The Relationship between Lean Mass and Contractile Properties of the Quadriceps in Elderly and Young Adults

Anett Mau-Moeller^{a, b} Sven Bruhn^b Rainer Bader^a Martin Behrens^b

^aDepartment of Orthopedics, University Medicine Rostock, and ^bDepartment of Exercise Science, University of Rostock, Rostock, Germany

Key Words

Muscle mass · Aging · Isometric twitch · Electrical stimulation · Force summation

Abstract

Background: Aging is associated with a loss of muscle mass (sarcopenia) and function. The twitch torque evoked by supramaximal electrical stimulation of peripheral nerves has been frequently used to analyse age-related modulations at the skeletal muscle level, such as changes in muscle mass. However, only one study has investigated the association between twitch contractile properties and skeletal muscle mass. A significant positive correlation between cross-sectional area and twitch parameters was found for the plantar flexors in young adults when using supramaximal doublet stimulation. It remains unclear whether this relationship exists for the quadriceps in elderly and young subjects when using single and doublet stimulation. **Objective:** The aim of the present study was to investigate the relationship between the lean mass of the thigh and evoked twitch properties of the quadriceps using single and doublet stimulation in two age groups. **Methods:** Fifteen young (aged 25.3 ± 3.6 years) and 15 elderly (aged 69.6 ± 3.1 years) subjects were recruited to participate in this study. The lean mass of the thigh was measured by dual-energy X-ray absorptiometry. Supramaximal single and doublet electrical stimulation was

used to assess the contractile properties of the quadriceps. **Results:** We observed no significant associations between lean mass and contractile properties when using single stimulation. Significant positive correlations were shown between lean mass and peak twitch torque evoked by doublet stimulation in young ($r = 0.56$; $p = 0.030$) and elderly ($r = 0.54$; $p = 0.040$) subjects. The analysis of twitch time and slope parameters demonstrated no significant correlations with lean mass. **Conclusion:** The peak twitch torque evoked by doublet electrical stimulation seems to be an appropriate measure to assess modulations in muscle mass in elderly and young subjects. The use of supramaximal single stimulation and the analysis of time and slope parameters may not be recommended for estimating changes in muscle mass. Consequently, the occurrence of muscle mass loss with aging can be identified from the twitch torque signal induced by doublet stimulation, which is a simple and favorable way to estimate sarcopenia.

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Introduction

Aging is associated with alterations at the skeletal muscle level [1, 2], which have frequently been evaluated by examining the twitch torque signal evoked by supramaximal electrical stimulation of peripheral nerves [1, 2]. The

twitch torque signal has generally been analysed regarding the highest value of the twitch torque (peak twitch torque; PTT), the average and highest rise of the torque-time curve (mean and maximal rate of torque development; RTD) and the time from onset to PTT (twitch contraction time; TCT). Furthermore, the average and peak fall rate of the twitch torque signal (mean and maximal rate of torque relaxation, RTR), the time from the PTT to one half of its peak value (twitch half-relaxation time; THRT) and the area under the torque-time curve (total twitch area; TTA) have been calculated and interpreted. These different parameters have commonly been considered to estimate age-related changes within the skeletal muscle system (e.g. modulations of muscle mass) [1, 2]. However, it is surprising that the association between twitch contractile properties and skeletal muscle mass has hardly been investigated. We found only one study addressing the relationship between these variables [3]. Ryan et al. [3] observed a significant positive correlation between cross-sectional area (CSA) and PTT ($r = 0.874$) as well as between CSA and maximal RTD (MRTD) ($r = 0.907$). Nevertheless, that study was carried out on the plantar flexor muscles in young adults. It remains unclear whether the association between muscle mass and twitch mechanical parameters is still present for the quadriceps, which is particularly important for many activities of daily living [4]. Furthermore, it has not yet been investigated whether this relationship can also be found in elderly subjects and when supramaximal single and doublet stimulation is used. If the occurrence of muscle mass loss with aging can be identified from the twitch torque signal, it might be a simple, non-invasive and favourable way to estimate sarcopenia.

Thus, the objective of the present study was to investigate the relationship between the lean mass of the thigh assessed by dual-energy X-ray absorptiometry (DXA) and evoked twitch properties of the quadriceps using supramaximal single and doublet stimulation in elderly and young adults. We hypothesized a positive correlation between lean mass and contractile properties in both age groups.

Materials and Methods

Subjects

Fifteen young (aged 20–34 years) and 15 elderly (aged 64–74 years) subjects with no history of neurological or musculoskeletal disorders or injuries volunteered to participate in this cross-sectional study. Physically active and healthy subjects were chosen in order to take account of the problem of disuse atrophy. Prior to

Table 1. Subject characteristics and lean mass of the thigh

	Young (n = 15)	Elderly (n = 15)	p
Men	8 (53.3)	8 (53.3)	
Age, years	25.3±3.6	69.6±3.1	0.000**
Weight, kg	71.3±11.6	72.6±9.9	0.750
Height, m	1.75±0.9	1.69±0.9	0.058
Physical activity, h/week	4.6±3.6	3.7±3.3	0.134
Lean mass, kg	5.94±1.45	5.27±1.09	0.028*

Values are presented as numbers (%) or means ± SD unless otherwise stated. Significant differences between groups: * $p \leq 0.050$, ** $p \leq 0.010$.

participation, written informed consent was obtained from the subjects. This study was conducted according to the Declaration of Helsinki and was approved by the Ethical Review Committee of the University Medicine Rostock (A 2009 52). Subject characteristics are displayed in table 1.

Experimental Procedure

The subjects participated in two experimental sessions. The first session included the measurement of body composition using DXA. In the second session, contractile properties were evaluated. The methods for carrying out lean body mass and contractile property measurements are only briefly outlined. A detailed description of electromyography (EMG), torque recordings and electrical stimulation has been given previously [2].

Lean Body Mass

The lean mass was measured using DXA (Lunar Prodigy densitometer; General Electric Medical Systems Lunar, Madison, Wisc., USA), with low exposure to radiation and a minimal radiation dose (<10 mSv, 76 kV, 0.15 mA, 0.4 µGy). Subjects were placed in a supine position on the scanning bed. The whole-body scan took 6–7 min in standard mode. Prior to each test, the quality assurance procedure was carried out using a cuboid-shaped calibration body (20 × 130 × 60 mm). Body composition was calculated for one manually determined region of interest (ROI) of the dominant leg using Lunar enCORE™ software. The upper limiting line of the ROI runs at an oblique angle above the trochanter major and minor and the lower limiting line horizontally through the knee joint line. The relevant parameter is the lean mass of the thigh, which was calculated using Lunar enCORE 2007 software (version 11.40.004). The lean mass represents the bone-free lean mass (appendicular lean soft tissue), composed primarily of the muscle mass as well as other parts such as ligaments, tendons, joint capsules and meniscal tissue. Levine et al. [5] demonstrated a high correlation between lean mass determined by DXA and the muscle mass of the thigh determined by means of computed tomography ($r = 0.86$, $p < 0.001$). The lean mass parameter can thus be used to estimate muscle mass. A systematic error of –0.62 to –4.68% was evidenced when measuring lean mass with the Lunar Prodigy densitometer compared to the 4-component model [6].

Table 2. Contractile properties evaluated by single and doublet stimulation at supramaximal intensity

	Single		p	Doublet		p
	young	elderly		young	elderly	
PTT, N·m	39.39±10.55	15.35±7.20	0.000**	69.05±14.67	41.47±13.91	0.000**
MRTD, N·m·s ⁻¹	1,814.41±449.65	673.54±359.21	0.000**	3,220.57±117.83	1,491.53±173.61	0.000**
MRTR, N·m·s ⁻¹	828.08±171.78	292.97±163.94	0.000**	1,326.27±80.82	526.93±42.76	0.000**
TCT, s	0.064±0.017	0.069±0.022	0.101	0.067±0.004	0.085±0.006	0.013*
THRT, s	0.084±0.015	0.073±0.019	0.106	0.090±0.004	0.083±0.005	0.290
TTA, N·m·s	4.37±0.55	2.15±0.44	0.001**	8.70±0.60	5.78±0.63	0.006**

Values are presented as means ± SD unless otherwise stated. Significant differences between groups: * $p \leq 0.050$, ** $p \leq 0.010$.

Contractile Properties

The measurement of contractile properties was carried out on the quadriceps of the dominant leg. Subjects were seated in a standardized position on a Cybex NORM dynamometer (Computer Sports Medicine®, Inc., Stoughton, Mass., USA). The tests were performed in a sitting position with a knee angle of 75° and a hip angle of 50° (0° = full extension). The surface EMG was recorded using bipolar EMG Ambu® Blue Sensor N electrodes (2-cm diameter). The electrodes were firmly attached to shaved, abraded and cleaned skin over the vastus medialis muscle of the dominant leg. Transcutaneous electrical stimulation was used to assess contractile properties of the quadriceps. The anode (self-adhesive electrode, 35 × 45 mm; Spes Medica, Italy) was placed over the greater trochanter. The cathode (ball electrode, 1-cm diameter) was fixed to the subject's femoral triangle, 3–5 cm below the inguinal ligament. One-millisecond rectangular pulses (400 V) were applied to the femoral nerve by a constant-current stimulator (Digitimer® DS7A; Hertfordshire, UK). The inter-stimulus intervals (10 s) were provided by a Digitimer train/delay generator (DG2A; Hertfordshire, UK). Different current intensities were delivered to the femoral nerve until identification of the maximal M wave ($M_{\max}$) of the vastus medialis was achieved. The evaluation of contractile properties was realized at a stimulation intensity of 40% above the level that was needed to elicit the maximal twitch response and concomitant $M_{\max}$. Resting twitch responses were evoked using single and doublet (inter-stimulus interval 10 ms) stimulation. Signals were amplified (×2,500), band-pass filtered (10–450 Hz) and digitized with a sampling frequency of 5 kHz through an analogue-to-digital converter (DAQ Card™-6024E; National Instruments, USA). The EMG and torque signals were analysed using a custom-built LabVIEW®-based program (Imago; Pfitec, Germany). The resting twitch torques were analysed regarding their PTT, MRTD, maximal RTR (MRTR), TCT, THRT and TTA. Furthermore, the ratio between doublet PTT and single PTT ($PTT_{\text{doublet}}/PTT_{\text{single}}$) was calculated to estimate the degree of contractile fusion that describes the contribution of the second pulse to the overall torque. A higher ratio indicates greater fusion, i.e. the second impulse contributed a considerable portion of the total torque [7].

Statistical Analysis

Data were checked for normal distribution using a Shapiro-Wilk W test. Age-related differences between the groups were tested for significance using unpaired Student's t tests or Mann-Whitney U

tests. Differences between single and doublet stimulation were tested for significance by paired Student's t tests or Wilcoxon tests. Pearson product-moment correlation coefficients (r) were used to quantify the relationships between lean mass and twitch contractile properties (PTT, MRTD, MRTR, TCT, THRT and TTA). According to Cohen, r was interpreted as follows: $r = 0.10$, small effect; $r = 0.30$, moderate effect, and $r = 0.50$, large effect [8]. The level of significance was established at $p \leq 0.050$. All data were analysed using SPSS statistical package 20.0 (SPSS Inc., Chicago, Ill., USA).

Results

Differences between Groups

The lean body mass of the thigh was significantly lower by 19.1% in elderly subjects (table 1). The PTT, MRTD, MRTR and TTA were significantly reduced in elderly subjects when single stimulation (61.0, 62.9, 64.9 and 50.9%, respectively) and doublet stimulation (39.9, 53.7, 60.3 and 33.6%, respectively) were used. The TCT evoked by doublet stimulation was significantly longer in elderly subjects (27.1%). No significant differences between the groups could be determined for THRT. The mean ± SD values of contractile properties are displayed in table 2.

Differences between Single and Doublet Stimulation

Doublet PTT, MRTD, MRTR and TTA were significantly higher ($p < 0.001$) in young (87.5, 82.6, 60.4 and 127.5%, respectively) and elderly (191.2, 142.3, 107.4 and 248.4%, respectively) subjects. The TCT was significantly lower when using single stimulation in elderly subjects (29.7%; $p = 0.006$), whereas no differences were found for TCT in young subjects or for THRT in both age groups. $PTT_{\text{doublet}}/PTT_{\text{single}}$ was significantly higher by 63.1% in elderly subjects ($p < 0.001$; mean ± SD: young, 1.79 ± 0.20 ; elderly, 2.91 ± 0.75).

Table 3. Pearson's correlation coefficients (r) between lean mass of the thigh and contractile properties evaluated by single and doublet stimulation at supramaximal intensity

	Lean mass			
	single		doublet	
	young	elderly	young	elderly
PTT, N·m	0.386	0.114	0.560*	0.535*
MRTD, N·m·s ⁻¹	0.092	0.009	0.165	0.286
MRTR, N·m·s ⁻¹	-0.044	0.262	0.126	0.413
TCT, s	-0.088	-0.036	-0.254	-0.450 [†]
THRT, s	0.011	-0.235	0.246	0.166
TTA, N·m·s	0.025	-0.073	0.055	0.367

Significant correlation: * $p \leq 0.050$. [†] Statistical tendency towards correlation ($p \leq 0.100$).

Correlation between Lean Mass and Contractile Properties

We observed no significant correlations between lean mass and contractile properties when supramaximal single stimulation was used. Significant positive correlations were only shown between lean mass and PTT evoked by doublet stimulation in young ($p = 0.030$) and elderly ($p = 0.040$) subjects. A statistical tendency towards a negative correlation was found for the TCT evoked by doublet stimulation in elderly subjects ($p = 0.092$) (table 3).

Discussion

Aging is associated with changes at the skeletal muscle level [1]. This could be confirmed by the current study as the lean mass of the thigh and contractile properties of the quadriceps were reduced in elderly compared to young subjects.

Nevertheless, the major objective of the present study was to evaluate the correlation between lean mass and contractile properties. Ryan et al. [3] found positive correlations between CSA and PTT as well as between CSA and RTD for the plantar flexors in young adults when using doublet stimulation. However, the relationship between lean mass and contractile properties has not yet been examined for the quadriceps in elderly and young subjects and when single and doublet stimulation is applied. No significant correlations between the variables were observed when using single stimulation. Thus, our hypothesis has only been confirmed for doublet stimula-

tion. We found a significant positive correlation between lean mass and PTT evoked by doublet stimulation in both elderly and young subjects. However, in contrast to Ryan et al. [3], we observed lower correlation coefficients and found that PTT only accounts for 31% (young; $r^2 = 0.314$) and 29% (elderly; $r^2 = 0.286$) of the variance in lean mass, respectively. The analysis of time and slope parameters showed no significant correlations with lean mass, which partly conflicts with previous results. The contrary findings may be related to methodological differences. A possible explanation for the larger correlation coefficients found by Ryan et al. [3] might be that the authors analysed CSA of the plantar flexors assessed by peripheral quantitative computer tomography, whereas we measured the lean mass of the total thigh using DXA. Furthermore, the authors used different stimulation parameters, i.e. doublets with an inter-stimulus interval of 5 ms.

Consequently, supramaximal doublet stimulation seems to be a more sensitive method to identify changes in muscle mass with aging compared to single stimulation. The doublet twitch torque responses are larger and rise faster in both age groups and are longer in elderly subjects. The primary causes of these are physiological differences in twitch force generation between single and doublet stimulation, i.e. an increase in muscle stiffness, changes in excitation-contraction coupling and potentiation of intracellular Ca^{2+} movements [7, 9]. The first response is most likely attributable to differences in the passive structure (tendon) of series elastic components while the second pulse can be primarily ascribed to excitation-contraction coupling processes [10]. Our results further indicate that supramaximal single stimulation as well as the variables MRTD, MRTR, TCT, THRT and TTA may be unsuitable to estimate morphological changes of the quadriceps in elderly and young subjects. RTD and in particular TCT are primarily indicators of contraction speed [11]. Possible underlying mechanisms of modulations in contraction speed are changes in muscle mass [3], muscle fibre distribution [12] and cross-bridge kinetics, i.e. Ca^{2+} release and binding to troponin or the binding of actin to myosin. Alterations in RTR and THRT could be ascribed to a modified efficiency in the sarcoplasmic reticulum function to re-uptake Ca^{2+} [13].

The results further demonstrate a greater ratio between doublet PTT and single PTT in elderly subjects, indicating a higher contractile fusion present within the doublet force recording. The force contribution of the second pulse is 1.8 times greater than that of the single twitch in young subjects. In contrast, a considerably higher doublet force contribution (2.9 times that of the single

PTT) was found in elderly subjects. Therefore, the first stimulus contributed the highest portion of the total force in young adults, whereas the contribution of the second stimulus was largest in elderly subjects. Tendon stiffness is reduced with aging and thus the first stimulus might 'take up the slack' and increase stiffness with a low force production in elderly subjects [10].

In conclusion, this is the first study to analyse the association between thigh lean mass and contractile properties of the quadriceps in two different age groups. The application of supramaximal single stimulation as well as the analysis of time and slope parameters may not be recommended for estimating changes in muscle mass. However, the analysis of PTT evoked by doublet stimulation

seems to be an appropriate measure to assess modulations of muscle mass in elderly and young subjects. Consequently, the occurrence of sarcopenia could be estimated from analysis of the twitch torque signal induced by doublet stimulation, which provides a simple, non-invasive and favourable way to identify muscle mass declines with aging.

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