SELF-REPORTED PHYSICAL ACTIVITY AND ULTRASOUND ASSESSED MUSCLE QUALITY IN ADULTS AND ADOLESCENTS FOUR TO NINE MONTHS FOLLOWING ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

By

Jessica Elizabeth Tolzman

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Kinesiology – Master of Science

2024

ABSTRACT

Purpose: Anterior cruciate ligament reconstruction (ACLR) is common in adolescents, however, there is little information highlighting the differences between adults and adolescents' post-surgery. This study aims to identify differences in quadriceps muscle quality between adults and adolescents post-ACLR and how self-reported physical activity is associated with muscle quality. **Methods:** A cross-sectional study was conducted with 24 participants aged 14-36 years, 4-9 months post-ACLR. Adolescents and adults were compared. Quadriceps muscle quality was assessed using static ultrasound for rectus femoris cross-sectional area (CSA) and echo-intensity, and dynamic ultrasound for vastus lateralis pennation angle changes during treadmill walking. A limb symmetry index (LSI) was calculated for CSA and echo intensity. Physical activity was assessed using the International Physical Activity Questionnaire and Muscle Strengthening Exercise Questionnaire. Independent t-tests compared CSA and echo intensity LSI, and pennation angle change between groups. Spearman's rank correlation quantified the association between physical activity and muscle characteristics. **Results:** Participants included 12 adolescents (age: 16.0±1.4 years, height: 171.7±8.5 cm, weight: 70.8±12.5 kg, maturity offset: 2.4±1.3 years) and 12 adults (age: 24.4±7.4 years, height: 174.0±7.0 cm, weight: 79.0±14.4 kg). No significant differences were found in rectus femoris CSA LSI (t_{22}=0.22, p=0.83), echo intensity LSI (t_{22}=-1.42, p=0.17), or vastus lateralis pennation angle change (t_{22}=0.41, p=0.68). Associations between muscle quality and physical activity were low to negligible. **Discussion**: Adolescents and adults show similar muscle quality 4-9 months post-ACLR. Associations between muscle quality and self-reported physical activity were minimal. Future studies should explore longitudinal changes post-ACLR in both populations.

This thesis is dedicated to my dogs Moose and Chicken for providing me emotional support throughout this process.

ACKNOWLEDGMENTS

I would like to thank Dr. Matthew Harkey for believing in me throughout this process and providing me with support in both my thesis and my development as a graduate student. Without his help and guidance, I would not have thrived as much as I have within this program. Additionally, I would like to extend my deepest gratitude to my thesis committee, Dr. Karin A. Pfeiffer and Dr. Katharine Currie, for their support and guidance throughout this project. Through their help in this thesis and in the classroom, I have become a stronger researcher and student. They both have provided me with vital skills needed to succeed in this field. Lastly, I would like to thank my lab mates within the Athletic Injury and Rehabilitation Lab for helping me through all aspects of this research project. I would not have been able to complete this project without their support.

TABLE OF CONTENTS

CHAPTER 1: Introduction

Each year, between 100,000 to 200,000 individuals experience an anterior cruciate ligament (ACL) tear, with about 75% of those individuals receiving reconstructive surgery (Sanders et al., 2016). ACL injuries occur in diverse age cohorts, frequently afflicting physically active individuals. However, 33% of ACL reconstructions (ACLR) are completed in high school or college-aged individuals within the United States (Mall et al., 2014). Research is scarce concerning the disparities between adult and adolescent populations following ACLR. Specifically, differences in muscle characteristics between these age groups may be important, given that adolescents undergo skeletal growth and maturation during the recovery phase, a developmental aspect not occurring in adults (Tanner, 1981). Previous studies have found that adults and adolescents differ in levels of physical activity post-ACLR; however, it is unknown if these differences can be seen in muscle quality as well (Kuenze, Collins, Triplett, et al., 2022). Specifically, there is limited knowledge about the differences in muscle quality and its association with physical activity in adults and adolescents.

Asymmetries in muscle quality between limbs can become apparent following surgery, which could lead to further injuries (Birchmeier et al., 2020). A study conducted in 2012 found that individuals experience atrophy as quickly as after three weeks of inactivity (Bosquet & Mujika, 2012). The muscular deficits following ACLR can also be related to altered walking biomechanics, decreased functional movement, and, eventually, thinning of the femoral articular cartilage (Birchmeier et al., 2020). Ultrasound offers a clinically accessible tool to assess both the size and overall quality of a muscle. Both strength and ultrasound-assessed echo intensity and the cross-sectional area (CSA) of the muscle serve as ways to measure muscle quality. Individuals following ACLR tend to show the smaller CSA of the vastus lateralis compared to the contralateral limb while also having a higher echo intensity or worse quality of muscle within the ACLR limb (Garcia, Moffit, et al., 2020). Nevertheless, there is a lack of research regarding the disparities in these outcomes between adults and adolescents.

Following ACLR, walking biomechanics are crucial for joint health, with muscle function significantly influencing walking dynamics. Portable ultrasound technology, such as a Clarius (Clarius, Burnaby, BC, Canada) unit, can be attached to an individual's limb to enable real-time assessment of muscle characteristics during functional activities like walking or jogging. This dynamic imaging approach, when paired with force-sensing insoles (e.g., loadsols), provides valuable insights into the relationship between muscle physiology and biomechanics in a functional state. Specifically, we can assess the changes in pennation angle throughout the gait cycle, to understand muscle behavior during activity, which is crucial for force production of a muscle. After an ACLR, individuals often exhibit decreased pennation angles in the affected limb compared to the contralateral limb, as seen in static MRI images (Noehren et al., 2016). This reduction in pennation angle correlates with diminished force production capabilities (Sopher et al., 2017). However, these studies utilize static ultrasound imaging so it is unclear whether similar alteration would be seen in the muscle during walking.

Following an ACLR, individuals are more than twice as likely not to meet the United States Department of Health and Human Services' physical activity guidelines (Kuenze, Collins, Pfeiffer, et al., 2022). Frequently, the ultimate objective for individuals recuperating from physical ailments is the resumption of physical activity (Webster & Feller, 2019). This is important because participating in physical activity contributes to a higher quality of life and may serve as a preventive measure against chronic conditions like osteoarthritis (Bell et al., 2017; Luc et al., 2014). The reduction in physical activity can lead to a decrease in overall muscle mass, strength, and power (Lepley et al., 2020; Marcus et al., 2010). Self-reported physical activity questionnaires offer an easy and accessible form of assessing an individual's activity level. Self-reported physical activity questionnaires are beneficial in understanding an individual's activity levels at a certain time point while also assessing why they believed their activity has changed since receiving an ACLR (COLLINS et al., 2011). Although it is known that adults participate in more physical activity following an ACLR than adolescents, the interaction between ultrasound-assessed muscle quality and self-reported physical activity remains unclear.

Purpose Statement

Previous literature has shown that adults are more physically active and have a greater vertical ground reaction force (vGRF) compared to adolescents following an ACLR; however, a gap remains within the literature on the interaction of ultrasound-assessed muscle quality and self-reported physical activity between adults and adolescents (Kuenze, Collins, Triplett, et al., 2022; Lisee et al., 2023). Therefore, the overall purpose of this study is to determine the muscle quality and physical activity differences between adolescents and adults using both static and dynamic diagnostic ultrasound and self-reported physical activity questionnaires 4- to 9-months post-ACLR.

Specific Aim 1: Determine how adolescents and adults differ in static ultrasound-assessed quadriceps muscle quality in participants between 4- and 9-months post-ACLR. Approach: Muscle quality will be defined as CSA and echo intensity of the rectus femoris. Poor muscle quality is defined as higher echo intensity and smaller CSA. The average limb symmetry index will be calculated to assess the differences between the ACLR and the contralateral limb. *Our* *hypothesis is that adolescents will experience greater asymmetry between their injured and contralateral limb CSA and echo intensity.*

Specific Aim 2: Determine how adolescents and adults differ in dynamic ultrasound-assessed quadriceps contractile behavior during treadmill walking in participants between 4- and 9- months post-ACLR. Approach: Dynamic quadriceps behavior will be assessed in the vastus lateralis as a participant walks on the treadmill during a ten-second interval. vGRF will be collected via loadsol inserts to allow us to identify key pennation angles during the gait cycle (i.e., initial contact and peak vertical ground reaction force). *Our hypothesis is that adolescents will have a smaller change in pennation angle change from initial contact to peak vGRF during the phases of walking when compared to adults.*

Specific Aim 3: Determine if there is an association between self-reported physical activity and static or dynamic ultrasound-assessed muscle quality in participants between 4- and 9-months post-ACLR. Approach: The International Physical Activity Questionnaire and Muscle-Strengthening Exercise Questionnaire Short-Form will be used to assess self-reported physical activity levels. Dynamic (pennation angle changes) and static (CSA and echo intensity) will be used to quantify muscle quality. *Our hypothesis is that those with a higher self-reported activity level will have a greater pennation angle change, CSA, and a lower echo intensity.*

This proposed study will advance the understanding of the ultrasound-assessed quadriceps muscle quality between adults and adolescents post-ACLR. The results of this proposed study will supply knowledge regarding the muscular change differences in adults and adolescents post-ACLR, which could potentially allow clinicians to alter rehabilitation programs based on age. Additionally, this will be the first study to our knowledge that determines if self-reported physical activity level is associated with ultrasound-assessed quadriceps muscle quality in patients post-ACLR.

CHAPTER 2: Review of Literature

Anterior Cruciate Ligament

Anatomy and Function

The anterior cruciate ligament (ACL) consists of dense connective tissue forming a band-like structure, containing bundles of wavy collagen fibers, all arranging in different directions (Strocchi et al., 1992). Collagen fibers are connective tissues consisting of collagen glycoproteins that are often arranged in bundles and are the most common type of fibers in the extracellular matrix (Miko & Varga, 2015). The ACL can be segmented into three separate regions based on its overall compensation. The regions are as follows (Duthon et al., 2006):

A. Proximal Region: The proximal region consists primary of collagen type II, glycoproteins, and fusiform fibroblasts. This region is highly cellular in it make up but less solid when compared to other areas of the ACL.

B. Middle Region: This region, also known as the fusiform zone due to its prevalence of fusiform cells, consists of spindle-shaped fibroblasts, collagen fibers, and elastic and oxytalan fibers. The middle region is characterized by the large amount of longitudinally oriented cells that contain fusiform-shaped nuclei.

C. Distal Region: The distal region is the most solid area of the ACL. This is due to the abundance of chondroblasts and ovoid fibroblasts while having a low concentration of collagen bundles.

In addition to the regions, the ACL can be broken into two fiber bundles: the anteromedial and posterolateral. When the knee enters extension, the posterolateral bundle tightens while the anteromedial bundle loosens. The roles of the bundles switch upon the start of flexion and are due to the attachment site on the femoral condyle (Duthon et al., 2006). The ACL originates on the medial wall of the posterior portion of the lateral femoral condyle and inserts on the intercondylar area of the tibia. Due to its origin on the femoral condyle during extension, the ACL will become taunt with tension (Petersen & Tillmann, 2002). In general, the ACL is not a static bundle of fibers, but rather a dynamic structure that continuously adjusts from tightness to looseness to facilitate optimal knee movement.

The primary function of the ACL is to restrain anterior tibial translation; the ACL accounts for 87% and 85% of the restraining force at 30 degrees and 90 degrees of flexion respectively (Dargel et al., 2007). Without the ACL, the knee would be left unstable resulting in a decrease in the roll-glide movement within the femorotibial joint (Dargel et al., 2007). These deteriorations lead to increased tibial translation in the anterior direction and internal tibial rotation (Dargel et al., 2007). The bundles mentioned previously provide support during different movements. The anteromedial bundle limits the forward translation of the tibia and the posterolateral bundle aids in stabilizing the knee during movements approaching maximum extension (Petersen & Zantop, 2007). While the ACL contributes to supporting extension and hyperextension, its primary function is to prevent excessive anterior translation of the tibia.

Mechanisms and Risk of ACL Injury

ACL injuries can occur during a wide range of activities and movements, such as noncontact and cutting movements. Most injuries transpire during competitive events, with a lesser number of injuries occurring during practice and leisure activities (Kobayashi et al., 2010). Sex, in many cases, does not significantly impact the occurrence of these injuries (Kobayashi et al., 2010). Although sex does not seem to play a role in the type of activity, it does seem to play a role in the mechanism of injury (Kobayashi et al., 2010). A study completed in 2010 found that females are at a higher risk of experiencing an injury during noncontact sports, then their male counterparts during the same noncontact activities. (Kobayashi et al., 2010). In the same study, researchers investigated the difference between injury mechanism and dynamic alignment at the time of the injury. When males and females tore their ACL, their knees were most often in the valgus position with toes abducted (Kobayashi et al., 2010). Although ACL injuries can occur at any time, they are mostly found in non-contact situations, while individuals are in knee valgus and when the toes are pointed out.

Approximately 78% of ACL injuries are reported to occur during noncontact events (Noyes et al., 1983). Females are at a higher risk to sustain an ACL injury during a non-contact activities when compared to males (Boden et al., 2010; Kobayashi et al., 2010). High energy motions are often the mechanism of injury during a non-contact method and are a result of sudden change of directions, rapid deceleration, or during landing activities (Takahashi et al., 2019). Sports such as basketball, soccer, and volleyball are considered high risk for an ACL tear for female athletes (Boden et al., 2010). ACL injuries that occurred in female soccer and basketball players happened during rapid changes of direction or deceleration (Boden et al., 2010). Improper landing mechanics contribute to a higher risk of ACL injury across both sexes and are highest in male and female volleyball players and female handball players (Boden et al., 2010). The risk is amplified by improper landing mechanics, such as when the lower leg and foot act as a single segment, impairing the calf's ability to absorb ground reaction forces during landings.

Griffin et al. (2006) focus on knowledge and prevention of ACL injuries during noncontact events and divide the risk of a non-contact injury into two different risk factors: extrinsic and intrinsic. Intrinsic factors are divided into four subcategories: environmental, anatomical, hormonal, and neuromuscular. Environmental factors focus on the type of surface as a potential risk factor for an ACL injury (Griffin et al., 2006). For example, turf, hard flooring, or grass could be potential environmental risks for an ACL injury during a sport. A prospective study of 8 high school football teams found that ACL injury rates have decreased with the addition of the newest generation of turf compared to grass (Meyers & Barnhill, 2004). However, it is important to note that this study did not include any information on footwear of participants which could be a confounding factor of these results. Although information regarding environmental factors as a mechanism of ACL injuries in a noncontact situation may not be clear, it is plausible to believe it could influence the injuries.

In addition to environmental factors impacting the risk of injury, it is possible that an individual's anatomical structure could increase the risk of injury (Griffin et al., 2006). Specific anatomical characteristics that elevate risk for ACL injury are the quadriceps' femoris angle or Q angle, knee valgus, foot pronation, body mass, width of femoral notch, and ACL geometry (Griffin et al., 2006). While an individual cannot change their overall anatomical structure, it is beneficial to understand who may be at a higher risk for injuries. For example, those with a wider Q angle could have altered biomechanics and the possibility of lower body injuries (Griffin et al., 2006).

ACL Injury and ACLR are Burdensome

Injury to the ACL is frequently studied due to its high prevalence. To understand the prevalence of ACL injury, a population-based historical cohort study was conducted in 2016 to achieve three different goals: (a) Define the population-based incidence of ACL injuries, (b) identify trends in ACL injuries, and (c) evaluate changes in the rate of surgical management (Sanders et al., 2016). The authors concluded that males have a significantly higher injury-rate compared to females in all ages (Sanders et al., 2016). Females reported the highest ACL injuries during the ages of 14 to 18 years, while males reported the highest rates during the ages of 19 to

25 (Sanders et al., 2016). The age ranges could be explained by this being a period where individuals are engaging in high activity sports. Sanders et al. (2016) provides the needed data for the understanding of the prevalence of ACL injuries and the ages that often experience these injuries.

Similarly, another study investigated the occurrence of ACL injuries and ACL reconstruction (ACLR) surgeries among individuals with health insurance living in the United States (Herzog et al., 2017). Between 2002 and 2014, there were a total of 283,810 ACLRs. Males and females aged 13 to 17 had the highest increase in ACLRs during this study's 13-year time frame (females: 169 to 268.7; males 146.8 to 211.7) (Herzog et al., 2017). This highlights the frequency of ACLRs and ACL injury within this age range. This same study found that the prevalence of ACL injury increased 22% in the last 10 to 20 years, which highlights the increasing frequency of ACL injury and ACLR (Herzog et al., 2017). It is important to note that ACL injuries can be costly to mend. A cost-utility analysis study in 2013 found that an ACLR is the most cost effective mode of treatment compared to rehabilitation on its own (Mather et al., 2013). While reconstruction is considered a cost-effective treatment option, it can still impose a financial strain on individuals (Luc et al., 2014).

Mall et al. produced a study in 2014 that found that the rates of ACL injury are increasing in the United States, with rates increasing about 66.8% from 1994 to 2006. The rates of ACLRs performed on individuals under the age of 20 increased during this time frame as well (Mall et al., 2014). ACLR is the preferred method of treatment of ACL injury compared to rehabilitation without surgery because of its effectiveness at repairing stability. Mather et al. found that individuals who only participated in rehabilitation for their ACLR experienced higher instability and lower use levels than those who received an ACLR (Mather et al., 2013). Over 33% of

ACLRs a year are performed on either high school or college athletes (Mall et al., 2014). This study supports that younger populations are at a higher risk of experiencing an ACL injury and receiving reconstructive surgery before reaching the age of 20. These statistics underscore the importance of including a younger population in research, thus providing a compelling rationale for their inclusion.

Individuals following an ACL injury are at a high risk of developing post-traumatic osteoarthritis (PTOA) (Wang et al., 2020). PTOA is a subsection of osteoarthritis which occurs following a ligament injury within a joint (Wang et al., 2020). Osteoarthritis (OA) occurs when there is a degradation of the articular cartilage along with inflammation within the joint space (Abramoff & Caldera, 2020). Individuals suffering from OA experience pain, stiffness, and functional impairments within their joint spaces (Cheung et al., 2020; Lohmander et al., 2007). Individuals with an ACL and ACLR have over an 80% chance of developing PTOA and subsequently OA following their injury (Luc et al., 2014; Wang et al., 2020). Due to younger individuals being at the highest risk for ACL injuries, a younger population accounts for a large portion of those with early-onset OA due to their traumatic knee injuries (Lohmander et al., 2004, 2007). The early-onset of OA in these younger individuals ultimately lead them to have a decreased quality of life as they age (Lohmander et al., 2007).

The studies mentioned above provide evidence that ACL injury and ACLRs are occurring at an increasing rate in both males and females of all ages, causing economic burden to individuals and the health care system. Despite the growing understanding of ACL injuries, the incidence of such injuries has been on the rise in recent years, affecting individuals across all age groups. This trend highlights the increasing significance of gathering comprehensive information on ACL injuries.

Physical Activity Following ACL Injury

Individuals following an ACLR are at a risk of decline in physical activity post-surgery (Kuenze, Collins, Pfeiffer, et al., 2022). This reduction or complete halt of activity may occur throughout the recovery process following an ACLR. Individuals often report not willing to return to physical activity due to the fear of new injury or not trusting their knee (Fomin et al., 2020). This fear may persist past the first year of surgery, where 79% of individuals who never return to sport or their pre-surgery level of physical activity report that it was due to their ACLR. knee (Filbay et al., 2017). Individuals not returning to activity can experience lower quality of life due to the reduction of physical activity (Filbay et al., 2017).

In addition to reduction in physical activity, individuals may demonstrate deficits in muscle strength and functional fitness following an ACLR. This loss of strength and fitness contributes to not returning to sport and lack of confidence due to the weakened performance of their knees. The lack of return to sport can eventually lead to the subsequent ACL injuries. Up to 6 months following ACLR, individuals have isokinetic strength deficiencies compared to their contralateral limbs. The deficiencies in strength were found to be approximately 24.1% lower compared to the individual’s contralateral limb. They can been seen across of all age groups, highlighting how the decreases in strength or physical activity are not specific to a certain age group (Csapo et al., 2020).

Individuals often go into their ACLRs with the expectation of eventually returning to their normal levels of physical activity. In one study, 88% of individuals expected to return to their pre-surgery levels of activity (Webster & Feller, 2019). These expectations were higher in those who were experiencing their first ACLR, while those who with two or more surgeries conveyed expectation to return to normal levels dropped to 63% (Webster & Feller, 2019). The number of individuals who actually return to their previous level of sport is even lower, with only 24% making the return (Webster & Feller, 2019). Those who undergo an ACLR struggle with returning to their previous levels of physical activity, even those who expected themselves to return to full function.

Importance of Physical Activity Following an ACL

Engaging in regular physical activity provides numerous benefits, including the maintenance of overall health, reduction in the risk of obesity, and the promotion of a sound mental health and well-being. (Melo et al., 2016; Waleh, 2016). The decrease of physical activity following ACLR leaves individuals at a higher risk of developing unhealthy habits and increases susceptibility to developing OA. Gaining a comprehensive understanding of the interplay between physical activity and OA is critical for individuals who have undergone ACLR, as they often fall within the criteria of early knee OA affliction (Harkey et al., 2022). Physical activity is vital in the management and control of OA. Physical activity can lead to decreased weight and better joint range of motion, while reducing the symptoms of OA (Bennell et al., 2005; Ettinger et al., 1997). Research has shown that those with OA have found pain reduction when completing both aerobic and strengthening exercises (Roddy, 2005). Given the increased risk of OA for individuals who have undergone ACLR and the recognized benefits of physical activity in managing and controlling OA, it becomes imperative for those recovering from ACLR to actively participate in physical activity after their reconstructive surgery.

Self-Reported Physical Activity

After undergoing ACLR surgery, physical activity is commonly evaluated through self-reported activity reports, primarily due to their convenience and ease of use when compared to quantitative measurements like accelerometers. The International Physical Activity Questionnaire (IPAQ) offers both a long and short form to assess an individual’s physical activity within the past week, allowing clinicians to understand if there is any concern to their current activity level (Craig et al., 2003). Additionally, a newly validated questionnaire, Muscle-Strengthening Exercise Questionnaire (MSEQ) assesses an individual's time participating in muscle-strengthening exercises (Shakespear-Druery et al., 2022). These tools provide the opportunity to assess activity levels following an ACLR to inform treatment and help individuals understand their activity level. They also promote activity level awareness, as continuing exercise after the injury is beneficial in maintaining their health and reducing the chance of developing OA (Cheung et al., 2020).

International Physical Activity Questionnaire Short Form

IPAQ both long and short form versions cover an individual's time and frequency in both moderate and vigorous activity, along with their time spent sitting or walking within the week, providing valuable information on whether individuals are engaging in activity and along with the intensity of that activity (Craig et al., 2003). The use of questionnaires allows for a quick and easy way to assess an individual's physical activity levels, especially following an ACLR. Previous literature has found that the IPAQ varying information on the correlation with accelerometers in several different populations (Grimm et al., 2012; Oliveira et al., 2023; Wolin et al., 2008). A study observing the physical activity levels in elderly individuals compared activity levels collected from the IPAQ short form and accelerometry data finding that there was significant relationship between walking assessed in both forms ($r= .28 - .39$, $p < .05$) (Grimm et al., 2012). Additionally, the IPAQ was found to have good test-retest reliability within an adult population; however, when translated to an adolescent population only a poor to moderate reliability was found. This is due to the fact the IPAQ originally was not created for that age population (Oliveira et al., 2023). Due to the IPAQ being a recall survey, there is always the possibility of a recall bias causing an overestimate in an individual's activity levels. One study found that when compared to the metabolic equivalent of task (METs) the self-reported activity data it is over estimating by 36% to 173% (Trost et al., 2011). Despite differing results, the IPAQ remains a pillar within the self-reported physical activity questionnaires, due to its inclusion of time, frequency, and intensity it does provide helpful information when determining if an individual is meeting physical activity guidelines.

Muscle Strengthening Exercise Questionnaire Short Form

Muscle strengthening exercises have the potential to change overall muscle quality and increase health within individuals (Shakespear-Druery et al., 2022). However, there are limited self-reported questionnaires that include strengthening activities making it difficult to track and assess an individual's time and frequency within these activities. A 9-item instrument was created in 2021 as a physical activity surveillance tool focusing on muscular training activities. The MSEQ collects information regarding an individual's type of muscular activity along with the frequency, duration, intensity, and the muscle groups used, providing researchers detailed information regarding an individual's activity (Shakespear-Druery et al., 2022). The MSEQ was found to have high test-retest reliability for frequency, duration, and intensity for the four types of muscle strengthening exercises (p range 0.76-0.91), additionally, it was shown to have a moderate to high reliability for muscle groups (k range 0.44 – 0.78) (Shakespear-Druery et al., 2022). In the same study MSEQ was found to have a moderate to high validity for frequency, duration, and intensity level (p range 0.30 – 0.77) (Shakespear-Druery et al., 2022). While the MSEQ is relatively new as a survey, it has shown promising results in assessing an individual's engagement in muscle-strengthening activities. This is particularly valuable when evaluating muscle quality post-injury, as these exercises can significantly influence overall muscle characteristics.

Quadriceps Muscle Changes Following ACLR

Quadricep Deficits Overview

Following an ACLR, there are acute strength deficits and overall changes in the muscle activation (Drechsler et al., 2006). A goal of rehabilitation is to improve these deficits while diminishing any asymmetries found between limbs following an ACLR. These deficits can be linked back to several different mechanisms from motor control, central activation failure (CAF), or morphological changes (Larsen et al., 2014; Mirkov et al., 2017; Pamukoff et al., 2018; Pietrosimone et al., 2022; Schmitt et al., 2015). Previous research examining muscle changes post-anterior cruciate ligament reconstruction (ACLR) frequently focuses on either a single quadriceps muscle or multiple muscles. The rectus femoris is commonly selected for studies, particularly those employing diagnostic ultrasound, due to its accessibility for researchers and participants alike (Garcia, Curran, et al., 2020; Paris et al., 2022) In contrast, the vastus lateralis is advantageous for evaluating muscle quality alterations related to knee injuries, as it exclusively spans the knee joint, unlike the rectus femoris, which also extends over the hip joint (*Muscle Atlas*, 2023). Given that the vastus lateralis is a uni-articular muscle that only crosses the knee joint, alterations within this muscle are more likely attributable to alterations occurring at the knee. Consequently, the vastus lateralis is especially pertinent for investigating dynamic muscular changes following an ACLR.

Neuromuscular Control

Motor Control

A study conducted in 2014 found that individuals continued to experience torque deficits in their contralateral limb as much as 9 to 12 months following surgery, despite being well past the return to sport time point (Larsen et al., 2014). Impairments can be found in the late phase of torque development when comparing an ACLR limb to a control limb of a non ACLR individual

(Pamukoff et al., 2018). Pamukoff et al., found a reduction in knee extension moment development in ACLR individuals who had been cleared for sports by clinicians. In conjunction with the Pamukoff results, researchers have found there is a decrease in the early phase of torque or contraction (Mirkov et al., 2017). The ability of limbs to produce explosive strength during the early phase of torque could be controlled by neural mechanisms, specifically within the central nervous system (Folland et al., 2014; Mirkov et al., 2017; Pietrosimone et al., 2022). Mirkov et al., found that individuals with an ACLR have deficits in both early and late phase of torque, limiting a person's ability to produce explosive strength. These results highlight a possible reduction in central nervous system response following an ACLR, causing deficiencies in strength and reduction in overall motor control for the quadriceps following an ACLR.

Central Activation Failure

In addition to a reduction of overall motor control following an ACLR, individuals may also experience central activation failure (CAF). CAF can be a result of the quadriceps inability to recruit all available motor units to produce maximal force (Kent-Braun & Le Blanc, 1996; Pietrosimone et al., 2022). A meta-analysis identified that 11% to 13% of individuals who had an ACLR experienced CAF in their ACLR and contralateral limbs (Hart et al., 2010). These percentages were based on the fact that the weight means of the quadricep activation for the ACLR limb and contralateral limb were 86.9% and 88.6% respectively (Hart et al., 2010). These results support that CAF is not limited to the injury limb and can be detected in the contralateral limb as well following an ACLR.

Morphological Changes

The quality and strength of the quadriceps and the rectus femoris muscle may decrease following an ACLR. This decrease can lead to poor function of the lower limb, changes in biomechanics, and overall reduction in joint health (Pamukoff et al., 2017; Schmitt et al., 2012, 2015). Typically, an individual will not return to sport or return to full activity until 6 to 12 months post-ACLR (Alswat et al., n.d.). During this 6-to-12-month period, an individual's physical activity can decrease as compared to their activity level prior to an ACLR (Alswat et al., n.d.). Typically, individuals can see a decline in muscle mass after only four to six weeks of inactivity (Campbell et al., 2013). Therefore, patients with an ACLR are faced with the potential of a massive decline in their muscle mass following their surgery. Muscle quality is often assessed through the overall echo intensity (EI) of a muscle, typically the rectus femoris. The tracking of EI can detect any major changes such as increases in adipose tissue and other fibrous tissues (Fukumoto et al., 2012). The evaluation of EI through image processing, such as an ultrasound, allows clinicians to have a quantifiable evaluation of a patient's muscle health without the need for the individual to complete any physical tasks. This can be beneficial to individuals early in their recovery process, as they may not be able to complete any of the physical tasks to assess their knee function.

Importance of Ultrasound

Ultrasound is a non-invasive clinically assessable tool that is reliable and validated to assess muscle quality and quality. Ultrasound is cost-effective when compared to the gold standard magnetic resonance imaging (MRI). When assessing skeletal muscle with ultrasound, it is important to select proper settings and probes to ensure the highest quality of image. Due to the quadriceps being a deep muscle group within the thigh, a low-frequency probe (frequency band 3.5 – 10 MHz) is beneficial in obtaining the images (Bianchi & Martinoli, 2007). A low-frequency allows for deeper penetration depth of the imaging (Whittingham, 1999). When assessing muscle with ultrasound, it is important that the proper settings and probes are used to ensure the highest quality image. The use of ultrasound to assess muscle quality allows clinicians to gain vital information regarding their patient's muscle health.

Echo Intensity

Muscle quality, or composition, is often assessed via echo intensity (EI). Muscles are composed of contractile proteins and non-contractile tissues. The non-contractile tissues could include adipose and fibrous tissue, which would be associated with a higher echo intensity (Stock & Thompson, 2020). EI in muscles is quantified as a mean grayscale histogram. The units are arbitrary with a scale between 0 and 255 where 0 is represented as white on ultrasound and 255 represents black. In 2014, Palmer et al. found that panoramic ultrasound is a reliable tool to assess overall muscle size and quality when assessing EI (Palmer et al., 2015). To evaluate the dependability of panoramic ultrasound, researchers conducted assessments of individuals' hamstring muscles on two distinct days (Palmer et al., 2015). The findings revealed that panoramic ultrasound provides a valuable tool for examining both muscle size and quality in individuals (Palmer et al., 2015; Stock & Thompson, 2020).

Fukumoto et al. collected muscle quality assessed via EI, specifically the rectus femoris, and concluded that EI was associated with muscle strength (Fukumoto et al., 2012). The results presented by Fukumoto et al. highlight the potential benefits of using EI to assess the muscle quality changes following an ACLR. When evaluating the quadriceps structure, Fukumoto et al. found that there was an age-related increase in EI (Fukumoto et al., 2012). The atrophy associated with an ACLR can be seen across all age groups. Additionally, strength can decrease up to 30% in the ACLR limb compared to the contralateral limb at 6-months following after completing surgery (Bryant et al., 2008). The reduction in strength can lead to a decrease in lower limb control and reinjury of the ligament. Therefore, it is important for individuals to gain more control before returning to sport or activity (Kellis et al., 2019). Although atrophy and strength deficits are prevalent in ACLR patients, an understanding of all factors that impact muscle quality following the surgery remains unknown.

A study focusing on association of muscle strength and muscle quality in elderly men (65-91 years) found that EI had a significant negative correlation with muscle strength, which could lead to a lower quality of life (Watanabe et al., 2018). Additionally, Garcia et al., found that a lower EI, or a higher quality of muscle, was associated with higher self-reported function in individuals with an ACLR (Garcia, Moffit, et al., 2020). Similarly, Fukumoto et al., concluded that the use of ultrasound EI was related to overly muscle strength in elderly women (Fukumoto et al., 2012). When compared to MRI, Young et al., found that the use of US to estimate intramuscular fat percentage was strongly correlated to MRI fat percentage (Young et al., 2015). The cost-effectiveness, versatility, and ease of use makes the diagnostic US a popular tool to observe muscle quality.

Muscle Size

Muscle size is a known determinant of overall strength, so the ability to assess and monitor changes to size can be vital knowledge for clinicians (Bamman et al., 2000). A study conducted in 2016 found that muscle size in recreationally active individuals was associated with knee flexor eccentric strength when size was assessed via MRI (Evangelidis et al., 2016). However, MRI is not the only way to assess muscle-size as panoramic US imaging can be used to assess larger muscle sizes to ensure the entire organ is within view (Franchi et al., 2020). Additionally, a group sought to investigate the validity of panoramic ultrasound to MRI in adolescent alpine ski racers (Franchi et al., 2020). This group focused on the cross-sectional area (CSA) of the hamstrings for their assessment. Their results highlighted that there was agreement between a trained rater and MRI for the CSA of the hamstrings (Franchi et al., 2020). The results presented by this group show the validity of use of ultrasound derived CSA to area detected by an MRI.

An additional study sought to assess the “reliability and validity of the panoramic brightness mode ultrasound method to detect training-induced changes in muscle CSA” when the results produced from the ultrasound were compared to results collected by an MRI (Ahtiainen et al., 2010). In young, healthy, and untrained males, they found that the validity of the “ultrasound method against MRI in assessing CSA in vastus lateralis produced an ICC of 0.905” (Ahtiainen et al., 2010). They also found agreement between the two methods in detecting changes within the CSA (ICC = 0.929) even though the CSA obtained via MRI was larger when compared to the ultrasound (Ahtiainen et al., 2010). The results of this study show that panoramic imaging via ultrasound of the CSA is valid and repeatable against MRI.

Evaluating muscle thickness can be beneficial when researchers are unable to collect panoramic imaging with ultrasound. Muscle thickness can be defined as the total distance between two fasciae of a muscle of interest (Strasser et al., 2013). A study in 2022 evaluated inter- and intra-rater reliability of ultrasound in the tensor fasciae latae and gluteus medius muscles (Lanza et al., 2022). This study found evidence of “excellent intra-rater ICC values” for both the tensor fascia latae and gluteus medius (Lanza et al., 2022). This means that there was high correlation in the degree of agreement when the same rater measures the muscle multiple times. Additionally, this study showed high inter-rater ICC values for both muscles examined, meaning that separate individuals assessed similar degree of agreement for the tensor fascia latae and gluteus medius thickness (Lanza et al., 2022). This highlights the usability of ultrasound assessed muscle thickness. Another study assessed muscle thickness and its relationship to frailty, and with the use of ultrasound, they found that muscle thickness of the vastus lateralis muscle and anterior tibialis decreased with increased frailty (Lv et al., 2022). The thickness of the muscle is a useful measurement when panoramic imaging is not available.

Fascicle Length

Fascicles, or bundles of muscle fibers, are correlated to the amount of force a muscle is able to generate and will affect the muscle's overall range of motion. The rectus femoris which is classified as a bipennate muscle, has fascicles located on both sides of the tendon. The arrangement is similar to a feather pattern (Biga et al., 2019). The fascicle length of a muscle is vital in its ability to produce force and can be connected to a muscles overall quality (Adkins & Murray, 2020). Ultrasound can often be used to assess fascicle length in an individual. There are multiple ways to assess fascicle length with ultrasound technology, such as using equations to predict the entire fascicle when only a portion is visible with the transducer or using multiple transducers to capture the entire image area (Brennan et al., 2017). Individuals following an ACLR have been found to have fascicles that performed at slower shortening velocities when compared to healthy controls (Davi et al., 2021). Additionally, those with an ACLR had fascicles that underwent less excursion, or the total length change of an fascicles during range of motion, as compared to healthy controls (Davi et al., 2021). Understanding fascicle length post-ACLR can offer an investment in overall muscle health.

Pennation Angle

Pennation angle of a muscle refers to the angle between the muscle fiber fascicles and the deep aponeurosis of insertion. It is possible that changes in pennation angle can affect overall CSA or overall force production of a muscle (Aagaard et al., 2001). A study focusing on the cellular and morphological alterations in the vastus lateralis following ACLR found that pennation angle decreased in the ACLR limb following reconstructive surgery (Noehren et al., 2016). Images after reconstruction were collected by MRI once the individuals return to sport. This reduction in pennation angle will limit the number of fibers within the area, leading to a decrease in force within the ACLR limb (Noehren et al., 2016). However, a different study assessing the architect of the quadriceps two years after an ACLR found no difference in the pennation angle between the ACLR and contralateral limb at two year mark (Longo et al., 2014). Both studies used different imaging techniques to capture the pennation angle and collected the angles at different time points following an ACLR. Therefore, time and imaging capturing techniques may influence pennation angle.

Ultrasound Assessment of Quadriceps Muscle after ACLR

The use of US following an ACLR can help clinicians to assess the quadriceps of individuals in a cost-effective and portable method. US allows clinicians to inspect the physical changes within the quadriceps after an ACLR, which is beneficial in understanding their healing process. Previous research often uses multiple of the quadricep muscles within their study design, however, the most commonly used on its own is the rectus femoris (Garcia, Curran, et al., 2020; J.-H. Lee et al., 2020; Paris et al., 2022). The use of panoramic imaging allows clinicians to have the entire quadricep in view. Garcia et al., assessed CSA and EI via US in individuals who received an ACLR. Their results showed that the CSA of the vastus lateralis was smaller in the ACLR limb as compared to the contralateral limb and the lateralis also contained a higher EI compared to the contralateral limb (Garcia, Moffit, et al., 2020). The higher EI in the ACLR limb implies that there are more non contractile fibers within the muscle, such as adipose. Garcia et al. concluded that larger vastus lateralis and lower EI was associated with higher self-reported function (i.e., International Knee Documentation Committee score), highlighting the importance in overall CSA and EI (Garcia, Moffit, et al., 2020). Similarly, Lee et al., sought to compare quadriceps thickness between an individual's ACLR limb and contralateral limb via US. For this study, the images were taken an hour before the reconstruction occurred and anywhere from 2 to 3 days after the surgery. Thickness was defined as the distance between the superficial and deep border of the muscles (J.-H. Lee et al., 2020). The results of this study highlighted that the vastus intermedius had a significant reduction in thickness compared to the contralateral limb from prior to surgery to only a few days after (J.-H. Lee et al., 2020). The use of portable US was effective in detecting thickness changes post-surgery in individuals with an ACLR.

A study conducted in 2019 sought to examine quadriceps thickness and biomarkers following an ACLR (Yang et al., 2019). The researchers assessed the rectus femoris and vastus intermedius, lateralis, medialis and medialis oblique with a US machine pre-ACLR and again two visits post-surgery (Yang et al., 2019). They found a significant reduction in thickness in all assessed muscles, and thickness was greater in the pre-surgery assessment than both posts (Yang et al., 2019). The vastus intermedius was the only muscle assessed that had additional reduction between the two post assessments (Yang et al., 2019). These studies highlight the benefits of assessing quadriceps following an ACLR with a US machine.

Association Between Physical Activity and Muscle Characteristics

Physical activity is associated with health benefits, and reduction of several different diseases such coronary heart disease and type 2 diabetes (Penedo & Dahn, 2005; Rostron et al., 2021). In addition, physical activity is associated with reduced stress and overall weight (Canning et al., 2014). On the opposite end of the spectrum, a decline in physical activity is connected with a decline in muscle characteristics, such as strength and overall muscle size (Penedo & Dahn, 2005). A study conducted in 2021 investigated the effects of a 16-week resistance training program on the muscle quality in older women, specifically in the thigh area. The thigh composition was assessed via computed tomography and the results found that the addition of resistance training can improve muscle quality and overall functional fitness within older women with sarcopenia (Seo et al., 2021). Hofmann et al., evaluated the effects of elastic band resistance training in older women. When defining muscle quality as the ratio between muscle strength and overall mass they found that the older women benefited in overall muscle quality in the lower limbs following a resistance band intervention (Hofmann et al., 2016). Due to resistance type exercises having an influence on overall muscle characteristics, the information collected by the MSEQ could provide insight on why there is a decline in muscle quality when comparing limbs. The MSEQ will identify individuals engaging in these resistance type exercises while providing a possible reason for their overall muscle quality (Shakespear-Druery et al., 2022). For those individuals not participating in resistance exercises the IPAQ Short-Form is able to capture information on frequency, duration and type, moderate or vigorous, exercise (Craig et al., 2003). These two questionnaires can collect a wide range of information regarding an individual's physical activity levels. A study in 2014 assessed muscle quality in terms of muscle quality index. Muscle quality index refers to the timed sit to stand, body mass, and leg length (Fragala et al., 2014; Takai et al., 2009). The results of this study found that a 6-week evolving resistance training program was able to increase the muscle quality index in older individuals. Physical activity can be beneficial to improving an individual's muscle quality and characteristics.

Adults' vs Adolescents:

Differences in Rate and Outcomes Related to ACLR

Adolescents, or those with an age ranging from 15 to 18 years, could be suspected to at higher risk of experiencing an ACL injury due to their involvement in physical activities such as sports. Both males and females suffer higher rates of ACL injuries within this age range (DiSanti et al., 2018; Herzog et al., 2017). It is unknown what differences occur in the musculoskeletal regions between adults and adolescents following an ACLR. Adolescents experience physical and psychological injury related to trauma different from their elderly peers (Disanti et al., 2018). Along with psychological and physical response to trauma, adolescents experience skeletal growth and maturation during these essential years of development (Tanner, 1981). This makes their recovery starkly different when compared to adults. Adolescents and adults are inherently different leading to the possibility that their recovery following an ACLR could have different effects on their quadriceps and their ability to return to sport. Understanding the differences in recovery of adolescents and adults allows clinicals to monitor and progress patients throughout the recovery process (Kuenze et al., 2023).

Adolescents are found to be less physically active when compared to adults following an ACLR. In as little as 8-months following an ACLR, adults were participating more in moderate to vigorous physical activity as compared to the younger population (Kuenze, Collins, Triplett, et al., 2022). Adolescents following an ACLR were participating in moderate to vigorous 33% less than adults, while taking 26% fewer steps compared to adults (Kuenze, Collins, Triplett, et al., 2022). Even though the adolescents reported higher physical activity levels before injury, following ACLR their activity levels are lower compared to adults (Kuenze, Collins, Triplett, et al., 2022). The decline in physical activity in adolescents is concerning since physical activity adopted during adolescence are a predictor of adult physical activity levels (Kuenze, Collins, Triplett, et al., 2022). The decline in physical activity following an ACLR in adolescents could potentially lead to obesity, decline in muscle characteristics, and risk of more injury.

When using self-reported questionnaires to assess physical activity post-ACLR there are limited data between adolescents and adults. However, a study completed in 2006 found that there was not a statistically significant difference from athletes' (ages: 17 to 50 years) pre-injury activity scale to their follow-up visit. However, the follow-up visit was 1 year post-operation, which may influence the lack of difference between the two assessments (Gobbi & Francisco, 2006). Additionally, when using the Tegner Activity Scale, a study found that individuals with an ACLR engaged in lower peak levels of physical activity during the past year when compared to control individuals of similar age (Kuenze et al., 2019). Self-reported activity provides a convenient method of assessing decreases of activity following an ACLR.

In addition to having a reduced level of physical activity as compared to adults, adolescents have also been found to have a slower habitual speed when compared to older individuals. Adolescents have a lower ground reaction force during stance phase, in addition to having a slower speed (Lisee et al., 2023). The slower walking speed could lead to concern of decrease knee health following ACLR, which could affect overall physical activity following injury. Adolescents and adults heal and recover following an ACLR differently, and understanding what causes these differences can provide clinicians with knowledge on how to care for and treat these different ages. The differences in muscle quality between adults and adolescents following an ACLR is unknown; however, both groups recover differently.

Adults' vs Adolescents: Differences in Muscle Characteristics

Adults and adolescents are inherently different from each other, especially in the muscular system. There is limited research on quadriceps changes between adults and adolescents in a healthy population. A study tracking growth changes within the elastic properties of the vastus lateralis tendon in males ranging from 10 to 24 years found that tendon structure in the younger age group was more compliant compared to the older population (Kubo et al., 2001). A study completed in 1993 assessed the determinants of peak muscle power in ages ranging from 8 to 65 years. Based on study findings, the authors concluded that age- and training-related changes seen in absolute peak muscle power are not connected with muscle high energy phosphate content. Additionally, they concluded that differences in peak power can be partially connected to the CSA of the muscle. Peak muscle power was significantly lower in children ages 8-13 years and adults ages 20-50 years. As to be expected, the CSA of children was significantly lower when compared to adults (Ferretti et al., 1994). Adults and adolescents have differences within their muscle characteristics; however, the drivers of these differences are not obvious.

Summary

Comprehending the disparities in muscle quality assessed via ultrasound, both static and dynamic images, between adults and adolescents after ACLR can empower clinicians and individuals to make well-informed decisions regarding the recovery process. Diagnostic ultrasound is a readily accessible tool to assess and quantify muscle characteristics. Past research has shown that adults and adolescents exhibit differentiating recovery patterns following ACLR, specifically adolescents displaying reduced levels of physical activity compared to adults. Thus, highlighting the importance on determining if adults and adolescents have large differences in muscle quality following an ACLR and if muscle quality is associated with self-reported physical activity within this demographic.

CHAPTER 3: Methods

Research Design

We conducted a cross-sectional study during a single laboratory visit to assess both static and dynamic quadriceps muscle quality using ultrasound and physical activity assessed via self-reported questionnaire to address three specific aims: Specific Aim 1 determined the differences between adults and adolescents using static ultrasound assessed muscle quality of the rectus femoris; Specific Aim 2 assessed the difference between adults and adolescents in dynamic quadriceps muscle contractile behavior during treadmill walking; and Specific Aim 3 determined the association between self-reported physical activity and both static and dynamic ultrasound-assessed muscle quality. For aim 1 and 2 we utilized different quadriceps muscles. Aim 1 utilized the rectus femoris due to its accessibility during imaging and past success within the laboratory. In Aim 2, the vastus lateralis was utilized because it only crosses one joint, making any changes to the muscle directly attributable to the knee joint. Continuous data were acquired by measuring the CSA and echo-intensity collected during a static ultrasound assessment. Additionally, dynamic ultrasound measurements of pennation angle changes from initial contact to peak vertical ground reaction force (vGRF) during walking were collected on the ACLR limb of participants. Ordinal data were gathered through participants responses to the IPAQ and MSEQ.

Subjects

The participants within this study were a part of an ongoing longitudinal study at Michigan State University assessing clinical outcomes following ACLR. This study was approved by the University’s Institutional Review Board, and all participants completed written informed consent. Individuals were eligible for this study if they have experienced an ACL injury and received reconstructive surgery between 4 to 9 months before enrolling in this study and between the ages of 14 to 36. To be included in this study individuals did not need to be cleared for full activity. Individuals were excluded from this study if they experienced a lower extremity fracture during the ACL injury or if they received multiple ligament surgeries during their ACLR. Based on age limit recommendations from a previously published paper, participants between the ages of 14 to 17.9 were considered adolescents, while participants between the ages of 18 to 36 were considered to be adults (Kuenze, Collins, Triplett, et al., 2022). If participants were under the age of 18 at the time of testing, a legal parent or guardian provided consent, and the participant under the age of 18 provided assent. If participants were above the age of 18, they provided written informed consent to participate in the study. Height (cm), sitting height (cm), and mass (kg) were collected for all participants.

To estimate age at peak height velocity in the adolescent population, two regression models were utilized based on sex (Moore et al., 2015):

Girls:

Maturity offset (years) = -7.709133 + (0.0042232 x (age x stature))

Boys:

Maturity offset (years) = -8.128741 + (0.0070346 x (age x sitting height))

Using sitting height, stature, and age we were able to predict years from age from peak height velocity (Moore et al., 2015). Peak height velocity is an important maturational time point and represents a time when yearly height increases are maximized (Tsutsui et al., 2022).

The same participants were used for all three aims of this study. For aims 1 and 2, we conducted an *a priori* power analysis using an effect size from a prior study that identified differences in physical activity levels between adult and adolescent patients following ACLR. This study found adults had 8,365 ± 2,294 steps per day while adolescents had 6,153 ± 1,765 steps per day effect size of 1.08 between groups (Kuenze, Collins, Triplett, et al., 2022). Therefore, with an *a priori* power analysis using an effect size of 1.08, power of 80%, and an alpha level of 0.05, we would need 12 participants in each group (24 total) to be powered to detect differences between groups. For aim 3, we again conducted an *a priori* power analysis using an effect size from a prior study that assessed the association between physical activity and the composition of muscles in older individuals (Varesco et al., 2022). This study found that steps per day and the percentage of type IIx fibers had an effect size of 0.57. Therefore, with an a priori power analysis using an effect size of 0.57, power of 80%, and alpha level of 0.05, we would need 21 participants to detect any association.

Specific Aim 1

Static Quadriceps Muscle Ultrasound Assessment

Ultrasound images were collected on the participant's ACLR and contralateral limbs using a GE LOGIQ P9 R3 ultrasound machine (GE Healthcare, Chicago, IL). We collected three panoramic transverse scans on each limb, capturing cross-sectional images of the quadriceps, particularly focusing on the rectus femoris (Figure 1). We focused on the rectus femoris muscle for our static quadriceps muscle assessment as it is the most superficial quadriceps muscle. Ultrasound images were acquired at a depth of 4 cm, with a frequency of 12 Hz and the gain set at 50. Participants were positioned supine on the examination table, with their knees flexed at 20-30 degrees and supported by a bolster (Figure 1). A reference mark was placed at 50% of the measured distance between the anterior superior iliac spine (ASIS) and the lateral knee joint line. Participants were instructed to keep their quadriceps muscles relaxed during the image collection process. The images were taken precisely at the 50% mark to encompass the entire cross-section of the rectus femoris. To ensure successful transmission of the ultrasound waves with minimal interference, an ample amount of ultrasound gel was applied. The imaging probe was situated on the medial aspect of the thigh and then moved laterally to capture a CSA of the rectus femoris, along with the overlying subcutaneous fat. Throughout the image collection, the transducer was kept perpendicular to the skin to maintain consistency and accuracy.

Static Ultrasound Image Segmentation

Ultrasound images were analyzed using the open-source ImageJ software (National Institutes of Health, Bethesda, MD) (Schneider et al., 2012). The aponeuroses encircling the rectus femoris were delineated, with exclusion of any hyperechoic tissue surrounding the muscle during the segmentation process (Figure 2A). The ultrasound reader had previously established high inter-rater reliability for CSAs compared to a trained reader in our lab on a reliability set of 30 images ($ICC_{2,k}$=0.99). For assessment of the muscle a different researcher than the one who collected the scans completed the segmentation of the rectus femoris. Therefore, they were blinded by which group the participants were in, i.e. if they were adults or adolescents. The area and mean echo-intensity was quantified for the segmented cross-section of the muscle. Echo-intensity, or brightness of the image, was averaged within the area on a scale from 0 - 255 (0 = black; 255 = white) (Figure 2B). Echo intensity measurements closer to 0 or a more hypoechoic color indicates a higher quality of muscle, while a mean closer to 255 or a more hyperechoic indicates a lower quality of muscle. For statistical analysis, the limb symmetry index (LSI) for both CSA and echo intensity were calculated. The equation that was utilized to assess LSI is as follows:

$$\text{LSI} = (\text{ACLR Limb} / \text{Contralateral Limb}) * 100$$

LSI was utilized in statistical analysis to quantify any differences in muscle quality between the ACLR limb and the contralateral limb.

Figure 1: Static ultrasound collection of the rectus femoris. Panoramic transverse scans of the rectus femoris are collected at 50% between the anterior superior iliac spine (ASIS) and the lateral joint line.

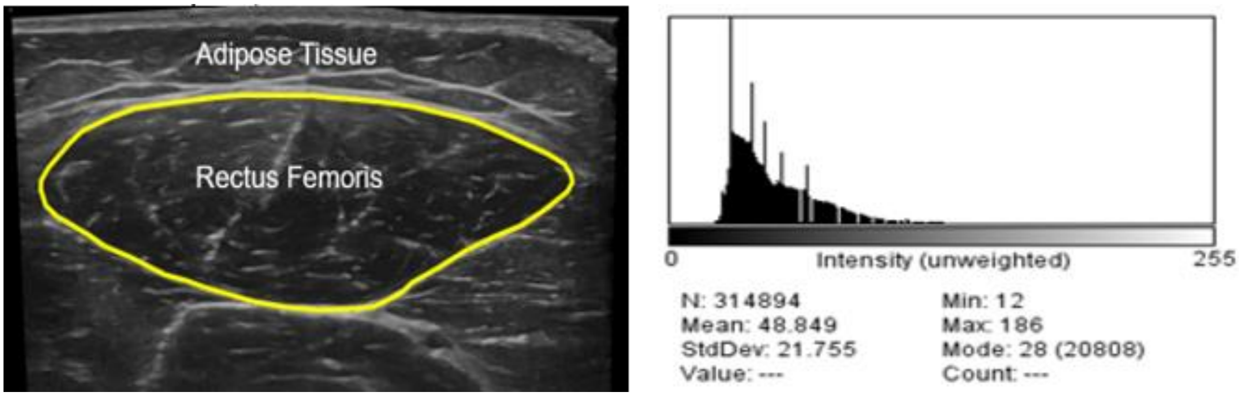


Figure 2: A. The cross-sectional area sectioned off in ImageJ allowing for the program to assess only the area of the rectus femoris for muscle quality. B. The histogram produced based off the cross-sectional area of the rectus femoris highlighting the mean and standard deviation of muscle quality (48.85 ± 21.76). A lower mean is associated with higher quality of muscle, a higher mean is associated with a lower quality of muscle.

Statistical Analysis

Statistical analysis was performed within SPSS for Windows (*IBM SPSS Statistics for Windows*, 2021). The statistical analysis for this study aimed to evaluate the differences in static ultrasound-assessed muscle quality LSI between adults and adolescents. Our data was checked for normality with a Sharipo Wilks, with a prior set at 0.05; therefore, we utilized an independent t-test to compare quadriceps CSA and echo-intensity LSI between adults and adolescents. As a post-doc sensitivity analysis, we defined outliers that were greater than two standard deviations away from the mean in any of the muscle ultrasound outcomes. If any outlier was identified, the outlier was removed, and analyses were then repeated. P-value was set *a priori* at <0.05. Figures and tables were created within Jamovi (*The Jamovi Project*, 2024). Cohen's *d* were interpreted as small ($d = 0.2$), medium ($d = 0.5$), and large ($d = 0.8$) (Lakens, 2013).

Specific Aim 2

Dynamic Quadriceps Muscle Ultrasound Assessment

In addition to the static muscle ultrasound assessment, we performed a dynamic ultrasound assessment to track how the muscle functions during a treadmill walking task. With recent advancements in a novel wireless ultrasound probe and a 3D printed Usono probe holder, we strapped a wireless ultrasound probe to a participant's thigh for a more convenient and easier setup to dynamically monitor how the muscle responds to a functional task. A wireless ultrasound unit was placed at 50% of the thigh length between the ASIS and lateral joint line of the vastus lateralis on the ACLR limb. For this dynamic quadriceps assessment, we chose to focus on the vastus lateralis as this quadriceps muscle only crosses one joint; thus, any variation in fascicle behavior can be attributed to what is occurring at the knee joint. A Clarius L15 linear ultrasound probe was utilized as the wireless ultrasound unit. The Clarius wireless ultrasound unit transmits images to an iPad via a direct WIFI connection, making it an easily accessible tool to assess dynamic movements. Clarius was attached at 50% of the vastus lateralis with the Usono mounting strap, on the longitudinal plane of the muscle (Figure 3). Participants were instructed to walk on the treadmill at their habitual overground walking speed (Figure 4), which was determined by having the participants complete at least five overground walking trials where their speed is monitored with timing gaits. Two ten second videos were collected using the Clarius video tool.

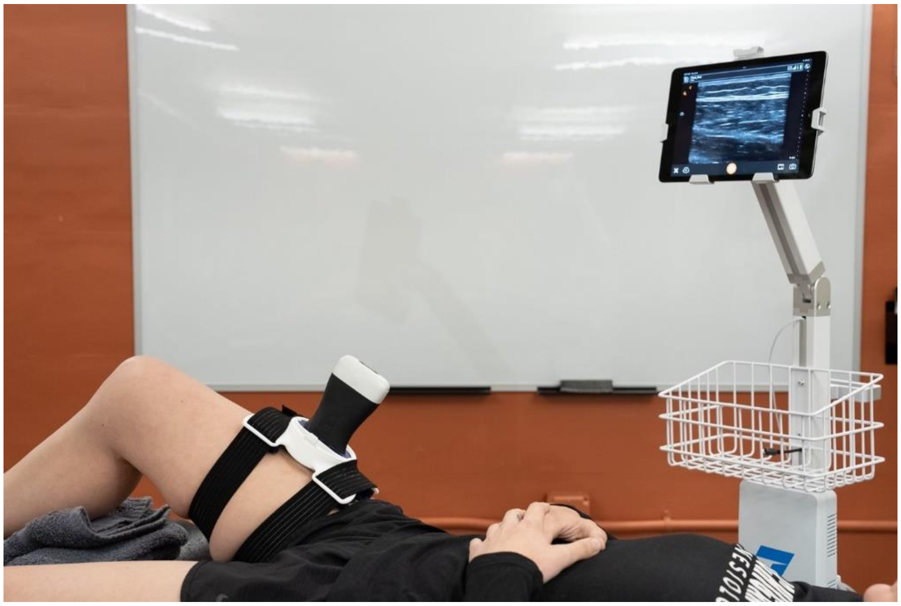


Figure 3: Wireless ultrasound placement for dynamic ultrasound assessment. The wireless Clarius ultrasound was attached at the 50% of the vastus lateralis with the Usono mounting strap with the participants were supine on the examination table. The probe was placed on the longitudinal plane.

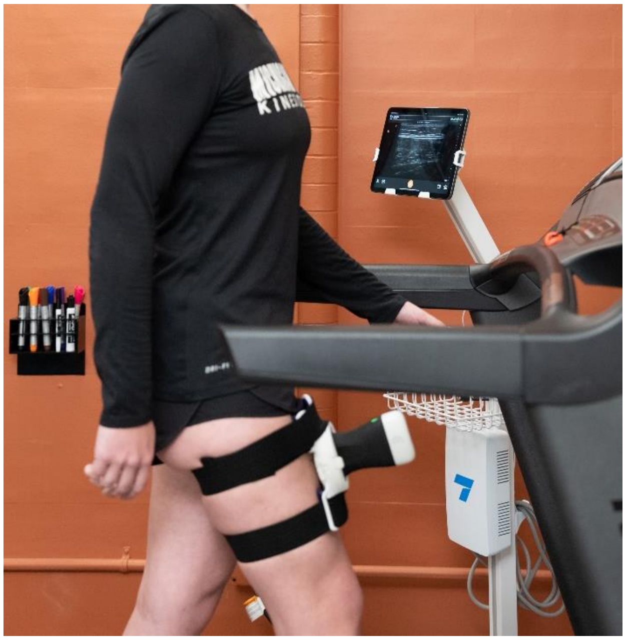


Figure 4: Dynamic ultrasound assessment of the vastus lateralis. Participants are instructed to walk at a specific speed while the Clarius wireless ultrasound unit collects 10 seconds videos of the vastus lateralis.

LoadSols

During the treadmill walking task, we placed LoadSol force-sensing inserts into participants' personal shoes to monitor the limb’s vGRF throughout the gait cycle. At the beginning of the visit, participants were equipped with LoadSol inserts (loadsol, Novel Electronics, St. Paul, MN, USA) in their chosen walking shoes. Their body weight was inputted into the LoadSol app, where it was converted to Newtons (N), with a maximum resolution set at 2.5 N, covering a force range of 7 to 2000 N. We utilized LoadSols recommended guidelines for the calibration of the sensors. We utilized this vGRF data to identify the participant’s initial contact and peak vGRF during each step of the trial. These points were then used to determine the corresponding frames on the Clarius ultrasound video. For each initial contact and peak vGRF within a 10-second period, the times were identified. With these times, frames were pinpointed in the Clarius video so that pennation angles at initial contact and peak vGRF could be included in the statistical analysis. Both the Clarius and Loadsol app were used simultaneously to easily identify key frames (Figure 5).

Figure 5: LoadSol starting point. Loadsol was used in conjunction with the Clarius ultrasound to pinpoint the timing of initial contact and peak vertical ground reaction force.

Pennation Angle Segmentation

Pennation angle is vital in a muscle's ability to produce force; therefore, understanding any differences within the angles following an ACLR could be vital in understanding the muscle quality post-surgery (Sopher et al., 2017). During the dynamic walking trials, we assessed pennation angle at initial contact and peak vGRF for each gait cycle included in a trial using the open-source ImageJ software (National Institutes of Health, Bethesda, MD) (Schneider et al., 2012). For assessment of the muscle, the researcher was blinded on which group the participant was in, i.e., if they were adults or adolescents. To begin the image processing of the vastus lateralis, the region of interest was identified as the area between the superficial and deep aponeurosis. We then traced a singular fascicle within the vastus lateralis muscle body at the ultrasound frame corresponding to the initial contact phase of gait. We utilized the angle function within ImageJ to determine the pennation angle between the deep aponeurosis and the selected fascicle (Figure 6). Next, we repeated these steps for the ultrasound frame corresponding to the peak vGRF. Change in pennation angle was calculated from initial contact to peak vGRF on the ACLR limb. For each gait cycle initial contact and peak vGRF was identified. The average difference of the pennation angle at all initial contacts and peak vGRFs of each gate cycle was utilized within the statistical analysis.

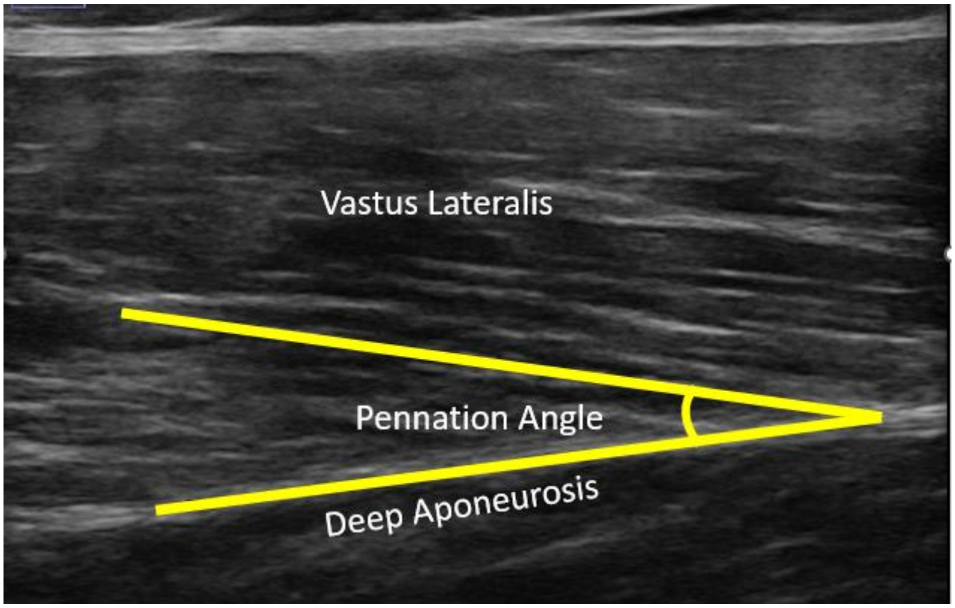


Figure 6: Pennation angle calculated within ImageJ. Pennation angle was calculated as the angle between the deep aponeurosis of the vastus lateralis and a selected fascicle within the muscle.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows (*IBM SPSS Statistics for Windows*, 2021). The statistical analysis in this study assessed the differences in the pennation angle change from initial contact to peak vGRF between two distinct age groups: adults and adolescents. Our data was checked for normality with a Sharipo Wilks, with a prior set at 0.05. An independent t-test was used to compare pennation angle change from initial contact to peak vGRF between the adults and adolescents. Figures and tables were created within Jamovi (*The Jamovi Project*, 2024). Cohen's *d* were interpreted as small ($d = 0.2$), medium ($d = 0.5$), and large ($d = 0.8$) (Lakens, 2013).

Specific Aim 3

Self-Reported Activity

The IPAQ and MSEQ short forms were used to assess participants' activity levels between 4- and 9-months post-ACLR. The IPAQ was utilized to assess participants' frequency and time spent in both vigorous and moderate activity in the past seven days. Vigorous activity ranges from activities like heavy lifting to aerobics, while moderate activities include bicycling or carrying light loads. Additionally, the IPAQ collects frequency and time spent walking, lastly, it does include time spent sitting whether that be at work or at home. The IPAQ provides information on the total metabolic equivalent of tasks (METs) an individual engages in throughout a typical week. METs per week collected via the IPAQ short form were utilized within the statistical analysis. MSEQ was utilized to collect information regarding the participants' frequency and time spent engaging in strengthening exercises in a usual week. This form includes types of exercise, for example weight machines, body weight, or resistance exercise, and what body part is primarily targeted. Both minutes per week spent engaging in muscle-strengthening exercises and the intensity of exercises were utilized within the statistical analysis. The previously mentioned muscle quality assessments were utilized within this aim. Static CSA and echo intensity of the rectus femoris and dynamic pennation angle changes of the vastus lateralis were included within the statistical analysis.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows (*IBM SPSS Statistics for Windows*, 2021). The statistical analysis for this specific aim determined the relationship between participants' activity levels, as determined by IPAQ (METs per week) and MSEQ (time spent and intensity) short forms, and their muscle quality, assessed through static and dynamic ultrasound measurements. To assess the relationship between muscle quality, defined as CSA, echo intensity, and pennation angle change, and self-reported physical activity levels, we utilized a Spearman’s rank order correlation. We used Spearman’s correlation because an *a priori* Shapiro Wilks test indicated that IPAQ and MSEQ data were non-normally distributed. The intensity score from the MSEQ includes missing data, as one adolescent did not fully complete the questionnaire during the visit (n=11). As a post-hoc sensitivity analysis, we defined outliers were greater than two standard deviations away from the mean in any of the muscle ultrasound outcomes. If any outlier was identified, the outlier was removed analyses were then repeated. Classification of association is as follows; very high (0.90 to 1.00), high (0.70 to 0.90), moderate (0.50 to 0.70), low (0.30 to 0.50), and negligible (0.00 to 0.30) (Mukaka, 2012). Figures and tables were created within Jamovi (*The Jamovi Project*, 2024).

Results

Twenty-four participants (10 females, 42%) were recruited for this study, 12 adolescents (4 females [33%], age: 16.01 ± 1.35 years, height: 171.68 ± 8.46 cm, weight: 70.81 ± 12.53 kg, maturity offset: 2.39 ± 1.30 years) and 12 adults (6 females [50%], age: 24.36 ± 7.42 years, height: 173.98 ± 6.99 cm, weight: 78.97 ± 14.41 kg). 21 (11 adolescents) individuals identified as Non-Hispanic or Latino, the remaining three identified as Unknown or not reported. Complete demographics can be found in Table 1. A previous study found that time of day, classified as morning or evening, has an effect on both resting (13.0 ± 5.1%, p = 0.05) and peak contracted (8.0 ± 3.1%, p = 0.04) pennation angle (Pearson & Onambele, 2005). In our study, 21% of adults and 17% of adolescent participants attended their visit during the morning (8-11:59AM), and 29% of adults and 33% of adolescents had their visit throughout the afternoon to evening (12-6pm).

Individuals' mean, median, and standard deviation of muscle quality characteristics can be found in Table 2. For aim 1, there were no significant differences between adolescents and adults for rectus femoris CSA LSI (t_{22} = 0.220, p = 0.828, *d* = .090) or echo intensity LSI (t_{22} = -1.417, p = 0.171, *d* = -0.578). However, an outlier was identified in an adolescent participant for echo intensity LSI. With the outlier removed, there was a significant difference between groups (t_{21} = -2.46, p = 0.023, *d* = -1.02). For aim 2, there were no significant differences between adolescents and adults for pennation angle change (t_{22} = 0.412, p = 0.684, *d* = 0.168). Individuals' mean, median, standard deviation, and interquartile range of physical activity questionnaires can be found in Table 3. For aim 3, we found negligible and low associations between rectus femoris CSA, echo intensity, and METs from the IPAQ short form (r = -0.023, p = .916) (r = 0.43, p = .840), as well as a negligible association between vastus lateralis pennation angle change and METs (r = 0.116, p = 0.590). The association between rectus femoris CSA and the intensity of muscle-strengthening exercises was also negligible (r = 0.154, p = 0.483). A low, negative association was observed between rectus femoris echo intensity and exercise intensity (r = -0.405, p = 0.055), with a low association between pennation angle change and exercise intensity (r = 0.354, p = 0.097). Lastly, negative, negligible associations were found between minutes of muscle-strengthening exercises and rectus femoris CSA (r = -0.226, p = 0.288) and echo intensity (r = -0.075, p = 0.728), with a negligible association for pennation angle change (r = 0.244, p = 0.250). Due to an outlier being identified in Aim 1, we again removed it and reran the associations between echo intensity, METs, intensity of muscle-strengthening exercises, and minutes of muscle-strengthening exercises (r = 0.209, p = 0.340; r = -0.174, p = 0.439; r = -0.083, p = 0.705). Complete statistics for Aim 3 can be found in Table 4.

Table 1: Demographics

	Age Groups	Mean	SD	Minimum	Maximum
Age (years)	Adolescents	16.01	1.35	14.02	17.91
	Adults	24.36	7.42	18.37	36.35
Height (cm)	Adolescents	171.68	8.46	157.00	188.00
	Adults	173.98	6.99	157.00	182.00
Weight (kg)	Adolescents	70.81	12.53	50.50	86.80
	Adults	78.97	14.41	61.90	104.10
Maturity Offset (years)	Adolescents	2.39	1.30	0.05	4.28
Time Since Surgery (months)	Adolescents	5.18	1.44	3.67	8.54
	Adults	6.15	1.53	4.10	9.40
Sex (n [%] female)	Adolescents	4 (33%)			
	Adults	6 (50%)			

Abbreviations: cm, centimeters; kg, kilograms; SD, standard deviation.

Table 2: Muscle Characteristics

	Age Groups	**Mean**	**Median**	**SD**	**Minimum**	**Maximum**
ACLR CSA (cm^2)	Adolescents	10.19	9.57	3.51	4.73	16.57
	Adults	10.44	9.02	3.33	6.67	15.82
Contralateral CSA (cm^2)	Adolescents	10.78	10.90	3.62	5.65	15.87
	Adults	11.44	10.46	4.36	6.04	22.07
CSA LSI (%)	Adolescents	96.46	99.78	19.88	66.45	130.17
	Adults	94.68	98.05	19.93	63.57	124.57
ACLR EI (au)	Adolescents	64.51	64.03	6.99	55.50	78.26
	Adults	65.14	63.94	6.67	57.75	79.01
Contralateral EI (au)	Adolescents	63.54	63.65	7.31	53.87	75.56
	Adults	61.39	59.76	6.87	55.00	74.97
EI LSI (%)	Adolescents	101.86	101.48	7.84	94.18	123.82
	Adults	106.47	105.19	8.11	95.06	121.41
Initial PA (degrees)	Adolescents	12.13	11.46	3.27	7.93	19.79
	Adults	11.84	12.18	2.41	7.77	15.67
Peak PA (degrees)	Adolescents	16.08	15.17	3.20	13.13	23.30
	Adults	15.48	14.99	3.48	11.41	21.94
PA (degrees)	Adolescents	3.96	3.66	2.12	0.80	9.01
	Adults	3.64	3.06	1.61	1.96	6.73

Abbreviations: CSA , Cross-sectional Area; EI, Echo Intensity ; PA, Pennation angle; cm^2, centimeters squared; LSI, Limb Symmetry Index; %, percentage; SD, standard deviation.

Table 3: Physical Activity Questionnaires

	Age Groups	**N**	**Mean / Median^**	**SD / IQR^**	**Minimum**	**Maximum**
Total METs Per Week (METs)	Adolescents	12	2792.96	2015.03	425	8052
	Adults	12	3776.75	2445.64	318	7482
Intensity MSEQ	Adolescents	11*	8^	0.50^	5	10
	Adults	12	7^	1.75^	3	10
Minutes Per Week MSEQ (mins)	Adolescents	12	218.33	161.90	0	630
	Adults	12	245.83	166.50	60	600

Abbreviations: MSEQ, Muscle Strengthening Exercise Questionnaire; N, number; SD, standard deviation; IQR, interquartile range.

*The Intensity score from the MSEQ is incomplete due to an adolescent not completing the entire survey.

Table 4: Correlation Matrix

		CSA LSI (%)	EI LSI (%)	Pennation Angle (degrees)	Total METs Per Week (METs)	Minutes Per Week MSEQ (mins)	Intensity MSEQ
CSA LSI (%)	Spearman's rho	—					
	p-value	—					
EI LSI (%)	Spearman's rho	-0.326	—				
	p-value	0.120	—				
Pennation Angle (degrees)	Spearman's rho	-0.077	-0.155	—			
	p-value	0.719	0.468	—			
Total METs Per Week (METs)	Spearman's rho	-0.023	0.043	0.116	—		
	p-value	0.917	0.840	0.589	—		
Minutes Per Week MSEQ (mins)	Spearman's rho	-0.226	-0.075	0.244	0.408 *	—	
	p-value	0.288	0.728	0.250	0.048	—	
Intensity MSEQ	Spearman's rho	0.154	-0.405	0.354	0.299	0.360	—
	p-value	0.483	0.055	0.097	0.166	0.092	—

Abbreviations: CSA , Cross-sectional Area; EI, Echo Intensity ; LSI, limb symmetry; MSEQ, Muscle Strengthening Exercise Questionnaire.

Discussion

The primary objective of this study was to compare static and dynamic quadriceps muscle quality between adults and adolescents between 4- and 9- months post-ACLR. Additionally, this study investigated the association between self-reported physical activity, assessed with the short forms of the IPAQ and MSEQ, and static and dynamic ultrasound measurements of muscle quality. Understanding these differences and associations can provide insight into age-specific recovery processes and inform tailored rehabilitation strategies. The results showed no significant differences in rectus femoris CSA and echo intensity between adults and adolescents post-ACLR. These results suggest similar muscle size and muscle quality changes in both groups following an ACLR. Additionally**,** there were no significant differences in the change in pennation angle of the vastus lateralis during treadmill walking between adults and adolescents. This indicates comparable dynamic muscle contractile behavior during gait in both groups. Low associations were found between METs per week, time spent in and intensity of muscle-strengthening activities, and ultrasound measures of muscle quality. Most correlations were non-significant, indicating that self-reported activity levels may not strongly relate to muscle quality, defined as CSA, echo intensity, and pennation angle changes, assessed by ultrasound.

Our results indicate no differences in LSI between adults and adolescents. When comparing raw CSA and echo intensity values with previous studies, we found that both our adult and adolescent groups exhibited larger CSA and lower echo intensity (Garcia, Curran, et al., 2020; Garcia, Moffit, et al., 2020). The absence of significant differences in rectus femoris CSA and echo intensity between adults and adolescents could be attributed to several factors. Firstly, the time frame post-ACLR (4-9 months) might not be sufficient for detecting age-related differences in muscle quality. Both age groups might be undergoing similar phases of muscle recovery and adaptation. Additionally, the sample size, though powered for detecting differences, might still have been insufficient to capture subtle variations. Previous studies have found that adolescents engage in less physical activity and produce less force during walking post-ACLR (Kuenze, Collins, Triplett, et al., 2022; Lisee et al., 2023). However, our results found no static or dynamic ultrasound measurement differences within these groups. It is important to note that the lack of differences could be due to the minimal age differences between our adult (24.36 ± 7.42 years) and adolescent (16.01 ± 1.35 years) groups. Future studies should consider including a wider age range to potentially detect age-related differences in these muscle outcomes.

Both adults and adolescents within our study had a greater pennation angle at initial contact than previously published data within a healthy adult female population (A. E. Munsch, Pietrosimone, et al., 2022). Additionally, our population saw an increase in angle from initial contact to peak vGRF, whereas previous research in ACL populations found a decrease between the two-time points (A. E. Munsch, Evans-Pickett, et al., 2022). The lack of significant differences in pennation angle changes between adults and adolescents suggests that dynamic muscle function during gait is not markedly different between these age groups post-ACLR. These findings are significant as they indicate that both groups will likely have similar muscle contractile efficiency and adaptability during functional activities like walking. To our knowledge, this is the first study to assess dynamic ultrasound muscle quality between adults and adolescents post-ACLR. Dynamic ultrasound can provide helpful information regarding an individual's contractile efficiency and overall function post-injury. Previous studies have found limited fascicle length change during weight acceptance in the ACLR limb; this lack of lengthening leads to an isometric muscle action, which is not seen in the contralateral limb (A. Munsch, Evans-Pickett, et al., 2022). The lack of lengthening affects overall knee extensor moments, which are critical for stability during dynamic tasks (A. Munsch, Evans-Pickett, et al., 2022). Dynamic ultrasound can provide vital information regarding the muscle's function and efficiency during functional tasks, something that a static ultrasound image limits. Our study's use of wireless ultrasound and 3D-printed probe holders provided a novel approach to capturing real-time muscle dynamics, potentially offering more detailed insights than traditional static assessments.

Our participants reported a lower moderate-to-vigorous physical activity a day from the IPAQ (61.22 ± 47.5 min/day) compared to previously published data on high school-aged individuals (130 ± 80 min/day) (Kuenze et al., 2020). To our knowledge, no previously published data utilizing the MSEQ in an ACLR population exists. The weak associations between self-reported physical activity and muscle quality could be due to numerous reasons. Although the IPAQ and MSEQ are validated surveys in the general population, the MSEQ has yet to be validated in an injured population such as ACLRs. Additionally, the reliance on self-reported physical activity data introduces bias and inaccuracies in results. Individuals could have over- or underestimated their physical activity levels, which would ultimately affect the results of this study (P. H. Lee et al., 2011). Integrating more objective measurements of physical activity, such as wearable activity trackers, could provide more accurate data and strengthen the overall findings. Additionally, we did not collect information about physical activity levels before ACLR; therefore, it is unknown if activity levels were affected by surgery. Furthermore, muscle adaptation is a complex process and is influenced by several factors beyond physical activity that could affect the strength of association seen within this study. The weak associations seen may suggest that while physical activity is vital for health and recovery, its overall impact on muscle quality as measured by ultrasound may not be straightforward. There is a need for a comprehensive rehabilitation program that includes physical activity and other factors such as nutrition and psychological support to optimize muscle recovery post-ACLR.

Due to similar muscle quality outcomes between adults and adolescents, a generalized approach to rehabilitation programs can initially focus on a gradual and safe reintroduction to physical activity. However, there is still a need for age-specific considerations for long-term recovery plans, such as growth spurts in adolescents and age-related decline in muscle mass in adults. This study highlights the use of both static and dynamic ultrasound measurements in monitoring muscle quality post-ACLR. Despite the lack of significance between adult and adolescent ultrasound measurements, CSA, echo intensity, and pennation angle remain important outcomes to monitor post-ACLR. Future studies are needed to see if there are differences between a healthy cohort and ACLR individuals. Additionally, there is a need to see if these ultrasound-assessed outcomes are associated with patient-reported outcomes. The use of static imaging provides valuable information on muscle morphology, while dynamic assessments offer insights into the muscle’s functional behavior (Garcia, Moffit, et al., 2020; Stock & Thompson, 2020). The ease and accessibility of ultrasound allows clinicians to leverage these tools to track rehabilitation progress and tailor interventions more precisely to specific individuals' needs.

The cross-sectional design of this study removes the ability to infer causality or track the longitudinal changes in muscle quality. Our sample size was adequate for detecting large effect sizes but may have lacked statistical power to identify subtle group differences due to the outcomes used in our power analysis. Ideally, the sample size calculation would have been based on prior studies directly comparing muscle quality between adults and adolescents post-ACLR.

However, the referenced studies for our estimation did not assess muscle-related variables specifically. This may have limited our ability to detect potential muscle quality differences between adult and adolescent populations post-ACLR. Consequently, caution is warranted when interpreting negative findings, and consideration of effect sizes alongside statistical significance is crucial in our analysis. Additionally, we did not match patients within groups, matching individuals based on months post-ACLR could be beneficial when trying to detect any differences between groups. While ultrasound imaging is highly informative, it is operator-dependent and requires standardization to ensure image consistency. Any variations in probe placement, image acquisition, and segmentation techniques could cause variability. Additionally, pennation angle tracking can be complex and vary between frames. Although precautions were taken to ensure accurate measurements, human error is possible. Therefore, future studies should aim to explore the use of automated image analysis techniques to reduce variability in assessments. Additionally, previous research has identified a time-of-day effect on pennation angle changes, and the varied appointment times across both groups in our study may have influenced our results (Pearson & Onambele, 2005). Future studies should control the time of day when assessing pennation angle changes to ensure accurate and consistent results. Longitudinal studies are needed to track changes in muscle quality over time better to understand the trajectory of recovery in different age groups. A larger sample size or multicenter trials could also provide more generalizable findings.

This study found no significant differences in static and dynamic quadriceps muscle quality between adults and adolescents post-ACLR. Additionally, there were low to negligible associations between self-reported physical activity and muscle quality measurements. These findings contribute to the growing body of knowledge on overall muscle recovery post-ACLR and highlight a need for age-specific, comprehensive rehabilitation strategies, especially for long-term care plans. The insights gained from this study support the complexity of muscle recovery post-ACLR and the multifaceted nature of rehabilitation. Continued research is essential to improve and support recovery protocols and improve long-term outcomes for individuals across different age groups undergoing ACLR.

REFERENCES

Aagaard, P., Andersen, J. L., Dyhre-Poulsen, P., Leffers, A.-M., Wagner, A., Magnusson, S. P., Halkjær-Kristensen, J., & Simonsen, E. B. (2001). A mechanism for increased contractile strength of human pennate muscle in response to strength training: Changes in muscle architecture. *The Journal of Physiology*, *534*(Pt 2), 613–623. https://doi.org/10.1111/j.1469-7793.2001.t01-1-00613.x

Abramoff, B., & Caldera, F. E. (2020). Osteoarthritis: Pathology, Diagnosis, and Treatment Options. *Medical Clinics of North America*, *104*(2), 293–311. https://doi.org/10.1016/j.mcna.2019.10.007

Adkins, A. N., & Murray, W. M. (2020). Obtaining Quality Extended Field-of-View Ultrasound Images of Skeletal Muscle to Measure Muscle Fascicle Length. *Journal of Visualized Experiments : JoVE*, *166*, 10.3791/61765. https://doi.org/10.3791/61765

Ahtiainen, J. P., Hoffren, M., Hulmi, J. J., Pietikäinen, M., Mero, A. A., Avela, J., & Häkkinen, K. (2010). Panoramic ultrasonography is a valid method to measure changes in skeletal muscle cross-sectional area. *European Journal of Applied Physiology*, *108*(2), 273–279. https://doi.org/10.1007/s00421-009-1211-6

Alswat, M. M., Khojah, O., Alswat, A. M., Alghamdi, A., Almadani, M. S., Alshibely, A., Dabroom, A. A., Algarni, H. M., & Alshehri, M. S. (n.d.). Returning to Sport After Anterior Cruciate Ligament Reconstruction in Physically Active Individuals. *Cureus*, *12*(9), e10466. https://doi.org/10.7759/cureus.10466

Bamman, M. M., Newcomer, B. R., Larson-Meyer, D. E., Weinsier, R. L., & Hunter, G. R. (2000). Evaluation of the strength-size relationship in vivo using various muscle size indices. *Medicine and Science in Sports and Exercise*, *32*(7), 1307–1313. https://doi.org/10.1097/00005768-200007000-00019

Bell, D. R., Pfeiffer, K. A., Cadmus-Bertram, L. A., Trigsted, S. M., Kelly, A., Post, E. G., Hart, J. M., Cook, D. B., Dunn, W. R., & Kuenze, C. (2017). Objectively Measured Physical Activity in Patients After Anterior Cruciate Ligament Reconstruction. *The American Journal of Sports Medicine*, *45*(8), 1893–1900. https://doi.org/10.1177/0363546517698940

Bennell, K. L., Hinman, R. S., Metcalf, B. R., Buchbinder, R., McConnell, J., McColl, G., Green, S., & Crossley, K. M. (2005). Efficacy of physiotherapy management of knee joint osteoarthritis: A randomised, double blind, placebo controlled trial. *Annals of the Rheumatic Diseases*, *64*(6), 906–912. https://doi.org/10.1136/ard.2004.026526

Bianchi, S., & Martinoli, C. (2007). *Ultrasound of the Musculoskeletal System*. Springer.

Biga, L. M., Bronson, S., Dawson, S., Harwell, A., Hopkins, R., Kaufmann, J., LeMaster, M., Matern, P., Morrison-Graham, K., Oja, K., Quick, D., & Runyeon, J. (2019). *Anatomy & Physiology*. https://open.oregonstate.education/aandp/chapter/11-2-explain-the-organization-of-muscle-fascicles-and-their-role-in-generating-force/

Birchmeier, T., Lisee, C., Kane, K., Brazier, B., Triplett, A., & Kuenze, C. (2020). Quadriceps Muscle Size Following ACL Injury and Reconstruction: A Systematic Review. *Journal of Orthopaedic Research*, *38*(3), 598–608. https://doi.org/10.1002/jor.24489

Boden, B. P., Sheehan, F. T., Torg, J. S., & Hewett, T. E. (2010). Non-contact ACL Injuries: Mechanisms and Risk Factors. *The Journal of the American Academy of Orthopaedic Surgeons*, *18*(9), 520–527.

Bosquet, L., & Mujika, I. (2012). Detraining. In *Loss of training-induced physiological and performance adaptations. Part I: short term insufficient training stimulus* (Vol. 30, pp. 100–106).

Brennan, S. F., Cresswell, A. G., Farris, D. J., & Lichtwark, G. A. (2017). In vivo fascicle length measurements via B-mode ultrasound imaging with single vs dual transducer arrangements. *Journal of Biomechanics*, *64*, 240–244. https://doi.org/10.1016/j.jbiomech.2017.09.019

Bryant, A. L., Kelly, J., & Hohmann, E. (2008). Neuromuscular adaptations and correlates of knee functionality following ACL reconstruction. *Journal of Orthopaedic Research*, *26*(1), 126–135. https://doi.org/10.1002/jor.20472

Campbell, E. L., Seynnes, O. R., Bottinelli, R., McPhee, J. S., Atherton, P. J., Jones, D. A., Butler-Browne, G., & Narici, M. V. (2013). Skeletal muscle adaptations to physical inactivity and subsequent retraining in young men. *Biogerontology*, *14*(3), 247–259. https://doi.org/10.1007/s10522-013-9427-6

Canning, K. L., Brown, R. E., Jamnik, V. K., Salmon, A., Ardern, C. I., & Kuk, J. L. (2014). Individuals Underestimate Moderate and Vigorous Intensity Physical Activity. *PLoS ONE*, *9*(5), e97927. https://doi.org/10.1371/journal.pone.0097927

Cheung, E. C., DiLallo, M., Feeley, B. T., & Lansdown, D. A. (2020). Osteoarthritis and ACL Reconstruction—Myths and Risks. *Current Reviews in Musculoskeletal Medicine*, *13*(1), 115–122. https://doi.org/10.1007/s12178-019-09596-w

COLLINS, N. J., MISRA, D., FELSON, D. T., CROSSLEY, K. M., & ROOS, E. M. (2011). Measures of Knee Function. *Arthritis Care & Research*, *63*(0 11), S208–S228. https://doi.org/10.1002/acr.20632

Craig, C. L., Marshall, A. L., Sj??Str??M, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F., & Oja, P. (2003). International Physical Activity Questionnaire: 12-Country Reliability and Validity: *Medicine & Science in Sports & Exercise*, *35*(8), 1381–1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB

Csapo, R., Pointner, H., Hoser, C., Gföller, P., Raschner, C., & Fink, C. (2020). Physical Fitness after Anterior Cruciate Ligament Reconstruction: Influence of Graft, Age, and Sex. *Sports*, *8*(3), 30. https://doi.org/10.3390/sports8030030

Dargel, J., Gotter, M., Mader, K., Pennig, D., Koebke, J., & Schmidt-Wiethoff, R. (2007). Biomechanics of the anterior cruciate ligament and implications for surgical reconstruction. *Strategies in Trauma and Limb Reconstruction*, *2*(1), 1–12. https://doi.org/10.1007/s11751-007-0016-6

Davi, S. M., Brancati, R. J., DiStefano, L. J., Lepley, A. S., & Lepley, L. K. (2021). Suppressed quadriceps fascicle behavior is present in the surgical limbs of those with a history of ACL reconstruction. *Journal of Biomechanics, 129*, 110808. https://doi.org/10.1016/j.jbiomech.2021.110808

DiSanti, J., Lisee, C., Erickson, K., Bell, D., Shingles, M., & Kuenze, C. (2018). Perceptions of Rehabilitation and Return to Sport Among High School Athletes With Anterior Cruciate Ligament Reconstruction: A Qualitative Research Study. *The Journal of Orthopaedic and Sports Physical Therapy*, *48*(12), 951–959. https://doi.org/10.2519/jospt.2018.8277

Drechsler, W. I., Cramp, M. C., & Scott, O. M. (2006). Changes in muscle strength and EMG median frequency after anterior cruciate ligament reconstruction. *European Journal of Applied Physiology*, *98*(6), 613–623. https://doi.org/10.1007/s00421-006-0311-9

Duthon, V. B., Barea, C., Abrassart, S., Fasel, J. H., Fritschy, D., & Ménétrey, J. (2006). Anatomy of the anterior cruciate ligament. *Knee Surgery, Sports Traumatology, Arthroscopy*, *14*(3), 204–213. https://doi.org/10.1007/s00167-005-0679-9

Ettinger, W. H., Burns, R., Messier, S. P., Applegate, W., Rejeski, W. J., Morgan, T., Shumaker, S., Berry, M. J., O'Toole, M., Monu, J., & Craven, T. (1997). A randomized trial comparing aerobic exercise and resistance exercise with a health education program in older adults with knee osteoarthritis. The Fitness Arthritis and Seniors Trial (FAST). *JAMA*, *277*(1), 25–31.

Evangelidis, P. E., Massey, G. J., Pain, M. T. G., & Folland, J. P. (2016). Strength and size relationships of the quadriceps and hamstrings with special reference to reciprocal muscle balance. *European Journal of Applied Physiology*, *116*(3), 593–600. https://doi.org/10.1007/s00421-015-3321-7

Ferretti, G., Narici, M. V., Binzoni, T., Gariod, L., Le Bas, J. F., Reutenauer, H., & Cerretelli, P. (1994). Determinants of peak muscle power: Effects of age and physical conditioning. *European Journal of Applied Physiology and Occupational Physiology*, *68*(2), 111–115. https://doi.org/10.1007/BF00244022

Filbay, S. R., Ackerman, I. N., Russell, T. G., & Crossley, K. M. (2017). Return to sport matters—Longer-term quality of life after ACL reconstruction in people with knee difficulties. *Scandinavian Journal of Medicine & Science in Sports*, *27*(5), 514–524. https://doi.org/10.1111/sms.12698

Folland, J. P., Buckthorpe, M. W., & Hannah, R. (2014). Human capacity for explosive force production: Neural and contractile determinants. *Scandinavian Journal of Medicine & Science in Sports*, *24*(6), 894–906. https://doi.org/10.1111/sms.12131

Fomin, S., Gauffin, H., & Kvist, J. (2020). Short-term recovery of physical activity and knee function after an acute knee injury. *BMJ Open Sport & Exercise Medicine*, *6*(1), e000950. https://doi.org/10.1136/bmjsem-2020-000950

Fragala, M. S., Fukuda, D. H., Stout, J. R., Townsend, J. R., Emerson, N. S., Boone, C. H., Beyer, K. S., Oliveira, L. P., & Hoffman, J. R. (2014). Muscle quality index improves with resistance exercise training in older adults. *Experimental Gerontology*, *53*, 1–6. https://doi.org/10.1016/j.exger.2014.01.027

Franchi, M. V., Fitze, D. P., Hanimann, J., Sarto, F., & Spörri, J. (2020). Panoramic ultrasound vs. MRI for the assessment of hamstrings cross-sectional area and volume in a large athletic cohort. *Scientific Reports*, *10*, 14144. https://doi.org/10.1038/s41598-020-71123-6

Fukumoto, Y., Ikezoe, T., Yamada, Y., Tsukagoshi, R., Nakamura, M., Mori, N., Kimura, M., & Ichihashi, N. (2012). Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *European Journal of Applied Physiology*, *112*(4), 1519–1525. https://doi.org/10.1007/s00421-011-2099-5

Garcia, S. A., Curran, M. T., & Palmieri-Smith, R. M. (2020). Longitudinal Assessment of Quadriceps Muscle Morphology Before and After Anterior Cruciate Ligament Reconstruction and Its Associations With Patient-Reported Outcomes. *Sports Health*, *12*(3), 271–278. https://doi.org/10.1177/1941738119898210

Garcia, S. A., Moffit, T. J., Vakula, M. N., Holmes, S. C., Montgomery, M. M., & Pamukoff, D. N. (2020). Quadriceps Muscle Size, Quality, and Strength and Self-Reported Function in Individuals With Anterior Cruciate Ligament Reconstruction. *Journal of Athletic Training*, *55*(3), 246–254. https://doi.org/10.4085/1062-6050-38-19

Gobbi, A., & Francisco, R. (2006). Factors affecting return to sports after anterior cruciate ligament reconstruction with patellar tendon and hamstring graft: A prospective clinical investigation. *Knee Surgery, Sports Traumatology, Arthroscopy*, *14*(10), 1021–1028. https://doi.org/10.1007/s00167-006-0050-9

Griffin, L. Y., Albohm, M. J., Arendt, E. A., Bahr, R., Beynnon, B. D., Demaio, M., Dick, R. W., Engebretsen, L., Garrett, W. E., Hannafin, J. A., Hewett, T. E., Huston, L. J., Ireland, M. L., Johnson, R. J., Lephart, S., Mandelbaum, B. R., Mann, B. J., Marks, P. H., Marshall, S. W., … Yu, B. (2006). Understanding and preventing noncontact anterior cruciate ligament injuries: A review of the Hunt Valley II meeting, January 2005. *The American Journal of Sports Medicine*, *34*(9), 1512–1532. https://doi.org/10.1177/0363546506286866

Grimm, E. K., Swartz, A. M., Hart, T., Miller, N. E., & Strath, S. J. (2012). Comparison of the IPAQ-Short Form and Accelerometry Predictions of Physical Activity in Older Adults. *Journal of Aging and Physical Activity*, *20*(1), 64–79. https://doi.org/10.1123/japa.20.1.64

Harkey, M. S., Baez, S., Lewis, J., Grindstaff, T. L., Hart, J., Driban, J. B., Schorfhaar, A., & Kuenze, C. (2022). Prevalence of Early Knee Osteoarthritis Illness Among Various Patient-Reported Classification Criteria After Anterior Cruciate Ligament Reconstruction. *Arthritis Care & Research*, *74*(3), 377–385. https://doi.org/10.1002/acr.24809

Hart, J. M., Pietrosimone, B., Hertel, J., & Ingersoll, C. D. (2010). Quadriceps Activation Following Knee Injuries: A Systematic Review. *Journal of Athletic Training*, *45*(1), 87–97. https://doi.org/10.4085/1062-6050-45.1.87

Herzog, M. M., Marshall, S. W., Lund, J. L., Pate, V., Mack, C. D., & Spang, J. T. (2017). Incidence of Anterior Cruciate Ligament Reconstruction Among Adolescent Females in the United States, 2002 Through 2014. *JAMA Pediatrics*, *171*(8), 808–810. https://doi.org/10.1001/jamapediatrics.2017.0740

Hofmann, M., Schober-Halper, B., Oesen, S., Franzke, B., Tschan, H., Bachl, N., Strasser, E.-M., Quittan, M., Wagner, K.-H., & Wessner, B. (2016). Effects of elastic band resistance training and nutritional supplementation on muscle quality and circulating muscle growth and degradation factors of institutionalized elderly women: The Vienna Active Ageing Study (VAAS). *European Journal of Applied Physiology*, *116*(5), 885–897. https://doi.org/10.1007/s00421-016-3344-8

IBM SPSS Statistics for Windows (28.0). (2021). [Computer software]. IBM Corp.

Justin Disanti, Caroline Lisee, Karl Erickson, David Bell, Michael Shingles, & Christopher Kuenze. (2018). *Perceptions of Rehabilitation and Return to Sport Among High School Athletes With Anterior Cruciate Ligament Reconstruction: A Qualitative Research Study*. https://doi.org/10.2519/jospt.2018.8277

Kellis, E., Galanis, N., & Kofotolis, N. (2019). Hamstring-to-Quadriceps Ratio in Female Athletes with a Previous Hamstring Injury, Anterior Cruciate Ligament Reconstruction, and Controls. *Sports*, *7*(10), 214. https://doi.org/10.3390/sports7100214

Kent-Braun, J. A., & Le Blanc, R. (1996). Quantitation of central activation failure during maximal voluntary contractions in humans. *Muscle & Nerve*, *19*(7), 861–869. https://doi.org/10.1002/(SICI)1097-4598(199607)19:7<861::AID-MUS8>3.0.CO;2-7

Kobayashi, H., Kanamura, T., Koshida, S., Miyashita, K., Okado, T., Shimizu, T., & Yokoe, K. (2010). Mechanisms of the Anterior Cruciate Ligament Injury in Sports Activities: A Twenty-Year Clinical Research of 1,700 Athletes. *Journal of Sports Science & Medicine*, *9*(4), 669–675.

Kubo, K., Kanehisa, H., Kawakami, Y., & Fukanaga, T. (2001). Growth changes in the elastic properties of human tendon structures. *International Journal of Sports Medicine*, *22*(2), 138–143. https://doi.org/10.1055/s-2001-11337

Kuenze, C., Cadmus-Bertram, L., Pfieffer, K., Trigsted, S., Cook, D., Lisee, C., & Bell, D. (2019). Relationship Between Physical Activity and Clinical Outcomes After ACL

Reconstruction. *Journal of Sport Rehabilitation*, *28*(2), 180–187. https://doi.org/10.1123/jsr.2017-0186

Kuenze, C., Collins, K., Pfeiffer, K. A., & Lisee, C. (2022). Assessing Physical Activity After ACL Injury: Moving Beyond Return to Sport. *Sports Health*, *14*(2), 197–204. https://doi.org/10.1177/19417381211025307

Kuenze, C., Collins, K., Triplett, A., Bell, D., Norte, G., Baez, S., Harkey, M., Wilcox, L., & Lisee, C. (2022). Adolescents Are Less Physically Active Than Adults After Anterior Cruciate Ligament Reconstruction. *Orthopaedic Journal of Sports Medicine*, *10*(2), 23259671221075658. https://doi.org/10.1177/23259671221075658

Kuenze, C., Lisee, C., Triplett, A., Birchmeier, T., Straus, M. W., Wilcox, C. L., Schorfhaar, A., Joseph, S., & Shingles, M. (2020). PATIENT-REPORTED AND OBJECTIVE MEASURES OF PHYSICAL ACTIVITY ARE NOT RELATED 6-MONTHS AFTER ACL RECONSTRUCTION. *Orthopaedic Journal of Sports Medicine*, *8*(4 suppl3), 2325967120S00156. https://doi.org/10.1177/2325967120S00156

Kuenze, C., Weaver, A., Grindstaff, T. L., Ulman, S., Norte, G. E., Roman, D. P., Giampetruzzi, N., Lisee, C., Birchmeier, T., Triplett, A., Farmer, B., Hopper, H., Sherman, D. A., Ness, B. M., Collins, K., Walaszek, M., Baez, S., Harkey, M. S., Tulchin-Francis, K., … Hart, J. M. (2023). Age-, Sex-, and Graft-specific Reference Values From 783 Adolescent Patients At 5-7 months After ACL Reconstruction: IKDC, PEDI-IKDC, KOOS, ACL-RSI, Single-leg Hop, and Thigh Strength. *Journal of Orthopaedic & Sports Physical Therapy*. https://doi.org/10.2519/jospt.2023.11389

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*, 863. https://doi.org/10.3389/fpsyg.2013.00863

Lanza, M. B., Rock, K., Marchese, V., Gray, V. L., & Addison, O. (2022). Ultrasound measures of muscle thickness and subcutaneous tissue from the hip abductors: Inter- and intra-rater reliability. *Musculoskeletal Science & Practice*, *62*, 102612. https://doi.org/10.1016/j.msksp.2022.102612

Larsen, J. B., Farup, J., Lind, M., & Dalgas, U. (2014). *Muscle strength and functional performance is markedly impaired at the recommended time point for sport return after anterior cruciate ligament reconstruction in recreational athletes | Elsevier Enhanced Reader*. https://doi.org/10.1016/j.humov.2014.10.008

Lee, J.-H., Cheon, S., Jun, H.-P., Huang, Y.-L., & Chang, E. (2020). Bilateral Comparisons of Quadriceps Thickness after Anterior Cruciate Ligament Reconstruction. *Medicina*, *56*(7), Article 7. https://doi.org/10.3390/medicina56070335

Lee, P. H., Macfarlane, D. J., Lam, T. H., & Stewart, S. M. (2011). Validity of the International Physical Activity Questionnaire Short Form (IPAQ-SF): A systematic review. *The International Journal of Behavioral Nutrition and Physical Activity*, *8*, 115. https://doi.org/10.1186/1479-5868-8-115

Lepley, L. K., Davi, S. M., Burland, J. P., & Lepley, A. S. (2020). Muscle Atrophy After ACL Injury: Implications for Clinical Practice. *Sports Health*, *12*(6), 579–586. https://doi.org/10.1177/1941738120944256

Lisee, C. M., Bjornsen, E., Horton, W. Z., Davis-Wilson, H., Blackburn, J. T., Fisher, M. B., & Pietrosimone, B. (2023). Differences in Gait Biomechanics Between Adolescents and Young Adults With Anterior Cruciate Ligament Reconstruction. *Journal of Athletic Training*, *57*(9–10), 921–928. https://doi.org/10.4085/1062-6050-0052.22

Lohmander, L. S., Englund, P. M., Dahl, L. L., & Roos, E. M. (2007). The Long-term Consequence of Anterior Cruciate Ligament and Meniscus Injuries: Osteoarthritis. *The American Journal of Sports Medicine*, *35*(10), 1756–1769. https://doi.org/10.1177/0363546507307396

Lohmander, L. S., Ostenberg, A., Englund, M., & Roos, H. (2004). High prevalence of knee osteoarthritis, pain, and functional limitations in female soccer players twelve years after anterior cruciate ligament injury. *Arthritis and Rheumatism*, *50*(10), 3145–3152. https://doi.org/10.1002/art.20589

Longo, U. G., Rizzello, G., Frnaceschi, F., Campi, S., Maffulli, N., & Denaro, V. (2014). The architecture of the ipsilateral quadriceps two years after successful anterior cruciate ligament reconstruction with bone–patellar tendon–bone autograft. *The Knee*, *21*(3), 721–725. https://doi.org/10.1016/j.knee.2014.02.001

Luc, B., Gribble, P. A., & Pietrosimone, B. G. (2014). Osteoarthritis Prevalence Following Anterior Cruciate Ligament Reconstruction: A Systematic Review and Numbers-Needed-to-Treat Analysis. *Journal of Athletic Training*, *49*(6), 806–819. https://doi.org/10.4085/1062-6050-49.3.35

Lv, S., Ling, L., Shi, H., Chen, X., Chen, S., Zhu, S., Lin, W., Lv, R., & Ding, G. (2022). Application of Muscle Thickness and Quality Measured by Ultrasound in Frailty Assessment in China. *Frontiers in Medicine*, *9*, 859555. https://doi.org/10.3389/fmed.2022.859555

Mall, N. A., Chalmers, P. N., Moric, M., Tanaka, M. J., Cole, B. J., Bach, B. R., & Paletta, G. A. (2014). Incidence and Trends of Anterior Cruciate Ligament Reconstruction in the United States. *The American Journal of Sports Medicine*, *42*(10), 2363–2370. https://doi.org/10.1177/0363546514542796

Marcus, R. L., Addison, O., Kidde, J. P., Dibble, L. E., & Lastayo, P. C. (2010). Skeletal muscle fat infiltration: Impact of age, inactivity, and exercise. *The Journal of Nutrition, Health & Aging*, *14*(5), 362–366. https://doi.org/10.1007/s12603-010-0081-2

Mather, R. C., Koenig, L., Kocher, M. S., Dall, T. M., Gallo, P., Scott, D. J., Bach, B. R., Spindler, K. P., & MOON Knee Group. (2013). Societal and economic impact of anterior cruciate ligament tears. *The Journal of Bone and Joint Surgery. American Volume*, *95*(19), 1751–1759. https://doi.org/10.2106/JBJS.L.01705

Melo, M. C. A., Daher, E. D. F., Albuquerque, S. G. C., & De Bruin, V. M. S. (2016). Exercise in bipolar patients: A systematic review. *Journal of Affective Disorders*, *198*, 32–38. https://doi.org/10.1016/j.jad.2016.03.004

Meyers, M. C., & Barnhill, B. S. (2004). Incidence, causes, and severity of high school football injuries on FieldTurf versus natural grass: A 5-year prospective study. *The American Journal of Sports Medicine*, *32*(7), 1626–1638. https://doi.org/10.1177/0363546504266978

Miko, M., & Varga, I. (2015). *Histologic Examination of Peripheral Nerves | Elsevier Enhanced Reader*. https://doi.org/10.1016/B978-0-12-410390-0.00006-8

Mirkov, D. M., Knezevic, O. M., Maffiuletti, N. A., Kadija, M., Nedeljkovic, A., & Jaric, S. (2017). Contralateral limb deficit after ACL-reconstruction: An analysis of early and late phase of rate of force development. *Journal of Sports Sciences*, *35*(5), 435–440. https://doi.org/10.1080/02640414.2016.1168933

Moore, S. A., Mckay, H. A., Macdonald, H., Nettlefold, L., Baxter-Jones, A. D. G., Cameron, N., & Brasher, P. M. A. (2015). Enhancing a Somatic Maturity Prediction Model. *Medicine & Science in Sports & Exercise*, *47*(8), 1755. https://doi.org/10.1249/MSS.0000000000000588

Mukaka, M. (2012). A guide to appropriate use of Correlation coefficient in medical research. *Malawi Medical Journal : The Journal of Medical Association of Malawi*, *24*(3), 69–71.

Munsch, A. E., Evans-Pickett, A., Davis-Wilson, H., Pietrosimone, B., & Franz, J. R. (2022). *Quadriceps Muscle Action and Association With Knee Joint Biomechanics in Individuals with Anterior Cruciate Ligament Reconstruction*. https://doi.org/10.1123/jab.2021-0381

Munsch, A. E., Pietrosimone, B., & Franz, J. R. (2022). Walking speed does not affect net vastus lateralis fascicle length change on average during weight acceptance. *Journal of Biomechanics*, *144*, 111300. https://doi.org/10.1016/j.jbiomech.2022.111300

Munsch, A., Evans-Pickett, A., Davis-Wilson, H., Pistrosimone, B., & Franz, J. (2022). *Quadriceps Muscle Action and Association With Knee Joint Biomechanics in Individuals with Anterior Cruciate Ligament Reconstruction in: Journal of Applied Biomechanics Volume 38 Issue 5 (2022)*. https://journals-humankinetics-com.proxy2.cl.msu.edu/view/journals/jab/38/5/article-p328.xml

Muscle Atlas. (2023). UW Radiology. https://rad.washington.edu/muscle-atlas/vastus-lateralis/

Noehren, B., Andersen, A., Hardy, P., Johnson, D. L., Ireland, M. L., Thompson, K. L., & Damon, B. (2016). Cellular and Morphological Alterations in the Vastus Lateralis Muscle as the Result of ACL Injury and Reconstruction. *The Journal of Bone and Joint Surgery. American Volume*, *98*(18), 1541–1547. https://doi.org/10.2106/JBJS.16.00035

Noyes, F. R., Mooar, P. A., Matthews, D. S., & Butler, D. L. (1983). The symptomatic anterior cruciate-deficient knee. Part I: The long-term functional disability in athletically active individuals. *JBJS*, *65*(2), 154.

Oliveira, J. M., Spositon, T., Rugila, D. F., Pitta, F., & Furlanetto, K. C. (2023). Validity of the International Physical Activity Questionnaire (short form) in adults with asthma. *PLOS ONE*, *18*(2), e0282137. https://doi.org/10.1371/journal.pone.0282137

Palmer, T. B., Akehi, K., Thiele, R. M., Smith, D. B., & Thompson, B. J. (2015). Reliability of Panoramic Ultrasound Imaging in Simultaneously Examining Muscle Size and Quality of the Hamstring Muscles in Young, Healthy Males and Females. *Ultrasound in Medicine & Biology*, *41*(3), 675–684. https://doi.org/10.1016/j.ultrasmedbio.2014.10.011

Pamukoff, D. N., Montgomery, M. M., Choe, K. H., Moffit, T. J., Garcia, S. A., & Vakula, M. N. (2018). Bilateral Alterations in Running Mechanics and Quadriceps Function Following Unilateral Anterior Cruciate Ligament Reconstruction. *Journal of Orthopaedic & Sports Physical Therapy*, *48*(12), 960–967. https://doi.org/10.2519/jospt.2018.8170

Pamukoff, D. N., Pietrosimone, B., Ryan, E. D., Lee, D. R., Brown, L. E., & Blackburn, J. T. (2017). Whole-Body Vibration Improves Early Rate of Torque Development in Individuals With Anterior Cruciate Ligament Reconstruction. *The Journal of Strength & Conditioning Research*, *31*(11), 2992. https://doi.org/10.1519/JSC.0000000000001740

Paris, M. T., Bell, K. E., Avrutin, E., Rosati, K., & Mourtzakis, M. (2022). Influence of Subcutaneous Adipose Tissue and Skeletal Muscle Thickness on Rectus Femoris Echo Intensity in Younger and Older Males and Females. *Journal of Ultrasound in Medicine: Official Journal of the American Institute of Ultrasound in Medicine*, *41*(9), 2355–2364. https://doi.org/10.1002/jum.15922

Pearson, S. J., & Onambele, G. N. L. (2005). Acute Changes In Knee-Extensors Torque, Fiber Pennation, and Tendon Characteristics. *Chronobiology International*, *22*(6), 1013–1027. https://doi.org/10.1080/07420520500397900

Penedo, F. J., & Dahn, J. R. (2005). Exercise and well-being: A review of mental and physical health benefits associated with physical activity. *Current Opinion in Psychiatry*, *18*(2), 189.

Petersen, W., & Tillmann, B. (2002). [Anatomy and function of the anterior cruciate ligament]. *Der Orthopade*, *31*(8), 710–718. https://doi.org/10.1007/s00132-002-0330-0

Petersen, W., & Zantop, T. (2007). Anatomy of the anterior cruciate ligament with regard to its two bundles. *Clinical Orthopaedics and Related Research*, *454*, 35–47. https://doi.org/10.1097/BLO.0b013e31802b4a59

Pietrosimone, B., Lepley, A. S., Kuenze, C., Harkey, M. S., Hart, J. M., Blackburn, J. T., & Norte, G. (2022). Arthrogenic Muscle Inhibition Following Anterior Cruciate Ligament Injury. *Journal of Sport Rehabilitation*, *31*(6), 694–706. https://doi.org/10.1123/jsr.2021-0128

Roddy, E. (2005). Aerobic walking or strengthening exercise for osteoarthritis of the knee? A systematic review. *Annals of the Rheumatic Diseases*, *64*(4), 544–548. https://doi.org/10.1136/ard.2004.028746

Rostron, Z. P., Green, R. A., Kingsley, M., & Zacharias, A. (2021). Associations Between Measures of Physical Activity and Muscle Size and Strength: A Systematic Review. *Archives of Rehabilitation Research and Clinical Translation*, *3*(2), 100124. https://doi.org/10.1016/j.arrct.2021.100124

Sanders, T., Kremers, M., & Bryan, A. (2016). *Incidence of anterior cruciate ligament tears and reconstruction: A 21-year population-based study*.

Schmitt, L. C., Paterno, M. V., Ford, K. R., Myer, G. D., & Hewett, T. E. (2015). Strength Asymmetry and Landing Mechanics at Return to Sport after Anterior Cruciate Ligament Reconstruction. *Medicine and Science in Sports and Exercise*, *47*(7), 1426–1434. https://doi.org/10.1249/MSS.0000000000000560

Schmitt, L. C., Paterno, M. V., & Hewett, T. E. (2012). The Impact of Quadriceps Femoris Strength Asymmetry on Functional Performance at Return to Sport Following Anterior Cruciate Ligament Reconstruction. *Journal of Orthopaedic & Sports Physical Therapy*, *42*(9), 750–759. https://doi.org/10.2519/jospt.2012.4194

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). *NIH Image to ImageJ: 25 years of image analysis.* [Computer software].

Seo, M.-W., Jung, S.-W., Kim, S.-W., Lee, J.-M., Jung, H. C., & Song, J.-K. (2021). Effects of 16 Weeks of Resistance Training on Muscle Quality and Muscle Growth Factors in Older Adult Women with Sarcopenia: A Randomized Controlled Trial. *International Journal of Environmental Research and Public Health*, *18*(13), 6762. https://doi.org/10.3390/ijerph18136762

Shakespear-Druery, J., De Cocker, K., Biddle, S. J. H., & Bennie, J. (2022). Muscle-Strengthening Exercise Questionnaire (MSEQ): An assessment of concurrent validity and test–retest reliability. *BMJ Open Sport — Exercise Medicine*, *8*(1), e001225. https://doi.org/10.1136/bmjsem-2021-001225

Sopher, R. S., Amis, A. A., Davies, D. C., & Jeffers, J. R. (2017). The influence of muscle pennation angle and cross-sectional area on contact forces in the ankle joint. *The Journal of Strain Analysis for Engineering Design*, *52*(1), 12–23. https://doi.org/10.1177/0309324716669250

Stock, M. S., & Thompson, B. J. (2020). *Echo intensity as an indicator of skeletal muscle quality: Applications, methodology, and future directions*. https://doi.org/doi.org/10.1007/s00421-020-04556-6

Strasser, E. M., Draskovits, T., Praschak, M., Quittan, M., & Graf, A. (2013). Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *Age*, *35*(6), 2377–2388. https://doi.org/10.1007/s11357-013-9517-z

Strocchi, R., de Pasquale, V., Gubellini, P., Facchini, A., Marcacci, M., Buda, R., Zaffagnini, S., & Ruggeri, A. (1992). The human anterior cruciate ligament: Histological and ultrastructural observations. *Journal of Anatomy*, *180*(Pt 3), 515–519.

Takahashi, S., Nagano, Y., Ito, W., Kido, Y., & Okuwaki, T. (2019). A retrospective study of mechanisms of anterior cruciate ligament injuries in high school basketball, handball, judo, soccer, and volleyball. *Medicine*, *98*(26), e16030. https://doi.org/10.1097/MD.0000000000016030

Takai, Y., Ohta, M., Akagi, R., Kanehisa, H., Kawakami, Y., & Fukunaga, T. (2009). Sit-to-stand Test to Evaluate Knee Extensor Muscle Size and Strength in the Elderly: A Novel Approach. *Journal of PHYSIOLOGICAL ANTHROPOLOGY*, *28*(3), 123–128. https://doi.org/10.2114/jpa2.28.123

Tanner, J. M. (1981). Growth and maturation during adolescence. *Nutrition Reviews*, *39*(2), 43–55. https://doi.org/10.1111/j.1753-4887.1981.tb06734.x

The jamovi project (2.5). (2024). [Computer software]. https://www.jamovi.org

Trost, S. G., Loprinzi, P. D., Moore, R., & Pfeiffer, K. A. (2011). Comparison of accelerometer cut points for predicting activity intensity in youth. *Medicine and Science in Sports and Exercise*, *43*(7), 1360–1368. https://doi.org/10.1249/MSS.0b013e318206476e

Tsutsui, T., Iizuka, S., Sakamaki, W., Maemichi, T., & Torii, S. (2022). Growth until Peak Height Velocity Occurs Rapidly in Early Maturing Adolescent Boys. *Children*, *9*(10), 1570. https://doi.org/10.3390/children9101570

Varesco, G., Coudy-Gandilhon, C., Lapole, T., Decourt, A., Gueugneau, M., Barthélémy, J.-C., Roche, F., Bechet, D., Féasson, L., & Rozand, V. (2022). Association Between Physical Activity, Quadriceps Muscle Performance, and Biological Characteristics of Very Old Men and Women. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *77*(1), 47–54. https://doi.org/10.1093/gerona/glab239

Waleh, M. Q. (2016). Impacts of Physical Activity on the Obese. *Primary Care: Clinics in Office Practice*, *43*(1), 97–107. https://doi.org/10.1016/j.pop.2015.08.014

Wang, L.-J., Zeng, N., Yan, Z.-P., Li, J.-T., & Ni, G.-X. (2020). Post-traumatic osteoarthritis following ACL injury. *Arthritis Research & Therapy*, *22*(1), 57. https://doi.org/10.1186/s13075-020-02156-5

Watanabe, Y., Ikenaga, M., Yoshimura, E., Yamda, Y., & Kimura, M. (2018). *Association between echo intensity and attenuation of skeletal muscle in young and older adults: A comparison between ultrasonography and computed tomography*. https://doi.org/10.2147/CIA.S173372

Webster, K. E., & Feller, J. A. (2019). *Expectations for Return to Preinjury Sport Before and After Anterior Cruciate Ligament Reconstruction. 47*(3). https://doi.org/10.1177/0363546518819454

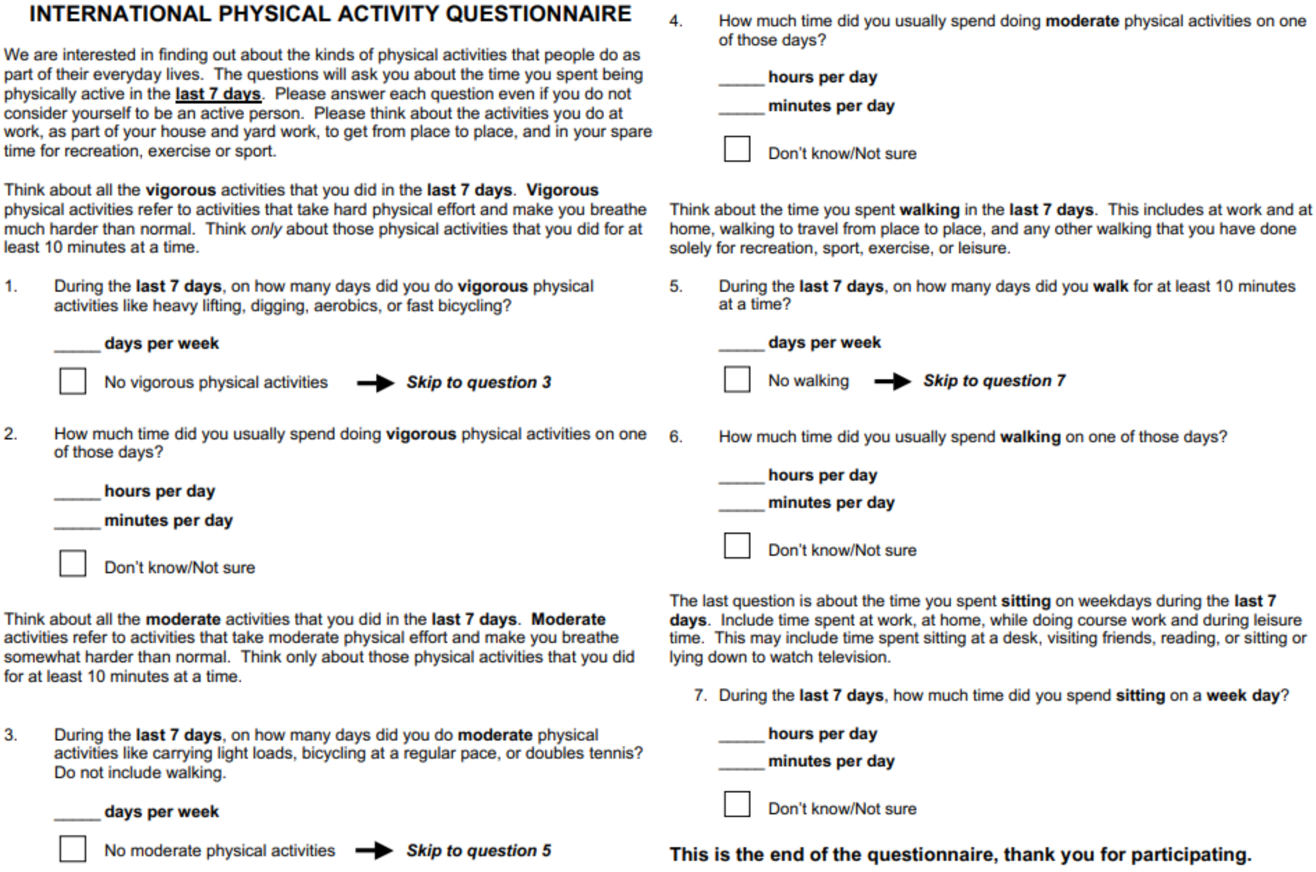
Whittingham, T. A. (1999). Broadband transducers. *European Radiology*, *9*(3), S298–S303. https://doi.org/10.1007/PL00014060

Wolin, K. Y., Heil, D. P., Askew, S., Matthews, C. E., & Bennett, G. G. (2008). Validation of the International Physical Activity Questionnaire-Short Among Blacks. *Journal of Physical Activity and Health*, *5*(5), 746–760. https://doi.org/10.1123/jpah.5.5.746

Yang, J.-H., Eun, S.-P., Park, D.-H., Kwak, H.-B., & Chang, E. (2019). The Effects of Anterior Cruciate Ligament Reconstruction on Individual Quadriceps Muscle Thickness and Circulating Biomarkers. *International Journal of Environmental Research and Public Health*, *16*(24), 4895. https://doi.org/10.3390/ijerph16244895

Young, H.-J., Jenkins, N. T., Qun, Z., & Kevin K., M. (2015). *Measurement of Intramuscular Fat by Muscle Echo Intensity*. https://doi.org/doi:10.1002/mus.24656.

APPENDIX A: IPAQ SHORT FORM



INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

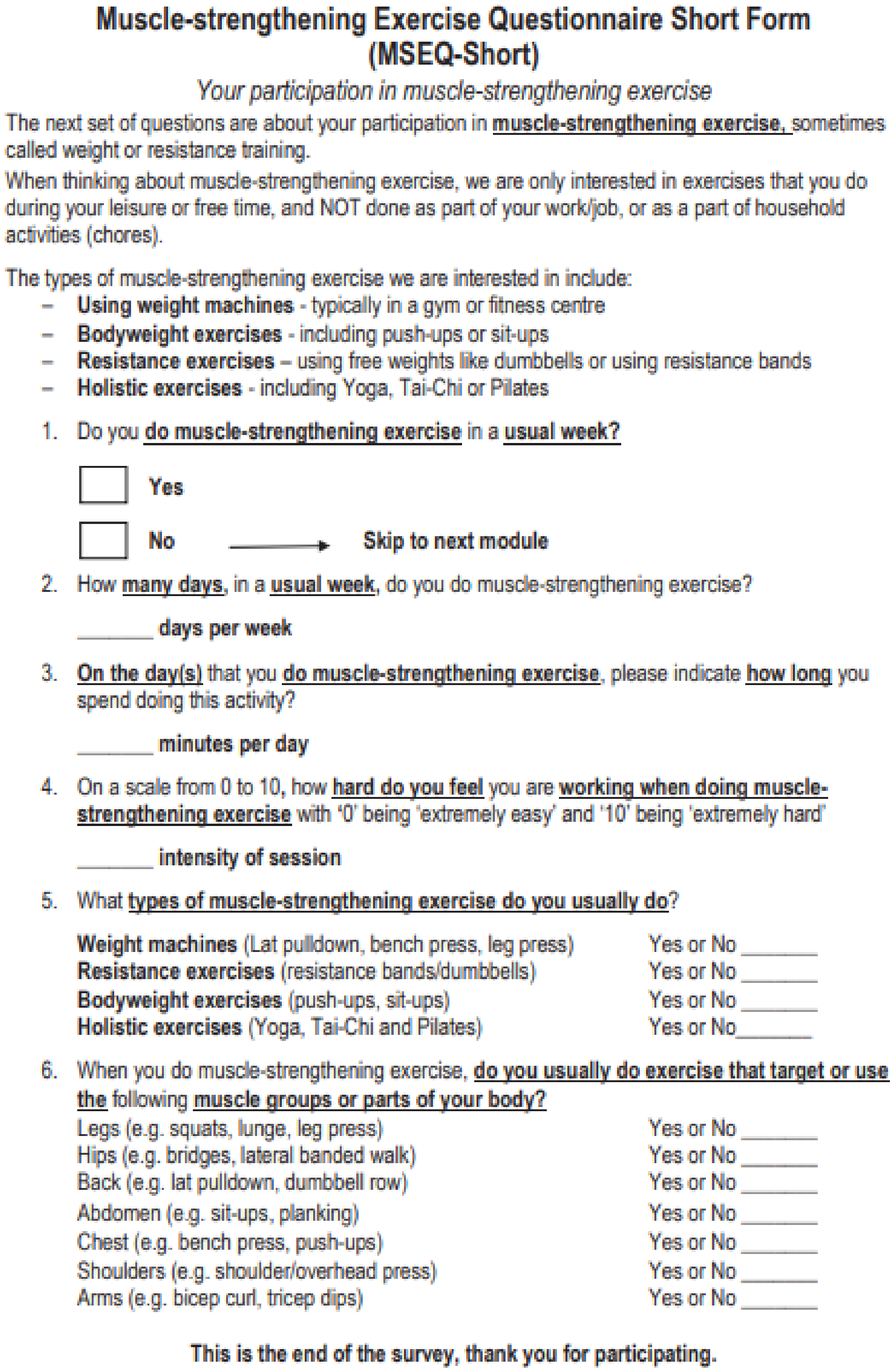
_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

APPENDIX B: MUSCLE-STRENGTHENING EXERCISE QUESTIONNAIRE SHORT FORM



Muscle-strengthening Exercise Questionnaire Short Form (MSEQ-Short)

Your participation in muscle-strengthening exercise

The next set of questions are about your participation in **muscle-strengthening exercise,** sometimes called weight or resistance training.

When thinking about muscle-strengthening exercise, we are only interested in exercises that you do during your leisure or free time, and NOT done as part of your work/job, or as a part of household activities (chores).

The types of muscle-strengthening exercise we are interested in include:

- **Using weight machines** - typically in a gym or fitness centre
- **Bodyweight exercises** - including push-ups or sit-ups
- **Resistance exercises** – using free weights like dumbbells or using resistance bands
- **Holistic exercises** - including Yoga, Tai-Chi or Pilates

1. Do you **do muscle-strengthening exercise** in a **usual week?**

   ☐ **Yes**

   ☐ **No** ⟶ **Skip to next module**

2. How **many days,** in a **usual week,** do you do muscle-strengthening exercise?

   _______ **days per week**

3. **On the day(s)** that you **do muscle-strengthening exercise**, please indicate **how long** you spend doing this activity?

   _______ **minutes per day**

4. On a scale from 0 to 10, how **hard do you feel** you are **working when doing muscle-strengthening exercise** with '0' being 'extremely easy' and '10' being 'extremely hard'

   _______ **intensity of session**

5. What **types of muscle-strengthening exercise do you usually do**?

   Weight machines (Lat pulldown, bench press, leg press) Yes or No _______
   Resistance exercises (resistance bands/dumbbells) Yes or No _______
   Bodyweight exercises (push-ups, sit-ups) Yes or No _______
   Holistic exercises (Yoga, Tai-Chi and Pilates) Yes or No_______

6. When you do muscle-strengthening exercise, **do you usually do exercise that target or use the** following **muscle groups or parts of your body?**

   Legs (e.g. squats, lunge, leg press) Yes or No _______
   Hips (e.g. bridges, lateral banded walk) Yes or No _______
   Back (e.g. lat pulldown, dumbbell row) Yes or No _______
   Abdomen (e.g. sit-ups, planking) Yes or No _______
   Chest (e.g. bench press, push-ups) Yes or No _______
   Shoulders (e.g. shoulder/overhead press) Yes or No _______
   Arms (e.g. bicep curl, tricep dips) Yes or No _______

This is the end of the survey, thank you for participating.