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Research article

Ultrasound Determined Muscle Quality is Associated with Neuromuscular Fatigue and Mobility in Older Adults-A Pilot Study

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Abstract

The purpose of this study was to examine the relationships of ultrasound-derived muscle quality with the onset of neuromuscular fatigue (NMF) and functional mobility in older adults. Fifteen older men and women (age: 70.7±7.3y; BMI: 27.3+5.6 kg.m^{-2}) volunteered for this study. Cross-sectional area (CSA) and muscle thickness (MT) of the vastus lateralis (VL) were determined from ultrasound imaging, and echo intensity (EI) was determined by grayscale analysis using the standard histogram function in ImageJ. NMF was determined during a discontinuous incremental cycle ergometer test. Functional mobility was assessed using the get-up-and-go test (GUG). Data were evaluated using Pearson correlation coefficients, partial correlations, and stepwise regression analyses. Significant correlations ($p<0.05$) were observed between EI and GUG (r=0.62; $p<0.05$) and NMF (r= -0.68; $p\leq0.01$). After controlling for age and BMI, significant correlations remained between EI and GUG (r=0.69, $p\leq0.01$) and NMF (r= -0.66; $p<0.05$). Stepwise regression indicated EI to be the single best predictor of NMF (R=0.67, SEE=22.0 watts, $p<0.01$), and EI and age were the best predictors of GUG (R=0.86, SEE=1.3 seconds, $p<0.001$). The findings of the present study indicated that muscle quality (EI) of the vastus lateralis was related to the onset of NMF and functional mobility, independent of age and BMI, in this sample of older men and women. In addition, it appears that muscle quality, not quantity (CSA, MT), was the strongest predictor of functional mobility and neuromuscular fatigue.

Keywords: Ultrasound; Echo Intensity; Neuromuscular Fatigue; Functional mobility; Older Adults

Abbreviations

NMF: Neuromuscular Fatigue;
CSA: Cross-Sectional Area;
MT: Muscle Thickness;
EI: Echo Intensity;
GUG: Get-Up-And-Go Test;
IMAT: Intramuscular Adipose Tissue;
PWCFT: Physical Working Capacity at Fatigue Threshold;
VL: Vastus Lateralis;
MQ: Muscle Quality;
RMS: Root Mean Square

Introduction

The loss of muscle mass and function observed with aging, termed sarcopenia, has been shown to be associated with deficits in muscular strength and functional mobility [1-3]. However, it has been suggested that other factors, such as muscle quality, may provide an important component to muscle health with regard to aging [4-6]. Recent evidence suggests that even without a decrease in muscle mass related to aging, alterations to the composition of skeletal muscle, such as an increase in intramuscular adipose tissue (IMAT), may be a confounding factor in muscle function in older adults [3,5]. Past research examining changes to muscle morphology has relied on either expensive (e.g., MRI and CT scans), or invasive methods (e.g., muscle biopsy), when investigating the effects of sarcopenia in older individuals [7-12]. Alternatively, the use of echo intensity (EI) to determine skeletal muscle composition is an emerging approach which provides an assessment of intramuscular infiltration of non-contractile tissues [1,3,13-17]. This inexpensive and non-invasive method to assess muscle quality in older adults may provide an additional screening tool to help assess the risk of sarcopenia.

Functional mobility has been defined as the balance, gait speed and maneuverability of an individual, and has been used to assess older adults' ability to perform activities of daily living (ADLs) [10,18-20]. White and colleagues [19] recently demonstrated that in previously healthy older adults, individuals who experience a more marked decrease in functional mobility experience a higher rate of mortality. Furthermore, a study by Nikolaus and colleagues [21] demonstrated that the get-up-and-go (GUG) test, a common assessment of functional mobility, was one of the best predictors of mortality in older adults. With sarcopenia, the ability to perform ADLs decreases which may increase the probability of falls [22].

Compounding the effect on strength and mobility, the age related loss of muscle function has also been associated with a decrease in resistance to fatigue, which may lead to a deterioration in motor coordination and result in greater risk of falls in older adults [23,24]. Previous investigators have suggested that most ADLs consist of a series of submaximal activities in which continued independence for older adults is dependent upon their ability to delay fatigue [25,26]. The physical working capacity at fatigue threshold (PWC_{FT}) is a non-invasive, sub-maximal test that is a valuable alternative to measures, which require a maximal effort (i.e. VO_2max) for older adults [23,27-29]. In addition, a recent study has shown that PWCFT in older adults demonstrates an association with both functional ability and sarcopenia-related changes to body composition in older adults [27]. However, the relationship between the PWC_{FT}, the estimated onset of neuromuscular fatigue (NMF), and muscle quality, as determined by ultrasound-derived EI, has yet to be examined.

As previous research has demonstrated, ultrasound may offer a diagnostic tool sensitive enough to provide an alternative to the more costly CT and MRI imaging when examining muscle morphology [14,30]. To the best of our knowledge, no studies have examined the relationship between skeletal muscle quality and the onset of neuromuscular fatigue in older adults. It is therefore the aim of the current study to examine the efficacy of the use of ultrasound, PWCFT and GUG to determine relationships among neuromuscular fatigue, functional mobility and muscle quality in healthy older adults.

Methods

Participants

Fifteen older, but healthy, men and women (Table 1) volunteered to participate in this study. All participants provided written informed consent and all procedures involving human subjects in this study were approved by the Institutional Review Board at the University of Central Florida.

	Mean ± SD	Range
Physical Characteristics		
Age (years)	70.73 ± 7.27	61-85
BMI	27.26 ± 5.62	20.49-39.00
Ultrasound Measures		
EI (au)	91.87 ± 10.02	74.1-108.8
CSA (cm^2)	14.67 ± 4.63	8.87-25.10
MT (cm)	1.41 ± 0.45	0.80-2.00
Neuromuscular Fatigue		
PWC_{FT} (W)	65 ± 26.66	35-125
Mobility Measure		
GUG (sec)	8.92 ± 2.17	6.70-15.33

Table 1. Physical characteristics, ultrasound measures, neuromuscular fatigue, and mobility of participants (*n*=15).

Ultrasound measurement

Participants were asked not to perform vigorous exercise 72 h prior to image collections. In addition, a rest period of 15 minutes was required immediately prior to the scan to allow fluid shifts to occur [1]. To capture images of the vastus lateralis (VL) muscle in the right leg, the participant lied supine on an examination table, allowing for a 10º bend in the non-dominant knee as measured by a goniometer and with toes angled approximately 45 degrees in relation to the frontal plane. A 12 MHz linear probe scanning head (General Electric LOGIQ P5, Wauwatosa, WI, USA) with a gain of 50dB and a dynamic range of 72 was used to optimize spatial resolution [31]. The probe was coated with water soluble transmission gel (Aquasonic 100 ultrasound transmission gel, Parker Laboratories, Inc. Fairfield, New Jersey) and positioned on the surface of the skin to provide acoustic contact without depressing the dermal layer to collect the image. VL was measured at 50% of the distance from the most prominent point of the greater trochanter to lateral condyle (Abe et al., 1998). For echo intensity (EI), muscle thickness (MT), and cross-sectional area (CSA), the probe was held perpendicular to the axis of the muscle. Three consecutive images were taken to analyze EI, CSA and MT. The same investigator performed all ultrasound measurements. Test-retest reliability for the ultrasound technician measures was determined from 10 participants measured at least 1 day apart. The intraclass correlation coefficient (ICC) of the ultrasound technician for EI was 0.93 (SEM = 5.1 au), for CSA ICC was 0.99 (SEM=1.26 cm^2), and for MT, ICC was 0.89 (SEM= 0.12 cm).

Echo intensity of the VL was determined by grayscale analysis using the standard histogram function in ImageJ [1]. Using the manual tracking tool, a region of interest for the VL was selected containing as much muscular tissue as possible, excluding the fascia. Echo intensities in the region of interest are expressed as values between 0-255 (0: black; 255: white); where a higher score indicates an increase in EI or muscle quality (MQ), see Figure 1. Muscle thickness of the VL was measured in ImageJ using a digital caliper at the site of the muscle image's greatest diameter. The cross sectional area scans were taken by a sweep in LV (logiq view) mode, medial to lateral to obtain the entire muscle, transverse to the muscle tissue interface. Mean EI, MT, and CSA were calculated from the average of three images. VL measures were used to examine muscle morphology in an area localized to EMG signal acquisition.

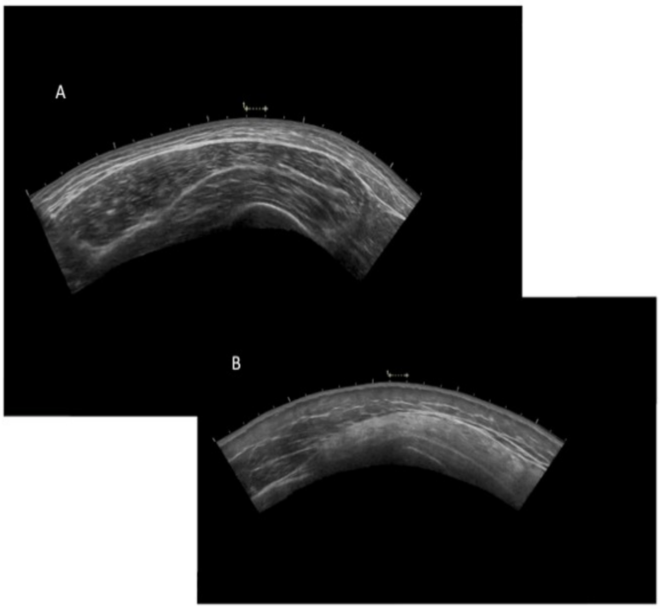

Figure 1. Higher quality muscle representedvia ultrasound(A)and lower quality muscle(B). Higher quality muscle demonstrates as a lower score on histograph analysis.

Electromyography (EMG) measurements

A bipolar (4.6 cm center-to-center) surface electrode (Quinton Quick-Prep silver-silver chloride) arrangement was placed over the VL muscle of the right leg at 60% of the distance from the lateral portion of the patella and the greater trochanter. A reference electrode was placed at the lateral epicondyle of the distal femur. Inter-electrode impedance was kept below 5,000 ohms with abrasion of the skin beneath the electrode. Raw EMG signals were pre-amplified through a differential amplifier (MP150 BIOPAC Systems, Inc., Santa Barbara, CA), sampled at 2,000 Hz. All EMG signals were saved to a personal computer (Dell Latitude E6530, Dell Inc., Round Rock, TX) for later off-line analysis. The EMG signals were expressed as root mean square (RMS) amplitude values (μVrms) by software (AcqKnowledge v4.2, BIOPAC Systems, Inc., Santa Barbara, CA).

Determination of Neuromuscular Fatigue

de Vries and colleagues [26] previously described in detail the procedures for determining PWCFT for the VL using the discontinuous protocol. In summary, testing was performed on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) with all participants using toe clips. Participants first performed a warm up with a work rate set at 30 watts and the participant pedaling at 50 rpm. The first stage of the test was performed at a work rate of 30W. During this stage, and all subsequent stages, pedaling cadence remained consistent at 50 rpm. Each stage of the discontinuous test lasted two-minutes. Following each stage, the EMG signal was analyzed utilizing a custom-written software (LabView, National Instruments Corporation, Austin, TX). When a stage did not produce a statistically significant, positive slope for the relationship between RMS and time ($p < 0.05$), an increase in work rate of 20 watts was implemented for the subsequent stage. Once a statistically significant, positive slope was reached, one final stage was performed at 10 watts less than the resistance that produced the statistically significant, positive slope. If a stage resulted in the participant achieving 75% of their age-predicted maximal heart rate, or surpassing a rating of perceived exertion (RPE, Borg scale) of 13 the test was halted. The PWC_{FT} was estimated to be the mean power output of the highest non-statistically significant positive slope and the lowest statistically significant positive slope. Test-retest reliability for the PWCFT test was determined from 7 participants measured 6 weeks apart. The ICC was 0.989 (SEM = 3.87 W). No significant difference ($p>0.05$) was noted between the mean PWC_{FT} values from trial 1 (76.7 ± 35.4 W) to trial 2 (71.7 ± 38.8 W).

Mobility Measurement

For this test, participants were asked to perform a modified version of the timed get up and go test described by Podsiadlo[20]. Individuals were required to stand from a seated position, without using their arms to push off, walk ten feet turn, return to the chair and sit. Time to complete the task was measured in seconds. The ICC for GUG was 0.81 (SEM = 0.41 s).

Statistical Analysis

Descriptive statistics and measurement results are reported as mean ± SD. Pearson's product moment correlation coefficients were calculated to assess the relationship between EI, CSA, MT, PWC_{FT}, age, BMI and GUG. Partial correlations were employed to investigate the association of EI and GUG and between EI and PWCFT when age and BMI were used as controlling variables. To determine the variables (EI, MT, CSA, Age, BMI) with the highest predictive value for PWCFT and

GUG a stepwise regression analyses were performed. Data were analyzed using SPSS version 20 software (IBM Corp., Armonk, NY). Prior to all statistical analyses, the alpha level was set to p≤0.05 to determine statistical significance.

Results

The participant descriptive characteristics, ultrasound measures (EI, MT, CSA), PWC_{FT} and GUG values are presented in Table 1. In addition, correlation coefficients between EI, MT, CSA, PWC_{FT}, age, BMI and GUG values are presented in Table 2. EI demonstrated significant relationships with PWC_{FT} (p=0.008) and GUG (p=0.0.018) while CSA revealed a significant association to PWC_{FT} (p= 0.011) and BMI (p= 0.036). MT displayed a significant correlation only with BMI (p= 0.032).

	EI	CSA	MT	PWC_{FT}	Age	BMI	GUG
EI	-	-0.44	-0.30	-0.68**	0.21	0.02	0.62*
CSA		-	0.25	0.64*	-0.29	0.55*	0.05
MT			-	-0.07	0.11	0.57*	0.05
PWCFT				-	-0.36	0.04	-0.45
Age					-	0.07	0.72**
BMI						-	0.13
GUG							-

Statistical Significance: *p < 0.05, **p < 0.01

Table 2. Correlation coefficients between ultrasound measures, neuromuscular fatigue, physical characteristics, and mobility of the participants (n=15).

EI was not significantly related to CSA, MT, age, or BMI. CSA was not significantly correlated to MT, age, or GUG. MT was not significantly correlated with PWCFT, age, or GUG. PWCFT demonstrated no significant correlation to age, BMI or GUG. Age showed a significant, positive correlation to GUG (p=0.003) only.

Table 3 shows the partial correlation coefficients between EI, PWC_{FT} and GUG when controlling for age and BMI. A significant partial correlation exists between EI compared to PWCFT and GUG.

Stepwise regression analysis (Table 3) indicated EI was the single best predictor of PWCFT (R=0.67, SEE=22.0 W, p<0.01). Additionally, EI and age were identified as the best predictors of GUG (R=0.86, SEE=1.3 s, p<0.001).

Dependent variables	Independent variables	Coefficient	Standardized coefficient	t value	p value	95% Confidence interval	
						Lower	Upper
PWC_{FT}	EI	-1.87	-0.67	4.2	0.002	-3.2	-0.05
R^2 = 0.45							
GUG	Age	0.19	0.62	3.7	0.004	0.08	0.31
R^2 = 0.74	EI	0.10	0.46	2.8	0.02	0.02	0.19

Table 3. Factors associated with mobility and Neuromuscular Fatigue .

Discussion

The unique findings in the present study were that MQ as determined by EI of the VL was related to the onset of NMF and functional mobility, independent of age and BMI in this sample of older men and women. In addition, it appears that MQ, not quantity (CSA, MT), was the strongest predictor of functional mobility and NMF.

EI has been shown to be a valid determinant of MQ (see Figure 1) in a variety of neuromuscular disorders [14,32,33]. The loss of muscle with aging (sarcopenia) has been associated with an increase in intramuscular fat and connective tissue which have been related to an increase in EI indicating a lower MQ [16,34,35]. In support, recent studies have reported EI values to be significantly correlated to cardiovascular performance (WVT1r=−0.46, p=0.013; WVT2 r=−0.50, p=0.009) and muscle strength (range from r=−0.48 to r=−0.64; p<0.05; r=-0.40; p<0.01) in older men and women [1,3].

In addition to age related increase in EI, or decrease in MQ, changes in muscle architecture may also result in a decrease in neuromuscular function [25,36]. Cadore and colleagues [1] suggested that the decrease in MQ as a result of increased intramuscular connective tissues may also negatively affect cardiovascular function due to impaired blood supply to muscle tissue. It has been hypothesized that increased infiltration of non-contractile tissue with ageing is associated with a decrease in capillary density that may adversely affect blood supply to skeletal muscles [1,37]. If true, this would affect oxygen supply to working muscles and fatigue would ensue at a much lower exercise intensity. In support, Cadore and colleagues [1] reported that the decrease in rectus femoris MQ was associated with a decrease in workload at ventilatory threshold (VT). The VT value is used as a physiological measure to evaluate cardiorespiratory fitness and may be a predictor of cardiovascular morbidity and mortality [38,39].

The current study demonstrates a significant relationship (Table 2) between the onset of NMF, as measured by PWCFT, and MQ. In addition, stepwise regression indicated that MQ, not muscle quantity, was the best predictor of the onset of NMF (Table 3). Although a small sample size was utilized in this pilot study, the validity of the cycle ergometry submaximal PWC_{FT} test to determine the onset of NMF value in older men and women has been established in previous studies[23,29]. Previous research has reported strong relationships between VT, onset of blood lactate accumulation, and PWCFT in younger men and women indicating that both tests are reflective of cardiorespiratory fitness [26,40,41]. In support of Cadore and colleagues [1], our data suggest a strong relationship (r=-0.68; p<0.01) between MQ and cardiorespiratory fitness, as measured by the PWCFT test.

The current study also demonstrated a significant correlation between EI and functional mobility (GUG) in older adults (r=0.62; p<0.05). Furthermore, stepwise regression revealed that age and EI were the best predictors of GUG (see Table 3) in this population. These findings support the normative data findings for the GUG that suggest age, as opposed to other factors (i.e. gender) provides a more valuable reference for mobility determined via GUG[42]. A recent study by Marcus and colleagues [5] demonstrated that functional mobility in older adults was associated with intramuscular adipose tissue (IMAT). Previous research has shown that an increase in intramuscular fat as assessed via MRI is associated with a decrease in MQ[10]. Lang and colleagues [43] also reported that increased IMAT of thigh muscle was associated with increased risk of hip fracture in older men and women of both Caucasian and African American decent. Although ultrasound measures appear to be a useful approach to examine MQ, the inability to assess the type of tissue (IMAT, connective) does pose a potential limitation. A further limitation in this study includes the small sample size, which acts as a detriment to the statistical power of the analysis performed. To correct for this, future studies developed from this pilot study should include a larger population.

Conclusion

Our results from this pilot study support previous findings, which suggest ultrasound provides a viable, inexpensive diagnostic tool to analyze muscle quality of the vastus lateralis [44]. Also, this study suggests a possible relationship between an ultrasound-derived measure of MQ and a suggested marker of mortality, functional mobility in older adults that further research with a larger sample should investigate. An expanded research study on the use of ultrasound to evaluate skeletal muscle EI may result in noninvasive methodology to examine interventions that affect MQ, NMF and functional mobility.

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