For publication in *Case Reports in Orthopedics*

Please note: this is the final draft of the accepted article:

Mechelli F, Bayford R, Garelick H, Stokes M, Agyapong-Badu S.

Clinical utility of ultrasound imaging for measuring anterior thigh thickness after anterior cruciate ligament injury in an individual patient to assess post-surgery outcome

Accepted: 05th October 2023

Please use the following link for the final, fully proofed and peer-reviewed journal article online:

https://www.hindawi.com/journals/crior/

1 # Clinical utility of ultrasound imaging for measuring anterior thigh thickness after
2 anterior cruciate ligament injury in an individual patient to assess post-surgery
3 outcome
4
5
6 **Filippo Mechelli,[1,2] Richard Bayford,[2] Hemda Garelick,[2] Maria Stokes,[3,4,5] Sandra Agyapong-Badu, [6]**
7 [1] PT, MSc, Private Practice, Urbino, Italy.
8 [2] School of Science and Technology, Department of Natural Sciences, Middlesex University, London, UK.
9 [3] School of Health Sciences, University of Southampton, Southampton, UK.
10 [4] Centre for Sport, Exercise and Osteoarthritis Versus Arthritis, Southampton, UK.
11 [5] Southampton NIHR Biomedical Research Centre.
12 [6] School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, UK.
13
14 Correspondence should be addressed to Filippo Mechelli; fmechelli@gmail.com
15

16 **Abstract**
17 The present study investigated the clinical utility of ultrasound imaging (USI) for assessing changes in an
18 individual's quadriceps muscle and subcutaneous fat (SF) thickness of the anterior thigh and their relative
19 proportions. A patient was studied prior to and after anterior cruciate ligament reconstruction (ACLR) surgery
20 and during rehabilitation.
21 This case study involved an 18-year-old female recreational athlete with a complete tear of the anterior cruciate
22 ligament (ACL). Tissue thickness (SF and quadriceps muscle) was measured from transverse USI images of
23 the anterior thigh before surgery, at weekly intervals during 12 weeks of post-surgery and then every 2 weeks
24 for the following 12 weeks (total of 21 measurement sets).
25 Statistically significant differences pre-surgery to post-rehabilitation were found for muscle thickness (p=0.04)
26 and SF tissue thickness (p=0.04) measurements. There was no difference in muscle to fat ratio (p=0.08).
27 Changes in measurements greater than reported minimal detectable change (MDC) demonstrate the
28 sensitivity of the USI technique as an objective tool to assess clinically useful changes in an individual's anterior
29 thigh muscle thickness post-ACLR surgery and during rehabilitation.
30

31 **Keywords:** anterior cruciate ligament reconstruction; rectus femoris; subcutaneous fat thickness; ultrasound
32 imaging; vastus intermedius
33

34 ## 1. Introduction
35

36 Rupture of the anterior cruciate ligament (ACL) is among the most common and economically costly sport
37 injuries [1]. Injuries to the ACL frequently require surgery and extensive rehabilitation resulting in an economic
38 burden on society caused by absence from work, reduced productivity, and associated health care costs [2,3].
39 Surgical management is currently the preferred treatment for ACL injuries in the UK, with a conservative
40 estimated £63 million (*n* = 15,000) in costs for ACL reconstruction to the NHS in 2015 [4]. Prehabilitation before
41 considering surgery, particularly with isolated ACL tears without comorbidity, is reported to reduce ACL surgery
42 by up to 50% [5].
43 Quadriceps muscle atrophy and weakness are usually reported in patients after knee surgery and may persist
44 postoperatively for long periods [6,7], causing a reduction of physical function [8-12], a possible dysfunction of
45 movement patterns [13-18], and an increased risk of re-injury [19-21]. There are inconsistencies in guidelines

for ACL reconstruction (ACLR) rehabilitation [22,23], and 80% of hospital orthopaedic departments within London, UK, have their own ACL rehabilitation guidelines [24], resulting in significant variations and little data on the effect of rehabilitation regimens. ACLR rehabilitation progression should be tailored according to objective data and measurements and not merely on time or protocols.

Physical therapy plays a key role in the rehabilitation process to achieve beneficial clinical outcomes [25]. The clinical assessment of quadriceps femoris muscle bulk has been traditionally performed visually, observing the contours of the thigh and by measuring limb girth with a tape measure [26,27]. Visual observation and comparison of the thighs is known to underestimate loss of quadriceps' cross-sectional area (CSA) by 22-33% on the injured side [26]. Specifically, measuring limb girth with an anthropometric tape measure to estimate quadriceps' size involves considering all muscles of the thigh, as well as bone, subcutaneous fat (SF), and all the other anatomical structures not related to the anterior thigh compartment. This approach could lead to errors in estimating muscle size [26-28], in addition to effects of inter- and intra-operator reliability [29]. An accurate and objective assessment of quadriceps muscle atrophy is a powerful tool to guide physiotherapy care [30].

Musculoskeletal ultrasound imaging (USI) is used to assess the morphology, CSA and thickness of muscles and other neuromusculoskeletal structures [29,31]. The technique provides a rapid, accurate, safe, portable, noninvasive method of obtaining objective measurements, and it is much less expensive than computed tomography (CT) and magnetic resonance imaging (MRI).

Reliability of measurements between investigators [32], and test re-test reliability [32] and criterion validity against the gold standard of MRI [33] were recently reported for USI in measuring quadriceps muscle and SF thickness of the anterior thigh. The sensitivity of the USI measurements over a 2-year period has also been reported [34], advancing the application of the USI technique to longitudinal studies.

As highlighted earlier, there is little consensus on the management of ACL injuries and the present study aims to demonstrate how USI can provide objective evidence regarding the clinical outcomes following ACLR surgery and inform care for acute ACL patients. Specifically, the study implemented and assessed the clinical utility of USI for measuring anterior thigh tissue thickness after ACL injury, ACLR intervention and during rehabilitation, to indicate how USI can be used to monitor an individual patient (n of 1) objectively [35].

2. Materials and Methods

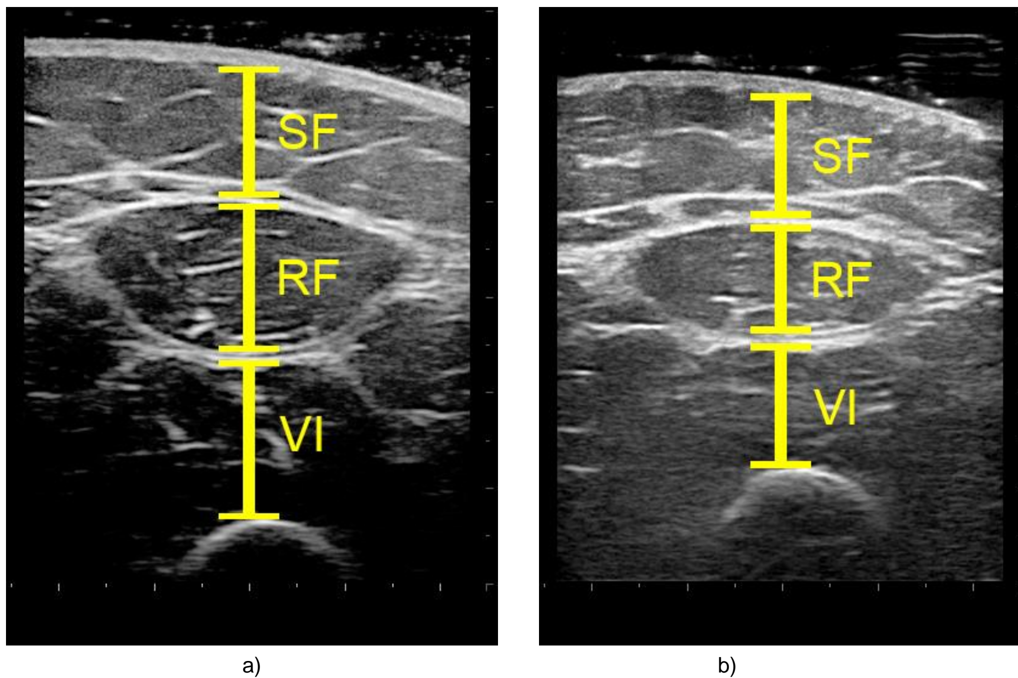
2.1 Participant

An 18-year-old female recreational athlete who suffered a complete tear of the left ACL and underwent ACLR surgery was studied. The protocol was approved by Middlesex University Research Ethics Committee (#14872/2020). The study was conducted in accordance with the ethical standards of the Declaration of Helsinki, as revised in 2013 [36]. Written informed consent was obtained from the participant after full explanation of the aims and procedures, and after providing her with a written information sheet. The participant's rights were protected at all times during this study.

2.2 Procedure

Transverse B-mode images of the anterior thigh were acquired using an ultrasound scanner (MyLab25 Gold; Esaote, Genova, Italia) with a 7.5 MHz linear transducer (40 mm length). Ultrasound images were obtained

1 with the participant lying in a supine position with the hips in neutral and knees fully extended, with the support
2 of sandbags at the ankles to avoid rotation. Measurements were performed at a site two-thirds of the distance
3 between the antero-superior iliac spine and the superior pole of the patella in the sagittal plane [32], and the
4 site was marked on the skin with a non-toxic pen. Images were acquired coating the transducer with a generous
5 amount of ultrasound transmission gel and applying minimal pressure to the contact point with the skin to avoid
6 compression of the underlying tissues [34].
7 Thigh circumference measurements were taken at the same site using a tape measure.
8 Ultrasound images and thigh circumference measurements were acquired at 4, 5 and 6 weeks after injury pre-
9 surgery (6 weeks after the injury and 1 week before surgery was the same day), at weekly intervals post-ACLR
10 surgery to 12 weeks, then every 2 weeks for the following 12 weeks. In all a total of 21 measurement sets were
11 taken.
12
13 *2.3 Ultrasound Imaging Data Processing*
14 Ultrasound images were analysed offline using ImageJ software (available from https://imagej.nih.gov/ij/). SF
15 thickness was included between the skin and the outside edge of the superficial fascial layer of rectus femoris
16 (RF); thickness of RF and vastus intermedius (VI) were determined between the inside edges of each muscle
17 border, excluding perimuscular fascia (Fig.1).
18



19
20 a) b)
21 Fig.1 Ultrasound images of the anterior thigh 3 weeks post ACLR of the a) uninjured limb and b) injured limb. SF=Subcutaneous fat,
22 RF=Rectus Femoris muscle, VI=Vastus Intermedius muscle
23
24 *2.4 Study design*
25 This was a longitudinal n of 1 study [35] over a period of 27 weeks.
26
27 *2.5 Data Analysis*
28 Data were analysed using SPSS 25 (SPSS Inc, Chicago, IL). Student's t-test was used to compare mean
29 differences in the thickness of the anterior thigh tissues between the 3 measurements acquired before ACLR
30 surgery, and the last 3 measurements of the final period of rehabilitation.

Holm's correction was used to adjust the p-values for multiple testing within an individual positive rate to 5% [37]. Differences were considered statistically significant for p values less than 0.05.

Visual observation analysis of data presented graphically was performed to evaluate changes in trend across the different phases.

Changes in measurement values between different phases were compared to minimal detectable change (MDC) values reported in the literature [32], to assess if differences were greater than the error associated with the measurement technique. The MDC values were generated in a previous study [32] by the lead investigator (FM), who acquired all the scans and established between-day reliability [32], with intraclass-correlation coefficients of: ICC3.2 values of 0.96 for muscle and 0.98 for SF. MDC values of the same reliability study were 3.6 mm for total muscle thickness (RF + VI) and 1.3 mm for SF [32]. Retrospective analysis of the data of the same test-retest reliability study [32] , produced intraclass-correlation coefficients ICC3.2 values of 0.93 for RF and 0.89 for VI, and MDC values of 2.5 mm for RF, and 3.3 mm for VI.

Percentage difference between uninjured and injured side was calculated as [(Uninjured limb - Injured limb) / Uninjured limb] x 100.

3. Results

Changes in anterior thigh tissue thickness between pre-ACLR surgery to 24 weeks post-ACLR were evident on all data presentation and analysis methods used, which concurred with one another.

3.1. *Statistical comparisons over time*

Total muscle thickness (RF+VI) decreased from pre-ACLR to 3-weeks post-ACLR, then began increasing at 6-weeks post-ACLR, with a constant increment to the end of the study period (Table 1). SF increased gradually post-surgery and recorded a dip towards the end of the study period.

From pre-ACLR until 3-weeks post-ACLR, VI muscle thickness decreased, then remained almost stable until 12-weeks post-ACLR. VI thickness then increased during the last 12-weeks of the study period exceeding the pre-intervention value at the final measurement. For RF muscle thickness, a decrease from pre-intervention to 1-week post-intervention was observed, which then slowly began to increase and remained stable between 6 to 12-weeks post-intervention, with a further increase in size during the last 12-weeks of the rehabilitation period that exceeded the pre-operative measurement.

A separate evaluation of the two muscles (RF and VI), rather than the sum of both, enables assessment of selective atrophy of one of the two muscles. There was a greater decrease in VI pre to post-ACLR in comparison to RF. Both RF and VI thickness exceeded the pre-surgery values by the end of the study period. A more pronounced, and persistent atrophy was evident in VI rather than in RF and a faster recovery of RF was observed.

Thigh girth measurements in the present study (Table 1) remained fairly consistent throughout the study.

Table 1. Anterior thigh tissue thickness measurements on the injured and uninjured limbs following ACLR.

18-year-old female, Height 1.7m; Weight 58kg; BMI 20.1kg/m^2; Dominant side - Right lower limb (uninjured)

	Uninjured lower limb (cm)					**Injured lower limb (cm)**				
	SF	RF	VI	M/F	TG	SF	RF	VI	M/F	TG
Pre-ACLR (T3)	1.11	1.71	1.79	3.15	47	1.26	1.11	1.28	1.89	45
1 wk post ACLR (T4)	1.12	1.56	1.60	2.82	47	1.37	1.11	1.15	1.65	45
3 wks (T6)	1.25	1.55	1.66	2.57	47	1.39	1.19	1.14	1.68	45
6 wks (T9)	1.26	1.52	1.70	2.56	47.5	1.51	1.47	1.26	1.81	46
12 wks (T15)	1.25	1.57	1.79	2.69	47.5	1.52	1.50	1.28	1.83	46.4
24 wks (T21)	1.22	1.61	1.79	2.79	47.5	1.37	1.57	1.58	2.29	47.2

BMI=body mass index, Wks= weeks, T=time points of interest, SF=Subcutaneous fat tissue, RF=Rectus femoris, VI=Vastus intermedius, M/F=Muscle to fat ratio calculated as [(Rectus femoris + Vastus intermedius)/subcutaneous fat] no units, TG= Thigh girth

Statistical differences in anterior thigh tissue thickness measurements between pre-ACLR surgery to post-rehabilitation are shown in Table 2. There were statistically significant differences between muscle thickness (p=0.04) and SF tissue thickness (p=0.04) and thigh girth (p=0.03) measurements taken prior to ACLR intervention and post-rehabilitation, while there was no statistically significant difference in muscle to fat ratio (p=0.08).

Table 2. Examination of differences in anterior thigh tissue thickness between pre-ACLR surgery to post-rehabilitation on the injured limb.

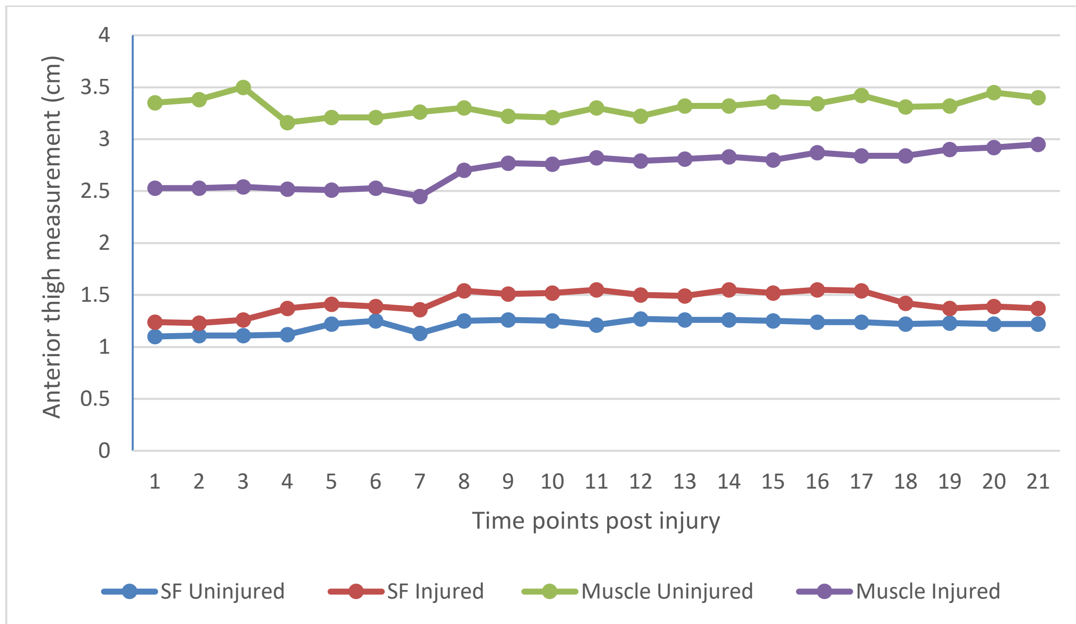
Tissue thickness (cm)	**Pre-surgery**	**Post-rehab**	**Paired mean diff**	**SD**	**SEM**	**Lower**	**Upper**	**t**	**df**	**Holm adjusted p- value**
SF thickness (cm)	1.24 ± 0.02	1.38 ± 0.01	-0.14	0.03	0.01	-0.19	-0.07	-9.18	2	0.04*
Muscle thickness (cm)	2.48 ± 0.08	3.09 ± 0.05	-0.61	0.13	0.07	-0.93	-0.29	-8.34	2	0.04*
Muscle to fat ratio	1.99 ± 0.08	2.24 ± 0.05	-0.25	0.13	0.08	-0.58	0.07	-3.33	2	0.08
Thigh girth (cm)	45.17± 0.29	47.2 ± 0.01	-2.03	0.29	0.17	-2.75	-1.32	-12.2	2	0.03*

SD=standard deviation, SEM=Standard error of the mean, df=degrees of freedom, *significant 2-tailed; p<0.05, Pre-surgery=last 3 measurements prior to ACLR, Post-rehab=last 3 measurements at the final period of rehabilitation

3.2 *Trends in outcome measures*

The trend in anterior thigh muscle and SF tissue thickness of the injured and uninjured limb measurements across the whole study period can be observed in Fig. 2. At the baseline (time point 1), there was evident atrophy of quadriceps in the injured limb compared with the uninjured one. A decrease in muscle thickness at 1-week post-ACLR (time point 4), is more evident for the uninvolved limb (healthy control) than the injured limb, the latter being already atrophied due to the ACL injury itself. Muscle thickness of the injured limb began to increase after 4-weeks post-ACLR (time point 7), while muscle thickness in the uninjured began to increase from 2 weeks post-ACLR (time point 5). Muscle thickness of the injured limb exceeded the pre-operative value at 24 weeks post-ACLR (time point 21), and that of the uninjured limb returned to the pre-operative value.

SF tissue thickness of the anterior thigh of the injured limb was greater compared with the contralateral uninjured limb throughout the study period, and both remained stable (Fig. 2)



1
2 Figure 2. Trend in anterior thigh muscle and subcutaneous tissue thickness measurements following anterior cruciate ligament injury
3 across the study period showing the healthy control level and injured limb. Muscle= Vastus intermedius + Rectus Femoris,
4 SF=Subcutaneous fat
5

6 3.3 *Comparison with MDC*

7 The changes in anterior thigh measurements of the injured and uninjured limb, compared with MDC values
8 between pre-operative to post-operative, pre-operative to post-rehabilitation (24-weeks post-ACLR), and post-
9 operative to post-rehabilitation are shown in Table 3.

10 Measurements greater than the MDC were found in the injured limb pre-ACLR surgery to post-rehabilitation,
11 for total muscle thickness (RF+VI), and for RF, while post-ACLR surgery to post-rehabilitation measurements
12 greater than the MDC value were found for total muscle thickness (RF+VI), and for both VI and for RF
13 measured separately (Table 3).

14 Differences in measurements in the injured limb between pre-surgery to post-surgery for muscle tissue
15 thickness (total and separately for RF and VI), and differences in SF tissue thickness measurements in all the
16 periods assessed, were less than MDC values and so inside the error associated with the measurement
17 technique (Table 3).

18 For the uninjured limb, measurements greater than the MDC were only found for total muscle thickness
19 (RF+VI) pre-ACLR surgery to post-ACLR surgery and post-ACLR surgery to post-rehabilitation.

20
21

1 **Table 3.** Anterior thigh tissue thickness measurements of the injured and uninjured lower limbs compared to MDC values obtained from
2 a reliability study[32]
3

			Difference compared to MDC (cm) **VI=0.33 RF=0.25 MT=0.36; SF=0.13**
Injured limb	**Pre-surgery (cm)**	**Post-surgery (cm)**	
VI	1.28	1.15	0.13 < MDC
RF	1.11	1.11	0 < MDC
MT	2.39	2.26	0.13 < MDC
SF	1.26	1.37	0.11< MDC
	Pre-surgery (cm)	**Post-rehabilitation (cm)**	
VI	1.28	1.58	0.3 < MDC
RF	1.11	1.57	**0.46 > MDC***
MT	2.39	3.15	**0.76 > MDC***
SF	1.26	1.37	0.11 < MDC
	Post-surgery (cm)	**Post-rehabilitation (cm)**	
VI	1.15	1.58	**0.43 > MDC***
RF	1.11	1.57	**0.46 > MDC***
MT	2.26	3.15	**0.89 > MDC***
SF	1.37	1.37	0 < MDC
Uninjured limb	**Pre-surgery (cm)**	**Post-surgery (cm)**	
VI	1.79	1.60	0.19 < MDC
RF	1.71	1.46	0.25 < MDC
MT	3.50	3.06	**0.44 > MDC***
SF	1.11	1.12	0.01 < MDC
	Pre-surgery (cm)	**Post-rehabilitation (cm)**	
VI	1.79	1.79	0 < MDC
RF	1.71	1.61	0.10 < MDC
MT	3.50	3.40	0.10 < MDC
SF	1.11	1.22	0.11 < MDC
	Post-surgery (cm)	**Post-rehabilitation (cm)**	
VI	1.60	1.79	0.19 < MDC
RF	1.46	1.61	0.15 < MDC
MT	3.06	3.40	**0.44 > MDC***
SF	1.12	1.22	0.10 < MDC

4 VI=Vastus intermedius, RF=Rectus femoris, M=Vastus intermedius + Rectus femoris, MT=Muscle thickness, SF=Subcutaneous fat,
5 MDC=minimal detectable change, * and bold = values greater than MDC.
6

7 Differences in anterior thigh tissue thickness that were greater than MDC values between injured and uninjured
8 lower limb were found for muscle at pre-surgery and post-surgery but not post-rehabilitation (Table 4).
9 Differences greater than MDC values in SF tissue thickness between injured and uninjured limbs were found
10 pre-surgery and post-surgery (Table 4). Percentage between-side difference in muscle thickness was greatest
11 pre-operatively (-32%) and reduced post-operatively until reducing below 10% at the end of rehabilitation,
12 when the difference was also below the MDC. Large percentage (>10%) between-side differences in SF tissue
13 thickness were found, with the involved limb being greater, returning to near 10% post-rehabilitation but still
14 above the MDC.
15

Table 4. Differences in anterior thigh tissue thickness between injured and uninjured lower limb compared to MDC values.

Anterior thigh thickness (cm)	Injured (cm)	Uninjured (cm)	Difference compared to MDC MT = 0.36; SF = 0.13	Percentage between-side difference
MT				
T3	2.39	3.50	**1.11 > MDC***	-32%
T4	2.26	3.16	**0.90 > MDC***	-28%
T21	3.15	3.40	0.25 < MDC	-7%
SF				
T3	1.26	1.11	**0.15 > MDC***	+14%
T4	1.37	1.12	**0.25 > MDC***	+22%
T21	1.37	1.22	**0.15 > MDC***	+12

MT=Muscle thickness: Vastus intermedius + Rectus femoris, SF=Subcutaneous fat MDC=minimal detectable change, * and bold = values greater than MDC, T3=1-week pre-surgery, T4=1-week post-surgery, T21=24-weeks post-surgery

4. Discussion

The present findings confirm the presence of quadriceps muscle atrophy that is known to occur rapidly after ACLR surgery [6,38,39] and how USI can be used to monitor recovery. A separate assessment of RF and VI (Table 1) revealed selective atrophy, where reduced thickness was more marked in VI than RF post-operatively. The use of existing MDC values of tissue thickness enabled objective assessment of abnormality and recovery using USI in the individual patient.

The finding of selective atrophy of VI confirmed a recent study [38] where quadriceps muscle thickness was measured using USI in 14 patients aged 30.4±5.9 years. Measurements were taken 1 hour prior and 48-72 hours after ACLR, which showed a significant decrease in VI thickness compared with pre-surgery values and compared with the other heads of quadriceps femoris muscle. The underlying mechanism of selective atrophy of VI after ACLR surgery is unknown, therefore further studies are needed to investigate and clarify the possible causes to minimise VI atrophy using targeted rehabilitation approaches.

To understand the slower recovery of VI compared to RF after ACLR surgery, the anatomy and function of the quadriceps muscle can be considered [40]. It can be observed that VI is a mono-articular muscle and acts just at knee level, while RF is a bi-articular muscle and acts both as a knee extensor and hip flexor. It may be possible that the dual joint actions of RF cause it to be stimulated consistently during rehabilitation even when the knee is kept at full extension, thus resulting in earlier recovery of muscle thickness than VI. The surgical access through the knee joint capsule could play a role inducing an inhibition of the articularis genus muscle that inserts into the synovial membrane of the joint capsule, the suprapatellar bursa and occasionally its distal muscle fibres are blended with the suprajacent fibres of VI [41,42]. The articularis genus muscle shares the same blood supply with VI via the deep circumflex branch of the femoral artery and the same innervation via the deep intermuscular branches of the femoral nerve [41,42]. The close anatomical links between articularis genus and VI could explain the interaction, with the underlying mechanism between the two muscles being more complicated than has been previously assumed [41,42]. Further studies are required to better understand the role of this mechanism of interaction and to investigate its possible implication during surgical knee procedures.

The present study also found a diminished quadriceps thickness on the contralateral uninjured side from the pre- to post-surgery period, which may be attributed to reduced mobility in the perioperative period. Another possible cause of a transient bilateral lower limb weakness is spinal anaesthesia, due to motor and sensory inhibition, anaesthetic neurotoxicity and neuroendocrine stress response [43]. The mechanism by which anaesthesia could induce muscle weakness/atrophy, by influencing the neuroendocrine stress response, is unclear [43]. However, reduced mobility is the more likely explanation for bilateral atrophy.

Thigh girth measurements (Table 1) remained consistent throughout the study period, despite changes in muscle and SF thickness measurements, demonstrating limitations in the sensitivity of thigh girth as an outcome measure to monitor SF and/or muscle changes, confirming previous studies [26-28]. SF tissue thickness (Table 4) was greater on the injured side contributing to thigh circumference, also demonstrating inaccuracies in thigh girth for estimating differences in muscle size. It is logical that by measuring thigh girth using a tape measure, the measurement includes all anatomical structures of the thigh and not only the specific structures of interest. At best, the measure provides an estimate of the global state of the entire thigh compared to the uninjured limb but is far from being an accurate measurement.

The MDC values used to compare the data from the present case study participant were derived from a test-retest reliability study [32], which involved 24 participants (12 females, 12 males) aged 48.91±9.78 (36-64) years. Changes in values smaller than the MDC, 3.6 mm for total muscle thickness (RF+VI), and 1.3 mm for SF thickness [32], are likely caused by random measurement error. The retrospective analysis of data from the same between-day reliability study [32] to obtain MDC values for RF (2.5 mm) and VI (3.3 mm) allowed specific evaluation of each muscle separately, highlighting a selective atrophy, which was intended to enable specific and customized physiotherapy care. A selective change in RF thickness of the injured lower limb between the pre-ACLR surgery and post-rehabilitation periods was observed (Table 3), with changes in the other periods being either below or above the MDC in both VI and RF muscles.

The present 18-year-old participant was younger than the group from which the MDC values were generated. The age of the participant, quality of the ultrasound image that is associated with the echogenicity of the individual's tissues and the error associated with the measurement technique itself, represent important variables in determining MDC values. In a recent study [44] of 12 young male adults aged 26.5±3.9 years, the MDC value of RF thickness (VI and SF were not measured), for the test-retest reliability was 2.0 mm. This MDC value from younger people may be more appropriate but the MDC was not for total muscle thickness and the number of participants studied [44] was smaller (n=12) compared to the study (n=24) used for MDC values in the present study [32]. Further studies could investigate the MDC values using USI in measuring anterior thigh tissue thickness in a younger age group and also differentiate the values for both RF and VI.

A recent study using USI in 26 patients who underwent ACLR surgery revealed a reduction in RF CSA from pre-surgery to 9 weeks post-surgery ($p < 0.01$), followed by an increase of CSA from 9 weeks to 9 months post-surgery ($p = 0.03$) [39].

Reduction in RF CSA was also recorded in the uninjured limb from surgery to 9 weeks post-surgery ($p < 0.01$), with a complete return to the pre-operative CSA at 9 months post-surgery, when the injured limb failed to recover [39]. Differences between the cited study [39] and the present study, are that we measured VI as well as the RF muscle (showing selective changes) and SF tissue of anterior thigh, and measured muscle thickness, which is easier and faster than measuring CSA, although less reflective of muscle mass.

Limitations of the present study are mainly intrinsic to the type of study design (n of 1) [35], concerning external validity, replicability and providing low level of evidence. The investigator conducting the USI imaging underwent training and established their reliability [32]. External validity and generalisability were not addressed but these were not part of the aims of the present study. Rather, the aim was to provide clinically useful measurements to enable personalized patient care that could be delivered with precision. Another limitation was that muscle strength was not measured directly and USI only provides an indication of force. It is generally accepted that the relationship between muscle size and strength is positive but the level of

correlation varies between muscles and also in response to strength training [45]. This dissociation between the two variables with training involves neural motor control and/or cellular and molecular adaptations of muscle fibres [46]. Such neural adaptation could possibly explain the lack of increase in muscle thickness found between 6-12 weeks of rehabilitation (Table 1), at a time when strength would be expected to increase. Another possibility is that the rehabilitation programme may not have provided sufficient stimulus to induce continued increase muscle size or, indeed, strength, which would need to be measured to determine this. However, strength testing does not allow selective changes between muscles to be recognised, as demonstrated by the present findings using USI.

Potential clinical implications of the present study are that the USI technique could be used to assess clinically useful changes of RF and VI muscle thickness in an individual patient post-ACLR surgery, enabling individualized and tailored optimal clinical care.

Skeletal muscle wasting and atrophy are commonly reported in critically ill patients and occur rapidly during the first week of critical illness, having significant implications on patient outcomes [47-50]. Critical illness patients suffer severe muscle atrophy, impaired muscle function, with increased morbidity and health care costs, and poorer quality of life [51,52]. Monitoring skeletal muscle size using USI in critically ill patients at the bedside, is increasingly used, as it has proved to be an accurate and reliable tool to assess muscle changes [47-54].

The RF muscle is typically monitored but the present observations suggest VI muscle could be more sensitive to atrophy than RF (Table1), so it may be preferable to include VI in the assessment. However, the disuse in intensive care patients without lower limb injuries may result in atrophy through a different mechanism to that seen with ACL injuries, which may involve inhibitory reflex responses from articular/periarticular receptors [55]. Further studies are needed to investigate a greater susceptibility to atrophy of VI compared to RF with different causal mechanisms and the potential use of USI as an indicator of the early muscle atrophy process.

5. Conclusions

The present findings demonstrate that it is possible to measure statistically significant differences in USI measurements of anterior thigh muscle and SF tissue thickness in an individual over time, using comparison with MDC values. Measurements taken prior to ACLR surgical intervention and post-rehabilitation showed greater reductions in VI than RF muscle thickness, indicating selective atrophy. These findings confirm the utility of the USI technique as an accurate tool with good sensitivity for monitoring effects of surgery and physiotherapy rehabilitation in an individual patient.

Conflicts of interest: The authors declare that they have no competing interests.

Funding: The received no financial support for conducting the research or for authorship. Publication of this article was supported by funding from the University of Southampton.

Acknowledgments: The authors thank the participant for the case study and Dr Vasiliki Koutra for statistical support.

1 **References**

2 [1] C. C. Kaeding, B. Leger-St-Jean, and R. A. Magnussen, "Epidemiology and Diagnosis of Anterior Cruciate
3 Ligament Injuries," *Clinics in sports medicine*, vol. 36, no. 1, pp. 1-8, 2017.

4 [2] R. C. Mather, L. Koenig, M. S. Kocher, T. M. Dall, P. Gallo, D. J. Scott, B. R. Bach, K. P. Spindler, and
5 MOON Knee Group, "Societal and economic impact of anterior cruciate ligament tears," *The Journal of bone*
6 *and joint surgery.American volume*, vol. 95, no. 19, pp. 1751-1759, 2013.

7 [3] B. Moses, J. Orchard, and J. Orchard, "Systematic review: Annual incidence of ACL injury and surgery in
8 various populations," *Research in sports medicine (Print)*, vol. 20, no. 3-4, pp. 157-179, 2012.

9 [4] L. Davies, J. Cook, J. Leal, C. M. Areia, B. Shirkey, W. Jackson, H. Campbell, H. Fletcher, A. Carr, K.
10 Barker, S. E. Lamb, P. Monk, S. O'Leary, F. Haddad, C. Wilson, A. Price, and D. Beard, "Comparison of the
11 clinical and cost effectiveness of two management strategies (rehabilitation versus surgical reconstruction)
12 for non-acute anterior cruciate ligament (ACL) injury: study protocol for the ACL SNNAP randomised
13 controlled trial," *Trials*, vol. 21, no. 1, pp. 405-y, 2020.

14 [5] R. B. Frobell, H. P. Roos, E. M. Roos, F. W. Roemer, J. Ranstam, and L. S. Lohmander, "Treatment for
15 acute anterior cruciate ligament tear: five year outcome of randomised trial," *BMJ (Clinical research ed.)*, vol.
16 346, pp. f232, 2013.

17 [6] A. C. Thomas, E. M. Wojtys, C. Brandon, and R. M. Palmieri-Smith, "Muscle atrophy contributes to
18 quadriceps weakness after anterior cruciate ligament reconstruction," *Journal of science and medicine in*
19 *sport*, vol. 19, no. 1, pp. 7-11, 2016.

20 [7] R. L. Mizner, S. C. Petterson, J. E. Stevens, K. Vandenborne, and L. Snyder-Mackler, "Early quadriceps
21 strength loss after total knee arthroplasty. The contributions of muscle atrophy and failure of voluntary
22 muscle activation," *The Journal of bone and joint surgery.American volume*, vol. 87, no. 5, pp. 1047-1053,
23 2005.

24 [8] A. S. Lepley, D. R. Grooms, J. P. Burland, S. M. Davi, J. M. Kinsella-Shaw, and L. K. Lepley, "Quadriceps
25 muscle function following anterior cruciate ligament reconstruction: systemic differences in neural and
26 morphological characteristics," *Experimental brain research*, vol. 237, no. 5, pp. 1267-1278, 2019.

27 [9] L. K. Lepley and R. M. Palmieri-Smith, "Quadriceps Strength, Muscle Activation Failure, and Patient-
28 Reported Function at the Time of Return to Activity in Patients Following Anterior Cruciate Ligament
29 Reconstruction: A Cross-sectional Study," *The Journal of orthopaedic and sports physical therapy*, vol. 45,
30 no. 12, pp. 1017-1025, 2015.

31 [10] A. S. Lepley, B. Pietrosimone, and M. L. Cormier, "Quadriceps function, knee pain, and self-reported
32 outcomes in patients with anterior cruciate ligament reconstruction," *Journal of athletic training*, vol. 53, no. 4,
33 pp. 337-346, 2018.

34 [11] M. P. Ithurburn, M. A. Longfellow, S. Thomas, M. V. Paterno, and L. C. Schmitt, "Knee Function,
35 Strength, and Resumption of Preinjury Sports Participation in Young Athletes Following Anterior Cruciate
36 Ligament Reconstruction," *The Journal of orthopaedic and sports physical therapy*, vol. 49, no. 3, pp. 145-
37 153, 2019.

38 [12] M. Lindstrom, S. Strandberg, T. Wredmark, L. Fellander-Tsai, and M. Henriksson, "Functional and
39 muscle morphometric effects of ACL reconstruction. A prospective CT study with 1 year follow-up,"
40 *Scandinavian Journal of Medicine & Science in Sports*, vol. 23, no. 4, pp. 431-442, 2013.

41 [13] B. D. Roewer, S. L. Di Stasi, and L. Snyder-Mackler, "Quadriceps strength and weight acceptance
42 strategies continue to improve two years after anterior cruciate ligament reconstruction," *Journal of*
43 *Biomechanics*, vol. 44, no. 10, pp. 1948-1953, 2011.

1 [14] M. P. Ithurburn, M. V. Paterno, K. R. Ford, T. E. Hewett, and L. C. Schmitt, "Young Athletes With
2 Quadriceps Femoris Strength Asymmetry at Return to Sport After Anterior Cruciate Ligament Reconstruction
3 Demonstrate Asymmetric Single-Leg Drop-Landing Mechanics," *The American Journal of Sports Medicine*,
4 vol. 43, no. 11, pp. 2727-2737, 2015.

5 [15] C. Kuenze, J. Hertel, A. Weltman, D. R. Diduch, S. Saliba, and J. M. Hart, "Jogging biomechanics after
6 exercise in individuals with ACL-reconstructed knees," *Medicine and science in sports and exercise*, vol. 46,
7 no. 6, pp. 1067-1076, 2014.

8 [16] M. V. Paterno, B. Huang, S. Thomas, T. E. Hewett, and L. C. Schmitt, "Clinical Factors That Predict a
9 Second ACL Injury After ACL Reconstruction and Return to Sport: Preliminary Development of a Clinical
10 Decision Algorithm," *Orthopaedic journal of sports medicine*, vol. 5, no. 12, pp. 2325967117745279, 2017.

11 [17] M. V. Paterno, L. C. Schmitt, K. R. Ford, M. J. Rauh, G. D. Myer, B. Huang, and T. E. Hewett,
12 "Biomechanical measures during landing and postural stability predict second anterior cruciate ligament
13 injury after anterior cruciate ligament reconstruction and return to sport," *The American Journal of Sports*
14 *Medicine*, vol. 38, no. 10, pp. 1968-1978, 2010.

15 [18] B. Tayfur, C. Charuphongsa, D. Morrissey, and S. C. Miller, "Neuromuscular Function of the Knee Joint
16 Following Knee Injuries: Does It Ever Get Back to Normal? A Systematic Review with Meta-Analyses,"
17 *Sports medicine (Auckland, N.Z.)*, vol. 51, no. 2, pp. 321-338, 2021.

18 [19] M. V. Paterno, M. J. Rauh, L. C. Schmitt, K. R. Ford, and T. E. Hewett, "Incidence of contralateral and
19 ipsilateral anterior cruciate ligament (ACL) injury after primary ACL reconstruction and return to sport,"
20 *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine*, vol. 22, no. 2,
21 pp. 116-121, 2012.

22 [20] M. V. Paterno, M. J. Rauh, L. C. Schmitt, K. R. Ford, and T. E. Hewett, "Incidence of Second ACL
23 Injuries 2 Years After Primary ACL Reconstruction and Return to Sport," *The American Journal of Sports*
24 *Medicine*, vol. 42, no. 7, pp. 1567-1573, 2014.

25 [21] H. Grindem, L. Snyder-Mackler, H. Moksnes, L. Engebretsen, and M. A. Risberg, "Simple decision rules
26 can reduce reinjury risk by 84% after ACL reconstruction: the Delaware-Oslo ACL cohort study," *British*
27 *journal of sports medicine*, vol. 50, no. 13, pp. 804-808, 2016.

28 [22] R. Lobb, S. Tumilty, and L. S. Claydon, "A review of systematic reviews on anterior cruciate ligament
29 reconstruction rehabilitation," *Physical therapy in sport : official journal of the Association of Chartered*
30 *Physiotherapists in Sports Medicine*, vol. 13, no. 4, pp. 270-278, 2012.

31 [23] R. W. Wright, A. K. Haas, J. Anderson, G. Calabrese, J. Cavanaugh, T. E. Hewett, D. Lorring, C.
32 McKenzie, E. Preston, G. Williams, and MOON Group, "Anterior Cruciate Ligament Reconstruction
33 Rehabilitation: MOON Guidelines," *Sports health*, vol. 7, no. 3, pp. 239-243, 2015.

34 [24] A. Ajuied, C. Smith, F. Wong, S. Hoskinson, D. Back, and A. Davies, "A Survey of Rehabilitation
35 Regimens Following Isolated ACL Reconstruction," *JMED Research*, vol. 2014, pp. 1-9, 2014.

36 [25] J. T. Cavanaugh and M. Powers, "ACL Rehabilitation Progression: Where Are We Now?" *Current*
37 *reviews in musculoskeletal medicine*, vol. 10, no. 3, pp. 289-296, 2017.

38 [26] A. Young, I. Hughes, P. Russell, M. J. Parkers, and P. J. Nichols, "Measurement of quadriceps muscle
39 wasting by ultrasonography," *Rheumatology and rehabilitation*, vol. 19, no. 3, pp. 141-148, 1980.

40 [27] G. A. Arangio, C. Chen, M. Kalady, and J. F. Reed, "Thigh muscle size and strength after anterior
41 cruciate ligament reconstruction and rehabilitation," *The Journal of orthopaedic and sports physical therapy*,
42 vol. 26, no. 5, pp. 238-243, 1997.

43 [28] S. Mathur, K. P. Takai, D. L. Macintyre, and D. Reid, "Estimation of thigh muscle mass with magnetic
44 resonance imaging in older adults and people with chronic obstructive pulmonary disease," *Physical*
45 *Therapy*, vol. 88, no. 2, pp. 219-230, 2008.

1 [29] J. L. Whittaker, D. S. Teyhen, J. M. Elliott, K. Cook, H. M. Langevin, H. H. Dahl, and M. Stokes,
2 "Rehabilitative ultrasound imaging: understanding the technology and its applications," *The Journal of*
3 *orthopaedic and sports physical therapy*, vol. 37, no. 8, pp. 434-449, 2007.

4 [30] J. M. Dias, B. F. Mazuquin, F. Q. Mostagi, T. B. Lima, M. A. Silva, B. N. Resende, R. M. Borges da
5 Silva, E. L. Lavado, and J. R. Cardoso, "The effectiveness of postoperative physical therapy treatment in
6 patients who have undergone arthroscopic partial meniscectomy: systematic review with meta-analysis," *The*
7 *Journal of orthopaedic and sports physical therapy*, vol. 43, no. 8, pp. 560-576, 2013.

8 [31] J. L. Whittaker, R. Ellis, P. W. Hodges, C. OSullivan, J. Hides, S. Fernandez-Carnero, J. L. Arias-Buria,
9 D. S. Teyhen, and M. J. Stokes, "Imaging with ultrasound in physical therapy: What is the PT's scope of
10 practice? A competency-based educational model and training recommendations," *British journal of sports*
11 *medicine*, vol. 53, no. 23, pp. 1447-1453, 2019.

12 [32] F. Mechelli, L. Arendt-Nielsen, M. Stokes, and S. Agyapong-Badu, "Inter-rater and intra-rater reliability of
13 ultrasound imaging for measuring quadriceps muscle and non-contractile tissue thickness of the anterior
14 thigh," *Biomedical physics & engineering express*, vol. 5, no. 3, pp. 037002, 2019.

15 [33] F. Mechelli, L. Arendt-Nielsen, M. Stokes, and S. Agyapong-Badu, "Validity of Ultrasound Imaging
16 Versus Magnetic Resonance Imaging for Measuring Anterior Thigh Muscle, Subcutaneous Fat, and Fascia
17 Thickness," *Methods and protocols*, vol. 2, no. 3, pp. 58, 2019.

18 [34] F. Mechelli, L. Arendt-Nielsen, M. Stokes, and S. Agyapong-Badu, "Ultrasound imaging for measuring
19 muscle and subcutaneous fat tissue thickness of the anterior thigh: a 2 year longitudinal study in middle
20 age," *JCSM clinical reports*, vol. 5, no. 1, pp. 3-7, 2020.

21 [35] K. W. Davidson, Y. K. Cheung, T. McGinn, and Y. C. Wang, "Expanding the Role of N-of-1 Trials in the
22 Precision Medicine Era: Action Priorities and Practical Considerations," *NAM Perspectives*, 2018.

23 [36] World Medical Association, "World Medical Association Declaration of Helsinki: ethical principles for
24 medical research involving human subjects," *Jama*, vol. 310, no. 20, pp. 2191-2194, 2013.

25 [37] S. Y. Chen, Z. Feng, and X. Yi, "A general introduction to adjustment for multiple comparisons," *Journal*
26 *of thoracic disease*, vol. 9, no. 6, pp. 1725-1729, 2017.

27 [38] J. H. Lee, S. Cheon, H. P. Jun, Y. L. Huang, and E. Chang, "Bilateral Comparisons of Quadriceps
28 Thickness after Anterior Cruciate Ligament Reconstruction," *Medicina (Kaunas, Lithuania)*, vol. 56, no. 7, pp.
29 10.3390/medicina56070335, 2020.

30 [39] S. A. Garcia, M. T. Curran, and R. M. Palmieri-Smith, "Longitudinal Assessment of Quadriceps Muscle
31 Morphology Before and After Anterior Cruciate Ligament Reconstruction and Its Associations With Patient-
32 Reported Outcomes," *Sports health*, vol. 12, no. 3, pp. 271-278, 2020.

33 [40] A. C. Waligora, N. A. Johanson, and B. E. Hirsch, "Clinical anatomy of the quadriceps femoris and
34 extensor apparatus of the knee," *Clinical orthopaedics and related research*, vol. 467, no. 12, pp. 3297-3306,
35 2009.

36 [41] J. Caterson, M. A. Williams, C. McCarthy, N. Athanasou, H. T. Temple, T. Cosker, and M. Gibbons, "The
37 articularis genu muscle and its relevance in oncological surgical margins," *Bone & joint open*, vol. 1, no. 9,
38 pp. 585-593, 2020.

39 [42] K. Grob, H. Gilbey, M. Manestar, T. Ackland, and M. S. Kuster, "The Anatomy of the Articularis Genus
40 Muscle and Its Relation to the Extensor Apparatus of the Knee," *JB & JS open access*, vol. 2, no. 4, pp.
41 e0034, 2017.

42 [43] J. P. Desborough, "The stress response to trauma and surgery," *British journal of anaesthesia*, vol. 85,
43 no. 1, pp. 109-117, 2000.

1 [44] Y. Takahashi, Y. Fujino, K. Miura, A. Toida, T. Matsuda, and S. Makita, "Intra- and inter-rater reliability of
2 rectus femoris muscle thickness measured using ultrasonography in healthy individuals," *The ultrasound*
3 *journal*, vol. 13, no. 1, pp. 21-8, 2021.

4 [45] S. J. Dankel, S. L. Buckner, M. B. Jessee, J. Grant Mouser, K. T. Mattocks, T. Abe, and J. P. Loenneke,
5 "Correlations Do Not Show Cause and Effect: Not Even for Changes in Muscle Size and Strength," *Sports*
6 *medicine (Auckland, N.Z.)*, vol. 48, no. 1, pp. 1-6, 2018.

7 [46] C. Reggiani and S. Schiaffino, "Muscle hypertrophy and muscle strength: dependent or independent
8 variables? A provocative review," *European journal of translational myology*, vol. 30, no. 3, pp. 9311, 2020.

9 [47] M. Tillquist, D. J. Kutsogiannis, P. E. Wischmeyer, C. Kummerlen, R. Leung, D. Stollery, C. J. Karvellas,
10 J. C. Preiser, N. Bird, R. Kozar, and D. K. Heyland, "Bedside ultrasound is a practical and reliable
11 measurement tool for assessing quadriceps muscle layer thickness," *JPEN.Journal of parenteral and enteral*
12 *nutrition*, vol. 38, no. 7, pp. 886-890, 2014.

13 [48] V. Hadda, G. C. Khilnani, R. Kumar, A. Dhunguna, S. Mittal, M. A. Khan, K. Madan, A. Mohan, and R.
14 Guleria, "Intra- and Inter-observer Reliability of Quadriceps Muscle Thickness Measured with Bedside
15 Ultrasonography by Critical Care Physicians," *Indian journal of critical care medicine : peer-reviewed, official*
16 *publication of Indian Society of Critical Care Medicine*, vol. 21, no. 7, pp. 448-452, 2017.

17 [49] F. V. Valla, D. K. Young, M. Rabilloud, U. Periasami, M. John, F. Baudin, C. Vuillerot, A. Portefaix, D.
18 White, J. A. Ridout, R. Meyer, B. Gaillard Le Roux, E. Javouhey, and N. Pathan, "Thigh Ultrasound
19 Monitoring Identifies Decreases in Quadriceps Femoris Thickness as a Frequent Observation in Critically Ill
20 Children," *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World*
21 *Federation of Pediatric Intensive and Critical Care Societies*, vol. 18, no. 8, pp. e339-e347, 2017.

22 [50] D. O. Toledo, D. C. L. E. Silva, D. M. D. Santos, B. J. Freitas, R. Dib, R. L. Cordioli, E. J. A. Figueiredo,
23 S. M. F. Piovacari, and J. M. Silva, "Bedside ultrasound is a practical measurement tool for assessing
24 muscle mass," *Revista Brasileira de terapia intensiva*, vol. 29, no. 4, pp. 476-480, 2017.

25 [51] R. Y. Li, H. W. He, J. H. Sun, Q. Li, Y. Long, and H. P. Liu, "Clinical value of early bedside ultrasound
26 measurement of quadriceps femoris in diagnosis of ICU-acquired weakness," *Zhonghua yi xue za zhi*, vol.
27 100, no. 25, pp. 1967-1972, 2020.

28 [52] C. A. Galindo Martin, E. Monares Zepeda, and O. A. Lescas Mendez, "Bedside Ultrasound
29 Measurement of Rectus Femoris: A Tutorial for the Nutrition Support Clinician," *Journal of nutrition and*
30 *metabolism*, vol. 2017, pp. 2767232, 2017.

31 [53] A. Sabatino, G. Regolisti, L. Bozzoli, F. Fani, R. Antoniotti, U. Maggiore, and E. Fiaccadori, "Reliability of
32 bedside ultrasound for measurement of quadriceps muscle thickness in critically ill patients with acute kidney
33 injury," *Clinical nutrition (Edinburgh, Scotland)*, vol. 36, no. 6, pp. 1710-1715, 2017.

34 [54] M. C. de Andrade-Junior, I. C. D. de Salles, C. M. M. de Brito, L. Pastore-Junior, R. F. Righetti, and W.
35 P. Yamaguti, "Skeletal Muscle Wasting and Function Impairment in Intensive Care Patients With Severe
36 COVID-19," *Frontiers in physiology*, vol. 12, pp. 640973, 2021.

37 [55] B. Sonnery-Cottet, A. Saithna, B. Quelard, M. Daggett, A. Borade, H. Ouanezar, M. Thaunat, and W. G.
38 Blakeney, "Arthrogenic muscle inhibition after ACL reconstruction: a scoping review of the efficacy of
39 interventions," *British journal of sports medicine*, vol. 53, no. 5, pp. 289-298, 2019.

40