Sarcopenia is associated with clinical outcomes and physical function in children with end-stage liver disease pre-and-post liver transplantation

by

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Abstract

Background & Aims: Sarcopenia is defined as reduced skeletal muscle mass (myopenia), muscle strength and physical performance. In adults with end stage liver disease (ESLD), sarcopenia is highly prevalent and associated with adverse outcomes. Emerging data have shown that sarcopenia is prevalent in children before and after liver transplantation (LTx). Little information is available regarding the associations of sarcopenia with clinical outcomes and the risk factors influencing the expression of sarcopenia. The study aims were to determine the prevalence of sarcopenia in children with ESLD before and after LTx and to study associations of sarcopenia with clinical outcomes. In **study 1**, we described myopenia in ESLD children awaiting LTx and associations with adverse clinical outcomes up to 1 year post-LTx. In **study 2**, we determined sarcopenia using adult consensus definitions which included skeletal muscle mass (SMM), muscle strength and physical performance measures and explored lifestyle factors (dietary quality and physical activity) that may influence sarcopenia risk/expression.

Methods: Study 1 was a retrospective review study involving SMM and adipose tissues measurements in ESLD children (n=30) with MRI/CT images performed during LTx assessment. Age and gender matched healthy control (HC; n=24) with MRI/CT was included. Clinical outcomes studied include growth, neuro-development, medical complications (e.g. infection, hospitalization). In **study 2**, body composition was measured using Dual Energy X-ray absorptiometry and multiple skinfolds. Muscle strength/function was assessed using handgrip, sit-to-stand, push-up, stair climb tests, 6 minute walk tests in post-LTx children (n=22) and age matched HC (n=47). Habitual

physical activity and dietary intake were assessed using accelerometer (2 weeks) and 3-day food records, respectively.

Results: In **study 1,** myopenia and low subcutaneous adipose tissues were highly prevalent in ESLD children affecting up to 37% of children prior to LTx. Males and older age (>2 years) were the major risk factors. These conditions were associated with gross motor delay, longer hospitalization and increased risk for infection. In **Study 2,** sarcopenia was prevalent in up to 36% of children post-LTx and was associated with deficits in lower limb muscle strength/function (sit to stand, push-ups, and stair climb tests) and lower level of vigorous physical activity. Diet quality was poor but unrelated to sarcopenia expression.

Conclusions: Myopenia associated with low subcutaneous fat deposition and reduced peripheral limb strength/function was highly prevalent in children pre-and-post-LTx and associated with adverse clinical outcomes. Development of rehabilitation strategies to identify and treat sarcopenia is important to optimize clinical outcomes in children with ESLD pre-and-post LTx.

Preface

Chapter 1 of this thesis has been published as "Ooi Poh Hwa, Amber Hager, Vera C Mazurak, Khaled Dajani, Ravi Bhargava, Susan M Gilmour, Diana R Mager. Sarcopenia in chronic liver disease: impact on outcomes. Liver Transpl. 2019;25(9):1422-1438". Ooi Poh Hwa and Amber Hager reviewed the literature/prepared tables. Ooi Poh Hwa, Amber Hager, Diana R Mager drafted the manuscript. Vera Mazurak, Khaled Dajani, Ravi Bhargava, Susan M Gilmour critically reviewed the paper. All authors approved the final version of manuscript prior to submission.

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interpretation, review/final approval of manuscript. Abha Dunichand-Hoedl; reviewed MRI/CT scans, review/final approval of manuscript. Rocio Ayala Romero; data collection, data entry, review/final approval of manuscript. Susan M. Gilmour; study design, data analysis/interpretation, review/final approval of manuscript. Jason Yap YK; study design, data analysis/interpretation, review/final approval of manuscript. Diana R. Mager: study design, data collection/analysis/interpretation, co-wrote with Ooi PH/review/final approval of manuscript. Funding for this study was supported by Hazel McInytre Summer Research Award/ Dr. Elizabeth A Donald MSc Fellowship in Human Nutrition Scholarship, University of Alberta (PHO).

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List of Abbreviation (in alphabetical order)

AIMS; Alberta Infant Motor Scales

ALM; Appendicular lean mass

AWM: Abdominal wall muscle

AWMI: Abdominal wall muscle index

BCAA; Branched chain amino acid

BIA; Bioelectrical impedance analysis

BMI; Body mass index

BMI-z; Body mass undex-z score

BMR; Basal metabolic rate

BOT-2; Bruininks-Oseretsky Test of Motor Proficiency, Second Edition

CMD; Cardio-metabolic dysregulation

CST; Corticosteroid Therapy

CT; Computed tomography

D; Day

DAT; Diet as tolerated

DQ; Diet Quality

DXA; Dual Energy X-ray absorptiometry

EN; Enteral nutrition

ESLD; End-Stage Liver Disease

EWGSOP; European Working Group on Sarcopenia in Older People

FFM; Fat-Free Mass

FFMI; Fat-Free Mass Index

FFMI-z; Fat-Free Mass Index z-scores

HBV; Hepatitis B virus

HCV; Hepatitis C virus

HC; Heathy Control

HEI-C; Healthy eating index for children

Height-z; Height-z score

HRQoL; Health related quality of life

IBD; Inflammatory bowel disease

ICU; Intensive care unit

IMAT; Intermuscular muscular adipose tissue

INR; International normalized ratio

L; Lumbar

L3; Third Lumbar vetrebrate

L4; Fourth Lumbar veterbrate

LBM; Lean body mass

LOS; Length of hospital stay

LTx; Liver Transplantation

MELD; Model End-stage Liver Disease

MF; Muscle function

MQ; Muscle Quality

MRI; Magnetic resonance imaging

MS; Muscle Strength

mTOR; mammalian target of rapamycin.

MUFA; Monounsaturated fatty acid

NPO; Nil per os

PA; Physical activity

PDMS-2; Peabody Developmental Motor Scales-2

PELD; Pediatric End-stage Liver Disease

PM; psoas muscle

PMI; Psoas muscle index (PMI)

PN; Parenteral nutrition

PP; Physical Performance

PSI; Paraspinal muscle index

PTT; partial thromboplastin time

PUFA: Polyunsaturated fatty acid

RAI; Rejection activity index

SAT; Subcutaneous adipose tissue

SATI; Subcutaneous adipose tissue index

SCT; Stair Climb Test

SD; Standard deviation

SDS; Standard deviation score

SGNA; Subjective Global Nutrition Assessment Tool adapted for Pediatrics

SMM; Skeletal muscle mass

SMMA; Skeletal muscle mass area

SMMI: Skeletal muscle mass index

SMM-z; Skeletal muscle mass z-scores

SO; Sarcopenic obesity,

STS; Sit-to-Stand

TAT; Total adipose tissue

TATI; Total adipose tissue index

UPP; Ubiquitin–proteasome pathway

VAT; Visceral adipose tissue

VATI; Visceral adipose tissue

VO2 max; Maximum rate of oxygen consumption measured during exercise

Weight-z; Weight z-score

WHO; World Health Organization

25-OH Vitamin D; 25-Hydroxy Vitamin D

6MWT; Six Minute Walk Test

Publications related to MSc thesis

- Mager DR, Hager A, **Ooi PH**, Siminoski K, Gilmour SM, Yap J. Persistence of sarcopenia after pediatric liver transplantation is associated with poorer growth and recurrent hospital admissions, JPEN J Parenter Enteral Nutr. 2019;43(2):271-280.

- **Ooi Poh Hwa**, Amber Hager, Vera C Mazurak, Khaled Dajani, Ravi Bhargava, Susan M Gilmour, Diana R Mager. Sarcopenia in chronic liver disease: impact on outcomes. Liver Transpl. 2019;25(9):1422-1438.

- **Ooi PH**, Hodgetts S, Pritchard-Wiart L, Gilmour SM, Mager DR. Pediatric sarcopenia: a paradigm in the overall definition of malnutrition in children? JPEN J Parenter Enteral Nutr. 2019. doi: 10.1002/jpen.1681.

- **Ooi Poh Hwa**, Vera C. Mazurak, Kerry Siminoski, Ravi Bhargava, Jason Yap YK, Susan M. Gilmour, Diana R. Mager. Deficits in muscle strength and muscle quality influence physical activity in pediatric liver transplant recipients with sarcopenia. Submitted to Liver Transplantation Journal on September 2019, in review (Manuscript ID LT-19-513).

- **Ooi Poh Hwa**, Vera C. Mazurak, Ravi Bhargava, Abha Dunichand-Hoedl, Rocio Ayala Romero, Jason Yap YK, Susan M. Gilmour, Diana R. Mager. Sarcopenia and reduced subcutaneous adiposity in children with liver disease are associated with adverse outcomes. Submitted to Journal of Hepatology on December 2019, in review (Manuscript ID HEP-20-0001).

Publications not related to MSc thesis

- **Ooi PH**, Gilmour S, Yap J, Mager D, Effects of branched chain amino acid supplementation on patient care outcomes in adults and children with liver cirrhosis: A systematic review. Clin Nutr ESPEN. 2018; 28:41-51.

Other publications

Clinical Nutrition Pathways: Co-reviewer for Practice-Based Evidence in Nutrition (PEN) pathway, Dietitians of Canada on the following topics

- November 2017: Protein intake in adult liver disease
- January 2018: Branched-chain amino acid use in pediatric liver disease

- April 2018: Fat and water soluble vitamins and mineral supplementations in chronic liver disease

Abstract presentations

- Mager DR, Hager A, **Ooi PH**, Robert C, Tang-Wai R, Snyder T, Gilmour S, Yap J. Branched Chain Amino Acid (BCAA) Supplementation in infants with cholestatic liver diseases awaiting liver transplantation: preliminary results from an RCT. ID#2830582. Presented (oral) in American Society of Parenteral and Enteral Nutrition (ASPEN) Nutrition Science & Practice Conference, Las Vegas, Nevada, USA, Jan 21-25, 2018.

- **Ooi PH**, Hager A, Siminoski K, Gilmour S, Yap J, Mager D. Cardio-metabolic dysregulation and sarcopenia in children and youth post-Liver Transplantation. Presented (poster) in Alberta Transplant Institute Research Day, University of Alberta, May 31, 2018.

- Hager A, **Ooi PH**, Siminoski K, Yap J, Gilmour S, Mager DR. Suboptimal vitamin D status and sarcopenia in children post-liver transplantation. Presented (poster) in Faculty of Medicine and Dentistry Undergraduate Research Day, University of Alberta, Oct 24, 2018.

- **Poh Hwa Ooi**, Amber Hager, Kerry Siminoski, Jason Yap ,Susan Gilmour, Diana Mager. Frailty is influenced by poor diet quality in children who have undergone liver transplantation: preliminary findings from a pilot study. Presented (poster) in ASPEN 2019 Nutrition Science & Practice Conference, Phoenix, USA, March 23-26, 2019.

- Amber Hager, **Poh Hwa Ooi**, Vera Mazurak, Jason Yap, Ravi Bhargava, Susan Gilmour, Diana Mager. Sarcopenia and nutritional considerations in children and infants with end-stage liver disease undergoing liver transplantation. Presented (poster) in Annual Poster Symposium Nutritional Science Research Project 2019, Edmonton Clinic Health Academy, University of Alberta, March 27, 2019.

- Diana Mager, **Poh Hwa Ooi**, Daniel Fung, Cheri Robert, David Nicholas, Susan Gilmour. Longitudinal Changes in Health Related Quality of Life in Pediatric Transplant Recipients. Presented (oral) in 10th Congress of the International Pediatric Transplant Association, Vancouver, Canada, May 4-7, 2019.

- **Poh Hwa Ooi**, Amber Hager, Kerry Siminoski, Jason Yap ,Susan Gilmour, Diana

Mager. Frailty is influenced by poor diet quality in children who have undergone liver transplantation: preliminary findings from a pilot study. Presented (poster) in Alberta Transplant Institute (ATI) Research day, University of Alberta, June 10, 2019.

- Diana Mager, **Poh Hwa Ooi**, Kerry Siminoski, Susan Gilmour. Deficits in muscle strength and muscle function may influence expression of cardio-metabolic dysregulation in children post-liver transplantation (LTx). Presented (poster) in American Association for the Study of Liver Diseases (AASLD), The Liver Meeting, November 8-12, 2019.

- Diana Mager, **Poh Hwa Ooi**, Rocio Ayala Romero, Ravi Bhargava, Vera Mazurak, Jason Yap, Susan Gilmour. Does sarcopenia in children with end-stage liver disease (ESLD) adversely impact post-operative clinical outcomes? Accepted for an oral presentation at the 2020 ASPEN Nutrition Science & Practice Conference at the Tampa Convention Center in Tampa, FL from March 28-31,2020. International Abstract of Distinction.

Chapter 1: Literature Review

This chapter represents the work from two review articles that have been published in Liver Transplantation Journal[1] and Journal of Parenteral and Enteral Nutrition[2]. The present chapter included literature review, figures, tables and some components of discussion from those papers. These were used with permission from the publisher.

1.1 Introduction

Sarcopenia is a muscle disease that is characterized by reduced skeletal muscle mass (SMM) and declined muscle strength (MS) and muscle function (MF)[3,4]. Sarcopenia is a component of malnutrition that may occur across a spectrum of body habitus. When relative body fat mass is disproportionately larger relative to reduced SMM, this condition is known as sarcopenic obesity (SO; **Figure 1.1**). The presence of sarcopenia with and without obesity in adults with end-stage liver disease (ESLD) has been well documented and associated with adverse patient outcomes[5-8]. However, the longitudinal evolution of sarcopenia and the lifestyle factors (diet, physical activity) influencing sarcopenia expression and evolution have not been well defined in adults pre-and-post liver transplantation (LTx). While suboptimal nutrient intake due to malabsorption, altered nutrient utilization and hypermetabolism have been implicated in the etiology of sarcopenia in adults with ESLD[9], little is known regarding the impact of these factors in the post-LTx period. This is important to understand to ensure effective rehabilitation programs to treat sarcopenia can be developed. In pediatrics, even less is known regarding the prevalence, etiology, progression of sarcopenia and lifestyle factors

influencing sarcopenia in children with ESLD and liver recipients. Recent evidence has identified sarcopenia in children pre-and-post-LTx[10-12]. However, these studies are limited in measures of muscle strength(MS) or muscle function (MF), that are important components within the definition of adult sarcopenia by the European Working Group on Sarcopenia in Older People (EWGSOP)[3,4]. In fact, a major gap in the pediatrics literature is a lack of a standardized definition of sarcopenia, hindering the ability to accurately diagnose sarcopenia in children.

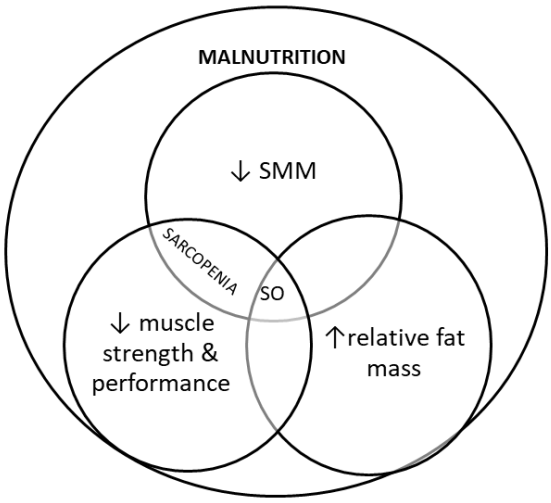

Figure 1.1. The overlapping concept of sarcopenia and sarcopenic obesity. SO: sarcopenic obesity.

Another challenge related to the sarcopenia literature in liver disease is the different methods used to evaluate body composition. A wide plethora of methods have been employed to measure body composition including computed tomography (CT)/Magnetic resonance imaging (MRI) which have advanced the field in body composition analysis in adults with ESLD. These techniques offer an objective measure of nutritional status and body composition without the inherent limitations in other methods (bioelectrical impedance analysis/BIA, Dual Energy X-ray absorptiometry/DXA)

related to the presence of fluid overload[13]. In pediatrics, the use of radiological imaging for body composition assessment may be limited by lack of normative data, radiation exposure (in CT) and the potential limitations related to fluid overload. This review evaluates the literature related to the prevalence of sarcopenia with and without obesity in adults and children with ESLD, the methodological considerations required to assess for sarcopenia, including the need to standardize sarcopenia definition in adults and children with ESLD.

1.2 Sarcopenia in adults pre-and-post liver transplantation

A comprehensive review of the adult literature was conducted to evaluate the presence of sarcopenia with and without obesity in pre-and-post-LTx adults (n=35; **Table 1.1**). The search terms and flow chart of articles screened and reviewed based on the inclusion and exclusion criteria were presented in **Appendix A-1 and Appendix A-2**. The majority of studies (n=29/35) focused on pre-LTx sarcopenia, which included studies that characterized patients with sarcopenia (n=26)[5,8,13-36], SO (n=1)[6] as well as both sarcopenia and SO (n=2)[7,37]. Five studies longitudinally tracked changes in pre-LTx sarcopenia into the post-LTx period (up to 19.3 months)[38-42]. Only 1 article evaluated post-LTx SO[43]. Most of the studies were performed retrospectively (n=30/35)[6-8,13-33,36-40,43]. The sample size ranged from 40 to 795 with subjects aged between 50 to 61 years. The model for end-stage liver disease (MELD) score ranged 14 to 22, with hepatocellular carcinoma, hepatitis C virus (HCV) and alcoholic liver disease being the top 3 indications for LTx.

Table 1.1 Study characteristics of pediatric and adult liver transplantation articles.

Author, year	Study design	N	Sex (M/F, %)	Age (years)	Liver etiology (%)	PELD/ MELD	Study quality
Pediatric studies that assessed pre-LTx sarcopenia							
Lurz et al., 2018[10]	CC	23	39/61	1 (0.5, 4)	BA 65%, AS17%, Low YGT cholestasis 10%, AIH 4%, B-cell ALL 4%	12 (2 , 24)	5
Mangus et al., 2017[12]	CC	35	37/63	8 ± 1	BA 54%, hepatoblastoma14%, Others 32%	14 (10-47)	5
Pediatric study that assessed post-LTx sarcopenia on post-LTx outcomes							
Mager et al., 2018[11]	R	41	41/59	2 (0.5-8)	BA 73%, AS 5%, PSC7%, Others 15%	15 ± 11	8
Adults studies that assessed pre-LTx sarcopenia on waitlist outcomes							
Montano-Loza et al., 2015[28]	R	669	68/32	57 ± 1	HCV40%, Alcohol 23%, NASH23%, Autoimmune 8%, HBV6%	14 ± 0.3	6
Carey et al., 2017[15]	R	396	70/30	58 (51, 62)	HCV 48%, Alcohol 17%, NASH 12%, PSC/PBC/AIH 10%, Others 7%	15 (11, 21)	6
Tandon et al., 2012[31]	R	142	60/40	53 (47, 57)	HCV±Alcohol 38%, AIH 25%, Alcohol 20%, Cryptogenic/NAFLD11%, Others 6%	15 (12, 22)	5
Yadav et al., 2015[36]	R	213	61/39	55 ± 9	HCV 44%, Alcohol 16%, PBC/PSC 8%, NASH 14%, Cryptogenic 6%, Other 12%	16 ±6	7
Van Vugt et al., 2017[33]	P	585	69/31	56 (48, 62)	Alcohol 16%, HBV 3%, HCV 6%, PSC/PBC 22%, HCC/PHC33%, NASH 5%, Cryptogenic 5%, AIH 3%, Others 7%	14 (9, 19)	7
Shirai et al., 2018[30]	R	207	52/48	55 (18-69)	HCC 36%, HBV/ HCV 19%, PBC&PSC 17%, BA 9%, ALF (unknown) 3%, Alcohol 4%, Metabolic 3%, BCS 1%, Others 8%	17 (5-41)	5
Dolgin et al., 2018[18]	R	136	66/34	≥55: 58%, <55: 42%	HCV 47%, Alcohol 24%, Others 29%	21 (11, 29)	7
Wang et al., 2016[35]	P	292	66/34	61 (55, 65)	HCV 60%, Alcohol 10%, NAFLD 8%, Cholestatic 10%, Others 12%	15 (12, 18)	7
Adults studies that assessed pre-LTx sarcopenia on post-LTx outcomes							
Dimartini et al., 2013[17]	R	338	66/34	55 ± 10	HCV/HBV 27%, Alcohol 23%, Alcohol + HCV/HBV 9%, NASH 14%, Autoimmune/PSC 12%, FHF 4%, Other 11%	20 ± 9	6
Masuda et al., 2014[8]	R	204	50/50	54 ± 10	HBV 13%, HCV 51%, PBC 13%, Alcohol 5%, Others 18%	<20: 83% ≥20: 17%	8
Aby et al., 2018[14]	R	146	42/58	58 ± 10	NASH 73%, Cryptogenic 27%	35±7	6
Englesbe et al., 2010[20]	R	163	63/37	53 ± 9	Alcohol 12%,HCC 13%,HCV 35%,PBC 6%,PSC10%, Others 24%	19± 8	7

Montano-Loza et al., 2014[29]	R	248	68/32	55 ± 1	HCV: 51%, Alcohol 19%, Autoimmune 15%, HBV 8%, Other 7%	18±1	4
Harimoto et al, 2017[5]	P	102	44/56	56 (54, 58)	HCV 24%, HCC 42%, Others 34%	16 (15, 18)	8
Hamaguchi et al., 2017[22]	R	250	49/51	54 (43, 62)	HCC 33%, HBC/HCV 20%, PBC& PSC 17%, BA 8%, ALF(unknown) 4%, Alcohol 5%, Metabolic 2%, BCS 2%, Others 9%	17 (14, 22)	8
Chae et al., 2018[16]	R	408	70/30	52 ± 9	HBV 58%, alcohol 20%, HCV 8%, toxins and drugs 6%, AIH 3%, HAV1%, Cryptogenic hepatitis 4%	16±1	7
Golse et al., 2017[21]	R	256	77/23	53 ± 11	Alcohol 45%, HCV 35%, HBV 7%, NASH 2%,Autoimmune 2%,Biliary 6%,Other 3%	19 ±10	7
Kalafateli et al,2017[25]	R	232	70/30	54 (22-70)	AIH 20% , Viral 35%, Alcohol 24%, Others 21%	14 (6-42)	7
Hamaguchi et al., 2014[13]	R	200	48/53	54 (18-69)	HCC 34%, HBV/ HCV 19%, PBC & PSC 17%, BA 10%, ALF (unknown) 4%, Alcohol 3%, Metabolic 3%, BCS 2%, Others 8%.	18 (5-55)	6
Izumi et al., 2016[23]	R	47	51/49	54 (26-66)	PBC 20%, FHF 7%, HCC 19%, HCV 29%, HBV 10%, NASH 3%, Alcohol 5%, Unknown 2%, AIH 2%, Others 3%	19 (5-48)	6
Kaido et al., 2013[24]	R	124	48/52	54 (19-69)	HCC 32%, HBV/HBC 23%, PBC/PSC 17%, Alcohol 5%, Metabolic 5%, BA 4%, Others 14%	19 (7 - 41)	5
Krell et al., 2013[27]	R	207	62/38	52 ± 10	HCV 24%, HBV 4%, HCC 23%, Alcohol 13%, PSC 9%, PBC 7%, AIH 5%, NASH 3%, FHF 2%, A1AD 1%, Wilson's disease 1%, Others 8%	20 ± 7	6
Kim et al., 2018[26]	R	92	100/0	53 (50, 57)	HBV 85%, HCV 9%, Alcohol 3%, Unknown 3%	≥20: 11%	7
Underwood et al., 2015[32]	R	348	62/38	52 ± 10	HCV 38%, Alcohol 41%, HCC 27%	19 ± 8	5
Itoh et al., 2016[6]	R	153	56/44	58 (34-70)	HCC 100%	≥15: 31%	7
Hammad et al., 2017[37]	R	200	48/53	54 (18-69)	HCC 34%, HCV/HBV 19%, PBC/PSC 17%, ALF 4%, BA 10%, Metabolic 3%, Alcohol 3%, BCS 2%, Others 8%	18 (5-55)	7
Kamo et al., 2018[7]	R	277	48/52	54 (18-69)	HCC 27% HBV/HCV 22%, Cholestatic 20%, Others 31%	17 (4-55)	5
Adults studies that assessed pre-LTx sarcopenia on wait list and post-LTx outcomes							
Engelmann et al., 2018[19]	R	795	71/29	54 ± 9	Alcohol 62%, HBV & HCV 10%, NASH 6%, Others 22%	16 ± 7	6
Van Vugt et al., 2018[34]	R	224	67/33	56 (48, 62)	Alcohol 13%, HBV 3%, HCV 7%, PSC/PBC 29%, HCC 33%, Cholangiocarcinoma 1%, NASH 3%,Cryptogenic 4%, AIH 2%, Other5%	16 (11, 20)	6
Adults studies that assessed longitudinal evolution of sarcopenia pre-and post-LTx							
Jeon et al., 2015[40]	R	145	80/20	50 ± 8	HBV 84% and/or HCC 66%	14 ± 8	8

Tsien et al, 2014[42]	P	53	77/23	57 ± 8	HCC 64%, Cirrhosis without HCC 28%, Cholestasis 8%	13 ± 5	7
Kaido et al., 2017[41]	P	72	53/47	55 (21–68)	HCV/HBV 22%, HCC 22%, PBC &PSC 22%, Alcohol 10%, BA 8%, NASH 4%,Others 12%	18 (6–41)	6
Bergerson et al,2015[38]	R	40	65/35	57 ± 11	Alcohol 23%, NASH 53%, PSC 24%	15 ± 6	5
Carias et al., 2015[39]	R	182	69/31	54 ± 8	Alcohol 31%, HCV 31%, NASH 18%, HCC 20%	21 ± 8	5
Choudhary et al, 2015[43]	R	82	84/16	51 ± 11	Alcohol 30%, HCV 22%, HBV 17%, Cryptogenic 24%, Others 7%	N/A	6

Data expressed as mean+/-SD or median (Q1, Q3) or median (range x-y) or percentage (%)
LTx: Liver transplantation, CC: Case Control, R: Retrospective, P: prospective, BA: Biliary atresia, AS: Alagile syndrome, YGT: Gamma glutamyl transferase, AIH: Autoimmune hepatitis, ALL: Acute lymphoblastic leukemia, PSC: Primary sclerosing cholangitis, HCV: Hepatitis C virus, NASH: Nonalcoholic Steatohepatitis, HBV: Hepatitis B virus, PBC: Primary biliary cholangitis, NAFLD: Nonalcoholic fatty liver disease, HCC: Hepatocellular carcinoma, PHC: Perihilar cholangiocarcinoma, ALF: Acute liver failure, BCS: Budd-Chiari Syndrome, FHF: Fulminant hepatic failure, HAV: Hepatitis A virus, A1AD:Alpha-1 antitrypsin deficiency, NSarc: non sarcopenia, Sarc: sarcopenia, N/A: Not available.
Study quality was assessed by Newcastle Ottawa Scale based on 3 subscales (selection, comparability, outcome). Maximum score of 9. . A score of ≥7 is considered high quality study.
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1.2.1 Body Composition Methods used to define sarcopenia in adults

In the studies reviewed, CT was the most commonly used tool to measure body composition method (n=30/35)[5-8,13,15-23,25-28,30,32-40,42,44], followed by BIA (n=3/35)[24,41,43] and concurrent use of CT and MRI (n=2/35)[14,31] (**Table 1.2**). There were inconsistencies in terms of the landmarks used to measure SMM (62% at L3[5-8,14,15,22,23,25,26,28-31,33-36,38,39]; 16% at L4[18,27,32,40,42], 16% at L3/L4[16,17,19-21] and 6% at umbilical level[13,37]), muscle types and number of muscle groups to assess SMM (50% of studies defined sarcopenia using total skeletal muscle area[5-7,15,17,22,28,29,31,33-36,38,39,42], 44% based on psoas muscle area[8,13,14,16,18,20,21,23,25,27,30,32,37,40], 3% used 3 muscle parameters [paraspinal, abdominal wall muscle area, skeletal muscle area][19] and 3% measured psoas muscle thickness[26]). Some studies indicated that L3 should be used as the landmark because this will result in better representations of whole body SMM, but others have used L4 as a landmark[45]. There were studies which cited total SMM area as a more complete measure than psoas muscle (PM) area alone, as it is closely related to total body protein and waitlist mortality[46,47]. Psoas muscle thickness as an alternative indicator to assess for sarcopenia was also used, but requires further validation[26].

Cross sectional CT/MRI, segmental DXA using appendicular measures and phase angle BIA have been shown to be less influenced by over-hydration than whole body measures[48-50]. While ascites may be a confounding factor in determinations of SMM, a recent study indicates that total body fluid status rather than the presence of ascites may be the determining factor[47]. Even though DXA and BIA have been reported to overestimate muscle mass due to the assumption of constant tissue hydration, use of these

methods may be clinically warranted to assess for reduced SMM when CT/MRI are unavailable[49,51,52].

1.2.2 Sarcopenia prevalence in adults pre-and post-liver transplantation

The prevalence of pre-LTx sarcopenia, pre-LTx SO, post-LTx sarcopenia and post-LTx SO were 14% to 78%, 2% to 42%, 30-100% and 88%, respectively (**Table 1.2**). There was a wide heterogeneity in the cut off values for lean body mass (LBM) used to define sarcopenia. These included (1) cut offs defined from cancer populations (n=8)[17,28,29,31,35,36,38,39], (2) published healthy adult data (n=6)[5,7,8,22,30,37], (3) sex specific lowest quartile/tertile (n=10)[6,13,16,18,25-27,32,34,40], (4) cut offs specific to patients with ESLD awaiting LTx (n=3)[14,15,21], and (5) cut offs from both cancer and LTx populations (n=1)[33]. In studies that used BIA (n=3), the cut offs were determined by predictive equations[24,41,43]. Four studies included a control group from trauma patients (n=2)[19,20], liver donors (n=1)[23] and healthy adults with CT done due to unspecified abdomen pain (n=1)[42] in defining sarcopenia. There was only 6% of the studies (n=2/35)[5,41] that adhered to the European Working Group on Sarcopenia in Older People (EWGSOP) sarcopenia definition by including reduced SMM and muscle strength or function[3]. This was related to study design where majority of the studies were retrospective review with no information on muscle strength or physical performance provided.

Table 1.2. Definition and prevalence of sarcopenia in pediatric and adult liver transplantation articles.

Authors, year	Body composition method	Muscle measured	Sarcopenia or Sarcopenic obesity definition/ cut off	Time frame of body composition measurement	% prevalence of sarcopenia	% prevalence of SO
Pediatric studies that assessed pre-LTx sarcopenia						
Lurz et al., 2018[10]	CT; L3/4& L4/5	PMA	Compared with controls (Trauma patient with CT)	N/A	N/A	N/A
Mangus et al., 2017[12]	CT; L2/L3	PMA	Compared with controls (Trauma patient with CT)	6-mo pre-LTx	N/A	N/A
Pediatric study that assessed post-LTx sarcopenia on post-LTx outcomes						
Mager et al., 2018[11]	DXA	SMM	SMM-z < -2SD	1–13-yr post-LTx	41	N/A
Adults studies that assessed pre-LT sarcopenia on waitlist outcomes						
Montano-Loza et al., 2015[28]	CT, L3	SMA	F <41cm^2/m^2, M < 53cm^2/m^2	N/A	45	N/A
Carey et al., 2017[15]	CT, L3	SMA	F < 39cm^2/m^2 , M < 50cm^2/m^2	3-mo of listing	45	N/A
Tandon et al., 2012[31]	CT/MRI,L 3	SMA	F <38.5cm^2/m^2, M <52.4cm^2/m^2	1.5-mo of listing	41	N/A
Yadav et al., 2015[36]	CT, L3	SMA	F ≤38.5 cm^2/m^2 and M ≤52.4 cm^2/m^2	6-mo pre-LTx	22	N/A
Van Vugt et al., 2017[33]	CT, L3	SMA	(BMI ≥25) F ≤41 cm^2/m^2 & M ≤53 cm^2/m^2 (BMI<25) ≤43 cm^2/m^2	3-mo of listing	43	N/A
Shirai et al., 2018[30]	CT, L3	PMA	<mean -2 SD: F: 3.9 cm^2/m^2, M: 6.4cm^2/m^2	1-2-wk pre-LTx	N/A	N/A
Dolgin et al., 2018[18]	CT, L4	PMA	>1 SD below average LPA, M: 1488.4mm^2, F: 974.8mm^2	≤ 3-mo & ≥7-d pre-LTx	50	N/A
Wang et al., 2016[35]	CT, L3	SMA	(BMI <25) F <41 cm^2/m^2, M <43 cm^2/m^2, (BMI ≥25) M<53 cm^2/m^2	3-mo pre-LTx	38	N/A
Adults studies that assessed pre-LTx sarcopenia on post-LTx outcomes						
Dimartini et al., 2013[17]	CT, L3/4	SMA	F <38.5cm^2/m^2, M <52.4cm^2/m^2	80-d pre-LTx	68	N/A
Masuda et al., 2014[8]	CT, L3	PMA	<5th percentile: F ≤ 380 cm^2, M ≤ 800 cm^2	1-mo pre-LTx	47	N/A
Aby et al., 2018[14]	CT/MRI; L3	PMA	F <1464mm^2 , M <1561mm^2	6-mo pre-LTx	62	N/A
Englesbe et al., 2010[20]	CT, L4	PMA	By quartile. Reference area: 1.91 cm^2	3-mo pre-LTx	N/A	N/A
Montano-Loza et al.,2014[29]	CT, L3	SMA	(BMI≥25) F ≤41 cm^2/m^2 & M ≤53 cm^2/m^2 (BMI<25) ≤43 cm^2/m^2	6-mo pre-LTx	45	N/A
Harimoto et al, 2017[5]	CT, L3	SMA	<75% SMA of healthy Japanese adults (gender specific formula) AND weak muscle strength	Pre-LTx	24	N/A

			(handgrip OR gait speed)			
Hamaguchi et al.,2017[22]	CT, L3	SMA	<2SD of mean. F:30.9cm^2/m^2, M: 40.3cm^2/m^2	Pre-LTx	21	N/A
Chae et al., 2018[16]	CT, L3-4	PMA	PMI change before LTx to POD7.Cut off: ≤25th quartile/ <-11.7%	1-mo pre-LTx	25	N/A
Golse et al., 2017[21]	CT, L3-4	PMA	F: 1464 mm^2, M: 1561 mm^2	4-mo pre-LTx	22	N/A
Kalafateli et al,2017[25]	CT, L3	PMA	Lowest sex-stratified quartiles. F: 264mm^2/m^2, M: 340mm^2/m^2	≤3-mo pre-LTx/ 1-wk post-LTx	25	N/A
Hamaguchi et al., 2014[13]	CT, UL	PMA	PMI (F: 4.117, M: 6.868)	1-2-wk pre-LTx	N/A	N/A
Izumi et al., 2016[23]	CT, L3	PMA	< First quartile of PMI of donors. F: 442.9 mm^2/m^2, M: 612.5mm^2/m^2	2-mo pre-LTx	N/A	N/A
Kaido et al., 2013[24]	BIA	SMM	<90% of the standard value analyzed by BIA	Pre-LTx	38	N/A
Krell et al., 2013[27]	CT, L4	PMA	Sex specific lowest tertile	3-mo pre-LTx	33	N/A
Kim et al., 2018[26]	CT, L3	PMT	<15.5 mm/m	2-mo pre-LTx	78	N/A
Underwood et al., 2015[32]	CT, L4	PMA	Lowest tertile	3-mo pre-LTx	34	N/A
Itoh et al., 2016[6]	CT, L3	SMA	**SO**: Lowest quartile of SMM-to-visceral fat area ratio	Pre-LTx	N/A	25
Hammad et al., 2017[37]	CT, UL	PMA	**SO:** BMI ≥25 & PMI <−2 SD below the mean of matched sex young healthy LT donors (F<3.92 cm^2/m^2, M<6.36cm^2/m^2)	1-2-wk pre-LTx	36	5
Kamo et al., 2018[7]	CT, L3	SMA	**SO:** F<30.88cm^2/m^2, M <40.31cm^2/m^2 AND VFA≥100cm^2 or BMI ≥25	1-mo pre-LTx	N/A	2-3 (based on VFA & BMI)
Adults studies that assessed pre-LTx sarcopenia on wait list and post-LTx outcomes						
Engelmann et al., 2018[19]	CT, L3/L4	PSMA, AWMA, SMA	Lower quartile. PSMI:F-19.2cm^2/m^2; M-22.4cm^2/m^2, AWMI: F-15.0cm^2/m^2, M-18.5cm^2/m^2, SMI: F-35.3cm^2/m^2, M- 41.9 cm^2/m^2	200-d of LTx assessment	N/A	N/A
Van Vugt et al., 2018[34]	CT, L3	SMA	Lowest sex-specific quartile	3-mo from listing	25	N/A
Adults studies that assessed longitudinal evolution of sarcopenia pre-and post-LTx						
Tsien et al, 2014[42]	CT, L4	SMA	Gender & age specific 5th percentile	Pre & post-LTx	Pre-LTx: 63, Post 13-mo LTx: 87	N/A
Jeon et al., 2015[40]	CT, L4	PMA	<5th percentile. M: 7.7cm^2/m^2 (20-50yr), 6.6 cm^2/m^2 (>50yr), F: 4.6 cm^2/m^2 (20-50yr), 4.4	0.3-mo pre-LTx, 12-mo post-LTx	Pre-LTx: 36, Post 1-yr	N/A

			cm^2/m^2 (>50yr)		LTx: 46	
Kaido et al., 2017[41]	BIA	SMM	<90% of lower limit of standard SMM (calculated based on sex and height by BIA) AND low grip strength (M <26 kg, F<18 kg)	Pre & post LTx	Pre-LTx: 14. SMM declined post-LTx	N/A
Bergerson et al,2015[38]	CT, L3	SMA	F<38.5 cm^2/m^2 , M <52.4 cm^2/m^2	Pre &12-48-mo post-LTx	Pre-LTx: 55, Post LTx: 30	N/A
Carias et al., 2015[39]	CT, L3	SMA	**Sarc**: muscle mass >2 SD below normal. F≤38.5cm^2/m^2, M ≤52.4cm^2/m^2. **SO:** obesity class I, II, or III and sarc	3-mo pre-LTx	Pre-LTx: 59, Post 1-yr LTx: 100	Pre-LTx: 42
Choudhary et al, 2015[43]	BIA	Muscle mass	**Sarc:** muscle mass< normal range. **SO**: BMI >25 and visceral fat mass >normal range (normal range predetermined by BIA)	N/A	N/A	Post-LTx: 88

LTx: Liver transplantation, CT: Computed tomography, PMA: Psoas muscle area, N/A: Not assessed, ESLD: End stage liver disease, mo: Month, DXA: Dual-energy X-ray absorptiometry, SMM: Skeletal muscle mass, SD: Standard deviation, Yr: Year, Mo: Month, Wk: week, d: day, SMA: Skeletal muscle area, F: Female, M: Male, MRI: Magnetic resonance imaging, UL: umbilical level, LPA: Lean psoas area = Total psoas area× [mean density+85]/170, mm^2, POD: Post-operative day, PMI: Psoas muscle index, BIA: Bioelectrical impedance analysis, Sarc: Sarcopenia, SO: Sarcopenic obesity, PMT: Psoas muscle thickness, PSMA: Paraspinal muscle mass area, AWMA: Abdominal wall muscle area, VFA: Visceral fat area, UL: Umbilical level.
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1.2.3 Sarcopenia and clinical outcomes in adults pre-and-post-liver transplantation

The presence of sarcopenia has negatively impacted clinical outcomes in both pre-and-post-LTx periods. In the pre-LTx period, 71% (n=5/7) of the studies reviewed showed an increased waitlist mortality risk associated with sarcopenia[15,19,28,31,33](**Table 1.3**). The pre-LTx sarcopenia was also associated with increased infection rates[19], decline in functional status[18] and pulmonary function[30], but not to health related quality of life (HRQoL) during time on the waitlist[36].

A total of 75% (n=15/20) of the studies reported an association of sarcopenia with higher post-LTx mortality risk[5-8,13,16,17,20-25,37,41]. In those studies that reported greater mortality rates, n=2/15 were conducted in patient with SO[6,7]. Mortality rates were similar in patients with sarcopenia and SO at 1, 3 and 5 years (**Appendix B-1**). Eight out of 9 studies that examined infection showed a greater risk in sarcopenic liver recipients[5,8,21,25,27,29,32,37] compared to non-sarcopenic patients, although one study showed a reduced risk of post-operative bacteremia in patients with SO when compared to sarcopenia alone[37]. Variable results were found related to sarcopenia and hospitalization; with half of the studies (n=4/8) associating the presence of sarcopenia with longer LOS[5,17,25,29], and the other half (n=4/8) demonstrated no association[14,16,21,34]. In studies that reported the effects of sarcopenia on intensive care unit (ICU) stay (n=5)[16,17,21,25,29] and ventilator dependency (n=3)[16,17,21], all studies consistently illustrated an association of longer ICU stay and ventilator needs with sarcopenia. Post-operative complications such as respiratory, renal, graft failure and cardiac events were investigated in 5 studies[5,16,23,32,37]. Of these, 4 studies showed higher rate of these co-morbid conditions in sarcopenic patients than non-sarcopenic patients[5,23,32,37], while the

other study reported that SO patients had a lower incidence of neurological, surgical, respiratory and cardiovascular complications compared to those with sarcopenia alone[37]. Only 1 study investigated the presence of sarcopenia on total hospital cost and found a small effect size on increased total hospital cost as determined by Cohen's *d*[34,53].

Of the 5 studies that assessed longitudinal evaluation of sarcopenia, 4 studies revealed an average 24% increment of post-LTx sarcopenia prevalence as compared to pre-LTx sarcopenia[39-42], while one study showed a 25% reduction in sarcopenia prevalence post-LTx[38] (**Table 1.2**). Sarcopenia appeared to wax and wane through the pre-and post-LTx period in two studies[38,42], where some of the subjects with pre-LTx sarcopenia had normalized muscle mass in post-LTx period or subjects with normal muscle mass before LTx had sarcopenia post-operatively. Two of these studies (n=2/5) examined the association of post-LTx sarcopenia on survival[40,42], with one study shown increased mortality risk with newly developed post-LTx sarcopenia[40]. Only 1 study investigated post-LTx SO and found higher incidence of cardio-metabolic dysregulation (CMD) in those with SO[43].

Table 1.3. Clinical outcomes of sarcopenia in pediatric and adult liver transplantation articles.

Authors. Year	Mortality/ Survival	Infection	LOS/ hospitalization	Others
Pediatric study that assessed post-LTx sarcopenia on post-LTx outcomes				
Mager et al., 2018[11]	N/A	N/A	↑ hospital & ICU stay, readmission, readmission LOS	↓weight velocity SD scores, ↓wt-z/ht-z, ↑ventilator dependency, ↑emergency care
Adults studies that assessed pre-LTx sarcopenia on waitlist outcomes				
Montano-Loza et al., 2015[11]	↓ survival	N/A	N/A	N/A
Carey et al., 2017[15]	↑wait-list mortality	N/A	N/A	N/A
Tandon et al., 2012[31]	↑wait-list mortality	N/A	N/A	N/A
Yadav et al., 2015[36]	Not predictor of wait-list mortality	N/A	N/A	ND in HRQoL
Van Vugt et al.,2018 [33]	↑ 1-mo, 3-mo, 1-yr wait list mortality	N/A	N/A	N/A
Shirai et al., 2018[30]	N/A	N/A	N/A	↓pulmonary function in males
Dolgin et al., 2018[18]	N/A	N/A	N/A	↑risk of being severely impaired functionally[a]
Wang et al., 2016[35]	Muscle function[b] & quality[c] were associated with wait list mortality, but not muscle mass.	N/A	N/A	N/A
Adults studies that assessed pre-LTx sarcopenia on post-LTx outcomes				
Dimartini et al., 2013[17]	Predictor of survival only in males	N/A	↑hospital & ICU stay	↑ ventilator dependency
Masuda et al., 2014[8]	↓OS, 3-yr, 5-yr survival	↑ rate of sepsis	N/A	N/A
Aby et al., 2018[14]	ND in 1yr survival/OS	N/A	ND in hospital stay	N/A
Englesbe et al., 2010[20]	↑ mortality	N/A	N/A	N/A
Montano-Loza et al.,2014[29]	ND in survival	↑ bacterial infection. ND in overall, viral &fungal infections	↑hospital & ICU stay	N/A
Harimoto et al,2017[5]	↑ 6-mo mortality	↑ post-op sepsis	↑ hospital stay	↑ post-op complications[d]
Hamaguchi et al., 2017[22]	↓OS	N/A	N/A	N/A
Chae et al., 2018[16]	↓OS	ND in all-cause infection	ND in hospital stay. ↑ ICU stay	ND in complications[e], thrombosis, ↑ventilator duration
Golse et al., 2017[21]	↓3-mo, 1-yr, 5-yr OS rates & ↑ mortality	↑ severe sepsis	ND in hospital stay. ↑ICU stay	↑ventilator need
Kalafateli et al,2017[25]	↑1-yr mortality	(n=10)↑ infection	(n=10)↑hospital & ICU stay	N/A
Hamaguchi et al.,2014[13]	↓ OS	N/A	N/A	N/A
Izumi et al., 2016[23]	↓4-mo survival rates	N/A	N/A	↑complications[f]

Kaido et al., 2013[24]	↓OS	N/A	N/A	N/A
Krell et al., 2013[27]	N/A	↑ infection	N/A	N/A
Underwood et al., 2015[32]	N/A	↑ sepsis, bacterial infection	N/A	↑complication[g]& FTR[h] rates
Itoh et al., 2016[6]	↓OS	N/A	N/A	N/A
Hammad et al., 2017[37]	Sarc pt had ↓OS than NSarc pt. SN pt ↓OS than SO.	Sarc pt had ↑ bacteremia than NSarc pt. SO pt had ↓ bacteremia than SN.	N/A	Sarc pt had↑ complication[i] than NSarc pt. SO pt had ↓ complications than SN.
Kamo et al., 2018[7]	↓1 and 5-yr OS in SN and SO pt	N/A	N/A	N/A
Adults studies that assessed pre-LTx sarcopenia on wait list and post-LTx outcomes				
Engelmann et al., 2018[19]	PSMI was predictor for death within 1-yr after LTx listing. PSMI, SMI, AWMI were not associated with post-LTx 1-yr survival	Low PSMI predicted bacterial infection, SBP on wait list	N/A	N/A
Van Vugt et al., 2018[34]	N/A	N/A	ND in hospital stay	↑total hospital costs
Adults studies that assessed longitudinal evolution of sarcopenia pre-and post-LTx				
Tsien et al, 2014[42]	ND in pre-LTx sarc on mortality. Post-LTx sarc had trend toward ↑mortality (p=0.08)	N/A	N/A	N/A
Jeon et al., 2015[40]	Newly developed sarc post-LTx ↑mortality	N/A	N/A	N/A
Kaido et al., 2017[41]	(pre-LT sarc)↓OS post-LTx	N/A	N/A	N/A
Carias et al., 2015[39]	SO pt had a trend toward ↓survival (p=0.40)	N/A	N/A	N/A
Choudhary et al, 2015[43]	N/A	N/A	N/A	SO pt had ↑ metabolic syndrome[j]

LOS: Length of stay, LTx: Liver transplantation, N/A: Not assessed, ICU: Intensive care unit, SD: Standard deviation, wt-z: Weight-z score, ht-z: Height-z score, ND: No difference, HRQoL: Health related quality of life, mo: Month, yr: Year, OS: Overall survival, Pt: Patient, Sarc: Sarcopenia, NSarc: Non sarcopenia, SN: Sarcopenic non obesity, PSMI: Paraspinal muscle mass index, SMI: Skeletal muscle mass index, AWMI: abdominal wall muscle index SBP: Spontaneous bacterial peritonitis.

[a]Severely impaired: Functional status; Karnofsky Performance Status/KPS 'C',[b]Muscle function: Grip strength and short physical performance battery,[c]Muscle quality: Defined by the mean hounsfiled units/fat infiltration for total skeletal muscle area at L3,[d]Complications: Complications of Clavien-Dindo grade IV, including amount of ascites and total bilirubin on POD 14,[e]Complications: acute cellular rejection, biliary complications,[f]Complications: Defined as grade ≥3 according to the Clavien–Dindo classification (condition requiring surgical, endoscopic, or radiological intervention), [g]Complications: Included renal failure, sepsis, bacterial infection, multisystem organ failure, bleeding, bile leak, pneumonia, respiratory failure, cardiac event, biliary stricture, graft failure, thrombosis, Clostridium difficile infection, acute rejection, and pulmonary embolus,

[h]FTR: Failure to rescue; if patient experienced one of the complications[g] within one yr of LT and died within one yr of LTx,

[i]Complications: Clavien-Dindo score of ≥3a,includes neurological, surgical, respiratory, cardiovascular, vascular complications,

[j]Metabolic syndrome: Defined as >/= 3 ATP III criteria,

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1.3 Sarcopenia in children pre-and-post liver transplantation: prevalence and clinical outcomes

There were only two studies examining sarcopenia in children pre-LTx[10,12] and one post-LTx[11]. Two studies investigated sarcopenia pre-LTx using psoas muscle area obtained from L3/L4, L4/L5 and L2/3 CT images[10,12]. These case-control studies demonstrated that children with ESLD have a lower SMM than healthy children, but no data was available regarding prevalence, measures of muscle strength/muscle function or peri-and post-operative clinical outcomes[10,12]. One retrospective cohort study conducted by our group explored sarcopenia prevalence post-LTx (for up to 10 years) and examined associations with clinical outcomes[11]. Body composition was measured using DXA[11]. Sarcopenia was defined as SMM-z scores <-2 standard deviation (SD) of published healthy norms and found that 41% of children had sarcopenia up to 8 years post-LTx (**Table 1.2**). This study showed a large effect size related to sarcopenia and peri-operative LOS (longer ICU/total), longer ventilator dependency, poorer growth and re-hospitalization in the post-LTx period[11] (**Table 1.3 & Appendix B-1)**.

1.4 Research gap and considerations in sarcopenia diagnosis in children

1.4.1 Body composition measurement

There are a variety of body composition methods to assess muscle mass with pros and cons (**Table 1.4**). In pediatrics, the important considerations in body composition assessment include radiation exposure and presence of normative data. The high radiation exposure is the major drawback with routine CT use for sarcopenia assessment in children[51]. Even though MRI is radiation free, it is more expensive than CT which may limit its use in serial measurements[51]. Imaging (CT/MRI) is often performed during LTx

assessment and routine follow-up, therefore they may be opportunistically applied to evaluate SMM in pediatric liver population without additional cost and radiation risk. In children, exposure to additional radiation imposed by DXA scan and the requirement for sedation in young children (<3 years) may outweigh the benefits of using DXA to measure SMM. While emerging normative data for body composition analysis using CT exists (1-20 years), the data was only derived from psoas muscle area and not total muscle area[54]. At present, there is no known pediatric normative data for MRI and there is a lack of data for infants and children <18 months for DXA, limiting the ability to diagnose low SMM in children using these methodologies.

Table 1.4. Body composition measurement methods in pediatrics.

Methods	Components measured	Strengths	Limitations
Skinfold**,** Mid upper arm circumference (MUAC), calf circumference (CC)[55-57]	**Skinfold**: Subcutaneous fat **MUAC &CC**: surrogate measure of muscle mass	- Inexpensive - Simple to perform - Non invasive	- Intra- and inter observer variability - Prone to errors in obese children and children with edema - Predictive equations may not be valid in populations other than those from which they were derived.
Bioelectrical impedance analysis (BIA)[56,58]	Fat mass, Fat free mass	- Inexpensive - Quick - Simple to perform - Non invasive	- Potential errors due to generalized predictive equations used - Less suitable for children with altered hydration - Underestimates total body fat in leaner children and overestimates in obese children - Conflicting validity against DXA&isotope dilution in children
Densitometry: a) Air displacement plethysmography[55,59]	Fat mass, fat free mass	- Quick, Noninvasive - Validated in infants & children against isotope dilution & DXA	- Less suitable for subjects with altered tissues density and hydration
b) Hydrostatic weighing[55,56]		- Gold standard in density measurement	- Extensive equipment - Not suitable for children (require complete submersion in water)
Dual-energy X-ray absorptiometry (DXA)[55,56,60,61]	Fat mass, lean tissue mass, bone mineral content	- Precise - Quick - Validated against MRI - Validated equation to determine SMM present	- Trained technician required - Potential errors for subjects with altered tissues density and hydration - Weight, height, width limits (200 kg, 197cm, 66cm) - Low dose of radiation
Magnetic resonance imaging (MRI)[56]	Total, subcutaneous, intramuscular and visceral adipose tissues, skeletal muscle mass	- Gold standard (distinguish visceral & subcutaneous fat), Very precise - No radiation	- High cost - Trained analyst required - Lack of normative data
Computed tomography **(**CT)[54,56]		- Gold standard (distinguish visceral& subcutaneous fat)	- High cost - Trained analyst required

		- Very precise - Normative data present	- Radiation
Ultrasound[56]		- Quick - No radiation - Portable - Relatively inexpensive	- Lack of standardized measurement procedures - Not well validated in pediatrics (conflicting findings against CT and MRI) - Lack of normative data
Total body potassium[57,62]	Body cell mass	- Relatively simple - No radiation	- High cost - Limited availability
Stable isotope dilution/ deuterium dilution[57,63]	Total body water	- Gold standard to estimate fat free mass - Easy to carry out - Low compliance required, suitable for infants and toddlers	- Expensive equipment - Labor intensive for analyses

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1.4.2 Muscle strength and muscle function assessments

A major gap in the literature related to sarcopenia in ESLD children and adults is the lack of muscle strength (MS) and muscle function (MF) measures. Defining sarcopenia without assessing muscle function is not optimum as muscle strength is not linearly related to muscle mass[3]. In children, functional impairment has been associated with higher waitlist and post-LTx mortality, highlighting the importance of thorough assessment of muscle functionality in this population[64]. In younger children (<3 years), considerations of gross and fine motor skills to assess MF/ MS are warranted. However, it is challenging to assess muscle function in a standardized way in infants as their motor performance is determined by a variety of factors, including the development of postural control, coordination, core stability and ability to perform purposeful and isolated movements. Decreased muscle function may contribute to delays in gross and fine motor performance, but it is challenging to isolate the specific effects of decreased muscle function in infants and young children. In addition, the presence of diseases may also limit the ability to perform specific muscle tests. For instance, hyperammonemia in children with ESLD may impair neurocognitive development, a potential confounding variable in muscle function assessment[65]. Hence, it is currently unknown the extent to which sarcopenia in infants and very young children occurs. This area warrants further investigation. Muscle tests need to be tailored to age-appropriate levels of function with consideration of clinical conditions, time, psychometric properties and resources (interdisciplinary team expertise) required to implement muscle function assessments in in-patient and out-patient settings.

There is currently no gold standard tool to assess motor function impairment for the diagnosis of sarcopenia in children[66]. **Table 1.5** represents some validated tools/ tests that may be useful for the MS and MF assessments in pediatrics. In infants and young children, validated tools that include items related to strength and consider the relative developmental age and stage of gross motor and fine motor development may be used[66,67]. These tools involve scoring by examiners, often pediatric physical or occupational therapists who can compare individual child scores to published normative data. In older children (school-aged children/adolescents), handgrip and 6-minute walk test are well documented to determine MS and MF, respectively[68,69]. These tests are in line with the recommended tests to assess for potential deficits in muscle functionality within the adult definitions of sarcopenia[3,4]. While other muscle strength tests (e.g. sit to stand, push-up, pull up, standing broad jump) and physical performance tests (e.g. stair climb test) have been used in pediatric populations[68,70-74], these tests are constrained by the absence of standardized protocols and reference values that often been generated from small sample sizes. In ESLD children, especially those with advanced ascites, concern should be focused on ability to perform muscle tests that required core muscles (**Table 1.5**)[1].

Table 1.5 The potential muscle strength and muscle function assessments in infants and children with ESLD.

Age	Muscle function assessments	Components tested
<6 years	Alberta Infant Motor Scales (AIMS; 0-18 months)	Gross motor skill
	Peabody Developmental Motor Scales (PDMS-2; birth-5 years)	Strength, gross and fine motor skill
	Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2; 4-21 years)	Strength, gross and fine motor skill
6-18 years	**Muscle strength**: - Hand grip strength - Sit to stand* **Muscle function**: - 6 minutes walk test* - Stair climb test*	 Upper limb strength Lower limb strength Endurance Power and balance

**May be difficult to perform in ESLD patients with advanced ascites*

1.4.3 Growth in children

Another important consideration in the evaluation of body composition in pediatrics includes the careful evaluation of overall growth which may impact the assessment of sarcopenia in children (**Figure 1.2**). In pediatric clinical populations, pubertal and growth delay due to malnutrition, alterations in nutrient utilization, energy expenditure may be induced by underlying liver disease and/or medical therapies[75]. A recent retrospective study conducted by our group illustrated that while children post-LTx with sarcopenia may grow within normal reference ranges, growth was lower than children with sarcopenia compared to the children without sarcopenia[11]. Reference data specific to age, gender, ethnicity, pubertal stages and clinical population should be used to determine SMM cut off to avoid misclassification of sarcopenia in children. Hence, sarcopenia in children may not represent the loss of muscle mass, but rather the reduction

in lean body mass accrual and muscle development which may translate to reduction or lack of development in MS and MF.

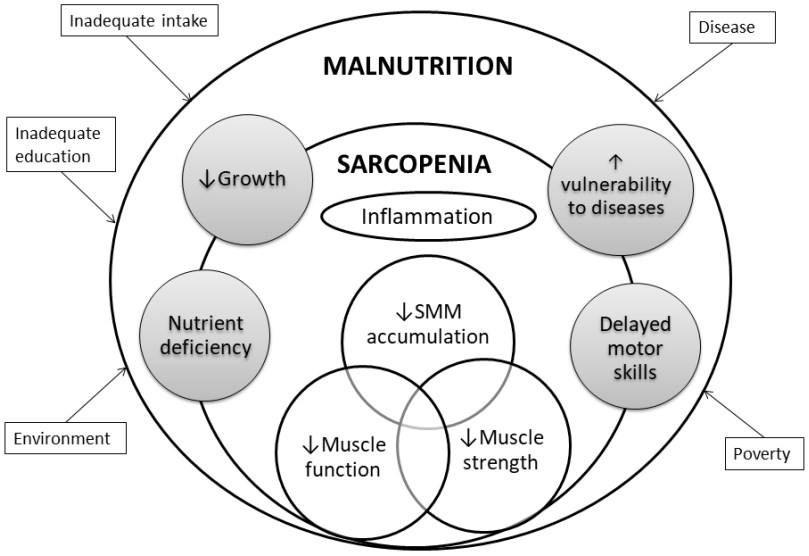


Figure 1.2 Conceptual model for childhood sarcopenia diagnosis. SMM: Skeletal muscle mass. Used with permission by the publisher.

1.5 Factors influencing sarcopenia prevalence and outcomes in adults and children pre-and-post liver transplantation

1.5.1 Gender, Age, Ethnicity and Liver Disease (Type and severity)

The important confounding factors on sarcopenia prevalence in adults and children are sex, age, race and liver disease etiology/severity. Adults males have a higher incidence of sarcopenia than females post-LTx[28,29,39,40]. However, adult females were found to have worse outcomes associated with sarcopenia when compared to males post-LTx[15]. This may due to the earlier age of sarcopenia presentation in women induced by menopausal hormonal changes. These changes may rapidly and adversely influence

protein turnover over shorter periods than in males where declines in testosterone may induce slower changes[76]. In contrast, female children (<10 years) post-LTx had a higher sarcopenia prevalence than older females[11]. While there is limited data addressing gender difference in pediatrics, lower lean mass in young females during early life (1 year), higher proteolysis and protein oxidation in pre-puberty children may be contributing factors to increased expression of sarcopenia in young female children with chronic disease[77,78]. Healthy adults of Asian ancestry were found to have a higher risk of developing sarcopenia than Caucasians given the lower baseline SMM and lifestyle (diet and physical activity) differences[79]. Differences in sarcopenia prevalence and outcomes may be due to variations in liver disease type, severity and/or the emergence of co-morbid diseases such as coinciding inflammatory bowel disease (IBD) with primary sclerosing cholangitis. Variability in liver disease type may be one of the factors for the inconsistent findings related to LOS and sarcopenia found in adults, with the most consistent findings occurring in adults with HCV. Studies with body composition measurement taken closer to LTx are likely to represent sicker patients with higher MELD score, and hence leading to higher sarcopenia prevalence[17,26].

1.5.2 Lifestyle Factors: Diet and Physical Activity

Reduced dietary intake induced by malabsorption, anorexia associated with hyperammonemia and abdominal ascites, and hypermetabolism have been largely implicated in pre-LTx malnutrition and sarcopenia in adults with ESLD[25,80]. Suboptimal protein and vitamin D intake as well as sedentary lifestyles have been recognized as key variables contributing to sarcopenia in adults with ESLD[9]. Data in cirrhosis adults indicated that branched-chain amino acid (BCAA) supplementation may be beneficial in

improving muscle strength[81], while a late evening snack containing carbohydrates has been shown to improve lean body mass in a 12 months randomized trial[82]. Preoperative nutrition and post-operative early enteral nutrition are reported to be beneficial in reducing mortality and sepsis in sarcopenic adult liver recipients[8,24]. In children, there is limited information available regarding whether diet plays a role in sarcopenia etiology in the pre-and-post-LTx periods. A study conducted in newly diagnosed IBD youths demonstrated that suboptimal vitamin D status is associated with higher sarcopenia prevalence[83]. Also, a pediatric study demonstrated that BCAA requirements pre-and-post-LTx are significantly higher than healthy children, indicating that BCAA supplementation to treat sarcopenia may be warranted[84]. While there is evidence on poor dietary quality in post-LTx children[85], little is known regarding the association of dietary intake and sarcopenia in the post-operative period.

Impaired aerobic capacity is common in patients with liver cirrhosis due to sedentary lifestyle[86]. Even though physical activity generally increases post-LTx, physical inactivity remains highly prevalent in adult and children liver recipients[87]. Suboptimal aerobic fitness, chronic and early fatigue and reduced self-efficacy to participate in routine physical activity are often cited as barriers to low physical activity[88-90]. In post-LTx children, parental concerns on physical vulnerability and risk of graft injury also limit their participation in physical activity[88]. Aerobic and resistance exercises are the potential interventions to treat sarcopenia by reducing muscle loss and promoting muscle synthesis[91]. In a randomized trial conducted in cirrhotic adults, aerobic exercise along with leucine supplementation have been shown to improve physical fitness, muscle mass and health related quality of life (HRQoL)[92]. Recent evidence also

demonstrated that resistance exercise is helpful in increasing muscle strength and size in compensated cirrhosis adult patients[93-95]. Besides the beneficial effects targeted on muscle health, physical fitness has been shown to be predictive of waitlist and post-transplant survival in children and adults with liver disease[64,96]. Given scarcity of pediatric data, the role of physical activity in childhood sarcopenia remains to be explored through control trials before effective rehabilitation strategies can be developed.

1.5.3 Post-operative complications and use of immunosuppression drugs

Liver transplantation corrects biochemical and metabolic abnormalities without improving sarcopenia for at least a year[39-42], however one study reported sarcopenia improved after LTx[38]. This difference could be due to the exclusion of subjects with confounding conditions (e.g. infection, kidney failure) that may potentially contribute to post-operative SMM loss and/or the potential reversal of sarcopenia post-LTx[38]. The mechanism of post-LTx sarcopenia is not currently well understood, with some data suggesting immunosuppressant use, inflammation (e.g. post-operative sepsis) and physical inactivity as the contributing factors[38,40]. Sarcopenia pre-and post-LTx seems to share similar cellular mechanisms even though the underlying contributing factors may differ (**Figure 1.3**). The common pathways are upregulation of myostatin expression and inhibition on mTOR signalling leading to a reduction in protein synthesis[97,98]. Concurrently, activation of ubiquitin–proteasome pathway by inflammation and physical inactivity has caused protein degradation[97,98]. The emerging data demonstrated that sarcopenia appears to wax and wane through the pre-and post-LTx period[38,42], which may be related to alteration of immunosuppressive regimen or inflammation. The immunosuppressant calcineurin inhibitor (e.g. Tacrolimus), corticosteroid and

mammalian target of rapamycin (mTOR) inhibitor (e.g. Rapamycin) have been shown to impact muscle morphology type, protein turnover and inflammation; key factors thought to influence sarcopenia prevalence[97] **(Figure 1.3).** Future studies are needed to understand the mechanism of persistent sarcopenia so that targeted long term interventions can be developed.

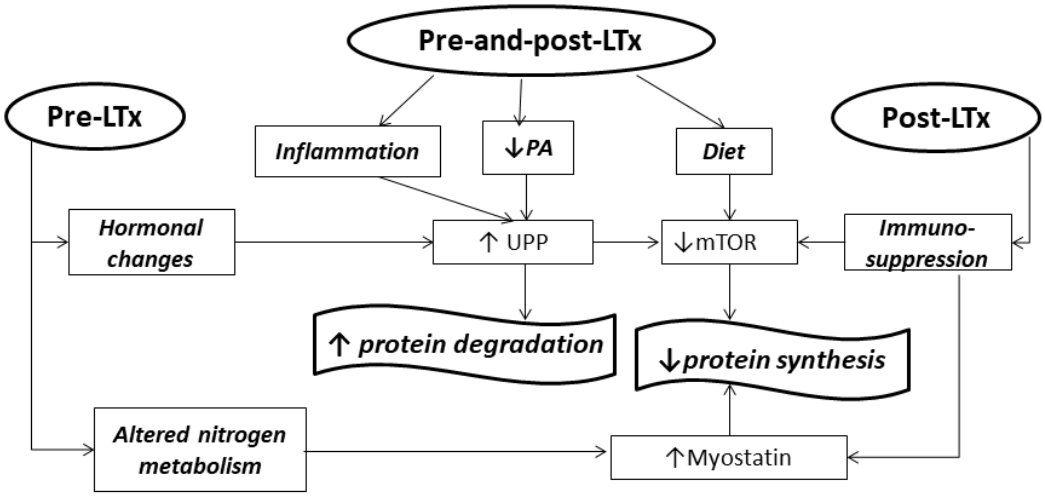


Figure 1.3 Mechanisms of sarcopenia in pre-and-post liver transplantation in skeletal muscle. LTx: Liver transplantation, PA: Physical activity, UPP: Ubiquitin–proteasome pathway, mTOR: mammalian target of rapamycin. Used with permission by the publisher.

1.6 Conclusion

The present review found that sarcopenia in ESLD adults is highly prevalent and is associated with adverse outcomes pre-and-post LTx. Methodological considerations in sarcopenia diagnosis, consistency in approaches to assess body composition and muscle function to define sarcopenia are warranted in adults with ESLD. The emerging longitudinal data illustrates that sarcopenia in the post-LTx period may wax and wane in its presentation[38,42]; which may be related to changes in immunosuppression and

highlight the need for ongoing screening and evaluation. In pediatrics, the presence of sarcopenia is an emerging and important finding that has implications to pre-and-post-LTx outcomes and hence warrants further investigation. Sarcopenic obesity in adults occurs pre-and-post-LTx and may be a significant co-morbid condition contributing to adverse patient outcomes and cardio metabolic dysregulation. In children, SO in pre-and-post LTx children has not been documented, and warrants further investigation.

A major gap in the pediatric liver literature is the lack of uniform definitions for sarcopenia diagnosis, absence of normative data for radiological imaging body composition technique assessment, shortage of muscle strength and muscle function assessments to diagnose sarcopenia and the unavailability of information regarding the potential lifestyle factors that may influence sarcopenia expression in children with ESLD. This information is critically needed to ensure that effective treatment strategies can be developed for children pre-and-post LTx. Hence, this thesis will examine 1) low skeletal muscle mass (myopenia) in infants and children with ESLD and the associations with dietary intake and clinical outcomes in the pre-and-post-LTx period (**Chapter 3**) and 2) the prevalence of sarcopenia in post-LTx children using measures of skeletal muscle mass, muscle strength and muscle function and the association of lifestyle factors (diet, physical activity) with sarcopenia in this population (**Chapter 4**). Results from these studies will inform future research related to the development of rehabilitation strategies to treat sarcopenia in children and youth pre-and-post LTx.

Chapter 2: Research Plan

2.1 Study Rationale

Malnutrition is a common complication in adults and children with end-stage liver disease (ESLD)[99,100]. Sarcopenia is a component of malnutrition focusing on muscle health, characterized by reduced skeletal muscle mass (SMM), muscle strength or muscle function[4]. In adults with ESLD, the presence of sarcopenia is highly prevalent and has been associated with increased waitlist mortality and adverse post-LTx outcomes such as reduced survival, longer hospital stay, higher infection and post-operative complication risks[5,8,31]. The additional adverse outcome includes reduced health-related quality of life[101]. While there is emerging evidence related to the presence of reduced SMM in children with ESLD[10,12], no study has been performed to investigate the association of SMM depletion in the pre-LTx period with pre-and-post-LTx clinical outcomes in this vulnerable clinical group.

Liver transplantation corrects biochemical and metabolic abnormalities in ESLD patients, but in adults it does not appear to reverse sarcopenia consistently for at least the first year after LTx[1]. In pediatrics, sarcopenia in the post-LTx period appears to last up to 8 years post-LTx in approximately 40% of children[11]. Sarcopenia in children post-LTx has been showed to adversely impacted growth, extended hospital stay (total/intensive care unit [ICU]), increase dependency on ventilator support and increase risk for hospital readmission[11]. One major limitation in this research, however, was the lack of data related to muscle strength and physical performance, which is needed for a thorough sarcopenia diagnosis. In addition, the literature lacks any information in pediatric sarcopenia related to the potential lifestyle factors (diet, physical activity) that may

contribute to post-LTx sarcopenia in childhood. This information is needed so evidence-based rehabilitation strategies to treat and prevent sarcopenia in children with ESLD can be developed.

Given limited pediatric data related to sarcopenia in children with ESLD, along with improved post-operative survival with medical advancement[2,102], there is a need to expand the knowledge related to sarcopenia in pre-and-post-LTx children. The purpose of this thesis was to fill the knowledge gap in clinical outcomes associated with pre-LTx sarcopenia, establish the prevalence of post-LTx sarcopenia using definitions of sarcopenia that include measures of muscle strength and physical performance and to examine and the lifestyle factors (dietary intake and physical activity) influencing the risk of sarcopenia in pediatric LTx recipients. Only one component of sarcopenia (reduced skeletal muscle mass/ myopenia) was assessed in **chapter 3** due to the retrospective study design that reflects standard clinical care and no muscle function data was available. In **chapter 4**, prospective design allows comprehensive assessment of sarcopenia which include muscle mass, muscle strength and physical performance. The findings from this thesis will serve as an important foundation for future clinical trials and development of nutritional intervention and rehabilitation strategies targeted to combat this muscle disease in children with ESLD.

2.2 Objectives & Hypotheses

Study 1 (Chapter 3)

- **Title:** Myopenia in children with end-StAge liver disease awaiting Liver Transplantation- 2 factor evaluation (SALT-2)

- **Objective 1:** To evaluate the prevalence of reduced skeletal muscle mass (myopenia) in infants and children with ESLD at the time of LTx assessment using Magnetic resonance imaging (MRI)/Computed tomography (CT) technology.

- **Objective 2:** To evaluate the associations of reduced skeletal muscle mass (myopenia) in infants and children with ESLD on clinical outcomes (growth, gross motor skills, hospitalization, medical complications e.g. infection, biliary and vascular complications, rejection) in the pre-and-post-LTx periods.

- **Objective 3:** To evaluate the associations of reduced skeletal muscle mass (myopenia) in infants and children with ESLD with nutritional intake (energy and protein) in the pre-and-post-LTx periods.

- **Hypothesis 1**: Reduced skeletal muscle mass (myopenia) is highly prevalent in infants and children with ESLD.

- **Hypothesis 2**: Reduced skeletal muscle mass (myopenia) in infants and children with ESLD at time of LTx assessment will be associated with reduced growth, delayed gross motor, increased length of stay and increased risk for

complications (vascular, and biliary, infection and rejection) in pre-and-post-LTx periods..

- **Hypothesis 3**: Reduced skeletal muscle mass (myopenia) in infants and children with ESLD at time of LTx assessment will be associated with decreased energy and protein intakes in the pre-and-post-LTx periods.

Study 2 (Chapter 4)

- **Title**: Contribution of lifestyle factors and functional measures of muscle strength and physical performance and sarcopenia in pediatric liver transplant recipients.
- **Objective 1:** To determine the prevalence of sarcopenia in children post-LTx using a definition that included the evaluation of skeletal muscle mass, muscle strength and muscle function.
- **Objective 2:** To describe the lifestyle factors (diet and physical activity) associated with sarcopenia prevalence.
- **Hypothesis 1:** Sarcopenia (as defined by reduced SMM and reduced muscle strength and physical function) is highly prevalent in post-LTx children.
- **Hypothesis 2:** Sarcopenia in post-LTx children is related to poor diet quality and physical inactivity.

Chapter 3: Study 1

Title: Myopenia in children with end-StAge liver disease awaiting Liver Transplantation- 2 factor evaluation (SALT-2)

This manuscript has submitted to Journal of Hepatology on December 2019, currently in review (Manuscript ID HEP-20-0001).

3.1 Abstract

Background/Aims: Sarcopenia is a component of malnutrition defined as reduced skeletal muscle mass, muscle strength and physical performance. While low skeletal muscle mass (myopenia) has been recently identified in children pre-and-post liver transplantation (LTx), clinical outcomes associated with myopenia remain unknown. We hypothesized that myopenia would be prevalent in children with end-stage liver disease (ESLD) and related to suboptimal nutritional intake contributing to gross motor and growth delay, increased hospitalization and medical complications. **Methods**: This retrospective study involved evaluation of MRI/CT scans for skeletal muscle mass (SMM) indices (total, psoas, paraspinal, abdominal wall muscle; $cm^2/height^2$) and adipose tissues indices (total, visceral, subcutaneous (SAT), intermuscular fat) at L3 and L4 vertebrates at LTx assessment. ESLD children (n=30) were age and gender matched to healthy controls (HC; n=24). Myopenia was defined as SMM index z-score <-2 and low SAT was defined as SAT index z-score <-1.5. Anthropometrics, biochemical data, clinical outcomes (hospitalization, complications, growth, neurodevelopment, energy/protein intake) were collected at LTx assessment, LTx, post-LTx (first hospitalization, 6 & 12

months). **Results:** Four distinct body composition phenotypes in infants and children with ESLD awaiting LTx were found, a) myopenia with low SAT (17%), b) myopenia (3%), c) low SAT (20%), d) normal muscle mass and SAT (60%). Myopenia in the presence of low SAT was prevalent in older (>2 years), male children and was associated with gross motor delay, reduced energy intake, increased hospitalization and infections (total/viral/fungal). **Conclusions:** Myopenia, accompanied by low SAT in children with ESLD is associated with adverse clinical outcomes. Rehabilitation strategies aimed at combating sarcopenia in children prior to LTx is important.

3.2 Introduction

Malnutrition secondary to suboptimal intake, altered nutrient utilization, elevated resting energy expenditure and malabsorption is highly prevalent in children with end-stage liver disease (ESLD) awaiting liver transplantation (LTx)[103]. Emerging data indicate that sarcopenia is an important component of malnutrition in adults with ESLD[104]. Sarcopenia is a muscle disease that is defined as loss of skeletal muscle mass (SMM), muscle strength and physical performance[4]. In adults with cirrhosis, there is substantial evidence that sarcopenia is associated with increased morbidity and mortality pre-and-post-LTx[5,8,29]. Many factors influence sarcopenia risk including reduced dietary intake, physical inactivity, inflammation and use of immunosuppressive medications (e.g. corticosteroids, calceneurin inhibitors)[1,80,97] which may adversely impact skeletal muscle physiology.

Sarcopenia in children with ESLD has been recently identified, but has not been related to patient outcomes[10-12]. We identified that children post-LTx have recurrent sarcopenia that is associated with increased hospitalization (total/ICU) and ventilator dependency in the peri-operative period and associated with reduced muscle strength, muscle functioning and growth, along with increased frequency of re-hospitalization in the longer term[11,105]. This highlights the importance of understanding the evolution of sarcopenia in children with ESLD and the factors that may influence sarcopenia expression.

The study purpose was to determine the prevalence of one component of sarcopenia, which is low skeletal muscle mass (myopenia) in children with ESLD at the time of LTx assessment using cross sectional abdominal imaging and to evaluate the

associations with clinical outcomes including growth, gross motor skills, hospitalization, medical complications and nutritional outcomes pre-LTx and up to one year post-LTx. We hypothesized that myopenia is highly prevalent in children with ESLD and would be associated with reduced growth, malnutrition, delayed gross motor development, increased medical complications and length of stay.

3.3 Methods

A retrospective cohort study was conducted in children with ESLD who underwent LTx assessment (2013-2019) at the Pediatric Liver Transplant Clinic, Stollery Children's Hospital in Edmonton, Alberta. Children aged 2 months to 18 years with CT/MRI scan during LTx assessment were included (n=40). Charts were excluded if children were <2 months of age or >18 years or who underwent multi-visceral transplants or re-transplantation or had metabolic defects, deceased (n=4), inability to access medical charts due to confidential issue (n=1) and those without available abdominal imaging at time of LTx assessment (n=5)**,** leaving a total of 30 children for review **(Figure 3.1)**.

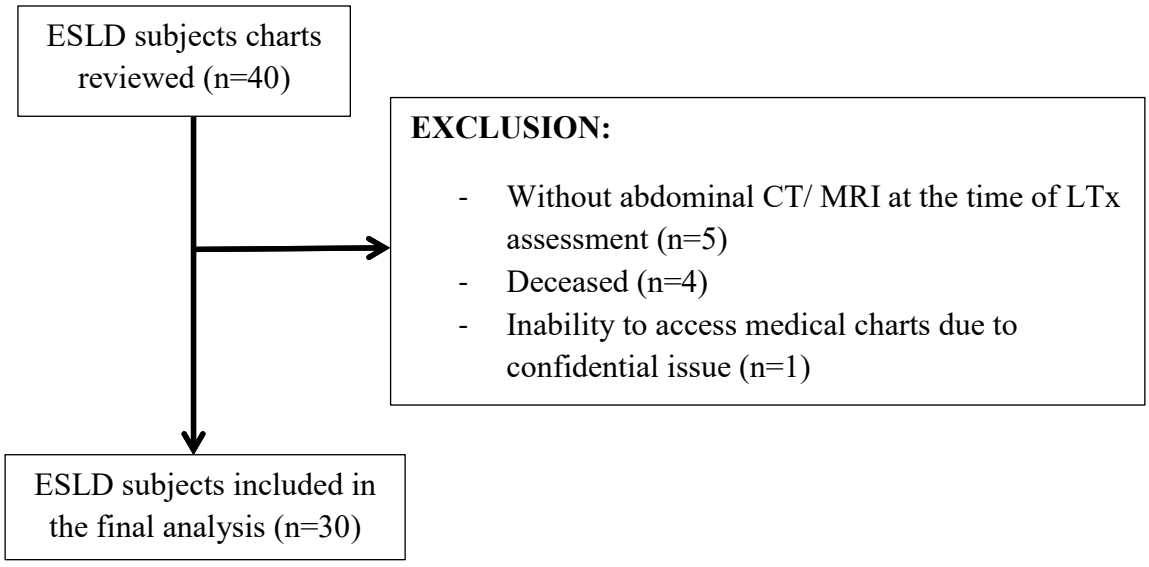


Figure 3.1 Flow chart of patient selection.

Age and gender matched healthy controls (HC) with abdominal CT/MRI (2012-2018) performed for investigational purposes that were found to have normal CT/MRI findings were selected from the hospital radiology database (n=30). HC were excluded if underlying medical conditions such as Crohn's disease (n=3), kidney disease (n=1), hemangioma (n=1), heart disease (n=1) were documented; leaving a total of 24 scans. HC with CT imaging were matched with ESLD children with CT scans and the same with children with MRI imaging. Data were obtained through the electronic medical record (eClinician and Organ Transplant Tracking Record [OTTR]) and from paper medical chart review. Data retrieved covered 5 time points, which included during LTx assessment, LTx, immediate post-LTx (during ICU and hospital stay) until time of first discharge, at six-month and one-year follow-up **(Appendix C-1)**. This study was approved by the Health Research Ethics Board at the University of Alberta (PRO0078499).

3.3.1 Demographic, Anthropmetric, Laboratory, Growth and Neurodevelopment data

Demographic and anthropometric data, including age, gender, weight, height at the time of MRI/CT scans were collected. Weight-z, height-z and BMI z-scores were calculated according to World Health Organization standards using the Canadian Pediatric Endocrine Guideline[106]. Growth as indicated by absolute weight/height gain (g/day or mm/day) and weight/height velocity standard deviation score (SDS) was determined in ESLD children using weight and height at LTx, at 6 month and 1 year post-LTx based on published reference data[107]. Medical variables such as liver disease etiology, immunosuppressive medications (type/dose), liver disease severity scores

(Pediatric End-Stage Liver Disease scores [PELD]/ Model for End-Stage Liver Disease [MELD]) were collected. PELD and MELD were calculated according to the United Network for Organ Sharing/Organ Procurement and Transplantation Network[108]. Laboratory parameters including liver function test (alanine transaminase [ALT], aspartate aminotransferase [AST], Gamma-glutamyltransferase [GGT], albumin, bilirubin), international normalized ratio [INR], partial thromboplastin time [PTT], tacrolimus though level, C-reactive protein [CRP], white blood cell, hemoglobin, 25-hydroxy vitamin D, urea, creatinine were measured by the Core Laboratory at Alberta Health Services using validated methodologies[11]. Data for developmental assessments (Vineland Adaptive Behavior Scales-II) and brain MRI reports performed at LTx assessment were collected where available[109]. Individual (communication, daily living skill, socialization, motor skill) and composite domain (adaptive behaviour composite score) were recorded by actual score, standard deviation, percentile and age-appropriate motor function (yes/no). Brain MRI performed was scored as normal vs abnormal findings based on radiologist assessment.

3.3.2 Body composition measurement

Body composition was measured using abdominal MRI/CT images performed at time LTx assessment (ESLD) and assessment (HC). Abdominal MRI (n=46)/CT (n=8) scans in axial view were retrieved from the Picture Archiving and Communication System (PACS) without subjects' identifiers in Digital Imaging and Communications in Medicine (DICOM) file format and analysed using Sliceomatic software (5.0 Rev- 5f, Tomovision). All images were analyzed by trained investigators and re-reviewed with the team radiologist who was blinded to patient disease condition.

The landmark for body composition analysis was at the third (L3) and fourth (L4) lumbar veterbrate levels, with the presence of anterior and posterior body, pedicle and lamina of veterbrate bone for both CT/MRI **(Figure 3.2)**. Landmarks chosen for cross sectional imaging included a) inferior margin of the liver, b) position of the portal vein/superior mesenteric vein and c) location of the iliac crests. At times, only L3 slices were available to review due to anatomical differences in the individual child that precluded available data at the L4 vertebrate level (n=47 with L3 + L4 slices [n=23 ESLD, n=24 HC], n=7 ESLD with L3 slices only). The assessment on two levels was done to allow comparison to ensure a better representation of whole body composition. No major differences in CT or MRI values for total or segmental muscle tissue and adipose tissue were observed ($p>0.05$), and there were no major differences in the length of time between individual scan and LTx between methods ($p=0.63$).

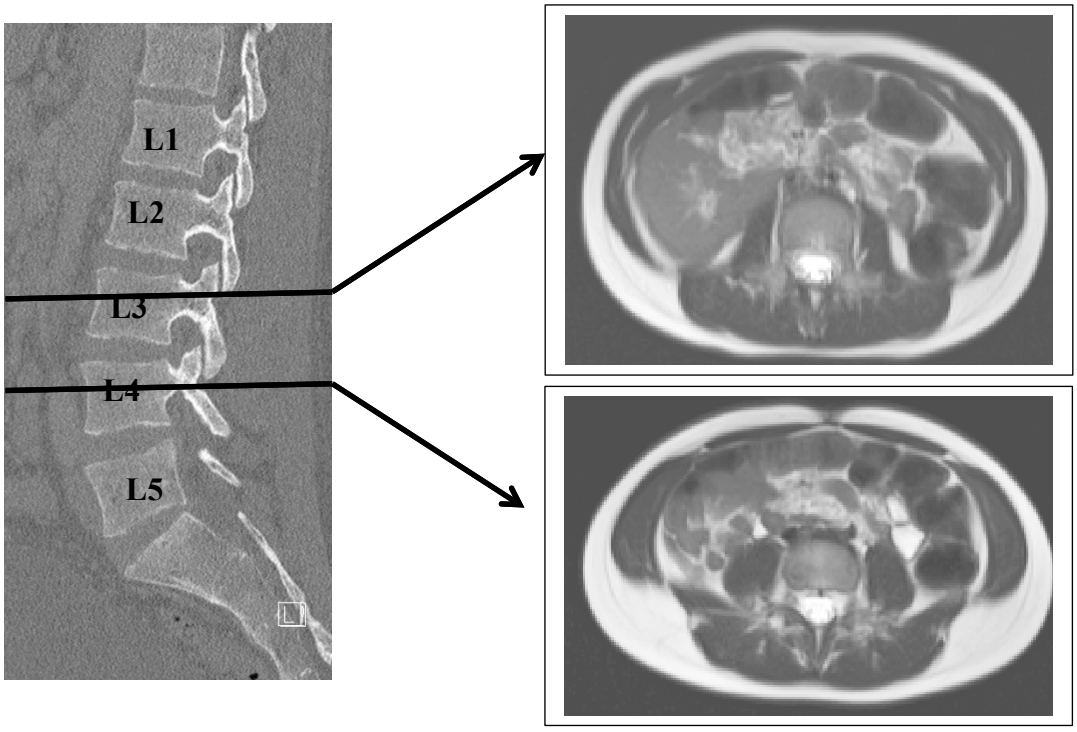


Figure 3.2. Body composition measurement landmarks at lumbar veterbrate 3 (L3) and lumbar veterbrate 4 (L4) using MRI or CT modality in post-liver transplantation children and healthy controls.

3.3.3 Muscle and Fat Cross-Sectional Area

Cross-sectional area (in cm^2) of 3 muscle sites, including psoas, paraspinal (quadratus lumborum & erector spinae) and abdominal wall muscles (AWM; rectus abdominus, internal and external obliques, transverse abdominus) were measured. Total skeletal muscle mass area (SMMA) was defined by the sum of all muscle groups **(Figure 3.3)**. Similarly, individual adipose tissue area was analysed, including visceral adipose tissue [VAT], subcutaneous adipose tissue [SAT] and intermuscular muscular adipose tissue [IMAT]. Total adipose tissue [TAT] was referred to the sum of VAT, SAT and IMAT **(Figure 3.3)**. Mesenteric fat was excluded from analysis to minimize the potential

of overestimation of VAT. This was done as ESLD children often presented with abdominal ascites which engorges the mesenteric fat. Besides, tendency of gut motion during MRI scan causing blood vessels and fat blur together, making it difficult to differentiate mesenteric fat. The retroperitoneal fat surrounding the aorta and kidneys was included as a part of VAT **(Figure 3.4)**.

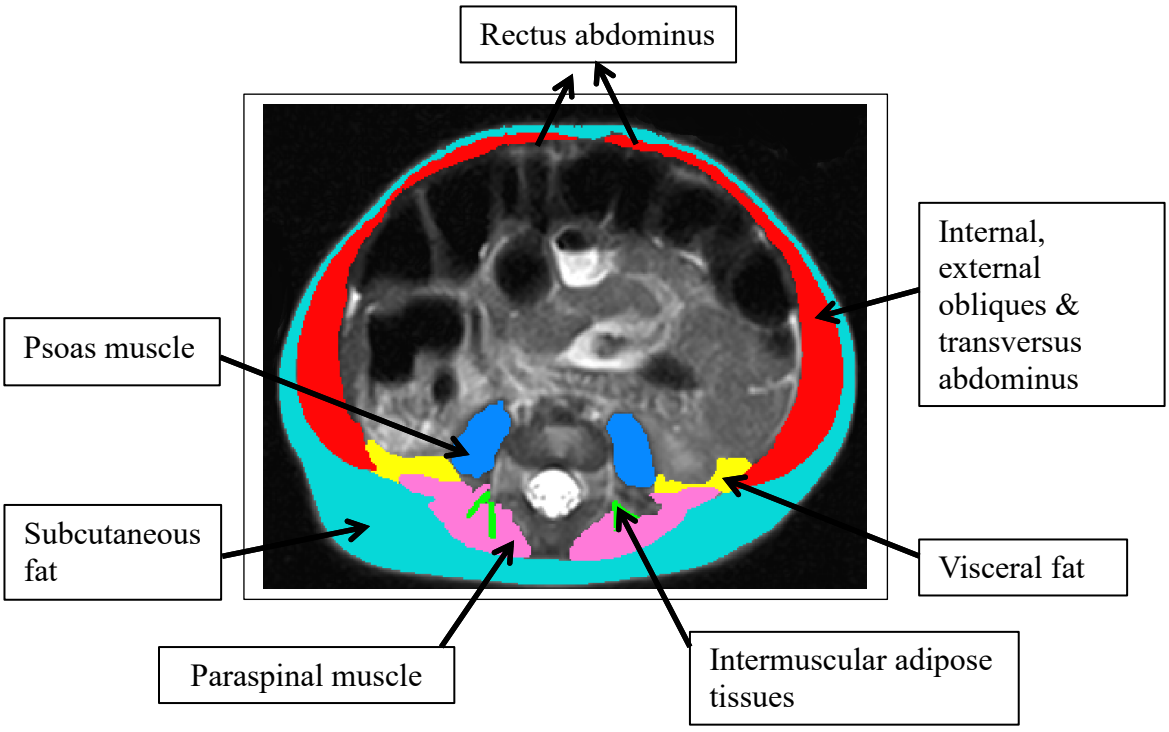


Figure 3.3 The parameters of skeletal muscle and adipose tissue measured. *Blue*: Psoas muscle, *Pink*: paraspinal muscle (Quadratus lumborum & Erector spinae), *Red*: Adominal wall muscles (Rectus abdominus, internal and external obliques and transversus abdominus), *Green*: Intermuscular adipose tissue, *Yellow*: Visceral adipose tissues, *Cyan*: Subscutaneos adipose tissues. Total skeletal muscle area (cm^2) refers to sum of red, blue and pink surface area. Total adipose tissue area (cm^2) refers to sum of yellow, cyan and green surface area

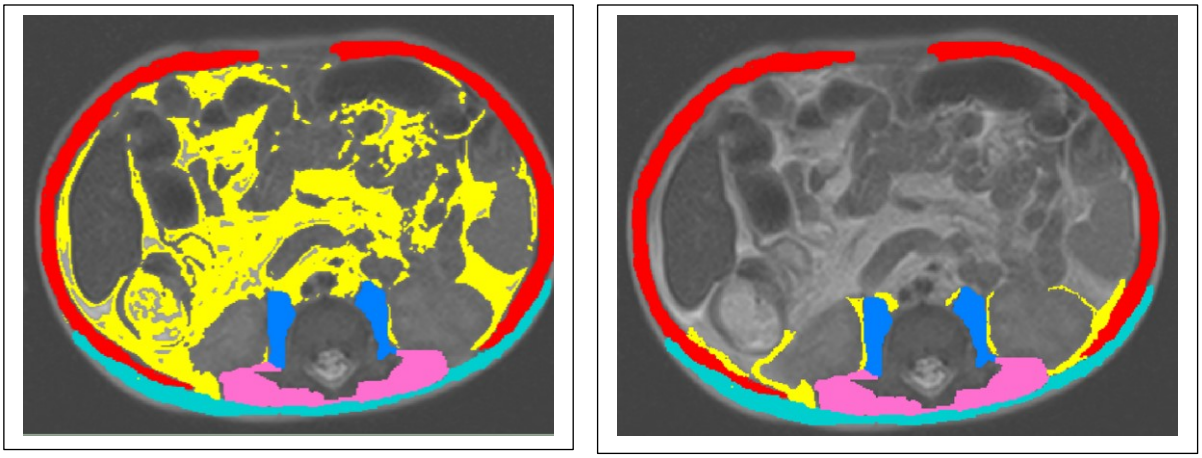


Figure 3.4 MRI image with and within inclusion of mesenteric fat. An example of 0.29 years old baby girl with Biliary Atresia, with (left) and without (right) inclusion of mesenteric fat (in yellow). Mesenteric fat was excluded in the analysis to minimize the potential of overestimation of visceral fat.

In CT, body composition is quantified using tissue density measured in Hounsfield units (HU). Both upper and lower limit HU were set prior to analysis, where HU for muscles (-29 to 150), Intermuscular adipose tissue (IMAT; -190 to -30), visceral adipose tissue (VAT; -150 to -50), subcutaneous adipose tissue (SAT; -190 to -30). For subjects with CT scans (n=8), the HU for individual muscle site and total SMM was retrieved from the software. MRI scans were analysed by comparing 2 images with and without fat saturation to determine adipose tissue area. In the majority of the MRI scans, T2 sequence was selected due to better visual acuity to discrimate between muscle mass and fat mass.

3.3.4 Definition of myopenia/ low muscle mass and low subcutaneous fat

Total SMMA (cm^2) was corrected for height square (m^2) to generate SMM index (SMMI) in both groups and for L3 & L4 slices. The individual muscle groups were also corrected for height square to generate psoas muscle index (PMI), paraspinal muscle index (PSI), AWM index (AWMI) and compared between ESLD and HC groups. Using HC data as the reference points, myopenia was defined as SMMI <2.5th percentile (-2 z-score). ESLD children were divided into 2 groups (±myopenia). Similarly, VAT, SAT and TAT were corrected for height square, resulting in VAT index (VATI), SAT index (SATI) and TAT index (TATI). Data was divided into two groups (± low SAT) based on SATI <7% percentile (<-1.5 z-score) as defined by HC data. Results presented in this paper will focus on data obtained from L3 slice. Data from L4 slice will be presented in **Appendix C-2, C-3, C-5, C-6**.

3.3.5 Pre-and-post-liver transplantation clinical outcomes

Clinical outcomes included number/length of pre-LTx hospitalization, length of post-operative hospital stay (total/ intensive care unit [ICU]), number/length of post-LTx hospitalization (from discharge up to 1 year), duration of ventilator dependence and number/type of post-operative complications (vascular and biliary complications, rejection and infection). Vascular complications included portal vein thrombosis/stenosis and hepatic venous occulsion, while biliary complications covered anastomotic bile leak, biliary stricture, cholangits and cholestasis. Episode and severity of rejection were also recorded. Severity of rejection was indicated by rejection activity index (RAI), where 3-4, 5-6 and >6 were indicative of mild, moderate and severe, respectively. The incidence

and type of infection (fungal/bacterial/viral) were determined by reviewing pathology reports.

3.3.6 Nutritional status, energy and protein intakes in ESLD children

Nutritional status was based upon the Subjective Global Nutrition Assessment Tool adapted for Pediatrics (SGNA scores ≥ 3 reflective of moderate-severe malnutrition; <3 minimal-no risk) at the time of LTx assessment[110]. Nutritional intake was extracted from daily nursing flow sheets (input-output [IO]) and compared against nutrition prescriptions at the time of LTx assessment and during the post-operative stay (medical floor stay for post-LTx up to discharge). Data including route of administration (enteral nutrition [EN]/ parenteral nutrition [PN]/ oral), quantity (total volume of EN/PN), type and concentration of formula (e.g. brand and kcal/oz), use of modular formula were collected daily from post-ICU transfer up to hospital discharge. Data related to days of Nil per os (NPO) post-LTx, number of days on PN/EN and number of days to start oral feeding were included. Energy and protein intakes (cumulative intakes throughout hospital stay and average energy [kcal/kg/day] and protein intake [gram/kg/day]) were calculated from PN, EN, oral feeding and IV maintenance fluid (e.g. dextrose 5% or 10%) collected. When available, fluid/food quantity (mL or gram) such as clear fluids, full fluids, baby foods (e.g. rice cereals) was recorded and energy and protein intakes were calculated based on Canadian Nutrient File database. Days with patients on diet as tolerated (DAT) and on day passes (away from hospital) were excluded from analysis as full day intake was not available. Basal metabolic rate (BMR) was determined using Schofield equation based on age, gender and discharge weight from first hospital admission post-LTx[111]. The discharge weight was selected due to the rationale of best estimate of dry weight to

prevent overestimation of BMR. Calculated BMR and 120% of BMR were compared with subjects' energy intake per day (kcal/day)[112-114]. For children ≤ 2 years, 120kcal/kg body weight was also used as a cut off for energy requirement[115]. Protein intake was compared with recommended protein requirements for post-LTx children, where 2g/kg for children ≤ 2 years and 1.5g/kg for children >2 years[84,114].

3.3.7 Statistical Analysis

Data analysis was completed using the SAS 9.0 statistical software (SAS, Version 9.4; SAS Institute Inc., Cary, NC, USA). Data were expressed as mean ± standard deviation (SD) or median and inter-quintile range (IQ), unless otherwise specified. The Shapiro-Wilk test was conducted to assess the normality of distribution. Non-parametric data was analysed using Mann-Whitney. Repeated measures analysis of variance was performed to assess the effects of time on primary outcomes. Univariate and multivariate were conducted to assess potential relationships between primary outcomes of interest (body composition and pre-and-post-operative clinical outcomes). Analysis of co-variance was performed to adjust for any variables influencing primary outcomes (e.g. age, gender, PELD/MELD scores). Chi-square tests were used to measure differences in categorical data (± myopenia). A p-value≤0.05 was indicative of statistical significance.

3.4 Results

3.4.1 Demographic, Anthropometric and Laboratory Data

Demographic, anthropometric and laboratory data at time of LTx assessment are presented in **Table 3.1.** The median (interquartile range) for age, weight-z, height-z and BMI-z at LTx were 1.5 (0.6, 7.7 years), -0.81(-1.17, -0.19), -1.35(-2.38,-0.43) and 0.26 (-0.60, 0.51), respectively.

Table 3.1 Demographic, anthropometric, medical and laboratory variables in ESLD and HC groups during scan.

Variables	ESLD (n=30)	HC (n=24)	p-value
Age (years)	1.0 (0.4, 7.4)	0.7 (0.4, 6.7)	0.97
Gender	18M /12F	14M/10F	0.90
Weight (kg)	8.8 (6.5, 22)	8.6 (6.8, 21)	0.38
Weight-z	-0.55 ± 1.33	0.56 ± 1.60	**0.009**
Height (cm)	72.3 (63.0, 118.5)	73.5 (60.30, 133.80)	0.68
Height-z	-1.02 ± 1.67	0.26 ± 2.21	**0.02**
BMI	16.6 (15.7, 18.4)	17.7 (16.0, 19.4)	0.10
BMI-z	0.06 ± 1.26	0.57 ± 1.63	0.21
Liver etiology, n (%) - BA - PFIC - A1AD - Hepatoblastoma - Others*	 12 (40%) 4 (13%) 3 (10%) 3 (10%) 8 (27%)	-	-
PELD	12.4 (5, 20)	-	-
MELD	19.8 (5.1, 24.3)	-	-
AST (IU/L)	241 (100, 353)	-	-
ALT (IU/L)	145 (63, 269)	-	-
GGT (IU/L)	111 (36, 438)	-	-
Albumin (g/L)	32 ± 8	-	-
Total bilirubin (µmol/L)	240 ± 172	-	-
INR	1.4 (1.1, 2.2)	-	-
PTT (seconds)	39.5 (35, 58)	-	-
Ammonia (µmol/L)	54 (37, 62)	-	-
CRP (mg/L)	8 (3, 19)	-	-
Vitamin D (nmol/L)	33 (14, 74)	-	-
Urea (mmol/L)	3.1 (2.0, 3.7)	-	-
Creatinine (µmol/L)	21 (15, 25)	-	-

*Others liver etiology included Cringler-Najjar (n=1), Primary Sclerosing Cholangitis (n=1), Giant cell hepatitis (n=1), Alagille syndrome (n=1), Glycogen storage disease (n=1), Unknown (n=1), Fulminant hepatic failure (n=1), Autoimmune cirrhosis (n=1).

MELD was calculated for children >12 years (n=4).

Normal ranges for biochemical data 2 months-18 years: AST (10-75U/L), ALT (<60U/L), GGT (10-150 U/L), Albumin (30-50g/L), Total bilirubin (<21umol/L), INR (0.8-1.2), PTT (27-39 seconds), Ammonia (25-55 µmol/L), CRP (0-10mg/L) , Vitamin D (>75nmol/L), Urea (2-7mmol/L), Creatinine 10-120 µmol/L.

ESLD: End-stage liver disease, HC: Healthy control, BMI: Body mass index, BA: Biliary atresia, PFIC: progressive familial intrahepatic cholestasis, A1AD: alpha-1-antitrypsin deficiency, PELD: Pediatric end-stage liver disease score, MELD: Model for End-Stage Liver Disease. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: gamma-glutamyl transpeptidase, INR: International normalized ratio, PTT: Partial thromboplastin time, CRP: C-reactive protein.

Data expressed in mean ± SD or median (IQR) or percentage (%).

P-values ≤0.05 is considered statistically significant.

3.4.2 Absolute Surface Area and Indices of Skeletal Muscle Mass

L3 & L4 SMM (total, psoas, paraspinal and AWM) absolute surface area (cm^2) and indices (cm^2/ht^2) are presented in **Tables 3.2** (L3) and **Appendix C-2** (L4). With the exception of PSI (L3 [p=0.006], L4 [p=0.02]), no significant differences in total SMM, psoas and AMW surface areas or indices were observed between groups (L3 and L4). However, when adjusted for age (> and < 2 years), ESLD children > 2 years had significantly lower absolute muscle surface areas (cm^2) for total (p=0.02), AWM (p=0.04), paraspinal (p=0.04) and psoas muscle (p=0.04) than HC >2 years (L3 and L4). While males had higher muscle surface areas at L3 and L4 for total SMM, psoas and AWM in both groups, males only had higher paraspinal muscle surface area in the HC group ($p<0.05$). When adjusted for height, males had lower L3 and L4 AWMI, PMI and SMMI than females in the ESLD group only ($p<0.05$).

3.4.3 Absolute Surface Area and Indices of Adipose Tissues

ESLD children had lower L3 SAT/SATI (p=0.005) and TAT/TATI (p=0.005) than HC **(Table 3.2)**. Older children (>2 years) had higher SAT, TAT, but lower SATI and TATI in both groups (L3 and L4). Male children (>2 years) with ESLD had lower L3 SATI (21.2 ± 14.4 [males] vs 36.3 ± 2 [females] cm^2/ht^2; p=0.002) and L3 TATI (26.4 ± 15.2 [males] vs 42.7 ± 23 [females] cm^2/ht^2; p=0.02). With the exception of higher VAT in males in the healthy group, no differences in L3/L4 SAT/SATI, TAT/TATI, VATI or IMAT were observed between males/female children ($p>0.05$).

Table 3.2. Skeletal muscles and adipose tissues in end-stage liver disease and healthy children at third lumbar level (L3).

Variables	≤2 years			>2 years			Overall group p-value
	ESLD (n=18)	HC (n=15)	p-value	ESLD (n=12)	HC (n=9)	p-value	
SKELETAL MUSCLE							
Psoas muscle (cm^2)	2.5 (1.9, 3.3)	2.7 (1.8, 3.9)	0.20	7.1 (3.1, 8.4)^	13.4 (6.8, 15.9)*	**0.02**	0.16
Psoas muscle index (cm^2/m^2)	5.7 (4.3, 7.8)	6.4 (5.4, 7.2)	0.44	3.5 (3.2, 4.6)^	5.7 (4.9, 6.0)	**0.02**	0.16
Abdominal wall muscle (cm^2)	12.2 (10.3, 14.9)	11.6 (10.3, 14.5)	0.86	26.5 (22.4, 31.9)^	45.5 (24.6, 59.6)*	**0.05**	0.19
Abdominal wall muscle index (cm^2/m^2)	27.0 (24.1, 38.1)	27.1 (25.1, 33.0)	0.81	17.9 (13.9 19.7)^	21.2 (19.6, 21.4)*	**0.002**	0.56
Paraspinal muscle (cm^2)	5.4 (4.2, 7.5)	6.1 (4.6, 7.2)	0.46	20.7 (14.1, 26.5)^	36.5 (19.3, 54.9)*	**0.03**	0.09
Paraspinal muscle index (cm^2/m^2)	13.2 (10.5, 14.7)	13.7 (12.6, 15.8)	0.39	12.6 (8.8, 15.1)	16.5 (16.0, 19.0)*	**0.002**	**0.006**
Total SMM (cm^2)	20.7 (16.4, 25.0)	21.2 (17.3, 24.3)	0.74	54.8 (38.5, 65.1)^	95.6 (48.9, 130.1)*	**0.03**	0.16
Total SMM index (cm^2/m^2)	47.8 (39.0, 56.1)	48.3 (47.6, 54.2)	0.81	35.6 (24.1, 37.3)^	42.5 (41.9, 47.1)	**0.003**	0.11
ADIPOSE TISSUES							
VAT (cm^2)	2.6 (1.3, 3.2)	1.8 (1.6, 3.9)	0.89	5.9 (3.9, 9.4)^	11.5 (5.5, 23.1)*	0.09	0.11
VAT index	6.0 (2.7, 7.8)	4.4 (4.0, 5.6)	0.78	3.8 (2.6, 4.9)	5.0 (4.1, 9.4)	0.11	0.36

(cm^2/m^2)							
SAT (cm^2)	15.8 (8.4, 21.8)	24.1 (18.1, 46.0)	**0.006**	23.4 (13.2, 29.7)^	68.9 (48.1, 171.0)*	**0.006**	**0.003**
SAT index (cm^2/m^2)	38.5 (21.5, 50.3)	57.0 (43.9, 86.0)	**0.008**	13.9 (9.1, 18.9)^	43.7 (24.1, 71.2)	**0.01**	**0.03**
TAT (cm^2)	17.7 (9.7, 24.0)	27.4 (19.5, 50.8)	**0.006**	28.3 (17.5, 40.5)^	74.2 (54.4, 195.2)*	**0.01**	**0.005**
TAT index (cm^2/m^2)	46.3 (25.8, 59.9)	61.2 (49.6, 101.5)	**0.003**	20.9 (12.3, 23.3)^	51.7 (28.2, 81.3)	**0.01**	**<0.001**

(^) indicated significant difference between ESLD ⩽2 years vs >2 years. (*) indicated significant difference between HC ≤2 years vs >2 years. Overall group p-value referred to ESLD vs HC groups difference without age and gender effects.

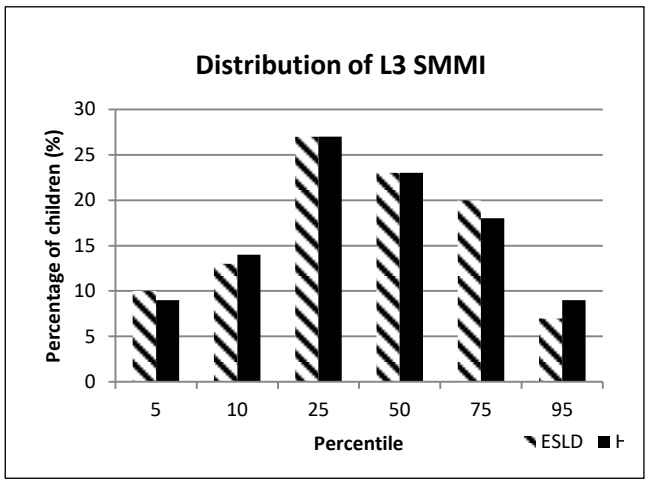
Index: Skeletal muscle/ adipose tissues area corrected for height= area (cm^2)/height (m^2). Height available for n=30 ESLD and n=22 HC.

Total skeletal muscle mass referred to sum of psoas, abdominal wall muscle (rectus abdominus, internal, external oblique, transverse abdominus) and paraspinal muscles (quadratus lumborum and erector spinae). Total adipose tissues referred to sum of IMAT, VAT and SAT. ESLD: End stage liver disease, HC: Healthy control, SMM: Skeletal muscle index, IMAT: Intermuscular adipose tissues, VAT: Visceral adipose tissues, SAT: Subcutaneous adipose tissue, TAT: Total adipose tissues. Data expressed in median (IQR). P-values ≤0.05 is considered statistically significant.

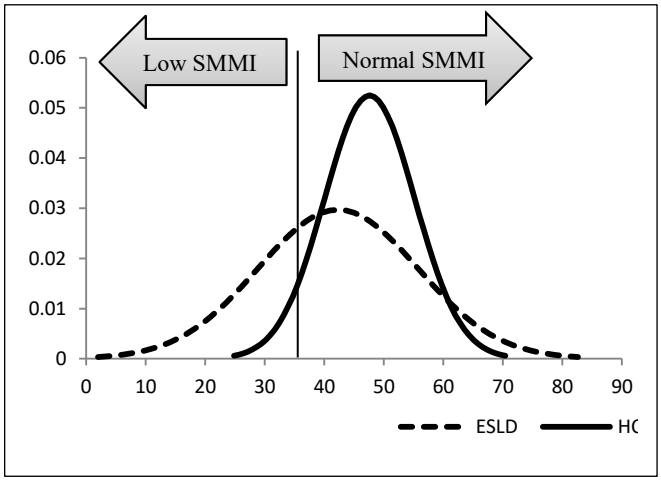
3.4.4 Myopenia and Subcutaneous adiposity

Distributions of SMMI and SATI are presented in **Figure 3.5 (L3) and Appendix C-3 (L4)**. Distributions of L3-PMI, AWMI and PSI are included in **Appendix C-4.** A total of six children with ESLD had L3-SMMI indicative of myopenia (20%). A total of 11 ESLD children (37%) had L3-SATI z scores<-1.5, indicative of low SAT. Out of the 11 ESLD children with low SATI, 5 had myopenia (45%) (**Figures 3.6A/ Appendix C-5)**. One ESLD child had myopenia with normal SATI (**Figure 3.6C);** with the remaining ESLD children (60%) had normal L3-SATI and SMMI **(Figure 3.6B).** ESLD children with both myopenia and low SATI were all above 2 years of age (p=0.03) and were all male (p=0.0008).

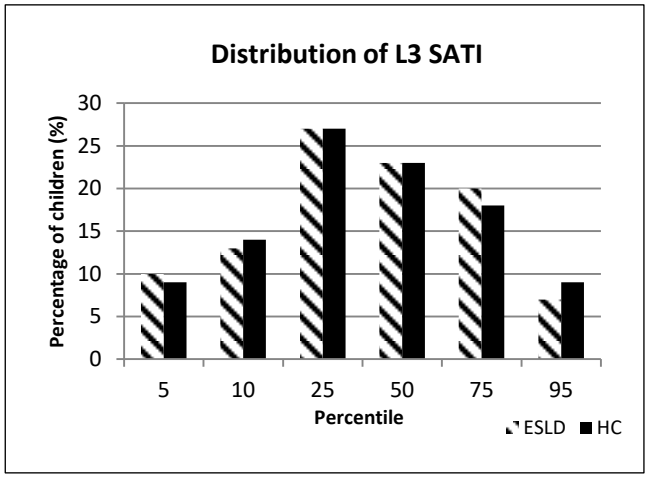
(A)



(B)



(C)



(D)

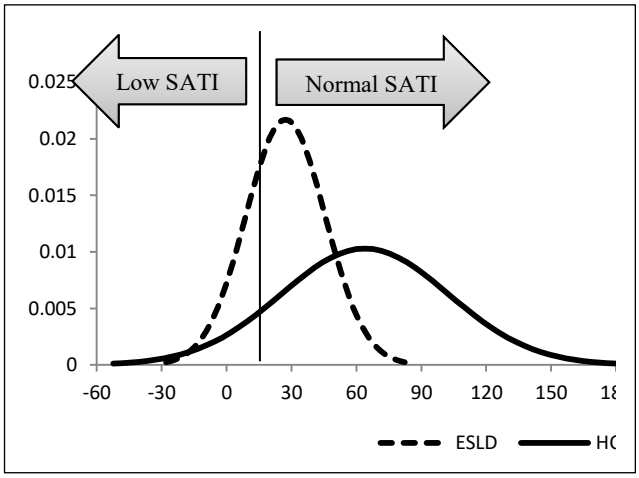


Figure 3.5. Distribution of skeletal muscle mass index (A & B) and subcutaneous adipose tissues index (C & D) at L3 in end-stage liver disease children and healthy controls. The cut off for low skeletal muscle mass index is 34.74cm^2/m^2 and subcutaneous adipose tissues index is 15.60 cm^2/m^2. ESLD: End stage liver disease, HC: Healthy control, SMMI: Skeletal muscle mass index, SATI: Subcutaneous adipose tissues index.

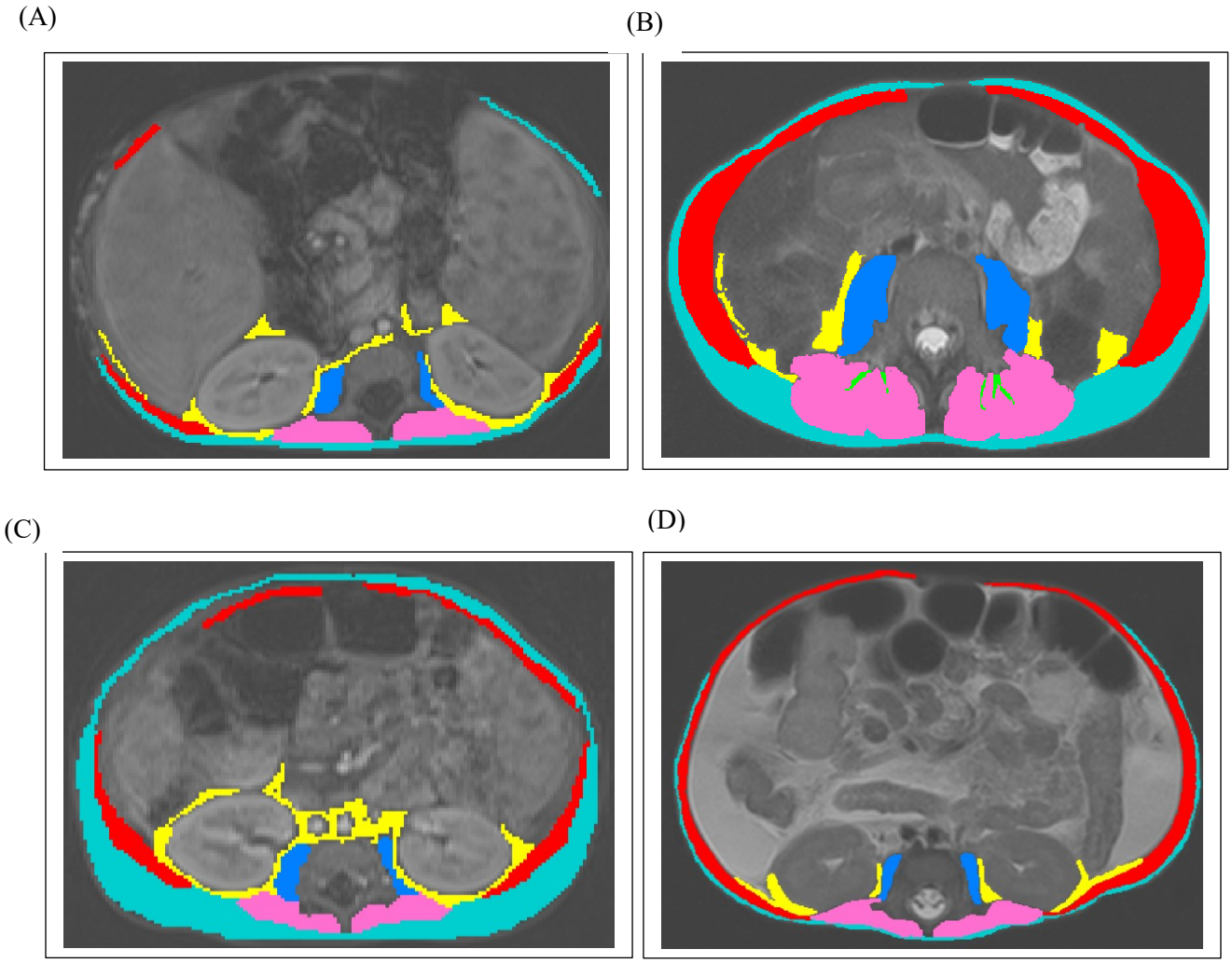


Figure 3.6. Phenotypes related to skeletal muscle mass and adiposity in infants and children with end stage liver disease in MRI images at third lumbar (L3) vertebrate. (A) Low SMMI + low SATI, (B) normal SMMI + normal SATI, (C) low SMMI+ normal SATI, (D) normal SMMI+ low SATI. SMM: Sum of *red* (Abdominal wall muscle: rectus abdominus, internal and external obliques and transversus abdominus), *blue* (psoas muscle) and *pink* (paraspinal muscle) surface area. SAT: c*yan* surface area. SMMI=SMM/height^2, SATI= SAT/height^2. SMM: Skeletal muscle mass, SAT: Subcutaneous adipose tissues, SMMI: Skeletal muscle mass index, SATI: Subcutaneous adipose tissue index.

3.4.5 Anthropometric and Laboratory Variables

Within ESLD children, there was no difference in weight-z, height-z, BMI-z, PELD and time from LTx Ax to LTx between females and males. However, male ESLD children were significantly older at LTx assessment (5.9 ± 6.1 years [males] vs 1.8 ± 2.6 years [Females], p=0.03) and had a trend toward older age at the time of LTx (6.0 ± 6.1 years [males] vs 2.3 ± 2.7 years [Females], p=0.06) than females. No significant associations between liver disease diagnosis, PELD/MELD and the presence of low L3 SMMI or low SATI was observed (p>0.05). **Table 3.3** demonstrated liver disease diagnosis in ESLD children with and without myopenia and low or normal subcutaneous fat. With the exception of higher creatinine, no significant associations between laboratory variables and the presence of myopenia or low SATI were observed (p>0.05) pre-and-post-LTx. Myopenia ± low SATI (L3 and L4) was not related to tacrolimus (dose/serum) or corticosteroid dose (p=0.42). In ESLD children with CT images (n=4), none of them was myopenic. There was no significant difference in total SMM density as indicated by HU between ESLD and HC with CT images.

Table 3.3. Liver disease diagnosis in ESLD children with or without myopenia and low or normal subcutaneous fat

Myopenia	n=2 (33%) Alpha-1-antitrypsin deficiency, n=1 (17%) Biliary Atresia, n=1 (17%) Primary Sclerosing Cholangitis, n=1 (17%) Progressive familial intrahepatic cholestasis, n=1 (17%) Glycogen storage disease
Non-myopenia	n=11 (46%) Biliary Atresia, n=3 (13%) Progressive familial intrahepatic cholestasis, n=3 (13%) Hepatoblastoma, n=1 (4%) Crigler-Najjar, n=1 (4%) Giant cell hepatitis, n=1 (4%) Alagilles syndrome, n=1 (4%) unknown, n=1(4%) Fulminant liver failure, n=1 (4%) Alpha-1-antitrypsin deficiency, n=1 (4%) Autoimmune cirrhosis
Low subcutaneous fat	n=3 (30%) Alpha-1-antitrypsin, n=2 (20%) Progressive familial intrahepatic cholestasis, n=1 (10%) for Biliary atresia, n=1 (10%) Crigler-najjar, n=1 (10%) Primary Sclerosing Cholangitis, n=1 (10%) Giant cell hepatitis, n=1 (10%) Glycogen storage disease
Normal subcutaneous fat	n=11 (55%) Biliary atresia, n=3 (15%) Hepatoblastoma, n=2 (10%) Progressive familial intrahepatic cholestasis, n=1 (5%) Alagilles disease, n=1 (5%) Unknown, n=1 (5%) Fulminant liver disease, n=1 (5%) Autoimmune cirrhosis

3.4.6 Growth

Children with low L3-SATI (± myopenia) had lower weight (p=0.002), weight-z (p=0.03), height (p<0.001), height growth (p=0.02), and BMI-z (p=0.001) than children with normal L3-SATI (z scores>-1.5) at all time points (LTx assessment, LTx, hospital discharge and at 6 and 12 months post-LTx). No differences in overall weight-SDS (p=0.76), height-z (p=0.33) and height SDS (p=0.69) was observed (at all time points). However, children with low L3-SATI (±myopenia) appeared to experience greater rates of weight gain between LTx and six month F/U visit (p<0.05) (**Figure 3.7A/Appendix**

C-6). Children with myopenia had lower height ($p<0.001$) and weight ($p=0.01$), but when adjusted for age (> and <2 years), differences in height were no longer apparent. Myopenia was not associated with differences in weight-z ($p=0.91$), height-z ($p=0.63$), BMI ($p=0.60$), BMI-z ($p=0.12$), height gain ($p=0.07$), height SDS ($p=0.36$), weight gain ($p=0.16$) and weight SDS ($p=0.83$) over all time points studied.

3.4.7 Low subcutaneous adipose tissue and myopenia and associations with markers of neurodevelopment

Neurodevelopment

A total of 27% of ESLD children had adaptive behaviour composite scores below 1SD of normative data, indicating mild developmental delay. Of the 4 domains, majority of children (42%) had delay in motor skills **(Table 3.4).** No differences in developmental scores (total, percentile) for socialization, communication, daily living or adaptive behaviour composite scales and the presence of myopenia/low SATI were observed. However, the percentage of ESLD children with noted gross motor delay was significantly higher in children with low SATI (±myopenia) (67%) when compared to children with normal SATI (8%) ($p=0.004$) (**Figure 3.7B)**. Body composition was not related to brain MRI (normal/abnormal) findings ($p>0.05$).

Table 3.4. Scores on Vineland Adaptive Behaviour Scales -II.

Subscales	Mean ± SD	>1SD below mean (n (%))	>1.5SD below mean (n (%)	>2SD below mean (n (%))
Communication	97.65 ± 9.13	3 (18)	3 (18)	1 (6)
Daily living	92.19 ± 11.71	3 (19)	1 (6)	0 (0)
Socialization	99.44 ± 8.53	1 (6)	0 (0)	0 (0)
Motor skills	93.35 ± 8.20	7 (42)	2 (12)	0 (0)
Adaptive behaviour composite	93.80 ± 6.14	4 (27)	1 (7)	0 (0)

Vineland Adaptive Behavior Scales-II was performed at liver transplant assessment was collected where available for LTx children <7 years[109].
>1 SD below mean: mild, >1.5SD below mean: moderate delays, > 2 SD below mean: significant delays. Communication and motor skill subcales were available for n=17, Daily living and socialization were available for n=16, Adapative behaviour composite was available for n=15 ESLD subjects.

3.4.8 Clinical Outcomes

The three most common reasons for pre-LTx hospitalization included LTx assessment (37%), worsening of liver function (24%) and for nutrition rehabilitation (13%). In the post-LTx period, infection (51%), gastrointestinal issues (15%) and admission for procedure (9%) were the three most common reasons for admission **(Table 3.5)**. There were significant associations between increased numbers of fungal ($p<0.001$), viral ($p=0.005$) and the total number of infections ($p=0.005$) in children with low SATI (±myopenia) than children with normal SATI at hospital discharge, 6 and 12 months follow-up visits (**Figure 3.7C**). In addition, children with low SATI (±myopenia) had significantly longer length of inpatient stays pre-and-post-LTx (**Figure 3.7D**) when compared to children with normal SATI. No associations between number of biliary ($p=0.40$) or vascular complications ($p=0.60$), total number/type of infections ($p=0.80$) or hospitalization (LOS, inpatient LOS, outpatient, emergency visits) in the pre-and-post-LTx periods was observed in children with-or-without myopenia alone ($p>0.05$). Number

of rejection episodes and rejection severity (RAI) was not related to any body composition measures ($p>0.05$).

Table 3.5 Reasons for inpatient admission pre-and-post liver transplantation

Pre-LTx	Post-LTx
• Inpatient liver transplant assessment • Worsening of liver function • Failure to thrive/ nutritional rehabilitation • Management of electrolyte imbalances • For procedures e.g. placement of a left subclavian central line, chemotherapy , esophagogastroduodenoscopy • Gastrointestinal issues e.g. nausea/ vomiting	• Infections • Failure to thrive/ nutritional rehabilitation/ feeding related issues • Management of electrolyte imbalances • For procedures e.g. cholangiogram and t-tube removal, removal of Broviac catheter, for liver biopsy • Gastrointestinal issues e.g. nausea/vomiting, sore throat, abdominal pain, developed intestinal ileus, gastrointestinal bleed • Rejection • Others e.g. leg fracture

Pre-LTx covers the period between LTx assessment to LTx. Post-LTx covers the period between post-LTx hospital discharge up to 1 year post-LTx.
LTx: Liver transplantation

3.4.9 Energy and protein intakes and nutritional status

ESLD children met 96% protein and 98% of energy requirement as compared to the prescription. Eight-nine percent and 74% of children met estimated BMR (+20%) and protein requirements (>2g/kg/d for children ≤2 years; and >1.5g/kg/d for children>2 years), respectively. Children with low SATI had lower energy intakes (kcal/d) than children with normal SATI (p=0.03). Children with myopenia and low SATI received lower energy intake (kcal/kg) (p=0.04) than children with normal SMMI and SATI at the time of LTx assessment and post-LTx (**Figure 3.7E**). Children with myopenia and low SATI also had higher average daily protein intake (29.5±13.2 g/d [myopenia and low SATI] vs 18.5±5.6 g/d [normal SMMI and SATI]; p=0.04). However, when expressed on a per kg basis, protein intake was trending towards lower protein intakes (1.8±0.7 g/kg/d [myopenia and low SATI] vs 2.4±0.8 [normal SMMI and SATI]; p=0.06) in children with myopenia **(Figure 3.7F)**. These relationships remained significant after adjusting for the potential confounding effects of sex and age. Duration of NPO status (p=0.62), modes of nutrition delivery (day to oral intake (p=0.18), day on EN (p=0.48), PN (p=0.19) or the number of days with available nutrition information in hospital (p=0.15) were not different between children ±myopenia or in children with low L3-SATI.

Lower L3-SATI (± myopenia) was associated with higher SGNA scores (≥ 3 indicative of high risk for malnutrition) when compared to children with normal L3-SATI (p=0.04). No differences in SGNA scores were observed for children ± myopenia (p=0.13) or when treated as a continuous variable to any body composition measure (p>0.05).

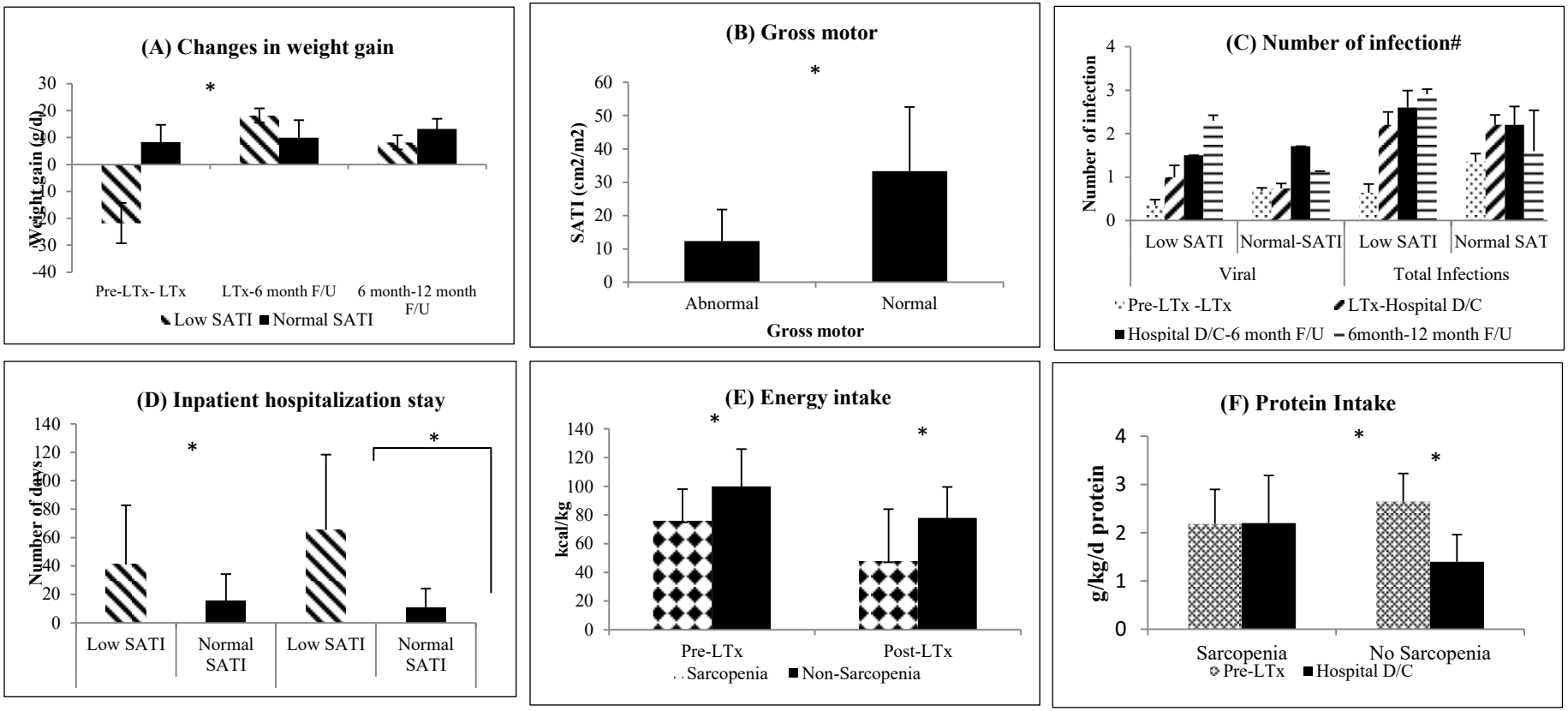


Figure 3.7. Growth, gross motor, complications and nutritional intake in children with end-stage liver disease at different time points. ##Children with low SATI (±myopenia) had significant higher viral, fungal and total infections than normal SATI end-stage liver disease children at hospital discharge, 6 and 12 months follow-up visits. Low SATI is defined by SATI<-1.5SD and myopenia is defined by SMMI<-2SD. LTx: Liver transplantation, F/U: Follow up post-Tx, D/C: discharge, SATI: Subcutaneous adipose tissues index, SMMI: Skeletal muscle mass index.

3.5 Discussion

This is the first study that has examined the prevalence and associations of myopenia and low subcutaneous adipose tissues (SAT) in ESLD children with pre-and-post-LTx clinical outcomes. Our findings indicate that there are four distinct body composition phenotypes in children with ESLD awaiting LTx, a) myopenia with low SAT (17%), b) myopenia alone (3%), c) low SAT alone (20%), d) normal muscle mass and SAT (60%). The presence of myopenia and low SAT were more prevalent in older male (>2 years) than younger (<2 years) female ESLD children. Low SAT rather than myopenia was related to gross motor delay, higher risk of total/viral/fungal infections, longer hospitalization post-LTx. These appeared to be largely driven by deficits in energy intake, rather than protein intake, particularly in the older children. These findings highlight that suboptimal SAT accretion in children with ESLD may be sacrificed to preserve or optimize lean mass accretion and this may be more pronounced in older children with longer disease duration.

Males had the highest rates of myopenia and low SAT. This is in line with earlier work that reported older ESLD children (13-18 years) had less SMM and SAT than younger children with ESLD[12]. However, this does conflict with earlier findings that younger female children (<10 years) had a higher prevalence of myopenia post-LTx[11]. These differences may be due to the older age of male children at the time of LTx assessment, which suggests liver disease duration may directly influence lean body mass/fat accretion. In addition, higher proportions of total body fat mass in female children pre-and-post-puberty may confer an increased benefit related to SAT depletion.

These findings remain controversial as other studies suggest that males may have similar SAT mass as female children[116,117].

Similar to adults with cirrhosis[118], low SAT, was associated with adverse clinical outcomes: increased infection risk (total, viral, fungal), hospitalization and gross motor delay. While the underlying mechanism related to SAT depletion in children and adults with ESLD is not fully understood, it is likely that several factors in addition to undernutrition play a role. Excessive free fatty acid and low serum leptin level in the circulation may to lead to adipocyte dysfunction, insulin resistance, increase secretions of proinflammatory cytokines such as TNF-α and IL-6[119]; all of which could lead to increased infection risk. Low leptin level is an independent predictor of poor clinical outcomes in adults with cirrhosis[120]. Gender differences in plasma leptin level may also influence the expression of low SAT and its associations with adverse clinical outcomes[121]. While some data has attributed lower serum leptin in males to the suppressive effect of androgens during puberty, differences in leptin concentrations between sexes may exist even in newborn infants[122,123]. A higher concentration of serum leptin in females seems to confer protective effects against adverse clinical outcomes[118]. Similar to adults with ESLD, myopenia expression and its impact on clinical outcomes may vary depending on the sex and age of children[11,15,40]. In the post-LTx period, alterations in body composition may also be markedly influenced by the use of immunosuppression and/or pre-existing alterations in body composition experienced in the pre-LTx period[11].

The beneficial effects of SAT may be related to its role as an energy reservoir and endocrine organ[118]. In our cohort, children with low SAT had lower energy intake than

children with normal SAT. In the early stages of SAT depletion, it is likely that lean body mass would have been preserved at the expense of total body fat. When combined with the potential for increased resting energy expenditure and malabsorption, it may have led to negative energy balance. ESLD children with low SAT coincided with low weight-z, height, suboptimal energy intake and a trend toward lower SNGA scores than children with normal SAT. This is in line with data that shows low SAT stores in children are associated with delayed motor development, rather than changes in muscle size[124]. In adult cancer patients, there is also evidence that rapid fat mass depletion occurs even prior to lean body mass depletion with cancer disease progression, leading to an increased risk for morbidity and mortality[125]. In the post-LTx period, catch-up weight gain was noted in the children with low SAT, which may have reflected normalization of resting energy expenditure post-LTx and improved overall absorption[126]. However, it is unclear whether or not this 'weight' gain represented predominantly SAT accretion vs lean body mass accretion.

This study has several strengths, including the evaluation of SMM from multiple muscle sites and total SMM area, the assessment of body fat and its association with clinical outcomes. While both MRI and CT technologies were used to assess body composition, we evaluated both L3 and L4 slices which enabled a comprehensive evaluation of body composition and did not find any differences between the two modalities at each vertebrate level (data not shown). Recent evidence indicates that CT and MR abdominal imaging for the evaluation of body composition in children and adults are interchangeable[127,128]. Children with ESLD were age and gender matched with HC and also matched in scan type (CT or MRI). Body composition data from ESLD and HC

groups are comparable to published data, indicating the representative of healthy and ESLD children in our study[10,12,54,129,130]. In addition, our ESLD cohort is comparable to other pediatric liver centers in term of demographic, anthropometric and liver disease severity[131]. We evaluated three major muscle groups (psoas, paraspinal, AWM) and fat compartments (VAT, IMAT and SAT) which enabled us to determine differences in deficits in each muscle/fat group. Psoas muscle is the main flexor of hip, AWM help with trunk movement, while paraspinal muscle supports, stabilize and move spine. All are important for gross motor function. Interestingly, paraspinal muscle index was the only muscle group that showed significances differences between HC and ESLD. In adults with ESLD, both PMI and PSI, but not total SMMI have been shown to be independent predictors of clinical outcomes[19]. In these studies, individual muscle group types seem to be depleted in an unequal manner[19]. This is comparable with the older ESLD children who had greater deficits in PMI and PSI than AWMI and SMMI when compared with HC > 2 years. Similarly, the deficit in SATI was more than VATI and TATI **(Table 3.6)**. These findings have potential implications in terms of overall gross motor skill development and movement in children and warrant further exploration.

Table 3.6: Percentage of skeletal muscle and adipose tissue indices difference between end-stage liver disease children and healthy controls

Variables	Percentage (%) difference between end-stage liver disease and healthy children	
	≤2 years	>2 years
Psoas muscle index (cm2/m2)	10.94	38.60
Abdominal wall muscle index (cm2/m2)	0.37	15.57
Paraspinal muscle index (cm2/m2)	3.65	23.64
Total SMM index (cm2/m2)	1.04	16.24
VAT index (cm2/m2)	-36.36	24.00
SAT index (cm2/m2)	32.46	68.19
TAT index (cm2/m2)	24.35	59.57

Percentage difference was calculated by (value of HC- value of ESLD)/ value of HC *100. SMM: Skeletal muscle mass, VAT: Visceral adipose tissues, SAT: Subcutaneous adipose tissues, TAT: Total adipose tissues.

To date, there is limited data on the sensitivity of individual muscle groups to changes in nutritional intake. One adult study demonstrated an association of reduced psoas muscle density with nutrition inadequacy as early as 3 days, highlighting the ability to detect acute nutrition changes[132]. This was not apparent in this study. This may be due to smaller sample size and the fact that low SAT was more prevalent (~40%) than myopenia (20%). Hence, SAT depletion may be the over-riding concern related to adverse clinical outcomes in children with ESLD. This has not been reported and warrants further investigation.

This study has some limitations. Data represents standards of clinical practice, hence may have some missing data at specific time points. Given the lack of information on the other components of sarcopenia (e.g. muscle strength/physical performance), we used a more conservative cut off (SMMI-z score<-2) to define myopenia. Hence, there is a potential for underestimation of myopenia prevalence. Nutrition data was collected in the post-ICU period, but did reflect 87% of the total available data; providing a

'snapshot' of post-operative nutrition support. While the sample size is relatively small, the inclusion of age and gender matched HC had allowed sufficient power to detect differences in SMMI and SATI (post-hoc power analysis $\beta>0.8$). Lastly, wide variability of liver disease etiology and the lack of longitudinal data beyond one year post-LTx are important to evaluate in future studies as the impact of health related quality of life are important post-LTx. Examination of the potential mechanisms contributing to myopenia and low SAT would enable effective screening and interventions to be developed.

Conclusions

Infants and children with ESLD presented with four body composition phenotypes. These include myopenia, myopenia+low SAT, low SAT and body composition within healthy reference ranges. These presentations appear to be independent of liver disease type. Myopenia with low SAT was more prevalent in older male children with longer disease duration and was associated with increased risk for gross motor delay, infection and suboptimal energy intake. Development of effective screening tools for the assessment of myopenia and low SAT is crucial to prevent increased risk for adverse clinical outcomes.

Chapter 4: Study 2

Title: Contribution of lifestyle factors and functional measures of muscle strength and physical performance and sarcopenia in pediatric liver transplant recipients.

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4.1 Abstract

Background & Aims: Sarcopenia is a muscle disease characterized by reduced skeletal muscle mass (SMM), muscle strength and physical performance. Reduced SMM has been identified in children after liver transplantation (LTx), but no information related to muscle strength/physical performance or lifestyle factors contributing to sarcopenia is available. We hypothesized that sarcopenia, as determined by measures of SMM, muscle strength and physical performance is highly prevalent in post-LTx children and related to poor diet quality (DQ) and physical inactivity. **Methods**: A cross-sectional study in post-LTx children (n=22) and age-matched healthy controls (HC, n=47) between the ages of 6-18 years examining body composition (Dual Energy X-ray absorptiometery, multiple skinfold), measures of muscle strength (handgrip, sit-to-stand, push-ups), physical performance (6-minute walk test, stair climb test), diet (3-day food intake) and physical activity (accelerometer) was conducted. Low muscle strength/physical performance and SMM (SMM-z scores≤-1.5) were defined by values 2 standard deviation below mean values for age-and-gender matched HC. **Results**: Sarcopenia occurred in 36% of LTx children. LTx children had significantly lower scores for muscle strength (sit-to-stand,

push-up) and physical performance (stair climb test) than HC ($p<0.05$). Deficits in physical performance in children with sarcopenia were predominantly revealed by longer stair climbing times ($p=0.03$), with no differences in other muscle tests. Low SMM, muscle strength and physical performance were associated with lower amount of time spent in fairly and very active physical activity but no associations with DQ were found. **Conclusions:** Sarcopenia is highly prevalent in children after LTx and is related to lower moderate and vigorous physical activity. Development of effective rehabilitation strategies to treat sarcopenia is urgently needed in post-LTx children.

4.2 Introduction

Malnutrition is prevalent in children and adults with end-stage liver disease (ESLD) awaiting liver transplantation (LTx)[1]. Common factors influencing malnutrition include alterations in nutrient utilization, dietary intake, physical debilitation, hypermetabolism and chronic inflammation[1]. A common co-morbid condition of malnutrition that has been identified in adults with ESLD is a condition called sarcopenia[80]. Sarcopenia is a muscle disease, characterized by reduced muscle strength (MS), loss of skeletal muscle mass (SMM) and low physical performance (PP)[4]. Sarcopenia in adults with ESLD has been associated with increased risk of mortality and morbidity such as increased length of hospital stay, post-operative complications and risk for cardio-metabolic dysregulation[5,43]. In contrast, in pediatrics, sarcopenia has only been recently identified in children with liver disease pre-and-post-LTx and other clinical disorders such as Inflammatory Bowel Disease[1,2,10-12,83,133,134]. Sarcopenia post-LTx has been reported to be up to 40% in children and has been associated with reduced rates of growth, prolonged peri-operative length of stay, ventilator dependency and re-hospitalization in the longer term[11]. However, the definition of sarcopenia in children has not been well defined[2]. The interpretation of sarcopenia in pediatrics has been limited to SMM deficiency without consideration of alterations in MS or PP; important features in adult sarcopenia definitions[4,10-12]. The European Working Group on Sarcopenia in Older People (EWGSOP2) defines sarcopenia as having reduced MS accompanied by reduced SMM and/or PP **(Figure 4.1)**[4].

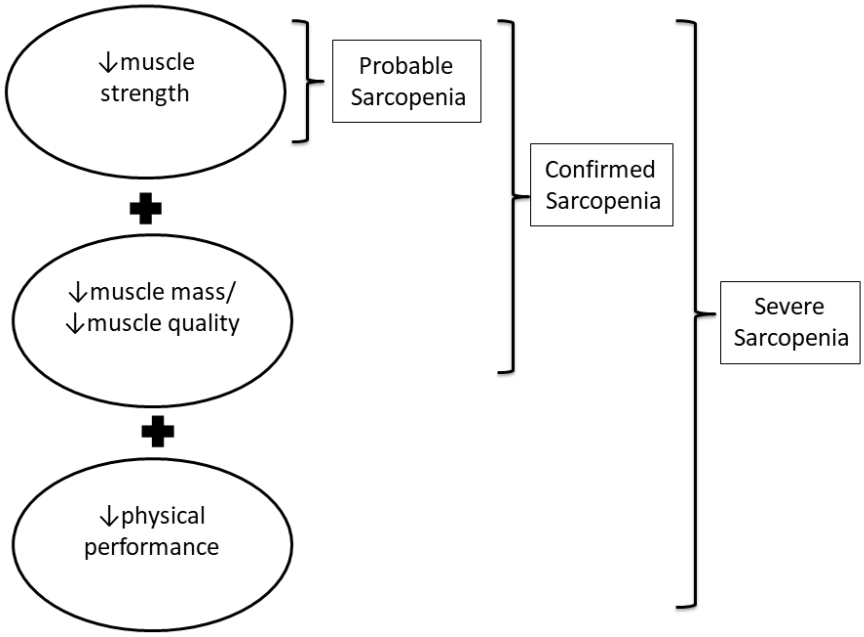

Figure 4.1 Sarcopenia based on current adult definitions[4]. Probable sarcopenia is defined by low muscle strength, confirmed sarcopenia is defined by low muscle strength and low muscle quantity/ quality, severe sarcopenia is defined by low muscle strength, low muscle quantity/ quality and low physical performance. Muscle quality refers to the ratio between muscle strength and muscle mass.

There are several factors that may influence the expression of sarcopenia in children post-LTx. These factors include the use of immunosuppressive drugs (e.g. tacrolimus, corticosteroid) that may adversely impact protein metabolism, pre-LTx nutritional status and lifestyle factors (diet, physical inactivity) which may contribute to suboptimal nutritional status and skeletal muscle function[1,135,136]. In adult clinical populations, reduced protein and vitamin D intake have been identified as important dietary factors that contribute to sarcopenia risk[1]. A recent study in newly diagnosed

pediatric patients with inflammatory bowel disease has identified suboptimal vitamin D status to be more prevalent in those with sarcopenia[83]. Poor diet quality (DQ), physical inactivity and reduced aerobic fitness levels have been frequently observed in children post-LTx which may also be contributing factors to sarcopenia risk[88,135,137].

There is a need to explore sarcopenia prevalence based on broader definitions in pediatric LTx recipients and to evaluate the contribution of modifiable lifestyle factors to sarcopenia risk. The study objectives were to a) describe the prevalence of sarcopenia in children post-LTx using a definition of sarcopenia that includes the evaluation of skeletal muscle mass, muscle strength and physical performance and b) to describe the lifestyle factors (diet and physical activity) associated with sarcopenia prevalence. We hypothesized that sarcopenia is highly prevalent in post-LTx children and related to poor diet quality and physical inactivity.

4.3 Methods

We prospectively recruited participants aged 6-18 years (n=22) from the Pediatric Liver Transplant Clinic, at the Stollery Children's Hospital who had undergone LTx for a minimum of 1 year, presented with normal liver allograft function and no acute organ rejection within 3 months. The exclusion criteria were participants younger than 6 years, with any known episodes of acute rejection within the past 3 months or with bone or muscular disorder/injuries that precluded the ability to participate in muscle function assessments or who underwent LTx due to inborn error of metabolism. Age-matched healthy controls (HC; n=47) were recruited from the community. Selection bias was minimal as 2 LTx patients (8%) who met the inclusion criteria declined participation due to large geographical distance from centre. Informed consent and assent were obtained

from participants and parents prior to study entry. This study was approved by the Human Research Ethics Board, University of Alberta (Pro00076244).

Primary outcomes included body composition measurements using dual-energy x-ray absorptiometry (DXA; Hologic QDR 4500A/Apex System 2.4.2, Hologic Inc., Waltham, MA), multiple skinfolds and five muscle function tests assessing MS and PP. Demographic data (gender, age, Pediatric End-stage Liver Disease [PELD], Model End-stage Liver Disease [MELD], age at LTx, etiology of LTx, immunosuppressant, laboratory data (e.g. liver function test, tacrolimus/sirolimus though level, 25-OH vitamin D, urea, creatinine, estimated Glomerular filtration rate[138]), anthropometric data (weight, height, BMI) were collected at LTx, LTx assessment and at time of study. PELD and MELD were calculated according to the United Network for Organ Sharing/Organ Procurement and Transplantation Network[108].

4.3.1 Anthropometric

Weight and height were recorded to the nearest 0.1kg/cm with participants wearing light clothing and no shoes by trained personnel. Body mass index (BMI) was calculated by weight to height squared ratio (kg/m^2). Weight-z, height-z and BMI-z were determined based on World Health Organization (WHO) standards[106]. Weight-z and height-z scores in LTx children were examined at LTx assessment, LTx, and annually until the time of study.

4.3.2 Body Composition

Body composition was evaluated using two methods: multiple skinfold measures and DXA. Due to ethical restraints related to radiation exposure associated with DXA, only LTx children underwent DXA which is performed as a part of routine clinical care.

Total and segmental body composition for fat mass and lean soft tissue mass (absolute and z-scores) were reviewed. Skeletal muscle mass (SMM) as determined by DXA was calculated against age-gender matched normative data and SMM z-scores (SMM-z) were determined[139]. Low muscle quantity was defined as SMM-z≤-1.5.

Skinfold measurement (triceps, biceps, subscapular, supraspinal, suprailiac, abdominal and calf) was performed by 1 trained investigator according to the International Society for the Advancement of Kinanthropometry methodology using a Lange skinfold caliper[140]. The intra-subject coefficient of variation for all of the individual skinfolds measured for LTx and HC groups ranged between 1-3% (p=0.78). Fat-free mass (FFM) from 4 skinfolds (biceps, triceps, subscapular, and suprailiac) were calculated according to predictive equation[141]. Bland-Altman was used to assess agreement in FFM determinations between DXA and multiple skinfold measures[142]. FFM index (FFMI=FFM/height^2 [absolute/z-score]) was determined according to normative data[143]. A FFMI-z score ≤-1.5 was defined as reduced FFM[144].

4.3.3 Measures of Muscle Strength and Physical Performance

Muscle strength was assessed by handgrip, knee bent push up and sit to stand, while physical performance was evaluated by six minute walk test and stair climb test. All muscle tests were performed by 1 trained investigator using standardized protocols, order of test and rest time (1 min) between muscle test repetitions. The five muscle tests were demonstrated by investigator, followed by participant practice to ensure technique was consistent and performed accurately. Test and retest reliability of each muscle test was performed in an independent sample of healthy children (n=9) on 2 separate occasions within a median 0.9 (IQR 0.7, 1.1) weeks. Reliability testing for muscle tests

was evaluated using intra-class correlation coefficient (ICC), where ICC <0.50, 0.50-0.75, 0.75-0.90, >0.90 were indicative of poor, moderate, good and excellent reliability respectively[145].

a) **Handgrip strength** was assessed using JAMAR® Hand Dynamometer according the standardized methodology[146]. Subjects sat with feet flat on floor with their shoulder adducted and neutrally rotated, elbow flexed at 90º, forearm and wrist in neutral position (wrist between 0 and 30 degree dorsiflexion and between 0 and 15 degree ulnar deviation). Three measurements were taken from each hand (dominant and non-dominant hands) in an alternative manner with brief breaks (<1 min) between testing. The average of three scores was used to evaluate handgrip strength.

b) Prior to testing, a standardized picture of **knee bent push up** was showed to participants **(Appendix D-1)**, followed by 3 minutes warm up session (forward and backward arm circles, biceps stretch and triceps stretch; 1 minute each)[147]. Participants were asked to face down on a soft non-movable mat, hands place underneath shoulders, elbows slightly apart, arm straight, and knee touching the map (up position)[148]. Then, subjects were instructed to lower their upper body until touch a soft object (sponge; height 5.2 cm) that was placed underneath the chest area with elbows reach a 90° angle (down position; **Appendix D-2**). The soft object was to ensure consistency of down position. Participants were given 30 seconds to complete as many push-ups as possible and the number of executed push-ups was recorded. The average push-ups from 2 measurements were used to evaluate muscle strength.

c) **Sit to stand test (STS)** was done on a straight back chair without arm rests (seat 43 cm high). Participants were asked to sit on the chair with hip and knee flexed and feet flat on

the floor. Participants were instructed to fold their arm across the chest, stand up and sit down as quickly as possible in 30 seconds, while ensuring the trunk and lower extremity to be fully extended **(Appendix D-3)**[72,149]. For younger children who were unable to perform STS with their feet position directly on the floor (n=4 LTx and n=8 HC), an accommodation that included the use of a stool (height 20 cm) was used **(Appendix D-4)**. This was done to ensure that technique was consistent between children. Number of repetitions in 30 seconds was recorded and the average number from 2 measurements was taken. No differences were noted in the number of repetitions between children using either method were observed ($p<0.05$).

d) **Six minute walk test (6MWT)** was performed in 30m indoor track on a flat and hard surface according to standard methodologies[150]. Blood pressure, heart rate, SPO_2 and perceived exertion (using modified Borg scale) were measured and rated before and after test. Walk distance (in meter) was measured at the end of the test.

e) For **stair climb test (SCT)**, participants were instructed to walk up and down the stair cases (12 steps, 17cm height) as fast as they could without running, jumping or skipping steps from a standardized starting point. Participants were free to choose to use handrail for support when ascending and descending the staircases. Use of handrail was recorded by the investigator (with handrail; LTx n=4 vs HC n=2, p=0.07). The time taken (seconds) to walk up, turn around on the top platform and walk down the stairs with both feet touching the starting point was recorded[72,74,149]. Three measurements were taken and the average of three scores was used to evaluate PP. A shorter time taken to complete the test represents better PP[74].

4.3.4 Definition of Sarcopenia

There is no established definition of sarcopenia in children. Hence, we adopted the adult definition of sarcopenia by the revised European consensus 2019: a) probable sarcopenia (low MS assessed by handgrip or sit-to-stand or push-up test), b) sarcopenia (low MS + low muscle quantity) and c) severe sarcopenia (low MS + low muscle quantity + low PP assessed by 6MWT or stair climb test)[4]. Low MS/PP was defined by values 2 SD below the mean values for age and gender matched HC. Low muscle mass was defined as SMM-z ≤-1.5 for LTx (only). In addition, low FFM was defined by using FFMI-z ≤-1.5 in both groups.

4.3.5 Muscle Quality (MQ)

The assessment of muscle quality (MQ) could be an alternative component in sarcopenia diagnosis as MQ can be impaired in sarcopenia[4]. MQ was defined as the ratio between measures of MS (handgrip) and total (SMM/LBM/FFM/Appendicular lean mass [ALM]) and segmental (lean peripheral limbs, trunk) muscle mass determined by DXA and/or multiple skinfold measures (FFM)[151].

4.3.6 Dietary Intake

Dietary intake was assessed using 3-day food records; 2 weekdays, 1 weekend day. Food records were analyzed using food processor software (ESHA Research 2015; version 10.15.41) and diet quality (DQ) was determined using healthy eating index for children (HEI-C)[152]. HEI-C scores of <60, 60-80, >80 were referred to poor, needs improvement and good DQ respectively. Under-over reporting of intake was determined by the ratio of energy intake to basal metabolic rate, where energy intake/basal metabolic rate values outside the 95% confidence intervals indicative of misreporting[153].

4.3.7 Physical Activity: Accelerometry

Habitual physical activity (number of steps, distance walked, floor climbed, active minutes, heart rate) and sleep patterns (total sleep/stages) were assessed using accelerometer (*Fitbit Charge 2*™). Participants were instructed to wear accelerometer around their wrist for 2 weeks (24 hours). Hourly reminders to move by accelerometer was turn off to ensure habitual physical activity was captured. Accelerometers were synced with an electronic device to the web-based server and data was downloaded by investigators through a de-identified email. The number of steps, active minutes (sedentary, lightly, moderate and very active minutes), total sleep hours and sleep stages (percentages of awake, rapid eye movement, light, deep stage) and resting heart rate were compared to normative data[154-157]. Moderate and vigorous physical activity minutes was determined using the sum of fairly active and very active minutes and compared to current guidelines[154]. Cardio fitness score is an estimate of VO2 max was pre-determined by the device using resting heart rate, age, gender and weight and classified into poor (score 1) to excellent (score 6) fitness levels based on published data[158]. As Fitbit only records heart rate if it is placed on participants' wrist, adherence rate was determined by the presence of heart rate data for at least 20 hours/day of wear time[159]. Any day within 2 weeks with heart rate data <20 hours/day was removed from the analysis.

4.3.8 Statistical analysis

Data analysis was completed using the SAS 9.0 statistical software (SAS, Version 9.4; SAS Institute Inc., Cary, NC, USA). Data were expressed as mean ± standard deviation (SD) or median and inter-quintile range (IQR), unless otherwise specified. The Shapiro-Wilk test was conducted to assess the normality of distribution. Independent t-

tests or Kruskal–Wallis test with post-hoc Dunn tests were conducted to compare the differences between HC and LTx children for body composition, MS, PP, anthropometric and demographic data. Chi-square/Fisher exact tests were used to measure differences in categorical data. Multivariate analysis (ANOVA) was conducted to examine the potential interrelationships between measures of muscle mass (SMM-z or FFMI-z), tests of MS, PP and lifestyle factors (diet, physical activity). A p-value ≤0.05 was considered significant.

4.4 Results

4.4.1 Demographic, Anthropometric, Clinical Characteristics and Body Composition

Demographic and anthropometric characteristics are presented in **Table 4.1.** The majority of children had weight-z, height-z and BMI-z within normal reference ranges (>95%). LTx children had significant lower height-z (p=0.02), but higher BMI-z than HC (p=0.01). LTx children with low SMM-z (≤-1.5) had lower weight-z than those with SMM-z within normal reference ranges (>1.5). There were no significant differences in demographic and clinical characteristics (age, gender, liver disease type, PELD/MELD scores, height-z, weight-z or BMI-z at LTx, laboratory parameters or immunosuppressive therapies (type/dose/trough levels) between children with SMM-z above or below -1.5 (p>0.05). All LTx children had normal albumin, international normalized ratio (INR) and partial thromboplastin time (PTT), indicative of normal liver synthetic function. The majority of LTx children had height-z (80%) and weight-z (100%) within normal reference ranges, indicative of normal growth patterns. No differences in adipose indices were noted in the LTx children ± sarcopenia **(Appendix D-5).**

Table 4.1Demographic, anthropometric, relevant medical and laboratory variables in liver transplantation children and healthy children.

Variables	LTx cohort (n=22)	Healthy Controls (n=47)	p-value	Sarcopenia (n=8)	Non-sarcopenia (n=14)	p-value
Gender	11M/ 11F	20M/27F	0.56	6M /2F	5M/9F	0.08
Age (years)	12.1 ± 3.5	12.2 ± 3.5	0.88	11.1 ± 3.5	12.7 ± 3.5	0.32
Weight-z	0.28 ± 0.85	0.11 ± 1.03	0.51	-0.24 (-0.51, 0.63)	0.53 (-0.13,0.68)	0.37
Height-z	-0.19 ± 1.01	0.44 ± 1.04	0.02	-0.30 ± 1.27	-0.13 ± 0.87	0.71
BMI-z	0.52 ± 0.98	-0.15 ± 1.05	0.01	0.37 ± 1.12	0.60 ± 0.92	0.61
Weight-z at LTx	-0.34 ± 1.09	-	-	-0.50 ± 1.00	-0.25 ± 1.17	0.63
Height-z at LTx	-0.36 ± 1.63	-	-	-0.77 ± 1.78	-0.01 ± 1.53	0.43
BMIz-at LTx	0.11 ± 0.85	-	-	0.20 ± 0.60	0.02 ± 1.06	0.72
PELD score	9 (6, 16)	-	-	8 (0, 16)	10 (8, 19)	0.40
Age at LTx (years)	1.5 (0.9, 8.0)	-	-	4.6 (1.0, 9.4)	1.3 (0.7, 3.8)	0.47
Year of post-LTx	8.0 ± 4.4	-	-	5.7 ± 3.5	9.1 ± 4.5	0.08
Liver etiology, n (%) - BA - ALD - Others	 12 (55) 2 (9) 8 (36)	-	-	 5 (63) 1 (13) 2 (24)	 7 (50) 1 (7) 6 (43)	0.63
Immunosuppression, n(%) - Tacrolimus - Sirolimus - Tacrolimus+ MMF - Tacrolimus+ CST	 19 (85) 1 (5) 1 (5) 1 (5)	-	-	 7 (88) 0 0 1 (12)	 12 (86) 1 (7) 1 (7) 0	0.41
Re-LTx, n (%)	3 (14)	-	-	1 (12)	2 (14)	0.47
Number of rejection	0 (0, 1)	-	-	0 (0, 0.5)	0 (0, 0)	0.79
Tacrolimus trough (µg/L)	4.4 (3.5, 4.8)	-	-	4.4 (3.9, 4.8)	3.8 (3.1, 4.8)	0.36
AST (U/L)	31(22, 29)	-	-	31 (30, 36)	32 (21, 47)	0.95
ALT (U/L)	27 (21, 29)	-	-	27 (22, 29)	27 (18, 31)	0.89
GGT (U/L)	17 (11, 38)	-	-	13 (6, 46)	20 (14, 38)	0.41
Total bilirubin (µmol/L)	9 (6, 11)	-	-	11 (7, 12)	8 (6, 11)	0.61
Albumin (g/L)	43 ± 4	-	-	43 ± 4	43 ± 3	0.93
INR	1.0 (1.0, 1.1)	-	-	1.1(1.0, 1.1)	1.0 (1.0, 1.1)	0.18
PTT (seconds)	33 ± 3	-	-	34 ± 3	32 ± 3	0.30
25-OH vitamin D(nmol/L)	90 ± 13	-	-	87 ± 9	92 ± 15	0.37
Urea (mmol/L)	3.8 ± 1.4	-	-	3.8 ± 1.4	3.9 ± 1.5	0.92
Creatinine (µmol/L)	50 ± 15	-	-	44 ± 13	54 ± 15	0.14
eGFR (ml/min/1.73m^2)	92 ± 14	-	-	97 ± 15	89 ± 12	0.20

Sarcopenia refers to probable sarcopenia, defined as low muscle strength (low hand-grip/ sit-to-stand/ push-ups) based on revised European consensus on sarcopenia 2019[4].

Others liver etiology: Children with probable sarcopenia [Crigler-Najjar (n=1), Alpha-1-antitrypsin deficiency (n=1)], Children without probable sarcopenia [Cringler-Najjar (n=2), Primary sclerosing cholangitis (n=2), Progressive familial intrahepatic cholestasis type 3 (n=1), Unknown(n=1)]
Immunosuppression: n=1 on Sirolimus 1mg OD with level of 3.7ug/L. n=1 on tacrolimus 4mg once daily and mycophenolate mofetil 8.5ml twice daily, n=1 on tacrolimus 0.8mg twice daily and prednisolone 15mg daily.
MELD score (n=2) mean± SD: 23 ± 5, children with probable sarcopenia (n=1) MELD 19, children without probable sarcopenia (n=1) MELD 26.
Normal ranges for AST (<40U/L), ALT (<50U/L), GGT (<70 U/L), Total bilirubin (<20umol/L), Albumin (35-50g/L), INR (0.8-1.2), PTT (27-39 seconds), 25-OH vitamin D (>75nmol/L).eGFR was calculated according to creatinine-cystatin C CKID equation, 2012[138].All tacrolimus trough levels were within therapeutic range (3-5 ug/L).
LTx: Liver Transplantation, PELD: Pediatric end-stage liver disease score, MELD: Model for End-Stage Liver Disease. MELD was calculated for children >12 years, BA: Biliary atresia, ALD: Acute liver disease, MMF: mycophenolate mofetil, CST: Corticosteroid, Re-LTx: Liver re-transplantation in children with first graft failure, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: gamma-glutamyl transpeptidase, INR: International normalized ratio, PTT: Partial thromboplastin time, eGFR: estimated glomerular filtration rate.
Data expressed in mean±SD or median (IQR) or %. P-values ≤0.05 is considered statistically significant.

4.4.2 Muscle Strength (MS), Physical Performance (PP) and Muscle Quality (MQ)

Table 4.2 and **Figure 4.2** illustrate the differences in MS and PP between LTx and HC. LTx children had lower mean performance in all 5 muscle tests than HC with significant differences observed in 3 out 5 tests (push up, sit to stand and stair climb test). There were no differences (pre vs post-test) in blood pressure, heart rate, SPO_2 and perceived exertion (Borg scale) related to 6MWT in LTx vs HC and in LTx children with or without sarcopenia **(Table 4.3).** LTx children had lower MQ than HC (0.62 ± 0.16 kg/kg [LTx] vs 0.76 ± 0.17 kg/kg [HC]; p=0.002). With the exception of STS (ICC 0.66; moderate), test–retest reliability of all the muscle tests showed good to excellent repeatability (ICC 0.84-0.99; **Table 4.4**). For each muscle test, the intra-subject reliability in LTx and HC groups was good to excellent (ICC 0.81-0.98; **Table 4.5**).

Table 4.2 Percentage of muscle strength and physical performance tests in liver transplantation children as compared to healthy controls.

Muscle tests	Mean values of LTx	Mean values of HC	Percentage (%)*	Interpretations
Push Up (number in 30s)	11.23	17.85	63	LTx performance was 63% of HC group
Sit to stand test (number in 30s)	16.20	22.26	73	LTx performance was 73% of HC group
6 minute walk test (m)	508.34	561.57	91	LTx performance was 91% of HC group
Dominant handgrip (kg)	20.77	24.76	84	LTx performance was 84% of HC group
Non-dominant handgrip (kg)	19.44	23.47	83	LTx performance was 83% of HC group
Stair climb test (seconds)	11.78	8.95	132	LTx was 32% slower than HC in staircase climb test

*Percentage of performance was calculated by: mean values of liver transplantation/ mean values of healthy control x100%.
LTx: Liver transplantation, HC: Healthy control

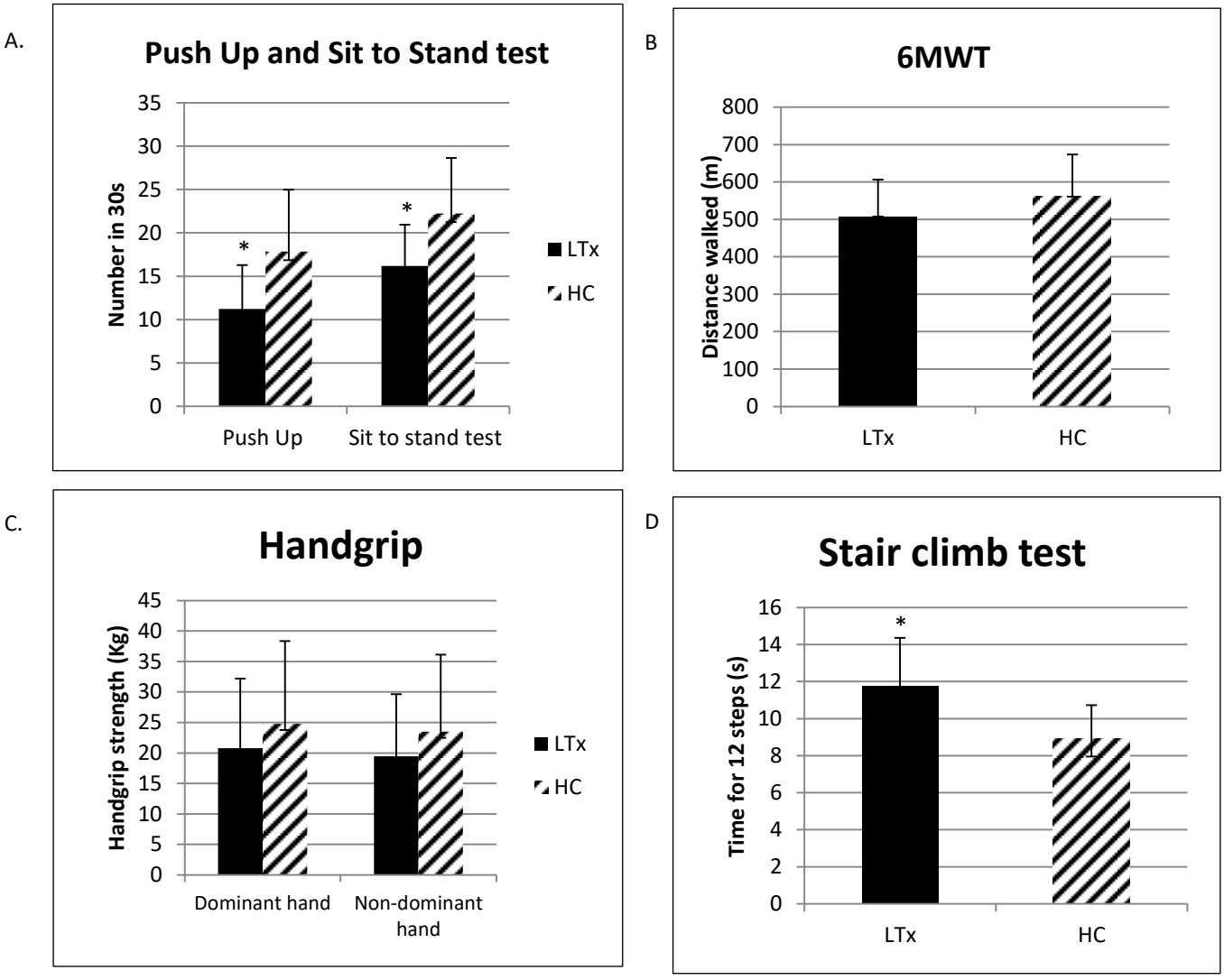

Figure 4.2 Muscle tests in post-liver transplantation (LTx) children and age-matched healthy controls (HC). The differences in LTx and HC cohorts on (A) Number of push up ($p=0.0002$) and sit to stand ($p=0.0002$) performed in 30 seconds, (B) Distance (meters) walked over 6 minutes continuously on a flat and hard surface ($p=0.06$), (C) Handgrip strength for dominant hand ($p=0.34$) and non-dominant hand ($p=0.27$), (D) Time taken (seconds) to climb up and down 12 step of stairs ($p<0.0001$). Values represent mean ± SD. *Values significantly different at $P<0.05$. LTx: Liver transplantation cohort (n=22); HC: healthy control cohort (n=47); 6MWT: 6-minute walk test.

Table 4.3 Changes in blood pressure, heart rate, SPO2 and Borg scale in liver transplantation children and healthy controls before and after 6 minute walk test.

Changes in variables *	LTx cohort	HC cohort	p-value	Sarcopenia	Non-sarcopenia	p-value
Systolic BP-z score	1.0± 1.1	1.4 ± 1.2	0.17	0.7 ± 1.0	1.2 ± 1.2	0.40
Diastolic BP-z score	0.6 ± 0.5	0.6 ± 0.8	0.99	0.5 ±0.5	0.7 ±0.6	0.51
HR (bpm)	10(4,16)	9 (3, 20)	0.75	8 ± 8	11± 9	0.43
SPO2	0 (-1, 0)	0 (-1, 0)	0.86	-1 (-2, 0)	0 (-0.5, 0)	0.14
Borg scale	1 (0,2)	1 (0, 2)	0.76	1.4 ± 1.3	1.1 ± 1.0	0.57

*Changes in blood pressure, heart rate, SPO2 and Borg scale were determined by the difference between post-6 minutes walk test and pre-6 minute walk test (post-pre).
Sarcopenia refers to probable sarcopenia, defined as low muscle strength (low hand-grip/ sit-to-stand/ push-ups) based on revised European consensus on sarcopenia 2019[4].
BP: Blood pressure, HR: Heart rate, bpm: beat per minute, SPO2: Oxygen saturation

Table 4.4 The test-retest intraclass correlation coefficients (ICC) of muscle tests.

Muscle tests	ICC of test-retest reliability	Classifications
Push up	0.84	Good
Sit to stand	0.66	Moderate
6-minute walk test	0.94	Excellent
Dominant handgrip	0.99	Excellent
Non dominant handgrip	0.99	Excellent
Stair climb test	0.84	Good

Test-retest reliability of each muscle test was performed in an independent sample of healthy children (n=9) on 2 separate occasions within a median 0.9 (IQR 0.7, 1.1) weeks.
Intraclass correlation coefficients (ICC) <0.50, 0.50-0.75, 0.75-0.90, >0.90 were indicative of poor, moderate, good and excellent reliability respectively[145].

Table 4.5 The intra-subject intraclass correlation coefficients (ICC) of muscle tests in post-liver transplantation and healthy children.

Muscle tests	LTx cohort (n=22)	Classification	HC cohort (n=47)	Classification
Push up	0.89	Good	0.91	Excellent
Sit to stand	0.93	Excellent	0.87	Good
Dominant handgrip	0.97	Excellent	0.98	Excellent
Non-dominant handgrip	0.96	Excellent	0.98	Excellent
Stair climb test	0.88	Good	0.81	Good

Two trials of push up and sit to stand, three trials of handgrip and stair climb test by each participant were used to determine intra-subject reliability. 6 minute walk test was done once in each participant, hence no intra-subject reliability was determined.
Intraclass correlation coefficients (ICC) <0.50, 0.50-0.75, 0.75-0.90, >0.90 were indicative of poor, moderate, good and excellent reliability respectively[145].
LTx: Liver transplantation, HC: healthy control

4.4.3 Sarcopenia Prevalence

The prevalence of sarcopenia by EWGSOP2 definition was 36%. Out of the 8 children with sarcopenia, two children were classified as severe sarcopenia, with the remaining classified as 'probable'. None of the HC had sarcopenia. Of the LTx children, 29% had low SMM defined by SMM-z≤-1.5 and 55% had low MS and low PP. There were no significant differences in demographic (age, sex, liver disease diagnosis/severity, tanner stage), anthropometric, laboratory variables or immunosuppressive therapy (type/dose) in LTx children with and without sarcopenia ($p>0.05$). Deficits in PP in children with sarcopenia were predominantly revealed by longer stair climbing times ($p=0.03$), with no differences in 6MWT, STS, and number of push-ups performed **(Table 4.6)**. Handgrip was significantly higher in children with FFMI-z >-1.5 vs FFMI-z ≤-1.5 (27.9 ± 13.7kg vs 17.8 ± 9.3kg; $p=0.02$). LTx children with sarcopenia had lower MQ (as defined by the ratio of handgrip/lean body mass) in the peripheral limbs (particularly the legs) and axial skeleton (appendicular lean body mass) ($p<0.05$).

Table 4.6 Muscle strength and physical performance tests in sarcopenia and non-sarcopenia post-liver transplantation children.

Muscle tests	Sarcopenia (n=8)	Non-sarcopenia (n=14)	p-value
Push Up (number in 30s)	8.81 ± 2.19	12.61 ± 5.76	0.09
Sit to stand test (number in 30s)	14.75 ± 4.17	17.04 ± 4.99	0.29
6 minute walk test (m)	479.43 ± 116.93	526.13 ± 83.81	0.30
Dominant hand (kg)	15.57 ± 10.49	23.73 ± 11.18	0.11
Non-dominant hand (kg)	15.06 ± 9.02	21.94 ±10.30	0.13
Stair climb test (seconds)	13.22 ± 2.67	10.95 ± 2.21	0.03

Sarcopenia refers to probable sarcopenia, defined as low muscle strength (low hand-grip/ sit-to-stand/ push-ups) based on revised European consensus on sarcopenia 2019[4].

4.4.4 Lifestyle Factors and Sarcopenia

4.4.4a Diet

The majority of LTx (68%) and HC (66%) children had diet quality (DQ) characterized by 'needs improvement', while 27% of LTx children and 28% HC had intake characterized by poor DQ. There were 5% and 6% of children had good DQ in LTx and HC groups, respectively **(Table 4.7).** A greater proportion of LTx children (77%) were on vitamin D supplements (single preparation or multi-vitamin preparations) containing 200-1000 IU/day as compared with HC (32%; p=0.0004). LTx children with sarcopenia had lower fat, PUFA, and MUFA intakes (absolute, % kcal) and higher protein intake (% kcal, g/kg [2.4 ± 0.6 g/kg (+sarcopenia) vs 1.7 ± 0.4 g/kg/d (-sarcopenia); p=0.005) than children without sarcopenia. These differences were independent of age, sex, liver disease diagnosis or misreporting of dietary intake.

Table 4.7 Dietary intake per 1000kcal in post-liver transplantation and healthy children.

Variables	LTx cohort (n=22)	HC cohort (n=47)	p-value	Sarcopenia (n=8)	Non-sarcopenia (n=14)	p-value
Protein (g)	37 (33, 43)	39 (35, 45)	0.31	42 ± 8	36 ± 7	0.07
Carbohydrate (g)	130 ± 15	133 ± 15	0.60	135 ± 18	128 ± 13	0.28
Fat (g)	37 ± 7	35 ± 7	0.32	33 ± 6	39 ± 6	0.03
Saturated fat (g)	13 ± 3	12 ± 3	0.15	13 ± 3	14 ± 3	0.42
MUFA (g)	13 ± 3	12 ± 3	0.78	11 ± 3	14 ± 3	0.04
PUFA (g)	6 (5, 8)	6 (5, 8)	0.64	5 ± 1	7 ± 2	0.02
Sugar (g)	55 ± 16	44 ± 14	0.01	56 ± 12	54 ± 19	0.80
Dietary vitamin D (IU)	97 (67, 164)	75 (43, 107)	0.07	160 ± 86	98 ± 76	0.09
% of participants on vitamin D supplement	17 (77%)	15 (32%)	0.0004	7 (88%)	10 (71%)	0.30
Total vitamin D (including supplement; IU)	509 (209, 747)	95 (52, 332)	<0.0001	650 ± 282	390 ± 272	0.05
Dietary calcium (mg)	563 ± 209	467 ± 162	0.04	640 ± 128	519 ± 237	0.20
% of participants on calcium supplement	3 (14%)	5 (11%)	0.28	1 (12%)	2 (14%)	0.47
Total calcium (including supplement; mg)	575 ± 211	471 ± 162	0.03	641 ± 127	537 ± 243	0.27
Diet quality	65 ± 12	66 ± 11	0.73	68 (64, 72)	66 (58, 69)	0.41

Sarcopenia refers to probable sarcopenia, defined as low muscle strength (low hand-grip/ sit-to-stand/ push-ups) based on revised European consensus on sarcopenia 2019[4].

All nutrients were expressed on a per 1000kcal basis, except diet quality that based on absolute energy intake.

Diet quality (DQ) was calculated from 3-day food records. DQ is a scoring system based on dietary adequacy, moderation and variety. DQ scores range from 0-100, in which <60, 60-80, >80 referred to poor, need improvement and good DQ respectively.

Data expressed in mean±SD or median (IQR) or %. P-values ≤0.05 is considered statistically significant

4.4.4b Habitual Physical Activity and Sleep

Accelerometers were worn for 13 (12-14) days in excess of 20 hours/day. Only 11% of LTx and 22% of HC (p=0.19) met guidelines for the recommended number of steps/d for age and gender[155] **(Table 4.8a& 4.8b)**. None of the LTx children and 16% of HC met age-related guidelines for time spent in moderate and vigorous physical activity (p=0.08)[154]. LTx children had higher resting heart rate (79 ± 8 beats/min vs 69 ± 5 beats/min; p<0.0001) and lower cardio fitness scores as compared to HC (3 ± 1 [LTx]vs 4 ± 1 [HC]; p=0.03). Time spent in moderate and vigorous physical activity and average fairly active minutes and very active minutes were positively correlated with measures of MS (handgrip) (p<0.001)/ MQ and inversely correlated with FFMI-z scores (p<0.05). In particular, FFMI-z scores≤-1.5 was associated with less time spent in moderate and vigorous physical activity (15.6 ± 16.7 min/d [FFMI-z scores≤-1.5] vs 39.5± 30.5 min [FFMI-z scores>-1.5]; p=0.006), fairly active minutes (12.5 ± 13.5 min/d [FFMI-z≤-1.5] vs 28.3 ± 22 min/d [FFMI-z> -1.5]; p=0.002) and very active minutes (3.1 ± 0.6 min/d [FFMI-z≤-1.5] vs 11.2 ± 11.5 min/d [FFMI-z>-1.5]; p=0.002) in both groups. No other major differences in physical activity (sedentary) or sleep patterns (total, % of light, deep and rapid eye movement sleep or number of awakenings) were observed between groups.

Table 4.8a Habitual physical activity assessed by accelerometer.

Variables	LTx cohort (n=18)	HC cohort (n=47)	p-value	Sarcopenia (n=8)	Non-sarcopenia (n=10)	p-value
Number of step/day	9236 ± 2609	10011 ± 2706	0.23	8447 ± 2402	9867± 2715	0.26
Distance walked(km)/d	5.1 (4.1, 6.9)	6.4 (4.9, 7.5)	0.16	5.3 ± 2.4	5.8 ± 1.6	0.61
Number of floor climbed/d	9 (5, 16)	11 (6, 16)	0.60	8 (5, 10)	12 (5, 17)	0.29
Resting heart rate(bpm)	79 ± 8	69 ± 5	<0.0001	80 ± 9	77 ± 7	0.47
Sedentary minutes/d	622 ± 104	604 ± 117	0.59	653 ± 112	596 ± 95	0.26
Lightly active minutes/d	299 ± 87	297 ± 77	0.91	275 ± 75	318 ± 95	0.31
Fairly active minutes/d	16 (4, 19)	15 (7, 36)	0.12	11 ± 9	15 ± 11	0.45
Very active minutes/d	2 (0, 7)	2 (1, 11)	0.32	2 (0, 17)	3 (0, 7)	0.93
Moderate and vigorous activity minutes/d	21 (5, 30)	24 (9, 48)	0.16	20 ± 19	19 ± 13	0.91
Cardio fitness scores	3 (2, 4)	4 (3, 6)	0.03	3 ± 2	3 ± 1	0.33

Table 4.8b Percentage of participants meeting age specific physical activity and sleep recommendations.

Variables	LTx cohort (n=18)	HC cohort (n=47)	p-value	Sarcopenia (n=8)	Non-sarcopenia (n=10)	p-value
Number of step/d (%)	11	22	0.19	12	10	0.52
Moderate and vigorous physical activity minutes/d (%)	0	16	0.08	0	0	-
Total sleep (%)	6	9	0.38	0	10	0.56
Sleep stages (%)						
- Awake	94	100	0.28	100	89	0.53
- REM	100	98	0.72	100	100	-
- Light sleep	100	100	.	100	100	-
- Deep sleep	88	86	0.33	75	100	0.21

Data available for n=18 LTx participants. Sarcopenia refers to probable sarcopenia, defined as low muscle strength (low hand-grip/ sit-to-stand/ push-ups) based on revised European consensus on sarcopenia 2019[4].

Adherence to wearing accelerometer device was determined using heart rate data≥20 hours/day. Any days within 2 weeks with heart rate data<20 hours will be eliminated from data analysis.

Sedentary time refers to inactivate for 10 consecutive minutes.

Active minutes (lightly, fairly active and very active) was recorded if participants participated in continuous activities at or above 3 METs (indicator of exercise intensity based on heart rate) for 10 minutes.

Moderate and vigorous physical activity minutes/d refers to fairly active minutes + very active minutes and compared to guideline.

Cardio fitness score is an estimate of VO2 max that pre-determined by accelerometer using resting heart rate, age, gender and weight. Score classification: 1=poor, 2=fair, 3=average, 4=good, 5=very good, 6=excellent.

Recommendations: Steps: ≥12000 steps/day (Colley et al., 2012)[155], Moderate and vigorous physical activity minutes: ≥ 60minutes/day (Canadian Society for Exercise Physiology, 2011)[154], Total sleep: 6-13 years: 540-660 min/d, 14-17years: 480-600min/d (National Sleep Foundation, 2015)[156], Sleep stages based on age specific recommendations (Ohayon et al., 2004)[157].

Min: Minutes, D: Day, REM: Rapid eye movement

Data expressed in mean±SD or median (IQR) or %. P-values ≤0.05 is considered statistically significant.

4.5 Discussion

This is the first study that has examined the prevalence of sarcopenia in pediatric LTx recipients using definitions that included measures of body composition, MS, PP and examined lifestyle factors (diet and physical activity) that may contribute to sarcopenia. We confirmed that sarcopenia is highly prevalent in children up to 10 years post-LTx[11] and characterized by deficits in MS and PP, particularly in the lower limbs. In addition, children with sarcopenia had low habitual levels of moderate and vigorous physical activity. Diet quality is poor following LTx, but not related to sarcopenia.

Poor DQ in pediatric transplant recipients is not an unexpected finding. DQ in LTx recipients is similar to HC and often does not meet current dietary guidelines[135,137]. While suboptimal protein and vitamin D intake has been associated with sarcopenia prevalence in adults, we did not see any associations. Children with sarcopenia had higher protein intake (>2.5g/kg/d) when compared to children without sarcopenia (1.7g/kg/d) and were equally sufficient in vitamin D due to the high prevalence of vitamin D supplementation (>95% in LTx). However, both groups had protein intakes in excess of recommended protein requirements and no major differences in the absolute gram amount of protein consumed. Differences may have been due to the increasing emphasis on protein intake by families with children who have experienced protein-energy malnutrition in the pre-LTx period. Vitamin D deficiency is known to increase oxidative stress in the skeletal muscle that lead to mitochondrial dysfunction and muscle atrophy[160]. Emerging evidence in adult population suggested that vitamin D supplementation increases vitamin D receptor concentration in skeletal muscle, increases muscle fiber size and contributes to myoblast proliferation[160,161]. We have previously

shown that branched-chain amino acid (BCAA) requirements are significantly higher in LTx recipients when compared to HC[162], suggesting that routine BCAA supplementation may be an important component of rehabilitation in children with sarcopenia[162]. Despite the exact mechanism of vitamin D and sarcopenia is not currently well understood, emerging evidence suggested that vitamin D supplementation increases vitamin D receptor concentration in skeletal muscle, increases muscle fiber size and contributes to myoblast proliferation[160,161,163].

Suboptimal aerobic fitness in children post-LTx has been well documented, with early muscle fatigue and reduced self-efficacy related to participation in physical activity commonly reported[88,164]. Findings provide preliminary evidence that reductions in MS and PP in pediatric sarcopenia may influence physical activity in LTx children. In particular, children with sarcopenia spent less time in 'active' physical activity than children without sarcopenia and levels of active physical activity were related to measures of MQ and MS, particularly in the lower limbs. Reductions in lower limb muscle mass would potentially exacerbate physical inactivity potentially posing barriers to participating in routine physical activity. This has important implications for the development of effective rehabilitation strategies to treat sarcopenia in pediatric LTx recipients. Recent evidence in adults with cirrhosis and sarcopenia indicate that the utilization of resistance exercise to treat sarcopenia is a safe, feasible and effective method to elicit improvements in MS and MQ[93-95,165]. Evaluation of this approach as a potential rehabilitation strategy is warranted in children with sarcopenia.

We used multiple skinfold measures to determine FFM in HC and LTx and found a high level of agreement between these two measures (**Figure 4.3**). While use of

multiple skinfold measures is simple and easy to perform, it depends on technical precision and the use of normative equations to calculate FFM[2]. In addition, these equations may not be the best measure to determine muscle mass in some clinical populations. While use of imaging (MRI or CT) has been identified as a gold standard in sarcopenia assessment; they have the potential to expose the child to radiation, are expensive, require specialized technical support and may not be routinely available[1,2].

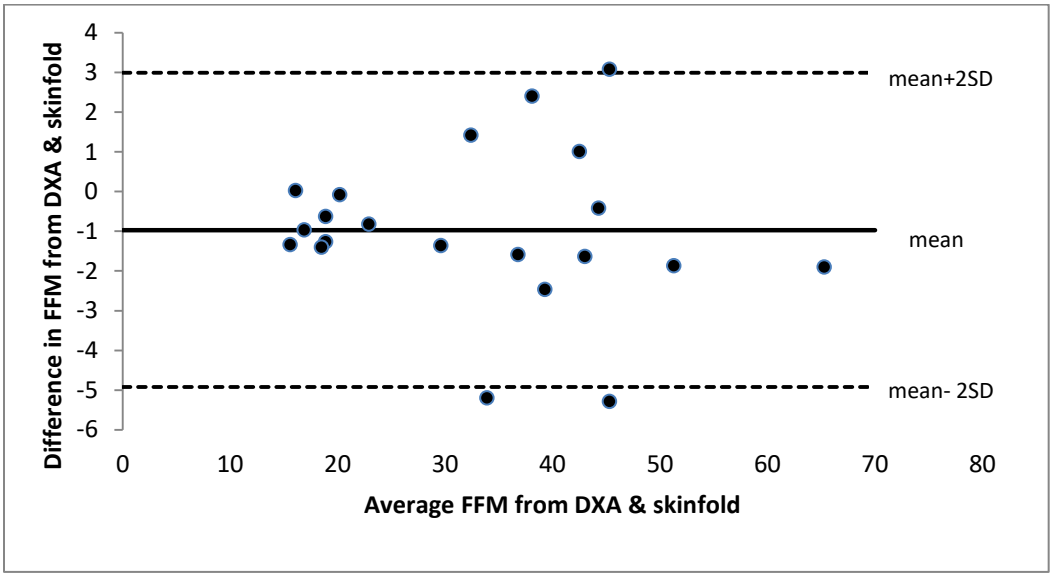

Figure 4.3 Agreement between fat free mass from DXA and skinfold measurements. FFM: Fat free mass, DXA: Dual-energy X-ray absorptiometry

Age and gender did not have any observable effects on the categorization of sarcopenia based on the functional tests or body composition in this cohort. However, variations in age may potentially affect the performance of muscle function tests, suggesting the inclusion of more than one muscle test in sarcopenia diagnosis in children may be important. Our present findings contrast with previous data, where younger (<10 years) female post-LTx children are at higher risk of developing sarcopenia[11]. Data have shown that males have higher lean body mass than female post-puberty[166], which may

confer benefit related to lean mass depletion in older male children. However, controversies exist for lean body mass difference between genders in the pre-puberty period[116,167]. There is a need to develop age and gender specific cut off for low skeletal muscle[2]. Moreover, parameters such as growth, gross motor and neurocognitive development may need to be considered for the diagnosis of sarcopenia in children[2]. Other factors that may have influenced study findings include the use of immunosuppressive therapies such as tacrolimus and corticosteroid (CST), which have been shown to influence protein turnover and induce muscle fiber changes that may potentially contribute to changes in muscle strength and performance[1]. Administration of tacrolimus or CST was found to upregulate myostatin expression in skeletal muscle, an inhibitor to muscle synthesis and transformations of slow to fast twitch muscle fibers[168-170]. Similar to our earlier findings[11], both tacrolimus and CST therapy was not associated with sarcopenia prevalence likely due to the fact that all patients had stable tacrolimus concentrations that were within targeted therapeutic levels (3-5 μg/L) and only one patient with autoimmune hepatitis was on CST. Uniquely, our centre implemented a CST minimization protocol in year 2007 which has effectively reduced CST use, enabling the study of skeletal muscle physiology in a relatively CST-free environment.

Inclusion of MS and PP measures that covered different muscle groups and component of muscle functions was a conferred strength in this study **(Appendix D-6)**. The MS and PP from HC group were well within the range of published normative data, indicative the representativeness of healthy children[72,74,171]. The reliability testing demonstrated a high concordance with low intra-subject and test-retest variation; suggesting that the testing environment was reproducible and consistent. All muscle tests

used were simple to perform, reliable, with short duration and minimal equipment needed. Assessment of physical activity using accelerometer was an important consideration of examining habitual physical activity. While Fitbit has shown validity and reliability in step counts with comparable heart rate estimates in children, there is a potential for under or overestimate in total sleep time, sleep stages, distance walked and vigorous physical activity[172-174]. Fitbit also tends to underestimate physical activity in participants who participated in water sport or other physical training that required removal of device (e.g. soccer, hockey). Despite its limitations, use of accelerometer is easy and low cost and could be a satisfactory measure than self-report physical activity level and sleep pattern[174]. In addition, the potential for variations in adherence to wearing the device may be a challenge for children and youth. In this study, participants wore the device for two weeks to account for variations in activity due day of the week (weekend/weekday). Our cohorts had good compliance rate in excess of 90%.

Our study was limited by a smaller sample size and participants with wide age range and number of year post-LTx. Despite a smaller sample size, post-hoc analysis revealed sufficient power (β>95%) to detect differences in primary outcomes of interest (muscle strength/ physical performance) between groups. When adjusted for age (above/below median age of 12.5 years) and years of post-LTx (above/below 7.6 years (median) and > and <10 years), we did not find any difference in the prevalence of sarcopenia. This is in line with our earlier longitudinal review, where sarcopenia in children occurred 1-10 years post-LTx[11]. While heterogeneity of liver disease diagnosis may have influenced sarcopenia expression, the majority of children had Biliary Atresia (>50%), which is typically the most common reason for pediatric LTx. In addition, our

LTx cohort is generalizable to other liver centres in North America in terms of demographic, anthropometric, liver disease type and severity[175,176].

4.6 Conclusions

In summary, post-LTx children have a high prevalence of sarcopenia that included deficits in lean mass, muscle mass and physical performance that was associated with low level of moderate and vigorous physical activity. These findings highlight the importance of examining childhood sarcopenia from a broader perspective beyond muscle quantity measures, as variations in routine physical activity may play a large impact on overall muscle development, strength and physical functioning in children. High quality randomized control trials examining the impact of different rehabilitation approaches to prevent and treat sarcopenia post-LTx is warranted in children[165].

Chapter 5: Conclusions and General Discussion

5.1 Introduction

Sarcopenia is a muscle condition characterized by reduced muscle strength, muscle mass and physical performance[4]. While classically, sarcopenia has been described in aging adults or adults with chronic diseases such as liver disease, emerging evidence indicates that sarcopenia or myopenia (reduced skeletal muscle mass) occurs in children with chronic diseases[4]. One of the greatest concerns related to the emerging pediatric sarcopenia literature; is the lack of a standardized pediatric definition for sarcopenia[2]. In adults, the current definition of sarcopenia includes reduced muscle strength (probable sarcopenia), reduced muscle mass plus reduced muscle strength (confirmed sarcopenia), and reduced physical performance combined with reduced muscle mass and muscle strength (severe sarcopenia; **Figure 4.1**)[4]. However, consensus regarding a pediatric definition for sarcopenia has not been reached. Incorporation of features related to muscle growth and development, along with neurodevelopmental features may be important to include in any pediatric sarcopenia definition because delays in overall development would likely influence muscle strength and muscle function in childhood, Currently, most studies focus on reduction in skeletal muscle mass (myopenia) as the defining feature of sarcopenia in childhood[2,10-12,83,133,134,177,178].

In this thesis, we have examined sarcopenia from two perspectives a) measurement of skeletal muscle mass in infants and children with end-stage liver disease (ESLD) (Chapter 3) and b) measurement of skeletal muscle mass along with measures of muscle strength and physical performance in post-liver transplantation children (Chapter 4). The purpose of this thesis was to study the pre-and-post-operative clinical outcomes

associated with myopenia and to explore the gaps related to sarcopenia risk factors (diet, physical activity) in children post-LTx. We hypothesized that sarcopenia would be highly prevalent in children and infants with ESLD pre (Chapter 3)-and-post LTx (Chapter 4) and associated with adverse clinical outcomes and suboptimal protein and energy intake.

5.2 Summary of overall research findings and clinical implications

5.2.1 Myopenia in children and infants with end-stage liver disease: relationships to clinical outcomes in the pre-and-post liver transplantation period (Chapter 3).

This is the first study that has examined the associations between myopenia (reduced skeletal muscle mass) and clinical outcomes in infants and children with ESLD awaiting LTx. We demonstrated that myopenia as measured by magnetic resonance imaging/computed tomography is prevalent, affecting up to 20% infants and children with ESLD awaiting liver transplantation (**Hypothesis 1, Chapter 3**). Children with ESLD had more than a 50% reduction in absolute total, psoas, paraspinal and abdominal wall muscle surface area when compared to age matched healthy children, with more marked reductions in overall skeletal muscle mass index in older children (> 2years of age). Myopenia was accompanied predominantly by deficits in subcutaneous and total adipose tissues when compared to healthy controls. However, myopenia in children with ESLD also presented in the absence of reduced subcutaneous adiposity tissue index (SATI) and reduced SATI also presented without myopenia; indicating that a spectrum of phenotypes related to skeletal muscle mass and adiposity in infants and children with ESLD exists.

Subcutaneous adipose tissue (SAT) plays an important role in insulin mediated functions, including overall insulin sensitivity and leptin mediated physiology in the

body[118,179,180]. Potentially, low levels of SAT may contribute to increased insulin resistance, altered glucose/lipid metabolism, inflammation and altered immune responses[118,181]. This could translate into increased rates of adverse outcomes such as reduced growth, delayed gross motor development, increased risk for hospitalization and complication rates (e.g. infection) after transplantation. Our studies uniquely support this hypothesis in that we found that children with low SAT experienced increased rates of fungal/viral infections, increased length of hospitalization, gross motor delay that coincided with lower rates of energy delivery. These findings are also comparable to evidence in adults with cirrhosis whereby low SAT has been identified and associated with increased mortality particularly in females, while myopenia predicts mortality in ESLD male adults[118].

Although we anticipated that low SMM/myopenia would have been associated with clinical outcomes (hospitalization, ventilator dependency, infections, protein and energy intake), most of the associations with body composition and clinical outcomes were related to low SAT, rather than specifically to low SMM (**Hypothesis 2 & 3, Chapter 3**). One of the reasons for this may have been that the majority of patients with low skeletal muscle mass index (SMMI) /low SATI were older children (> 2 years of age) and fewer numbers of children in this age range were present in our cohort. Moreover, children > 2 years may have experienced substantially longer periods of suboptimal intake before being assessed for LTx which may have resulted in significantly lower subcutaneous fat/skeletal muscle mass accumulation than those children who went to LTx assessment and LTx at earlier ages. While Mangus et al, 2017 found higher amounts of SAT in children with ESLD and myopenia in overall[12]; the authors reported

youth between 13-18 years had lower SMMI and SATI than healthy controls or younger children with ESLD. Similar to our study, low SATI was found in older male children prior to LTx, indicating that older (> 2 years), male children may be at the highest risk for adverse clinical outcomes than younger, female children when low SATI is found. Interestingly, this contrasts with earlier work by our group that illustrated that younger (<10 years) female children had a greater risk for myopenia (as measured by DXA)[11]. However, in this study, sex was not observed to be an independent predictor of adverse clinical outcomes. This suggests that similar to adults with liver disease, expression of sarcopenia and its effects on clinical outcomes may vary depending on the sex and age of children[11,15,40]. However, more work needs to be done to confirm these findings.

5.2.2 Sarcopenia in youth post-LTx: Implications of poor diet quality and reduced physical activity and impairments in muscle strength and muscle function (Chapter 4).

Uniquely, this study incorporated components of the adult definition of sarcopenia (muscle mass with measures of muscle strength and function) and explored potential associations with lifestyle factors with sarcopenia. Findings from this chapter showed that sarcopenia was highly prevalent in children up to 10 years post-LTx related to reductions in muscle strength/muscle quantity and physical performance. Few children had severe sarcopenia (**Hypothesis 1, Chapter 4**). Uniquely, we also demonstrated that children post-LTx had deficits in physical performance when compared to healthy children as a main component in the expression of sarcopenia. Deficits in physical performance occurred with and without deficits in muscle strength or muscle mass and represented more than 70% of the post-LTx children. This suggests that the definition of

sarcopenia in children with ESLD pre and post-LTx may be need to be explored from a broader perspective; including evaluating physical performance earlier in the definition of sarcopenia. This warrants further study across larger pediatric clinical populations.

We also showed that sarcopenia in children post-LTx was associated with reductions in some components of physical activity (moderately active, vigorous), but no major associations between dietary intake (diet quality, energy, protein) with sarcopenia in children post-LTx (**Hypothesis 2, Chapter 4**) were found. These findings are similar to data related to adults with ESLD pre-and-post LTx with sarcopenia; whereby adults with sarcopenia often experience early fatigue related to routine physical activity and reduced aerobic capacity[182,183]. In children, findings are similar, children post-LTx often experience early fatigue, reduced aerobic capacity and reduced self-efficacy to perform routine physical activity[88,89]. However, in these studies, body composition was not examined and hence the contribution of altered muscle strength/muscle mass or altered physical function to reduced physical activity was not identified[88,89]. Our current findings suggest that in the post-LTx period, this may be the case given that children with sarcopenia spent substantially lower amounts of time engaged in moderately active and vigorous activity when compared to those children without sarcopenia. Whether this is due to reduced muscle strength/aerobic capacity and/or impaired physical performance is currently unknown.

We also did not find any strong associations with energy and protein intakes or overall diet quality and sarcopenia prevalence in children post-LTx. This may be due to the higher protein intake in the post-LTx children (>1.5 g/kg/d). The diet of post-LTx children typically need significant improvements in overall diet quality (overall fat and

sugar intake), but are similar to the diet of age-matched healthy control children. While our study was not powered to address dietary intake and sarcopenia prevalence, post-hoc analysis revealed sufficient power (β>0.75) to detect any significant differences in diet quality between groups.

Interestingly, we also did not see any associations with corticosteroid use and/or tacrolimus serum levels and sarcopenia in either study, medications that are known to adversely influence skeletal muscle metabolism in adults[169]. This may be due to the very low use of corticosteroid therapy in this region, and the fact that all patients at the time of measurement had tacrolimus trough levels within targeted therapeutic ranges (3-5 µg/L). This was likely the case as all children had normal synthetic liver function and had not experienced any episodes of rejection for at least six months. In children with recurrent or chronic rejection, it is possible that higher trough levels of tacrolimus may result in larger alterations in skeletal muscle metabolism contributing to increased risk of sarcopenia. Calcineurin-inhibitors are known to inhibit the gene expression related to the synthesis of slow-twitch fibers which are important in postural support and locomotion/movement spanning a broad range of intensity[168]. This may explain in part why children post-LTx performed fewer timed sit-to-stand and number of push ups and took a longer time for stair climb test than their age-matched healthy counterparts as these muscle fiber types are important to perform these activities. Lower lean muscle mass measured within the peripheral limbs found in the LTx children also suggests that tacrolimus may have been a contributing factor to sarcopenia etiology. Research aimed at examining underlying skeletal muscle biology in children with sarcopenia and the impact of

immunosuppressive therapies on muscle biology is warranted. Summary of overall research findings and clinical implications were presented in **Figure 5.1**.

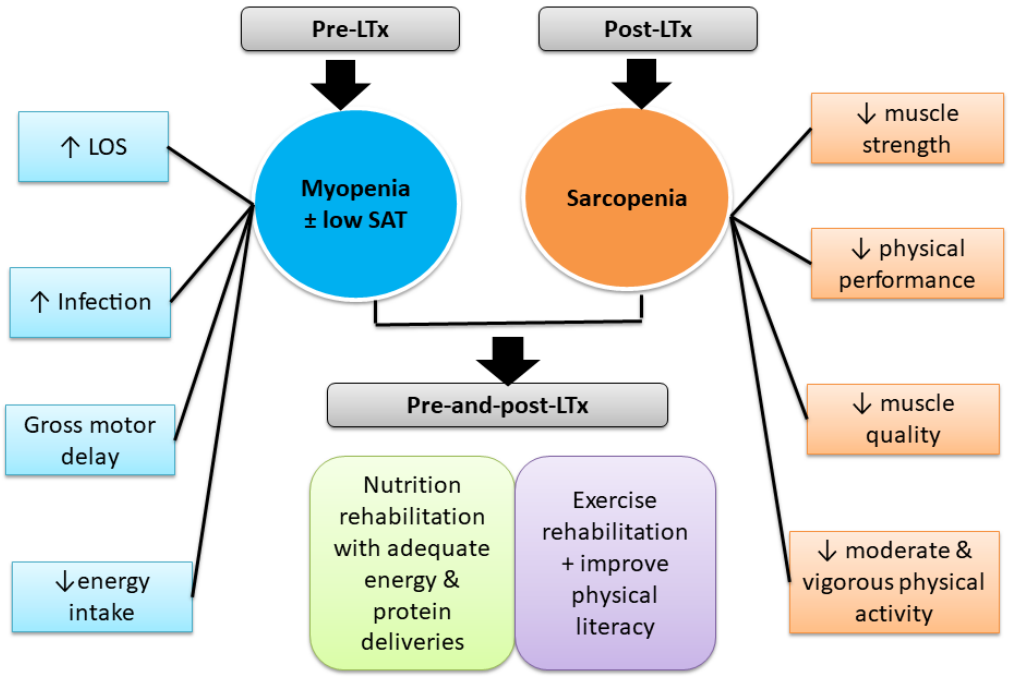

Figure 5.1. Myopenia and/or low subcutaneous fat pre-liver transplantation and sarcopenia in post-liver transplantation. LTx: Liver transplantation, LOS: Length of stay.

5.3 Strengths and limitations

One of the strengths in these studies included the comprehensive evaluation of sarcopenia in a vulnerable population of children with ELSD before and after LTx. Sarcopenia was defined from the perspective of body composition alone **(Chapter 3, Study 1)** and body composition plus comprehensive measures of muscle strength and physical performance **(Chapter 4, Study 2)**. Body composition was evaluated using three methods: Magnetic resonance imaging (MRI)/ Computed tomography (CT) (in LTx

/healthy controls), Dual-energy X-ray absorptiometry (DXA) (in LTx) and multiple skinfold measures (LTx/healthy controls), but was limited in that healthy controls in Study 2 were not allowed to undergo DXA in this region due to concerns related to potential radiation exposure. Although both CT/MRI (predominantly) was used to determine SMM in Study 1, recent evidence has shown that findings related to body composition have excellent agreement[127,184]. MRI has the conferred benefit of lack of radiation exposure to children. This has important practical and clinical implications in terms of measuring body composition in vulnerable pediatric populations. This thesis also included a comprehensive evaluation of the lifestyle (diet and physical activity) and medical risk factors that may contribute to sarcopenia risk and the impact that sarcopenia may have on longer term clinical outcomes in children. This has not been reported within the pediatric literature in children with ESLD. However, thesis findings do not prove cause and effect for pediatric sarcopenia in ESLD. In particular, study 1 was a retrospective clinical review of infants and children with ESLD. As with any retrospective review, data represents standards of clinical practice, and hence may have missing data at specific time points of study. While this may have been a factor in this study, the Pediatric Liver Transplant program has instituted protocolized approaches to care (e.g. routine DXA at annual clinic appointments) that provide unique opportunities to study body composition in infants and children with ESLD longitudinally. Understanding the underlying skeletal physiology and morphology related to sarcopenia in children with ESLD is also important to ensure that the determinants of sarcopenia can be more readily understood in this population. This is currently under study by our group.

The sample sizes in both studies were relatively small, but were comparable to those found in the pediatric literature[10,12]. Use of age-matched healthy controls in both studies may have mitigated this to some extent since post-hoc power analysis demonstrated sufficient power ($\beta > 0.8$) to detect differences in SMMI and SATI. Study of more homogenous liver populations (e.g. Biliary Atresia) with larger sample sizes may be important as prevalence and expression of sarcopenia may differ between liver disease types. While we evaluated diet and physical activity in older children post-LTx, we do not have any information related to habitual physical activity prior to LTx or in children< 6 years. This is important to understand as the majority of children who receive LTx in North America are typically lower than 6 years of age. In addition, delays in muscle growth and development over prolonged periods of time in early childhood may contribute to gross motor and neurocognitive delays that may last throughout life. Hence, early identification of sarcopenia in infants and children with ESLD are critically important to ensure the development of effective treatment of pediatric sarcopenia in liver disease occurs.

5.4 Future Research

Thesis findings support that sarcopenia is highly prevalent in children with ESLD before and after LTx and associated with adverse clinical outcomes. One of the most important avenues of research that should be focused on is the development of a universal definition for pediatric sarcopenia. Considerations of parameters such as growth, gross motor and neurodevelopment may need to be included in sarcopenia definition in children. Puberty has a huge impact in body composition and gender differences in lean mass during growth spurts may affect the classification of low skeletal muscle mass. In

addition, gross motor and neurodevelopment are the important components as they will influence muscle strength and physical performance of a child. Although we comprehensively studied components of the adult definitions (SMM, measures of muscle strength and physical performance), there is little information regarding the best functional measures across different age groups to include in a definition of sarcopenia. There is also lack of age and gender-specific normative data for SMM, particularly in young children and infants. Specifically, to date, there is no SMM reference value for MRI and DEXA for younger children or infants. While some reference data are available for muscle strength/function tests (e.g. handgrip and six minute walk test), other tests such as sit to stand, push up, stair climb test are constrained by non-standardized protocols and normative data generated from a small sample size. Moreover, assessments of other important components that are applicable to the definition of sarcopenia in children (fatigue, health related quality of life) were not included in this study. Health related quality of life domains such as emotional, social and school functioning are worth to explore as these domains might be impacted by sarcopenia in children. This is important to establish to ensure that study findings are comparable across pediatric sarcopenia.

Future research should also include a focus on a) the underlying physiological characteristics of sarcopenia (currently in progress) and b) the development of effective rehabilitation strategies for the treatment and prevention of sarcopenia in children with ESLD. Resistance and aerobic exercises have been shown to be safe, feasible and beneficial in adults with ESLD and sarcopenia[92-96]. Physical exercise was found to increase muscle strength, muscle size and health-related quality of life in adult patients

with cirrhosis[92,93]. In children, there is no information regarding the efficacy of resistance or aerobic exercise on treatment of sarcopenia. However, there is evidence in overweight and obese children that resistance exercise may be beneficial at improving muscle mass and muscle strength[185-187]. One challenge in developing pediatric rehabilitation is long term adherence to interventions and access to health care services providing these services. This is particularly important in Alberta because the pediatric LTx program at the Stollery Children's Hospital covers the largest geographic catchment areas for LTx in North America. This challenge might be overcome with the use of home-based exercise and diet interventions that interface with the home and community settings. Consideration of the use of internet-based programming (e.g. use of applications [Apps]) within these settings might result in higher rates of adherence to lifestyle interventions, particularly if the entire family is involved in rehabilitation programming. Also, consideration of the components of physical literacy (motivation factors, self-efficacy and the ability to perform physical activity) is also critically important to address when developing home-based clinical trials in children. This could be done by the development of interactive web applications that include the opportunity for child-parent goal setting and intervention feedback. Principles of machine learning may also offer novel methods to develop web-based learning applications. Randomized clinical trials examining the efficacy of home-based rehabilitation programs are worthy in children with ESLD undergoing transplantation to ensure that children in remote communities without routine access to health care can receive treatment to prevent worsening of their clinical condition.

Development of effective rehabilitation programs should also address the potential dietary deficits facing by children and how this may impact risk for sarcopenia. Future research examining protein quality and sarcopenia may be important, as there is evidence that branched-chain amino acid requirement is higher in children pre-and-post-LTx when compared to healthy children[84,162]. Vitamin D is also another key nutrient that has been identified with sarcopenia risk in adults[188], but no association was found in this study. This was likely due to the high rates of vitamin D supplementation in the post-LTx children leading to optimal vitamin D status. Further examination of vitamin D status and sarcopenia is warranted in the pre-LTx period because the prevalence of vitamin D insufficiency in children with ESLD is substantial due to malabsorption and suboptimal vitamin D intake. While children are routinely supplemented with other fat soluble vitamin preparations (A, E and K), there is evidence that vitamin K in particular plays a role in lean body mass accumulation, muscle strength and physical performance in adults[189-191]. Vitamin K, in particular, is a nutrient at high risk for deficiency in children with ESLD and has been shown to have a synergistic effect on the absorption and utilization of vitamin D[192], and hence consideration of overall vitamin K status in children with sarcopenia is important.

5.5 Overall conclusions

Sarcopenia in children with ESLD is highly prevalent pre-and-post-LTx and is related to adverse clinical outcomes before and after LTx. While diet (energy and protein) appears to play a role in the early expression of sarcopenia in children with ESLD pre-LTx, more works need to be done to clearly establish the factors that influence sarcopenia expression before and after LTx. The longer term consequences of sarcopenia expression

post-LTx appear to be related to reduced muscle strength and participation in vigorous physical activity. All of these factors can influence health-related quality of life, overall growth, gross motor and neurocognitive development. Early identification of sarcopenia in children with chronic disease is warranted to prevent the progression of the disorder and to identify children who warrant additional rehabilitation support of an interdisciplinary health care team. This will ensure that children with sarcopenia can receive the needed therapies to prevent lifelong disability.

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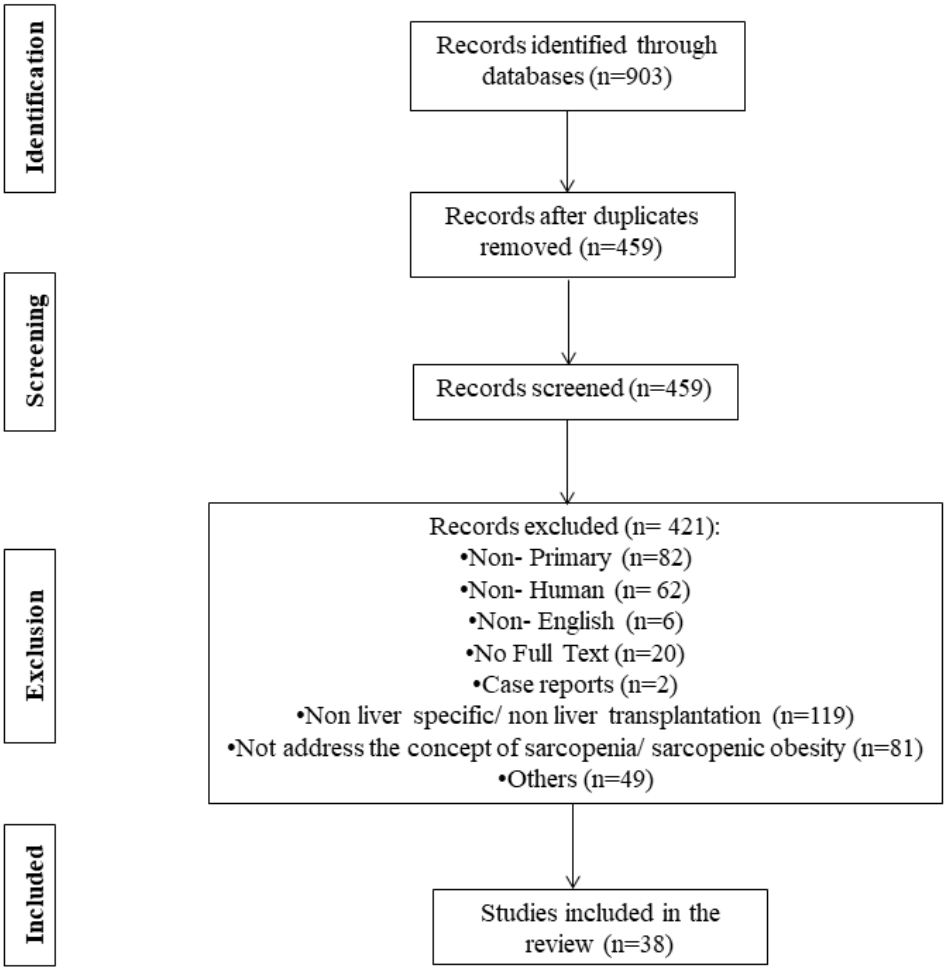
APPENDIX

Appendix A-1. Methods for literature search using a systematic approach

Databases	PubMed and Web of Science
Keywords	sarcopenia, sarcopenic obesity, muscle depletion, muscle loss, reduced skeletal muscle mass, low muscle mass, reduced muscle strength or muscle function, anthropometry, obesity, clinical outcome, outcomes, liver transplant, liver transplantation and one of: childhood, children, pediatric or adults



Appendix A-2: Flow chart of articles screened and reviewed based on the inclusion and exclusion of study methods.

APPENDIX B-1: Effect sizes (Cohen's *d* and Cohen's *h*) in adult and pediatric studies

	Mortality	Infection	LOS	ICU stay	Post-op complications	Ventilator dependency	Hospital cost
Adult studies							
Pre-LTx sarcopenia on waitlist outcomes	**No effect- medium** 1mo[e]: n=1 small (0.31)[33] 3mo: n=2 small (0.36±0.04)[28,33] 6mo: n=1 small (0.49)[28] 1 yr[f]: n=3 small-medium (0.41±0.23)[28,31,33] 2 yr: n=2 no effect-small (0.27±0.30)[31,36] 3 yr: n=2 no effect- small (0.27±0.17)[31,33]	**Small** n=1 (0.20)[19]	N/A	N/A	N/A	N/A	N/A
Pre-LTx sarcopenia on post-LTx outcomes	**No effect- large** 3mo: n=2 no effect- small (0.29±0.16)[5,29] 4 mo: n=1 medium (0.73)[23] 6 mo: n=2 no effect- small (0.26±0.30)[5,29] 1 yr: n=7 no effect-large (0.38±0.35)[14,16,20-22,24,29] 2 yr: n=1 no effect (0.09)[40] 3 yr: n=4 no effect-large (0.45±0.43)[8,16,20,39] 5 yr: n=8 no effect-large (0.43±0.29)[8,13,16,21,22,29,37,40]	**No effect-large** n=1 no effect (0.09)[16] n=3 small (0.33±0.12)[5,8,29] n=1 medium (0.60)[21] n=1 large (1.12)[37]	**No effect-large** n=2 no effect (0.04±0.03)[14,21] n=2 small (0.25±0.05)[16,34] n=1 medium (0.52)[5] n=2 large (3.20±1.47)[25,29]	**Small-large** n=1 small (0.34)[16] n=2 medium (0.59±0.08)[2,1,25] n=1 large (3.79)[29]	**No effect-medium** n=2 no effect (0.15±0.06)[16,21] n=3 medium (0.58±0.06)[5,32,37]	**Small-medium** n=1 small (0.37)[16] n=1 medium (0.51)[21]	**Small** n=1 small (0.44)[34]
Pre-LTx SO on post LTx outcomes	**Small-large** 1 yr: n=3 small-large (0.65±0.75)[6,7,39] 3 yr: n=2 small (0.27±0.04)[6,39] 5 yr: n=4 small-large (0.68±0.82)[6,7,37,39] 10 yr: n=1 small (0.27)[6]	N/A	N/A	N/A	N/A	N/A	N/A
Pediatric studies							
Post-LTx sarcopenia on post-LTx outcomes	N/A	N/A	**Large** n=1 large (4.70)[11]	**Large** n=1 large (3.54)[11]	N/A	**Large** n=1 large (2.44)[11]	N/A

Cohen's *d* effect size was calculated using mean difference between groups and divided by the pooled standard deviation. Cohen's *h* effect size was calculated in studies that reported proportion with formula 2arcsin √P1 - 2arcsin √P2. The effect size of ≤0.1 was classified as no effect, 0.2 to 0.4 as small effect, 0.5 to 0.7 as medium effect and ≥0.8 as large effect. Effect sizes calculation was based on the available data. LOS: Length of stay, ICU: Intensive care unit, Post-op: Post-operative, LTx: Liver transplantation, mo: Month, yr: Year, N/A: Not available, SO: Sarcopenic Obesity. There were no statistical significant in pre-LTx sarcopenia and pre-LTx SO on 1yr (p=0.72), 3yr (p= 0.22) and 5 yr (p=0.57) mortality using Chi Square test. Pediatric study (Mager et al., 2018)[11]: Effect size for number of hospital readmission: 3.0 (large), number of emergency visit: 0.41 (small), total LOS for readmission: 6.86 (large), height velocity: 1.51 (large), weight velocity: 3.15 (large).

Appendix C-1: Availability of variables during different time points.

Time points/ Variables	**Pre-LTx**	**Perioperative**			**Post-LTx**	
	LTx assessment	*At the time of LTx*	*ICU discharge*	*Hospital discharge*	*6 month post-LTx*	*1 year post-LTx*
Body composition data from MRI/CT	✓					
Anthropometric data	✓	✓	✓	✓	✓	✓
Biochemical data	✓		✓	✓	✓	✓
Nutrition data	✓		✓			
Neurocognitive data	✓					
Medications	✓		✓	✓	✓	✓
LOS (total post-LTx hospital/ICU)		✓(ICU LOS)				
		✓(Total post-LTx hospital LOS)				
Hospitalization data*	✓			✓		
Growth data						
• Absolute weight (g/day)/height gain (mm/day)	✓					
		✓				
					✓	
• Growth velocity standard deviation score		✓				
					✓	
Complications						
• Ascites	✓	✓				
				✓		
					✓	
• Infections		✓				
				✓		
					✓	
• Rejection		✓				
				✓		
					✓	
• Encephalopathy	✓					

• Biliary		✓
• Vascular		✓

*Hospitalization data referred to number of inpatient admission, outpatient visit, emergency visits and inpatient length of stay. LTx: Liver transplantation, MRI: Magnetic resonance imaging, CT: Computed tomography, ICU: Intensive care unit, LOS: Length of stay

Appendix C-2 Skeletal muscles and adipose tissues in end-stage liver disease and healthy children at fourth lumbar level (L4).

Variables	≤2 years			>2 years			Overall group p-value
	ESLD (n=17)	HC (n=15)	p-value	ESLD (n=6)	HC (n=9)	p-value	
SKELETAL MUSCLE							
Psoas muscle (cm^2)	2.9 (2.4, 3.9)	3.7 (3.0, 4.6)	0.20	8.8 (6.9, 11.8)^	16.7 (8.8, 25.0)*	**0.007**	**0.05**
Psoas muscle index (cm^2/m^2)	7.6 (6.2, 9.4)	8.2 (7.8, 9.6)	0.20	6.3 (5.9, 6.7)	7.4 (6.6, 8.9)	**0.03**	0.17
Abdominal wall muscle (cm^2)	11.4 (10.5, 13.8)	10.3 (9.3, 11.7)	0.19	23.7 (20.4, 31.8)^	54.6 (24.1, 59.4)*	**0.004**	0.08
Abdominal wall muscle index (cm^2/m^2)	26.8 (25.2, 35.0)	27.3 (22.3, 29.9)	0.65	16.8 (14.7, 18.4)^	22.3 (20.0, 23.2)*	**0.02**	0.71
Paraspinal muscle (cm^2)	5.2 (4.4, 8.4)	6.6 (5.5, 8.3)	0.23	24.6 (15.6, 34.0)^	33.4 (21.2, 52.9)*	**0.01**	0.08
Paraspinal muscle index (cm^2/m^2)	13.6 (11.8, 15.8)	15.3 (14.1, 19.8)	0.10	15.9 (14.1, 16.7)	18.2 (16.0, 19.7)	**0.006**	**0.02**
Total SMM (cm^2)	20.0 (18.2, 25.0)	20.8 (18.2, 23.4)	0.82	58.6 (42.8, 74.5)^	104.7 (50.9, 136.4)*	0.11	0.07
Total SMM index (cm^2/m^2)	45.8 (42.3, 64.9)	50.1 (45.6, 55.5)	0.64	39.1 (37.2, 40.4)^	46.5 (43.5, 52.2)	**0.008**	0.49
ADIPOSE TISSUES							
VAT (cm^2)	2.8 (1.7, 5.4)	3.5 (2.4, 4.9)	0.72	9.4 (5.0, 10.9)^	22.1 (5.5, 25.7)*	0.44	0.11
VAT index (cm^2/m^2)	7.4 (3.9, 10.2)	7.3 (6.1, 12.2)	0.70	5.1 (3.9, 8.0)	7.0 (4.1, 10.7)	0.44	0.87

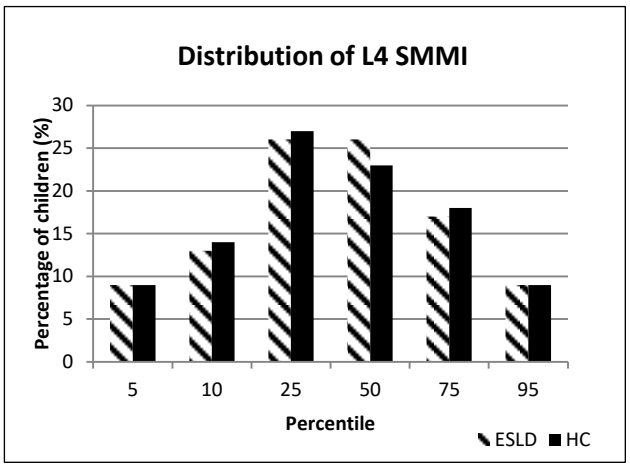
SAT (cm^2)	19.3 (12.0, 23.2)	31.7 (24.2, 55.0)	**0.003**	36.0 (27.7, 39.2)^	82.3 (73.4, 196.8)*	**0.01**	**0.005**
SAT index (cm^2/m^2)	37.4 (30.2, 67.2)	72.9 (57.9, 105.4)	**0.004**	21.2 (16.6, 23.1)^	61.8 (36.0, 82.0)	**0.02**	**0.002**
TAT (cm^2)	23.1 (14.2, 27.7)	34.2 (26.0, 61.8)	**0.008**	44.3 (33.2, 51.2)^	109.2 (79.3, 224.1)*	**0.02**	**0.006**
TAT index (cm^2/m^2)	47.8 (36.4, 75.9)	79.6 (62.5, 112.1)	**0.007**	28.1 (26.1, 29.2)^	68.5 (40.5. 93.3)	**0.02**	**0.006**

(^) indicated significant difference between ESLD ≤2 years vs >2 years. (*) indicated significant difference between HC ≤2 years vs >2 years. Overall group p-value referred to ESLD vs HC groups difference without age and gender effects.

Index: Skeletal muscle/ adipose tissues area corrected for height= area (cm^2)/height (m^2). Image at L4 available for n=23 ESLD and n=24 HC. Height available for n=23 ESLD and n=22 HC.

Total skeletal muscle mass referred to sum of psoas, abdominal wall muscle (rectus abdominus, internal, external oblique, transverse abdominus) and paraspinal muscles (quadratus lumborum and erector spinae). Total adipose tissues referred to sum of IMAT, VAT and SAT. ESLD: End stage liver disease, HC: Healthy control, SMM: Skeletal muscle index, IMAT: Intermuscular adipose tissues, VAT: Visceral adipose tissues, SAT: Subcutaneous adipose tissue, TAT: Total adipose tissues. Data expressed in median (IQR). P-values ≤0.05 is considered statistically significant.

(A)



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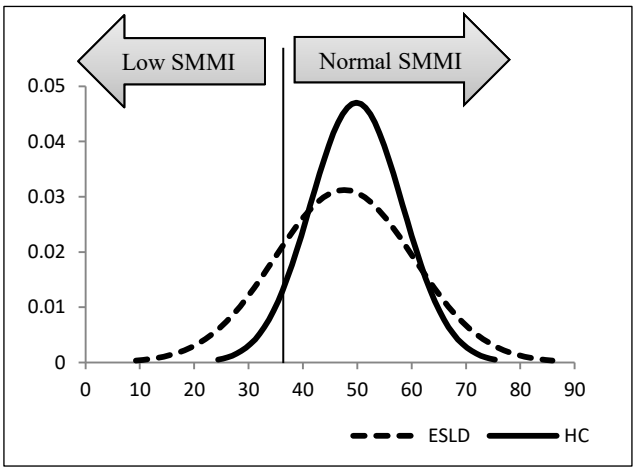
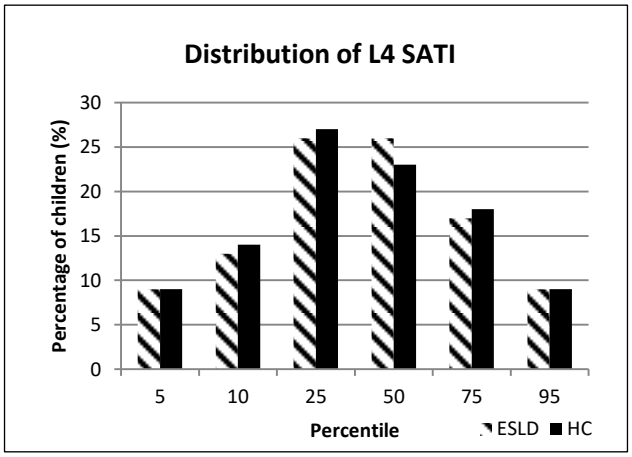
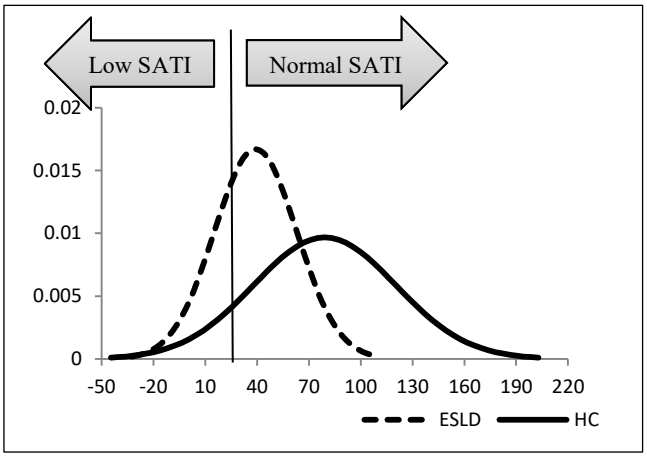


(C)

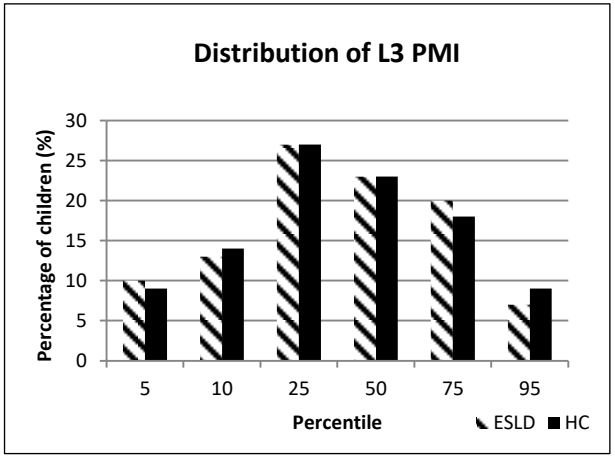


(D)

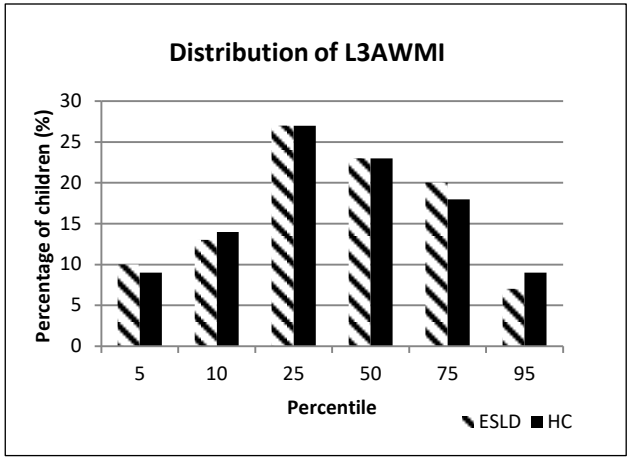


Appendix C-3 Distribution of skeletal muscle mass index (A & B) and subcutaneous adipose tissues index (C & D) at L4 in end stage liver disease children and healthy controls. The cut off for low skeletal muscle mass index is 37.80 cm^2/m^2 (z score<-2) and subcutaneous adipose tissues index is 26.02 cm^2/m^2 (z score <-1.5). ESLD: End stage liver disease, HC: Healthy control, SMMI: Skeletal muscle mass index, SATI: Subcutaneous adipose tissues index.

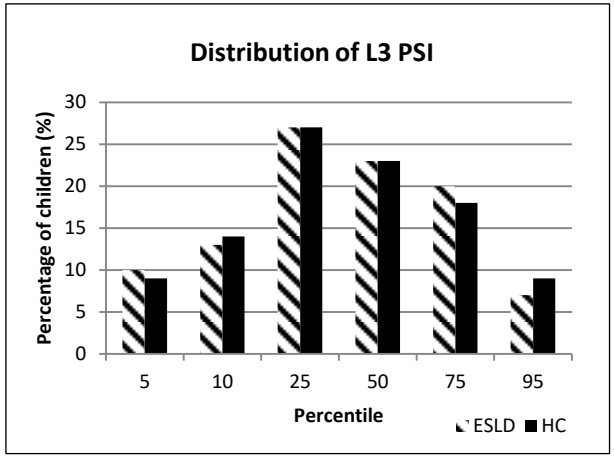
(A)


Distribution of L3 PMI
Percentage of children (%)
30
25
20
15
10
5
0
5
10
25
50
75
95
Percentile
ESLD
HC

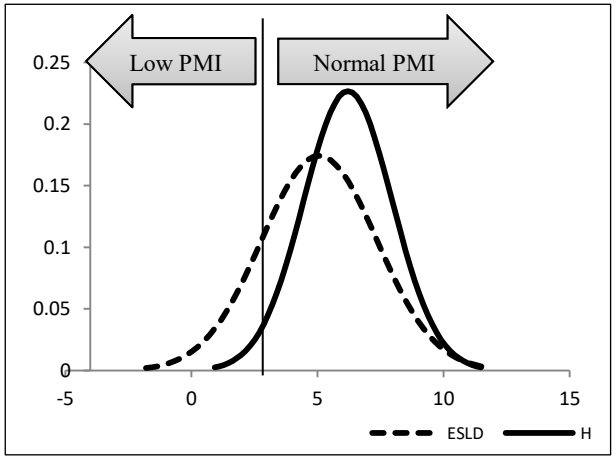
(C)


Distribution of L3AWMI
Percentage of children (%)
30
25
20
15
10
5
0
5
10
25
50
75
95
Percentile
ESLD
HC

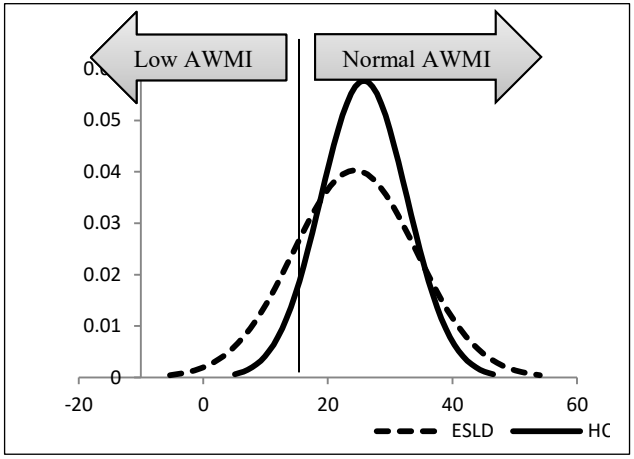
(E)


Distribution of L3 PSI
Percentage of children (%)
30
25
20
15
10
5
0
5
10
25
50
75
95
Percentile
ESLD
HC

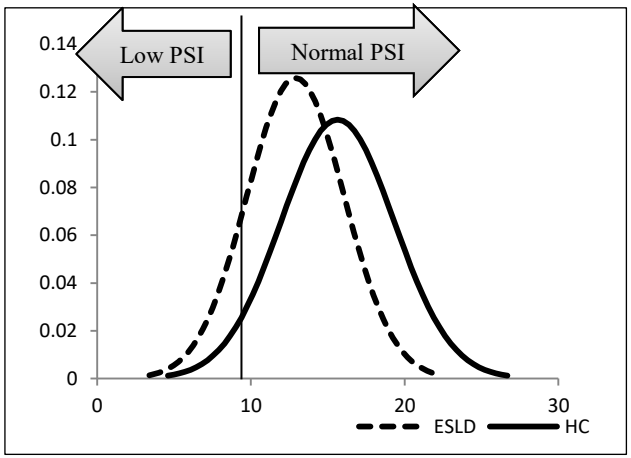
(B)


Low PMI
Normal PMI
0.25
0.2
0.15
0.1
0.05
0
-5
0
5
10
15
ESLD
H

(D)

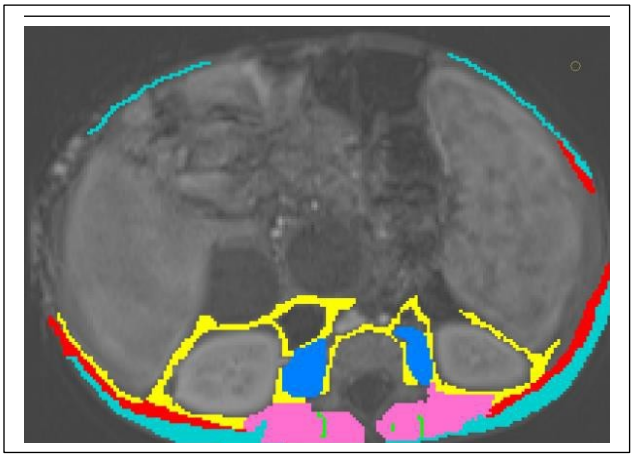

Low AWMI
Normal AWMI
0.05
0.04
0.03
0.02
0.01
0
-20
0
20
40
60
ESLD
HC

(F)

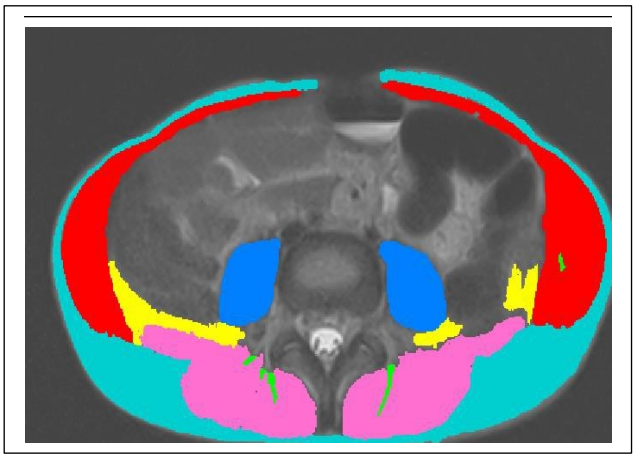

Low PSI
Normal PSI
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
10
20
30
ESLD
HC

Appendix C-4 Distribution of psoas (A&B), abdominal wall muscle (C&D) and paraspinal (E&F) muscle index at L3 in end stage liver disease children and healthy controls. The cut off for psoas muscle index is 3.64 cm^2/m^2 (z score<-2), abdominal wall muscle index is 18.01 cm^2/m^2 (z score <-2) and paraspinal muscle index is 9.93 cm^2/m^2 (z score<-2). ESLD: End stage liver disease, HC: Healthy control, PMI: Psoas muscle index, AWMI: Abdominal wall muscle index, PSI: Paraspinal muscle index.

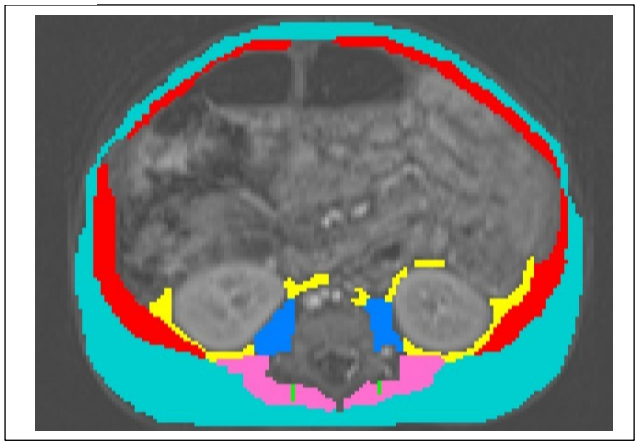
(A)



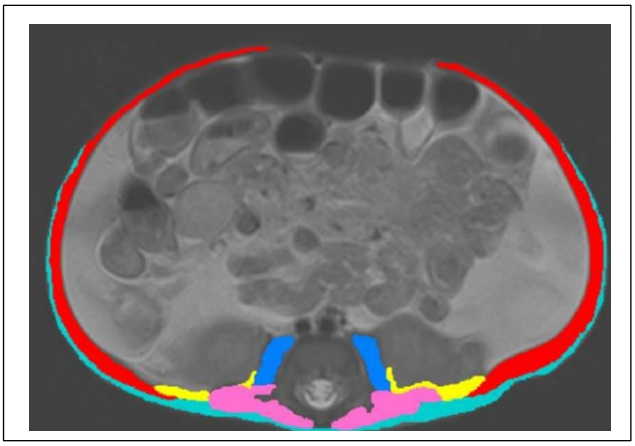
(B)



(C)



(D)



Appendix C-5 Phenotypes related to skeletal muscle mass and adiposity in infants and children with end stage liver disease in MRI images at fourth lumbar (L4) vertebrate. (A) Low SMMI + low SATI, (B) normal SMMI + normal SATI, (C) low SMMI+ normal SATI, (D) normal SMMI+ low SATI. SMM: Sum of *red* (Abdominal wall muscle: rectus abdominus, internal and external obliques and transversus abdominus), *blue* (psoas muscle) and *pink* (paraspinal muscle) surface area. SAT: *cyan* surface area. SMMI=SMM/height2, SATI= SAT/height2. SMM: Skeletal muscle mass, SAT: Subcutaneous adipose tissues, SMMI: Skeletal muscle mass index, SATI: Subcutaneous adipose tissue index.

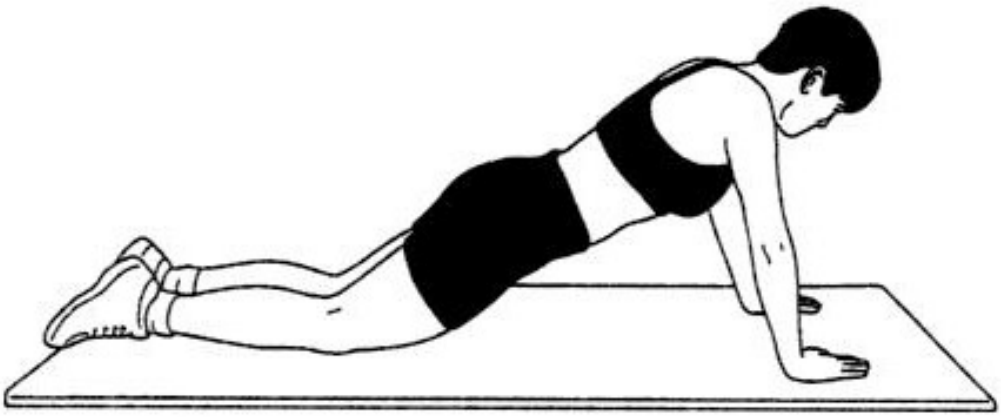
Appendix C-6 Sarcopenia and low subcutaneous adiposity derived from L4 and clinical outcomes in ESLD children.

Variables	Sarcopenia	Non-Sarcopenia	p-value	Low SATI	Normal SATI	p-value
Growth						
- Weight gain (g/day)	19.1 (5.6, 32.6)	9.8 (3.6, 13.5)	0.12	12.9 (-0.5, 18.2)	9.2 (4.8, 13.0)	0.21
- Height gain (mm/day)	0.25 (0.2, 0.3)	0.31 (0.2, 0.4)	0.86	0.17 (0.05, 0.3)	0 (0.2, 0.4)	**0.04**
- Weight SDS	1.6 (-0.3, 2.1)	1.0 (-0.5, 2.4)	0.88	0.9 (-1.2, 2.2)	0.9 (-0.3, 2.2)	0.89
- Height SDS	1.4 (-1.1, 2.8)	0.03 (-0.9, 1.1)	0.61	0.9 (0.3, 2.7)	-0.18 (-1.8, 0.9)	0.04
Developmental assessment						
- Communication	97 (78, 105)	100 (91, 112)	0.60	98.5 (89, 100)	100 (89, 115)	0.51
- Daily living skill	100 (100,100)	89.5 (85,94)	0.20	94 (88, 97)	92.5 (88, 100)	0.91
- Motor skill	85 (83,111)	97.5 (83.5, 103)	0.83	84 (80, 91)	103 (84, 106)	0.06
- Socialization	95 (86, 104)	100 (96.5, 106)	0.48	97 (97, 100)	100 (96, 104)	0.74
- ABC	90 (85, 106)	95.5 (89, 75)	0.67	88 (84, 93)	98 (89, 107)	0.19
Length of inpatient stays (days)						
- Pre-LTx	5 (2,31)	16 (0 , 39)	0.41	9 (0, 21)	9 (0 , 39)	0.99
- Post-LTx-Hospital D/C	26 (25, 56)	40 (24, 71)	0.96	5 (0, 27)	5 (1, 18)	0.75
ICU length of stay (days)	8 (4,40)	6 (5,7)	0.57	8 (4, 12)	6 (4, 29)	0.84
Ventilator days	3.5 (2, 19)	0 (0, 0.5)	**0.02**	1 (0, 5)	3 (1,19)	0.25
Number of infections						
- Fungal	0 (0,0)	0 (0. 0)	0.12	0 (0, 0)	0 (0, 0)	0.39
- Viral	0 (0,1)	1 (0, 2)	0.33	1 (0, 2)	1 (0, 2)	0.46
- Bacteria	0 (0,1)	0 (0, 1)	0.56	0 (0, 1)	0 (0, 1)	0.61
- Total	0.5 (0, 2)	2 (0, 3)	0.18	1 (0, 3)	1, (0, 3)	0.44
Nutritional intake						
- Energy (kcal/kg)	77 (55, 88)	79 (69, 106)	0.44	76 (49, 123)	99 (97, 106)	0.28
- Protein (g/kg)	1.5 (1.4, 2.6)	2.1 (1.9, 2.9)	0.23	2.1 (1,7, 3.2)	2.9 (2.1, 3.3)	0.49

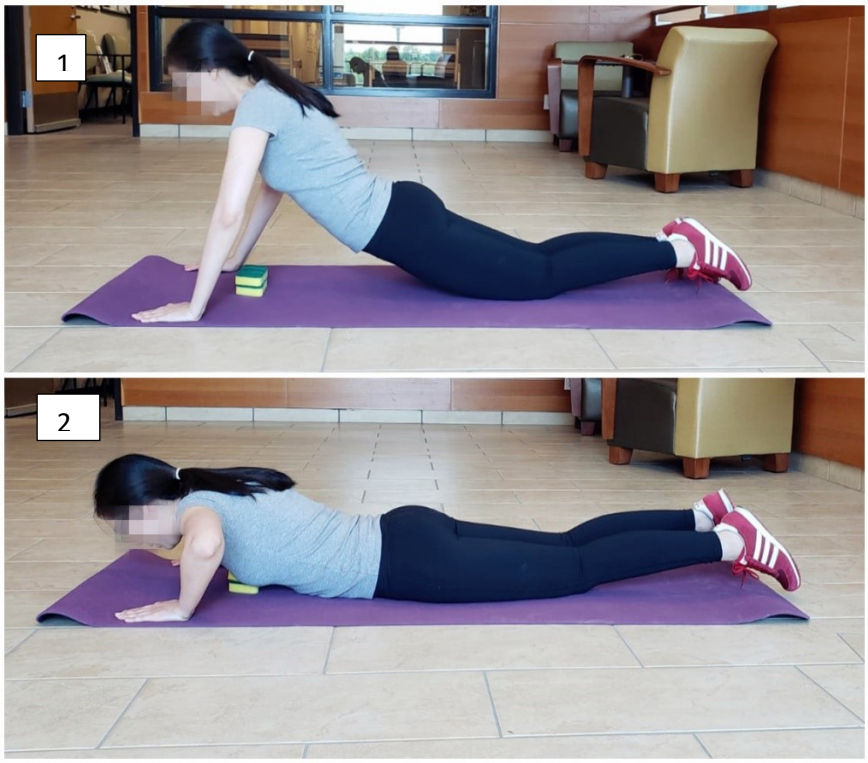
Sarcopenia is defined by skeletal muscle mass index<-2 z-score and low SATI is defined by SATI<-1.5 z-score derived from healthy controls at L4 vertebrate level. Weight and height SDS was calculated based on based on published reference data[107]. Developmental assessment was based on Vineland Adaptive Behavior Scales-II performed at liver transplant assessment was for LTx children <7 years[109].

SATI: Subcutaneous adipose tissues index, SDS: Standard deviation scores, ABC: Adaptive behaviour composite, LTx: Liver transplantation, D/C: Discharge, ICU: Intensive care unit.

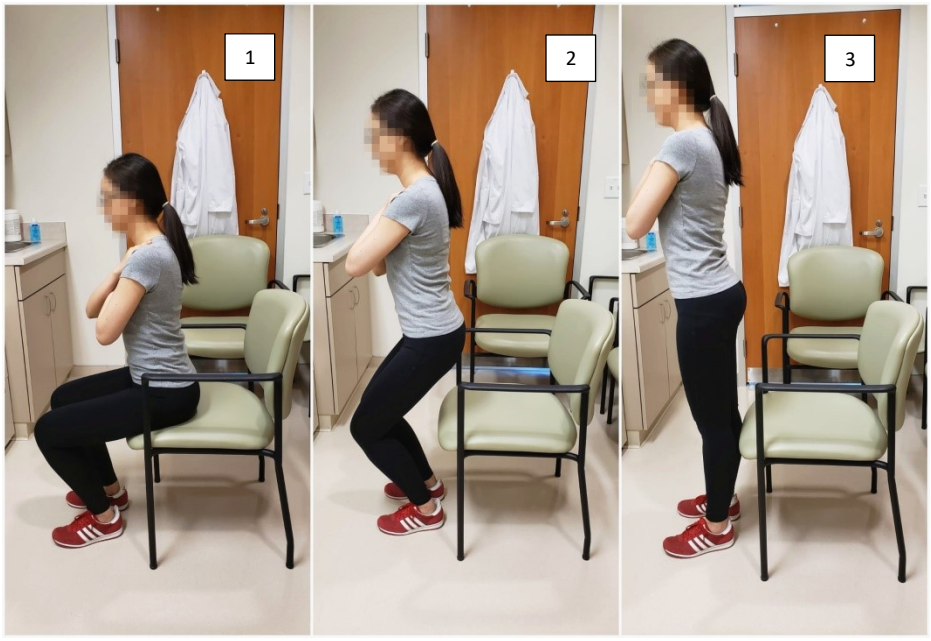
Data expressed in median (IQR). P-values ≤0.05 is considered statistically significant.



Appendix D-1 A standardized picture of knee bent push up that was showed to participants.



Appendix D-2 Knee bent push up: up position (1), down position (2).



Appendix D-3. Sit to stand test.

Appendix D-4. Sit to stand test. Inclusion of stool (height 20cm) for younger children who were unable to perform sit to stand test with their feet position directly on the floor.

Appendix D-5. Indices of lean body mass and adipose tissue as measured by dual-energy x-ray absorptiometry (DXA) in children post-liver transplantation.

Variables	Total LTx cohort with DXA reports (n=21)	Sarcopenia (n=7)	Non-sarcopenia (n=14)	p-value
% Fat free mass				
Arm	66.59 ± 9.35	68.25 ± 11.13	65.76 ± 8.67	0.58
Legs	63.58 ± 7.75	65.18 ± 7.11	62.78 ± 8.19	0.52
Trunk	74.57 ± 6.18	75.96 ± 6.02	73.88 ± 6.37	0.48
Android	69.68 ± 7.43	72.08 ± 6.38	68.48 ± 7.84	0.31
Gynoid	65.07 ± 7.12	66.22 ± 7.57	64.49 ± 7.11	0.61
Whole body	69.93 ± 6.36	71.55 ± 5.90	69.12 ± 6.63	0.42
Lean indices				
Lean/height^2 (kg/m^2)	12.60 (11.80, 14.60)	12.50 (11.40, 13.70)	13.95 (11.80,15.00)	0.41
Lean-height z-score	-0.20 (-0.90, 0.18)	-0.80 (-0.90, 0.10)	-0.10 (-0.75, 0.20)	0.57
Appendicular lean/height^2 (kg/m^2)	5.66 ± 1.15	5.33 ± 1.23	5.83 ± 1.12	0.36
Appendicular lean height^2-z score	-0.62 ± 0.82	-0.85 ± 0.35	-0.50 ± 0.97	0.41
SMM	17.44 ± 8.92	15.98 ± 9.00	18.18 ± 9.12	0.61
SMM-z score	-0.91 ± 0.89	-1.05 ± 0.53	-0.85 ± 1.04	0.64
Adipose indices				
Total body fat %	30.08 ± 6.36	28.44 ± 5.89	30.90 ± 6.64	0.42
Total body fat % T-score	-0.03 ± 1.07	0.29 ± 0.84	-0.19 ± 1.16	0.35
Android/gynoid ratio	0.87 ± 0.14	0.83 ± 0.12	0.89 ± 0.15	0.38
% Fat trunk/ %Fat legs	0.70 ± 0.07	0.69 ± 0.06	0.70 ± 0.08	0.61
Trunk/limb fat mass ratio	0.69 ± 0.09	0.71 ± 0.07	0.68 ± 0.10	0.49
Trunk/limb fat mass ratio T-score	-1.26 ± 0.69	-1.67 ± 0.82	-1.06 ± 0.55	0.05
FMI/ LMI indices				
FMI	6.22 ± 1.99	5.52 ± 1.64	6.57 ± 2.11	0.27
FMI >75th (%)	5	0	8	0.68
LMI	12.56 (11.84, 14.66)	12.26 (11.53, 13.72)	14.07 (11.84,14.99)	0.41
LMI<5th (%)	0	0	0	.

Body composition data available for n=21 LTx participants.

Sarcopenia refers to probable sarcopenia, defined as low muscle strength (low hand-grip/ sit-to-stand/ push-ups) based on revised European consensus on sarcopenia 2019[4].
SMM calculated from healthy children normative data from Weber et al., 2012[139].
FMI/LMI calculated from fat mass and lean mass measured from DXA and percentiles from age-specific and sex-specific reference ranges from Weber et al., 2013[193].
LTx: Liver transplantation, SMM: Skeletal muscle mass, FMI: Fat mass index; LMI: lean mass index
Data expressed in mean±SD or median (IQR) or %. P-values ≤0.05 is considered statistically significant.

Appendix D-6. Major muscle groups and components tested in muscle function tests

Muscle tests	Components tested	Major muscle groups involved
Upper limb		
Handgrip	Strength	Flexor and extensor muscles of forearm
Push up	Strength and muscular endurance	Pectoral, triceps and deltoids muscles
Lower limb		
Sit to stand	Strength and power	Quadriceps, hamstrings and core muscles
Stair climb test	Power and balance	Quadriceps, hamstrings, gluteal muscles, hip flexors and calf muscles
6MWT	Cardiorespiratory Endurance	Quadriceps, hamstrings, gluteal muscles and calf muscles