# Influence of diet on cardiometabolic dysregulation and sarcopenia in children after liver transplantation

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Nutrition and Metabolism

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## Abstract

**Background/Aim:** Sarcopenia is a muscle disease involving reduced skeletal muscle mass and decreased muscle strength and physical performance. Recent evidence has shown high prevalence of sarcopenia in children with end-stage liver disease. Pediatric transplant populations are also at risk of suffering from complications related to cardiometabolic dysregulation (CMD). Mechanisms such as insulin resistance, myosteatosis, oxidative stress and inflammation are present in sarcopenia and CMD. Dietary patterns, such as high consumption of carbohydrates, fat, and saturated fat as well as low intake of protein, antioxidant, and anti-inflammatory micronutrients, have been linked to sarcopenia and cardiometabolic risk. The study aims were to assess the association between diet and the prevalence of sarcopenia and CMD in children after liver transplant (LTx).

**Methods:** Secondary analysis of a cross-sectional study in children after LTx (n=22) and age-matched healthy controls (HC, n=47) between ages of 6-18 years was performed. Dietary intake was assessed for both groups using 3-day food records. Sarcopenia was determined using modified adult consensus definition based on the European Consensus Statement for Sarcopenia, and CMD was defined by three different pediatric definitions (modified Adult Treatment Panel-III, World Health Organization and Magnussen et al. [2010]) for children after liver transplant.

**Results:** Sarcopenia was present in 36% of LTx population, while prevalence of CMD ranged from 4.5-50% according to the different criteria. Patients with CMD had significantly higher calcium intake (p=0.05). The sarcopenia group had significantly higher protein (p=0.04), lower fat (p=0.02) and PUFA intake (p=0.02).

**Conclusions:** Children who underwent LTx are at risk of developing sarcopenia and cardiometabolic alterations. Additional research exploring the relationship between dietary intake, cardiometabolic dysregulation and sarcopenia is warranted.

# Preface

This thesis is a secondary data analysis of a previous project performed by Dr Diana Mager's group. The primary research project was approved by the Human Ethics Board, University of Alberta (Pro00076244).

This thesis is an original work by Rocio Ayala Romero. No part of this thesis has been previously published. Rocio Ayala Romero: secondary statistical data analysis/interpretation and thesis writing under the supervision of Dr Diana R Mager. Poh Hwa Ooi: responsible for subject recruitment, data collection, data analysis related to sarcopenia diagnosis in participants, conducting all study visits. Ashley Wilmott RA: data auditing, data input and organization of data spreadsheet for RAR analysis. Vera C Mazurak: on supervisory committee, review/approved thesis. Susan M Gilmour: on supervisory committee, review/approved thesis. Diana R Mager (supervisor): study design, data collection, data analysis and interpretation, thesis preparation for Ooi PH and Ayala RA. Funding for original project was supported by Vitamin Fund Graduate Student Award, University of Alberta (PHO).

# Dedication

To

**Antonio Romero Montoya**

I want to dedicate this thesis to my uncle, Antonio. We lost you and life will not be the same. You are gone, but I see you in everything. I love you.

**My parents, Isaias and Rosa Icela, and my siblings, Isamar and Isaias**

Thank you for always saying "yes" whenever I want to move far from you. I would not be here if it were not for you. I love you immensely.

**Nana and Tata**

Thank you for showing me your love and support even in distance. Thank you for always telling me you are proud of me. I miss you.

**Ariel Contreras Orrego**

My best friend and my love. You have helped me in a million ways. Thank you for keeping up with my stress. Thank you for taking care of me.

**My friends**

Cristina, Dannitza, Ana, Rogelio, Martha, Ceci and Jose: thank you for making me laugh, for supporting me and encouraging me. You are spark in my life.

**My Chilean-Canadian family**

Pabla, Carlos and Tony: thank you for adopting me, helping me, taking care of me. I am so grateful to have you in my life. My life has been happier since I met you.

# Acknowledgements

My graduate program has been challenging and unique in many ways. I had the opportunity to meet brave children, loving parents, and a healthcare team who is both smart and compassionate.

I want to thank my supervisor Dr Diana Mager, for giving me the opportunity to work under her supervision and guiding me through every step. Thank you for your encouragement and support throughout my program. Thank you to Dr Susan Gilmour and Dr Vera Mazurak who always had kind and constructive opinions to make my work better.

Thank you to every person in Dr Mager's lab: Samantha Cyrkot, Chelsea Kamprath, Amber Hager, Andrea Razcon, Amanda Liu, Kelsey Gordulic, Christine Lirette and Ashley Wilmott. I learned a lot from working with all of you. My gratitude to Poh Hwa (Rachel) Ooi and Abha Dunichand-Hoedl, who very patiently taught me what I know body composition analysis through MRI/CT scans. My sincere gratitude to the liver transplantation team, especially nurse coordinators Cindy Dziwenko-Cox and Cynthia Guavreau with whom I had the opportunity to work closely. Thank you to Dr Jason Yap for his assistance in patient recruitment.

Thank you to the Consejo Nacional de Ciencia y Tecnologia (CONACYT) for providing me with the financial support needed during my program. I also want to acknowledge the Canadian Liver Foundation and CDTRP (Canadian National Transplant program) for providing financial support to studies I was involved with.

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# List of Abbreviations (alphabetical order)

1RM; one-repetition maximum strength test

ADP: air displacement plethysmography

ALD: Acute liver disease

AMDR: Acceptable Macronutrient Distribution Range

ANGCY: Alberta Nutrition Guidelines for Children and Youth

ASM: appendicular skeletal muscle mass

ATP-III: Adult Treatment Panel III

BA: Biliary atresia

BIA; bioelectrical impedance analysis

BMI; body mass index

BP: blood pressure

CMR; cardiometabolic risk

CRP; C-reactive protein

CST: Corticosteroid

CT; computed tomography

CVD; cardiovascular disease

DBP: diastolic blood pressure

DBP-z: diastolic blood pressure z-score

DM; diabetes mellitus

DRIs: Daily Reference Intakes

DXA; dual-energy X-ray absorptiometry

EAR: Estimated Average Requirement

ESLD; end-stage liver disease

EWSOP2; European Working Group on Sarcopenia in Older People

FAC: flexed arm circumference

FFM; fat-free mass

FFMI: fat-free mass index

FFMI-z: fat free mass index z-score

FM: fat mass

HbA1c: hemoglobin A1c

HC: healthy controls

HDL; high-density lipoprotein

ht-z; height z-score

HU; Hounsfield units

ICU; intensive care unit

IMAT; intramuscular fat

IMET; intermuscular fat

INR: international normalized ratio

IR: insulin resistance

IRS-1; insulin receptor substrate 1

LDL; low-density lipoprotein

LOS; length of stay

LTx; liver transplantation

MAC: midarm circumference

MAC-z: midarm circumference z-score

MELD: Model End-stage Liver Disease

MMF: mycophenolate mofetil

MQ; muscle quality

MRI; magnetic resonance imaging

MS; muscle strength

MyHC; myosin heavy chain

Omega-3: n-3 polyunsaturated fatty acids

Omega-6: n-6 polyunsaturated fatty acids

PELD: Pediatric End-stage Liver Disease

Post-LTx; post liver transplant

PP; physical performance

Pre-LTx : pre liver transplant

PTT: partial thromboplastin time

RA; rectus abdominis

RDA: Recommended Daily Allowance

RN: reactive nitrogen species

ROS: reactive oxygen species

SBP: systolic blood pressure

SBP-z: systolic blood pressure z-score

SF; saturated fat

SMM; skeletal muscle mass

SMM-z: skeletal muscle mass z-score

TG; triglyceride

Tricep-z: tricep z-score

VLDL: very-low density lipoprotein

WC: waist circumference

WC-z: waist circumference z-score

WHO: World Health Organization

wt-z; weight z-score

**Publications not related to MSc thesis**

- Ooi PH, Mazurak V, Bhargava R, Dunichand-Hoedl A, **Ayala Romero R**, Gilmour SM, Yap JK, Mager DR. Sarcopenia and reduced subcutaneous adiposity in children with liver disease are associated with adverse outcomes. *Journal of Parenteral and Enteral Nutrition.* 2020, DOI: 10.1002/jpen.1963

**Abstract presentations**

- Mager DR (presenter), Ooi PH, **Ayala Romero R**, Mazurak V, Bhargava R,Yap JK, Gilmour SM. Does sarcopenia in children with end-stage liver disease impact post-operative clinical outcomes? Clinical Nutrition Week, American Society of Parenteral and Enteral Nutrition Annual Meeting, Tampa Florida. March 27-30,2020 Accepted for oral presentation (International Abstract of Distinction)
- **Ayala Romero R**, Ooi PH, Mazurak V, Simonesk K, Bhargava R, Gilmour SM, Mager DR. Somatotype measures may be useful in predicting deficits in muscle strength and physical performance in children who have undergone liver transplantation (LTx). Presented at the Canadian Nutrition Society meeting, May 2020.
- Mager DR (presenter), Dunichand-Hoedl A, Mazurak V, Dajani K, Ooi PH, **Ayala Romero R**, Shapiro J, Bigam D, Kneteman, NM, Noga M, Montano-Loza A, Yap J, Gilmour SM. Histological characterization of skeletal muscle in children with end-stage liver disease and myopenia at time of liver transplantation. Presented at the Canadian Society of Transplantation Fall Virtual Forum, November 2020

# Chapter 1: Literature Review

**This chapter includes a figure that has been published in Medical Journal of Australia[1]. This was used with permission from the publisher.**

## 1.1 Introduction

Sarcopenia is the loss of skeletal muscle mass and function accompanying aging and chronic illness.[2] The European Working Group on Sarcopenia in Older People (EWGSOP2) defines sarcopenia as a progressive muscle disease presenting as decreased skeletal muscle mass (SMM) and muscle quality (MQ), associated with reduced muscle strength (MS) and physical performance (PP).[3] When low MS is detected, sarcopenia is considered probable.[3] Sarcopenia is confirmed by low muscle quality or quantity.[3] Muscle quality is defined as muscle strength or power per unit of muscle mass.[4] It is considered severe when low MS, decreased muscle quality or SMM, and low PP concurrently present (**Figure 1.1**).[3] Sarcopenia is a component of malnutrition characterized by skeletal muscle mass decrease and function and is correlated with physical disability.[3] Sarcopenia appears in people with all body habitus spanning from underweight to obese.[3, 5] In some chronic conditions, there may be loss of muscle mass and increased weakness, while fat mass is preserved or increased.[6] This condition is known as sarcopenic obesity. [6] To date, no consensus exists on the definition of pediatric sarcopenia. The major differences between the adult and pediatric definition of sarcopenia include the importance of assessing and defining body composition cut-offs that consider the individual's age, as children are growing individuals.[7] Muscle function tests should consider stability, purposeful movements, and coordination in small infants.[7]

Sarcopenia has mostly been identified as a geriatric disease due to its association to age-related factors such as hormonal changes, oxidative stress, neuromuscular

degeneration, and decreased muscle protein turnover.[4] However, recent studies reported sarcopenia in younger populations.[5, 6] Sarcopenia can appear acutely or as a result of chronic illness, such as end-stage liver disease (ESLD).[7]

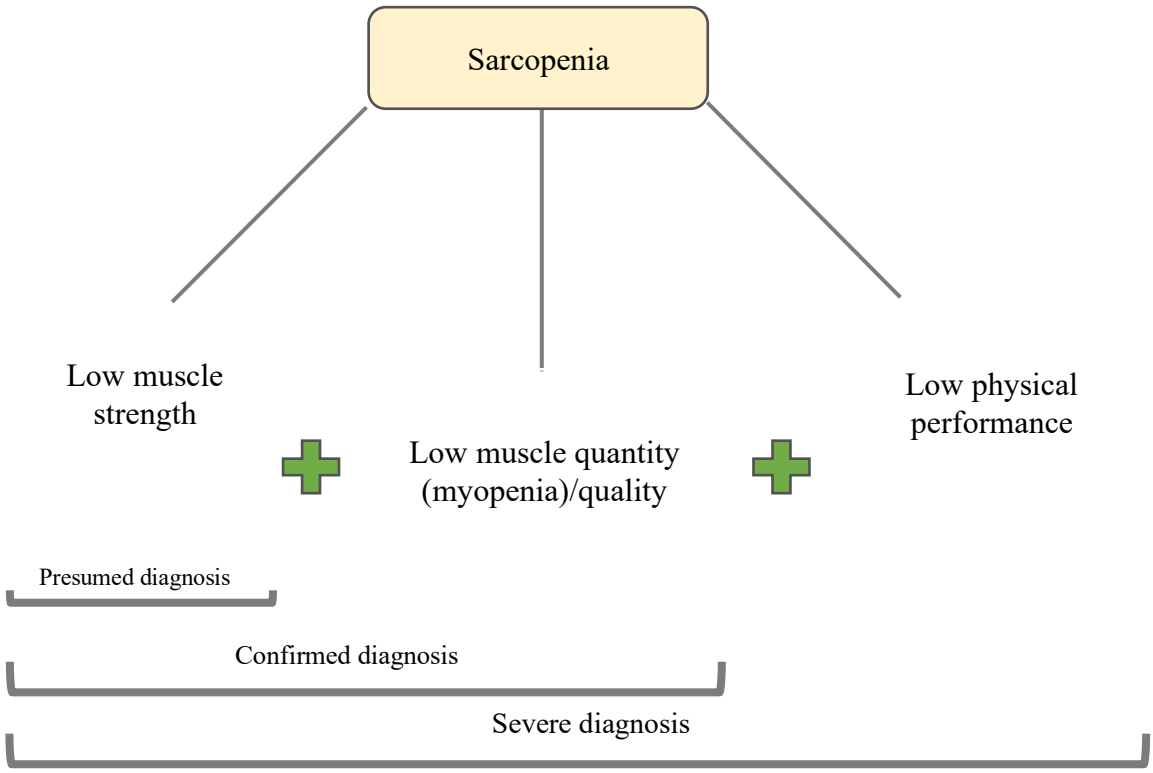


**Figure 1. 1** Revised definition of sarcopenia.[3] Sarcopenia is probable when low muscle strength is detected. It is confirmed by presence of decreased muscle quantity (also known as myopenia) or quality and considered severe when low physical performance is present.

In adult populations, sarcopenia lasting less than 6 months is considered acute.[3] Sarcopenia is usually related to acute disease or injury, where there is muscle disuse atrophy, prolonged bed rest, or ICU admission.[2, 8] Sarcopenia lasting more than 6 months is classified as chronic.[3] The development of sarcopenia in chronic diseases, such as liver disease, can be caused by chronic inflammation, endocrine disorders, and advanced organ failure.[9] Acute sarcopenia that does not recover completely could also lead to chronic sarcopenia.[8] Major surgery, such as pediatric liver transplant, is related to increased

cortisol and systemic inflammatory response.[9] Increased muscle protein degradation is activated by pro-inflammatory cytokines, and hypercortisolaemia exacerbates muscle mass loss during bedrest leading to acute sarcopenia.[9] Acute sarcopenia secondary to hospitalization has been associated with a rapid decline in muscle mass and physical functionality, and can lead to chronic sarcopenia in adults and pediatrics.[8,9] To date, there is no consensus on the definition for sarcopenia in pediatric populations.[10]

In adults, risk factors for sarcopenia include increasing age, lower body mass index (BMI), low protein intake, micronutrient deficiencies (e.g vitamin D) and low physical activity.[1] Sarcopenia is linked to hospitalization and higher healthcare costs.[11] It is a leading cause of physical disability and lower quality of life, as well as death.[2,11] Recent evidence suggests sarcopenia has a negative effect on disease recovery in adult and pediatric populations (liver transplant); isolation, prolonged rehabilitation, longer stay and higher number of admissions in hospital psychologically and economically affect these patients.[3,12-14] Additionally, declines in SMM are associated to lower ability to perform daily activities such as walking, rising from a chair and climbing stairs, affecting patients' independence.[2]

In addition to sarcopenia, research has shown adults and children who underwent solid organ transplantation have a higher risk of presenting complications linked to cardiometabolic dysregulation.[15,16] Adult LTx recipients have an increasing prevalence of obesity. Research has shown sarcopenic obesity and cardiometabolic disease is becoming more common in adult populations.[16,17] Studies involving pediatric LTx patients have found a higher risk of cardiovascular disease when compared to healthy controls.[15,18,19] These metabolic changes can lead to cardiovascular disease and diabetes mellitus in the long-term.[20] In addition, there are several mechanisms connecting sarcopenia and cardiometabolic dysregulation, including myosteatosis (fatty

accumulation in the muscle), inflammation, insulin resistance, oxidative stress, and dyslipidemia. For instance, accumulating evidence shows sarcopenic obesity is characterized by muscle fiber atrophy and lipid deposition resulting in fat accumulation in the muscle, impaired energy metabolism and skeletal muscle atrophy.[21] Sarcopenic obesity is also linked to release of proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-α) which can result in insulin resistance.[21] Evidence shows TNF-α and interleukins can promote skeletal muscle insulin resistance by impairing insulin action.[22] Interleukin-1 and interleukin-6 activate pathways (i.e. promoting the expression of cytokine signaling inhibitor 1 and signaling inhibitor 3) that impair insulin receptor substrate 1 activity thus decreasing insulin signaling in the skeletal muscle.[23] Insulin resistance along with sarcopenic obesity can lead to metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis. [16, 21]

Sarcopenia and cardiometabolic disease are associated to suboptimal nutritional intake. Diet is linked to sarcopenia and cardiometabolic dysregulation positively and negatively.[24, 25] High intake of sugar can result in impaired glucose metabolism, as well as increased visceral fat accumulation.[26, 27] Total fat and saturated fat intake increase the risk of high triglycerides and total cholesterol, which can result in dyslipidemia.[26 28] Protein is a crucial nutrient for muscle synthesis, has been associated to preservation of muscle function, and can help reduce inflammation.[24] Micronutrients such as vitamin D, selenium, omega-3 fatty acids and calcium can help reduce inflammation and oxidative stress. [24, 29, 30]

This review explores the literature in relation to prevalence of cardiometabolic dysregulation and sarcopenia in adults and children with ESLD before and after transplant, and the lifestyle factors that may contribute to increased risk for sarcopenia and cardiometabolic dysregulation.

## 1.2 Sarcopenia in end-stage liver disease

### Pre-liver transplant in adults

Assessment of disease severity in patients with ESLD is essential to define eligibility for liver transplantation (LTx). Common complications considered during evaluation are the presence of hepatic encephalopathy, variceal bleeding, and ascites.[31] Sarcopenia is not specifically considered and remains a frequently undiagnosed condition associated to decreased survival, higher infections, and lower quality of life.[31]

During the pretransplant period, sarcopenia is more frequent in adults with hepatic encephalopathy.[11] This suggests the association between HE and mortality could be explained in part by the presence of sarcopenia.[11] Given that the liver, the immediate pathway for ammonia detoxification, is dysfunctional, the muscle is a vital alternative for detoxification in patients with cirrhosis.[32] The muscle contains glutamine synthetase, which turns ammonia into glutamine via amidation of glutamate.[32] Additionally, ammonia could be a contributing factor to the development of sarcopenia by interference with protein modeling.[33] This could create a cycle in which sarcopenia promotes HE and high levels of ammonia worsen sarcopenia. [11] HE, significant muscle wasting, inflammation and oxidative stress in patients with ESLD could induce a decline in multiple physiological systems and predispose patients to worse outcomes.[22, 34, 35] Studies involving waiting-list outcomes have observed an increased mortality rate among ESLD patients with sarcopenia (**Table 1.1**).[10] Lower skeletal muscle mass index has been associated with waitlist mortality in women and men. [12] Pre-LTx sarcopenia has been associated to higher risk of severe disability and higher hospital costs.[36, 37] During the post-LTx period, pre-LTx sarcopenia has been related to higher risk of infection and longer ICU stay after transplant (**Table 1.1**).[34, 38]

**Post-liver transplant in adults**

Higher skeletal muscle mass index is associated with a decrease in total hospital costs independent of total time on waiting list.[37] Evidence from longitudinal studies show there is a significant loss of fat-free mass (FFM) during the first year after LT.[13, 31, 39, 40] Emerging data on post-LTx outcomes has shown pre-LTx sarcopenia increases risk of infections and mortality.[41-43] A retrospective study, found lower FFM is correlated to a higher incidence of severe infections in the first month post-LTx.[39] A study conducted in adults with liver disease (including hepatocellular carcinoma, primary sclerosing cholangitis, hepatitis C virus), found patients with decreasing total psoas area was associated with increased risk of developing any type of infection, and those who experienced severe infections within 180 days after transplant had higher mortality. [41] Existing evidence shows male patients diagnosed with sarcopenia by computed tomography (CT) measurement, have higher rates of death at 1 year (86 vs 95%) and 3 years (73% versus 95%) posttransplant. [42] Longer length of stay (LOS) and intensive care unit (ICU) stay, higher rate of infections and mortality was associated to the presence of sarcopenia in studies evaluating outcomes after transplant. [34, 43] Additionally, adult studies suggest components of CMD, such as diabetes mellitus (DM), are correlated to chronic rejection and graft failure, which can increase LOS and hospital costs after LTx.[44]

**Pre-liver and post- liver transplant in pediatrics**

The term "pediatrics" is commonly used to define individuals younger than 21 years.[45] Infancy is the period from birth to 2 years, childhood is from 3-12 years, and adolescence is 13-19 years.[46] Authors also use the term "young adults" to define individuals aged 18-21 years.[46] A liver transplant in this population is indicated to those who are at risk of dying from serious liver issues if the ill liver is not replaced with a new one. Common clinical indications for liver transplant in this populations are cholestatic

biliary atresia (≈31%), metabolic disorders (≈15%), acute liver failure (≈4%) and hepatoblastoma (≈3%).[47] Patients aged 1-5 account for approximately 40% of transplants performed, 25% are children <1 year, and 11-17 years account for 21%.[47]

Factors contributing to unintentional muscle loss in children with chronic disease can include nutritional deficiencies, physical limitations, and systemic inflammation.[3, 14] In pediatrics with chronic disease, higher protein oxidation and higher proteolysis may contribute to increased risk of sarcopenia.[10] Pediatrics with ESLD require 2-3 g/kg daily of protein as needs are higher.[10, 48] In children post-LTx, use of immunosuppressive drugs that negatively impact protein metabolism can also contribute to the development and worsening of sarcopenia.[10] For instance, sirolimus inhibits the activation of the AKT/mTOR pathway which is essential for muscle protein synthesis.[49] Calcineurin-inhibitors, such as tacrolimus and cyclosporine, may contribute to impairment of muscle growth. [10] Prednisone can increase myostatin expression, inhibiting muscle protein regeneration and synthesis.[50]

In pediatrics, evidence regarding the presence of sarcopenia on clinical outcomes is limited. A recent publication in children by our group, found myopenia (41%) lasted up to 8 years after liver transplantation; with prevalence being the highest in female children younger than 10 years.[13] A study conducted in pediatrics (0-18 years [n=23]) observed total psoas muscle area measured by CT was significantly smaller in ESLD participants compared to healthy controls (n=46). [51] Sarcopenia was linked to less weight gain and linear growth, increased LOS and ICU stay and higher ventilator dependency in this study.[13] Sarcopenia in children post-LTx was associated to decreased muscle strength and physical performance as well as lower muscle quality when compared to healthy controls (**Table 1.1**).[52] Deficits were found in MS and PP, especially in the lower body. Sarcopenia was associated to low levels of moderate-to-vigorous physical activity.[52]

Follow up studies examining the muscle mass in children pre-LTx also showed that myopenia and low subcutaneous adipose tissue measured through pretransplant CT and magnetic resonance imaging (MRI) scans were related to longer LOS, more infections, lower energy intake and gross motor delay in children.[14] Additionally, studies have shown that subcutaneous adipose tissue can store excess triglycerides, but when it is unable to accommodate overfeeding, visceral fat deposition can occur.[53] Even though visceral adipose tissue has not been linked to sarcopenia, evidence suggests it can contribute to increased atherosclerosis and cardiometabolic dysregulation.[53] Sarcopenia has been characterized by increased adipocytes and lipid deposition in the muscle. Increased fat accumulation leads to impaired energy metabolism in the muscle, resulting in skeletal muscle atrophy and a catabolic status.[21]

Myopenia is defined as relevant muscle wasting associated with increased risk of morbidity and mortality.[79] To date, most studies in pediatrics only focus on myopenia and do not include aspects of muscle function to define sarcopenia in children.[38] Infant and child studies looking to identify sarcopenic subjects, use measures of muscle mass and function adjusted by height, weight, or body mass index (BMI). Some studies report unadjusted skeletal muscle cross-sectional areas in diseased populations and compare those to age and gender-matched controls because of the lack of a consensus statement with age-specific cut-off values for sarcopenia.[78] Additionally, inclusion of alterations in MS and PP in the diagnosis of sarcopenia in children has been limited.[10] Myopenia measurement can be beneficial in pediatric populations where muscle contractile function is difficult to assess.[54] Muscle mass in children with ESLD can provide objective measures of growth given that other anthropometric measurements (weight, BMI, skinfolds, body circumference) can be altered by ascites, peripheral edema, and organomegaly.[54]

Few studies have examined sarcopenia in children pre-LTx and post-LTx.[13, 14, 51, 52, 55] These studies observed lower SMM in children with ESLD when compared to healthy children.[14, 51, 52, 55] Two studies conducted by our group included association to pre-LTx and post-LTx clinical outcomes, and one included measure of muscle function. However, in our studies prevalence ranged from 20-41% measured by DXA and CT scans. [13, 14, 52] This range is similar to that found by Woolfson et al. (2021), where they found a 40% prevalence of sarcopenia in children with ESLD.[56] Takeda et al. (2020) found a sarcopenia prevalence of 23% in children diagnosed with biliary atresia.[57] These studies have found pre-LTx sarcopenia is associated to longer ICU duration after transplant, higher infection rate, and higher blood loss in the post-LTx period.[56, 57] **Table 1.1** presents a summary of studies assessing the association of sarcopenia and clinical outcomes in adults and children with ESLD.

**Table 1. 1 Sarcopenia and clinical outcomes in adults and children with ESLD**

| Authors, Year | Sarcopenia[a] | Period for outcome collection[b] | Outcomes | % prevalence of sarcopenia |
|---|---|---|---|---|
| **Adults** | | | | |
| Dolgin et al., 2019[36] | Pre-LTx | Pre-LTx | ↑ risk of severe disability/impairment | 50 |
| Durand et al., 2014[58] | Pre-LTx | Pre-LTx | ↑ wait-list mortality | N/A |
| Carey et al., 2017[59] | Pre-LTx | Pre-LTx | ↑ wait-list mortality | 45 |
| Lattanzi et al., 2019[60] | Pre-LTx | Pre-LTx | Independent predictor of mortality | N/A |
| Hanai et al., 2015[61] | Pre-LTx | Pre-LTx | ↑ mortality | 68 |
| Bhanji et al., 2018[32] | Pre-LTx | Pre-LTx | ↑ risk of HE | N/A |
| Montano-Loza et al., 2015[62] | Pre-LTx | Pre-LTx | ↓ survival | 45 |
| van Vugt et al., 2018[37] | Pre-LTx | Pre-LTx | ↑ hospital costs | 43 |
| Tandon et al., 2012[63] | Pre-LTx | Pre-LTx | ↑ mortality | 41 |
| Krell et al., 2013[41] | Pre-LTx | Post-LTx | ↑ risk infections, ↑ mortality | 33 |
| DiMartini et al., 2013[38] | Pre-LTx | Post-LTx | ↑ LOS, ↑ ICU stay, ↑ ventilator days for males and females<br>Predictor of survival in males | 68 |
| Jeong et al., 2018[64] | Pre-LTx | Pre-LTx | ↓ survival | N/A |
| Lindqvist et al., 2017[39] | Pre-LTx | Post-LTx | Risk factor for sepsis | |
| Kuo et al., 2019[42] | Pre-LTx | Post-LTx | ↑ mortality in males | N/A |
| Montano-Loza et al., 2014[34] | Pre-LTx | Post-LTx | ↑ LOS and ICU stay, ↑ frequency of bacterial infections | 45 |

| | | | | |
|---|---|---|---|---|
| Masuda et al., 2014[65] | Pre-LTx | Post-LTx | ↓ survival, ↑ risk of sepsis | 47 |
| Kalafateli et al., 2017[43] | Pre-LTx or within one week post-Ltx | Post-LTx | ↑ LOS, ↑ ICU stay, ↑ infections, ↑ mortality | 25 |
| **Pediatrics** | | | | |
| Woolfson et al., 2021[56] | Pre-LTx | Pre-and-Post-LTx | ↑ nutrition support pre-LTx, ↑ ICU duration post-LTx | 40 |
| Ooi et al., 2020[14] | Pre-LTx | Post-LTx | ↑ LOS, ↑ infection, gross motor delay, ↓ energy intake | 20 |
| Takeda et al., 2020[57] | Pre-LTx | Post-LTx | ↑ operation duration time,↑ blood loss, ↑ PV stenosis, ↑ bacterial infection, ↓ patient and graft survival | 23 |
| Ooi et al., 2020[52] | Post-LTx | Post-LTx | ↓ muscle strength, ↓ physical performance, ↓ muscle quality | 36 |
| Mager et al., 2018[13] | Post-LTx | Post-LTx | ↑ LOS, ↑ ICU stay, ↑ readmissions, ↓wt-z/ht-z, ↑ ventilator use | 41 |

[a]Refers to period in which sarcopenia was assessed. [b]Refers to period in which outcomes were collected
[*]Pre-LTx: 3 weeks-1 year, Post-LTx: 1 month-8 years
Pre-LTx: pre-liver transplant, Post-LTX: post-liver transplant, HE:hepatic encephalopathy, LOS:length of stay, ICU:intensive care unit, ht-z:height z-score, wt-z:weight z-score, N/A:not available, PV stenosis: pulmonary valve stenosis

### 1.3 Methods to assess sarcopenia

Sarcopenia is diagnosed according to the amount and quality of muscle mass, muscle strength and physical performance.[3] Studies calculating reference ranges for skeletal muscle mass in children and adolescents through different body composition methods have been conducted.[66-68] A range of techniques and tools are available to identify sarcopenia. The method to utilize will depend on access to equipment and resources, as well as the patient's health status.[3] There are different tools used to quantify muscle mass, such as anthropometry, bioelectrical impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA), computer tomography (CT) scans and magnetic resonance imagining (MRI) scans. All these methods have different accuracy and precision.[31, 69] CT and MRI scans have better accuracy and precision than DXA, while DXA has better accuracy and precision than anthropometry.[69] For muscle strength, widely used tests include handgrip strength, sit-to-stand test and one-repetition maximum strength.[3, 69] Gait speed, the Short Physical Performance Battery, and Timed-Up and Go test are commonly used to measure physical performances in various age populations.[3, 31] Muscle function tests (push up, sit-to-stand, stair climb, handgrip, 6-minute walk test) conducted in children post-LTx reported good and excellent reliability (intraclass correlation coefficients: 0.84-0.99).[10]

#### 1.3.1 Muscle quantity

Methods to evaluate body composition have been developed and are constantly validated in research and clinical practice. Multiple techniques can estimate skeletal muscle quantity and results are reported as skeletal muscle mass (SMM), appendicular skeletal muscle mass (ASM), or muscle cross-sectional area of specific body locations.[3] Results can be adjusted for height or BMI.[3]

BIA does not measure SMM directly, but rather estimates the volume of fat mass and FFM based on differences in electrical conductivity.[3, 31] Many commercially available systems use DXA as reference and rely on prediction equations to report results as ASM or whole-body SMM.[3] The lack of radiation exposure, short time required to obtain results, low cost and easy to use technique make BIA an affordable method to use in clinical settings.[3, 31, 70] Good correlations between BIA-reported ASM and DXA lean soft tissue estimates has been reported in children and adults.[71] Nevertheless, hydration alteration, such as dehydration or ascites, can cause estimates to be inaccurate.[72] This method's precision and specificity may not be as reliable as that of other methods, and more studies are necessary to validate prediction equations.[3]

DXA depicts whole-body and regional (trunk, head, arms, legs) fat mass and FFM, and can provide information on bone mineral content and fat across the entire age populations.[39, 71, 72] This method is non-invasive and relatively inexpensive when compared to CT and MRI.[39] Regional and whole-body fat and bone estimates can also be used to evaluate the musculoskeletal system and adiposity levels. [33] Certified personnel are needed to administer this test with a low dose of radiation.[72] Natural background radiation in a day is approximately 10 μSv. Studies of radiation dose from DXA confirm dose is around 0.08-4.6 μSv.[73] This method is largely dependent on water, and does not differentiate between intracellular water and extracellular water.[74] Evidence suggests validity of DXA would be better if combined with measurement of fluid volume in patients with ESLD as hydration status can be altered.[39, 72] DXA measurement of FFM assumes a constant water content of FFM (0.73± 0.03), and values outside these could be defined as abnormal. Any abnormal hydration status (dehydration or overhydration) can lead to changes in lean tissue mass coefficient, thus creating errors in the amount of lean tissue attributed per pixel.[75] For instance, studies assessing body composition through

DXA found hypohydration reduced FFM estimates.[75] Underestimation of FFM may result in wrongly classifying individuals as having low muscle mass.[76] A method to measure fluid volume could be bioimpedance spectroscopy. Bioimpedance spectroscopy has been used before to quantify more accurately fluid volumes in sarcopenia, helping correct estimates of extracellular water, intracellular water, and total body water.[74, 77] This allows to identify excess fluid in the extracellular space and attain a better estimate for lean tissue. [74]

Air displacement plethysmography (ADP) is used to measure body volume through air displacement inside a chamber. ADP has only been validated in infants from birth to approximately 6 months and children over the age of 5, leaving a gap for children between these ages. ADP works with an assumption of hydration constant for FFM.[72] If hydration status is not following the assumption, FFM can be underestimated or overestimated.[72]

CT scans and MRI scans are considered gold standards to quantify total body and regional SMM, subcutaneous adipose tissue, and visceral adipose tissue in adults and children.[31, 54] High-resolution images of slices can be analyzed using automated software and hand segmentation.[71] In CT scans, each image pixel is defined by a linear attenuation coefficient or CT# reported in Hounsfield units (HU).[71] Compared to DXA, CT has a smaller margin of error in the assessment of total and FFM.[69] CT and MRI are more sensitive to small changes throughout time in SMM than other methods.[69] Cons of these two methods include cost, accessibility, plus patient exposure to radiation in CT.[39, 69]

A number of research studies use transverse images from L3 and L4 to determine muscle mass in both sexes.[3, 14 51, 55] Some authors report using L4 to determine SMM to evaluate validity of measurements and also for additional quality control.[51] These regions encompass psoas, erector spinae, quadratus lumborum, transverse abdominis, external

and internal obliques, and rectus abdominis (RA) muscle.[31] Current evidences suggests L3 is the best site to assess SMM, correlates significantly with whole-body muscle, and most precise to measure visceral fat in adults.[69, 70, 78] Additionally, mid-thigh imaging has been reported as a good predictor of whole-body muscle mass and strongly correlates with total body muscle volume.[69, 78] Recent studies have used psoas muscle to determine sarcopenia and support the use of psoas muscle as a more accurate assessment of muscle mass in children between ages 1-16 years. [54]A description of these methods is presented in **Table 1.2**.

**Table 1. 2 Comparison of skeletal muscle mass quantification methods**

| Method | Data obtained | Advantages | Limitations |
|---|---|---|---|
| **Anthropometry**[10, 71] | Skinfolds (subcutaneous fat), mid upper arm circumference (estimate measure of muscle mass), calf circumference (estimate measure of muscle mass) | Low cost, easy to perform, non-invasive | Prior training required, variability between observers, influenced by overhydration, relies on predictive equations |
| **BIA**[71, 79] | Segmental impedance, resistance and reactance (FM and FFM) | Easy to use, inexpensive, non-invasive, portable, useful for repeated measurements through time, no highly trained personnel needed | Sensitive to hydration status and physical activity, results can vary depending on device. Predictive equations are needed |

| | | | |
|---|---|---|---|
| **Ultrasound**[10, 71, 80] | Muscle width and area (total, subcutaneous, intramuscular, visceral adipose tissues, skeletal muscle mass) | Easy and quick to perform, no radiation exposure, low cost, portable, useful for repeated measures over time | No standard procedure for measurement, reproducible measurement sites to be identified, skilled personnel needed, influenced by physical activity<br>Not validated in critically ill children |
| **ADP**[10, 71] | FM, FFM | Easy to perform, non-invasive | High cost, not validated for ages 6 months to 5 years, hydration and tissue density can cause wrong estimates |
| **CT**[10, 71, 79] | Cross-sectional muscle area, muscle attenuation (total, subcutaneous, intramuscular, visceral adipose tissues, skeletal muscle mass) | High precision, muscle tissue composition can be quantified by muscle density, gives regional and whole-body data. | Low availability, high cost, trained personnel needed, radiation exposure, subject size limits |

| | | | |
|---|---|---|---|
| **MRI**[10, 71, 79] | Cross-sectional muscle area (total, subcutaneous, intramuscular, visceral adipose tissues, skeletal muscle mass) | High resolution, three-dimensional reconstruction, low radiation, can accommodate all subject sizes | High-cost, limited to certain subject size, trained personnel needed |
| **DXA**[10, 71, 74, 79] | Lean soft tissue, fat and bone mineral content | Non-invasive, low radiation, high precision measures three body compartments | Elevated cost, not portable, subject size limited, estimates depend highly on water, fatty infiltration in the muscle is not assessed, results depend on device and software used |

BIA: bioimpedance analysis, FFM: fat free mass, FM: fat mass, ADP: air displacement plethysmography, CT: computed tomography, MRI: magnetic resonance imaging, DXA: dual-energy X-ray absorptiometry

### 1.3.2 Muscle quality

Muscle quality (MQ) is an essential determinant of muscle function.[4] MQ refers to microscopic and macroscopic changes in muscle composition, and to muscle function delivered per unit of muscle mass.[81] This concept includes anatomic structure, composition, metabolic and mechanical performance.[71] MS quantifies the amount of force a muscle can generate, but bigger muscles may not be stronger than smaller muscles. Smaller muscles could be more effective at movement due to more contractile proteins and less fat infiltration; this phenomenon may be explained by MQ.[82] Different factors mediate MQ, including fatty infiltration, and neurological changes. Patients with ESLD can suffer from neurological complications such as mild encephalopathy, severe headaches, seizures and strokes. [83] These neurological complications can indirectly affect muscle quality by promoting lower motor nerve conduction velocity and defects in contraction coupling.[4] Evaluation of fat content in the muscle is complicated, and may only be obtained by analyzing muscle biopsies or using imagining techniques.[84]

## 1.4 Cardiometabolic risk in transplant populations

Cardiometabolic risk (CMR) is a set of conditions that increase the possibility of developing type 2 diabetes mellitus (DM) and cardiovascular disease (CVD). [85, 86] CMR risk factors include hypertension, hyperglycemia, dyslipidemia, abdominal obesity, insulin resistance, inflammation measured by high sensitivity C-reactive protein (CRP) levels, and sedentary life.[85] Previous findings reveal pediatric organ transplant recipients present metabolic changes leading to increased risk for CVD and DM.[20, 87] Long-term mortality and morbidity in organ transplant population are associated to cardiovascular events, with atherosclerosis considered the main cause of death in these patients.[88] Cardiovascular complications usually appear 10 years after surgery in adult liver transplant (LTx) recipients.[89] Meanwhile, hyperglycemia and DM are common

complications of organ transplantation.[90] These changes may negatively affect graft longevity.[87] For instance, DM is associated with decreased graft function caused by vascular damage.[15]Immunosuppressant drugs, especially tacrolimus, are identified as primary factors for carbohydrate metabolism dysfunction and insulin resistance leading to DM after pediatric LTx.[91] Patients may enter adulthood with a higher risk of developing other comorbidities (i.e. nonalcoholic fatty liver disease, hypertension, obesity) and lower survival rates.[15, 87]

In pediatric populations, risk factors contributing to CMR include obesity after LTx, immunotherapy and DM.[16] A longitudinal study from our site observed CMR to be independent from body composition and more associated to tacrolimus and/or mycophenolate mofetil therapy.[16] Additionally, several studies conducted in pediatric transplant recipients found high prevalence of hyperglycemia, hypercholesterolemia, and hypertriglyceridemia, as well as low glucose tolerance.[15, 18, 87, 88, 92]

**Cardiometabolic risk in pediatric transplant populations**

A study conducted in kidney-transplant pediatric patients (n=77) reported 28% of participants presented hypertension 6 months post-transplant. A percentage of 50, 47 and 6 of patients presented hypertriglyceridemia, hypercholesterolemia and hyperglycemia 6 months after transplant, respectively.[15] A multicenter study conducted in pediatric LTx survivors (n=461) found 7% and 10% of 5-year survivors presented hypercholesterolemia and hypertriglyceridemia, while 13% had evidence of glucose intolerance. Five percent of survivors were on insulin therapy or receiving antihyperglycemic drugs.[87] Another study conducted in children 1-year post-LTx concluded recipients (n=44) had high atherosclerotic risk, confirmed by inflammatory profiles and endothelial biomarkers (high-sensitive C-reactive protein, soluble intercellular adhesion molecule-1, adiponectin, plasminogen activator inhibitor 1, myeloperoxidase).[18] The prevalence of

markers of metabolic syndrome such as hypertension, low HDL and impaired glucose intolerance was significantly higher in a cohort of 83 pediatric liver transplant patients when matched to healthy controls even after adjusting for overweight, obesity and glucocorticoid use.[19] Authors hypothesized long-term exposure to calcineurin inhibitors (tacrolimus and cyclosporine) contributed to impaired glucose tolerance, and could have also been associated to higher prevalence of hypertension and low HDL.[19] Baskar et al. (2015) also looked at the prevalence of metabolic syndrome in pediatric LTx recipients. [93] In their study group (n=58), 41% were obese or overweight and in ongoing prednisone therapy. Thirty-two patients presented metabolic syndrome, approximately 10 patients had left ventricular hypertrophy and 3 patients had evidence of pulmonary hypertension.[93]

Researchers from The Vienna Cohort (n=24) found a prevalence of 25% of elevated cholesterol levels and 91% of elevated triglyceride levels in pediatric LTx recipients at 1-year follow up.[88] In addition, researchers found approximately 13% (n=214) of pediatric LTx patients in their study presented glucose intolerance or post-transplant diabetes mellitus; if developed one year after transplant these conditions had longer duration.[92] A study conducted by our group using longitudinal data (n=64) from liver transplant recipients found healthy body weight and age-appropriate growth on corticosteroid-free therapy have a low prevalence of cardiometabolic dysregulation over 10 years.[16] Alterations in glycemic levels, blood pressure, lipid metabolism appeared to be strongly related to immunosuppressants.[16] Medications such as tacrolimus, sirolimus, cyclosporine and prednisone have been observed to increase the risk of hypertension, hyperglycemia, weight gain, and dyslipidemia.[18, 20, 93] The low prevalence of CMR in this study align with the low rates of pediatric obesity. [16] Similarly, Roblin et al. (2016) concluded cardiovascular risk was mostly nonexistent in their pediatric liver transplant cohort (n=31), but they did find hypocholesterolemia (LDL) is frequent and related to

immunosuppressants.[89] This evidence highlights post-transplant risk for cardiometabolic dysregulation and the importance for studies assessing dietary intake and body composition in order to develop lifestyle treatment and prevention strategies.

### 1.4.1 Cardiometabolic risk and sarcopenia

Evidence suggests sarcopenia and cardiometabolic dysregulation are connected through the following main mechanisms: insulin resistance, myosteatosis, inflammation and oxidative stress.[1, 23, 94] Lipid accumulation, inflammatory adipokines, and high levels of reactive oxygen species (ROS) are linked to decreased skeletal muscle contractions and contribute to metabolic dysregulation of skeletal muscle. [22] These conditions have all been associated to decreased skeletal muscle contractive activity, low cardiorespiratory fitness and muscle strength.[22] Sarcopenia is characterized by loss of muscle mass and function, thereby decreasing skeletal muscle insulin sensitivity, which creates a so-called vicious cycle.[23] Moreover, increased lipid metabolites in the skeletal muscle result in impaired insulin signaling.[21] Mitochondrial dysfunction can result from myosteatosis – lipid accumulation causes lipotoxicity and promotes ROS production. Further lipid accumulation can also result from mitochondrial dysfunction.[21] **Figure 1.2** illustrates the connection between sarcopenia and IR.

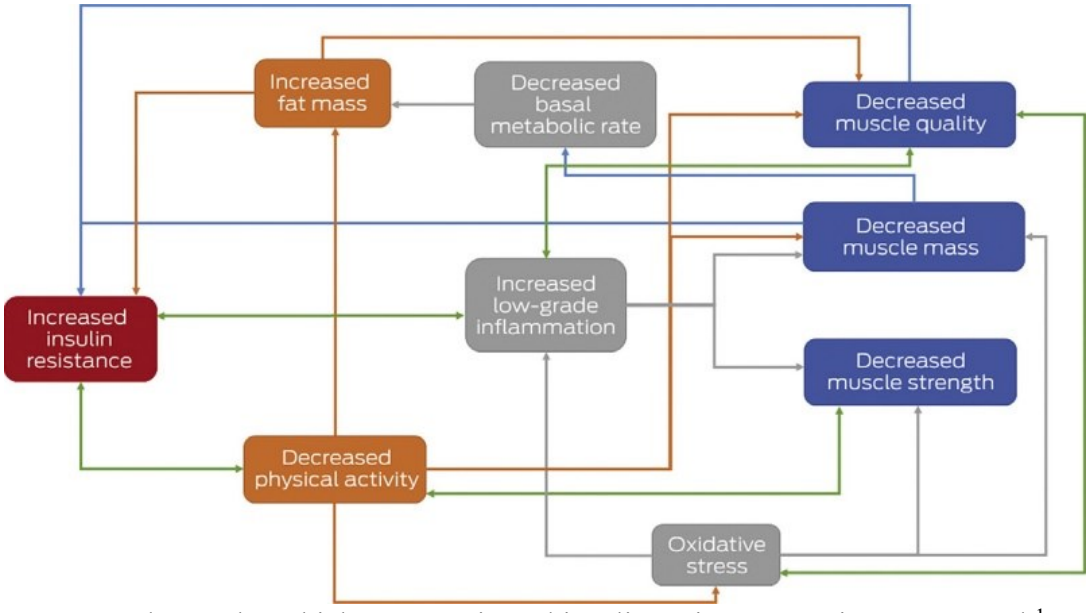


**Figure 1. 2** Pathways by which sarcopenia and insulin resistance are interconnected.[1] Figure used with permission by publisher (John Wiley and Sons).

Liver transplant patients can suffer insulin resistance, myosteatosis, inflammation and oxidative stress before and after transplant. Insulin resistance can stem from peripheral resistance, impaired glucose oxidation, reduced beta cell secretion and reduced glycogen synthesis.[95] Lipid accumulation in the muscle can be secondary to insulin resistance and hyperglycemia and can trigger inflammation.[96] Liver inflammation can cause release of pro-inflammatory cytokines.[96] Simultaneously, oxidative stress can result from liver damage, and can lead to production of ROS.[96] In the post-transplant period, immunotherapy is highly related to complications such as hyperglycemia, hypertension, and dyslipidemia which can lead to further insulin resistance, inflammation, oxidative stress and lipid accumulation.[19, 20]

Factors contributing to cardiometabolic dysregulation such as central adiposity, impaired glycemic control, inflammation, dyslipidemia and hypertension can lead to micro-and macro-vascular complications, neuropathy, and myopathy.[94] Inflammation, neuropathy, myopathy and vascular complications are negatively associated to

components of sarcopenia like muscle mass, strength and quality.[94] Low muscle mass has been linked to higher prevalence of type 2 diabetes mellitus (DM), and type 2 DM has also been reported to contribute to loss of skeletal muscle mass.[94] Additionally, sarcopenia is proposed as a cause and consequence of impaired glucose metabolism.[1]

Authors speculate that decreases in muscle mass leads to a reduced resting metabolic rate and physical activity. Reduced physical activity and resting metabolic rate accompanied by overfeeding (excess consumption of macronutrients) can cause increases in fat.[97] This elevation in fat stores can cause further loss of muscle mass by protein catabolism directly via cytokines and indirectly by insulin resistance.[97] Increased fat mass promotes inflammatory processes that in turn increase insulin resistance and triglyceride infiltration into the muscle.[97] Decreases in muscle mass also reduce available insulin-responsive target tissue, promoting insulin resistance, which is also involved in abnormal lipid metabolism leading to dyslipidemia.[97] These could explain the development of sarcopenia and cardiometabolic dysregulation in obese patients.[97]

Skeletal muscle is recognized as a vital tissue involved in the body's metabolism: key in glucose uptake, glycogen storage, lipid oxidation, amino acid release, and energy production.[98] Dietary intake, especially sugar and fat consumption, could be involved in several mechanisms involved in cardiometabolic dysregulation and development of sarcopenia. For instance, diet can influence insulin resistance and dyslipidemias, which affect the skeletal muscle's normal function, while inducing or worsening the presence of sarcopenia. [29, 30] Complex and interdependent mechanisms such as physical inactivity, insulin resistance, oxidative stress, mitochondrial dysfunction and inflammation are responsible for sarcopenia.[98]

**Intramyocellular lipid accumulation**

Muscle mass loss and muscle quality (MQ) is believed to be key in metabolic dysregulation and development of cardiometabolic disease.[99] MQ, indicated by density, rather than muscle area may be more important in terms of CMR.[99] Muscle density derived from CT has been inversely associated with total body fat, lipid accumulation in the muscle, muscle strength and function. [99] Sarcopenia is characterized by decreased MQ.[3] Low MQ can derive from increase infiltration of ectopic fat: intramyocellular lipids and adipocytes located between muscle groups, known as intermuscular fat (IMET), or between muscle fascicles, known as intramuscular fat (IMAT).[1] All these fat deposits in the skeletal muscle have been implicated in insulin resistance.[1] Adipocytes derived from intermuscular fat can affect muscle metabolism and insulin sensitivity by increasing local secretion of pro-inflammatory adipokines.[1] Intermuscular fat can impair insulin action by reduction of blood flow to the muscle.[1]

Myosteatosis or intramyocellular lipid accumulation can promote skeletal muscle insulin resistance by impaired insulin signaling, impaired protein synthesis and glucose metabolism.[23, 30, 100] As lipids accumulate, levels of ceramides and diacylglycerols increase, negatively affecting insulin signaling pathway, such as inhibiting P13K activation through increased serine phosphorylation of IRS-1.[22, 23] Furthermore, insulin resistance is also considered responsible of severe fat infiltration in the skeletal muscle. [30] Myosteatosis is therefore related to both insulin resistance and sarcopenia.[23]

**Inflammation**

Chronic inflammation is important in insulin resistance. Inflammation reduces insulin signaling by activating inflammation-related factors, thereby increasing the risk of insulin resistance and sarcopenia.[23] Skeletal muscle and adipose tissue appear to be capable of production and secretion of cytokines.[22] Increased migration of inflammatory

cells into adipose tissue, as observed in patients with obesity, could be the primary source of several cytokines (interleukin-1, interleukin-6, tumor necrosis factor-α, adiponectin).[22] Increased macrophages have also been found in skeletal muscle biopsies from patients with type 2 DM. [22]

Evidence shows tumor necrosis factor-α, CRP and interleukins can promote skeletal muscle insulin resistance by impairing insulin action.[22] Interleukin-1 activates pathways that reduce insulin receptor substrate 1 activity thus impairing insulin signaling in the skeletal muscle.[23] Interleukin-6 degrades IRS-1 by promoting the expression of cytokine signaling inhibitor 1 and signaling inhibitor 3. [23] Dyslipidemia activates cellular stress signalling pathways, inducing apoptosis and atrophy in the skeletal muscle. Saturated fat can activate pro-inflammatory macrophages in cultured myotubes.[100] Myosteatosis has also been associated to inflammatory pathway: increased levels of inflammatory factors such as tumor necrosis factor-α, Toll-like receptor 2 and interleukin-1β.[23]

**Oxidative stress**

Oxidative stress is the imbalance between the production of oxidants (ROS and reactive nitrogen species [RNS]) and the capacity of cellular antioxidant defenses to eliminate these molecules.[22] Several mechanisms contribute to the overproduction of ROS, such as mitochondrial electron leak due to excess glucose and free fatty acid metabolism.[22] Hyperglycemia, dyslipidemia, and high levels of inflammatory cytokines increase the production of ROS and RNS that may be linked to the exacerbation of skeletal muscle insulin resistance.[22] High levels of ROS can damage mitochondrial DNA, as well as proteins and lipids, and induce skeletal muscle oxidative stress, degeneration and dysfunction.[23, 101] Available data shows that as production of skeletal muscle ROS increases, oxidative levels increase, and subsequently damage skeletal muscle

mitochondrial DNA more.[23] ROS modulate transcription factors, thus increasing the production of proinflammatory cytokines.[23] Skeletal muscle oxidative stress activates pathways that lead to the degradation of IRS-1 and inhibition of insulin signaling pathways.[23] Oxidative stress may also decrease insulin sensitivity by impairing mitochondrial function.[23] Additionally, fibre atrophy and fibre loss secondary to oxidative stress can boost protein breakdown and apoptosis.[26]

### 1.4.2 Nutrients, sarcopenia and cardiometabolic risk

Sarcopenia is multifactorial and includes poor nutritional intake, as well as influence from nutrients regarding cardiometabolic disturbances.[102] Several dietary patterns have been recognized as a risk factor for cardiometabolic diseases.[27, 102] For instance, different types of studies have linked high intakes of sugar with increased total, LDL cholesterol and triglycerides (TG), as well as high blood pressure and sugar levels.[27] Meanwhile, reductions in saturated fat (SF) consumption are linked to decreases in both blood cholesterol levels and subsequent risk of cardiometabolic disease. [28]

Inflammation and oxidative stress are known causes of sarcopenia. Nutrients such as SF and sugars are involved in both processes.[26, 100] Saturated fatty acids, such as palmitic acid and stearic acid, increase production of inflammatory cytokines involved in muscle protein breakdown.[103] Sugary drinks are associated with cellular aging and shorter telomeres, which is a risk factor for aging. Authors hypothesize sugary drinks lower muscle strength due to accelerated skeletal muscle aging.[104] This could be related to the known effects of sugary drink consumption on increasing oxidative stress and systemic inflammation which affect telomere length.[104] Additionally, the fructose component of sugary drinks can upregulate lipid production, causing an increase in liver fat content.[105] Excess consumption of high-fructose corn syrup promotes higher hepatic total lipid and triglyceride content, which results from increased lipolysis and reduced lipid disposal due

to decreased β-oxidation without higher production of triglyceride-rich lipoproteins.[106] Interventions aimed at correcting inflammatory pathways to preserve skeletal muscle mass and function are therefore important to consider. Consumption of nutrients that have anti-inflammatory and antioxidant properties could suppress muscle degradation and enhance muscle synthesis.[107] Nutrients such as protein, calcium, selenium, vitamin D and omega-3 fatty acids are examples of dietary factors that have a positive effect on cardiometabolic risk and sarcopenia.[24, 26, 100, 101, 103] For instance, vitamin D stimulates insulin secretion and regulates production of inflammatory cytokines.[24, 108] Omega 3-fatty acids decrease inflammation. [24, 109] Their anti-inflammatory benefits have been connected to competing with arachidonic acid for the enzymes needed to synthesize pro-inflammatory mediator molecules [110]

Adequate protein intake is necessary for the synthesis of muscle protein, and preservation of muscle mass and function.[103] Cohort studies have associated sarcopenia and low protein intake to loss of lean body mass by DXA and reduced grip strength.[24] Research suggests an increase of dietary protein consumption can help reduce inflammation by increasing IGF-1 levels. [103]

**Anti-inflammatory and antioxidant micronutrients**

Antioxidants are suggested to combat the development of sarcopenia and CMR by inhibiting the production of ROS. [26] Selenium exerts most of its biological effects through selenocysteine; this amino acid is incorporated into selenoproteins functioning as oxidoreductase enzymes and protects against oxidative damage.[24] Additionally, even though the exact mechanisms are not clear, selenium is believed to be a marker of sarcopenia and muscle loss. [101] Studies have observed a positive association of muscle mass, physical performance and selenium.[24, 101] The Hertforshire Cohort study (n=628) found higher intakes of selenium were associated with faster 3 m walk times in men.[24]

Serum selenium was positively associated with grip strength in the Women's Health and Aging Study I (n=626).[24] The InCHIANTI study (n=891) observed that participants in the lowest quartile of plasma selenium had lower hip strength, knee strength, and grip strength.[24, 111]

Another mineral that has been linked to sarcopenia and CMR is calcium. Current evidence suggests calcium deficiency is associated to sarcopenia by modulation of calpains, cysteine proteases responsible for regulating new muscle synthesis.[24] A study conducted in Korean adults observed daily calcium intake was positively associated to appendicular skeletal muscle mass.[97] Low calcium intake has been associated to slower gait speed.[24] Several studies have linked inadequate calcium intakes are associated with dyslipidemia, insulin resistance, and inflammatory status.[112] Low-calcium diets have been found to result in insulin resistance via phosphorylation of glucose transporter type 4 which can affect glucose uptake mediated by insulin.[112] High-calcium diets have been observed to lower inflammatory markers (tumor necrosis factor-α, interleukin-6).[112] Calcium deficiency in liver transplant recipients is closely related to diminished metabolic capacity of the liver and reduced liver blood flow.[113] Calcium supplementation is routinely combined with vitamin D supplementation to prevent future bone disease in these patients.[113]

Vitamin D deficiency is related to limitation of muscle protein synthesis and muscle weakness.[26] Chronic reduction of the expression of muscle vitamin D receptors could affect muscle strength and functional capacity.[103] In addition, vitamin D regulates blood pressure and modulate inflammatory systems by regulation the generation of inflammatory cytokines and inhibition of proliferation of proinflammatory cells. [114] Deficiency is associated with stiffening of the arteries, vascular dysfunction, left ventricular hypertrophy and hyperlipidemia.[114] This vitamin stimulates the secretion of

insulin via the vitamin D receptor on pancreatic beta cells, plus reducing insulin resistance through vitamin D receptors in the muscles and liver.[115] Additionally, adult and child liver transplant recipients are at risk of vitamin D deficiency related to malabsorption and impaired hepatic 25-hydroxylation of vitamin D.[116] Supplementation of vitamin D and analogues (paricalcitol and doxercalciferol) have shown to be useful to prevent bone disease in this population.[116]

Omega-3 fatty acids are also nutrients with anti-inflammatory properties, which could help combat sarcopenia. [24] Omega-3 fatty acids decreased inflammatory response acting as lipid mediators while decreasing inflammatory response.[103] Higher dietary levels of omega-3s are linked to lower risk of heart failure and coronary disease.[103]

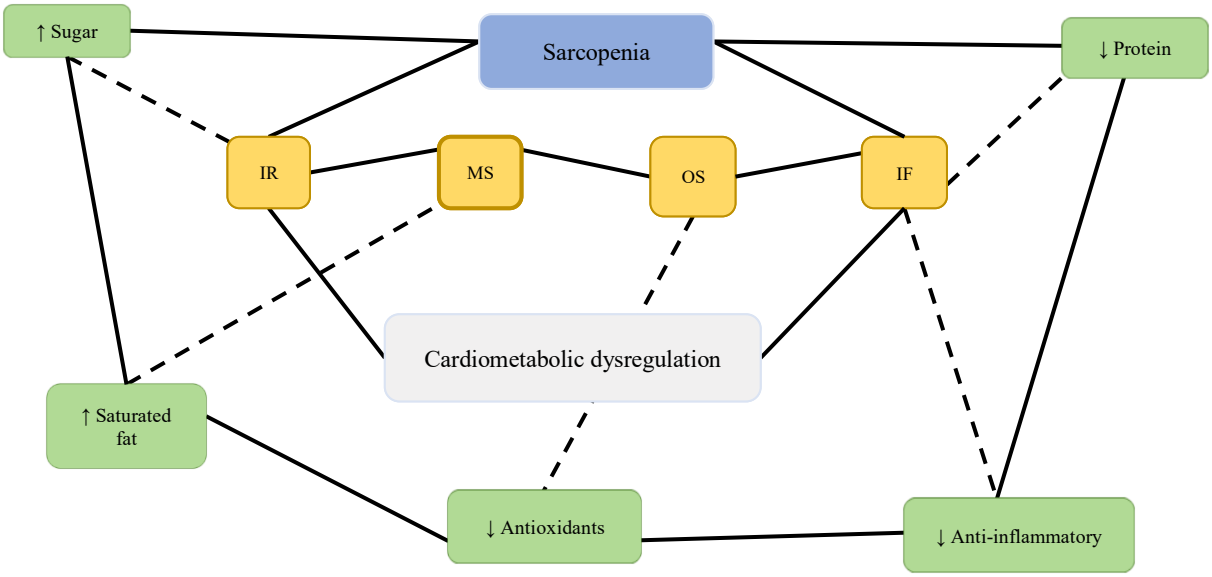


**Figure 1. 3** Dietary elements can influence mechanisms involved in the development of sarcopenia and cardiometabolic dysregulation.[1, 23] IR: insulin resistance, MS: myosteatosis, OS: oxidative stress, IF: inflammation

### 1.4.3 Liver transplantation: diet and cardiometabolic dysregulation

There is limited research exploring diet and cardiometabolic dysregulation in children who underwent liver transplantation. Previous studies have reported post-LTx

children do not have significant differences in dietary intake when compared to healthy peers.[117, 118] However, a study conducted by our group reported pediatric LTx recipients consumed insufficient amounts of vitamin D and calcium.[117] Another conducted in this population observed recipients had excess consumption of carbohydrates, fat, sugar and sodium. [118] This population also had higher prevalence of hypertension and were more like to have glucose intolerance than matched peers.[118] Chambers et al. (2019) were also the first ones to report alcohol use in LTx pediatric recipients ≥12 years old.[118] Further studies are needed to understand the impact of diet in order to prevent negative effects on health, since this population has a higher risk for developing components of cardiometabolic disturbances. [118]

### 1.4.4 Other Factors Influencing Risk for Cardiometabolic Dysregulation in Transplant Recipients

Long-term antirejection therapies are a contributing factor for increased risk of cardiometabolic disturbances in patients post-LTx. Immunosuppressive drugs prescribed to transplant patients have secondary effects on risk factors for CMR.[20] Dyslipidemia related to immunosuppressive drugs is well known after solid-organ transplant.[88] Corticosteroids and tacrolimus affect insulin synthesis, increasing the risk for insulin resistance.[20] Calcineurin inhibitors (cyclosporine and tacrolimus) induce hypertension, while mTOR inhibitors can cause dyslipidemia.[20, 87, 119] Studies show mTOR inhibitors reduced β-cell insulin secretion, thus inducing hyperglycemia.[120] A study evaluating the effect of immunosuppressive drugs on cardiovascular risk in post-kidney transplant children found those treated with sirolimus and cyclosporine had higher prevalence of hypertension, hypercholesterolemia, hypertriglyceridemia and lower serum HDL-cholesterol (HDL) compared to those taking tacrolimus. Those taking cyclosporine also had significantly greater LDL-cholesterol (low-density lipoprotein) levels.[20]

Immunosuppressive therapy also contributes to excess weight gain and body composition abnormalities after transplantation, which can result in overweight, obesity, and loss of muscle mass.[10, 87] Excess weight and loss of muscle mass may contribute to adverse patients outcomes and comorbid conditions. [10] **Table 1.3** gives more detail on the potential adverse effects of immunotherapy on cardiometabolic risk.

**Table 1. 3 Common immunosuppressants used after liver transplantation and their potential effects on cardiometabolic risk**

| Drug | Adverse effects | Possible mechanisms |
|---|---|---|
| **Tacrolimus**[121, 122] | DM, HP | DM: decreased insulin secretion, toxic effect on beta cells, mitochondrial swelling in pancreatic isografts. HP: inhibition of endothelial function |
| **Cyclosporine**[121, 123] | HP, DL, DM | DL: inhibition of bile acid synthesis from cholesterol, decreased transport of cholesterol to the intestines; increases in LDL levels by binding to the LDL receptor; increased hepatic lipase activity, decreased lipoprotein lipase activity resulting in deficits in clearance of VLDL and LDL cholesterol. HP: vascular dysfunction due to cytotoxic effects on the endothelium, impaired NO release, increased production of ET-1. DM: binds to calmodulin which may inhibit insulin secretion |
| **Sirolimus**[121, 124] | DL, IR | DL: rise in triglyceride production by inhibition of lipoprotein lipase and decreased lipolysis, increased secretion of VLDL. IR: altered insulin signaling pathway |
| **Corticosteroids**[20, 121, 122, 124] | DM, DL (decreased fat oxidation, increased fat deposition) | DM: induction of IR. Impaired peripheral glucose uptake in the muscle, decreased suppression of endogenous insulin production, activation of glucose/free fatty acid cycle. DL: IR induces reduction in triglyceride clearance, higher activity of acetyl-coenzyme A carboxylase and free fatty acid synthetase, higher hepatic synthesis of VLDL, down-regulation of LDL receptor activity, and impaired action of lipoprotein lipase |

DM: diabetes mellitus, HP: hypertension, DL: dyslipidemia, LDL: low-density lipoprotein, VLDL: very-low-density lipoprotein, NO: nitric oxide, ET-1: endothelin 1, IR: insulin resistance

## 1.5 Conclusions

This review highlights the negative outcomes before and after transplant related to sarcopenia in adults with ESLD. Sarcopenia is a relatively new finding in infants and children with ESLD. However, emerging evidence suggests it is as negatively associated to outcomes in this population as it is in the adult population. Sarcopenia may stunt growth and development, further affecting children in adulthood. This would also create a burden in healthcare costs. Certain diet elements have been associated to higher risk of cardiometabolic disturbances, such as insulin resistance, dyslipidemia, inflammation and oxidative stress.[29, 30] These conditions are involved in the development of sarcopenia and can also worsen this disease.

Presence of insulin resistance and fat depots in the muscle are linked to muscle strength and function deficits, as well as increased risk of mobility deficits, reduced physical performance.[125] This thesis will look at the relationship between dietary factors in the risk for cardiometabolic disease and subsequent development of sarcopenia. Findings obtained from this study will help develop future rehabilitation strategies to treat sarcopenia in children before and after transplant.

# Chapter 2: Research Plan

## 2.1 Study Rationale

Sarcopenia is a muscle disease, characterized by low muscle mass combined with poor muscle function and physical performance.[3] Sarcopenia is linked to adverse pre-liver transplant (LTx) and post-LTx outcomes including increased waitlist mortality, higher infection rate and longer hospital stay in adults with cirrhosis.[42, 59, 62, 63] Data in children with end-stage liver disease (ESLD) and sarcopenia are scant. In adult disease, complications associated with cardiometabolic dysregulation, such as atherosclerosis, decrease long-term survival rates following organ transplant.[15, 87, 88] These complications usually appear 10 years after adult liver transplantation.[89] Pediatric organ transplant recipients are at risk for metabolic disturbances such as cardiovascular disease and diabetes mellitus (DM).[20, 87]

Cardiometabolic dysregulation risk factors, such as insulin resistance and dyslipidemia, overlap with mechanisms contributing to sarcopenia.[23, 30] Myosteatosis, inflammation and oxidative stress are linked to decreased skeletal muscle contractions affecting movement and contribute to abnormal functioning of the skeletal muscle.[22] For instance, fat accumulation in the skeletal muscle contributes to glucose abnormalities by impairment of insulin diffusion and action, which can lead to changes in local muscle metabolism. [126]

Risk factors contributing to cardiometabolic risk in pediatric populations following transplant include obesity, receiving immunotherapy, dyslipidemia and DM.[16, 18] A negative impact of poor diet quality on cardiometabolic dysregulation influences graft survival and mortality in children post-LTx.[117, 127] Studies evaluating dietary intake of pediatric LTx recipients have reported higher intakes of carbohydrates, fat, sugar, and

sodium, as well as insufficient micronutrient amount when compared to North American healthy controls.[117, 118] Additionally, dietary factors such as poor vitamin D status have been linked to increased risk for sarcopenia in adults with cirrhosis.[10] Observational studies have shown that inadequate micronutrient intake, specifically selenium, calcium and omega-3 fatty acids, is linked to muscle mass, muscle function, and prevalence of sarcopenia.[24, 101, 128]

Myopenia (reduced skeletal muscle mass) appears to last up to 8 years after transplant in 40% of children with ESLD.[13] A recent study by our group investigated the association of pre-LTx myopenia/low subcutaneous adiposity on post-LTx outcomes in children with ESLD, showing children with low SMM/low subcutaneous adiposity had more infections, longer length of stay, delayed gross motor skills and suboptimal energy intake after transplant.[14] Muscle strength, physical performance and muscle quality also are negatively affected in children post-LTx, adversely influencing time spent engaging in physical activity.[52] However, there is also a lack of evidence regarding the association of diet, cardiometabolic dysregulation and prevalence of sarcopenia in children after LTx. This thesis will address these gaps by examining dietary intake patterns in children after LTx with and without sarcopenia.

Based on the available data on pediatrics with ESLD and adverse outcomes, more research is needed to evaluate the impact of dietary intake during the post-LTx period in infants and children with ESLD. The objective is to assess associations between dietary intake and cardiometabolic dysregulation linking to sarcopenia after LTx (Chapter 3).

The findings in this thesis will help the effect of diet on body composition and cardiometabolic dysregulation in order to help develop rehabilitation strategies to benefit children with sarcopenia.

## 2.2 Objectives and Hypotheses

**Chapter 3 is a secondary analysis of a cross-sectional study in post-LTx children and healthy controls exploring dietary intake.**

- **Title: The effect of diet on cardiometabolic dysregulations in children with sarcopenia post-LTx**
- **Objective 1:** To examine the relationship between macronutrient intake (protein, carbohydrate, fat, saturated fat, polyunsaturated fat and monounsaturated fat) intake and markers of cardiometabolic dysregulation (glycemic control, hypertension, dyslipidemia, obesity) in the prevalence of sarcopenia in children post-LTx.
- **Objective 2:** To evaluate anti-inflammatory and antioxidant consumption (vitamin D, selenium, calcium, omega-3 fatty acids) with markers of cardiometabolic dysregulation (glycemic control, hypertension, dyslipidemia, obesity) in post-LTx children with sarcopenia.
- **Hypothesis 1:** High consumption of carbohydrates (>65%), fat (>35%) and saturated fat (>10%) and low consumption of protein (<10%) will be more prevalent in post-LTx children with cardiometabolic dysregulation and sarcopenia.
- **Hypothesis 2:** Low intakes of specific nutrients that have anti-inflammatory and antioxidant properties (vitamin D [<600 IU], selenium [<30 μg) , calcium [<1100 mg], omega-3 fatty acids [<0.9g]) will be more prevalent in post-LTx children with cardiometabolic dysregulation and sarcopenia.

# Chapter 3

**Title: The effect of diet on cardiometabolic dysregulations in children with sarcopenia post-LTx**

## 3.1 Abstract

**Background/Aim:** Sarcopenia is a muscle disease characterized by low skeletal muscle mass, decreased muscle strength and physical performance. Sarcopenia has recently been observed in pediatric liver transplant populations. Organ transplant recipients may also suffer from complications stemming from cardiometabolic dysregulation. Certain dietary patterns have been linked to higher risk of sarcopenia and cardiometabolic risk. It was hypothesized high consumption of carbohydrates and saturated fat and low consumption of protein as well as low intake of specific micronutrients with anti-inflammatory and antioxidant properties will be associated with CMD and sarcopenia in children after liver transplant (LTx). **Methods:** A secondary analysis of a cross-sectional study in children after LTx (n=22) and age-matched healthy controls (HC, n=47) aged 6-18 years, assessing dietary intake, prevalence of sarcopenia and prevalence of cardiometabolic dysregulation defined by three different criteria (modified ATP-III, WHO and Magnussen et al.). **Results:** Eight LTx participants had confirmed of sarcopenia, while prevalence of cardiometabolic dysregulation ranged from 4.5-50%. Patients with CMD had significantly higher calcium intake (p=0.05). The sarcopenia group had significantly higher protein and lower fat intake (p=0.04 and p=0.02, respectively). **Conclusions:** LTx children are at risk of developing cardiometabolic diseases and sarcopenia. More studies

in transplant populations exploring the prevalence of CMD, sarcopenia and the effect of diet are needed.

### 3.2 Introduction

Pediatrics (individuals <21 years) with end-stage liver disease (ESLD) awaiting transplantation usually suffer from malnutrition contributed by ascites, gastrointestinal bleeding, inadequate dietary intake, inflammation, and low physical activity.[10, 46] Sarcopenia has been identified in adults with ESLD, and most recently in pediatric organ transplant populations.[13, 14] Sarcopenia is a muscle disease involving loss of skeletal muscle mass (SMM), decreased muscle strength (MS) and physical performance (PP). [3] Sarcopenia is a predictor of reduced mobility in daily living and poor quality of life independent of other comorbidities.[1] It is also associated with reduced growth, higher ventilator dependency and longer hospital stay in children post-liver transplant (LTx).[13, 14]

Complications linked to cardiometabolic risk (CMR) are frequent in both adult and pediatric organ transplant populations.[16, 89] CMR includes risk factors such as hypertension, hyperglycemia, insulin resistance, obesity and dyslipidemia.[85] Cardiovascular complications in adult LTx patients commonly appear 10 years post-surgery and reduce graft survival and increase mortality and morbidity.[88, 89] Studies in pediatric transplant recipients have observed high prevalence of hypertriglyceridemia and hyperglycemia, revealing negative metabolic changes associated with CMR.[15, 18, 87, 92] CMR can be increased by immunosuppressant drugs (tacrolimus, sirolimus, cyclosporine and prednisone) administered post-transplant.[20]

Mechanisms underlying sarcopenia are connected to cardiometabolic dysregulation (CMD) risk factors: fat accumulation in the muscle, inflammation, oxidative stress, insulin resistance (IR) and dyslipidemia.[23, 30] Loss of muscle mass may

contribute to reduction in metabolic rate (10-15 kcal/kg per day)[129], thus leading to fat accumulation.[97] Myosteatosis or lipid accumulation in the muscle, inflammation, and high levels of oxidative stress contribute to metabolic dysregulation of the skeletal muscle.[22] For instance, myosteatosis may promote muscle insulin resistance by impairing insulin signaling as well as reducing protein synthesis and glucose metabolism.[23, 30, 100] Proinflammatory cytokines can impair insulin action, while reactive oxygen species (ROS) can damage mitochondrial DNA and induce skeletal muscle degeneration and dysfunction. [22, 23, 101]

Certain dietary patterns are identified as risk factors for CMD. High intakes of sugar and saturated fat have shown to promote elevated total cholesterol and triglycerides, high blood pressure and high glycemic levels (fasting glucose >6.1 mmol/L).[27] Carbohydrate and saturated fat are also involved with higher levels of inflammation and oxidative stress.[26, 100] In contrast, dietary omega-3 fatty acid intake has been associated with reductions in inflammation and oxidative stress in adults with CVD.[109] Nutrients with anti-inflammatory and antioxidant activity, such as selenium, omega-3 fatty acids, vitamin D and calcium, may have a preventative role in the occurrence of sarcopenia and CMD by reducing proinflammatory factors and oxidative stress.[26, 100, 102, 103] Limited data is available regarding diet and cardiometabolic dysregulation in children post-LTx. Available studies have reported lower micronutrient intake and excess consumption of sugars and fat in this population when compared to healthy peers.[117, 118] Studies exploring modifiable factors related to CMR and sarcopenia in children post-LTx are needed. The study objectives were to 1) examine the relationship between macronutrient intake (protein, carbohydrate, fat, saturated fat, polyunsaturated fat and monounsaturated fat) and markers of cardiometabolic dysregulation (glycemic control, hypertension, dyslipidemia) in children with sarcopenia after LTx and 2) to examine the relationship

between intake of anti-inflammatory and antioxidant nutrients (vitamin D, selenium, calcium, omega-3 fatty acids) with markers of cardiometabolic dysregulation (CMD) (glycemic control, hypertension, dyslipidemia) and saecopenia in post-LTx children. It was hypothesized high consumption of carbohydrate and saturated fat and low consumption of protein as well as low intake of specific micronutrients with anti-inflammatory and antioxidant properties (selenium, calcium, vitamin D, omega-3 fatty acids) will increase the prevalence of CMD and sarcopenia in children after LTx.

### 3.3 Methods

This is a secondary data analysis of a cross-sectional study on sarcopenia and children who underwent LTx.[10, 52] In that prospective study, body composition, measures of muscle strength, physical performance, diet, and physical activity were collected. Participants included those in their childhood and adolescence stage[46] (aged 6-18 [n=22]) who had undergone LTx for a minimum of a year prior were recruited at the Stollery Children's Hospital in Edmonton, Canada. Patients presenting normal liver allograft function and no acute organ rejection within 3 months were included. Participants younger than 6 years, with inborn errors of metabolism, any known episodes of acute rejection within the past 3 months or that presented muscular, or bone disorders/injuries were excluded. Age-matched healthy controls (HC) (n=47) were recruited from the community with flyers and word-of-mouth. HC were included to allow a comparison between groups for anthropometric measurements, blood pressure and dietary intake. Dietary data from HC helped establish normative data for dietary analysis. Prior to recruitment, children in the control group and their parents/caregivers were asked to fill out a health history questionnaire to exclude history of liver disorders or prescription of medications that affects muscle metabolism.

Gender, age, Pediatric End-stage Liver Disease (PELD), Model End-stage Liver Disease (MELD), age at LTx, etiology of LTx, immunotherapy, biochemical data, anthropometric data were collected at LTx, LTx assessment and at the time of study. PELD and MELD calculations were based on the United Network for Organ Sharing/Organ Procurement and Transplantation Network.[130]

Informed consent/assent was obtained by participants and their parents and/or caregivers prior to enrollment into the study. The study was approved by the Human Ethics Board, University of Alberta (Pro00076244).

### 3.3.1 Anthropometric variables

Height (cm) and weight (kg) were measured without shoes and with light clothing to the nearest 0.1kg/cm by trained personnel using standard procedures.[131] Weight was measured with a Health o meter® Professional digital scale (Illinois, USA). Height was measured using a digital stadiometer (Measurement Concepts and QuickMedical, Washington, USA). Body mass index (BMI) was calculated as weight (kg)/ height ($m^2$). Weight, height, and BMI were converted into z-scores based on World Health Organization (WHO) guidelines.[132] Weight-z and height-z scores were collected at the time of study for both groups.

### 3.3.2 Laboratory variables

Biochemical parameters studied were serum triglycerides (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), hemoglobin A1c (HbA1c) and fasting/random glucose. Blood work was done as part of routine clinical care and was performed fasted in the Core Laboratory at Alberta Health Services through standardize methodologies.[52] Blood pressure was converted to percentiles and classified as normal or abnormal according to standards by the National High Blood Pressure Education Program Working.[133]

### 3.3.3 Definition of Cardiometabolic Dysregulation

Cardiometabolic dysregulation was defined according to the Adult Treatment Panel III (ATP-III) guidelines with modified age-specific criteria [16, 134-136], World Health Organization (WHO)[137, 138] and Magnussen et al. (2010)[139]. Metabolic syndrome is defined as having any 3 of the following: obesity, elevated TG, elevated BP, reduced HDL, and impaired fasting glucose. LTx children were classified as with cardiometabolic dysregulation or no cardiometabolic dysregulation according to the three criteria: modified ATP-III[16], Magnussen et al.[139], and WHO[137]. These definitions have different cut-offs for laboratory and anthropometric measures used to define CMD, which could impact prevalence. **Table 3.1** gives a summary of age-specific cut-offs for each parameter according to different definitions.

**Table 3. 1 Age-specific (5-18 years) cut-offs for cardiometabolic dysregulation parameters in children and adolescents**

| Parameter | ATP-III[16] | WHO[137] | Ferranti et al.[140] | Weiss et al.[141] | Magnussen et al.[139] |
|---|---|---|---|---|---|
| **Elevated serum TG (mmol/L)[1]** | >95$^{th}$ | ≥1.7 | ≥1.1 | ≥1.1 | ≥75$^{th}$ percentile |
| **Reduced HDL-cholesterol (mmol/L)[1]** | <5$^{th}$ percentile | M ≤0.9<br>F ≤1.01 | <1.3 | <1.3 | ≤25$^{th}$ percentile |
| **Obesity (BMI:kg/m$^2$)[2]** | >95$^{th}$ percentile | ≥95 percentile | - | >2 SD | >75$^{th}$ percentile |
| **WC percentile** | - | - | ≥75$^{th}$ percentile | - | - |
| **Hypertension (BP:mmHg)[3]** | >95$^{th}$ percentile | DBP: ≥ 85<br>SBP: ≥130 | ≥90$^{th}$ percentile | >90$^{th}$ percentile | >75$^{th}$ percentile |

| | | | | | |
|---|---|---|---|---|---|
| **Fasting glucose (mmol/L)[4]** | >6.1 | >6.1 | ≥6.1 | >5.6 | ≥5.6 |

[1]Pediatric reference ranged obtained from Daniels et al. (2008)[142]
[2] Determined using World Health Organization (WHO) anthropometric calculator[143]
[3]Classified using the National High Blood Pressure Education Program Working[133]
[4]Classified with criteria from Mager et al. (2019)[16]
ATP-III: Adult Treatment Panel III, WHO: World Health Organization, TG: triglycerides, BMI: body mass index, HDL: high density lipoprotein cholesterol, BP: blood pressure, DBP: diastolic blood pressure, SBP: systolic blood pressure, M: males, F: females

### 3.3.4 Body composition

Skinfolds

Multiple skinfold measures were taken from the right side to the nearest 0.5 mm using a Lange skinfold caliper (Beta technology, Santa Cruz, California, USA) following International Society for the Advancement of Kinanthropometry procedures.[131] Triceps skinfolds were measured at the most posterior part of the triceps at the midpoint between the acromion bone and the top of radius bone.[108, 131] Biceps skinfolds were measured at the most anterior part of the middle biceps muscle (between acromion and the top of the radius bone).[108, 131] Subscapular skinfolds were measured 2 cm along a line running laterally and obliquely downwards at a 45 degree angle from the lowest tip of the inferior angle of the scapula.[108, 131] The intersection point between two lines (a horizontal line from the most lateral edge of the iliac crest and a line from the axillary boarder to the most inferior part of the tip of the anterior superior iliac spine) was used to measure supraspinal skinfolds.[108, 131] Abdominal skinfold was measured 5 cm horizontally from the midpoint of the navel.[108, 131] Calf skinfold was measured on the point on the most medial aspect of the calf at level of maximum circumference.[108, 131] Triceps, biceps, subscapular, and suprailiac skinfolds were used to determine fat-free mass (FFM) calculated by a predictive equation.[10, 52, 144] FFM index (FFMI=FFM/height$^2$ [absolute/z-score]) was calculated according to normative data and was defined as reduced FFM when

z-score ≤-1.5.[52, 67] This interpretation was used to define low muscle mass in HC and LTx children as described previously.[52]

Body circumference

Body circumference were measured to the nearest 0.1 cm with a steel flexible tape (Rosscraft Innovations Incorporated, USA). Waist circumference (WC) was measured following WHO procedures (midpoint between the bottom of the rib cage and the highest point of the iliac crest).[108, 145] Mid-arm circumference (MAC) was assessed on the right side at the midpoint between the most lateral and highest top margin of the acromion bone and the proximal and lateral boarder of the head of the radius bone.[108, 131]

Dual-energy x-ray absorptiometry

Measurements for dual-energy x-ray absorptiometry (DXA; Hologic QDR 4500A/Apex System 2.4.2, Hologic Inc., Waltham, MA) were available for LTx patients. Measurements were only available for LTx patients who underwent this study as part of routine clinical care due to ethical concerns related to radiation exposure associated with DXA. Total and segmental body composition for fat mass and lean soft tissue mass (absolute and z-scores) were collected. Skeletal muscle mass (SMM) evaluated by DXA was calculated against age-gender matched normative data and SMM z-scores (SMM-z) were determined.[66] SMM-z≤-1.5 was defined as low muscle quantity. [52]

### 3.3.5 Defining sarcopenia

The adult definition of sarcopenia by the revised European consensus 2019 was adopted for this analysis to define sarcopenia since there is no consensus definition in children: a) probable sarcopenia (low muscle strength assessed by handgrip or sit-to-stand or push-up test) b) sarcopenia (low muscle strength + low muscle quantity) and c) severe sarcopenia (low muscle strength + low muscle quantity + low physical performance [assessed by 6-minute walk test or stair climb test]).[3, 52] Muscle strength and physical

performance tests were performed through standardized protocols described before.[10, 52] Low muscle strength/physical performance was defined by 2 SD below the mean values for age and gender matched healthy controls. Low muscle mass was SMM-z≤-1.5 for LTx only. Skinfolds were included to define low FFM in HC and LTx participants. Low FFM was defined by FFMI-z≤-1.5 in LTx and healthy controls.[10, 52] Only LTx children were classified as confirmed sarcopenia or non-sarcopenia.[3] **Figure 3.1** illustrates criteria for classification of sarcopenia.

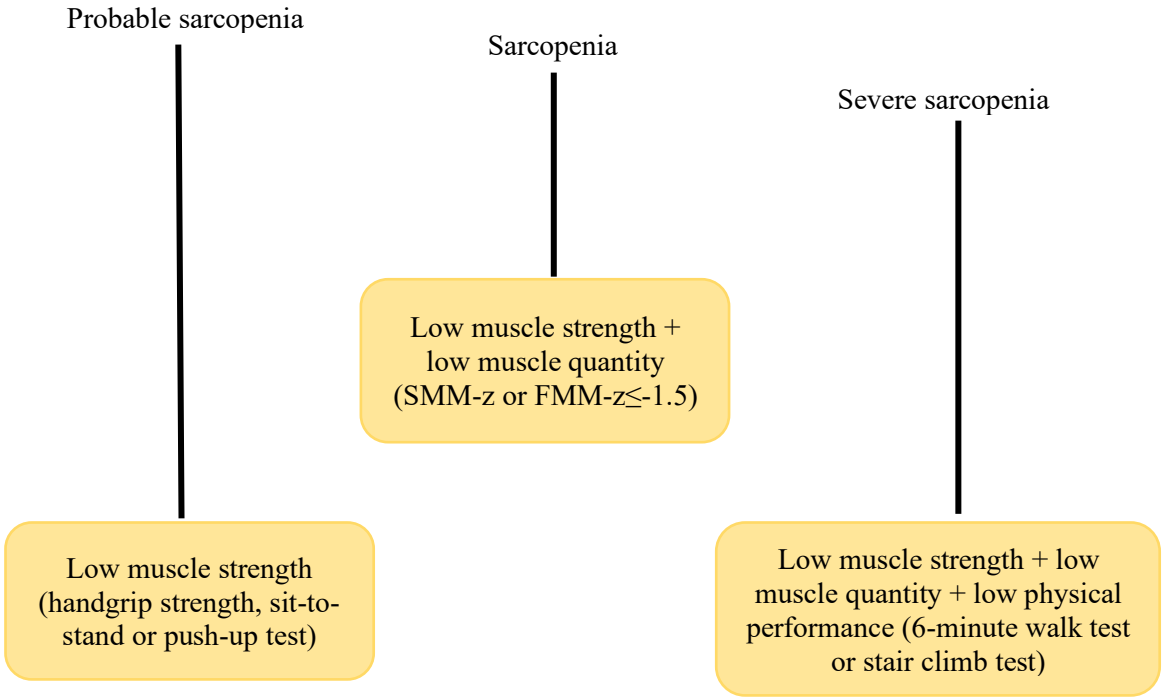


**Figure 3. 1** Diagnosis of sarcopenia[3]

### 3.3.6 Dietary Intake Analysis

Nutrient consumption was assessed through a three-day food record (2 weekdays and 1 weekend day) using food processor software (2015 ESHA Research, version 10.15.4, Salem, OR, USA) for both groups. The food processing software determined micro-and-macronutrient intake. Micronutrients analysed include calcium, vitamin D, selenium and omega-3 fatty acids. Accuracy of reporting was assessed calculating the ratio of energy intake divided by the estimate of basal metabolic rate.[146] Basal metabolic

rate specific for age, gender and weight was calculated using the World Health Organization equations. [147] Values below the 95% of confidence interval were indicative of under-reporting while values above the 95% of confidence interval indicated over-reporting.[146]

When necessary for brand foods, nutrient content was retrieved from the manufacturers' webpages or product labels.[108] Substitute foods were used when the exact product was not found in the food processing software and if macronutrient content was close to the same in both products.[117] This was done to ensure accuracy of macronutrient and micronutrient content. Age-and gender-specific DRI were used to compare macronutrient and micronutrient intake.[148-152] RDA (Recommended Daily Allowance) was used to assess nutritional adequacy as EAR (Estimated Average Requirement) is not useful as an estimate of nutrient adequacy in individuals.[153] The total intake for calcium and vitamin D included that obtained via diet and supplementation. Vitamin D supplementation included single preparation or multi-vitamin preparations. **Table 3.2** presents a summary of DRIs used for macronutrients and micronutrients.

Dietary data from HC was included to increase number of participants in the analysis. Additionally, HC and LTx children lived in the same geographical area, ensuring access to similar foods, as opposed to comparing LTx children data with international or national reference information.

**Table 3. 2 Dietary Reference Intakes recommended for children 6-18 years**

| **Nutrient** | **DRIs** |
|---|---|
| %Carbohydrate | 45-65[1] |
| %Protein | 10-30[1] |
| %Fat | 25-35[1] |
| %Omega-6 | 5-10[1] |

| | |
|---|---|
| %Omega-3 | 0.6-1.2[1] |
| Vitamin D (IU) | 600[2] |
| Calcium (mg) | 1100-1300[2] |
| Selenium (μg/d) | 6-8 y: 30[2]<br>9-13 y: 40[2]<br>14-18 y: 55[2] |

Dietary Reference Intakes from the Institute of Medicine: [1]AMDR:Acceptable Macronutrient Distribution Range[150], [2] RDA: Recommended Daily Allowance[150]
DRIs: Daily Reference Intakes, y: years, Omega-6: n-6 polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

### 3.3.7 Statistical analysis

Data was analysed using SAS 9.0 statistical software (SAS, version 0.4; SAS Institute Inc. Cary, NC, USA). Normality of distribution was assessed by the Shapiro-Wilk test. Data was expressed as mean ± standard deviation (range), median (IQR) or %. Univariate analysis (ANOVA) was performed to assess potential associations between sarcopenia, cardiometabolic dysregulation, anthropometric, demographic, and dietary data. Chi-square/Fisher exact tests were completed to measure differences in categorical data. Where necessary, primary outcome variables were adjusted for potential confounders (age, sex, diagnosis). HC were only included in tests assessing differences between groups in anthropometric, demographic, and dietary intake. Post-hoc power tests were performed to assess if sample size was adequate to detect statistically significant differences between groups (LTx and HC, sarcopenia and non-sarcopenia, CMD or no CMD).[154] A p-value $\leq 0.05$ was considered statistically significant.

## 3.4 Results

### Anthropometric, demographic, and clinical data

Anthropometric and demographic data are presented in **Table 3.3**. There were no significant differences in age and gender between LTx children and HC (p=0.56 and p=0.88 respectively). At the time of study, most children had weight-z, height-z and BMI-

z within healthy reference ranges. However, LTx children had significantly higher BMI-z (p=0.01), but lower height-z (p=0.02) than HC.

No LTx children were diagnosed as failure to thrive[155] (weight for age less than the 5$^{th}$ percentile, p=0.54). Two participants had a height-z score <2 (one from LTx group and one HC, p=0.53). Eight LTx children and 22 HC were classified as FFMI-z ≤1.5 (p=0.16). Six LTx children were classified as SMM-z ≤1.5. Females had significantly higher SMM-z in the LTx group (p=0.01). When adjusted from median age during study (12.5 years) and age during transplant (above/below median age 1.47 years) for the LTx group no associations to weight-z, height-z, weight circumference-z, tricep-z, MAC-z, FFMI-z and SMM-z were found.

**Table 3. 3 Anthropometric and demographic data in children who underwent liver transplantation and healthy controls**

| Variable | LTx children (n=22) | Healthy controls (n=47) | p-value |
|---|---|---|---|
| **Gender** | 11F/11M | 27F/20M | 0.56 |
| **Age (years)** | 12.1 ± 3.5 | 12.2 ± 3.5 | 0.88 |
| **Weight-z** | 0.28 ± 0.85 | 0.11 ± 1.03 | 0.51 |
| **Height-z** | -0.19 ± 1.01 | 0.44 ± 1.04 | 0.02 |
| **BMI-z** | 0.52 ± 0.98 | -0.15 ± 1.05 | 0.01 |
| **WC** | 69.3 ± 9.1 | 63.9 ± 9.6 | 0.03 |
| **WC-z** | 0.4 (-0.8,1.3) | -0.5 (-2.9,1.0) | 0.0002 |
| **Tricep-z** | 0.8 (-1.1, 1.7) | 0.01 (-1.2, 1.6) | 0.01 |
| **MAC-z** | -0.5 (-1.7, 0.8) | -0.8 (-2.8, 0.8) | 0.07 |
| **FFMI-z** | -1.1 (-2.2, 1.1) | -1.3 (-4.7, 1.0) | 0.14 |
| **SBP-z (mmHg)** | 0.8 (0.3, 1.2) | 0.2 (-0.3, 1.0) | 0.03 |
| **DBP-z (mmHg)** | 0.5 (0.3, 1.2) | 0.2 (-0.5,0.7) | 0.009 |
| **Years after LTx** | 8.0 ± 4.4 | - | - |
| **Liver diagnosis, n**<br>**- BA**<br>**- ALD**<br>**- Others** | <br>12<br>2<br>8 | - | - |
| **Immunotherapy, n**<br>**- Tacrolimus**<br>**- Sirolimus**<br>**- Tacrolimus + MMF** | <br>19<br>1<br>1 | - | - |

| | | | |
|---|---|---|---|
| **- Tacrolimus + CST** | 1 | | |
| **Re-LTx,n** | 3 | - | - |
| **Number of rejection** | 0 (0,1) | - | - |

Others liver etiology: Crigler-Najjar (n=3), Alpha-1-antitrypsin deficiency (n=1), Primary sclerosing cholangitis (n=2), Progressive familial intrahepatic cholestasis type 3 (n=1), Unknown(n=1)
LTx: Liver Transplantation, HC: healthy controls, BMI-z, body mass index-z score, WC, waist circumference, WC-z, waist circumference z-score; Tricep-z: tricep z-score. MAC-z: midarm circumference z-score, FFMI-z:fat free mass index z-score, SBP-z, systolic blood pressure z-score, DBP-z, diastolic blood pressure z-score, BA: Biliary atresia, ALD: Acute liver disease, MMF: mycophenolate mofetil, CST: Corticosteroid, Re-LTx: Liver re-transplantation in children with first graft failure
Data expressed in mean±SD or median (IQR) or %. P-values ≤0.05 is considered statistically significant.

## Laboratory data

**Table 3.4** presents a summary of laboratory values found in LTx children. All LTx children had normal liver synthetic function according to albumin, international normalized ratio (INR) and partial thromboplastin time (PTT). Three LTx children had elevated serum TG levels (mmol/L), while 2 had low HDL-cholesterol levels (mmol/L). Three participants were diagnosed with obesity (BMI >95$^{th}$ percentile). All had normal fasting glucose (FG, mmol/L), random glucose (RG, mmol/L), and hemoglobin A1c (HbA1c levels [%]).

Children >12.5 years at time of study had significantly higher values of TG (1.0 mmol/L vs 0.7 mmol/L [p=0.0001]) and LDL (1.9 mmol/L vs 1.5 mmol/L [p=0.04]), with children >12.5 years having significantly higher values. Children <1.47 years at time of transplant had significantly higher GGT values (p=0.02). Sex was not significantly associated to any abnormal laboratory values.

**Table 3. 4 Laboratory values and healthy reference ranges in LTx children**

| Parameter | LTx children (n=22) | Healthy reference ranges[1] |
|---|---|---|
| **TG (mmol/L)** | 0.7 (0.6, 0.9) | 0-9 y: <0.85<br>10-18 y: <1.02 |
| **TC (mmol/L)** | 3.5 ± 0.7 | <4.4 |
| **HDL (mmol/L)** | 1.4 ± 0.3 | >1.16 |
| **LDL (mmol/L)** | 1.7 ± 0.6 | <2.84 |

| | | |
|---|---|---|
| **RBG (mmol/L)** | 5.3 ± 0.9 | 3.3-11.0 |
| **FBG (mmol/L)** | 5.0 ± 0.4 | 3.3-6.0 |
| **HbA1c (%)** | 5.1 ± 0.3 | 4.3-6.1 |
| **Tacrolimus trough (µg/L)** | 4.4 (3.5, 4.8) | 3-5 |
| **Albumin (g/L)** | 43 ± 3.6 | 30-45 |
| **INR** | 1.0 (1.0, 1.1) | 0.8-1.2 |
| **PTT (seconds)** | 33 ± 3 | 27-39 |
| **AST (U/L)** | 31 (26, 40) | 6-9 y: 15-50<br>10-18 y: 10-45 |
| **ALT (U/L)** | 27 (22, 29) | <35 |
| **GGT (U/L)** | 17 (12,38) | <27 |
| **Total bilirubin (µmol/L)** | 9 (6,11) | <20 |
| **Urea (mmol/L)** | 3.8 ± 1.4 | 2.0-7.0 |
| **Creatinine (µmol/L)** | 50 ± 15 | 10-120 |

[1]Pediatric reference values obtained from Alberta Health services:
https://www.albertahealthservices.ca/assets/wf/lab/wf-lab-dth-meditech-hcis-reference-intervals.pdf
LTx children: liver transplant children, M: males, F: females, y: years TG: triglycerides, TC: total cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C, low density lipoprotein cholesterol, RG: random blood glucose, FG: fasting blood glucose, HbA1c: hemoglobin A1c, INR: International normalized ratio, PTT: Partial thromboplastin time, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: gamma-glutamyl transpeptidase,
Data expressed in mean±SD or median (IQR)

### Sarcopenia

A total of 4 LTx children (18%) had confirmed of sarcopenia, while 8 LTx children (36%) had probable sarcopenia. Please refer to Methods section 3.3.5 for details on sarcopenia definition. None of HC were classified with sarcopenia. Due to small sample size (8 sarcopenia vs. 14 non-sarcopenia), p-values could be difficult to interpret.

Probable sarcopenia was not significantly associated to anthropometric and demographic variables (**Table 3.5**). Blood pressure z-scores (systolic blood pressure z-score [SBP-z] and diastolic blood pressure z-score [DBP-z]) were not associated to sarcopenia. When adjusted for age at time of study (above/below median age of 12.5 years) and age at time of transplant (above/below median age of 1.47 years) no difference in the prevalence of sarcopenia was found. Sarcopenia was not associated with abnormal laboratory values in LTx children (**Table 3.6**). No significant differences when adjusting

for sex and diagnosis were found in anthropometric, demographic and laboratory variables.

**Table 3. 5 Anthropometric, demographic, and laboratory variables for sarcopenic and non-sarcopenic children after liver transplant**

| Parameter | Sarcopenia (n=8) | Non-sarcopenia (n=14) | p-value |
|---|---|---|---|
| **Age during study (y)** | 11.0±3.5 | 12.7±3.5 | 0.31 |
| **Age at LTx (y)** | 5.4±4.9 | 3.6±4.6 | 0.39 |
| **Sex** | 6M/2F | 5M/9F | 0.08 |
| **Weight-z** | 0.1±1.0 | 0.4±0.8 | 0.56 |
| **Height-z** | -0.3±1.3 | -0.1±0.8 | 0.70 |
| **BMI-z** | 0.4±1.3 | 0.6±0.9 | 0.61 |
| **WC-z** | 0.3±0.6 | 0.3±0.6 | 0.89 |
| **TG (mmol/L)** | 0.7±0.3 | 1.1±0.7 | 0.14 |
| **HDL (mmol/L)** | 1.4±0.3 | 1.4±0.3 | 0.94 |
| **LDL (mmol/L)*** | 1.5±0.6 | 1.9±0.6 | 0.13 |
| **TC (mmol/L)*** | 3.1±0.8 | 3.7±0.5 | 0.06 |

*LDL and TC values: 21 patients
y: years, LTx: liver transplant, M:males, F: females, weight-z: weight z-score, height-z: height z-score, BMI-z:body mass index-z score, WC-z: waist circumference z-score, TG: triglycerides, HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, TC: total cholesterol
Data expressed in mean±SD or median. P-values ≤0.05 is considered statistically significant.

**Table 3. 6 Percentage of participants with laboratory values outside reference ranges in children after liver transplant**

| Parameter | Sarcopenia (n=8) | Non-sarcopenia (n=14) | p-value | Healthy reference ranges[1] |
|---|---|---|---|---|
| **TG (mmol/L)** | 0 | 29 | 0.13 | 0-9 y:<0.85<br>10-18y: <1.02 |
| **HDL (mmol/L)** | 25 | 29 | 0.37 | >1.16 |
| **LDL (mmol/L)*** | 0 | 7 | 0.61 | <2.84 |
| **TC (mmol/L)*** | 13 | 21 | 0.38 | <4.4 |
| **AST (U/L)** | 0 | 21 | 0.23 | 6-9 y: 15-50<br>10-18 y: 10-45 |
| **ALT (U/L)** | 0 | 21 | 0.23 | <35 |
| **GGT (U/L)** | 38 | 29 | 0.32 | <27 |
| **Tacrolimus trough (µg/L)** | 25 | 36 | 0.36 | 3-5 |

[1]Pediatric reference values obtained from Alberta Health services: https://www.albertahealthservices.ca/assets/wf/lab/wf-lab-dth-meditech-hcis-reference-intervals.pdf
*LDL and TC values: 21 patients
TG: triglycerides, HDL: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, TC: total cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transpeptidase
Data expressed in %. P-values ≤0.05 is considered statistically significant

**Cardiometabolic dysregulation**

The overall prevalence of CMD according to ATP-III (n=2) was 9%, 50% by Magnussen et al. criteria (n=11) and 4.5% (n=1) according to WHO classification. Due to small sample size in ATP-III (2 CMD vs. 20 no CMD) and WHO (1 CMD vs 21 no CMD), p-values could be difficult to interpret. No differences in CMD prevalence according to ATP-III, WHO and Magnussen et al. criteria were observed when adjusted for age during study (above/below median 12.5 years) and age at transplant (above/below median 1.47 years). Association between laboratory markers, anthropometric and demographic parameters and CMD according to different criteria are illustrated in **Table 3.7** When adjusted for age during study **(**above/below median age of 12.5 years) significant associations in weight-z (p=0.04), BMI-z (p=0.05), WC-z (p=0.02), systolic blood pressure-z score (p=0.008), diastolic blood pressure z-score (p=0.04) and TG levels (p<0.0001) according to ATP-III definition of cardiometabolic dysregulation were observed, with children <12.5 years during study having significantly lower values for weight-z, BMI-z and WC-z. Systolic blood pressure z-score (p=0.006), diastolic blood pressure z-score (p=0.01) and TG levels (p=0.02) were significantly associated to cardiometabolic dysregulation according to Magnussen et al. definition when adjusted for age, with children <12.5 years during study having higher systolic blood pressure z-score and lower TG levels. Systolic blood pressure z-score (p=0.02) and TG levels (p=0.0009) had significant associations with cardiometabolic dysregulation according to WHO criteria when adjusting for age (above/below median age of 12.5 years). When adjusted for age during transplant (above/below median age of 1.47 years), children <1.47 had significantly higher systolic blood pressure z-score (p=0.002, p=0.01 and p=0.003 for ATP-III, Magnussen et. al and WHO criteria, respectively) and diastolic blood pressure

z-score ($p=0.02$, $p=0.05$ and $p=0.001$ for ATP-III, Magnussen et. al and WHO criteria, respectively), plus significantly lower TG levels ($p<0.0001$, $p<0.005$, $p=0.006$ for ATP-III, Magnussen et. al and WHO criteria, respectively). Children >1.47 years had significantly lower WC-z ($p=0.02$) according to ATP-III criteria.

**Table 3. 7 Anthropometric, demographic and laboratory values in children with and without cardiometabolic dysregulation according to ATP-III, Magnussen et al. and WHO criteria**

| Characteristic | ATP-III | | p-value | Magnussen et al. | | p-value | WHO | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | CMD (n=2) | No CMD (n=20) | | CMD (n=11) | No CMD (n=11) | | CMD (n=1) | No CMD (n=21) | |
| Age | 12.0±3.6 | 12.4±3.7 | 0.89 | 12.4±3.8 | 11.8±3.3 | 0.70 | - | 11.9±3.5 | 0.40 |
| Age at LTx | 4.3(0.7,8.0) | 1.5 (0.5,15.2) | 0.97 | 1.1 (0.5,12.8) | 2.2 (0.7,15.2) | 0.71 | - | 4.1±4.7 | 0.42 |
| Sex | 1M/1F | 10M/10F | 1.0 | 5M/6F | 6M/5F | 0.68 | 1M | 10M/11F | 0.33 |
| Weight-z | 0.17±0.76 | 1.43±1.07 | 0.04 | 0.60±1.05 | -0.04±0.43 | 0.07 | 0.67 | 0.26±0.87 | 0.65 |
| Height-z | -0.23±1.00 | 0.19±1.35 | 0.59 | -0.04±0.91 | -0.34±1.12 | 0.49 | -0.77 | -0.16±1.02 | 0.57 |
| BMI-z | 0.39±0.93 | 1.77±0.46 | 0.06 | 0.77±1.15 | 0.27±0.75 | 0.24 | 1.44 | 0.47±0.98 | 0.34 |
| WC-z | 1.2±0.20 | 0.22±0.52 | 0.02 | 0.41±0.68 | 0.20±0.45 | 0.41 | 1.02 | 0.27±0.56 | 0.21 |
| SBP-z | 2.2±0.2 | 0.6(-0.4,2.5) | 0.008 | 1.2(-0.0,2.5) | 0.3(-0.4,1.2) | 0.005 | 2.3 | 0.7 (-0.4,2.5) | 0.06 |
| DBP-z | 1.7±0.5 | 0.4(-0.7,2.0) | 0.03 | 1.3(-0.7,2.0) | 0.3(-0.0,0.7) | 0.02 | 2.0 | 0.4(-0.7,2.0) | 0.05 |
| TC (mmol/L) | 3.9±0.7 | 2.4±0.7 | 0.33 | 3.3±0.7 | 3.6±0.6 | 0.36 | 3.4 | 3.5±0.7 | 0.94 |
| TG (mmol/L) | 2.6±0.3 | 0.7±0.3 | <0.0001 | 1.2±0.7 | 0.6±0.2 | 0.01 | 2.8 | 0.8±0.5 | 0.004 |
| FG (mmol/L) | 4.8±0.3 | 5.0±0.5 | 0.59 | 4.9±0.3 | 5.0±0.5 | 0.53 | 5.0 | 5.0±0.5 | 0.94 |
| LDL (mmol/L) | 1.4±0.4 | 1.8±0.6 | 0.42 | 1.6±0.5 | 1.9±0.7 | 0.26 | 1.1 | 1.8±0.6 | 0.28 |
| HDL (mmol/) | 1.4±0.4 | 1.4±0.3 | 0.98 | 1.3±0.4 | 1.5±0.2 | 0.12 | 1.1 | 1.4±0.3 | 0.35 |
| Albumin (g/L) | 42.5±3.5 | 42.6±3.7 | 0.97 | 42.0±3.2 | 43.2±4.0 | 0.45 | 40.0 | 42.7±3.6 | 0.47 |
| GGT (U/L) | 31.5±9.2 | 36.2±63.2 | 0.92 | 24.4±19.7 | 14(5,292) | 0.39 | 2.0 | 16(5,292) | 0.97 |
| AST (U/L) | 31.5±0.7 | 34.9±14.0 | 0.74 | 34.1±16.2 | 35.0±10.6 | 0.88 | 31.0 | 34.7±13.7 | 0.79 |
| ALT (U/L) | 37.5±17.7 | 29.7±17.0 | 0.54 | 31.2±18.7 | 29.5±15.6 | 0.83 | 50.0 | 29.4±16.6 | 0.24 |

ATP-III: Adult Treatment Panel III[16], Magnussen et al (2010)[139] WHO: World Health Organization[137], CMD: cardiometabolic dysregulation, LTx: liver transplant, M: males, F: females, y: years, weight-z: weight z-score, height-z, height z-score, BMI-z: body mass index-z score, WC-z: weight circumference-z score, SBP-z:systolic blood pressure z-score, DBP-z: diastolic blood pressure z-score, TC: total cholesterol,TG: triglycerides, FG: fasting blood glucose, HDL: high density lipoprotein cholesterol, LDL:low density lipoprotein cholesterol, GGT: gamma glutamyl transpeptidase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

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

**Cardiometabolic dysregulation and sarcopenia**

No significant differences were observed in demographic, anthropometric and laboratory values by sarcopenia/no sarcopenia and CMD/no CMD according to the definition of CMD by ATP-III, Magnussen et al., and WHO criteria (**Table 3.8**). When adjusting age at time of study (above/below median age of 12.5 years) and age at transplant (above/below median age of 1.47 years) no significant association with the sarcopenia and cardiometabolic dysregulation according to ATP-III, Magnussen et al. and WHO was found. The association of CMD and sarcopenia in LTx children is presented in **Table 3.9**.

**Table 3. 8 Demographic, anthropometric and laboratory values by sarcopenia and CMD according to ATP-III, Magnussen et al. and WHO criteria**

| Variable | p-value | | |
|---|---|---|---|
| | ATP-III | Magnussen et al. | WHO |
| **Age** | 0.33 | 0.60 | 0.39 |
| **Sex** | 0.08 | 0.24 | 0.05 |
| **BMI-z** | 0.93 | 0.90 | 0.72 |
| **WC-z** | 0.65 | 0.84 | 0.93 |
| **SBP-z** | 0.95 | 0.80 | 0.72 |
| **DBP-z** | 0.68 | 0.90 | 0.83 |
| **FG** | 0.78 | 0.95 | 0.90 |
| **TG** | 0.28 | 0.27 | 0.20 |
| **HDL** | 0.95 | 0.61 | 0.82 |
| **LDL** | 0.07 | 0.21 | 0.08 |

ATP-III: Adult Treatment Panel III[16], Magnussen et al (2010)[139] WHO: World Health[137], BMI-z: body mass index-z score, WC-z: weight circumference-z score, SBP-z:systolic blood pressure z-score, DBP-z: diastolic blood pressure z-score, TG: triglycerides, FG: fasting glucose, HDL: high density lipoprotein cholesterol, LDL:low density lipoprotein cholesterol

Data expressed in p-values. P-values ≤0.05 is considered statistically significant.

**Table 3. 9 Cardiometabolic dysregulation and liver transplant children with and without sarcopenia**

| Criteria | Sarcopenia (n=8) | No sarcopenia (n=14) | p-value |
|---|---|---|---|
| **ATP-III** | 0 | 1M/1F | 0.39 |
| **WHO** | 0 | 1M | 0.63 |
| **Magnussen et al.** | 2M/1F | 3M/5F | 0.23 |

ATP-III: Adult Treatment Panel III[16], WHO: World Health Organization[137], Magnussen et al (2010)[139]

M:males, F:females

P-values ≤0.05 is considered statistically significant.

**Dietary data**

Dietary intake of LTx children and HC is presented in **Table 3.10**. Differences in dietary intake of fat (p=0.05), saturated fat (p=0.01), calcium (p=0.01) and vitamin D (p<0.0001) were noted. Dietary intake per 1000kcal in LTx and HC is presented in **Appendix A-1**. Percentage of participants in both groups who did not meet dietary intake requirements is presented in **Table 3.11**. The proportion of HC who did not meet calcium (72%) and vitamin D (74%) intake was significantly higher compared to the proportion of LTx children (36% for calcium and 36% for vitamin D). When adjusted for sex for comparison between groups, males had significantly higher selenium (p=0.01) and protein intake (g) (p=0.01). Males had significantly higher selenium intake (p=0.01) in the LTx group. LTx children >12.5 years during time of study had significantly higher calorie (p=0.05), omega-3 (p=0.02) and selenium (p=0.03) intake. LTx children >1.47 years during transplant had significantly higher protein intake (g) (p=0.04). No associations between dietary intake and diagnosis were found in the LTx group.

**Table 3. 10 Dietary intake (3-day food record) in liver transplant children and healthy controls**

| | LTx children (n=22) | HC (n=47) | p-value | DRIs |
|---|---|---|---|---|
| **Energy (kcal)** | 2174±453 | 1944±544 | 0.09 | - |
| **Protein (g)** | 84±27 | 80±34 | 0.66 | 13-52[1] |
| **% protein** | 15±3 | 16±3 | 0.28 | 10-30[2] |
| **Carbohydrate (g)** | 281±53 | 256±72 | 0.15 | 130[1] |
| **% carbohydrate** | 52±6 | 53±6 | 0.57 | 45-65[2] |
| **Fat (g)** | 81±26 | 69±25 | 0.05 | - |
| **% Fat** | 33±6 | 32±6 | 0.33 | 25-35[2] |
| **Saturated fat (g)** | 29±10 | 23±9 | 0.01 | - |
| **% Saturated fat** | 12±3 | 11±3 | 0.16 | <10[1] |
| **MUFA (g)** | 27±9 | 24±9 | 0.15 | - |
| **% MUFA** | 12±6 | 12±4 | 0.85 | 10[4] |

| | | | | |
|---|---|---|---|---|
| **PUFA** | 14±6 | 12±5 | 0.12 | - |
| **% PUFA** | 6±2 | 6±2 | 0.52 | 15[4] |
| **Omega-3 (g)** | 1.2±0.7 | 1.6±0.9 | 0.11 | 0.9-1.6[3] |
| **Calcium (mg) (total)*** | 1267±607 | 925±459 | 0.01 | 1100-1300[1] |
| **Vitamin D (IU) (total)*** | 1009±598 | 362±395 | <0.0001 | 600[1] |
| **Selenium (µg)** | 120±37 | 112±36 | 0.43 | 30-55[1] |

[1]RDA:Recommended Daily Allowance[150], [2]AMDR:Acceptable Macronutrient Distribution Range[150]. [3]AI:Adequate Intake[149-152]. [4]Obtained from American Heart Association[138]: https://www.heart.org/-/media/files/healthy-living/company-collaboration/inap/fats-white-paper-ucm_475005.pdf

*Total intake for calcium and vitamin D includes diet and supplementation. Supplementation: Vitamin D: 267-2000 IU daily, Calcium: 5-500 mg daily (single or multi-preparation)

LTx children: liver transplant children, HC: healthy controls, DRIs: Daily Reference Intakes, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids.

Data expressed in mean±SD. P-values ≤0.05 is considered statistically significant.

## Table 3. 11 Percentage of participants outside the range of dietary intake recommendations

| | LTx children (n=22) | HC (n=47) | p-value | DRIs |
|---|---|---|---|---|
| **% protein** | 5 | 0 | 0.31 | 10-30[2] |
| **% carbohydrate[a]** | 14 | 11 | 0.28 | 45-65[2] |
| **% Fat[b]** | 45 | 40 | 0.19 | 25-35[2] |
| **% Saturated fat** | 68 | 51 | 0.08 | <10[1] |
| **% MUFA** | 36 | 36 | 0.21 | 10[4] |
| **% PUFA** | 100 | 100 | - | 15[4] |
| **Omega-3 (g)** | 9 | 2 | 0.20 | 0.9-1.6[3] |
| **Calcium (mg) (total)** | 36 | 72 | 0.004 | 1100-1300[1] |
| **Vitamin D (IU) (total)** | 36 | 74 | 0.002 | 600[1] |
| **Selenium (µg)** | 0 | 0 | - | 30-55[1] |

[1]RDA:Recommended Daily Allowance[150], [2]AMDR:Acceptable Macronutrient Distribution Range[150]. [3]AI:Adequate Intake[149-152], [4]Obtained from American Heart Association[138]: https://www.heart.org/-/media/files/healthy-living/company-collaboration/inap/fats-white-paper-ucm_475005.pdf

[a]Participants below 45% daily intake

[b]One LTx participant below 25% daily intake, 4 HC under 25%

Total intake for calcium and vitamin D includes diet and supplementation. Supplementation: Vitamin D: 267-2000 IU daily, Calcium: 5-500 mg daily (single or multi-preparation)

LTx children: liver transplant children, HC: healthy controls, DRIs: Daily Reference Intakes, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

Data expressed in %. P-values ≤0.05 is considered statistically significant.

**Association between dietary components and cardiometabolic dysregulation according to ATP-III, WHO, and Magnussen et al. criteria in children post-liver transplant**

Due to small sample size in ATP-III (2 CMD vs. 20 no CMD) and WHO (1 CMD vs 21 no CMD), p-values could be difficult to interpret. Differences between total calcium intake and cardiometabolic dysregulation according to the definition by the ATP-III in children post-LTx (p=0.05) were observed (**Table 3.12**). No effects of age during study (above/below median age 12.5 years) and age during transplant (above/below median age 1.47 years) were observed on dietary intake and cardiometabolic dysregulation according to ATP-III and WHO criteria. Children >1.47 years had significantly higher saturated fat intake (p=0.02) and calorie intake (p=0.03) according to Magnussen et al. definition. Females had significantly lower calcium intake according to ATP-III criteria (p=0.05). Non-biliary atresia patients had significantly higher carbohydrate and calcium intake (p=0.008 and p=0.02 respectively) according to ATP-III. Association between dietary intake per 1000kcal and cardiometabolic dysregulation in children after LTx is presented in **Appendix A-2**.

**Table 3. 12 Dietary components in children after liver transplant classified as having cardiometabolic dysregulation**

| | ATP-III | | p-value | Magnussen et al. | | p-value | WHO | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | CMD (n=2) | No CMD (n=20) | | CMD (n=11) | No CMD (n=11) | | CMD (n=1) | No CMD (n=21) | |
| Energy (kcal) | 2492±510 | 2142±449 | 0.30 | 2235±583 | 2112±288 | 0.53 | 2131 | 2176±464 | 0.92 |
| Protein (g) | 88 (66, 109) | 83±28 | 0.83 | 87±31 | 80±23 | 0.58 | 66 | 84±27 | 0.52 |
| % protein | 14±2 | 15±3 | 0.43 | 15±3 | 15±4 | 0.94 | 12 | 15±3 | 0.31 |
| Carbohydrate (g) | 339 (296,382) | 275±50 | 0.10 | 292±61 | 271±45 | 0.36 | 296 | 281±54 | 0.78 |
| % carbohydrate | 55±1 | 52±6 | 0.58 | 53±6 | 51±6 | 0.51 | 55 | 52±6 | 0.64 |
| Fat (g) | 90 (77, 103) | 81±26 | 0.62 | 82±30 | 80±22 | 0.85 | 77 | 82±26 | 0.86 |
| % fat | 33±1 | 33±6 | 0.83 | 33±5 | 34±7 | 0.53 | 33 | 33±6 | 0.95 |
| Saturated fat (g) | 33 (21, 45) | 29±10 | 0.61 | 30±13 | 29±8 | 0.84 | 21 | 30±10 | 0.41 |
| % saturated fat | 12±4 | 12±3 | 0.79 | 12±3 | 12±3 | 0.54 | 9 | 12±3 | 0.26 |
| MUFA (g) | 29±3 | 27±10 | 0.81 | 27±10 | 28±9 | 0.79 | 27 | 27±10 | 0.96 |
| % MUFA | 11±2 | 12±6 | 0.67 | 13±7 | 11±4 | 0.59 | 9 | 12±6 | 0.57 |
| PUFA (g) | 16±4 | 14±6 | 0.68 | 13±5 | 15±7 | 0.41 | 19 | 14±6 | 0.43 |
| % PUFA | 6±3 | 6±2 | 0.92 | 5±2 | 6±2 | 0.19 | 8 | 6±2 | 0.31 |
| Omega-3 (g) | 1.5 (1, 2) | 1.2±0.7 | 0.56 | 1.3±0.6 | 1.2±0.8 | 0.76 | 2 | 1.2±0.7 | 0.25 |
| Calcium (mg) (total) | 2055±698 | 1188±556 | 0.05 | 1356±721 | 1178±485 | 0.50 | 1561 | 1253±618 | 0.63 |
| Vitamin D (IU) (total) | 1346±173 | 976±617 | 0.41 | 965±656 | 1054±563 | 0.73 | 1223 | 999±611 | 0.72 |
| Selenium (µg) | 118±26 | 120±39 | 0.93 | 117±47 | 122±26 | 0.75 | 99 | 120±38 | 0.58 |

Total intake for calcium and vitamin D includes diet and supplementation. Supplementation: Vitamin D: 267-2000 IU daily, Calcium: 5-500 mg daily (single or multi-preparation).

LTx children: liver transplant children, ATP-III: Adult Treatment Panel III[16], Magnussen et al.[139] WHO: World Health Organization[137], CMD: cardiometabolic dysregulation, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

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

**Association between dietary intake and sarcopenia**

Association between dietary data and sarcopenia in children post-LTx is presented in **Table 3.13.** Sarcopenia patients had significantly higher intake of protein (%, p=0.04), while non-sarcopenia patients had significantly higher fat (g, p=0.02), and PUFA (g, p=0.02; %, p=0.01) intake. Age during study was significantly associated to percentage protein (p=0.02) and sarcopenia, with children >12.5 years during study having significantly higher intake. Males had significantly higher MUFA (g) (p=0.04) intake. Sex was associated to PUFA (g and %, p=0.02 for both) and sarcopenia, with females having significantly higher intake. No associations between age at transplant (above/below median 1.47 years), dietary intake and sarcopenia were found. Diagnosis was not associated to dietary intake and sarcopenia. Association between dietary intake per 1000kcal and sarcopenia in LTx children is presented in **Appendix A-3**. **Table 3.14** presents percentage of participants that did not meet DRI requirements.

**Table 3. 13 Dietary data sarcopenia and non-sarcopenia children after liver transplant**

| Component | Sarcopenia (n=8) | Non-sarcopenia (n=14) | p-value | DRIs |
|---|---|---|---|---|
| Energy (kcal) | 2102±582 | 2214±379 | 0.30 | - |
| Protein (g) | 90±32 | 80±25 | 0.43 | 13-52[1] |
| % protein | 17±3 | 14±3 | 0.04 | 10-30[2] |
| Carbohydrate (g) | 281±65 | 281±48 | 0.99 | 130[1] |
| % carbohydrate | 54±7 | 51±5 | 0.27 | 45-65[2] |
| Fat (g) | 71±31 | 88±20 | 0.13 | - |
| % fat | 29±5 | 35±5 | 0.02 | 25-35[2] |
| Saturated fat (g) | 27±11 | 31±10 | 0.33 | - |
| % saturated fat | 11±3 | 12±3 | 0.33 | <10[1] |
| MUFA (g) | 23±11 | 30±8 | 0.10 | - |
| % MUFA | 12±8 | 12±3 | 0.77 | 10[4] |
| PUFA (g) | 11±4 | 16±6 | 0.02 | - |
| % PUFA | 5±1 | 7±2 | 0.01 | 15[4] |
| Omega-3 (g) | 0.9±0.6 | 1.4±0.6 | 0.06 | 0.9-1.6[3] |
| Calcium (mg) (total) | 1353±518 | 1218±665 | 0.62 | 1100-1300[1] |
| Vitamin D (IU) (total) | 1310±577 | 838±558 | 0.07 | 600[1] |
| Selenium (µg) | 127±45 | 115±33 | 0.46 | 30-55[1] |

[1]RDA:Recommended Daily Allowance[150], [2]AMDR:Acceptable Macronutrient Distribution Range[150]. [3]AI:Adequate Intake[149-152], [4]Obtained from American Heart Association[138]: https://www.heart.org/-/media/files/healthy-living/company-collaboration/inap/fats-white-paper-ucm_475005.pdf

Total intake for calcium and vitamin D includes diet and supplementation. Supplementation: Vitamin D: 267-2000 IU daily, Calcium: 5-500 mg daily (single or multi-preparation)

DRIs: Dietary Reference Intakes, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

Data expressed in mean±SD. P-values ≤0.05 is considered statistically significant.

**Table 3. 14 Percentage of sarcopenia/non-sarcopenia children outside the range of dietary intake recommendations**

| | Sarcopenia (n=8) | No-sarcopenia (n=14) | p-value | DRIs |
|---|---|---|---|---|
| **% protein** | 0 | 7 | 0.63 | 10-30[2] |
| **% carbohydrate[a]** | 13 | 14 | 0.47 | 45-65[2] |
| **% Fat[b]** | 38 | 50 | 0.29 | 25-35[2] |
| **% Saturated fat** | 63 | 71 | 0.32 | <10[1] |
| **% MUFA** | 50 | 29 | 0.21 | 10[4] |
| **% PUFA** | 100 | 100 | - | 15[4] |

| | | | | |
|---|---|---|---|---|
| **Omega-3 (g)** | 25 | 0 | 0.12 | 0.9-1.6[3] |
| **Calcium (mg) (total)** | 13 | 50 | 0.08 | 700-1300[1] |
| **Vitamin D (IU) (total)** | 13 | 50 | 0.08 | 600[1] |
| **Selenium (µg)** | 0 | 0 | - | 30-55[1] |

[1]RDA:Recommended Daily Allowance[150], [2]AMDR:Acceptable Macronutrient Distribution Range[150]. [3]AI:Adequate Intake[149-152], [4]Obtained from American Heart Association[138]: https://www.heart.org/-/media/files/healthy-living/company-collaboration/inap/fats-white-paper-ucm_475005.pdf

[a]Participants below 45% daily intake

[v]One sarcopenia participant below 25% daily intake

Total intake for calcium and vitamin D includes diet and supplementation. Supplementation: Vitamin D: 267-2000 IU daily, Calcium: 5-500 mg daily (single or multi-preparation)

DRIs: Dietary Reference Intakes, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

Data expressed in %. P-values ≤0.05 is considered statistically significant.

### 3.5 Discussion

Sarcopenia is a growing concern in pediatric transplant populations. Our group's previous studies reveal a high prevalence of sarcopenia in children who underwent liver transplant. [13, 14, 52] Few studies have examined the relationship between cardiometabolic dysregulation (CMD) and diet in children post-transplant. This is the first study to examine the association of diet and prevalence of CMD and sarcopenia in a pediatric LTx population. High prevalence of sarcopenia in children 10 years post-LTx had previously been confirmed.[52] A low prevalence of CMD was observed in our study population according to two definitions (ATP-III, WHO) and higher prevalence according to one definition (Magnussen et al.).[16, 137, 139] Our findings do not align with the first hypothesis because high consumption of carbohydrates, fat, and low protein did not increase the prevalence of CMD and sarcopenia. For our second hypothesis, an association between total calcium intake and presence of CMD according to ATP-III was observed when using absolute values. Interestingly, the CMD group had significantly higher calcium intake according to ATP-III criteria. This contrasts previous findings where HC were reported

to have significantly higher calcium intake when compared to children who underwent LTx.[118] Even though CMD prevalence for Magnussen et al. was higher, no associations were found between CMD and dietary intake when using this criteria. When adjusting for energy, no differences between the CMD and no CMD group were found. Association between dietary intake and sarcopenia when using absolute values were observed for protein (%), fat (%), and PUFA (%, g). The sarcopenia group had significantly higher protein intake, lower fat and PUFA intake. When adjusting for energy, the sarcopenia group had significantly higher protein intake (%).

Excess consumption of carbohydrates (>60% of total calorie intake) and fat (>30% of total calorie intake) have been reported in children post-LTx[117, 118] High intake of these macronutrients have been linked to abnormal cholesterol, triglyceride and blood glucose levels with subsequent risk of CMD.[27] We observed slightly higher (>30%) than recommended consumption (AMDR) of fat and saturated fat (>10%) but adequate carbohydrate intake when comparing between LTx children and HC.[150] Differences in the total fat (g) and saturated fat (g) intake were observed between LTx children and HC. These findings differ from a previous study, where investigators did not find a significant difference in fat (g) and saturated fat (g) intake between LTx children and HC.[118] Authors found HC had significantly higher carbohydrate intake (g), contrasting our findings.[118] Similar to our results, Chambers et al. (2019) noted LTx participants who met metabolic syndrome criteria did not have significantly different dietary intake compared to those who did not.[118] Even though slightly higher than recommended AMDR consumption of fat was found, this might not be clinically relevant and could help explain the lack of associations found between diet and CMD since cardiometabolic alterations have been observed at higher percentage intake.[28, 148, 156] Adequate carbohydrate consumption

according to AMDR recommendations in our population could have also contributed to the lack of associations.[27, 148, 150]

Adequate protein intake is vital for muscle protein synthesis and preservation of muscle function.[103] Suboptimal protein intake has been associated with sarcopenia in adult population[10], which is in contrast with our findings. Children with sarcopenia (>2.5 g/kg/d) and without sarcopenia (1.7 g/kg/d) had protein intakes according to recommendations, and differences were only found in percentage protein intake with children >12.5 years during study having significantly higher intake. However, participants who did not present sarcopenia had lower than recommended protein intake (AMDR) (7% of total non-sarcopenia population).[150] Adequate protein consumption could be related to higher concern from families and the clinical team regarding protein intake due to energy-protein malnutrition in the pre-LTx period.[10] Other studies have found no differences in protein intake (g and %) when comparing LTx children and HC, similar to our results.[117, 118] Adequate protein intake could explain the lack of associations between diet, sarcopenia and CMD in our population as well.[150]

Insufficient calcium or vitamin D intake have also been observed in children post-LTx.[117, 118] Vitamin D is related to muscle protein synthesis and muscle weakness, regulates blood pressure and generation of inflammatory cytokines.[26, 103] Calcium is a signaling molecule for muscle fibres and altered calcium status has been linked to muscle weakness.[24, 101] Most participants were taking vitamin D [267-2000 IU daily] and calcium supplements [5-500 mg daily], which could explain adequate dietary intake according to recommendations for both micronutrients and higher intake of these micronutrients in LTx. Chambers et al. (2019) observed LTx children consumed significantly less calcium than HC, but no differences in vitamin D intake.[118] Children >1.47 years during transplant had significantly higher saturated fat intake, likely linked to higher calorie intake after

infancy. Lower calcium intake according to ATP-III criteria in females is probably related to supplementation: female participant was not on supplementation while male participant was. Omega-3 fatty acids and selenium protect against oxidative damage and are considered important in reducing the risk for sarcopenia.[24, 103]. Studies examining sarcopenia have found selenium and calcium are associated to better muscle function test results, while low intake of omega-3 was associated to better protein synthesis.[24] No associations between micronutrients and sarcopenia were found in our study, and this could be due to our population having overall adequate micronutrients consumption.

We found similar prevalence of CMD by ATP-III and WHO criteria, with higher prevalence according to Magnussen et al. ATP-III and WHO are more conservative in their definitions, while Magnussen et al. have a wider range. Prevalence of CMD by ATP-III and WHO were lower than expected. We expected prevalence of CMD to be between what previous studies have reported which is 10-25% after organ transplantation. We found 4.5-9% by ATP-III and WHO criteria.[20, 88, 92, 118] Our findings contrast evidence from a previous study from 2019 conducted in LTx children where a higher prevalence of CMD (22%) with classification similar to ATP-III and WHO definitions was observed.[118] Laboratory markers of CMD were within normal ranges in most of our study population. This is different from existing literature, where high prevalence of hyperglycemia, hypercholesterolemia, and hypertriglyceridemia have been found in pediatric transplant populations.[18, 118 15, 87, 88, 92] These studies had a larger number of participants and/or longer follow-up after LTx (up to 24.5 years). Our group had previously explored cardiometabolic dysregulation in pediatric LTx participants and found recipients with healthy weights and free of corticosteroid therapy had low expression of CMD over 10 years.[16] This might help explain the differences found in comparison with previous evidence, where a larger proportion of participants were or had

been on corticosteroid therapy and had higher BMI. For instance, it is known corticosteroids increase fat deposition and affect insulin synthesis, while contributing to weight gain.[20, 121, 122, 124] Due to a corticosteroid minimization protocol implemented in our centre in 2007, most of our participants are on corticosteroid-free therapy. Our population of LTx children had a BMI within healthy reference ranges, with only one female participant with BMI >95$^{th}$ percentile. Even though only 11% of LTx participants met guidelines for recommended number of steps per day for age and gender,[10, 52] other studies have not found associations between CMD and physical activity measures.[118] Interestingly, children >9 years age at time of study presented abnormal lipid values. All children >9 years and with abnormal TG levels met CMD criteria by Magnussen et al. Age could be an important factor, since children may experience insulin resistance during puberty, which could potentially affect lipid blood levels.[16, 157] During puberty, growth hormone levels rise. Evidence shows growth hormone can cause insulin resistance.[158] Furthermore, studies suggest that during puberty lipolysis and fatty acid oxidation increase to preserve lean muscle mass and to maximize fat as energy.[158] Other studies in post-transplant pediatric populations have included older participants (up to 30 years old).[15, 19, 20, 90, 118] This could also account for CMD prevalence differences, since metabolic complications tend to appear after 10 years post-LTx.[89] The majority of our population (60%) was younger than 12 years, and 72% were less than 10 years after LTx.

This study has its limitations. The cross-sectional single-centre study approach does not permit to test causality and could limit generalization of results. One of the biggest limitations was smaller sample size, and therefore healthy controls were included to increase power. Our cohort is representative of LTx pediatric population, as it is alike other centres in terms of anthropometric, demographics and liver disease type, plus the majority of our LTx participants were diagnosed with Biliary Atresia (>50%) ensuring

heterogeneity of liver disease diagnosis[10]. However, post-hoc tests revealed insufficient power to detect differences in most primary outcome variables (**Appendix A-4 to Appendix A-11**). We did not have access to markers of inflammation such as CRP, or more specific markers such as serum adiponectin, thioredoxin-1 and pentraxin-3, as used in other studies exploring sarcopenia-related inflammation.[35] Having this information would have allowed for further analysis between diet and markers of inflammation impacting sarcopenia. We also did not have measures for insulin resistance, as insulin is not measured as part of routine clinical care in our centre. Having insulin values would have further enhanced the ability to detect associations between dietary patterns, CMD and sarcopenia. However, dietary influence on insulin resistance could have been hidden by antirejection therapy (i.e tacrolimus), whose side effects includes suppression of insulin secretion.[13] Additionally, laboratory tests to measure insulin and markers of inflammation would have required additional blood draws, making the procedure more invasive. Under and overreporting of dietary intake is one of the major challenges in nutrition research and could have affected our findings regarding association between diet, CMD and sarcopenia. Nonetheless, accuracy of reporting was examined prior to analysis and data per 1000 kcal analyzed only showed differences in protein intake (% and g). Inclusion of ATP-III[16], WHO[137] and Magnussen et al.[139] criteria allowed for comparison between definitions.

Our data analysis revealed there was a lack of association between dietary intake and prevalence of sarcopenia and cardiometabolic dysregulation. We did not observe an association between selenium, calcium, vitamin D or omega-3 fatty acids and prevalence of sarcopenia and cardiometabolic risk. Nonetheless, this does not mean diet does not have a crucial role in the development and worsening of these conditions. More research in this area is needed, and future studies should focus on mitigating our limitations. For

example, researchers should assure a bigger sample size (50-100+ participants in each group) and gather relevant laboratory data to measure both CMD and sarcopenia, keeping in mind some markers may be affected by drug therapies in transplant populations. Future studies could also try to obtain antioxidant and anti-inflammatory micronutrient levels in the blood. This data could be used to assess any potential associations between key micronutrients and sarcopenia and cardiometabolic risk.

### 3.6 Conclusions

In conclusion, children who underwent liver transplant are at risk of developing cardiometabolic diseases and sarcopenia. Healthy eating and physical activity counseling to prevent metabolic dysregulation is important in post-transplant period. More studies in transplant populations exploring the prevalence of CMD, sarcopenia and dietary patterns influencing both are needed. These studies should focus on a longitudinal approach with bigger sample size, multiple measurements for SMM and dietary intake.

# Chapter 4: Conclusions and General Discussion

## 4.1 Introduction

Sarcopenia is a muscle disease characterized by low skeletal muscle mass (myopenia), muscle strength and physical performance.[3] This disease has been identified in liver transplant populations.[13, 14, 52] Additionally, organ transplant populations are at higher risk of suffering other complications, including those linked to cardiometabolic disease.[89] To date, there is a lack of evidence regarding the relationship between cardiometabolic disease and sarcopenia in pediatric liver transplant population. An important component to explore in relation to cardiometabolic dysregulation and sarcopenia is lifestyle factors, such as diet. Nutrients influence sarcopenia and cardiometabolic disease both negatively and positively.[24] Higher than recommended (AMDR) fat and saturated fat intake is associated with increased blood lipids while high consumption of sugar is linked to high blood pressure.[27, 28, 150] Lower than recommended (AMDR) intake of protein has been linked to reduced muscle mass and muscle strength.[24, 150] Evidence suggests micronutrients such as vitamin D, omega-3 fatty acids, calcium and selenium help prevent sarcopenia and CMD by lower levels of inflammation and oxidative stress.[24, 26] The purpose of this thesis was to examine the role of diet in the prevalence of cardiometabolic dysregulation and sarcopenia. We hypothesized that high consumption of carbohydrate, fat and saturated fat, and low consumption of protein, vitamin D, omega-3 fatty acids, selenium and calcium would be more prevalent in post-LTx children with cardiometabolic dysregulation and sarcopenia.

## 4.2 Overall research findings

This study included three definitions of cardiometabolic dysregulation (WHO[137], ATP-III[16] and Magnussen et al[139].) along with components of the adult definition of

sarcopenia by the revised European consensus to classify participants: 1) probable sarcopenia (low muscle strength by handgrip or sit-to-stand or push-up test), 2) sarcopenia (low muscle strength + low muscle quantity) and 3) severe sarcopenia (low muscle strength + low muscle quantity + low physical performance determined by 6-minute walk test or stair climb test).[3] Other CMD definitions were considered (i.e. Ferranti et al., Weiss et al.)[140, 141]. However, these other definitions had similar cut-offs for elevated serum triglycerides, reduced HDL-cholesterol, obesity, hypertension, and fasting glucose to WHO and ATP-III, meaning that using different criteria would not have greatly changed our findings of prevalence.[16, 137] Magnussen et al. uses different cut-offs from ATP-III and WHO to define elevated triglycerides (≥75$^{th}$ percentile), reduced HDL-cholesterol (≤25$^{th}$ percentile), obesity (>75$^{th}$ percentile), hypertension (>75$^{th}$ percentile) and high fasting blood glucose (≥5.6 mmol/L), which influenced prevalence of CMD found in our population.[139] In their analyses, they found BMI >75$^{th}$ percentile was as good as dichotomous pediatric definitions in predicting type 2 diabetes and adult metabolic syndrome. A low prevalence of cardiometabolic dysregulation was observed by applying two definitions in our population (4.5-9%), in contrast to findings from other pediatric studies (10-25%).[16, 19, 89, 93] Current data suggests that pediatric liver transplant recipients present evidence of cardiometabolic dysregulation in the first months to 10 years after surgery.[15, 18, 88, 89, 93, 118] However, >72% of our patients were within healthy reference ranges for the parameters included in the definitions for CMD: triglycerides, HDL-cholesterol, LDL-cholesterol, obesity and fasting blood glucose. We observed adequate consumption of carbohydrate, protein, omega-3, calcium and vitamin D by DRIs for age and gender,[150], no associations to cardiometabolic prevalence in children post-liver transplant, while the sarcopenia group had significantly higher protein and lower fat intake, which is different from what was expected (hypothesis 1 and 2). Consumption

above the recommended by DRIs for age and gender of fat and saturated fat was observed[150, 152], but CMD patients had significantly higher calcium intake (hypothesis 2). Sarcopenia patients had significantly higher intake of protein, lower fat and PUFA intake (hypothesis 2). After evaluating our results, we could observe both hypotheses were disproved.

The only differences in dietary intake that were found between liver transplant recipients and healthy controls were in fat, saturated fat, calcium and vitamin D with recipients having higher intakes of these nutrients. Both groups exceeded RDA recommendations for saturated fat, whereas only LTx children exceeded RDA recommendations for calcium and vitamin D, likely due to supplementation.[150, 151] These findings are similar to those in previous studies comparing LTx children and healthy controls, where both reported similar dietary intake. [117, 118] We did not identify low vitamin D or calcium intake by RDA recommendation[149] in LTx children, and this is explained by calcium and vitamin D supplementation reported by this population. In a previous study conducted with HC and LTx children, HC reported significantly lower calcium intake.[118] Our results regarding association between diet and sarcopenia could have been affected by HC having lower calcium intake vs LTx children, since only LTx children were classified as sarcopenia or non-sarcopenia, presence of CMD or no presence of CMD. This fact, along with low prevalence of obesity in our cohort, could account for the low prevalence of CMD. There appeared to be sufficient power ($\beta$>70%) to detect differences related to sarcopenia, PUFA and fat intake only. Insufficient power may explain the lack of associations found between CMD and dietary intake, and sarcopenia and dietary intake.

Furthermore, the definition used to classify sarcopenia was adapted from the adult definition.[3] Given that there is no consensus on the best muscle function tests for

pediatrics and lack of normative data regarding SMM in this population, this could have influenced the number of participants categorized as having sarcopenia. Other components such as age and growth are not considered in the adult definition of sarcopenia but could also affect sarcopenia classification in pediatrics. **Figure 4.1** presents a summary of research findings.

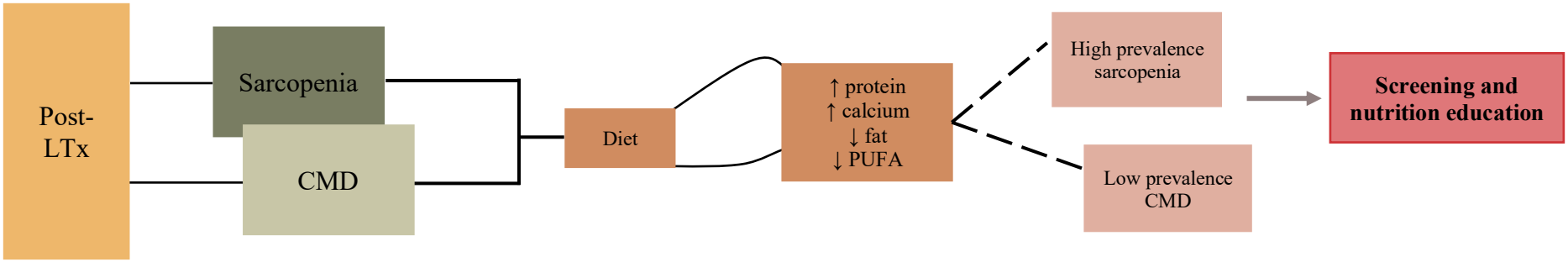


**Figure 4. 1** Relationship between diet and sarcopenia and cardiometabolic dysregulation children after liver transplant.

Post-LTx: post liver transplant, CMD: cardiometabolic dysregulation

### 4.3 Clinical implications

Evidence shows approximately 10-30% of children experience cardiometabolic dysregulation or increases in body weight five years after liver transplant, mostly related to antirejection therapy.[16, 117, 159] A previous study conducted at our centre observed children with healthy body weights and on corticosteroid (CST)-free antirejection therapy had a low prevalence of cardiometabolic dysregulation 10-years after liver transplant, mirroring our findings on prevalence.[16] These findings give further evidence on the long-term benefits of CST-free therapy, which could prevent unhealthy increases in body weight and metabolic disturbances in liver transplant recipients. This could be particularly important to consider in patients who exhibit features of metabolic syndrome in the pre-transplant period.[159] Laboratory testing to detect abnormal values of CMD markers are warranted to early identify and manage metabolic syndrome, especially in patients on CST-therapy. Developing nutrition programs aimed at this population is crucial to educate patients and their families on diet quality and how to meet dietary recommendations. In our LTx population, we observed a few patients with abnormal blood lipid (triglycerides, LDL-cholesterol, HDL-cholesterol) and blood pressure levels. We also observed higher than recommended fat and saturated fat intake in the LTx population. This type of patient would greatly benefit from having a personalized diet regime to help them adjust abnormalities. Registered dietitians should focus on identifying at-risk patients by routinely checking laboratory tests and designing a personalized diet regime according to their needs. For example, registered dietitians could consider an eating regime low in saturated fat and high in fibre for patients with high levels of triglycerides.[160, 161] Intensive Therapeutic Lifestyle Change Programs have been proposed as the gold standard for the management of obesity and metabolic syndrome. These programs consist of 14 individual or group sessions over 6 months.[161] The objective

of these programs is to promote changes in diet, physical activity and body weight.[161] Evidence has shown personalized diets, as well as nutrition education, are effective at preventing and treating metabolic syndrome.[161-163] Other pediatric transplant population would benefit from nutrition programs and proper screening for abnormal laboratory values, blood pressure and BMI-for-age since cardiometabolic disturbances have also been identified after surgery.[15, 20, 90]

At clinical level, sarcopenia-screening of patients undergoing or who underwent transplant is important. Since sarcopenia has been detected in the pre-transplant period and up to 10-years after transplant[13, 52], continuous testing for sarcopenia would help prevent further skeletal muscle mass decrease and/or deficits in muscle strength or physical performance which could lead to other type of complications.[14, 32, 34, 65] Routine follow-up visits after transplant should be considered an opportunity for health providers to implement strategies to detect decrease of muscle mass (DXA, MRI or CT scans, multiple skinfolds) and apply muscle function tests (i.e. sit-to-stand, grip strength, 6-minute walk test). For example, our centre has follow-up visits every 6 months after transplant, plus yearly DEXA testing for bone health. This would be a great opportunity to apply upper body and lower body functions tests (such as grip strength for upper and sit-to-stand for lower body) to help detect any decreases in muscle strength and check DEXA results for decreases in muscle mass. In the long-term, using this type of strategies would positively influence the patients' ability to engage in daily activities, hence impacting health-related quality of life.[164]

### 4.4 Future directions

More studies assessing dietary intake in liver transplant populations are needed to be able to develop recommendations and to have reference values. Studies should focus on gathering dietary information at various time-points, most importantly in the pre-

transplant, perioperative period, and several times after liver transplant. It is important that future studies have a larger sample size, since our study was not powered to address associations between most primary outcomes (diet and cardiometabolic dysregulation, diet and sarcopenia). These studies will also help registered dietitians and other health providers develop proper rehabilitation strategies impacting diet quality and nutrition knowledge. Additionally, there is no universal or best definition for cardiometabolic dysregulation (or metabolic disease) for pediatrics. Hence, we decided to use different criteria to define CMD. ATP-III and WHO criteria are commonly used in pediatric studies, while Magnussen et. al take a different approach.[139] It is important to define pediatric metabolic syndrome that takes into consideration different stages during childhood and adolescence.[118, 139]

One of the biggest issues for identifying sarcopenia in pediatrics is the absence of a standardized definition for this disease. Currently, the vast majority of studies define sarcopenia solely by low skeletal muscle mass without taking into consideration aspects of muscle function. [10] It is important to reach a consensus on the pediatric definition of sarcopenia which takes into account muscle strength, physical performance and neurodevelopment.[10] Future cross-sectional studies might help define the appropriate muscle function tests per age group in order to consider this evidence in the pediatric definition of sarcopenia. These studies could also help determine normative data for muscle function tests in children and adolescents particularly sit-to-stand, since there is existing reference data for other tests such as grip strength and 6-minute walk test.[10] Even though there is a wide range of muscle function tests, it is important to have normative data for those considered more practical in routine clinical care. Additionally, research has found children with sarcopenia have lower muscle quality in the lower limbs (especially legs).[10, 52] Moreover, longitudinal prospective studies exploring the effects of

cardiometabolic dysregulation and sarcopenia on health-related quality of life in children after liver transplant are warranted.[164] Healthy-related quality of life emotional and social domains could be indirectly impacted by sarcopenia and cardiometabolic dysregulation.[10, 164] Additionally, longitudinal prospective studies are needed to assess the effect of nutrients on the risk and treatment of sarcopenia in pediatric transplant populations. Even though we did not see many associations between diet and sarcopenia prevalence, previous studies have identified protein and vitamin D as two essential nutrients in the manifestation of this disease.[10, 24, 128] Examination of the relationship between protein quality and sarcopenia is relevant, since requirement of branched-chain amino acids before and after liver transplant in children has been observed previously.[10, 165]

To date, there is a lack of information regarding skeletal muscle histology in children and adolescents, both healthy and with end-stage liver disease. Evidence suggests sarcopenia can modify muscle fibre typing and contribute to lipid deposition in the muscle.[166] Atrophy and reduced sized of muscle fibres has been identified in sarcopenia affecting muscle movement.[125, 166, 167] Fat depot in muscle fibres contributes to fibre type shifting and reduced muscle function.[125] Furthermore, reduced skeletal muscle mass may increase the risk for metabolic disturbances since this is the major site for insulin-mediated glucose utilization.[168] Exploring the effects that sarcopenia and cardiometabolic dysregulation have on skeletal muscle is important since both conditions have common risk factors.[169] Studies involving biopsies would accurately characterize skeletal muscle (currently in progress by our group). Examining the physiological characteristics of the muscle in pediatric transplant patients with and without sarcopenia and/or cardiometabolic dysregulation will help understand if these contribute to modifications in the skeletal muscle and how to prevent negative changes. Understanding muscle physiology is essential to develop physical activity and nutrition programs.

## 4.5 Conclusions

Children after liver transplant are at risk of developing cardiometabolic disease and sarcopenia. More work is needed to establish the relationship between diet and the risk of developing these conditions. Patients should be monitored for any signs of cardiometabolic dysregulation and decrease in muscle mass and muscle function to prevent worsening conditions. Children exhibiting problems related to these conditions should undergo proper rehabilitation strategies.

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## Appendices

### Appendix A- 1. Dietary intake per 1000 kcal in liver transplant children and healthy controls (3-day food record)

| | LTx children (n=22) | HC (n=47) | p-value | DRIs |
|---|---|---|---|---|
| **Protein (g)** | 38±8 | 40±8 | 0.26 | 13-52[1] |
| **% protein** | 7±2 | 9±3 | 0.01 | 10-30[2] |
| **Carbohydrate (g)** | 131±15 | 133±15 | 0.62 | 130[1] |
| **% carbohydrate** | 25±7 | 30±9 | 0.05 | 45-65[2] |
| **Fat (g)** | 37±7 | 35±7 | 0.33 | - |
| **% Fat** | 16±4 | 17±6 | 0.20 | 25-35[2] |
| **Saturated fat (g)** | 13±3 | 12±3 | 0.14 | - |
| **% Saturated fat** | 6±2 | 6±2 | 0.60 | <10[1] |
| **MUFA (g)** | 13±3 | 12±3 | 0.81 | - |
| **% MUFA** | 5±1 | 6±2 | 0.09 | 10[4] |
| **PUFA** | 7±2 | 6±2 | 0.50 | - |
| **% PUFA** | 3±1 | 3±1 | 0.32 | 15[4] |
| **Omega-3 (g)** | 0.5 (0,1) | 0.8±0.4 | 0.01 | 0.9-1.6[3] |
| **Calcium (mg) (total)** | 575±211 | 471±162 | 0.02 | 700-1300[1] |
| **Vitamin D (IU) (total)** | 485±298 | 95 (10,728) | <0.0001 | 600[1] |
| **Selenium (µg)** | 55±13 | 58±13 | 0.34 | 30-55[1] |

[1]RDA:Recommended Daily Allowance[150], [2]AMDR:Acceptable Macronutrient Distribution Range[150]. [3]AI:Adequate Intake[149-152]. [4]Obtained from American Heart Association[138]: https://www.heart.org/-/media/files/healthy-living/company-collaboration/inap/fats-white-paper-ucm_475005.pdf

Total intake for calcium and vitamin D includes diet and supplementation.

LTx children: liver transplant children, HC: healthy controls, DRIs: Daily Reference Intakes, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids.

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

**Appendix A- 2. Dietary components and cardiometabolic dysregulation in LTx children (1000 kcal)**

| | ATP-III | | p-value | Magnussen et. al | | p-value | WHO | | p-value |
|---|---|---|---|---|---|---|---|---|---|
| | CMD (n=2) | No CMD (n=20) | | CMD (n=11) | No CMD (n=11) | | CMD (n=1) | No CMD (n=21) | |
| Protein (g) | 35±5 | 38±8 | 0.51 | 38±6 | 38±10 | 1.0 | 31 | 38±8 | 0.37 |
| % protein | 6±1 | 8±2 | 0.18 | 7±1 | 7±2 | 0.76 | 6 | 7±2 | 0.52 |
| Carbohydrate (g) | 137±4 | 130±16 | 0.58 | 133±16 | 128±15 | 0.47 | 139 | 130±15 | 0.58 |
| % carbohydrate | 23±5 | 26±7 | 0.58 | 26±9 | 25±5 | 0.68 | 26 | 25±7 | 0.92 |
| Fat (g) | 36±0 | 37±7 | 0.82 | 36±5 | 38±8 | 0.57 | 36 | 37±7 | 0.88 |
| % fat | 13±3 | 16±4 | 0.29 | 15±4 | 16±4 | 0.85 | 15 | 16±4 | 0.29 |
| Saturated fat (g) | 33±17 | 13±3 | 0.87 | 30±13 | 14±3 | 0.60 | 10 | 14±3 | 0.29 |
| % saturated fat | 5±1 | 6±2 | 0.32 | 5±2 | 6±2 | 0.49 | 4 | 6±2 | 0.33 |
| MUFA (g) | 12±1 | 13±3 | 0.78 | 12±2 | 13±4 | 0.24 | 13 | 13±3 | 0.87 |
| % MUFA | 4±0 | 6±1 | 0.17 | 5±2 | 5±1 | 0.77 | 4 | 5±1 | 0.35 |
| PUFA (g) | 7±4 | 7±2 | 0.97 | 6±2 | 7±3 | 0.31 | 9 | 6±3 | 0.32 |
| % PUFA | 3±2 | 3±1 | 0.70 | 3±1 | 3±1 | 0.30 | 4 | 3±1 | 0.22 |
| Omega-3 (g) | 1.5+0.7 | 1.2+0.7 | 1.0 | 1.3±0.6 | 1.19+0.75 | 0.68 | 1 | 0 (0,1) | 0.32 |
| Calcium (mg) (total) | 814±114 | 551±204 | 0.09 | 584±200 | 567±230 | 0.84 | 733 | 567±213 | 0.45 |
| Vitamin D (IU) (total) | 545±42 | 479±312 | 0.77 | 459±331 | 511±274 | 0.68 | 574 | 481±304 | 0.76 |
| Selenium (µg) | 47±1 | 55±13 | 0.36 | 52±47 | 58±11 | 0.26 | 99 | 56±13 | 0.48 |

Total intake for calcium and vitamin D includes diet and supplementation.

LTx children: liver transplant children, ATP-III: Adult Treatment Panel III[16], Magnussen et al.[139] WHO: World Health Organization[137], CMD: cardiometabolic dysregulation, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

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

## Appendix A- 3. Dietary data and sarcopenia in children after liver transplant (1000 kcal)

| Component | Sarcopenia (n=8) | Non-sarcopenia (n=14) | p-value | DRIs |
|---|---|---|---|---|
| Protein (g) | 42±8 | 36±7 | 0.07 | 13-52[1] |
| % protein | 9±2 | 7±2 | 0.03 | 10-30[2] |
| Carbohydrate (g) | 135±18 | 128±13 | 0.38 | 130[1] |
| % carbohydrate | 27±7 | 24±7 | 0.37 | 45-65[2] |
| Fat (g) | 33±6 | 39±6 | 0.03 | - |
| % fat | 15±5 | 16±3 | 0.37 | 25-35[2] |
| Saturated fat (g) | 27±11 | 14±3 | 0.34 | - |
| % saturated fat | 6±2 | 6±2 | 0.77 | <10[1] |
| MUFA (g) | 11±3 | 14±3 | 0.03 | - |
| % MUFA | 5±2 | 5±1 | 0.57 | 10[4] |
| PUFA (g) | 5±1 | 7±3 | 0.02 | - |
| % PUFA | 2±1 | 3±1 | 0.17 | 15[4] |
| Omega-3 (g) | 0.4±0.5 | 1.4±0.6 | 0.39 | 0.9-1.6[3] |
| Calcium (mg) (total) | 641±127 | 537±243 | 0.27 | 700-1300[1] |
| Vitamin D (IU) (total) | 1310±577 | 391±272 | 0.05 | 600[1] |
| Selenium (µg) | 60±11 | 52±13 | 0.20 | 30-55[1] |

[1]RDA:Recommended Daily Allowance[150], [2]AMDR:Acceptable Macronutrient Distribution Range[150]. [3]AI:Adequate Intake[149-152], [4]Obtained from American Heart Association[138]: https://www.heart.org/-/media/files/healthy-living/company-collaboration/inap/fats-white-paper-ucm_475005.pdf

Total intake for calcium and vitamin D includes diet and supplementation.

DRIs: Dietary Reference Intakes, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

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

## Appendix A- 4. Post-hoc power analysis for cardiometabolic dysregulation, anthropometric, demographic and laboratory data in LTx children

| Characteristic | Power | | |
|---|---|---|---|
| | ATP-III | Magnussen et. al | WHO |
| Age (y) | 3.5 | 5.9 | ND |
| Age at LTx (y) | 2.7 | 5.6 | ND |
| Weight-z | 57.2 | ND | ND |
| Height-z | ND | ND | ND |
| BMI-z | 54.5 | 22.6 | ND |
| WC-z | 100 | 13.4 | ND |
| SBP-z | 100 | 87.5 | ND |
| DBP-z | 81.4 | 72.3 | ND |
| TC (mmol/L) | 82.4 | 18.9 | ND |

| | | | |
|---|---|---|---|
| TG (mmol/L) | 100 | 78 | ND |
| FG (mmol/L) | 13 | 8.2 | ND |
| LDL (mmol/L) | 24.8 | 21.1 | ND |
| HDL (mmol/) | 2.7 | 31.7 | ND |
| Albumin (g/L) | 2.7 | 11.8 | ND |
| GGT (U/L) | 4.9 | 14 | ND |
| AST (U/L) | 18.7 | 3.5 | ND |
| ALT (U/L) | 8.6 | 4.2 | ND |

LTx children: liver transplant children ATP-III: Adult Treatment Panel III[16], Magnussen et al (2010)[139] WHO: World Health Organization[137], , ND: not determined, y: years, weight-z: weight z-score, height-z, height z-score, BMI-z: body mass index-z score, WC-z: weight circumference-z score, SBP-z:systolic blood pressure z-score, DBP-z: diastolic blood pressure z-score, TC: total cholesterol, TG: triglycerides, FG: fasting blood glucose, HDL: high density lipoprotein cholesterol, LDL:low density lipoprotein cholesterol, GGT: gamma glutamyl transpeptidase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

## Appendix A- 5. Post-hoc power analysis for sarcopenia, anthropometric, demographic and laboratory data in LTx children

| Variable | Power |
|---|---|
| Age (y) | 19.4 |
| Age at LTx (y) | 13.3 |
| Weight-z | 10.9 |
| Height-z | ND |
| BMI-z | 5.8 |
| WC-z | 2.7 |
| TG (mmol/L) | 46 |
| HDL (mmol/) | 3 |
| LDL (mmol/L) | 32.4 |
| TC (mmol/L) | 51.5 |
| GGT (U/L) | 10 |
| ALT (U/L) | 26.9 |
| AST (U/L) | 10.4 |
| Tacrolimus trough (µg/L) | 24.3 |

LTx: liver transplant, y: years, weight-z: weight z-score, height-z: height z-score, BMI-z:body mass index-z score, WC-z: waist circumference z-score TG: triglycerides, HDL: high density lipoprotein cholesterol, LDL:low density lipoprotein cholesterol, TC: total cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transpeptidase

## Appendix A- 6. Post-hoc power analysis for dietary intake in LTx children and HC

| Variable | Power |
|---|---|
| **Energy (kcal)** | 45.2 |
| **Protein (g)** | 7.6 |
| **Protein (g/kg)** | 8.9 |
| **% protein** | 25.2 |
| **Carbohydrate (g)** | 36.7 |
| **% carbohydrate** | 9.4 |
| **Fat (g)** | 44 |
| **% Fat** | 9.4 |
| **Saturated fat (g)** | 66.9 |
| **% Saturated fat** | 25.2 |
| **MUFA (g)** | 25.2 |
| **% MUFA** | ND |
| **PUFA** | 27.4 |
| **% PUFA** | ND |
| **Omega-3 (g)** | 52.1 |
| **Calcium (mg) (total)** | 65.1 |
| **Vitamin D (IU) (total)** | 99.6 |
| **Selenium (µg)** | 13.2 |

Total intake for calcium and vitamin D includes diet and supplementation.
LTx children: liver transplant children, HC: healthy controls, ND: not determined, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

## Appendix A- 7. Post-hoc power analysis for dietary components and cardiometabolic dysregulation in LTx children

| Variable | Power | | |
|---|---|---|---|
| | **ATP-III** | **Magnussen et al.** | **WHO** |
| Energy (kcal) | 15.3 | 9.5 | ND |
| Protein (g) | 3.9 | 8.7 | ND |
| % protein | 9.3 | ND | ND |
| Carbohydrate (g) | 29.8 | 14.9 | ND |
| % carbohydrate | 50.7 | 11.9 | ND |
| Fat (g) | 9.7 | 3.7 | ND |
| % fat | ND | 5.8 | ND |
| Saturated fat (g) | 5.1 | 4.1 | ND |
| % saturated fat | ND | ND | ND |
| MUFA (g) | 9.5 | 4.3 | ND |
| % MUFA | 7.4 | 12.8 | ND |
| PUFA (g) | 9.3 | 11.7 | ND |
| % PUFA | ND | 21.6 | ND |
| Omega-3 (g) | 8.2 | 5.2 | ND |

| | | | |
|---|---|---|---|
| Calcium (mg) (total) | 39.9 | 10 | ND |
| Vitamin D (IU) (total) | 51.9 | 5.7 | ND |
| Selenium (µg) | 3.1 | 4.9 | ND |

LTx children: liver transplant children , ATP-III: Adult Treatment Panel III[16], Magnussen et al (2010)[139] WHO: World Health Organization[137], ND: not determined, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

## Appendix A- 8. Post-hoc power analysis for dietary data and sarcopenia in children after liver transplant

| Component | Power |
|---|---|
| Energy (kcal) | 7.1 |
| Protein (g) | 11.5 |
| % protein | 61.7 |
| Carbohydrate (g) | 2.5 |
| % carbohydrate | 18.6 |
| Fat (g) | 28.6 |
| % fat | 77.3 |
| Saturated fat (g) | 13.3 |
| % saturated fat | 11.4 |
| MUFA (g) | 35.1 |
| % MUFA | 2.5 |
| PUFA (g) | 64.7 |
| % PUFA | 87.7 |
| Omega-3 (g) | 46.8 |
| Calcium (mg) (total) | 7.6 |
| Vitamin D (IU) (total) | 46.3 |
| Selenium (µg) | 9.7 |

Total intake for calcium and vitamin D includes diet and supplementation.
MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

**Appendix A- 9. Post-hoc power analysis for dietary intake in liver transplant children and healthy controls (per 1000 kcal)**

| Variable | Power |
|---|---|
| **Protein (g)** | 16.1 |
| **% protein** | 90.5 |
| **Protein (g/kg)** | 14.3 |
| **Carbohydrate (g)** | 7.4 |
| **% carbohydrate** | 71.1 |
| **Fat (g)** | 19.7 |
| **% Fat** | 12.7 |
| **Saturated fat (g)** | 25.2 |
| **% Saturated fat** | ND |
| **MUFA (g)** | 25.2 |
| **% MUFA** | 79 |
| **PUFA (g)** | 49 |
| **% PUFA** | ND |
| **Omega-3 (g)** | 69.5 |
| **Calcium (mg) (total)** | 53.5 |
| **Vitamin D (IU) (total)** | 53.5 |
| **Selenium (µg)** | 14.3 |

Total intake for calcium and vitamin D includes diet and supplementation.
ND: not determined, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

**Appendix A- 10. Post-hoc power analysis for dietary components and cardiometabolic dysregulation in LTx children (1000 kcal)**

| Variable | Power | | |
|---|---|---|---|
| | **ATP-III** | **Magnussen et.al** | **WHO** |
| Protein (g) | 11.5 | ND | ND |
| % protein | 66.7 | ND | ND |
| Carbohydrate (g) | 33.5 | 11.4 | ND |
| % carbohydrate | 11.8 | 5.1 | ND |
| Fat (g) | 9.3 | 10.4 | ND |
| % fat | 25.6 | 8.5 | ND |
| Saturated fat (g) | 38.3 | 97.8 | ND |
| % saturated fat | 22.2 | 21.6 | ND |
| MUFA (g) | 17.5 | 11.2 | ND |

| | | | |
|---|---|---|---|
| % MUFA | 100 | ND | ND |
| PUFA (g) | ND | 14.9 | ND |
| % PUFA | 2.5 | ND | ND |
| Omega-3 (g) | 8.2 | 5.7 | ND |
| Calcium (mg) (total) | 81 | 3.8 | ND |
| Vitamin D (IU) (total) | 13.8 | 6 | ND |
| Selenium (µg) | 76.2 | 6.1 | ND |

Total intake for calcium and vitamin D includes diet and supplementation.
ATP-III: Adult Treatment Panel III, Magnussen et al (2010)[139] WHO: World Health Organization, LTx children: liver transplant children, , ND: not determined, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Omega-3: n-3 polyunsaturated fatty acids

## Appendix A- 11. Post-hoc power analysis for dietary data and sarcopenia in children after liver transplant (1000 kcal)

| Component | Power |
|---|---|
| Protein (g) | 42.4 |
| % protein | 61.7 |
| Carbohydrate (g) | 16 |
| % carbohydrate | 16 |
| Fat (g) | 61.7 |
| % fat | 7.4 |
| Saturated fat (g) | 90.6 |
| % saturated fat | ND |
| MUFA (g) | 61.7 |
| % MUFA | 2.6 |
| PUFA (g) | 62.6 |
| % PUFA | 61.7 |
| Omega-3 (g) | 98.7 |
| Calcium (mg) (total) | 26 |
| Vitamin D (IU) (total) | 98.9 |
| Selenium (µg) | 33.5 |

Total intake for calcium and vitamin D includes diet and supplementation.
ND: not determined, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
Omega-3: n-3 polyunsaturated fatty acids

## Appendix B- 1. Preliminary analysis of skeletal muscle biology in infants and children with ESLD

**This appendix includes a figure that has been published in the Journal of Cachexia, Sarcopenia and Muscle[170]. This was used with permission from the publisher.**

### Literature review

### Skeletal muscle biology

Skeletal muscle consists of muscle fibres, which are polygonal and uniformly sized.[171] Type I fibres are also known as "slow twitch", responsible for long-duration, low-intensity activity and receive their energy from mitochondria and lipids.[172] Type II fibres are "fast twitch", responsible for short-duration, high-intensity activity and fast bursts of energy and receive their energy from glycogen.[172] Based on differential myosin heavy chain (MyHC) Type II fibres can be classified into three major subtypes (types IIA, IIX, IIB). [173] Hybrid MyHC expression allows more subtypes (I/IIA,IIA/IIX, IIX/IIB), with continuous range of ATP usage and muscle contraction speeds, the quickest is type IIB and slowest type I.[166] Energy use is not a predictor of fibre type; Type I and IIA fibres mostly use oxidative metabolism for energy, while type IIX and IIB fibres rely on glycolytic metabolism.[166]

Different human muscle groups have various proportions of fibre types. Muscle fibres can transition phenotypes to help muscles adapt to different use.[166] Type I fibres are better at decreasing energy utilization versus type II fibres, making them important at energy efficiency.[173] Disease can alter proportions of fibre types. Reduced muscle movement has been linked to atrophy of muscle fibre types, especially of type I fibres, and type I and IIA fibres shift to type IIX.[166] Long periods in bed appear to induce type I fibre atrophy, and increments in hybrid fibres have been observed after bed rest. In contrast, resistance training is link to hypertrophy of type II fibres.[173]

Type II fibres atrophy more quickly in response to muscle disuse.[173] Studies involving young adults have found trained individuals have a lower expression of MyHC hybrids. [174, 175] Sarcopenia has been characterized by reduced sized and greater atrophy of type II fibres.[166] However, there is little to no evidence on muscle fibre typing in healthy children and children with sarcopenia.

**Skeletal muscle characteristics in liver disease and cancer**

A study conducted with 20 adult patients undergoing liver transplantation (females n=10, males n=10) found a higher percentage of fibre type I in both females and males (58±5 and 42±6 respectively) in RA. The second highest percentage of muscle fibre type was IIA: 26±3 in females and 34±5 in males. They also observed a high percentage of IIA/D hybrids (13±4 in females, 19±5 in males). There was a higher prevalence of MyHC type IIA fibres in males when compared to females (55% vs 40%, p=0.009). [176]

In cancer patients, a study found skeletal muscle is characterized by muscle fibre atrophy in MyHC type I and IIA. In this same population, prevalence of muscle fibres were: MyHC type I 53%, type IIA 27%, hybrids IIA/X 13%, I/IIA/IIX 0.9% and IIX 0.7%.[177] Authors concluded cancer patients have a switch to more fast-twitch phenotypes, as well as an increased expression of IIA/X hybrids. They hypothesized this switch may be due to the need of maintaining muscle power output in response to declined force production secondary to atrophy and help preserve functionality.[177]

**Table 1. Muscle fibre typing in humans**

| Authors | Muscle studied | Population | Type I (%) | Type IIA (%) | Type IIX (%) | Type IIA/X | Type IIA/IIX (%) | Type I/IIA | Type I/IIA/IIX |
|---|---|---|---|---|---|---|---|---|---|
| **Staron et al., 2000**[178] | Vastus lateralis | Healthy | 41 | Men 41.2<br>Women 33.6 | N/A | N/A | N/A | N/A | N/A |
| **Murach et al., 2019**[174] | Vastus lateralis | Healthy | 62.1 | 24.6 | 0.4 | N/A | 8.1 | 3.7 | 1.0 |
| **Bagley et al., 2016**[179] | Vastus lateralis | Resistance trained men | 19.9 | 57.8 | 2.1 | N/A | 11.2 | 9.7 | 1.4 |
| **Toth et al.,** [177] | Vastus lateralis | Cancer | 53 | 27 | 0.7 | 13 | N/A | N/A | 0.9 |
| **Ebadi et al., 2020**[176] | Rectus abdominis | Cirrhosis | Men 42<br>Women 58 | Men 34<br>Women 26 | N/A | Men 19<br>Female 13 | N/A | N/A | N/A |
| **Henning et al., 2017**[180] | Vastus lateralis | McArdle disease | 44 | N/A | N/A | N/A | N/A | N/A | N/A |
| **Henning et al., 2017**[180] | Biceps brachii | McArdle disease | 38 | N/A | N/A | N/A | N/A | N/A | N/A |
| **Henning et al., 2017**[180] | Vastus lateralis | Healthy controls | 71 | N/A | N/A | N/A | N/A | N/A | N/A |
| **Henning et al., 2017**[180] | Biceps brachii | Healthy controls | 52 | N/A | N/A | N/A | N/A | N/A | N/A |

N/A: not available

**Central nuclei**

Nuclei in myofibres compartmentalize gene regulation, essential for proper muscle function.[167] These are positioned at the periphery of each myofibre. It is believed these peripheral nuclei are positioned to maximize the distance between nuclei.[167] Theories on why nuclei are positioned at the periphery are various and not exclusive, including that each nucleus sustains a portion of the muscle; if nuclei were clustered, some regions of the muscle would lack transcription and translation needed to maintain the myofibre.[167] Nuclei in the center of the cell could act as obstacles to contraction and impede muscle movement.[167] By staying at the periphery, nuclei could also be protected from the force of contraction.[167] Evidence on the role of nuclear positioning in disease progression and muscle function is not clear.[167] It is believed central nuclei are a consequence of continuing myofibre repair in muscle disease.[167]

Central nuclei have been observed in muscle biopsies from patients with muscle disorders (Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, Myotubular Myopathy).[167] For example, in patients with Myotubular Myopathy, there was >25% centrally positioned nuclei compared to <3% in unaffected individuals.[181] However, there is a lack of information regarding central nuclei in children, especially in RA.

**Myosteatosis**

Muscle quality is significantly reduced by myosteatosis.[84] Myosteatosis can be defined as an ectopic fat deposition producing negative clinical consequences such as fast aging and impaired metabolic and musculoskeletal health.[125] Fat depots can be grouped into two: intermuscular fat (IMET) – visible extracellular adipose tissues located beneath the muscle and within/between muscle groups, and fat infiltration within myocytes (intramuscular fat, IMAT), microscopic lipid droplets used as energy within the muscle. [125] IMAT and IMET increase with

age, and males have higher content.[84] Visceral fat and other ectopic fat depots (in the liver, pancreas and heart) are also associated to IMAT and IMET.[84]

Fat depot inside the muscle is strongly linked to frailty, cancer, obesity and diabetes.[84, 170] Myosteatosis is linked to increased levels fasting glucose and insulin, as well as higher prevalence of insulin resistance and type 2 diabetes.[126] Evidence suggests fat infiltration contributes to glucose and insulin abnormalities by impairing insulin diffusion capacity and insulin action, which could provoke changes in local muscle metabolism.[126] Additionally, insulin resistance may worsen fat accumulation in the muscle.[18] Please refer to section 1.5.1 for more details.

Increased levels of fat depot produces decreased muscle performance and physical movement.[125] Other reported effects of myosteatosis include decreased activation of quadriceps muscle in older adults, loss of mobility, decreased gait speed, lower number of repeated chair stands, and slower timed get-up and go tests.[125] In adults, IMAT has been negatively correlated to muscle function.[84]

Fat infiltration is not uniformly distributed across the muscle, and has been found in various muscle groups, such as soleus, tibialis anterior, vastus lateralis, and mid-thigh.[170] Muscle fibre orientation can be affected by fatty infiltration, affecting muscle strength.[84] Muscle fibre typing can also be changed by myosteatosis: type II fibres transition to type I. [125] This would decrease muscle power and impair contractile capacity, increasing risk of deficits in mobility.[125] IMAT can lead to systemic inflammation, muscle cells proliferation and muscle metabolism by secreting inflammatory cytokines.[41]

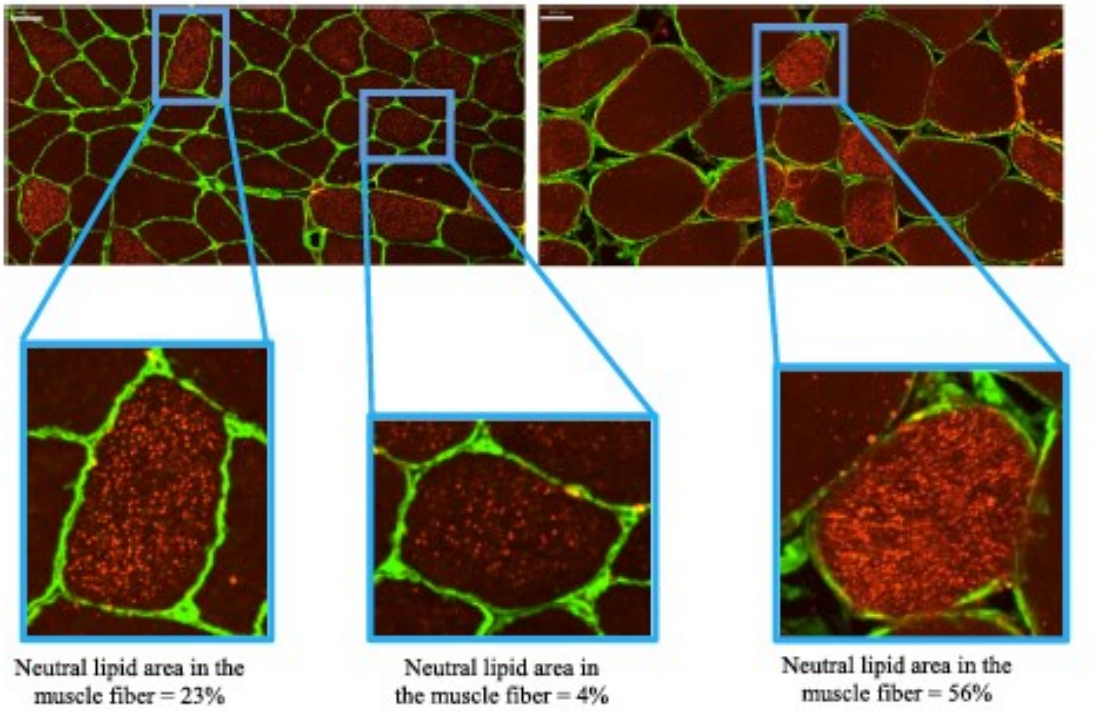


**Figure 1.** Lipid deposition inside rectus abdominis muscle fibre in cancer patients. Stained muscle sections with laminin and dystrophin (green) for cell membrane and Oil Red O (bright red) for neutral lipids. Different area percentage can be seen within fibres.[170] Figure used with permission by publisher (John Wiley and Sons).

**Radiodensity**

Hounsfield units (HU) are a unit widely used in CT scanning to express CT numbers in a standardized form, obtained from a linear transformation of the attenuation coefficient.[170, 182] Radiodensity is measured in HU: radiodensity of distilled water at standard pressure and temperature is defined as 0 HU and of air at standard pressure and temperature is -1000 HU.[170, 182] Evidence shows muscle radiodensity is directly correlated with triglyceride (TG) content evaluated by muscle biopsy.[183] Radiodensity decreases as fat myosteatosis increases.[183] Studies have related low muscle radiodensity is related to high TG content of rectus abdominis (RA) measured at L3.[170, 184] One limitation of CT is it not able to differentiate between intracellular

fat deposits or extracellular fat deposits.[183] Excess intracellular fat is related to insulin resistance, inflammation, and muscle dysfunction.[170] Extracellular fat is an accumulation of fat cells around the muscle fibres.[170] Muscle radiodensity has been linked to impairment in functional capacity, reduced contractile force of the muscle, low aerobic capacity, impaired lipolytic response, and lower survival.[170, 183] A review on the assessment of skeletal muscle radiodensity by CT found variations on abdominal region and muscle groups used to measure and no pattern among studies for low-radiodensity SM ranges.[183] The majority of studies selected the lumbar region to measure radiodensity (L3-L5), and most chose paraspinal muscles for muscle group. Authors established the interval from -29HU to +29HU as low-radiodensity, while high-radiodensity was stabled as +30HU to +150HU.[183]

**Summary and objectives**

Sarcopenia is a progressive muscle disease linked to decreased skeletal muscle mass (SMM) and muscle quality (MQ), accompanied with low muscle strength (MS) and physical performance (PP).[3] In recent studies conducted on infants and children, sarcopenia was observed in 36-41% of those who underwent liver transplantation and lasted up to 8-10 years post-LTx.[13, 14] Furthermore, our group associated sarcopenia to longer LOS and ICU stay and higher ventilator dependency post-liver transplant (LTx).[13] Myopenia and low SAT measured through routine CT and MRI scans before LTx were associated to longer LOS, more infections, lower energy intake and gross motor delay.[14] Additionally, decreased aerobic physical capacity and endurance have been reported in children post-LTx.[52] Children post-LTx have decreased lower body muscle strength and physical performance. [52]

Muscle fibre types can be altered by disease. Low physical movement can cause atrophy of muscle fibre types, especially of type 1 fibres, and shifts of type I and IIA fibres shift to type

IIX. [166] Type II fibres atrophy more quickly in response to muscle disuse.[173] Sarcopenia has been characterized by reduced sized and greater atrophy of type II fibres.[166]

Various studies involving patients with muscle disorders have found a higher percentage of central nuclei.[167] Since nuclei are positioned in the periphery to maximize the distance between nuclei, it is believed central nuclei may obstruct contraction and muscle movement.[167] Increased levels of fat depot in myofibres (myosteatosis) produce reduced muscle performance and physical movement.[125] Muscle fibre types can shift due to fatty infiltration: type II fibres transition to type I.[125] This would decrease muscle power, increasing risk of deficits in mobility. [125] Data in adults who underwent liver transplantation (females n=10, males n=10) suggests there is a higher percentage of fibre type I in females and males (58±5 and 42±6 respectively) in RA. A high percentage of IIA/D hybrids (13±4 in females, 19±5 in males) in this population can also be observed.[176] However, there is no data on muscle fibre typing, central nuclei and myosteatosis in healthy children and children with sarcopenia, especially in rectus abdominis (RA).

This pilot study will describe some of the histological features of skeletal muscle in children and infants with ESLD at time of LTx.

**Methods**

A prospective, pilot study was conducted in children with ESLD undergoing liver transplantation at the Pediatric Liver Transplant Clinic, Stollery Children's Hospital in Edmonton, Alberta. Patients ≤ 18 years were recruited (n=7). Exclusion criteria included liver retransplantation, small intestine and/or multi-visceral transplant. Demographic and anthropometric data was retrieved from electronic databases (ConnectCare, eClinician and Organ Transplant Tracking Record [OTTR]). This project was approved by the Health Research Ethics Board at the University of Alberta (Pro00078069).

**Muscle biopsy sample collection and processing**

Samples of rectus abdominis muscle and adipose tissue (VAT and SAT) were collected at the time of liver transplantation. This site is made of mixed fibre types and appears in images taken from the L3 region. Biopsies (0.5-1 g) were taken immediately after initial incision by sharp dissection from the edge of the surgical incision without the use of electrocautery, placed in empty containers and on top of ice for transportation to laboratory. The average time between biopsy removal and arrival to the laboratory was 20 minutes. Visible fat and connective tissue were removed from samples while processing. For most samples, one piece of rectus abdominis was frozen in liquid nitrogen and stored at -80°C, while another piece was frozen in isopentane cooled at -160°C in liquid nitrogen and stored for cryostat sectioning and immunohistochemical staining at -80°C.[170] Processing for samples was finished within 1.5 hours of the arrival of the specimen to the laboratory. All procedures were performed under sterile conditions. The instructions handed to surgeons for collection are illustrated in Figure 1.

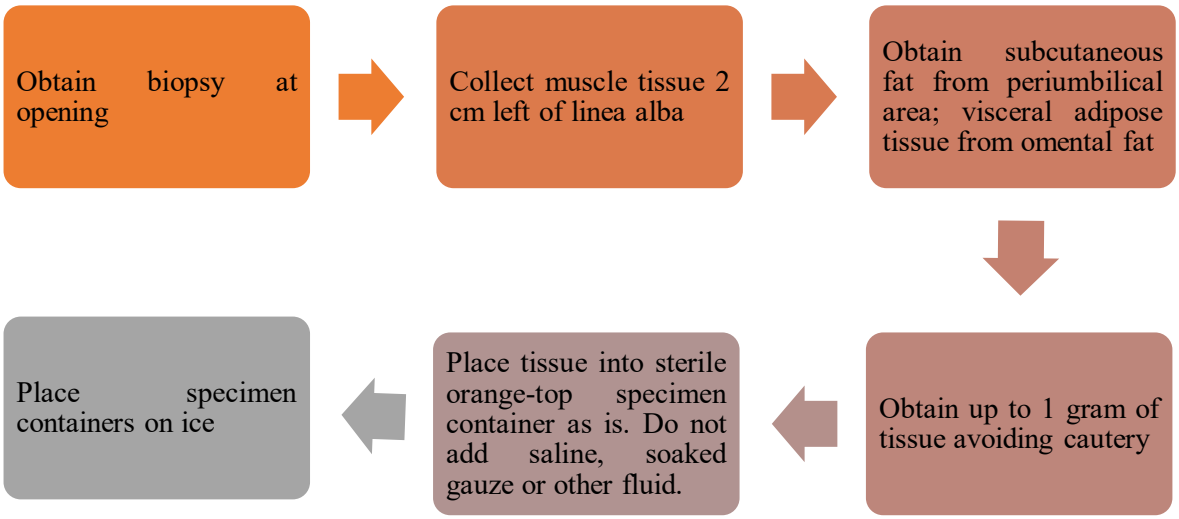


**Figure 2.** Procedure for biopsy collection at time of liver transplant. Instructions handed to surgeons prior to surgery for sample collection. [170]

**Immunofluorescence for muscle fibre typing, confocal microscopy and histological analysis**

Muscle samples were cryosectioned transversely (10 µm- cryostat Leica model CM300) at -22°C and stored at -80°C for staining. Analyses were performed with one slide for nuclear stain and one slide for muscle fibre area assessment. After fixing tissue slides (Apex™ superior adhesive slides, Leica Biosystems) using acetone at -20°C, slides were washed various times with a phosphate buffered saline (PBS) and incubated using a blocking solution (PBS-Tween 20, 1% bovine serum albumin and 10% normal goat serum) for 60 minutes. Slides were then washed in PBS before incubation with primary antibodies at 4°C overnight. Sections were washed once in PBS-Tween 20 and six times in PBS before applying the secondary antibodies. MyHC (Myosin heavy chain) isoforms were identified by binding specific monoclonal

antibodies against various isoforms: I (BAD5), IIA (SC71), and IID (6H1).[185] A detailed description of primary and secondary antibodies utilized for MyHC staining are described in Table 1. Slides were washed six times in PBS after two hours of secondary incubation at room temperature. DAPI (nuclear stain- 4'6,-diamidino-2-phenylindole) was added for 2 minutes and washed. ProLong® Diamond Antifade medium were used to mount slides in, then covered with 1.5 thick coverslips and left to dry for twelve hours.

A 20x/0.85 oil lens with a spinning disk confocal microscope (Quorum Wave FX Spinning Disc Confocal System, Quorum technologies) was used to take images for tissue sections. Individual Z-stacked images were assembled together and plane-merged to create a composite image of a whole-tissue cross section. A software script previously established by our team helped identify muscle fibre types (I, I/IIA, IIA, IIA/D, and D). The script used the intensity of the MyHC stains for identification and was quantified automatically by the software (Volocity 6.3 software, PerkinElmer).[170] The percentage of fibres with central nuclei was counted manually through selection of muscle fibres with mispositioned nuclei (separated from sarcolemma, equidistant or not) in a tissue cross-section. Muscle fibre CSA (μm) and mean fibre CSA value were calculated.[186]

**Table 2. Primary and secondary antibodies used for muscle fibre type staining[170]**

| Primary antibody | Dilution factor- primary | Secondary antibody | Dilution factor- secondary | Isotype | Species |
|---|---|---|---|---|---|
| Laminin | 1:200 | AlexaFluor® 647 | 1:400 | IgG | Rabbit |
| Dystrophin | 1:25 | AlexaFluor® 647 | 1:200 | IgG | Rabbit |
| MyHC I (BAD5) | 1:400 | AlexaFluor® 568 | 1:400 | IgG2b | Mouse |
| MyHC IID (6H1) | 1:400 | AlexaFluor® 488 | 1:400 | IgM | Mouse |
| MyHC IIA (SC71) | 1:400 | AlexaFluor® 405 | 1:200 | IgG1 | Mouse |
| DAPI | 300 nM | N/A | N/A | N/A | N/A |
| Wash buffer- phosphate-buffered saline | | | | | |

**Statistical analysis**

Only descriptive data is presented due to small sample size. Data were expressed as percentages or median and inter-quintile range (IQ). The Shapiro-Wilk test was conducted to assess the normality of distribution.

**Results**

Study population

Anthropometric and demographic information is presented in Table 2. All patients who were approached consented to intraoperative biopsy. (n=6). All patients were diagnosed with ESLD and were awaiting LTx. Liver disease diagnosis varied in our population; Table 2

presents these details. Mean and standard deviation (SD) for age and median for weight-z, height-z and BMI-z at LTx assessment were 4.5±3.9, 0.7 (0.2, 1.0), -1.0 (0.7, 1.2) and 1.2 (1.0, 1.8) respectively. The median for PELD during the time of LTx assessment was 5 (2, 11).

**Table 3. Demographic and anthropometric information in ESLD children during liver transplant assessment**

| **Variables** | **ESLD (n=6)** |
|---|---|
| **Age** | 4.5±3.9 |
| **Gender** | M=5<br>F=1 |
| **Weight (kg)** | 19.6±11.7 |
| **Weight-z** | 0.7 (0.2, 1.0) |
| **Height (cm)** | 98.6.6±29.8 |
| **Height-z** | -1.0 (0.7, 1.2) |
| **BMI** | 18.7±1.8 |
| **BMI-z** | 1.2 (1.0, 1.8) |
| **Liver etiology, n**<br>- **PFIC**<br>- **BA**<br>- **Others*** | <br>- 1<br>- 1<br>- 4 |
| **PELD** | 5 (2, 11) |
| **ALT (IU/L)** | 156 (84,230) |
| **AST (IU/L)** | 174 (154, 372) |
| **GGT (IU/L)** | 144.5 (93.3, 242.3) |

| | |
|---|---|
| **Albumin (g/L)** | 32.3±4.9 |
| **Total bilirubin (µmol/L)** | 98.0 (19.5, 188.5) |
| **INR** | 1.4±0.5 |
| **PTT (seconds)** | 52.0±1.4 |
| **Ammonia (µmol/L)** | 67.3±22.7 |
| **CRP (mg/L)** | 1.7 (0.9, 14.0) |
| **Vitamin D (nmol/L)** | 35.0 (21.3, 48.5) |
| **Urea (mmol/L)** | 2.8±1.5 |
| **Creatinine (µmol/L)** | 22.5 (18.3, 28.3) |

*Others liver etiology included: Alagille's syndrome (n=1), argininosuccinic aciduria (n=1), Alpha-1-antitrypsin deficiency (n=1), autoimmune cirrhosis (n=1)
Normal ranges for biochemical data 2 months- 18 years: ALT (<60U/L), AST (10-75 U/L), GGT (10-150 U/L), Albumin (30-50 g/L), Total bilirubin (<21 µmol/L), INR (0.8-1.2), PTT (27-39 seconds), Ammonia (25-55 µmol/L), CRP (0-10mg/L), Vitamin D (>75 nmol/L), Urea (207 mmol/L), Creatinine (10-120 µmol/L)
PFIC: progressive familial intrahepatic cholestasis, BA: biliary atresia, PELD: Pediatric end-stage liver disease, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, INR: International normalized ratio, PTT: Partial thromboplastin time, CRP: C-reactive protein
Data expressed in mean±SD or median (IQR)

Rectus abdominis characterization

A total of 6 muscle biopsies were collected. Central nuclei was not performed on one sample (n=5) due to dysfunctional staining. Data on fibre typing, fibre area and central nuclei for L3 is available in Table 3.4. It was determined through analysis of myosin heavy chain (MyHC) isoforms that MyHC type I was more abundant, followed by MyHC type IIA. Additionally, 12.1% of fibres were hybrids, which is a higher percentage than MyHC IID fibres (8.2%). For individual fibre types, type I fibres had the greatest percentage (52.3), followed by fibre type IIA (35.7%) and hybrid IIA/D (8.2%). Presence of hybrid type I/IIA was low (3.9%), and type IID fibres were not found in any of the samples (0%). Muscle fibre area for type I (51.2%) was larger than the rest of fibre types. Type IIA had the second largest percentage fibre

area (38.3). For central nuclei, an average of 627.6±144.8 of fibres were analyzed per participant. Central nuclei were observed in 3.8% of fibres.

**Table 4. Rectus abdominis myosin heavy chain content, mean muscle fibre area and central nuclei in ESLD children**

| **MyHC content (n=6)** | |
|---|---|
| **MyHC isoforms (%)** | |
| **MyHC type I** | 60.0 (47.1, 63.4) |
| **MyHC type IIA** | 41.2 (36.6, 57.5) |
| **MyHC type IID** | 8.0 (6.3, 10.9) |
| **Hybrids*** | 9.1 (6.3, 15.5) |
| **Individual fibre types** | |
| **Fibre type I** | 58.8 (42.5,63.4) |
| **Fibre type I/IIA** | 1.2 (0.0, 4.6) |
| **Fibre type IIA** | 33.6 (30.5, 42.0) |
| **Fibre type IIA/D** | 8.0 (6.1, 10.9) |
| **Fibre type IID** | 0.0 |
| **Muscle fibre area (n=6)** | |
| **Individual fibre types (%)** | |
| **Fibre type I** | 53.8 (43.4, 64.6) |
| **Fibre type I/IIA** | 1.3 (0, 2.9) |
| **Fibre type IIA** | 39.0 (30.2, 45.6) |
| **Fibre type IIA/D** | 6.7 (5.6, 8.2) |
| **Fibre type IID** | 0.0 |

| **Individual fibre types** | |
|---|---|
| **Fibre type I** | 52.3±16.2 |
| **Fibre type I/IIA** | 3.9±6.1 |
| **Fibre type IIA** | 35.7±7.7 |
| **Fibre type IIA/D** | 8.2±3.2 |
| **Fibre type IID** | 0 |
| **Muscle fibre area (n=6)** | |
| **Individual fibre types (%)** | |
| **Fibre type I** | 53.8 (43.4, 64.6) |
| **Fibre type I/IIA** | 1.3 (0, 2.9) |
| **Fibre type IIA** | 39.0 (30.2, 45.6) |
| **Fibre type IIA/D** | 6.7 (5.6, 8.2) |
| **Fibre type IID** | 0.0 |
| **Central nuclei (n=5)** | |
| **Mean number of fibres analyzed** | 681.0 (638, 695) |
| **Total of fibres with central nuclei** | 16.0 (12.0, 30.0) |
| **Fibres with central nuclei (%)** | 2.1 (1.7, 6.5) |

MyHC: myosin heavy chain
*Hybrids refers to fibres of mixed myosin heavy chain isoforms MyHC type I/IIA and MyHC type I
Data is expressed in mean±SD or percentage (%)

Muscle fibre type and muscle cell nuclei

Fibre type I (38.5%) and type IIA (5.4%) were higher in our female patient in comparison to male participants (6.3.3 [54.3, 63.5] and 32.9 [29.7, 34.3] respectively). Central nuclei percentages were 6.5 for our female participant and 1.9 (1.5, 3.6) for males. Table 4. presents information on the characteristics for muscle fibre typing separated by sex.

**Table 5. Characteristics for muscle fibre typing in children with ESLD**

| Variable | Female<br>N=1 | Male<br>N=5 |
|---|---|---|
| **Muscle fibre typing** | | |
| **Fibre type I (%)** | 38.5 | 63.3 (54.3, 63.5) |
| **Fibre type I/IIA (%)** | 5.4 | 0.0 (0.0, 2.3) |
| **Fibre type IIA (%)** | 44.6 | 32.9 (29.7, 34.3) |
| **Fibre type IIA/D (%)** | 11.5 | 6.8 (5.8, 9.1) |
| **Fibre type IID (%)** | 0.0 | 0.0 |
| **Central nuclei** | | |
| Fibres with central nuclei (%) | 6.5 | 1.9 (1.5, 3.6) |

ESLD: end-stage liver disease.
Data is expressed in median (IQR) or percentage (%)

## Discussion

This analysis was the first to look at muscle histology in RA in children with ESLD. We observed type I muscle fibres represent the highest percentage in these children at LTx, which is in line with previous evidence suggesting muscles in infants and children contain higher type I muscle fibres.[171] Children with PFIC and BA did not present type I/IIA hybrids, while children with other disease types presented percentages between 2.4-15.5. Type IIA/D hybrids were observed in all disease types; children diagnosed with argininosuccinic aciduria and autoimmune cirrhosis presented the highest percentages (12.0% and 11.5% respectively). These children also had the highest percentage of type IIA fibres with 45.6% in argininosuccinic aciduria and 44.6% in autoimmune cirrhosis, with other children presenting percentages between 27.1-34.3. We did not find type IID muscle fibres in our cohort.

Infants between 1 and 2 years old had similar type I muscle fibre content (63.3 and 63.5%). These infants also had close results regarding type IIA, with 29.7% for 1-year old and 32.9% for 2-years old. For hybrids, there was only presence of type IIA/D in these infants. Children 8 years of age had comparable results to these infants, with 67.2% for type I, 27.1% for type IIA and 5.8% for type IIA/D. Children who were 5 and 9 years old at the time of sample collection were the only who presented both type I/IIA and type IIA/D hybrids. Our findings are similar compared to those found in adults with ESLD, where researchers found a higher presence of type I and type IIA, with little presence of type I/IIA and type IIA/D fibre hybrids.[176] Additionally, researchers found a higher proportion of type I in male adults with ESLD.[176] This differs from our results, where we found our female patient had lower percentage of type I fibres.

Children with argininosuccinic aciduria and autoimmune cirrhosis had the highest number of fibres with central nuclei. These represented a total of 7.9% fibres for patients with arginonosuccinic aciduria and 6.5% fibres for patients with autoimmune cirrhosis. These percentages are higher than those found in individuals without muscle disease.[181] Interestingly, these patients had high levels of ammonia which could explain differences in muscle fibre types and centrally positioned nuclei. High levels of ammonia have been linked to interference of protein remodeling in the muscle.[187, 188] Ammonia causes and inactivation of mTORC1, which results in repression of mRNA translation and decrease in protein synthesis in the skeletal muscle.[33] Research shows that in cirrhosis, high skeletal muscle ammonia concentrations result in reduced protein synthesis by hampering translation initiation resulting from low ATP concentrations. [33] Children with other disease types had a lower percentage of muscle fibres with central nuclei. No descriptive age differences could be observed in terms of central nuclei presence.

This study is the first to examine RA composition in infants and children with ESLD. Even though our population had a low PELD, the medical team decided to go ahead with transplantation when an organ became available in order to prevent further complications for the patients. Information on muscle features in healthy and unhealthy young populations is extremely limited, mostly due to ethical considerations. Our team followed a standard procedure to obtain, process and analyze samples.

The biggest limitation to our study is sample size. We only had one female in our muscle biopsy sample cohort. We obtain limited clinical data. We had a small sample size but did have a variety of liver disease diagnosis. Sample size did not permit further statistical analysis. We could observe in our sample that disease type was a stronger factor for fibre typing differences. Hence, future studies should evaluate differences between disease type and disease duration. Even though these results are promising, we present them as observational rather than conclusive, due to our small sample size. Future research should also examine nutritional intake, such as protein quality and vitamin D which have been associated to sarcopenia risk.[10]