Body Composition Assessment after Pediatric Liver Transplant

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Alissa De'Anna Perteet-Jackson

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Carrie Penland Earthman

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
LIST OF TABLES	iv
LIST OF FIGURES.....	v
LIST OF ABBREVIATIONS	vi
CHAPTER 1: INTRODUCTION.....	1
CHAPTER 2: LITERATURE REVIEW – ALTERED BODY COMPOSITION POST PEDIATRIC LIVER TRANSPLANT: IMPLICATIONS AND ASSESSMENT	4
Introduction	5
Malnutrition and Failure to Grow Pre-Liver Transplant	6
<i>Prevalence of malnutrition.....</i>	<i>6</i>
<i>Definition of malnutrition</i>	<i>8</i>
<i>Prevalence of sarcopenia</i>	<i>9</i>
Nutritional Implications Post-Transplant.....	9
<i>Linear growth.....</i>	<i>10</i>
<i>Prevalence of obesity.....</i>	<i>11</i>
<i>Evidence of sarcopenia.....</i>	<i>12</i>
Body Composition Assessment Tools Appropriate for the Pediatric Post- Transplant Population.....	14
<i>Reference body composition assessment techniques.....</i>	<i>14</i>
Air Displacement Plethysmography	15
Dual-energy X-ray Absorptiometry	16
Deuterium Dilution	17
Total Body Potassium	18
<i>Bedside body composition assessment technologies.....</i>	<i>19</i>
Bioimpedance Techniques.....	19

Ultrasound	24
Summary and Conclusion	27
CHAPTER 3: BODY COMPOSITION ASSESSMENT AFTER PEDIATRIC LIVER TRANSPLANT	29
Synopsis	30
Introduction	32
Methods	33
<i>Subjects.....</i>	33
<i>Study protocol.....</i>	34
Anthropometric measurements	34
Body composition by multiple-frequency bioelectrical impedance analysis	34
Muscle and fat thickness by US	34
Body composition by ADP.....	36
<i>Statistical Analysis</i>	36
Results	36
<i>Descriptive characteristics of subjects</i>	36
<i>Comparison of body composition data to MF-BIA measurements</i>	37
<i>Comparison of body composition data to US measurements</i>	37
<i>Reliability</i>	38
Discussion	44
CHAPTER 4: CONCLUSIONS AND FUTURE RESEARCH	48
REFERENCES.....	51

LIST OF TABLES

Table 2-1: Search strategy for review of literature	6
Table 2-2: Summary of body composition assessment reference techniques	14
Table 3-1: Descriptive characteristics of study subjects at data collection	39
Table 3-2: Descriptive characteristics of study subjects pre-transplant	39
Table 3-3: Changes in anthropometric measures from transplant through 12 months post-transplant.....	40
Table 3-4: Mean z-score comparisons from time of transplant.....	40
Table 3-5: Body composition measures by Air Displacement Plethysmography	40
Table 3-6: Body composition measures by Multiple-Frequency Bioelectrical Impedance Analysis	41
Table 3-7: Mean body compositions measures by device	41
Table 3-8: Muscle and adipose thickness measures by ultrasound	43
Table 3-9: Intra-rater reliability of ultrasound measurements	43

LIST OF FIGURES

Figure 3-1: Ultrasound images of biceps and quadriceps	35
Figure 3-2: Scatterplot of the relationship between percentage body fat, fat mass and fat free mass grouped by age	42

LIST OF ABBREVIATIONS

ADP: air displacement plethysmography

BCM: body cell mass

BIS: bioimpedance spectroscopy

BMI: body mass index

CT: computed tomography

DXA: dual-energy x-ray absorptiometry

ECW: extracellular water

FM: fat mass

FFM: fat-free mass

ICU: intensive care unit

ICW: intracellular water

LOA: line of agreement

LST: lean soft tissue

MF-BIA: multifrequency bioimpedance analysis

MRI: magnetic resonance imaging

PA: phase angle

SD: standard deviation

SF-BIA: single-frequency bioimpedance analysis

SPLIT: Studies of Pediatric Liver Transplantation

TBW: total body water

US: ultrasound

CHAPTER 1: INTRODUCTION

Liver disease broadly describes any disorder of the liver resulting in a disruption of metabolic processes. Even though there are several types of liver diseases or conditions, liver disease can be generally categorized as communicable (viral hepatitis) and non-communicable (biliary atresia, metabolic disorders, etc.).¹ The incidence of liver disease in adults is studied thoroughly such that various health organizations publish statistics periodically. Liver disease in children has not been studied extensively, so there are no exact prevalence statistics. Neither the Centers for Disease Control nor the World Health Organization lists liver disease as a major cause of death for adults or children. Also consistent among adults and children is treatment for liver disease. A common treatment of liver disease is liver transplant, which involves replacement of a diseased or injured liver with healthy liver from another person. Transplantation may involve the whole liver or a section, and healthy livers can be obtained from living or deceased donors. It should be noted that the liver is the second most transplanted organ.² Liver disease and liver transplant have an extreme influence on growth and development of children.

Malnutrition is commonly seen in children with liver disease prior to transplant and is evidenced by underweight, stunted growth, and muscle loss.³⁻⁸ All of these consequences can be attributed to the disrupted metabolic pathways prior to transplant. A consensus on the definition of pediatric malnutrition is lacking, so malnutrition is not consistently diagnosed in children with liver disease. Within one year of liver transplantation, children tend to quickly recover with weight gain and linear growth.⁹⁻¹¹ In some children, obesity and sarcopenia have been observed as long-term post-transplant outcomes.¹²⁻¹⁴ An assessment of nutritional status prior to transplant would facilitate tailoring of nutritional therapy.

Body composition assessment tools have been utilized in diagnosing nutritional status for adults; yet, there are limited studies that utilize these tools in the pediatric liver transplant population. Technologies available to assess body composition include air displacement plethysmography (ADP), dual-energy X-ray absorptiometry (DXA), bioimpedance, and ultrasound (US). Total body potassium has been utilized for body composition assessment in adults and children post liver transplant; however, this method is not applicable in a clinical setting. Further, ADP and DXA are large, expensive devices that are not practical for clinical use. Alternatively, bioimpedance and US represent methods with greater accessibility in a clinical setting. Understanding body composition post-transplant could help clinicians diagnose and treat malnutrition, as well as reduce the incidence of obesity in this population. The primary objective of this thesis is to describe body composition and the quality of weight gain in children who have undergone liver transplant. Chapter 2 presents a literature review on the evidence specific to body composition of children undergoing liver transplant and a discussion of relevant body composition assessment methods for this population. Chapter 3 is a study investigating body composition in a sample of children who underwent a liver transplant and received care at the University of Minnesota Masonic Children's Hospital. Lastly, Chapter 4 provides an overall summary and suggestions for future research.

CHAPTER 2: LITERATURE REVIEW – ALTERED BODY COMPOSITION POST PEDIATRIC LIVER TRANSPLANT: IMPLICATIONS AND ASSESSMENT

Introduction

Liver transplant is a common treatment option to address impaired metabolic pathways for adults and children with liver failure. Over 7,100 liver transplants were performed in the United States in 2015 making it the second most commonly transplanted organ.¹⁵ Of these transplants, approximately 600 were pediatric transplants.² The most common reason for liver transplantation in the pediatric population is biliary atresia. The impaired metabolism from liver disease causes malnutrition from malabsorption of nutrients and profoundly impacts growth and development in children resulting in stunted growth and sarcopenia (defined as reduced skeletal muscle mass and strength). After transplantation, children tend to undergo rapid weight gain and growth recovery; however, some experience increased adiposity and may even develop obesity. In addition, long-term use of immunosuppressant drugs after transplantation contribute to growth issues for children often resulting in lifelong metabolic health concerns.^{9,10} There are a limited number of studies that have characterized body composition in children post liver transplant, and it is not clear which body composition assessment methods are most appropriate for this pediatric population. In this review, we discuss available evidence regarding the body composition of children undergoing liver transplant. Further, we provide an overview of the most commonly utilized body composition assessment technologies in this population, and outline opportunities for future research.

A literature search was completed via Ovid MEDLINE database using a modified systematic approach based on three concepts: liver transplant, body composition assessment and children. The search strategy is presented in Table 2-1.

Table 2-1: Search strategy for review of literature

1. liver transplant*	2. exp body composition 3. lean tissue or lean body mass or lean mass or muscle mass or muscle thickness or fat-free mass or fat free mass or fat mass or body fat or body cell mass 4. bioelectrical impedance or bioimpedance spectroscopy or bioelectrical impedance spectroscopy or electric* impedance or bioimpedance analysis or bioelectrical impedance analysis or bioimpedance or BIA or MF-BIA or MFBIA or BIS 5. air-displacement plethysmography or air displacement plethysmography or bod pod or bodpod 6. plethysmography, impedance 7. plethysmography, whole body 8. 2 or 3 or 4 or 5 or 6 or 7	9. exp Child 10. *Pediatrics 11. boy* or girl* or kid or kids or child or child* or pubescen* or pediatric* or paediatric* or peadiatric* 12. 9 or 10 or 11
13. 1 and 8 and 12		

Malnutrition and Failure to Grow Pre-Liver Transplant

Prevalence of malnutrition

Immediately prior to liver transplant, many children are underweight and have experienced muscle loss along with growth failure. Utterson et al. analyzed 755 patients under the age of 18 with biliary atresia listed in the Studies of Pediatric Liver Transplantation (SPLIT) database to identify risk factors associated with pre and post liver transplantation mortality and found 55% of the patients had growth disturbances

(where height/weight < -2 SD below the mean) prior to transplant.⁷ Furthermore, children are often diagnosable with overt malnutrition by the time they get to liver transplantation where the causes of poor nutritional status include inadequate intake, metabolic dysfunction, and depressed biochemical markers.^{3-6,8} Shepherd et al. analyzed clinical and nutritional features along with laboratory data of 37 children (aged 0.5 to 14.0 years) accepted for liver transplant,⁶ and concluded that severe nutritional issues, such as underweight, stunted growth, low serum albumin, and depleted cell mass based on total body potassium, negatively influence survival and are associated with complications before and after transplant.⁶ Trocki et al. performed a retrospective review of 186 children under the age of 3 with end-stage liver disease and suggested that anthropometric data is not enough to assess nutritional status.⁸ Based on height for age, Trocki et al. found that approximately 50% of the children were stunted (z-score < -2).⁸ Using total body potassium counting, Trocki et al. identified depleted cell mass in 75% of children.⁸ Chin et al. examined protein energy malnutrition in 27 children (aged 0.5 to 6.75 years) accepted for liver transplant and found severe malnutrition associated with depleted body cell mass using total body potassium and body fat and protein stores using skinfold thickness measures in all 27 patients.³ Roggero et al. analyzed the nutritional status of 49 children aged 2.5 months to 13.0 years with liver disease by reference to anthropometric indices and dietary intake and found evidence of malnutrition along with decrease in midarm muscle area and estimated lean body mass and higher adiposity.⁴ Roggero et al. separated the children into two groups by age: 1 year old and younger were in group 1 and age 2 and above were in group 2.⁴ The following measures were obtained for each subject: height/length; weight; biceps, triceps, subscapular, and suprailiac skinfold thicknesses. Using skinfold thickness measures, midarm muscle area and body density were calculated to assess lean body

mass and fat mass. The greatest reduction in midarm muscle area was observed in group 1, while the loss of calculated lean body mass was highest in group 2.⁴ Such findings supported the conclusion of chronic undernutrition resulting from prolonged liver disease children of group 2.⁴ Arvey et al. evaluated anthropometric measures, serum proteins, and body composition of 46 children aged 1 to 12 years with biliary atresia or Alagille syndrome and reported decreased height for age and stunted growth, respectively.⁵ Collectively, these study findings of pre-transplant children show that considerations beyond anthropometry are relevant as the underlying liver disease results in muscle loss and malnutrition.

Definition of malnutrition

When considering the diagnosis of malnutrition in children with liver disease, it is important to note that the definition and diagnostic criteria of malnutrition differs in adults and children. When assessing nutritional status in adults, “loss of muscle mass” and “loss of subcutaneous fat” have been identified by the Academy of Nutrition Dietetics/American Society for Parenteral and Enteral Nutrition as two characteristics that support a malnutrition diagnosis.¹⁶ Conversely, malnutrition in children is defined as “an imbalance between nutrient requirements and intake, resulting in cumulative deficits of energy, protein, or micronutrients that may negatively affect growth, development or other relevant outcomes.”¹⁷ Mehta et al. further concluded that anthropometric parameters, and both the etiology of malnutrition (non-illness related or illness related) and mechanism behind the nutritional imbalance should be considered in the definition of pediatric malnutrition.¹⁷ Although muscle and fat loss are not specifically evaluated, body composition is considered to be an important consideration in some definitions of pediatric malnutrition. Specifically, Becker et al. identified mid-upper arm circumference as an indicator in the diagnosis of pediatric malnutrition when a single data point is

available.¹⁸ In children with liver disease, body composition assessment methods along with anthropometry and laboratory data were utilized to examine nutritional status prior to transplant in a number of studies, suggesting that interest in obtaining body composition data in this patient population may be growing.^{3,5,6,19}

Prevalence of sarcopenia

Loss of muscle mass and or function (i.e. sarcopenia) is one consequence of prolonged malnutrition in children pre-transplant and may be associated with an increase in fat stores. Although sarcopenia has mostly been studied and reported as a long-term outcome of liver transplantation, one study in children with liver disease documented sarcopenia *prior to* transplantation. Mangus et al. analyzed muscle mass in 81 patients aged 18 and under with liver, kidney or intestinal failure and determined that children with end-stage liver disease demonstrated “profound sarcopenia” and increased visceral and subcutaneous fat stores when compared to healthy children.²⁰ These authors reported normal weight status for children with liver failure and no differences in BMI by gender or age group as compared to healthy children.²⁰ These findings underscore the occurrence of sarcopenic obesity in children with end-stage organ disease characterized by loss of muscle mass, and a simultaneous increase of fat stores with no change in weight and BMI. In addition, children with end stage liver disease often have ascites and edema leading to increased weight without increases in muscle mass. Comprehensively, findings of malnutrition and sarcopenia reinforce the relevance of body composition assessment in children with liver disease.

Nutritional Implications Post-Transplant

Many of the primary research studies involving pediatric liver transplant subjects have focused on growth factors or trends immediately following the surgery through at least 5 years post-transplant. Three themes emerged from research involving the

pediatric post-transplant population: adverse changes in linear growth, obesity prevalence, and evidence of sarcopenia.

Linear growth

One of the most consistent findings across reviewed studies was the potential for catch-up growth with respect to weight and height gains within 3 years of liver transplant with the highest level of growth noted in the first year following transplant.^{9–11} We found three studies that examined changes in growth using anthropometric data following a liver transplant. Ng et al. created a composite profile of an ideal 10 year survivor of liver transplant and observed that only 32% of the 167 liver transplant survivors satisfied the requirements, where the age at transplant was 13 years and younger.⁹ The composite profile created by Ng et al. was based on data from the SPLIT database and consisted of three broad categories, including success of surgery, conditions related to the use of immunosuppression drugs, and use of additional medications.⁹ Growth was analyzed as one variable in the immunosuppression drug related conditions category and was measured based on a z-score for height compared to normal growth, which was defined as greater than -2 SD.⁹ Ng et al. found growth impairment in 23% of the survivors at the 10-year follow-up and noted that long-term use of immunosuppression drugs adversely impacted catch-up growth after years 2 and 3 post-transplant.⁹ Cheng et al. also studied long-term outcomes post liver transplant, obtaining height and weight measures at time of transplant, 1 year, 5 years, 10 years and 15 years post-transplant.¹⁰ Using BMI and z-scores, these authors reported height recovery in 98 children aged 17 years and younger up to 10 years post-transplant.¹⁰ However, no changes in height were observed at 15 years post-transplant.¹⁰ Moghazy et al. measured long-term post transplantation changes in height and calculated z-scores for 237 children aged 17 years and younger.¹¹ A total of six measurements were recorded during the follow-up period: annually for the

first three years following transplant, then at years 5, 10 and 15.¹¹ The authors demonstrated three phases of growth after transplantation: a growth spurt within the first year, a plateau phase through 10 years and a late declining phase through year 15.¹¹ Determination of the growth spurts was based on the change in the z-score at each measurement with the greatest increase observed after year 1.¹¹ Even though a significant increase in height z-score was observed after year 1, the mean z-score of the post-transplant children was less than the mean z-score for the normal children.¹¹ Greater gains in catch-up growth were observed in children under age 2 at the time of transplant and those with higher growth retardation at time of transplant.¹¹ Overall, the findings with respect to catch-up growth and long-term growth impairment further supports malnutrition as a concern in this population. A consistent limitation in these three studies is the sole reliance on anthropometric data. Ng et al. indicated that use of ultrasound images would improve data analysis.⁹ Cheng et al. commented that better anthropometric measures and consensus on definitions would support effective assessment of risk for obesity.¹⁰ In addition, sample sizes were noted as small. More research with larger sample sizes is needed.

Prevalence of obesity

Contemporaneously, obesity can occur post-transplant and has been reported in this population.^{9,10,12,21,22} Sundaram et al. showed high prevalence of obesity in the pediatric liver transplant population ages 2 to 18 years: 19.2% after the first year, 17.5% after three years and 10.9% after 5 years.²¹ These investigators relied on BMI data from the SPLIT database and applied the definition of obesity as greater than the 95th percentile for age and sex.²¹ This study highlighted post-transplant medications and lack of activity as two important contributing factors to the prevalence of obesity. Further, this study revealed similar obesity prevalence statistics in a population perceived as

underweight compared to the general pediatric population.²¹ Sundaram et al. found that obesity at the time of transplant likely results in obesity post-transplant.²¹ Anastácio et al. measured 100 children aged 17 years and younger at two separate times post-transplant over a four year period and found obesity prevalence of 37%.¹² These investigators utilized body composition along with anthropometric measures to determine obesity prevalence.¹² Anastácio et al. also reported an increase in obesity prevalence in children over the four year period of the study.¹² Other post-transplant studies have reported obesity prevalence based on anthropometric data. Ng et al. reported obesity prevalence of 10% at the 10-year follow-up.⁹ Cheng et al. reported 9% were overweight (BMI 25-30 for age 18 and older or 90th-99th percentile for children less than 18 years of age) in the 10 to 15 year period; however, none had obesity.¹⁰ Sheikh et al. evaluated 21 patients post-transplant aged 7.1 to 15.9 years using anthropometrics and body composition measures and found 28.6% of the patients had obesity.²² Weight gain is an expected outcome post-transplant; however, researchers have not identified how to manage weight gain in this population. Catch-up growth through weight gain and linear growth should be closely monitored to avoid increasing prevalence of obesity in this population.

Evidence of sarcopenia

With prevalence almost equal to obesity, sarcopenia has been well-documented as a post-transplant outcome.¹²⁻¹⁴ However, there is not yet consensus on the criteria for diagnosis of sarcopenia across studies. Low muscle mass and low muscle function are most commonly utilized; however, some researchers rely only on low muscle mass to identify sarcopenia. In addition, body composition assessment methods vary by study. We found two studies that focused on the prevalence of sarcopenia in post-transplant children. Anastácio et al. reported sarcopenia prevalence of 19% at 3 years post-

transplant and 22% at 7 years post-transplant.¹² Using a 50 kHz Quantum X and RJL equations, Anastácio et al. identified an association with gender and sarcopenia, noting higher prevalence in female subjects.¹² Mager et al. documented the prevalence of sarcopenia in the pediatric liver transplant population through a retrospective chart review of DXA scans and hospital readmissions using weight, height, and z-scores of children aged 0.5 to 17 years.¹⁴ These investigators found sarcopenia up to 8 years post-transplant in children and observed higher prevalence in young females.¹⁴ Mager et al. also observed an association between sarcopenia and length of hospital stay (in the ICU) and ventilator dependence.¹⁴ These authors speculated that nutritional and physical therapies for patients with a z-score of -1 or -2 in the first few years post-transplant would reduce prevalence of sarcopenia.¹⁴ Dasarthy found evidence of sarcopenia in children post-transplant, including an increase in sarcopenic obesity.¹³ Dasarthy noted researchers have successfully described sarcopenia using DXA and bioimpedance techniques in the post-transplant population; however, the mechanisms resulting in post-transplant sarcopenia have not been thoroughly examined.¹³ Although studies investigating sarcopenia consistently utilize body composition assessment methods, there are significant limitations of using bioimpedance and other bedside methods without a gold standard or reference method for comparison purposes. Additional research on body composition post-transplant is needed to contribute to development of clinical criteria and diagnostic procedures with respect to sarcopenia. Provided that the post-transplant mechanisms can be identified, appropriate nutritional and physical therapies can be implemented to lessen the impact of metabolic syndrome and pathologic body composition changes including sarcopenic obesity.

Body Composition Assessment Tools Appropriate for the Pediatric Post-Transplant Population

Multiple body composition assessment methods such as ADP and DXA are applicable to children but are not portable and accessible in all clinical settings. Bioimpedance and US are two bedside methods that could be implemented in a clinical setting. Although there are several methods that could be used to measure quality of weight gain in children with liver disease after transplantation, it is not entirely clear which are most appropriate for application in the inpatient and outpatient setting in this population. Further, this area has not been pursued extensively to date.

Reference body composition assessment techniques

ADP, DXA, deuterium dilution and total body potassium are four commonly utilized reference techniques in clinical research settings for adults and children (Table 2-2).

Table 2-2: Summary of body composition assessment reference techniques

Technique	Principle	Measurements
Air Displacement Plethysmography (ADP)	Pressure and volume used to determine body's volume from which body density is calculated	FFM FM
Dual-energy X-ray Absorptiometry (DXA)	High and low energy beams passed through body to differentiate between soft tissue and bone	FFM LST FM Bone density
Deuterium Dilution	Compartment volume determined based on tracer or tracer concentration administered	TBW FFM FM
Total Body Potassium	Radioactive form of potassium administered and quantified based on consistency of intracellular potassium within body cell mass and fat free mass compartments	BCM

BCM – body cell mass; FFM – fat free mass, FM – fat mass; LST – lean soft tissue; TBW – total body water.

Air Displacement Plethysmography

ADP measurements determined by the Bod Pod (COSMED) are based on the displacement of air by the individual seated inside the chamber. The components of the Bod Pod include a plethysmograph, electronic weighing scale, and calibration weights and cylinders along with a computer and software. Calibration of the scale and Bod Pod must be completed before each use. The Bod Pod scale is calibrated using two 10 kg weights, and the Bod Pod chamber is calibrated using one of two cylinders of known volume. Gender, ethnicity, height and date of birth are required inputs for Bod Pod software prior to measurement. In addition to body volume, Bod Pod also measures thoracic gas volume. An option to predict thoracic gas volume is available. The test is a two-step process: weight using the Bod Pod scale and measurement of body volume inside the chamber. Body density is calculated as mass divided by volume with corrections for the thoracic gas volume, and then percent body fat is calculated from density using the Siri equation: $\% \text{ body fat} = (4.95/\text{body density} - 4.50) \times 100$. Fat mass and fat-free mass are then calculated by the software using the Siri equation for converting body density into percent body fat. Measurement by Bod Pod is straightforward, comfortable and convenient for subjects of all ages.

The majority of work to establish ADP as a reference technique has been done in adults. Early research on ADP involved comparison to hydrostatic weighing; it has now replaced hydrostatic weighing as a preferred reference method in healthy adults.²³ Millard-Stafford et al. compared hydrostatic weighing to ADP and found ADP measurements of body density and percentage body fat were highly correlated with measures by hydrostatic weighing, DXA, and deuterium dilution.²⁴ Bosy-Westphal et al. validated ADP measurements of FM and FFM against DXA in healthy elderly adults.²⁵ Noreen et al. determined reliability through repeated testing in a heterogenous sample of

adults and also concluded that ADP would be reliable in a subset of a larger population.²⁶ Anderson concluded there was high reliability with Bod Pod in healthy adults.²⁷

A limited number of studies have evaluated the validity of ADP in children. Fields and Goran compared hydrostatic weighing, DXA, ADP, and TBW to the four-compartment model and found ADP to be a valid technique for evaluating FM and FFM with no bias in healthy children where the mean age was 11.4 years.²⁸ Dewit et al. compared hydro-densitometric measurements of FM and FFM to ADP measurements of FM and FFM of healthy children aged 8 to 12 and found greater precision with ADP measurements for children than precision observed in adults.²⁹ To our knowledge, ADP has not been utilized in studies of post liver transplant patients (adults or children).

Dual-energy X-ray Absorptiometry

DXA is a noninvasive method to assess body composition that requires the subject to lie flat on a scanning table for regional or whole body measures. Low radiation x-rays from two different sources are passed through the bone and soft tissues of the subject and the differential amount of energy absorbed is measured to determine bone density, lean soft tissue, and fat mass. Even though a low level of radiation is involved, DXA is regarded as safe and is widely used in hospitals and clinics and is applicable to many populations.

DXA has been well validated and accepted as a standard reference technique in healthy adults since the 1980s. Haarbo et al. validated the use of DXA for body composition in healthy adults against dual-photon absorptiometry, which was used in the 1980s to estimate bone mineral density.³⁰ The accuracy between measurements from dual-photon absorptiometry and DXA resulted in DXA becoming the gold standard for diagnosis of osteoporosis and osteopenia. High reproducibility/reliability of DXA body

composition measurements is consistently observed in healthy adults and children, with precision estimates of approximately 1-2%.^{31,32} DXA has also been used to validate other body composition assessment tools, such as single-frequency bioelectrical impedance analysis (SF-BIA) in healthy children.³³

DXA has been used to assess body composition in adults post liver transplant to measure quality of life, diagnose sarcopenia, and to predict post-transplant outcomes; however, use in the pediatric post-transplant population has been limited.³²⁻³⁵ Two studies specific to pediatrics post liver transplant were identified. Sheikh et al. completed DXA scans of 21 children who were at least 5 years post-transplant with a median age of 10.6 years (range 7.1 to 15.9 years).²² BMI values post-transplant were recorded and compared to DXA scans.²² Sheikh et al. observed BMI values that indicate normal weight status but found that some children had overweight or obesity based on DXA scans.²² Ulivieri et al. completed DXA scans to measure bone density and body composition pre- and post-transplant in 9 children aged 6 to 29 months.¹⁹ Ulivieri et al. observed no significant changes in lean mass within the 6 month period following liver transplant; however, percentage fat mass and bone density increased significantly.¹⁹ While DXA has been used as a reference method for adults and children, more research comparing DXA scans to clinically accessible methods for this population is needed.

Deuterium Dilution

Based on the dilution principle, a tracer dose of deuterium labeled water is administered and two samples of body fluids (usually urine) are collected over a defined space of time, typically 3 to 5 hours. One sample of body fluid is collected before the tracer is administered, and the second is collected after the tracer has reached equilibrium. Total body water (TBW) is calculated based on the concentration of the

deuterium in the samples collected; from TBW, FFM can be calculated using the assumption that FFM is constantly hydrated at 73.2%.³⁸

Deuterium dilution is generally used as a reference method for TBW, FFM, and FM and has been widely used to validate other measurement techniques in healthy adults, such as SF-BIA algorithms and ADP.^{24,39} Deuterium dilution has also been utilized as a reference method for determining FFM and FM in healthy children.^{28,40}

No studies involving deuterium dilution and post liver transplant patients (adults or children) were identified through the literature searches.

Total Body Potassium

Total body potassium counting is an *in vivo* measurement where a scanning bed with detectors measure gamma rays emitted by the naturally occurring radioactive isotope ⁴⁰K within the cells of the subject. Given that more than 98% of total body potassium is inside cells, the measured total body potassium is used to calculate body cell mass, thought to be the most specific compartment reflecting protein rich tissue in the body including muscle. Intracellular water (ICW) can also be estimated from total body potassium measures.

Total body potassium is well accepted as a reference method for other body composition techniques, including DXA and bioimpedance techniques in healthy adults.^{30,41} For example, De Lorenzo et al. found a correlation between ICW calculated from total body potassium and ICW calculated from total body impedance in healthy adults.⁴²

Total body potassium has been used to measure body composition in adults and children following a liver transplant. Hussaini et al. evaluated 55 adults to identify changes post transplantation using DXA and total body potassium.³⁵ Hussaini et al. concluded that weight gain post liver transplant was likely due to an increase in total fat

where the specific outcomes were a decrease in lean tissue mass measured by DXA and lower total body potassium.³⁵ In children, Ee et al. utilized total body potassium to assess body composition changes at least 3 years post-transplant, where age at transplant was 0.38 to 10.92 years.⁴³ These investigators measured total body potassium in 32 patients and showed decreased body cell mass even after patients showed catch-up growth after transplant.⁴³ These results led the researchers to conclude that the weight gain in the subjects was due to increases in FM and not FFM.⁴³

In summary, although total body potassium provides specific measures of body cell mass, and ADP, deuterium dilution, and DXA provide measurements of FM and FFM, none of these methods is practical for the clinical setting. Conversely, bioimpedance and US represent tools that are relevant for daily use in a clinical setting.

Bedside body composition assessment technologies

Bioimpedance Techniques

Bioimpedance is the most widely used bedside technology for body composition assessment. Bioimpedance is a simple body composition assessment method that involves application of a small electrical current to the body to estimate body water and composition.⁴⁴ Bioimpedance devices measure resistance in the body when exposed to an electrical current, and body composition is indirectly predicted from the measured impedance.^{45–47} Whole body and segmental impedance measures are possible. The whole body can be measured with adhesive electrodes placed on the hand and the foot on one side of the body with the patient in a supine position, or adhesive electrodes can be placed on either end of a body segment of interest.^{45,46} Stand-on devices also measure the whole body through direct contact with the electrodes at the feet and hands.^{45,46}

There are three general approaches to bioimpedance based on the type of device used to collect measurements: single-frequency BIA (SF-BIA) and multiple-frequency BIA (MF-BIA), and bioimpedance spectroscopy (BIS).⁴⁶ SF-BIA devices measure impedance at 50 kHz, while MF-BIA devices typically measure at one low frequency (5 kHz) and several higher frequencies (commonly 50, 100, 200 and 500 kHz).^{45,46} Both SF-BIA and MF-BIA approaches use variable regression derived population specific equations to derive water compartments. While SF-BIA equations typically predict TBW, MF-BIA equations can estimate both TBW and extracellular water (ECW), from which ICW is determined.⁴⁶ FFM and FM can also be predicted by any bioimpedance approach. BIS differs from SF-BIA and MF-BIA by measuring impedance over a spectrum of frequencies from <5 to 1000-1200 kHz and using more complex algorithms based on biophysical models, rather than linear equations.^{45,46,48} The spectral data are fit to the Cole model, and model terms generated (including Re, resistivity of the extracellular compartment and Ri, resistivity of the intracellular compartment) are applied to complex equations based on the Hanai mixture theory that treats the body as a mixture of oil and water.^{49,50} These equations have been modified over time, and different constants may be applied.⁵¹ Typically body composition, including FFM can be predicted from the TBW and/or ICW estimates, depending on the approach.

All bioimpedance approaches require basic assumptions in the estimation of body composition. SF-BIA considers the human body as a “single, symmetrical cylinder with homogenous composition and cross-sectional area” and assumes a constant ratio between ICW and ECW with uniform conduction of the electrical current.⁴⁵ Accordingly, the accuracy of ICW and ECW from SF-BIA devices is highly questionable, as estimates are based on static relationships between fluid compartments. Although MF-BIA utilizes

very low and at least one high frequency and thus offers the advantage over SF-BIA of theoretically differentiating the ICW from ECW compartments, it also involves assumptions about consistency of tissue resistivity and shape. Primary among the assumptions in both SF-BIA and MF-BIA approaches is that the equations being utilized are appropriate to the person who is being measured. Ideally, impedance data from SF- and MF-BIA devices would be applied to equations that were developed *and cross-validated* using the same device, in the same age/gender/ethnicity category as the person being measured; this is rarely the case in practice and introduces errors to the estimates. However, in reality, many bioimpedance devices protect their programmed equations as proprietary information; this presents a significant challenge to clinicians utilizing these devices. BIS algorithms are quite complex and involve constants for body shape and tissue resistivity. These complex algorithms can be adjusted using specialized software to change resistivity constants, but this is an impractical expectation of clinicians using the device in practice.⁵¹ In addition to errors caused by inappropriate selection of equation/algorithm and violation of underlying assumptions about human physiology and physiognomy, deviations from optimal measurement protocol can also contribute to errors in estimates. For example, device or environment considerations, including measurement time, placement of the device on a conductive surface, extreme ambient temperatures, failure to calibrate device, along with patient specific issues, such as failure to fast, failure to avoid alcohol, failure to void bladder and incomplete separation of limbs all contribute to errors.^{41,52–54} Although easily used to obtain whole body composition estimates, MF-BIA devices often utilize segmental electrode placements, with recognition of the human body in five distinct cylinders, limbs and trunk.^{45,47}

In addition to the whole body and segmental measures, raw impedance data have been analyzed for their relationship to nutritional status and clinical outcomes. Specifically, researchers have evaluated phase angle (PA) as a potential marker of nutritional status.^{45,46,55} PA is defined as the ratio of the arc tangent of reactance to resistance and is purported to relate to important cellular characteristics where a lower PA is thought to reflect cell loss.^{45,46,55}

Generally speaking, bioimpedance techniques have been well studied for their validity in healthy and other populations; typically, they are well accepted as tools to assess population level body composition characteristics in adults, rather than to assess individuals at the bedside. Several large datasets have been utilized to establish reference cut-points from healthy adult data, including Swiss Caucasian, German, American and Brazilian populations.^{39,56–60} More recently, Kuchnia et al. developed reference cut-points for PA based on DXA-measured FFM in healthy adults using NHANES US population data.⁵⁵ The advantages of bioimpedance techniques include the ease of application in that measures can be conducted bedside or during a clinic visit, use of radiation is not required and the data obtained can provide information about fluid status, total and regional FM, FFM, and other lean tissue compartments, depending on the approach.⁴⁴ Kyle et al. provided specific recommendations for bioimpedance in a clinical setting applicable to adults that may preclude application of bioimpedance to children.⁶¹ Subsequently, Kyle et al. outlined specific considerations for use of SF-BIA in children, including validation against reference methods (such as DXA or densitometry), adjustment of the hydration fraction in reference method and SF-BIA equation, racial/ethnic differences, standardized measurement conditions and bioimpedance analyzer.⁴⁴ Recommended procedures for measurements are similar for children and adults, including 2 to 3 hour fasting and voiding prior to measurement and electrode

placement and body position during measurement. Ward noted inherent limitations regarding use of certain equations that may not be applicable to all populations, statistically significant differences should be distinguished from clinically relevant differences, and that more focus is needed to standardize impedance protocols.⁴⁷ In contrast to bioimpedance literature in adults, there have been very few studies focusing on children. These few studies have found that bioimpedance in general is a promising method for measuring body composition when used with other anthropometric measurements such as height and weight.^{33,44}

Use of bioimpedance in the liver transplant population is limited to adults and varies in application. Kyle et al. found that a published equation for estimating FFM using the 50-kHz data from a Xitron 4000B BIS device was valid compared to DXA in pretransplant and posttransplant in liver, lung and heart patients with use of the GENEVA equation for purposes of assessment of nutritional status pretransplant and posttransplant.⁶² Kyle et al. confirmed that weight gain post-transplant is related to higher fat mass.⁶² Parepully et al. used 50 kHz PA generated by an unspecified BIA device for nutritional assessment in liver transplant candidates and found that a low PA, presumed to indicate the presence of sarcopenia, was associated with adverse transplant outcomes, suggesting that this measure could be helpful as a pre-transplant evaluation tool.⁶³ Wagner et al. also used SF-BIA, 50 kHz BIA 101 device and RJL equations, to confirm malnutrition among adults 15 years after liver transplant and concluded that the additional nutritional support immediately following could reduce incidence of secondary disease.⁶⁴ Brito-Costa et al. used the Body Composition Monitor BIS device to examine the impact of the type and dosage of immunosuppressants on weight gain and changes in body composition in adults.³⁴ Ferreira et al. utilized indirect calorimetry and a SF-BIA RJL device and its equations in post-transplant adults to

understand the impact of liver transplant on metabolic function.⁶⁵ Ferreira et al. concluded that nutritional guidance to address inadequate intake resulting in hypometabolism and hypermetabolism should occur pre-transplant for improved outcomes in adults.⁶⁵ From these studies, it seems that bioimpedance can be an effective bedside technology for assessment of body composition in adults and children. Additional research on children is needed to establish various bioimpedance techniques as a reliable method for use in a clinical setting. Furthermore, clinical guidelines and procedures should be developed for children. The success achieved with measurement of post-transplant adults is promising and could serve as the basis for use in children post-transplant.

Ultrasound

Although not as widely used as bioimpedance techniques, there is growing interest in US as a bedside technology. US provides visualization of tissues through high-frequency sound waves that are partially reflected as an echo back to the transducer.^{46,48} The resulting image is the combination of the amplitude and the speed of the soundwaves.⁴⁸ Clinical use of US is quite common in medical settings specifically with certain injuries, location of adipose depots and neuromuscular disease.⁴⁶ Similar to bioimpedance techniques, US is simple, noninvasive and portable. Measuring muscle thickness in the ICU has become increasingly relevant as a potential biomarker of clinical outcomes, muscle strength and function, and nutritional status.

Because muscle wasting is a factor in diagnosing malnutrition in adults, quantifying muscle using US has relevant clinical application for metabolic, nutritional and functional assessment.⁶⁶ The quadriceps represents the largest muscle group in the body, and muscle mass directly impacts strength and function. Parry et al. used US to quantify muscle thickness of adults in intensive care from date of admission through

discharge and found quadriceps muscle wasting within the first 10 days of ICU admission.⁶⁷ Paris et al. compared quadriceps muscle US measurements in critically ill adults to CT scans and found a correlation between quadriceps and abdominal muscle cross-sectional analysis.⁶⁸ US measurements can also be used for whole body evaluation; the sum of measurements from 9 sites has been associated with whole body muscle mass determined by MRI and lean tissue determined by DXA.^{46,68} Beyond quantification, muscle quality is also relevant in critical care. US has been used to estimate quality of muscle through analysis of echogenicity, which is the characteristic of tissue to reflect ultrasound waves.⁴⁸ Puthucherry et al. measured the rectus femoris in adults and found myofibril necrosis in the first week of critical illness and concluded that echogenicity has a negative correlation with muscle strength.⁶⁹

Even though US is a ubiquitous clinical tool, there is a lack of consensus regarding the most effective protocol for measuring muscle characteristics, with the most contention around optimal site selection and the amount of compression to use. Challenges regarding the identification of tissue boundaries, variability in degree of tissue compression, and the difficulty in reproducing measurements between observers contribute to errors in US measurement.⁴⁶ Measurement of a patient in the supine position with relaxed muscles is the most common practice; however, compression with the transducer varies from no/minimal pressure to maximal pressure.^{46,66} Researchers have not been consistent in reporting transducer pressure in published studies; however, minimal pressure has been advocated by some.^{48,66,67} Paris et al. applied maximal pressure because of the likelihood that edema was present in their subjects.⁶⁸ Mourtzakis et al. recognized the potential variability in amount of gel applied, along with transducer pressure, as points where there is a lack of consensus on protocol, but noted that fluid status should be considered when determining the amount of pressure to

apply.⁶⁶ Many studies have focused on the quadriceps muscle, but there appears to not be a consistent measurement protocol across studies involving quadriceps muscles.^{46,67–}

⁶⁹ Campbell et al. reported a correlation between skinfold thickness measurements and US measurements based on five limb measurements and one trunk measurement in adults.⁷⁰ Campbell et al. also confirmed the US measurements through a comparison to DXA.⁷⁰ Comparability to CT scans, DXA and MRI suggest that US may be an appropriate tool for body composition assessment in a variety of populations.

US has been widely used in adults, but to a lesser extent in children. Leahy et al. evaluated the effectiveness of US in young adult men and women and found that US accurately predicted percent body fat in healthy young adults (ages 18 to 29).⁷¹ The researchers measured the thickness of subcutaneous adipose tissue and compared such measurements to percent body fat by DXA with the strongest correlation found between the abdominal adipose measurement and percent fat by DXA.⁷¹ Midorikawa et al. used ultrasound measurements of healthy children aged 6 to 12 years in Japan to validate regression-based prediction equations for total body fat mass.⁷² Nine sites were selected for ultrasound measurements to validate the estimates of total body and regional fat mass against DXA scans.⁷² Even though the subjects in some of the pediatric studies have been healthy, US could provide useful data for children with severe illness.

No studies specific to the pediatric post liver transplant population were identified. Because US is an emerging technology for body composition, additional pediatric research should be performed to address the challenges of US for body composition, including landmarking, measurement techniques, and identification of the locations for measurement. Future research should also include clear, reproducible protocols that can be used to develop clinical guidance. More research involving body

composition in children should ultimately yield sufficient data to determine normal ranges of muscle and adipose thickness.

Summary and Conclusion

Liver disease disrupts absorption of nutrients and metabolic function resulting in failure to grow in children and other adverse impacts. Liver transplant is a life-saving treatment option that generally normalizes growth and restores metabolic function. Prior to transplant, children are often malnourished and have sarcopenia. Post-transplant concerns include rejection, failure to grow, malnutrition, obesity and sarcopenia. Proper nutritional support before and after transplant could improve outcomes. Along with anthropometric measures, body composition assessment technology would allow clinicians to assess nutritional status in the post-transplant pediatric population. Determining the appropriate technology to describe body fat and FFM in this population is necessary. Several technologies have been studied; however, not all technologies are appropriate for use in a clinical setting.

Although not accessible in a clinical setting, DXA and ADP have been identified as reference methods for body composition in healthy and critically ill populations. Bioimpedance techniques and US are clinically accessible and should be studied further. US, bioimpedance techniques and ADP have been demonstrated as effective methods to assess body composition and could be beneficial in describing body composition in this population. Additional research could lead to use of US and bioimpedance pretransplant for nutritional assessment and intervention, as well as determination of relevant cut-points to define sarcopenia for the pediatric population. Future research will also contribute to development of standards for bioimpedance applications in the pediatric population. Establishment of a standard protocol for pediatrics for US measurements is also an area for future research. Body composition assessment

involving US and bioimpedance techniques in the pediatric post liver transplant population could prove to be useful in reducing adverse outcomes, including malnutrition, sarcopenia and obesity.

CHAPTER 3: BODY COMPOSITION ASSESSMENT AFTER PEDIATRIC LIVER TRANSPLANT

Synopsis

Background and Objectives

Children with chronic liver disease experience altered growth and development due to the disruption of metabolic function. Pediatric liver transplantation generally restores metabolic function and results in growth recovery; yet, some children who have undergone liver transplant continue to be malnourished, have increased adiposity and may develop obesity as a long-term outcome. Increases in fat mass are associated with adverse developmental and metabolic outcomes that could be addressed through nutritional intervention. The ability to describe weight gain post-transplant would allow clinicians to assess nutritional status beyond anthropometrics. Several body composition assessment technologies are applicable to children, including air displacement plethysmography, dual x-ray absorptiometry and total body potassium, but are not portable or suitable for the clinic setting. A cross-sectional study was conducted to describe the quality of weight gain in post liver transplant children between the ages of 2 and 17 using multiple assessment tools and to determine whether multiple-frequency bioelectrical impedance analysis and ultrasound will accurately describe body composition and quality of weight gain.

Methods

Ten children (n=10) with a median age of 9.5 years were recruited from the liver transplant program at the University of Minnesota Masonic Children's Hospital. Anthropometric measurements, multiple-frequency bioelectrical impedance analysis, air displacement plethysmography, and ultrasound measurements were conducted. Comparisons of z-scores for weight, height and BMI at the time of transplant and 6 and 12 months post-transplant were made by paired t-tests. The relationship between body composition methods was assessed using Wilcoxon signed rank test of the differences.

The relationship between body composition methods and ultrasound measurements of muscle and adipose thickness was estimated by Spearman's rank correlation rho. Intra-rater reliability was evaluated using deviance (absolute difference from mean).

Results

Mean percent body fat by air displacement plethysmography was 18.4% (± 3.3) and 19.0% (± 3.9) by multiple-frequency bioelectrical impedance analysis ($p > 0.99$). Positive differences in mean z-score comparisons from transplant indicate an increase over each time period: mean change in weight z-score at 6 months was 0.08 and 0.38 at 12 months; mean change in height z-score at 6 months was -0.56 and 0.09 at 12 months; mean z-score change in BMI at 6 months was 0.24 and 0.31 at 12 months. The combined muscle and adipose thickness measures by ultrasound were not a significant predictor for percent body fat determined by air displacement plethysmography or multiple-frequency bioelectrical impedance analysis. Ultrasound measures for biceps and quadriceps were not a significant predictor for segmental mass measures determined by multiple-frequency bioelectrical impedance analysis.

Conclusions

Percent body fat, fat mass and fat free mass measures determined by air displacement plethysmography and multiple-frequency bioelectrical impedance analysis were similar, which suggests the stand-on device used in this study is a useful body composition assessment tool for the pediatric population. There was no observed correlation between body composition variables by ultrasound, and air displacement plethysmography or multiple-frequency bioelectrical impedance analysis in children who have undergone a liver transplant. Including additional measurement sites and more study participants may improve the utility of ultrasound measurements.

Introduction

Liver disease and liver transplantation in the pediatric population result in altered growth and development often with lifetime nutritional implications. Malnutrition resulting in sarcopenia and growth failure is generally observed in children with liver failure where findings include low body weight for height and age, stunted growth, decreased fat stores, and muscle loss.³⁻⁶ Liver transplant is a common treatment for liver failure in adults and children. Researchers have documented malnutrition in children awaiting liver transplant based on inadequate intake, poor macronutrient metabolism, anthropometrics, and reduced biochemical markers.³⁻⁸ The diagnosis of malnutrition in children follows a broad definition of an imbalance of nutrient requirements and intake leading to cumulative deficits.^{17,18} Anthropometry alone can be inaccurate for identifying malnutrition in liver disease pre-transplant due to edema and ascites, and thus interest in the assessment of body composition has increased.

After liver transplant anthropometrics, including height, weight and BMI, improve with the highest level of growth noted in the first year after transplant.⁹⁻¹¹ While children demonstrate catch-up in growth post-transplant, body composition assessment demonstrates that these children have continued malnutrition, sarcopenia and even obesity in the long-term.^{12,21,22,43} Assessment of the quality of weight gain following a liver transplant may help reduce the prevalence of obesity as a long-term outcome.

Body composition assessment has been demonstrated as beneficial for diagnosing malnutrition in children with liver failure and may contribute to improved post-transplant outcomes where nutritional status is evaluated and treated before transplant. Identification of an appropriate clinical tool for assessing body composition is needed. Multiple body composition assessment methods exist and are applicable to children, including DXA and air displacement plethysmography (ADP). Both DXA and ADP have

been studied extensively and have been documented as reference methods in healthy and critically ill populations; however, neither technology is practical in the routine clinical setting. Conversely, bioimpedance and ultrasound (US) have been identified as accessible, portable tools, although research specific to liver failure or liver transplant has been limited to adults. A variety of bioimpedance approaches have been used to assess nutritional status, presence of sarcopenia pre-transplant, impact of immunosuppressant drugs, and metabolic function in adults.^{34,62–65} Similarly, US measurements have been well-correlated with skinfold thicknesses and whole body DXA body composition measures, suggesting that US is promising for quantifying muscle mass in adults.^{66,70–72} There is a paucity of research utilizing bioimpedance and US in children; studies are needed to establish optimal protocols and clinical guidelines.

The purpose of this cross-sectional study is to describe the quality of weight gain in post liver transplant children between the ages of 2 and 17 using multiple assessment tools and to describe the relationship between the assessment methods. We hypothesized that MF-BIA and US will accurately describe body composition and quality of weight gain compared to ADP in a pediatric post liver transplant population.

Methods

Subjects

Children aged 2 to 17 years who underwent liver transplantation and received care from the liver transplant program at the University of Minnesota Masonic Children's Hospital were recruited for this cross-sectional study to measure body composition. Participants were measured in a single visit that was approximately 30 minutes in duration. Demographic and patient characteristics pre- and post-transplant were gathered. Exclusion criteria included the following: history of multiple organ transplants (liver and another organ), body weight less than 10 kg, medical support that prevents

measurement by ADP, inability to sit unassisted for 5 minutes, and physical limitations that require support to stand on a MF-BIA scale. The study protocol was approved by the University of Minnesota Institutional Review Board.

Study protocol

At the beginning of the study visit, participants were encouraged to void their bladders and change into a plain tight fitting swimsuit.

Anthropometric measurements

Each participant's height was measured in triplicate to the nearest 0.1 cm using a wall-mounted stadiometer (Seca; Chino, CA) and weight was measured to the nearest 0.1 kg using the scale function of the MF-BIA device. BMI was calculated as $[(\text{weight in kg})/(\text{height in m}^2)]$ and z-score was calculated using the PediTools website (www.peditools.org).

Body composition by multiple-frequency bioelectrical impedance analysis

Body composition and extra- and intracellular body water were assessed by the InBody 770 (InBody USA; Cerritos, CA) MF-BIA device. The test instructions per the user manual and screen prompts on the device were followed. Bioimpedance measures were taken 1, 5, 50, 250, 500, and 1000 kHz at each of 5 segments (right arm, left arm, trunk, right leg, and left leg). Measurements were made immediately after anthropometric measurements as the participant stood on the device, in bare feet while holding the hand electrodes with arms horizontally abducted 30° from the body.

Muscle and fat thickness by US

US images were obtained with a GE Logiq e B-mode ultrasound with a 12MHz linear array transducer (GE Healthcare; Solingen, Germany). Each participant was measured using the Ped Abd option. Except for Depth, device settings were not changed for each participant. Images were obtained with the participant in a supine

position on an examination table. A water-soluble transmission gel was generously applied to the scanning head of the transducer for each measurement site. The transducer was placed perpendicular to the measurement site taking care not to compress the tissue. Three images at frequency of 10MHz were obtained of the anterior upper arm at approximately halfway between the lateral epicondyle of the humerus and the acromial process at the shoulder and the anterior upper leg approximately halfway between the lateral condyle of the femur near the knee and the greater trochanter at the hip. Muscle and adipose thickness were measured on each US image using electronic calipers (Figure 3-1). The mean of three measurements was used for analysis. If three measurements were not obtained, the mean of two measurements or one measurement was used instead.

Figure 3-1: Ultrasound images of biceps and quadriceps

Body composition by ADP

Body composition was assessed by ADP using the BOD POD Gold Standard Body Composition Tracking System (COSMED USA, Inc.; Concord, CA). The test sequence was executed per the user manual and screen prompts, as previously described.²⁸ The predicted option for thoracic gas volume and general population were selected for each participant. One test cycle was completed for each participant.

Statistical Analysis

Descriptive statistics were generated using R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). Comparisons of z-scores for weight, height and BMI at time of transplant and 6 or 12 months post-transplant were made by paired t-tests. Other comparisons of US measurements of muscle and adipose thickness between methods were made by Wilcoxon signed rank test of the differences, and correlation was estimated by Spearman's rank correlation rho. Bland-Altman plots were created using limits of agreement (LOA) defined by mean bias $\pm 1.96 \times$ standard deviation of bias. Deviances (absolute difference from mean) of multiple ultrasound measurements were calculated to examine reliability. Weight measure differences and hydration fraction ratios were calculated using Excel.

Results

Descriptive characteristics of subjects

A total of 10 children aged 5 to 16 years with a median age at data collection of 9.5 years were enrolled. Sixty percent were female, 90% prepubertal and right-handed. The most common reason for transplant was biliary atresia (n=6; 60%). Time since transplant ranged from 6 months to 14 years with a median of 7 years. Stunting or a decrease in linear growth pre-transplant was observed in 50% of the children (n=5), and growth failure was documented in 70% (n=7) (Tables 3-1 and 3-2).

Positive increases in height, weight and BMI occurred between 1 month post-transplant and 12 months post-transplant (Table 3-3). Table 3-4 shows changes in mean changes in z-score from transplant to 6 months and 12 months post-transplant. Comparison of mean z-scores indicated an increase in weight and BMI at 6 months post-transplant. The mean z-scores for weight, height and BMI increased at 12 months post-transplant.

Comparison of body composition data to MF-BIA measurements

Tables 3-5 and 3-6 show body composition data measured by Bod Pod (ADP) and InBody 770 (MF-BIA), respectively. Weight measures between ADP (fat mass, FM plus fat free mass, FFM) and MF-BIA (sum of water and mass measures) were within 0.4 kg where the weight determined by MF-BIA was higher for 4 of the 7 participants, MF-BIA was lower for 2 participants and MF-BIA agreed for 1 participant. The calculated hydration fraction ratios ranged from 0.730 to 0.738 for the study participants. Mean values for percentage body fat by device and p-value from the Wilcoxon signed rank test are shown in Table 3-7. Mean values for FM and FFM by device were also analyzed by the Wilcoxon signed rank test, and the p-values for FM and FFM are 0.58 and 0.81, respectively. The relationship between body composition measured by ADP and MF-BIA is shown in Figure 3-2.

Comparison of body composition data to US measurements

Additional analyses compared the relationships between ADP body composition measures and US muscle and adipose thicknesses (n=7). Muscle thickness and adipose thickness measures were analyzed for predictability for ADP body composition measures. None of the US measures for biceps and quadriceps were a significant predictor of FM or FFM determined by ADP.

Additional analyses comparing the relationships between MF-BIA and US variables were performed using the Spearman's rank correlation rho. Muscle thickness and adipose thickness measures were analyzed for predictability for MF-BIA FFM and FM measures, respectively. None of the US measures for biceps and quadriceps were a significant predictor of the segmental mass measures determined by MF-BIA.

Reliability

Intra-rater reliability of US measurements is shown in Table 3-9. The mean deviance for all US muscle and adipose thickness measures was 0.039 cm, for all adipose thickness measures 0.033 cm, and for all muscle thickness measures 0.046 cm.

Table 3-1: Descriptive characteristics of study subjects at data collection

Study subject	Sex	Age (years)	Height (cm)	Height z-score	Weight (kg)	Weight z-score	Puberty status	Handedness	Time since transplant (years)
1	Female	6	117.0	-0.73	20.2	-0.74	Prepubertal	Right	0.9
2	Male	8	129.5	-0.44	26.9	-0.20	Prepubertal	Right	7.6
3	Female	16	169.5	1.07	50.5	-0.42	Post-pubertal		14.3
4	Male	10	140.5	0.04	34.5	0.21	Prepubertal	Right	2.8
5	Female	9	139.1	0.83	27.3	-0.45	Prepubertal	Right	8.5
6	Male	12	146.9	-0.87	34.9	-1.26	Prepubertal	Right	3.9
7	Female	10	154.4	1.58	51.8	1.52	Prepubertal	Right	6.5
8	Female	8	134.2	0.94	30.0	0.73	Prepubertal	Right	7.5
9	Male	5	108.5	-1.06	18.5	-0.64	Prepubertal	Right	0.6
10	Female	9	146.6	1.47	37.2	0.75	Prepubertal	Right	8.3

Table 3-2: Descriptive characteristics of study subjects pre-transplant

Study subject	Diagnosis	Stunting or decrease in linear growth	Growth failure
1	Caroli disease	Yes	No
2	Biliary atresia	No	Yes
3	Congenital hepatic fibrosis		Yes
4	Unknown/idiopathic	No	No
5	Biliary atresia	Yes	Yes
6	Biliary atresia	Yes	Yes
7	Familial cholestasis	Yes	No
8	Biliary atresia	Yes	Yes
9	Biliary atresia	No	Yes
10	Biliary atresia	No	Yes

Table 3-3: Changes in anthropometric measures from transplant through 12 months post-transplant

	Transplant	1 month	6 months	12 months
n	10	10	10	10
Weight (kg)	14.0 (5.9, 35.1)	14.1 (6.0, 24.0)	16.05 (8.5, 28.7)	15.9 (10.0, 27.7)
z-score	-0.18 (-2.54, 1.77)	-0.99 (-3.08, 0.07)	-0.12 (-1.77, 0.81)	0.09 (-1.16, 1.26)
Height (cm)	92.3 (64.0, 146.5)	102.0 (63.0, 125.0)	106.9 (73.0, 128.5)	96.9 (78.5, 131.2)
z-score	-0.49 (-1.64, 3.02)	-1.30 (-2.43, -0.14)	-1.04 (-1.20, 0.08)	-0.32 (-0.97, 2.32)
BMI (kg/m ²)	16.1 (13.9, 18.2)	15.9 (14.4, 17.2)	16.5 (14.4, 19.5)	16.6 (14.5, 18.0)
z-score	0.29 (-2.40, 1.15)	-0.20 (-1.30, 0.64)	0.25 (-1.25, 1.57)	0.34 (-0.93, 1.24)

Values presented as median (range).

BMI, body mass index

Table 3-4: Mean z-score comparisons from time of transplant

	6 months	12 months
Weight (kg)	0.08 (0.86)	0.38 (0.51)
Height (cm)	-0.56 (0.32)	0.09 (0.91)
BMI (kg/m ²)	0.24 (0.56)	0.31 (0.47)

Values displayed as mean difference (p-value), by paired t-test. Positive difference means the value increased over the time period.

BMI, body mass index

Table 3-5: Body composition measures by Air Displacement Plethysmography

Study Subject	1	2	3	4	5	7	10
Body fat (%)	14.9	18.2	18.9	24.5	14.6	18.7	18.7
Fat mass (kg)	3.0	4.8	9.4	8.4	4.1	9.6	7.1
Fat free mass (kg)	17.2	21.7	40.6	25.8	23.7	41.9	30.8

Table 3-6: Body composition measures by Multiple-Frequency Bioelectrical Impedance Analysis

<i>Study Subject</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>	<i>10</i>
Body fat (%)	19.9	19.3	22.5	19.2	9.5	18.5	23.3	17.5	21.9	18.4
Body fat mass (kg)	4.0	5.2	11.3	6.7	2.6	6.4	12.1	5.3	4.0	6.9
Fat free mass (kg)	16.2	21.7	39.2	27.8	25.1	28.5	39.7	24.6	14.5	30.9
ECW/TBW (kg)	0.18	0.17	0.18	0.17	0.17	0.17	0.17	0.17	0.18	0.17
ICW (kg)	7.1	9.9	17.4	12.5	11.3	12.9	18.0	11.1	6.5	13.9
ECW (kg)	4.6	6.0	11.2	7.9	7.1	8.0	11.1	6.9	4.2	8.7
TBW (kg)	11.7	15.9	28.6	20.4	18.4	20.9	29.1	18.0	10.7	22.6
Phase angle (°)	3.6	5.3	3.7	4.8	4.6	4.6	5.3	4.5	4.1	4.7
Right arm lean mass (kg)	0.4	0.8	1.5	1.1	0.9	1.1	2.0	0.9	0.6	1.3
Left arm lean mass (kg)	0.4	0.7	1.6	1.1	0.9	1.1	1.9	0.9	0.6	1.3
Trunk lean mass (kg)	5.7	9.2	15.9	11.8	10.2	12.4	17.5	10.3	5.8	13.1
Right leg lean mass (kg)	1.7	2.7	6.4	3.6	3.3	4.0	5.5	2.9	1.5	4.1
Left leg lean mass (kg)	1.7	2.7	6.3	3.6	3.2	4.0	5.4	3.0	1.5	4.1
Right arm fat mass (kg)	0.4	0.4	0.8	0.5	0.3	0.5	0.7	0.4	0.3	0.5
Left arm fat mass (kg)	0.4	0.4	0.8	0.5	0.3	0.5	0.8	0.4	0.3	0.5
Trunk fat mass (kg)	0.6	1.3	4.9	2.3	0.1	2.2	5.9	1.5	0.6	2.6
Right leg fat mass (kg)	1.0	1.1	2.0	1.3	0.7	1.2	1.8	1.0	1.0	1.2
Left leg fat mass (kg)	1.0	1.1	2.0	1.2	0.7	1.2	1.8	1.0	1.0	1.2

ECW/TBW, extracellular water/total body water; ICW, intracellular water; ECW, extracellular water; TBW, total body water

Table 3-7: Mean body compositions measures by device

	ADP	MF-BIA	p-value [^]
Body fat (%)	18.4 (±3.3)	19.0 (±3.9)	>0.99

Values presented as mean (±SD).

ADP, Air Displacement Plethysmography; MF-BIA, Multiple-Frequency Bioelectrical Impedance Analysis

[^]Wilcoxon signed rank test

Figure 3-2: Scatterplot of the relationship between percentage body fat, fat mass and fat free mass grouped by age
• less than 10 years and ○ 10 years or more; years since transplant – △ less than 7 years and ▲ 7 years or more and
respective Bland-Altman plots of differences and means for percentage body fat, fat mass, and fat free mass.

Table 3-8: Muscle and adipose thickness measures by ultrasound

<i>Study Subject</i>	<i>1</i>	<i>2</i>	<i>3A</i>	<i>3B</i>	<i>4*</i>	<i>5</i>	<i>7</i>	<i>8</i>	<i>9</i>	<i>10B</i>	<i>10A</i>
Left quadriceps muscle thickness (cm)	2.65	2.89	3.12	3.30	2.78		4.48	3.34	1.92	2.72	3.10
Left quadriceps adipose thickness (cm)	0.69	0.78	1.09	1.08	1.07		1.07	1.00	0.82	0.84	0.99
Right quadriceps muscle thickness (cm)	2.73	2.37	3.52	3.31	2.89	3.71	4.92	3.55	1.69	2.18	3.49
Right quadriceps adipose thickness (cm)	0.74	0.86	1.24	1.21	1.05	0.65	1.18	0.97	0.67	0.48	1.31
Left biceps muscle thickness (cm)	0.74	1.21	1.04	1.51	1.31		2.43	1.29	1.38	2.07	1.79
Left biceps adipose thickness (cm)	0.26	0.50	0.70	0.59	0.59		0.46	0.79	0.28	0.42	0.59
Right biceps muscle thickness (cm)	0.94	1.51	1.22	0.89	1.71	1.66	2.28	1.34	1.32	3.14	1.81
Right biceps adipose thickness (cm)	0.42	0.41	0.70	0.83	0.51	0.28	0.51	0.54	0.51	1.02	0.52

*Values represent the mean of three measurements, except for subject 4 where the values represent the mean of two measurements (right quadriceps muscle and adipose and biceps muscle and adipose) or one measurement (left quadriceps muscle and left quadriceps adipose) A or B following the study subject number refer to the observer.

Table 3-9: Intra-rater reliability of ultrasound measurements

Measurement site	All Data		Researcher A		Researcher B	
	Deviance	Median	Deviance	Median	Deviance	Median
Left quadriceps muscle thickness	0.054 (± 0.049)	0.033	0.046 (± 0.043)	0.030	0.070 (± 0.059)	0.050
Left quadriceps adipose thickness	0.035 (± 0.023)	0.033	0.036 (± 0.022)	0.033	0.033 (± 0.026)	0.040
Right quadriceps muscle thickness	0.049 (± 0.060)	0.022	0.029 (± 0.032)	0.020	0.079 (± 0.080)	0.055
Right quadriceps adipose thickness	0.039 (± 0.032)	0.032	0.032 (± 0.021)	0.032	0.048 (± 0.042)	0.033
Left biceps muscle thickness	0.049 (± 0.042)	0.033	0.045 (± 0.046)	0.028	0.058 (± 0.033)	0.040
Left biceps adipose thickness	0.028 (± 0.026)	0.027	0.023 (± 0.024)	0.010	0.038 (± 0.028)	0.037
Right biceps muscle thickness	0.033 (± 0.026)	0.025	0.034 (± 0.023)	0.035	0.031 (± 0.031)	0.020
Right biceps adipose thickness	0.030 (± 0.026)	0.023	0.030 (± 0.026)	0.025	0.029 (± 0.026)	0.022
Sum of all muscle thickness	0.046 (± 0.046)	0.030	0.039 (± 0.037)	0.023	0.059 (± 0.057)	0.040
Sum of all adipose thickness	0.033 (± 0.027)	0.030	0.030 (± 0.023)	0.027	0.037 (± 0.032)	0.033
Sum of muscle and adipose thickness	0.039 (± 0.038)	0.030	0.034 (± 0.031)	0.027	0.048 (± 0.047)	0.037

Deviance is defined as absolute difference of each measure from the mean of the three measures in cm (which is treated as the true value). Values presented as deviance (mean ($\pm$ SD))

Discussion

Weight gain and linear growth are seen in pediatric patients after liver transplant; however, obesity and sarcopenia have been observed as long-term outcomes of liver transplant in children. Body composition assessment tools for children have been limited to reference methods, including ADP, DXA, deuterium dilution, and total body potassium.^{28,33,38,40,41} In this study, we measured body composition using ADP to assess the quality of weight gain following liver transplant and compared results from different assessment tools.

There was agreement between ADP and MF-BIA for measures of percent body fat, FM and FFM such that the differences observed were not statistically significant. Measures of percent body fat, FM, and FFM varied across the study participants, and there were no relationships between age at data collection and years since transplant. Percent body fat and FM measurements determined by MF-BIA were higher than ADP except for participants 4, 5, and 10 (1 male and 2 females); it was not possible to ascertain if this variability was related to the sex of the subject. The mean values for FFM were weakly correlated between ADP and MF-BIA. The ratio between total body water and FFM represents hydration fraction, and 0.732 is the average hydration constant for FFM in healthy adults.⁷³ The observed hydration fraction range in our subjects appears to suggest normal, healthy hydration; however, the water and FFM measures are not independently determined, and the InBody 770 algorithm is unpublished. Thus, it is not clear how appropriate it is for children in our population.

US technology for body composition is a growing area of research and has relevance for whole body evaluation. The sum of US measurements from 9 sites has been associated with whole body lean soft tissue determined by DXA, and has been reported to accurately predict percent body fat, and estimate whole body FM by DXA in

adults.^{68,71,72} In addition, a correlation has been found between US measurements and skinfold thicknesses with comparability to DXA scans, also in adults.⁷⁰ There were only 3 participants that had both US and ADP measures. In these subjects, there was a weak association between the sum of all US measures and ADP measures (not reported). We found a weak correlation between the sum of muscle thickness measures compared to FFM by ADP and the sum of adipose thickness measures compared to FM by ADP; however, the results were not statistically significant. A weak correlation was observed between FM and adipose thickness of the left quadriceps with a p-value of 0.08. US muscle and adipose thickness measures varied by participant, and visual analysis revealed no relationship between age at data collection and time since transplant. There was a weak association between the segmental measures determined by MF-BIA and the US measures for muscle and adipose thickness (not reported). There were 7 participants that had both US and MF-BIA measures. Our results contrast with those of Midorikawa et al. who found that predicted FM by US was similar to regional FM measured by DXA.⁷² Such differences are likely related to the population studied, the number of measurement sites, and the reference method used.

Linear growth following transplant and prevalence of obesity in the long-term are of concern with this population. Our anthropometric data indicate a catch-up in linear growth within 12 months of transplant as evidenced by positive changes in z-scores for height, weight and BMI. Mean z-score comparisons at 6 and 12 months show this increase in linear growth as well. These results are consistent with previous research specific to the pediatric liver transplant population where the highest level of growth has been consistently observed within the first year following transplant.^{9–11} In addition, our anthropometric data show no evidence of obesity or sarcopenia such that BMI values (not reported) for 9 of the 10 participants are <85th percentile and no height/weight z-

scores <-2 . It should be noted that subject 7 was categorized as overweight ($>85^{\text{th}}$ percentile and $<95^{\text{th}}$ percentile). Sarcopenia and obesity have been observed as long-term outcomes of liver transplant in children.^{9–12,21,22} Determination of sarcopenia was based on Mager et al. who found evidence of sarcopenia with z-scores <-2 in children who have had liver transplant.¹⁴ The results of this study indicate that anthropometric values alone are not sufficient to express or describe the quality of weight gain post-transplant.

Intra-rater reliability was assessed overall and by researcher and revealed similar results. There was minimal deviance with measurements ranging from 0.010 to 0.035 cm for Researcher A and 0.020 to 0.050 cm for Researcher B. Due to limited data and time constraints, we did not assess inter-rater reliability.

One major limitation of this study is the small number of participants, which represents approximately 13% of the total eligible patient pool. Recruitment was limited to one hospital, so the results are likely not representative of all children who have had a liver transplant. Another limitation is selection of US measurement sites that were practical and easily accessed with a child in the supine position. Lastly, we underestimated the degree to which the emotional status of the patients would interfere with measurements; some patients were not open to measurement by ADP, and not all patients agreed to the entire protocol. All patients agreed to MF-BIA measurements; we believe this was because the device we used is a stand-on device with hand grips that appears non-threatening to kids. We believe some patients opted out of US likely because of past experiences with US where pressure was applied to obtain images. A demonstration of the amount of pressure involved might have been helpful in obtaining more US measurements.

To our knowledge, this study is the first to measure body composition using multiple modalities and to correlate US measurements with whole body composition in children who have had a liver transplant. Percent body fat, FM, and FFM from MF-BIA using a specific stand-on device and algorithm and ADP were similar, suggesting that this MF-BIA approach is a useful technology for body composition in the pediatric population. No correlation was observed between US measurements and ADP, which may be related to the small sample size. Even though the muscle and adipose thickness measures did not predict percent body fat, FM and FFM, we have created a protocol that can be used in future studies of this population.

CHAPTER 4: CONCLUSIONS AND FUTURE RESEARCH

Children with liver disease experience impaired metabolism resulting in malnutrition and altered growth prior to liver transplant. Following transplant, children tend to immediately gain weight and recover from growth failure or stunting; however, some children may develop obesity as a long-term outcome or experience lifelong metabolic issues. Monitoring weight gain quality post-transplant may help clinicians decrease the risk for the development of obesity and other metabolic diseases while supporting growth recovery. A combination of anthropometry and body composition assessment technology would allow clinicians to assess nutritional status pre and post-transplant in the pediatric population. Use of body composition assessment technology for children has been limited to established reference methods, such as total body potassium and DXA, neither of which is practical in the clinic setting. US and bioimpedance techniques are portable, noninvasive, and can be used for routine measures; however, both require further exploration for application in children.

The purpose of this thesis research was to describe the quality of weight gain in post liver transplant pediatric patients using multiple assessment tools, including ADP, MF-BIA, and US measures of muscle and adipose thickness. Using ADP as a reference method, we found no statistically significant difference between percent body fat, FM and FFM determined by the MF-BIA device we used. When comparing body composition data by ADP to US measurements, we found that US measurements did not accurately predict body composition. Inclusion of additional measurement sites and more study subjects may improve predictability of US measures.

This thesis research provides a foundation for future studies focused on identification of an appropriate body composition technology for children. Development of a standard protocol for US measurements and bioimpedance techniques for children may contribute to the determination of cut-points to define sarcopenia and an expanded

definition of malnutrition in children. Future research will also contribute to pre-transplant nutritional assessment and intervention, such that there could be a reduction in adverse post-transplant outcomes of malnutrition, sarcopenia, and obesity.

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