For publication in the *Journal of Cachexia, Sarcopenia and Muscle* (*JCSM) Clinical Reports*

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

Mechelli F, Arendt-Nielsen L, Stokes M, Agyapong-Badu S.
Ultrasound imaging for measuring muscle and subcutaneous fat tissue thickness of the anterior thigh: A two-year longitudinal study in middle age.
JCSM Clinical Reports 2019; 4(2): In press

Accepted: 21st August 2019

Please use the following link for the final, fully proofed and peer-reviewed journal article online: doi: http://dx.doi.org/10.17987/jcsm-cr.v4i2.94

ORIGINAL ARTICLE

Ultrasound imaging for measuring muscle and subcutaneous fat tissue thickness of the anterior thigh: A two-year longitudinal study in middle age

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Abstract
Background Ultrasound (US) imaging technique is widely used in research and clinical settings to assess the morphology and morphometry of neuromusculoskeletal structures. The technique has reported validity and reliability in measuring the size of various muscles under controlled conditions. The aim of the present study was to assess anterior thigh thickness using US imaging, in a healthy cohort of middle-aged older adults.
Methods Participants included seventeen healthy older adults involved in regular moderate-vigorous activities (age range 39-66 years). US imaging scans of the anterior thighs two years since baseline measurements were performed. Images were analyzed off-line to compare US imaging measurements of muscle thickness and subcutaneous fat (SF) of the anterior thigh taken at baseline and after two years.
Results There was no significant difference between muscle thickness measurements taken at baseline and after two years (Mean, standard deviation; baseline=2.80±0.71cm; follow-up=2.77±0.72cm, p=0.33). There was also no significant change in SF thickness (baseline=1.04±0.41cm; follow-up=1.06±0.40, p=0.33).
Conclusions The results show there was no decline in anterior thigh muscle thickness or increase in SF in the healthy cohort studied using US imaging over a two-year period. These findings demonstrate the robustness of US imaging measurements over time.

Keywords: cohort study; muscle thickness; rectus femoris; ultrasound imaging; subcutaneous fat thickness; vastus intermedius

Introduction

The use of ultrasound (US) imaging in research and clinical settings to assess morphology and morphometry of neuromusculoskeletal structures is increasingly gaining acceptance [1]. An objective measure of muscle thickness, cross-sectional area, volume, fibres length and pennation angle provides the opportunity to obtain an indirect evaluation of muscle function and strength [2]. US imaging of skeletal muscles carries the advantage of providing a rapid, non-invasive, portable, safe and clinically useful method of obtaining objective measurements.

The US imaging technique is operator-dependent [3], so user repeatability of measurements need to be examined for muscle and perimuscular tissues [1]. The potential to use US for accurate and reliable measurement of muscle thickness as well as of non-contractile tissues is important for researchers and clinicians. Measurement of muscle characteristics longitudinally would be potentially useful to monitor changes with ageing. Agyapong-Badu et al [4] used US imaging to provide objective data on the effects of ageing and gender on relative anterior thigh thickness in healthy people. The authors also reported high intra-rater reliability for the measurements (ICC values from 0.88 for muscle thickness to 0.97 for subcutaneous fat [SF] thickness). Recently intra-rater and inter-rater reliability of US measurements of muscle and SF thickness of the anterior thigh were reported by Mechelli et al [5] in a healthy middle-aged cohort (intra-rater reliability ICC3,2 of 0.96 for muscle thickness, 0.99 for SF; inter-rater reliability ICC3,1 of 0.98 for muscle thickness, 0.81 for SF). Whittaker and Emery [6] have also reported excellent intra-rater reliability of US for measuring the vastus medialis muscle thickness (ICC values above 0.90). Regarding validity, muscle thickness measurements on US images were not significantly different to those obtained using magnetic resonance imaging (MRI), demonstrating criterion validity, for some muscles in the lower-limbs, including vastus medialis [7], rectus femoris and vastus intermedius [8], and anterior hip muscles [9].

Quadriceps muscle atrophy is common in patients with knee osteoarthritis [10] and other painful knee conditions [11,12]. Sudden muscle atrophy is almost an unavoidable consequence in critically-ill patients in intensive care units [13–17]. Objective assessment of muscle thickness at the bedside could be a useful marker for clinicians to assess relative contributions of muscle and fat, and balance nutritional support to attenuate such muscle mass loss.

Loss of muscle mass occurs over time with ageing due to sarcopenia [18] and also cachexia in chronic conditions [19]. Approximately 24–27% of muscle mass is lost between the second and seventh decades [20]. The functional consequences of sarcopenia include loss of mobility and physical independence [21]. Identifying robust ways of detecting changes in muscle in middle age may help to prevent premature frailty. Ultrasound studies of changes with age involve cross-sectional comparisons between age groups but documenting changes longitudinally in the same individuals appear to be lacking. It would be particularly helpful to know about the status of muscle changes in middle age, at the time when osteoarthritis commonly starts, so that the relative effects of joint disease and aging on muscle size could be better understood.

The purpose of the present study, the first to our knowledge, was to evaluate US imaging measurements of anterior thigh thickness (quadriceps muscle and non-contractile tissue), in a healthy middle-aged cohort at baseline and at two-year follow-up to assess changes over time.

The null hypothesis was that there would be no difference between US imaging measurements of anterior thigh SF tissue and muscle thickness taken at baseline and those after a two-year follow-up in a healthy middle-aged cohort.

Methods

Participants

Seventeen (9 females, 8 males) healthy, moderately-active adults [22], aged 51.58±10.29 (39-66) years (see Table 1 for other characteristics), underwent US imaging of their anterior thighs. The measurements were performed at baseline and at two-year follow-up. The two year follow-up period was chosen as the minimum length of time to enable changes in muscle thickness to occur, as it is known that after the age of 50 years, muscle mass decreases at an annual rate of approximately 1 to 2% [23]. Exclusion criteria applied were similar to that reported in Mechelli et al [5], to exclude conditions that affect muscle function, and a variation in diet and nutritional habits and/or in amount of weekly physical activity since baseline assessment. The investigator notified participants to refrain from vigorous exercise within the preceding 24 hours to study participation.

US imaging measurements

An ultrasound scanner (MyLab25; Esaote, Genova, Italia) with a 7.5 MHz linear transducer (40 mm length) was used to obtain images of the anterior thigh. Ultrasound scans, both baseline and at follow-up were acquired in the same setting with the participant in supine lying position with the knee fully extended and weight bags placed around the feet to keep hip in a neutral position, and ankles relaxed in slight plantar flexion (Fig1). B-mode transverse scans were obtained at two thirds of the distance measured from the antero-superior iliac spine to the superior pole of patella [5,8,24]. The scanning site was marked with a skin-marking pen. During image acquisition, the transducer was coated with a generous amount of ultrasound water-based transmission gel and was placed perpendicular to the skin applying the lightest contact pressure to ensure underlying tissues were not compressed [5,8]. Scanner parameters remained the same for all measurements, ensuring uniformity to the baseline measurements procedure. The same investigator (FM) with established reliability for the technique took all scans [5]. The between-day reliability was excellent (intraclass-correlation coefficients, ICC3,2, were 0.96 for muscle and 0.98 for SF). The minimal detectable change (MDC) from the reliability data for examining the precision of measurements was 3.6mm for muscle thickness and 1.3mm for SF.

US Imaging Data Processing

US images were analysed offline to compare the measurements taken at baseline and follow-up using ImageJ software (available from https://imagej.nih.gov/ij/). Each anonymized US scan was measured twice by the investigator (FM) and the mean used in the analysis. SF thickness was the distance from the skin to the outside edge of the superficial fascial layer, muscle thickness of rectus femoris (RF) and vastus intermedius (VI) were considered as the distance between the inside edges of each muscle border excluding perimuscular fascia (Fig2).

Data Analysis

SPSS 22 (SPSS Inc, Chicago, IL) was used to analyse the data. Normal distribution of the data was confirmed using the Shapiro-Wilk test.

Paired sample t-tests were used to assess differences between baseline and follow-up measurements. The alpha level was set at $p<0.05$ [25].

Results

The mean follow-up time was 26.55±0.65 (25.92-28.81) months. There were no significant differences between muscle thickness ($p=0.33$) and SF tissue ($p=0.33$) measurements taken at baseline and at follow-up (Table 2). The difference in mean muscle thickness over the two-year period (0.3mm) was below the MDC of 3.6mm reported previously in the same cohort [5]. Similarly, the difference in subcutaneous fat (0.2mm) was below the MDC of 1.3mm.

Fig1. The experimental set-up with a participant in supine lying. Placement of ultrasound transducer during image acquisition of anterior mid-thigh.

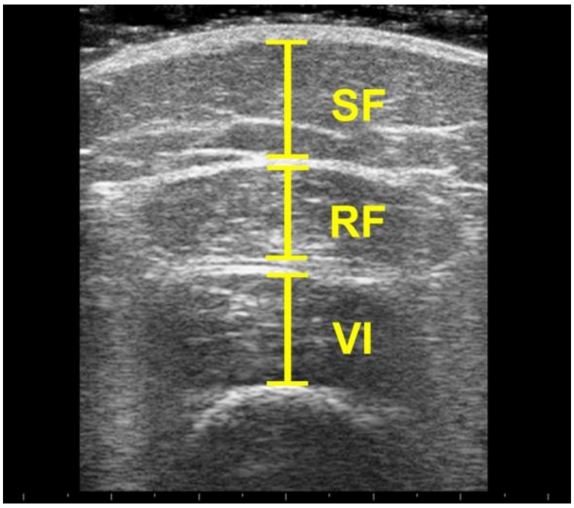

Fig2. Ultrasound scan of the anterior thigh showing subcutaneous fat (SF); rectus femoris (RF); vastus intermedius (VI)

Table 1 Participant Characteristics

Variable	Mean ± SD (n=17)	
	Baseline	2-year follow-up
Age (years)	49.6 ± 10.3	51.6 ± 10.3
Height (m)	1.7 ± 0.1	1.7 ± 0.1
Body mass (kg)	71.5 ± 12.2	72.0 ± 13.7

BMI (kg/m^2)	24.2 ± 3.8	24.3 ± 4.2

SD-standard deviation, BMI-body mass index

Table 2 Mean differences in anterior thigh thickness using paired sample t-tests

	Mean ± SD (n=17)				
Anterior thigh measurements	Baseline	2-year follow-up	t	df	p-value
Muscle thickness (mm)	28 ± 7.1	27.7 ± 7.2	0.98	33	0.33
Subcutaneous fat (mm)	10.4 ± 4.1	10.6 ± 4.0	-0.97	33	0.33

SD-standard deviation, df-degrees of freedom

Discussion

The present findings demonstrated that in the healthy middle-aged participants studied, there was no decline in quadriceps muscle thickness or increase in subcutaneous fat tissue that might be expected with advancing age [4]. The null hypothesis of no difference in US imaging measurements of SF tissue and muscle thickness of the anterior thigh taken at baseline and at two-year follow-up in a healthy middle-aged cohort was accepted. In addition to there being no statistically significant changes, differences in muscle and SF thickness at follow-up were within MDC from reliability testing [5], indicating that the difference was within the error of measurement and there was no real change over time. This consistency of measurements is important to demonstrate, as some measures can be statistically different over time but may not be clinically relevant if they are still within the MDC values. The consistency in muscle and SF thickness measurements demonstrates the robustness of US imaging measurements over the two-year follow-up period in a healthy middle-aged cohort.

The combined loss of strength and impaired physical performance is frequently reported in older people as a consequence of sarcopenia [18,20,21,26]. Loenneke et al [27] proposed using US imaging in addition to Dual X-ray Absorptiometry (DXA) to assess quadriceps muscle characteristics for early diagnosis of sarcopenia. The technique has been used to monitor lean mass loss at the bedside in critically-ill patients who experience quadriceps and skeletal muscle atrophy at a very early stage especially critically-ill children [28]. Clinicians can utilize objective data from US imaging to administer an appropriate metabolic support to improve muscle morphology and subsequently function [14,16,29]. The authors believe monitoring both muscle and subcutaneous thickness to assess their relative contributions are important.

The sensitivity of the US imaging technique for assessing anterior thigh thickness over a relatively longer period could be valuable for longitudinal assessment of the effects of nutrition during different diet protocols or weight loss/gain programs. For instance, the effects of amino acid or other metabolic supplementation for quadriceps wasting in patients with knee osteoarthritis [10] or other painful knee conditions [11,12]. Another potential use of US, could be to assess skeletal muscle atrophy for astronauts due to prolonged exposure to microgravity [30]. Indeed, such a longitudinal study is ongoing at the International Space Station (https://lsda.jsc.nasa.gov/Experiment/exper/13941), so the present results provide useful comparative data in terms of the expected consistency of quadriceps thickness throughout the one-year period of the project, involving middle-aged astronauts rather than the young and older age ranges that are typically included in ageing studies.

Longitudinal assessment of the lower-limb muscles could be potentially useful for research on athletes and in sports settings, monitoring effects of exercise interventions/training on quadriceps muscle mass and subcutaneous fat tissue, and helping to assess the type and dose of exercise that optimizes muscle hypertrophy and/or a decrease of subcutaneous fat.

Furthermore, the robustness of US imaging measurements over time can be valuable for longitudinally evaluating quadriceps thickness after knee arthroplasty, anterior cruciate ligament (ACL) reconstruction, and in general after surgical knee or hip procedures where muscle wasting may persist post-operatively [31].

A limitation of US muscle thickness measurements is the inability to measure the contribution of the fatty infiltration that occurs with ageing [21]. Likewise with some neuromuscular disorders, US thickness measurements need to be reported with caution, such as in Duchenne muscular dystrophy (DMD), which involves progressive atrophy of the skeletal muscles, together with a phenomenon known as pseudo hypertrophy, involving enlargement of muscles due to a replacement by connective tissue or fatty infiltration. In such cases, US imaging can be used to assess tissue quality, using specific techniques, such as grayscale analysis of echogenicity [32]. The technique has been used to assess diaphragm thickness in DMD patients with the potential to monitor disease progression [33].

The reliability of US imaging of muscle is well established [1] and the contribution of the present study was to demonstrate the robustness of US imaging measurements of the anterior thigh over a relatively longer period of time (two years), extending the use of ultrasound from research during observational cross-sectional studies to longitudinal studies.

Conclusions

The present findings indicate there was no statistically significant decline in muscle thickness or increase in anterior thigh subcutaneous fat tissue in a healthy cohort of middle-aged adults using US imaging over a two-year follow-up period. These novel findings also demonstrate the robustness of US measurements over time.

Ethical considerations The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle-Clinical Reports (von Haehling S, Ebner N, Morley JE, Coats AJS, Anker SD. Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle - Clinical Reports. J Cachexia Sarcopenia Muscle Clinical Reports 2016; 1;e28:1-2.). This study has been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed consent prior to their inclusion in the study.

Funding This research received no external funding.

Declaration of Conflict of interests The authors declare no conflict of interest.

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