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Title: **Anterior thigh composition measured using ultrasound imaging to quantify relative thickness of muscle and non-contractile tissue: a potential biomarker for musculoskeletal health**

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Short Title: Ultrasound measurement of thigh composition

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Abstract

This study aimed to use ultrasound imaging to provide objective data on the effects of ageing and gender on relative thickness of quadriceps muscle and non-contractile tissue thickness (subcutaneous fat, SF, combined with perimuscular fascia). In 136 healthy males and females (aged 18-90 years n=63 aged 18-35 years; n=73 aged 65-90). Images of the anterior thigh (dominant) were taken in relaxed supine using B-mode ultrasound imaging. Thickness of muscle, SF and perimuscular fascia were measured, and percentage thickness of total anterior thigh thickness calculated. Independent t-tests compared groups. Correlation between tissue thickness and BMI was examined using Pearson's coefficient. Muscle thickness was: 39±8mm in young males, 29±6mm in females, 25±4mm in older males and 20±5mm in females. Percentage muscle to thigh thickness was greater in young participants ($p=0.001$). Percentage SF and fascia was 17±6% in young and 26±8% in older males, 32±7% in young and 44±7% in older females. BMI was similar for age and correlated moderately with non-contractile tissue ($r=0.54$; $p<0.001$) and poorly with muscle ($r=-0.01$; $p=0.93$). In conclusion, this novel application of ultrasound imaging as a simple and rapid means of assessing thigh composition (relative thickness of muscle and non-contractile tissue) may help inform health status, e.g. in older people at risk of frailty and loss of mobility, and aid monitoring effects of weight loss or gain, deconditioning and exercise.

Key Words: Thigh composition; Ageing; Ultrasound imaging

List of Abbreviations

BMI- body mass index

MDC- minimum detectable change

MRI- magnetic resonance imaging

PASE- physical activity scale for the elderly

RF- Rectus femoris

RUSI- rehabilitative ultrasound imaging

SEM- standard error of measurement

SF- subcutaneous fat

USI- Ultrasound imaging

VI-Vastus Intermedius

Introduction

The need for accurate assessment of body composition was highlighted for various clinical conditions in a recent review by Wagner (2013). Assessment is important for several reasons, including to: identify health risk associated with excessively high or low body fat; monitor changes associated with certain diseases; aid weight loss or weight gain programs; assessing the effectiveness of nutrition and exercise interventions on muscle and fat contributions; and monitor the effects of deconditioning and age-related changes in body composition. The latter would be particularly important in frail older people at risk of losing mobility (Aaron et al. 2006). Measurement of body composition is also important in sport (Müller et al., 2013).

There are several ways of measuring body composition, which fall into two categories: laboratory and field based methods (Goodpaster, 2002; Lee & Gallagher, 2008; Wagner & Heyward, 1999). Laboratory methods include dual-energy X-ray absorptiometry (DXA), densitometry obtained from underwater weighing or air displacement plethysmography, hydrometry from isotope dilution, and *in vivo* measurement of the bone calcium/phosphorous (Ca/P) ratio (Fountos et al., 1999). Field methods, which are more portable, cost effective and require less skill include: skinfold thickness measurement, bioelectrical impedance (BIA), and anthropometric measurements including, weight height indices, such as body mass index (BMI). Field based measures can be less accurate than laboratory measures and their strengths and weaknesses have been discussed in reviews (Goodpaster, 2002; Lee & Gallagher, 2008; Wagner & Heyward, 1999). Ultrasound imaging (USI) can also be used to measure body composition but, as Wagner (2013) acknowledged in a review of USI to measure body fat, the technique has received relatively little attention for that purpose. Ultrasound provides a rapid, non-invasive, relatively inexpensive method for measuring body tissues, and is particularly useful for field settings. Wagner (2013) commented that USI has further advantages over other imaging devices and laboratory body composition techniques by having the ability to assess regional composition and allows for unique assessments of some clinical populations.

Muscle size is related to strength and the closeness of this relationship varies in different muscles but size is considered an indirect measure of strength i.e. force generating capacity (Ikai & Fukunaga, 1968). For example, quadriceps is an important muscle for mobility and protecting the knee joint from abnormal loading, so non-invasive clinical methods of measuring its functional capacity are needed to aid assessment in clinical and field settings. Quadriceps size is known to be closely related to strength in young and older females (Young et al 1984) and older males, although young males are stronger per unit area of muscle (Young et al 1985; Moore et al 2014).

There is a wealth of literature using USI reporting loss of muscle size with ageing (Ikezoe et al., 2011; Young et al., 1984 & 1985) and joint damage (Fukumoto et al., 2012; Staehli et al., 2010). More recently, USI is being promoted for measuring subcutaneous fat (Wagner, 2013). However, the relative proportions of muscle and fat have not been studied and may be a useful biomarker of body composition and health status

A recent study reported greater thickness in perimuscular fascia or connective tissue of the abdominal wall muscles in low back pain patients, which may have functional implications (Whittaker et al. 2013). This finding indicates the importance of considering non-contractile tissues when measuring muscle thickness. Intramuscular connective tissue is known to increase with ageing (Fukumoto et al., 2012; Tsubahara et al., 1995) but is difficult to quantify in routine practice, as it requires sophisticated software analysis programmes. Ultrasound imaging could be used to obtain thickness measurements of tissues to assess thigh composition but normative data are needed to characterise different groups in terms of age, gender and activity level. Once achieved, this would warrant further research to examine the responsiveness to change of such measures and then provide a potential means to assess changes in body composition, e.g. to assess health risk, aid weight management, monitor the effects of exercise programmes in clinical conditions and sport, and prevent frailty in older people at risk of losing independence. Thus the aim of the present investigation was to use ultrasound imaging as a rapid and simple technique to obtain normative data on the relative proportions of quadriceps muscle and non-contractile tissues (subcutaneous fat combined with perimuscular fascia) to total anterior thigh thickness in young and older healthy males and females.

Materials and Methods

Participants

A convenience sample of 136 self-reported healthy participants were studied, comprising 63 young participants (aged 18-35 years, mean 25.7 ± 4.8) recruited from the University, and 73 older participants (aged 65-90 years, 74.9 ± 5.9) recruited from the local community (Table 1). All participants gave their written informed consent. Young participants were included if they did not participate in sports or exercise more than three times per week, or competitively at university level or above. For older participants, activity was assessed using the Physical Activity Scale for the elderly (PASE) to ensure only sedentary or moderately active participants were included. Exclusion criteria for both age groups were: conditions known to affect muscle and function; lower limb pathology (fracture, surgery, neoplasm), skin disorder, neurological conditions and musculoskeletal injuries severe enough to require treatment or prevent activity for more than one week in the previous five years. Those taking medications, such as skeletal muscle relaxants, neuromuscular blocking drugs, and those unable to understand study requirements were excluded. The study was approved by the Faculty of Health Sciences, University of Southampton Ethics Committee and was conducted in accordance with the Helsinki Declaration of 1975. The data were collected as part of a larger study of non-invasive measures of muscle characteristics and motor function.

Table 1. Descriptive characteristics of participant groups

	Young males (n=36)	Young females (n=27)	Older males (n=30)	Older females (n=43)
Age (years)	24.9 ± 4.8	26.8 ± 4.6	74.1 ± 5.7	76.2 ± 6
Height (m)	1.7 ± 0.2	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Body Mass (kg)	55 ± 4.5	59 ± 7.9	65 ± 5.2	63.5 ± 7.6
BMI (kg/m^2) †	24.1 ± 4.1	23.2 ± 3.2	26.5 ± 3.5	27.1 ± 3.8*

† significant difference with ageing at 0.05 level (2tailed), *significant gender difference within age group at 0.05 level (2 tailed)

Ultrasound imaging

All imaging was performed by one operator (SA), an experienced physiotherapist trained in rehabilitative ultrasound imaging (RUSI), involving a one day introductory course (endorsed by the British Medical Ultrasound Society) followed by practising and mentoring prior to data collection. A real-time ultrasound scanner (Aquila; Esaote Spa, Genova, Italy) with a 6-MHz linear transducer array (60-mm footprint) was used to take B-mode transverse images of the anterior thigh of the dominant leg (assessed by kicking a ball). With the participant resting in supine lying (Figure 1), images were taken at a site two-thirds of the distance between the antero-superior iliac spine and the superior pole of the patella in the sagittal plane (Delaney et al. 2010).

Figure 1: The experimental set-up, with the participant lying supine and the ultrasound transducer placed over the anterior mid-thigh to obtain a transverse image trough the thigh.

A Matlab algorithm (written by co-author MW) was used to analyse the images offline. . Quadriceps thickness was defined as the distance between the inside edges of the rectus femoris (RF) and vastus intermedius (VI) muscle borders, to exclude perimuscular connective tissue (Whitaker et al. 2013). Thickness of the fascia was measured as the summed distances between the outside edges of the connective tissue layers superior to RF (superficial fascial layer) and between the RF and VI muscles (deep fascial layer). Subcutaneous fat thickness was the distance from the skin to the outside edge of the superficial fascial layer (Figure 2).

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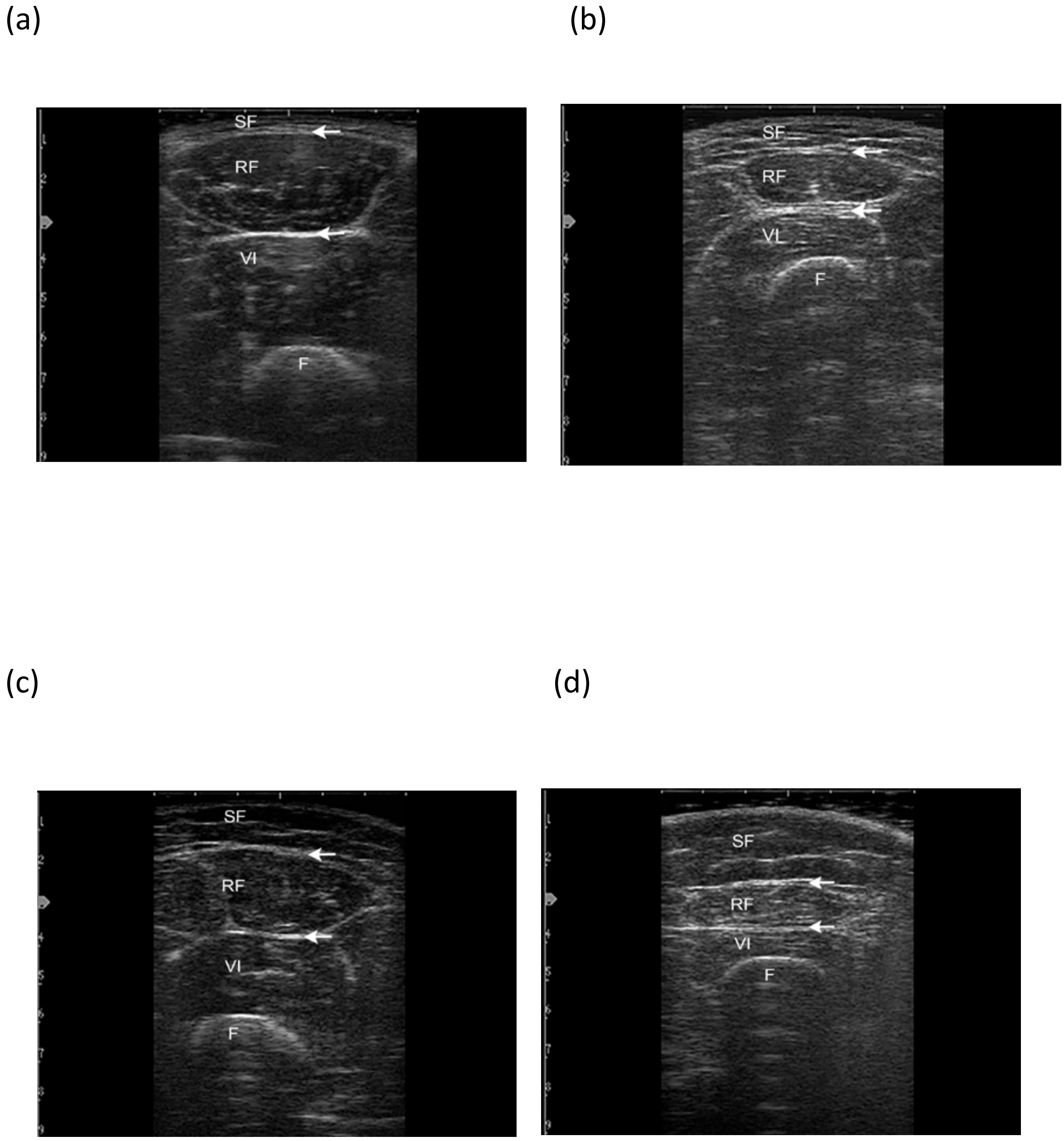

Figure 2

Example ultrasound scans from each group (a) young male, (b) older male, (c) young female, (d) older female, indicating tissues and landmarks: subcutaneous fat (SF), the quadriceps muscle layers (rectus femoris, RF; vastus intermedius. VI) and the femur (F). The superior and inferior perimuscular fasciae are indicated by the white arrows.

Reliability

A reliability study was conducted in 58 participants, to confirm reliability of the technique in the hands of the present investigator. Scans were taken on two days, one week apart.

Statistical Analysis

Data were imported from Microsoft Excel and analysed using SPSS 19 (SPSS Inc, Chicago, IL). The data were examined for normality using the Shapiro-Wilk test and found to be normally distributed. Descriptive statistics were used to summarise the data as means and standard deviations (SD). The mean values for each parameter (muscle, fascia and subcutaneous fat) thickness were calculated from the two images taken for each participant. Reliability of measurements repeated on a second day was examined using intraclass correlation coefficients (ICC 3,1) to assess agreement, using the classification of Fleiss (2007), in which ICC >0.75 is rated as excellent. Standard error of measurement (SEM) and minimal detectable change (MDC) were also used to assess precision of repeated measures. The percentage contributions of all three layers (muscle, and SF combined with fascia) to total thickness were calculated and also correlated with body mass index (BMI) using Pearson's correlation coefficient.

Results

Characteristics for participants are presented in Table 1. Young males and females had similar BMI (approximately 24 and 23 kg/m^2 respectively) and the older females had the highest BMI (27 kg/m^2) but the differences between groups were not significant ($p>0.05$).

Reliability of Measurements Repeated on Different Days

Reliability of muscle thickness measures was excellent, with ICC 3,1 of 0.89 (confidence intervals 0.78-0.95) in young participants (n=26) and 0.88 (0.77-0.94) in older participants (n=32). The SEM was 1.99mm and 2.11 in young and older participants respectively. The

MDC was 5.5 and 5.9 respectively. Subcutaneous thickness ICCs were 0.97 in both groups, with CIs of 0.93-0.98 and 0.94-0.99 respectively. The SEMs were 0.82 and 0.9, and MDCs were 2.28 and 2.49 respectively.

Relative contributions of contractile and non-contractile tissues to total thigh thickness

The total thickness of the anterior thigh was similar within age groups, approximately 45mm in the young group and 10mm smaller in the older groups (Tab 2). Thickness of SF and fascia was between 8-16mm, being smallest in young males (Tab 2), and the percentage contribution to total anterior thigh thickness is illustrated in Figure 3. Percentage thickness of fat in ascending order was young males, older males, young females then older females (Fig 3). Quadriceps thickness ranged from 20-39mm, being smallest in older females and greatest in young males (Tab 2). Despite similar total anterior thickness and BMI within each age group, the contributions of muscle thickness were significantly different (Tab 2). In the younger groups there was a 8% difference in total thigh thickness but a 26% difference in quadriceps size, which related to percentage contributions of muscle of 83±6% in males and 68±7 in females. In the older groups, a 3% difference in total thickness masked a 21% difference in quadriceps thickness, with contributions being 74±8% in males but only 56± 7% in females (Fig 3).

Table 2. Ultrasound thickness measurements of quadriceps muscle and non-contractile tissue (subcutaneous fat combined with perimuscular fascia)

	Young male (n=36)	Young female (n=27)	Older male (n=30)	Older female (n=43)
Total thickness (mm) †	46.4 ± 8.4	42.6 ± 7.6	34.8 ± 7.2	35.9 ± 8.3
Non-contractile tissue: subcutaneous fat and perimuscular fascia thickness (mm) †	7.8 ± 3. 4*	13.7 ± 4.2	9.4 ± 4.5*	15.8 ± 4.7
Percentage non-contractile tissue thickness †	17 ± 6*	32 ± 7	26 ± 8*	44 ± 7
SF thickness (mm) †	4.9 ± 3.5*	10.9 ± 4.2	7.1 ± 4.5*	13.8 ± 4.7
Percentage SF †	10 ± 6*	26 ± 7	19 ± 9*	38 ± 8
Muscle thickness (mm) †	38.7 ± 7.5*	28.8 ± 6.1	25.4 ± 4.4*	20 ± 5.2
Percentage muscle thickness †	83 ± 6*	68 ± 7	74 ± 8*	56 ± 7

† significant difference with ageing at 0.05 level (2 tailed), *significant gender difference within age group at 0.05 level (2 tailed); SF subcutaneous fat

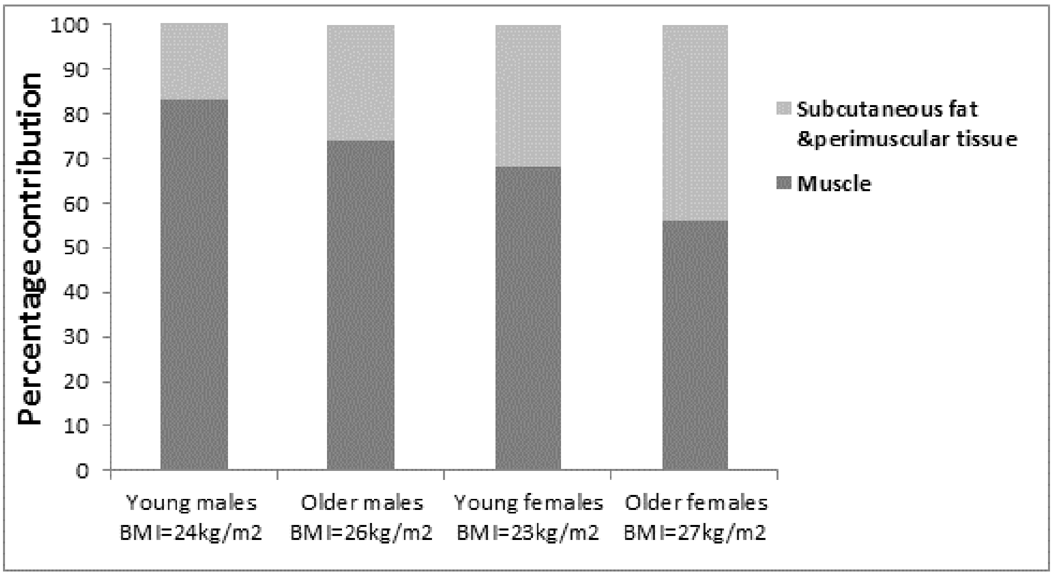

Figure 3: Percentage contribution of muscle and subcutaneous fat with perimuscular fascia thickness in healthy young and older participants. Note the gender difference in percentage contributions of muscle and non-contractile tissue to total anterior thigh thickness in young and older groups despite having similar values for body mass index.

Correlations of ultrasound parameters with BMI

There was a statistically significant ($p<0.001$) correlation ($r=0.54$) between BMI and subcutaneous fat combined with fascia thickness. There was no correlation between BMI and muscle thickness ($r=-0.01$; $p=0.93$) but there was a low correlation with total anterior thigh thickness ($r=0.31$; $p<0.001$).

Discussion

The contributions of resting thickness of quadriceps muscle and non-contractile tissues (subcutaneous fat combined with perimuscular), relative to total thigh thickness have been quantified for the first time using ultrasound imaging in healthy males and females of different ages. The present findings demonstrate a new application of ultrasound imaging to provide rapid assessment of thigh composition in field environments.

Quantified effects of ageing and gender on thigh tissue composition

Despite having similar BMI and total thigh thickness within each age group, thigh composition was significantly different between genders (Tab 2 and Fig 3). The findings therefore highlight that BMI can be deceiving, as the dramatic differences found in thigh composition between groups would be concealed if only BMI was considered. As expected, females had smaller muscles than males (Arts et al., 2010; Doherty, 2001) and older participants had smaller muscles (Aagaard et a, 2010; Goodpaster et al., 2006; Ikezoe et a., 2011; Young et al 1984 & 1985) and more fat (Fukumoto et al., 2012; Tsubahara et al., 1995) than younger participants. The age-related differences observed in the present study reflected those found in an MRI study of lower limb tissue compartment volume (Buford et al., 2012). The novelty of the present findings is providing objective data for the relative thickness of these tissues to total thigh thickness, providing an indicator of thigh composition, and possible biomarker for body composition.

Body composition can also be assessed using electrical impedance, by applying a painless electrical current to the skin (Zhu et al., 2005). Electrical impedance myography (EIM) for assessing the effects of normal ageing on muscle (Aaron et al. 2006) includes tissues other than muscle, such as subcutaneous fat and fascia, so is not as specific as ultrasound imaging for examining muscle.

Perimuscular fascia thickness was similar between groups but since measurements were only taken at one point in the thigh, this may not be representative of thickness around the muscle contour. However, the purpose of the present study was to use a measure that was fit for clinical purposes, i.e. rapid and easy to use, hence multiple measurements were not made to obtain a mean value.

When considering percentage thickness of subcutaneous fat without fascia, relative to young males, percentage thickness of fat was almost twice as much in older males, over two times thicker in young females and four times in older females (Table 2). Total anterior thigh thickness did not reflect relative proportions of muscle and non-contractile tissue. For example, relative to total anterior thigh thickness, young males had the greatest proportion of muscle and least proportion of non-contractile tissue than the other groups (Table 2). Also, total thickness in older males (mean 34.8mm) was smaller than in young females (42.6mm) but they had similar muscle thickness (25.4 and 28.8mm respectively), so therefore had leaner thighs i.e. greater proportion of muscle (74%) to total thickness than young females (68%). Conversely, the older males and females had similar total thickness (34.8mm and 35.9 mm respectively) but males had a much greater proportion of muscle (74%) than females (56%).

Young et al. (1980) used ultrasound imaging to demonstrate that a 5% difference in thigh circumference between the ipsilateral and contralateral sides measured with a tape measure could conceal a 22-33% difference in quadriceps cross-sectional area in patients with unilateral knee joint pathology. Similarities were seen with the present inconsistencies between smaller differences in total anterior thigh thickness between groups than muscle thickness, e.g. 8% vs 26% in young groups and 3% and 21% in older groups. Visual observation could be also misleading if thighs appear to be of similar size, such as in the present groups, where females and older groups had significantly less muscle and more subcutaneous fat. The clinical implication of these findings is that assessment of quadriceps atrophy to gain an understanding of functional capacity cannot rely on visual observation and either requires measurement of strength (which may not be possible) or muscle size from imaging.

The lack of correlation found in the present study between BMI and subcutaneous tissue thickness demonstrated that BMI underestimates the difference in subcutaneous fat contribution between groups, thus overestimating muscle content. These observations confirm reports about the poor sensitivity of BMI to assess body composition (Ackland et al., 2012). The fact that quadriceps thickness constituted 83% of total anterior thigh thickness in young males but only 56% in older females, illustrates the striking effects of age and gender on thigh composition. Research is needed to establish the critical threshold for quadriceps relative thickness that might be possible to use to assess when a frail older person is at risk of loss of mobility after a period of bed rest, which is known to accelerate sarcopenia (Coker & Wolfe, 2010).

Validity and reliability of ultrasound imaging to measure thigh tissue thickness

Real-time USI provides a rapid, non-intrusive, valid, reliable and relatively inexpensive method for measuring thigh tissue thickness in field environments outside the laboratory. Ultrasound imaging is being used increasingly in rehabilitation for measuring muscle size and providing visual biofeedback of muscle to aid re-education of contraction, and this application is termed rehabilitative ultrasound imaging (RUSI; see review by Whittaker & Stokes, 2011). Protocols for imaging various muscles have been developed and reliability of RUSI is generally good, although varies with muscles and experience of operators (Costa et al., 2009; Hebert et al., 2009), and was shown to be excellent by the present operator (ICC >0.88). The technique has been shown to be valid when compared with the gold standard of magnetic resonance imaging (MRI), e.g. vastus medialis (Worsley et al., 2014), multifidus (Hides et al., 1995), abdominal muscles (Hides et al., 2006), trapezius (O'Sullivan et al., 2009), masseter (Raadsheer et al 1994) and anterior hip muscles (Mendis et al., 2010). Ultrasound is being promoted to measure body fat and has been shown to be valid (Wagner,

2013) and more accurate than skinfold callipers for measuring subcutaneous fat (Muller et al 2013).

Limitations of the study

As well as subcutaneous fat, intramuscular fat is known to increase with ageing (Fukumoto et al., 2012; Tsubahara et al., 1995) but this factor is not accounted for in USI measures of muscle thickness, so the present data will overestimate the true amount of contractile tissue. Intramuscular fat cannot be measured rapidly using ultrasound and requires more sophisticated software.

The responsiveness to change of the relative tissue proportions was not examined in the present study, although the minimal detectable change (MDC) values reported from the reliability study indicate the differences needed to indicate a clinically meaningful change. Also, longitudinal studies have used USI successfully to monitor changes, e.g. in muscle thickness with exercise interventions (Koppenhaver et al., 2009); thicknesses of the preperitoneal and subcutaneous fat layers during pregnancy (Kinoshita & Itoh, 2006); and foetal thigh soft tissue to predict birth weight (O'Connor et al 2013).

Only a single site was measured, so results may not be representative of the whole thigh and, in turn, the thigh may not be representative of the whole body. However, Tanaka et al. (2012) found thigh fat thickness was correlated with whole body function. Multiple site measurements of fat thickness correlate with body composition (Wagner, 2013), so further sites in the thigh could be investigated to see how many are necessary to reflect body composition accurately. The thickness of the fascia, in particular, may vary within the thigh and a study is needed to determine the optimal number of measurements around the muscle contour. Furthermore, thigh composition alone is very relevant to mobility and assessing quadriceps function and effects of exercise programmes etc.

Potential uses of USI for measuring thigh composition and further research

Changes in relative fat and muscle thickness would provide important information for monitoring weight loss programmes to ensure preferential loss of fat without compromising muscle. Similarly, in managing weight gain, USI could help to ensure muscle and not subcutaneous fat is increased. Assessment of muscle atrophy in, e.g. joint disease or injury, deconditioning and sarcopenia with ageing could be aided by USI measurements of thigh composition to assess quadriceps functional capacity. The technique could monitor the effects of nutrition and exercise interventions on thigh composition.

It is well recognised that strength measurements are not always appropriate or possible, e.g. when pain is present, post-operatively or when a patient is unconscious in intensive care (Myers et al., 2013). Ultrasound therefore offers an accurate and reliable measure that does not require voluntary contraction to allow assessment of contractile capability or muscle health (Aaron et al. 2006).

Measurement of body composition is important in sports for optimising health and performance. Fasting in elite sport is common, e.g. to achieve weight limits for competition or improve performance which can involve rapid weight loss that may compromise health (Maughan, 2010). In ski jumping, for instance, BMI is used as a cut-off measure for applying penalties (shorter skis) to athletes whose BMI is considered too low (Müller, 2009), despite BMI not being a measure of body fat and only a very rough measure of 'relative weight' (Ackland et al., 2012). The International Olympic Committee (IOC) Working Group on Body Composition, Health and Performance has emphasised the need for improved body composition measurement techniques that are suitable for use in the field and provide sufficiently accurate and reliable data (Ackland et al., 2012; Meyer et al., 2013), and USI is being recommended to measure subcutaneous fat in sport (Müller et al., 2013). Investigation of relative thickness of muscle and fat may prove to be more informative than fat. There is a similar need to monitor the effect of weightlessness in astronauts during space flight and in research to develop effective countermeasures to aid recovery of atrophy and function to prevent musculoskeletal problems (Sayson et al., 2013). The relatively simple, economic and portable USI technique would be suitable for epidemiological studies of thigh composition in healthy cohorts in the general population and clinical groups in community settings.

The present findings warrant further research, e.g. to determine the relationship between thigh composition and whole body composition; to quantify thigh composition, perhaps establishing an index, in different healthy, clinical and sports cohorts. For example, does a more active lifestyle in older age produce a different profile in thigh composition to sedentary people that could be used to assess health status and be predictive of mobility and independence? The effects of ageing and pathology on perimuscular fascia, and the functional implications also require further investigation.

Conclusions

A relatively simple application of ultrasound imaging has provided reference data on the relative thickness of quadriceps muscle and non-contractile tissue (subcutaneous fat and perimuscular fascia), producing characteristic profiles for males and females of different ages. The present findings warrant further investigation to determine the utility of this ultrasound technique to assess thigh composition in field environments as a research tool, and for clinical and sports settings.

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References

Aagaard P, Suetta C, Caserotti P, Magnusson SP, Kjaer M. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. Scand J Med Sci Sport. 2010;20(1):49–64.

Aaron R, Esper GJ, Shiffman CA, Bradonjic K, Lee KS, Rutkove SB. Effects of age on muscle as measured by electrical impedance myography. Physiol Meas. 2006 Oct;27(10):953-959.

Ackland TR, Lohman TG, Sundgot-Borgen J, et al. Current status of body composition assessment in sport: review and position statement on behalf of the Ad Hoc Research Working Group on Body Composition Health and Performance, under the auspices of the I.O.C. Medical Commission. Sports Med 2012;42:227–249.

Arts IM, Pillen S, Schelhaas HJ, Overeem S, Zwarts MJ. Normal values for quantitative muscle ultrasonography in adults. Muscle Nerve 2010;41:32–41.

Buford T, Lott D, Marzetti E, Wohlgemuth S, Vandenborne K, Pahor M, Leeuwenburgh C and Manini T Age-related differences in lower extremity tissue compartments and associations with physical function in older adults. Experimental Gerontology 2012;47(1): 38-44.

Costa LOP, Maher CG, Latimer J, Smeets RJEM. Reproducibility of rehabilitative ultrasound imaging for the measurement of abdominal muscle activity: a systematic review. Phys Ther 2009;89(8):756–769.

Delaney S, Worsley P, Warner M, Taylor M, Stokes M. Assessing contractile ability of the quadriceps muscle using ultrasound imaging Muscle Nerve 2010;42:530–538

Doherty TJ. The influence of aging and sex on skeletal muscle mass and strength. Curr Opin Clin Nutr Metab Care. 2001;4(6):503-508.

Fleiss J (2007). Reliability of measurement In The Design and Analysis of Clinical Experiments. New York: Wiley and Sons 1-33

Fountos G, Tzaphlidou M, Kounadi E, Glaros D. In vivo measurement of radius calcium/phosphorus ratio by X-ray absorptiometry. Appl Radiat Isot. 1999;51(3):273-278.

Fukumoto Y, Ikezoe T, Tateuchi H, Tsukagoshi R, Akiyama H, So K, et al. Muscle mass and composition of the hip, thigh and abdominal muscles in women with and without hip osteoarthritis. Ultrasound Med Biol [Internet]. 2012;38(9):1540–1435.

Goodpaster BH. Measuring body fat distribution and content in humans. Curr Opin Clin Nutr Metab Care 2002; 5(5):481–487.

Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, Simonsick EM, Tylavsky FA, Visser M, Newman AB. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci. 2006;61(10):1059-1064.

Hebert JJ, Koppenhaver SL, Parent EC, Fritz JM. A systematic review of the reliability of rehabilitative ultrasound imaging for the quantitative assessment of the abdominal and lumbar trunk muscles. Spine 2009;34(23):E848–E856.

Hides JA, Richardson CA, Jull GA. Magnetic resonance imaging and ultrasonography of the lumbar multifidus muscle. Comparison of two different modalities. Spine 1995;20:54-58.

Hides J, Wilson S, Stanton W, et al. An MRI investigation into the function of the transversus abdominis muscle during “drawing-in” of the abdominal wall. Spine. 2006;31:E175-178.

Ikezoe T, Mori N, Nakamura M, Ichihashi N. Atrophy of the lower limbs in elderly women: is it related to walking ability? Eur. J. Appl. Physiol. [Internet]. 2011;111(6):989–995.

Ikai M, Fukunaga T. Calculation of muscle strength per unit cross-sectional area of human muscle by means of ultrasonic measurement. Int Z Angew Physiol. 1968;26:26-32

Kinoshita T, Itoh M. Longitudinal variance of fat mass deposition during pregnancy evaluated by ultrasonography: the ratio of visceral fat to subcutaneous fat in the abdomen. Gynecol Obstetr Invest 2006;61(2):115–118.

Koppenhaver SL, Hebert JJ, Parent EC, et al. Rehabilitative ultrasound imaging is a valid measure of trunk muscle size and activation during most isometric sub-maximal contractions: a systematic review. Aust J Physiother 2009;55:153–169.

Lee SY, Gallagher D. Assessment methods in human body composition. Curr Opin Clin Nutr Metab Care 2008; 11(5): 566–572.

Maughan RJ. Fasting and sport: an introduction. Br J Sports Med 2010;44(7):473-375.

Mendis MD, Wilson SJ, Stanton W, Hides JA. Validity of real-time ultrasound imaging to measure anterior hip muscle size: a comparison with magnetic resonance imaging. J Orthop Sport. Phys Ther 2010;40(9):577–581.

Meyer NL, Sundgot-Borgen J, Lohman TG, et al. Body composition for health and performance: a survey of body composition assessment practice carried out by the Ad Hoc Research Working Group on Body Composition, Health and Performance under the auspices of the IOC Medical Commission. Br J Sport Med 2013;47:1012–1022.

Moore AZ , Caturegli G, Metter EJ, Makrogiannis S, Resnick SM, Harris TB, Ferrucci L. Difference in Muscle Quality over the Adult Life Span and Biological Correlates in the Baltimore Longitudinal Study of Aging. JAGS 2014; 62:230–236

Müller W. Towards research based approaches for solving body composition problems in sports: ski jumping as a heuristic example. Brit J Sport Med 2009;43:1013–19.

Müller W, Horn M, Fürhapter-Rieger A, Kainz P, Kröpfl JM, Maughan RJ, Ahammer H. Body composition in sport: a comparison of a novel ultrasound imaging technique to measure subcutaneous fat tissue compared with skinfold measurement. Br J Sports Med 2013; 47: 1028-1035.

Myers H, Davis A, Lazicki R, Martinez C, Black D, Butler RJ. Sex differences in rectus femoris morphology across different knee flexion positions. Int J Sports Phys Ther 2013; 8(2):84-90.

O'Connor C, Farah N, O'Higgins A, Segurado R, Fitzpatrick C, Turner MJ, Stuart B, Kennelly MM. Longitudinal measurement of fetal thigh soft tissue parameters and its role in the prediction of birth weight. Prenat Diagn. 2013;33(10):945-951

O’Sullivan C, Meaney J, Boyle G, Gormley J, Stokes M. The validity of Rehabilitative Ultrasound Imaging for measurement of trapezius muscle thickness. Man Ther 2009;14(5):572–578.

Raadsheer MC, Van Eijden TM, Van Spronsen PH, Van Ginkel FC, Kiliaridis S, Prahl-Andersen B. A comparison of human masseter muscle thickness measured by ultrasonography and magnetic resonance imaging. Arch. Oral Biol. 1994;39:1079-1084.

Sayson JV, Lotz J, Parazynski S, Hargens AR. Back pain in space and post-flight spine injury: Mechanisms and countermeasure development. Acta Astronautica 2013;86:24–38.

Staehli S, Glatthorn JF, Casartelli N, Maffiuletti NA. Test-retest reliability of quadriceps muscle function outcomes in patients with knee osteoarthritis. J Electromyogr Kinesiol. 2010;20(6):1058-1065.

Tanaka S, Goto K, Yamamoto S, Arai A. Relationship between peak VO2 and subcutaneous fat thickness of the thigh measured by ultrasonography. Osaka City Med J 2012; 58(2):51-58.

Tsubahara A, Chino N, Akaboshi K, Okajima Y, Takahashi H. Age-related changes of water and fat content in muscles estimated by magnetic resonance (MR) imaging. Disabil. Rehabil.1995;17(6):298–304.

Wagner DR. Ultrasound as a tool to assess body fat. J Obesity 2013; Volume 2013: Article ID 280713, 9 pageshttp://dx.doi.org/10.1155/2013/280713

Wagner DR, Heyward VH, Techniques of body composition assessment: a review of laboratory and field methods. Res Quart Ex Sport 1999; 70(2):135–149.

Whittaker J, Stokes M. Ultrasound imaging and muscle function. (Review) J Orthop Sports Phys Ther 2011;41(8):572-580.

Whittaker J, Warner M, Stokes M. Comparison of the sonographic features of the abdominal wall muscles and connective tissues in individuals with and without lumbopelvic pain. J Orthop Sports Phys Ther 2013; 43(1):11-19.

Worsley P, Kitsell F, Samuel D, Stokes M. Validity of measuring distal vastus medialis muscle using rehabilitative ultrasound imaging versus magnetic resonance imaging. Manual Therapy. 2014; 19: 259-263.

Young A, Hughes I, Russell P, Parkers MJ, Nichols PJ. Measurement of quadriceps muscle wasting by ultrasonography. *Rheumatol Rehabil* 1980;19:141-148.

Young A, Stokes M, Crowe M. The size and strength of the quadriceps muscles of old and young women. Eur J Clin Investig 1984;14:282 287.

Young A, Stokes M, Crowe M. The size and strength of the quadriceps muscles of old and young men. Clin Physiol 1985;5:145 154.

Zhu F, Leonard EF, Levin NW. Body composition modeling in the calf using an equivalent circuit model of multi-frequency bioimpedance analysis. Physiol Meas 2005;26(2): S133-143.