, body composition is not routinely monitored in the NICU. While magnetic resonance imaging (MRI),[29] isotope dilution techniques, air displacement plethysmography (ADP), and dual energy x-ray absorptiometry (DXA) have been studied in premature infants, these methods are not feasible for routine use in the NICU because they require respiratory/medical stability, involve exposure to ionizing radiation, or are expensive.[119,120] Bedside methods such as skinfold thickness and bioimpedance analysis have been proposed to assess body composition in premature infants but have not been validated and have limitations that prevent their routine use in this population. In adolescent and adult populations, ultrasound (US) measurements of muscle and adipose tissue thickness have been used to predict whole body composition.[85,90,92] US is an ideal method for use in premature infants because it is portable, non-invasive,

involves no ionizing radiation, and is suitable for repeated measurements regardless of medical stability. US also has the potential for body composition assessment throughout the life cycle but has not been thoroughly examined in premature infants. In this study, we aimed to determine if US measurements of muscle and adipose tissue thickness are predictive of whole body composition (FFM, FM, percent body fat (%BF)) in premature infants.

METHODS

This study was approved by the University of Minnesota Institutional Review Board. Healthy premature infants were recruited from the University of Minnesota Masonic Children's Hospital Neonatal Intensive Care Unit between November 2018 and May 2019. Study inclusion criteria were birth between 25 and 34 + 6 weeks gestational age (GA), medical and respiratory stability, and independence from central intravenous catheters at time of measurement. Parents of the infants provided informed consent. During the recruitment period, 221 eligible infants were admitted to the NICU. Of the infants not consented to the study, 64 declined, 70 were unable to be consented, 18 transferred, and 1 died. Sixty-eight healthy premature infants were consented. Of these, one patient was transferred to an outside facility before measurements could be conducted. One infant had insufficient body fat for measurement via ADP, one infant was discharged before measurements were conducted, and two did not have complete ultrasound measurements available for analysis. Sixty-three (n=63) infants remained and were included in this study.

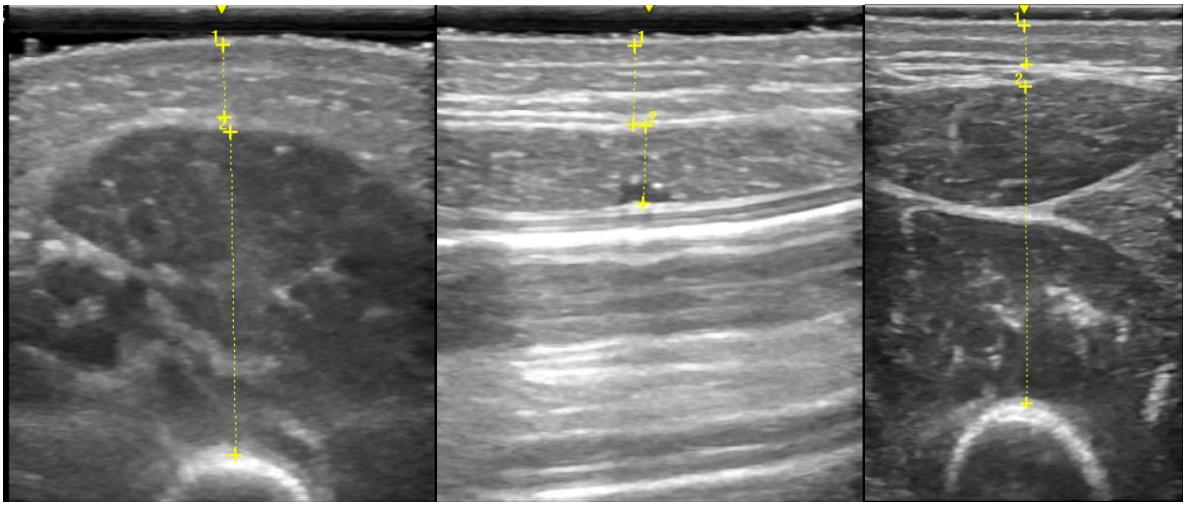
The Pea Pod (Cosmed Ltd, Concord, California) was used to conduct ADP. The Pea Pod's operating procedures have been described elsewhere.[44–46,121] Briefly, each infant's length was obtained to the nearest 0.1 cm using a recumbent length board. The

infant was then weighed on the Pea Pod's electronic scale to the nearest 0.0001 kg followed by an approximately 2-minute body volume measurement in the device's test chamber. The body volume measurement, the known density of fat (0.9007 g/mL), and Fomon's[34] or Butte's[35] age and sex specific densities for FFM were then used in the Pea Pod's calculation of %BF. All measurements were taken by a consistent study team.

US images of the biceps (brachii and brachialis), abdomen (rectus abdominus), and quadriceps (vastus intermedius and rectus femoris) and were taken in triplicate on the left side of the infant's body using a portable B-mode ultrasound device (NextGen LOGIQ e R7, GE Medical Systems, Chicago, IL) and high-resolution linear array transducer (LS 10-22-RS, GE Medical System, Chicago, IL). Ultrasound methodology for adults has been previously published but to our knowledge, no protocol for infants currently exists.[81–83,122] Therefore, the following methodology was adapted from adult guidelines. All measurements were taken by one trained operator while the infant was in a supine position. Measurements were conducted using zero compression by resting the transducer on a thick layer of US gel. Ultrasound measurements of the biceps were taken with the infant's palm facing upward and arm positioned slightly away from the body. Point of measurement was determined by visualizing the halfway point between the acromion and antecubital crease. US measurements of the abdomen were taken midway between the costal margin and anterior superior iliac crest, immediately to the left of umbilicus. Measurements were taken during the expiration portion of the respiratory cycle to prevent artificial inflation of abdominal adipose thickness. US measurement of the quadriceps were taken with the infant's knee extended and quadriceps muscle in a relaxed state. To obtain measurements, the transducer was placed approximately halfway between the anterior superior iliac spine and the superior

patellar border. After each US image was obtained, adipose tissue and muscle thickness were measured using electronic calipers (**Figure 1**). The mean of three measurements was used for calculations. If three measurements were unable to be obtained, the mean of two measurements or one measurement was used instead.

Figure 4.1: Ultrasound images of the biceps, abdomen, and quadriceps



a) Biceps b) Abdomen c) Quadriceps

Electronic calipers indicate adipose thickness (1) and muscle thickness (2)

Statistical Analysis

SAS 9.4 (SAS Institute, Cary, North Carolina) was used to perform statistical analyses. Intra-rater reliability was assessed by percent coefficient of variation (%CV) and intraclass correlation coefficient (ICC).[123] The relationship between US measurements and whole body composition was assessed using Pearson's Correlation Coefficient. To determine the ability of individual US measurements to predict FFM, FM, or %BF, simple linear regression analysis was first employed. Stepwise linear regression analysis was

then used to assess the ability of individual US measurements to predict outcomes of FM, FFM, and %BF in the presence of other predictors, including gestational age at birth, corrected age at measurement, weight, and length, and sex. The sum of US measurements was also considered as a predictor of outcomes in a separate analysis. The criterion for variable entry into a given model (significance level for entry, SLE) was set at $p \leq 0.15$, and the criterion for a variable to stay in the model (significance level for staying, SLS) was set at 0.05. The best model for a given outcome was that which explained the highest proportion of variance (R^2) with the lowest root mean square error (RMSE).

Because the study population included eleven sets of twins and three sets of triplets, a sensitivity analysis was performed. One member of each siblingship was selected for inclusion in the analysis, and the models were re-run and assessed for differences from the results of the original analysis.

RESULTS

Participant Characteristics

Characteristics of infants who participated in the study are described in Table 4.1. Of the 68 infants enrolled in the study, 63 had ultrasound measurements at all three sites and body composition values generated via ADP. The majority of infants were female (56%) and white (76%). Mean gestational age at birth was 32 weeks (± 2.24 weeks, SD). Mean weight at time of measurement was 2165 grams (± 360.7 grams, SD), while mean length was 44.1 cm (± 2.27 cm, SD). Mean corrected age at measurement was 35.1 weeks (±1.19 weeks, SD).

Reliability

Intra-rater reliability of ultrasound measurements is shown in Table 4.2. Ultrasound measurements at all sites had %CV between 4.8-13.5%, with the highest %CV reported for biceps adipose thickness. ICC for all ultrasound measurements ranged from 0.874-0.975.

Table 4.1: Descriptive characteristics of preterm infants (n=63)

Variable	Data[a]
Gestational age at birth (weeks)	32.0 (2.24)
Sex (male)	28 (44%)
Race	
White	48 (76.2%)
Black	6 (9.52%)
Other	9 (14.3%)
Corrected age at measurement (weeks)	35.1 (1.19)
Weight (grams)	2165 (360.7)
Length (cm)	44.1 (2.27)
FFM (grams)	1973.9 (325.3)
FM (grams)	191.5 (94.5)
%BF	8.72 (3.88)
Biceps adipose thickness (mm)	1.85 (0.49)
Biceps muscle thickness (mm)	7.90 (1.25)
Abdominal adipose thickness (mm)	2.21 (0.61)
Abdominal muscle thickness (mm)	2.46 (0.61)
Quadriceps adipose thickness (mm)	2.51 (0.80)
Quadriceps muscle thickness (mm)	11.3 (2.04)

[a]Continuous variables expressed as mean (standard deviation) and categorical variables as n (percentage)

Table 4.2: Intra-rater reliability of ultrasound measurements

Measurement Site	%CV[a]	ICC[b]
Biceps adipose thickness	13.5	0.873
Biceps muscle thickness	3.86	0.975
Abdominal adipose thickness	10.6	0.930
Abdominal muscle thickness	8.83	0.949
Quadriceps adipose thickness	4.8	0.963
Quadriceps muscle thickness	9.2	0.968

[a]Percent coefficient of variation
[b]Intraclass correlation coefficient

US measurements of muscle thickness and whole body FFM

Both biceps muscle (*r*=0.56) and quadriceps muscle (*r* = 0.51) were moderately but significantly correlated with FFM ($p < .05$) (**Table 4.3**). Table 4.6 shows the relationship between individual US measurements of muscle thickness and whole body FFM. All models had low R^2 values. Table 4.7 shows the results of stepwise regression analysis. The best linear regression model had an adjusted $R^2 = 0.94$ and included weight, length, and gestational age at birth. None of the US measurements of muscle were selected for model inclusion because they did not meet SLE requirements. Similarly, in a separate analysis, the sum of all muscle thickness measurements (USM) was not selected in stepwise regression analysis and was therefore excluded from the best-fitting model.

Table 4.3: Pearson's correlation coefficients for US measurements of muscle thickness and whole body FFM

Measurement site (mm)	FFM
Biceps muscle	*0.56
Abdominal muscle	0.23
Quadriceps muscle	*0.51

*indicates significance at $p < .0001$

US measurements of adipose tissue thickness and whole body FM and %BF

Biceps adipose tissue (*r* =0.43), abdominal adipose tissue (*r* =0.43), and quadriceps adipose tissue (*r* =0.40), were moderately but significantly correlated with whole body FM **(Table 4.4**). All three adipose sites were also weakly but significantly correlated with %BF. Table 4.5 shows models generated during simple linear regression of individual US measurements of adipose thickness on FM and %BF. All models had low R^2, and the models for %BF had greater RMSE values than the models for FM. In stepwise linear regression analysis, the final regression model for FM had a low R^2 value (0.34) and

included weight, length, and gestational age at birth (**Table 4.8**). In a separate regression analysis, the sum of all adipose thickness measurements (USA) was not selected in the final model, which included weight, length, and gestational age at birth. For %BF, biceps adipose tissue thickness and gestational age were included in the final model, which had a low R^2 (0.17) and high prediction error (RMSE = 3.54) (**Table 4.9**). When the sum of all US adipose thickness measurements (USA) was tested as a predictor in a separate analysis, the resulting model included USA and gestational age at birth but had low R^2 (0.16) and high RMSE (3.57).

Table 4.4: Pearson's correlation coefficients for US measurements of adipose tissue and whole body FM and %BF

Measurement site (mm)	FM	%BF
Biceps adipose	*0.43	*0.32
Abdominal adipose	*0.43	*0.34
Quadriceps adipose	*0.40	*0.28

*indicates significance at $p < .05$

Sensitivity Analysis

Results of the sensitivity analysis showed slight differences in models for prediction of FM and %BF from USA (**Supplemental Table 4.1**). When only one member of each siblingship was selected for inclusion in data analysis, USA and CGA were included in the final model for prediction of FM from the sum of US adipose thickness measurements USA and length were included in the final model predicting %BF from sum of US adipose thickness measurements

DISCUSSION

Body composition, while linked to neurodevelopmental and metabolic outcomes in preterm infants, is not routinely measured in the NICU due to limitations of currently available tools. Ultrasound, a non-invasive, portable device is highly desirable for measurement of body composition in a medically fragile population. In this study, we have shown that bedside ultrasound can detect and measure muscle and adipose tissue thickness in premature infants with good to excellent reliability (ICC). Variability relative to the mean of measurements (%CV) was highest for biceps adipose tissue thickness (13.5%), also the smallest of all muscle and adipose tissue thickness measurements. Our results are in contrast to those of McLeod and colleagues,[95] who found that %CV for US measurements in preterm infants was highest for thigh adipose tissue and lowest for midarm adipose tissue and mid-arm muscle tissue. This may be because their study population was slightly younger (mean gestational age of 27 weeks), and infants were older when measurements were taken (mean corrected age at measurement of 40 weeks). This likely resulted in larger mean measurements of muscle and adipose tissue at each site. While McLeod measured 6 different sites (abdomen, subscapular, anterior and posterior arm, mid-arm, posterior and anterior thigh, and mid-thigh), ultrasound measurements were not compared with whole body composition. The only other study examining ultrasound in premature infants did not report reliability.[93]

We found that ultrasound measurements of biceps and quadriceps muscle thickness were moderately and significantly correlated with whole body FFM but were not significantly predictive when covariates of weight, length, gestational age at birth, and corrected age at measurement were included. A preterm infant's weight is comprised predominantly of FFM and not surprisingly had the strongest effect on whole body FFM.[10] Gestational age was included in the model and has a strong association

with FFM; infants born at an earlier gestational age have less absolute FFM.[124] The sum of all muscle thickness measurements was not substantial enough to offset the effect of weight on FFM, likely explaining its exclusion from the best-fitting model.

Measurements of adipose tissue at all three sites were moderately and significantly correlated with whole body FM and weakly correlated with %BF, indicating the possibility that adipose thickness may be predictive of these outcomes. When competing against other, more easily measured anthropometric measures of body composition like weight and length, no US measurement of adipose thickness was retained in the final model, indicating none were predictive of whole body FM. However, biceps adipose thickness (BA) was selected in the regression model for %BF. Nonetheless, the low R^2 and moderately high RMSE of the model limits confidence in the predictive ability of these measures. Similarly, the sum of adipose tissue thickness (USA) at all three sites was selected for the model for %BF but had low R^2, again indicating low predictive ability.

Although the final models for prediction of FM and %BF from USA were slightly different upon inclusion of only one member of each set of twin and triplets in the data set, both models still had low variance explained and similar prediction error. Since no significant model improvement was seen with the inclusion of all members of each siblingship, the final data set contained no exclusions.

Our ultrasound sites were selected based on adolescent and adult studies which found that muscle and adipose tissue thickness of the abdomen and quadriceps were representative of whole body composition.[83,90] We also selected sites that would be simple to measure on a premature infant in the supine position. Our results are similar to those of Ahmad and colleagues, who found that the cross-sectional area of calf muscle

(r = 0.63) and adipose tissue (r = 0.67) but not thickness were significantly correlated with whole body composition.[125]

While other studies have found that FM differs based upon an infant's sex, we did not note any differences in body composition for males in comparison to females, likely because the mean corrected age of the infants in our study was 35 weeks, and sex-specific differences may not be noted until later in life.[126]

Our study was limited by the number of infants admitted to our NICU during the recruitment period, and our results may not be not representative of more diverse populations. Our study patients included twelve sets of twins and three sets of triplets, but we did not adjust for correlation of data due to lack of significant differences in our sensitivity analysis. We limited the number of ultrasound sites to three that were practical to obtain while the infant was in a supine position, but it is possible that inclusion of posterior region sites would have led to better-fitting models and should be considered in future studies. Furthermore, inclusion of measures of cross-sectional area may be more representative of whole body composition and should be explored.

While we examined intra-rater reliability of ultrasound measurements, we did not assess inter-rater reliability due to time constraints. Infants were measured between feeding and care times and needed to be transported to a research room for US and ADP measurements. Because of the need for transport, nursing staff preferred that measurements occur immediately before feedings to prevent emesis and loss of nutrition. Consequently, infants became fussy when measured for a prolonged period of time, which would most likely affect additional ultrasound measurements taken during a study session. In the future, inter-rater studies would strengthen the reliability of

ultrasound measurements and would help facilitate the development of a consensus protocol for the use of US as a bedside assessment tool in this population.

To our knowledge, our study is the first to correlate ultrasound measurements at multiple sites with whole body composition in premature infants. While measures of muscle and adipose thickness at the selected sites were not adequate proxies for whole body FFM, FM, or %BF, we were able to successfully modify adolescent and adult US protocols for use in premature infants, paving the way for future studies which may explore additional measures.

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Table 4.5: Association of individual US measurements of adipose tissue thickness with FM and %BF in 63 preterm infants measured at mean corrected age of 35 weeks

Variable	P-value	Regression equation for FM	Model Adj.R^2	Model RMSE (kg)
Biceps (BA)	0.0004	0.08*BA+0.04	0.18	0.09
Abdomen (AA)	0.0004	0.07*AA+0.04	0.17	0.09
Quadriceps (QA)	0.0012	0.05*QA+0.07	0.34	0.08
	P-value	Regression equation for %BF	Model Adj.R^2	Model RMSE (kg)
Biceps (BA)	0.0101	2.55*BA+4.0	0.09	3.70
Abdomen (AA)	0.0064	2.15*AA+3.96	0.10	3.68
Quadriceps (QA)	0.0250	1.37*QA+5.27	0.06	3.75

%BF, percent body fat; AA, abdominal adipose; BA, biceps adipose; FM, fat mass; GA, gestational age at birth; RMSE, root mean square error; US, ultrasound

Table 4.6: Association of individual US measurements of muscle thickness with FFM in 63 preterm infants measured at mean corrected age of 35 weeks

Variable	P-value	Regression equation for FFM	Model Adj.R^2	Model RMSE (kg)
Biceps (BM)	<.0001	0.15*BM+0.82	0.31	0.27
Abdomen (AM)	0.0584	0.12*AM+1.67	0.03	0.32
Quadriceps (QM)	<.0001	0.08*QM+1.06	0.25	0.28

AM, abdominal muscle; BM, biceps muscle; FFM, fat-free mass; gestational age at birth; QA, quadriceps muscle; RMSE, root mean square error; US, ultrasound

Table 4.7: Stepwise regression analysis for prediction of FFM from US measurements of muscle thickness and USM and weight, length, and GA in 63 preterm infants at mean corrected age of 35 weeks

Step and variable	Regression equation for FFM	Model Adj.R^2	Model RMSE (kg)
1. Weight	0.87*weight+0.08	0.93	0.08
2. Length	0.73*weight+0.03*length-0.77	0.94	0.08
3. GA	0.78*weight+0.02*length+0.01*GA-0.74	0.94	0.08

Step and variable	Regression equation for FFM	Model Adj.R^2	Model RMSE (kg)
1. Weight	0.87*weight+0.08	0.93	0.08
2. Length	0.73*weight+0.03*length-0.77	0.94	0.08
3. GA	0.78*weight+0.02*length+0.01*GA-0.74	0.94	0.08

FFM, fat-free mass; GA, gestational age at birth; RMSE, root mean square error; US, ultrasound, USM, sum of ultrasound muscle measurements

Table 4.8: Stepwise regression analysis for prediction of FM from US measurements of adipose tissue thickness and USM, weight, length, and GA in 63 preterm infants at mean corrected age of 35 weeks

Step and variable	Regression equation for FM	Model Adj.R^2	Model RMSE (kg)
1. Weight	0.13*weight-0.08	0.22	0.08
2. Length	0.27*weight-0.03*length+0.77	0.33	0.08
3. GA	0.22*weight-0.02*length-0.01*GA+0.74	0.34	0.08
Step and variable	Regression equation for FM	Model Adj.R^2	Model RMSE (kg)
1. USA	0.03*USA+0.01	0.22	0.08
2. Weight	0.02*USA+0.08*weight-0.09	0.27	0.08
3. Length	0.01*USA+0.22*weight-0.02*length+0.65	0.33	0.08
4. USA removed	0.27*weight-0.03*length+0.77	0.33	0.08
5. GA	0.22*weight-0.02*length-0.01*GA+0.74	0.34	0.08

FM, fat mass; GA, gestational age at birth; RMSE, root mean square error; US, ultrasound; USA, sum of ultrasound measurements

Table 4.9: Stepwise regression analysis for prediction of %BF from US measurements of adipose tissue thickness and USA and GA in 63 preterm infants at mean corrected age of 35 weeks

Step and variable	Regression equation for %BF	Model Adj.R^2	Model RMSE (%BF)
1. GA	-0.62*GA+28.7	0.12	3.65
3. Biceps (BA)	-0.54*GA+2.07*BA +22.0	0.17	3.54
Step and variable	Regression equation for %BF	Model Adj.R^2	Model RMSE (%BF)
1. USA	0.86*USA+3.05	0.12	3.64
2. GA	0.62*USA-0.44*GA+18.8	0.16	3.56

%BF, percent body fat; BA, biceps adipose; GA, gestational age at birth; RMSE, root mean square error; US, ultrasound; USA, sum of ultrasound adipose measures

Supplemental Table 4.1: Sensitivity Analysis: stepwise regression analysis for prediction of FM and %BF from the sum of US measurements of adipose tissue thickness, CGA, and length in 63 preterm infants at mean corrected age of 35 weeks

Step and variable	Regression equation for FM	Model Adj.R^2	Model RMSE (%BF)
1. USA	0.03*USA+0.01	0.21	0.08
2. CGA	0.02*USA+0.02*CGA-0.65	0.25	0.08
Step and variable	Regression equation for %BF	Model Adj.R^2	Model RMSE (%BF)
1. USA	0.74*USA+3.65	0.09	3.62
2. Length	1.0*USA-0.47*length+22.9		

FM, fat mass; GA, gestational age at birth; RMSE, root mean square error; US, ultrasound; USA, sum of ultrasound measuremen

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

CONCLUSIONS

Preterm infants experience altered growth patterns that may have later adverse effects on metabolic and neurodevelopmental health. Monitoring weight gain quality in response to nutritional provision as preterm infants grow may help clinicians decrease the risk for the development of obesity and other metabolic diseases while promoting optimal neurodevelopmental health.

Some researchers have proposed the use of weight for length indices to monitor growth, but an evaluation of these indices (W/L, BMI, PI) reveals that none is an adequate surrogate for adiposity in preterm infants from 30-63 weeks corrected age. Thus, a bedside method for assessing body composition in preterm infants is highly desirable and requires further exploration.

The primary purpose of this dissertation was to explore body composition methods in preterm infants, specifically ultrasound, a noninvasive bedside method which is relatively simple to conduct and does not require medical stability of the infant. When comparing ultrasound measures of biceps, abdomen, and quadriceps adipose and muscle thickness with whole body composition (FM, FFM, %BF) via ADP, we found that US measurements were not able to provide precise predictions of body composition due to low variation explained and/or high prediction error of resulting models. Inclusion of additional measurement sites or exploration of additional measures such as cross-sectional area may improve the predictive ability of ultrasound measures.

This dissertation work adds to previous research that found that weight for length indices were not predictive of adiposity in preterm infants within 72 hours after birth. This work demonstrates that weight for length indices cannot be used as proxies for adiposity in preterm infants ≥ 7 days after birth through 63 weeks corrected gestational age. However, it may be useful to monitor BMI, which is not currently tracked in infants or children less than 2 years old, as it was more predictive of FM and %BF than weight for length from 30-42 weeks corrected age.

While ultrasound measurements at the biceps, abdomen, and quadriceps were not adequate surrogates for whole body composition in preterm infants, we have shown that measurement protocol can be successfully adapted from adult and adolescent protocols. Additionally, there may be some prognostic value in individual ultrasound measurements, namely whether they influence factors related to NICU discharge, such as time to full oral feeds. Continued exploration of bedside methods for assessment of

neonatal body composition, including the pursuit of alternate ultrasound measurement sites, is warranted in order to find a suitable method for tracking weight quality in response to nutritional provision.

FUTURE DIRECTIONS

This dissertation work provides a foundation for future studies that further explore the use of ultrasound measurements for prediction of whole body composition and clinical outcomes. The following sections contain a brief glimpse of some of the work to be conducted.

Reliability of ultrasound measurements and longitudinal measurements

While this dissertation explored intra-rater reliability via ICC and CV%, inter-rater reliability was not assessed due to logical constraints, including limited tie for measurements with the infants. To strengthen the results of future studies and confirm the appropriateness of our protocol, inter-rater studies should be conducted. Furthermore, if the goal is to find a bedside tool for routine assessment of body composition, the feasibility of longitudinal measurements should be investigated.

Ability of ultrasound measurements of muscle thickness to predict neurodevelopmental outcomes

Previous research has shown that gains in FFM (but not FM) are associated with improved neurodevelopmental outcomes in preterm infants at 1 year corrected age.[8] Whether changes in muscle tissue thickness, a more specific component of FFM, are also associated with improved neurodevelopmental outcomes is unknown. Ultrasound measurements should be taken longitudinally in the NICU with at least one timepoint on an outpatient basis after the infant reaches term-corrected age to assess this relationship. If proven to be predictive of neurodevelopmental outcomes, changes in measurements of muscle thickness could be tracked in response to nutritional intervention. This may ultimately help clinicians to improve neurodevelopmental outcomes for preterm infants.

Ability of ultrasound measurements to predict metabolic outcomes and corrected age at discharge from the NICU

A preterm infant's discharge from the NICU is dependent on a myriad of factors such as respiratory stability, adequate growth, and the ability to consume oral feeds. We

conducted a secondary analysis of ultrasound measurements of muscle and adipose tissue thickness to predict systolic and diastolic blood pressure at discharge and corrected age at discharge.

Data from forty-six preterm infants originally recruited from the University of Minnesota NICU for a study on ultrasound measurements and whole body composition was used for this analysis. Ultrasound measurements of the biceps, abdomen, and quadriceps were taken when infants were medically stable. Stepwise linear regression analysis was used to determine the ability of ultrasound measurements to predict clinical outcomes with covariates of gestational age at birth, weight, length, and corrected age at measurement. We also evaluated the relationship between the sum of ultrasound measurements and outcomes. The best model for a given outcome was that with had the highest R^2 and lowest root mean square error (RMSE).

Preliminary Data

In linear regression analysis, biceps adipose thickness was a significant predictor of diastolic blood pressure (p=0.0386) but the final prediction model had low R^2 (0.07) and high error (RMSE = 8.59). Adipose tissue thickness was not predictive of SBP.

In linear regression analysis, abdominal muscle was significantly predictive of corrected age at discharge (p=0.0096) with R^2=0.30. For every 1 mm increase in abdominal muscle, corrected age at discharge decreased by 0.89 weeks. In a separate analysis, the sum of ultrasound measurements of muscle thickness was also a significant predictor of corrected age at discharge (p=0.0079) with R^2=0.26. For every 1 mm increase in the sum of muscle thickness measurements, corrected age at discharge decreased by 0.23 weeks. These results are shown in Table 5.1. Adipose tissue was not significantly predictive of corrected age at discharge (p=0.1124), but biceps adipose was included in the final prediction model (**Table 5.2**).

Should ultrasound measurements of adipose tissue thickness prove to be predictive of metabolic outcomes, they may be a useful in monitoring disease risk in preterm infants. While abdominal muscle and the sum of ultrasound muscle measurements were significantly predictive of corrected age at discharge and were included in final regression models, low R^2 and moderate RMSE limit their predictive ability. Evaluation of additional US sites is warranted to determine if they can yield increased R^2 and decreased RMSE. Should the ability of ultrasound measurements of

muscle thickness to predict corrected gestational age at discharge improve, ultrasound may be a useful tool for prediction of discharge from the NICU.

Table 5.1: Stepwise regression analysis for prediction of corrected age at discharge from US measurements of muscle thickness and USM, GA, weight, and length in 47 preterm infants at mean corrected age of 35 weeks

Predictor	Estimate ± SE	P-value	Model Adj.R^2	Model RMSE
			0.30	1.41
GA	-0.49 ± 0.12	0.0317		
CGA	0.95 ± 0.26	0.0326		
AM	-0.89 ± 0.34	0.0096		
Weight	-1.72 ± 0.90	0.0618		
Predictor	Estimate ± SE	P-value	Model Adj.R^2	Model RMSE
			0.26	1.45
GA	-0.23 ± 0.11	0.0317		
CGA	0.67 ± 0.20	0.0326		
USM	-0.23 ± 0.08	0.0079		

AM, abdominal muscle thickness; CGA, corrected gestational age at measurement; GA, gestational age at birth; RMSE, root mean square error; US, ultrasound; USM, sum of ultrasound muscle measurements

Table 5.2: Stepwise regression analysis for prediction of corrected age at discharge from US measurements of adipose thickness and GA, CGA, and weight in 47 preterm infants at mean corrected age of 35 weeks

Predictor	Estimate ± SE	P-value	Model Adj.R^2	Model RMSE
			0.24	1.47
GA	-0.36 ± 0.12	0.317		
CGA	0.85 ± 0.27	0.0326		
Weight	-2.61 ± 1.02	0.0475		
BA	0.86 ± 0.53	0.1124		

BA, biceps adipose thickness; CGA, corrected gestational age at measurement; GA, gestational age at birth; RMSE, root mean square error; US, ultrasound

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