AGE (2016) 38: 57
DOI 10.1007/s11357-016-9921-2

Effects of age and sex on neuromuscular-mechanical determinants of muscle strength

Rui Wu · Eamonn Delahunt · Massimiliano Ditroilo · Madeleine Lowery · Giuseppe De Vito

Received: 4 February 2016 / Accepted: 8 May 2016 / Published online: 17 May 2016

Abstract The aim of this study was to concurrently assess the effect of age on neuromuscular and mechanical properties in 24 young (23.6 ± 3.7 years) and 20 older (66.5 ± 3.8 years) healthy males and females. Maximal strength of knee extensors (KE) and flexors (KF), contractile rate of torque development (RTD) and neural activation of agonist-antagonist muscles (surface EMG) were examined during maximal voluntary isometric contraction (MVIC). Tissue stiffness (i.e. musculo-articular stiffness (MAS) and muscle stiffness (MS)) was examined via the free-oscillation technique, whereas muscle architecture (MA) of the vastus lateralis and subcutaneous fat were measured by ultrasonography. Males exhibited a greater age-related decline for KE (47.4 %) and KF (53.1 %) MVIC, and RTD (60.4 %) when compared to females (32.9, 42.6 and 34.0 %, respectively). Neural activation of agonist muscles during KE MVIC falls markedly with ageing; however, no age and sex effects were observed in the antagonist co-activation. MAS and MS were lower in elderly compared with young participants and in females compared with males. Regarding MA, main effects for age (young 23.0 ± 3.3 vs older 19.5 ± 2.0 mm) and sex (males 22.4 ± 3.5 vs females 20.4 ± 2.7 mm) were detected in muscle thickness. For fascicle length, there was an effect of age (young 104.6 ± 8.8 vs older 89.8 ± 10.5 mm), while for pennation angle, there was an effect of sex (males 13.3 ± 2.4 vs females 11.5 ± 1.7°). These findings suggest that both neuromuscular and mechanical declines are important contributors to the age-related loss of muscle strength/function but with some peculiar sex-related differences.

Keywords Ageing · Sex · Muscle strength · Muscle architecture · Stiffness

R. Wu (✉) · E. Delahunt · M. Ditroilo · G. De Vito
School of Public Health, Physiotherapy and Sports Science, University College Dublin, Dublin, Republic of Ireland
e-mail: rui.wu.1@ucdconnect.ie

E. Delahunt · M. Ditroilo · G. De Vito
Institute for Sport and Health, University College Dublin, Dublin, Republic of Ireland

M. Lowery
School of Electrical, Electronic and Communications Engineering, University College Dublin, Dublin, Republic of Ireland

Introduction

Deterioration in muscle strength/function is an inevitable consequence of the normal ageing process and can be associated with functional limitations and participation restrictions, such as an increased risk of falls (Wolfson et al. 1995), hip fractures (Langlois et al. 1998) and a loss of bone mineral density (Sinaki et al. 1986), making elderly persons more dependent on others in daily life. This is particularly relevant to females, who live longer, reach the threshold of dependence earlier and have lower physical capacities than males at all ages (Larsson et al. 1979; Lindle et al. 1997).

Loss of muscle mass represents the main factor associated with decreased muscle strength/function in elderly persons. This phenomenon is attributed to reductions in both the number and size of individual muscle fibres, especially type II fibres (Frontera et al. 2000; D'Antona et al. 2003). On the other hand, it is well known that muscle strength declines at a greater rate than muscle mass during the normal ageing process (Young et al. 1985; Jubrias et al. 1997). This disproportionate loss indicates that other variables are likely to contribute to the age-related deterioration in muscle strength/function, with both mechanical and neuromuscular factors requiring consideration. Changes in muscle architecture (MA) have been associated with decreased muscle strength/function in elderly persons (Kubo et al. 2003a; Narici et al. 2003; Strasser et al. 2013). Other relevant mechanical factors include tissue stiffness characteristics, which have been investigated in different biological structures (e.g. tendon, muscle and joint) involved in characteristic locomotion (Reeves et al. 2003; Bojsen-Moller et al. 2005; Ditroilo et al. 2012). Age-related decrease in muscle strength/function depends also on alterations in neural drive to agonist and antagonist muscles (Izquierdo et al. 1999; Macaluso et al. 2002; Macaluso and De Vito 2004). A number of studies have reported that maximal voluntary neural activation of agonist muscles was lower in groups of elderly individuals when compared to young and middle-aged groups (Häkkinen et al. 1998a; Macaluso et al. 2002; Billot et al. 2014). Ideally, to thoroughly investigate age-related decline in muscle strength/function, the changes manifest in both mechanical and neuromuscular factors should be concurrently quantified and assessed. To the best of our knowledge, no studies to date have concomitantly examined and contrasted age-related deterioration in muscle strength/function with respect to the contribution of MA, tissue stiffness and neural activities of agonist-antagonist muscles. Therefore, the aim of the present study was to simultaneously assess the age-related modifications of the aforementioned neuromuscular-mechanical factors by comparing young and older adults of both sexes.

Materials and methods

Participants

Twenty-four young adults (11 males and 13 females) and 20 older adults (11 males and 9 females) volunteered to participate in the study. The criteria for exclusion were applied through a general medical questionnaire based on the definition of 'medically stable' as proposed by Greig et al. (1994). Participants were excluded primarily for the following conditions: presence or/and history of cardiovascular, cerebrovascular, neurological, uncontrolled metabolic, respiratory and major systemic diseases. None of the participants had previously engaged in strength training or competitive sports within the last 5 years. In order to determine the reliability for the variables of interest, participants attended the laboratory on two occasions separated by at least 1 week. Each laboratory visit lasted approximately 2 h, with the same order of testing undertaken on both days: maximal voluntary isometric contraction (MVIC) of both the knee extensors (KE) and flexors (KF), musculo-articular stiffness (MAS) and muscle stiffness (MS), and MA. The study was approved by the University Human Research Ethic Committee, and all participants provided written informed consent to the procedures and aims of the study. The age and the main physical characteristics of participants are reported in Table 1.

Table 1 Participants characteristics

	Young		Older		Main effect		Interaction effect
	Male YM (n = 11)	Female YF (n = 13)	Male OM (n = 11)	Female OF (n = 9)	Age p value	Sex p value	Age × sex p value
Age (year)	23.7 ± 4.2	23.5 ± 3.4	66.8 ± 3.4	66.1 ± 4.4	<0.001	0.685	0.850
Stature (m)	1.80 ± 0.08	1.69 ± 0.07	1.77 ± 0.07	1.66 ± 0.05	0.150	<0.001	0.969
Body mass (kg)	81.2 ± 11.7	65.3 ± 12.9	82.6 ± 7.7	61.7 ± 7.7	0.742	<0.001	0.438
BMI (kg/m^2)	25.2 ± 3.1	22.7 ± 4.0	26.5 ± 2.6	22.3 ± 2.3	0.637	<0.01	0.366

Values are mean ± SD

YM young males, *YF* young females, *OM* older males, *OF* older females, *n* number of participants

MVIC

After a 5-min warm-up exercise performed on a cycle-ergometer at a light resistance (50–100 W), MVIC of both KE and KF (right leg) was measured using an adapted leg dynamometer (Technogym, Gambettola, Italy) equipped with a load cell (Leane International, Parma, Italy, measurement rage 0–500 kg, output 2.00 mV/V). Participants were seated comfortably in a rigid chair and firmly strapped at the pelvis and upper body with hip and knee flexion angles of 110° and 70° (where 0° represents full extension), respectively. The rotational axis of the dynamometer was aligned to the lateral femoral epicondyle, and the lower leg was strapped to the machine lever arm 2 cm above the lateral malleolus. After full familiarization with the testing procedures, each participant performed a minimum of three maximal attempts for KE and KF with 2 min of rest between attempts. The participants were required to exert force 'as strongly and as quickly as possible' for 2–3 s, while strong verbal encouragement and on-line visual feedback was provided. An additional attempt was allowed when force variation was higher than 5 % between the best two attempts. The highest force attained by each participant was chosen for further analysis.

MAS

MAS of the knee joint, which is a comprehensive measurement of stiffness of the whole joint including muscle-tendon unit, surrounding articular surfaces, ligaments and skin, was assessed using the free-oscillation technique as described in previously published studies (Watsford et al. 2010; Ditroilo et al. 2011a). The same dynamometer and position used for the MVIC assessment was adopted. Briefly, each participant was required to support a load corresponding to 20 % of KE MVIC on the anterior distal portion of the right lower leg. The weight of the right lower leg was estimated and added to the external load to incorporate the effect of gravity (Ditroilo et al. 2011a). A brief perturbation of 100-150 N was then applied perpendicular to the lever arm at the same distance as the load supported and the ensuing damped oscillations were recorded by a uniaxial accelerometer (Crossbow Technology, Milpitas, CA, USA). Each participant performed five trials separated by a 1-min rest period with the average of the best three trials being used for further analysis.

MS

Using the same setup as described for MAS assessment, MS of VL was measured via a myometry at a sampling rate of 3200 Hz (Myometer, Myoton-3, Mӳomeetra AS, Tallinn, Estonia, EU). This technique, similar to MAS, records the damped oscillations of a mechanically perturbed structure and, however, is a more localized and selective measurement of stiffness of an individual muscle (i.e. VL). The device's probe was positioned on the VL muscle at 2/3 of the distance between the anterior superior iliac spine and the lateral border of the patella. MS was assessed in a contracted condition (i.e. supporting a load =20 % KE MVIC). During testing, the probe was kept perpendicular to the skin surface and automatically tapped the muscle with a force of 0.3–0.4 N and a duration of 15 ms, as previously described (Ditroilo et al. 2012). The subsequent damped natural oscillations were recorded by an accelerometer incorporated in the device giving an instantaneous recording of the muscle deformation characteristics. Five consecutive measurements were performed and averaged for statistical analysis.

MA

MA was assessed in vivo by using B-mode real-time ultrasound imaging (DP-6600, Mindray Bio-Medical Electronics CO., LTD, Shenzhen, China) equipped with a 40-mm, 7.5-MHz linear-array probe. Images were taken at 60 % of the distance from the lateral femoral epicondyle to the greater trochanter (right leg), which has been previously demonstrated as the point corresponding to the largest quadricep cross-sectional area (Maden-Wilkinson et al. 2013). The measurements were taken with the participants in a supine position with the knee joint fully extended and muscles relaxed. Maps on transparency films were developed using anatomical reference points (i.e. border of patella) and skin marks (i.e. freckles and scars) to ensure that the same recording position was used on both experimental sessions. The head of the ultrasound probe was held perpendicular to the dermal surface to provide acoustic contact without depressing it. Two best quality images were selected and then averaged for further analysis. Muscle thickness, fascicle length (L*f*) and pennation angle ( ) were assessed using computer software (AutoCAD 2015, Autodesk, Inc., San Rafael, CA). Muscle thickness was defined as the distance between the deep and superficial aponeuroses of the VL.   was defined as the angle of insertion of muscle fibre fascicles into the deep aponeurosis and L*f* as the length of the fascicular path between the insertions of the fascicle into the superior and deep aponeuroses (Fig. 1). In addition, the subcutaneous fat was defined as the distance between skin surface and muscle belly (Nordander et al. 2003) and was measured adopting the same ultrasound procedure at the two sites corresponding to the position of the VL and biceps femoris (BF) surface EMG electrodes.

Surface EMG

Surface EMG was recorded during the assessment of MVIC from the VL and the long head of the BF muscles, as representative of the neural activation of KE and KF muscle groups, respectively (De Vito et al. 2003). Before the test, the skin was prepared (shaved, lightly abraded and cleaned with ethyl alcohol) and two Ag/AgCl bipolar electrodes (Blue Sensor N-00-S, Ambu Medicotest A/S, Ølstykke, Denmark) were positioned with 20 mm inter-electrode distance according to the SENIAM guidelines (Hermens et al. 1999). A ground electrode was attached on the patella. The same transparency films described above were used to mark the reference points to ensure that same examination sites were measured on the second testing day. Surface EMG signals were synchronized with force and acceleration signals that were all sampled at 1000 Hz, amplified with a gain of 1000 and stored on a PC using a 16-bit A/D converter data acquisition system (Biopac System, Inc., Goleta, CA, USA).

Data analysis

The signals were processed in Matlab R2014a (Mathworks, Natick, MA, USA). Force and acceleration signals were off-line low-pass filtered at 15 and 4 Hz, respectively, whereas EMG signals were band-pass filtered between 20 and 400 Hz using a fourth-order zero-phase Butterworth filter. Subsequently, force signals were converted into torque values (Nm). MVIC for knee extension and flexion was calculated as the highest 1000 ms average reached within any single force recording. The contractile rate of torque development (RTD) for KE was acquired by the linear slope of the torque-time profile (i.e. ΔTorque/ΔTime). The RTD was calculated over epochs 0–200 ms torque-time characteristics in line with previous studies (Aagaard et al. 2002; Andersen et al. 2010). The onset of torque development was defined as the instant when the torque value exceeded the baseline level by 7.5 Nm (Aagaard et al. 2002). To quantify the EMG amplitude and frequency components, the root mean square (RMS) and median frequency (MDF) were calculated over a period of 1000 ms corresponding to MVIC of the highest force attained. The EMG activation of agonist muscles and co-activation of antagonist muscles were calculated as previously described (Macaluso et al. 2002). Briefly, the RMS value from the antagonist muscle group was reported as a percentage of the RMS amplitude during its MVIC when acting as an agonist and used to express the level of antagonist co-activation during knee extension. The MDF of the EMG was estimated using the fast Fourier transform (FFT) calculated with overlapping Hamming windows of 500 ms duration and 75 % overlap.

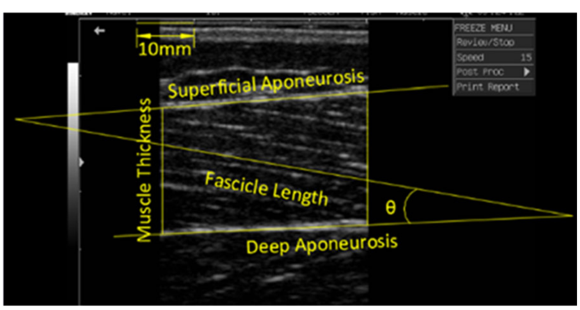

Fig. 1 Ultrasound image of the vastus lateralis (VL). , pennation angle

Statistical analysis

The statistical analysis was performed using SPSS 20.0 (IBM Ireland Ltd., Dublin, Ireland). All data are presented as mean ± SD. Normality of distribution was tested by a non-parametric test (one-sample Kolmogorov-Smirnov test), and all variables were normally distributed. Factorial analyses of variances (ANOVA) with two independent factors (age × sex) were adopted. When a significant interaction (age × sex) was detected, pairwise comparisons were performed using a Bonferroni adjustment. In addition, since the subcutaneous fat was correlated with MS ($p < 0.001$, $r = -0.69$), MDF ($p < 0.05$, $r = -0.30$), RMS EMG activation of agonist muscle (i.e. VL) ($p < 0.05$, $r = -0.32$) and co-activation of antagonist muscle (i.e. BF) ($p < 0.001$, $r = 0.54$), an analysis of covariance (ANCOVA) was performed adopting subcutaneous fat as a covariate. Effect size (ES) was also calculated using eta-squared (η^2) and interpreted as small (0.01), moderate (0.06) and large (0.14) (Cohen 1988). Intraclass correlation coefficients (ICC) with 95 % confidence intervals (CI) and coefficients of variation (CV) were applied to verify the between-session reliability performed on two separate days for all measurements. A paired sample *t* test was used to detect any systematic bias over the two testing sessions. The level of statistical significance was set at $p < 0.05$. For the purpose of the comparison, the data presented here refer only to the second testing session.

Results

MVIC and RTD

A significant interaction between age and sex was detected for both KE MVIC ($F_{(1, 40)} = 9.0$, $\eta^2 = 0.19$; $p < 0.01$) and KF MVIC ($F_{(1, 40)} = 7.0$, $\eta^2 = 0.15$; $p < 0.05$) (Table 2). The older participants in both sex groups exhibited lower levels of muscle strength than their younger counterparts for KE (males $F_{(1, 40)} = 45.6$, $\eta^2 = 0.53$, $p < 0.001$; females $F_{(1, 40)} = 5.9$, $\eta^2 = 0.13$, $p < 0.05$) and KF (males $F_{(1, 40)} = 44.9$, $\eta^2 = 0.53$, $p < 0.001$; females $F_{(1, 40)} = 8.3$, $\eta^2 = 0.17$, $p < 0.01$), with a more pronounced difference observed between OM and YM (KE by 47.4 % and KF by 53.1 %, respectively) with respect to OF and YF (KE by 32.9 % and by KF by 42.6 %, respectively) (Fig. 2). Similarly, an interaction (age × sex) was also observed for RTD ($F_{(1, 40)} = 12.8$, $\eta^2 = 0.24$; $p < 0.01$) (Table 2), showing a more pronounced reduction in OM (60.4 %) compared with 34.0 % observed in OF, respectively (Fig. 3). However, when RTD were normalized to KE MVIC (i.e. expressed as %MVIC), all the observed differences disappeared.

MAS and MS

Main effects for age and sex but with no interaction (age × sex) were observed for MAS (Table 2), showing a lower level of MAS in older participants compared to the young ($F_{(1, 41)} = 15.5$, $\eta^2 = 0.27$; $p < 0.001$) and in females compared to the males ($F_{(1, 41)} = 6.5$, $\eta^2 = 0.14$; $p < 0.05$). Moreover, MS was characterized by a main effect for sex due to a lower MS observed in females than males ($F_{(1, 41)} = 27.4$, $\eta^2 = 0.40$; $p < 0.001$) but not for age (Table 2). However, after controlling for the effect of subcutaneous fat over the VL (covering the area where the probe was applied) as a covariate ($F_{(1, 40)} = 18.3$, $\eta^2 = 0.31$; $p < 0.001$), the older participants demonstrated a lower MS than young participants ($F_{(1, 40)} = 6.8$, $\eta^2 = 0.15$; $p < 0.05$) and females were still characterized by lower MS values than males ($F_{(1, 40)} = 4.25$, $\eta^2 = 0.10$; $p < 0.05$).

MA

Concerning muscle thickness (Table 2), there were main effects for age ($F_{(1, 41)} = 22.4$, $\eta^2 = 0.35$; $p < 0.001$) and sex ($F_{(1, 41)} = 9.4$, $\eta^2 = 0.19$; $p < 0.01$). In regard to L*f*, a main effect for age indicated a 14.1 % shorter L*f* in the older compared with young participants ($F_{(1, 41)} = 24.6$, $\eta^2 = 0.38$; $p < 0.001$) but no sex effect. In contrast, the demonstrated a main effect for sex but not for age, which revealed a larger angle in males than females ($F_{(1, 41)} = 8.9$, $\eta^2 = 0.18$; $p < 0.01$).

Neural activation (surface EMG)

Figure 4 depicts the KE muscle torque signals and the associated EMG of VL of one older and one young participant recorded during the knee extension MVIC test. Main effects for both age and sex characterized RMS EMG VL amplitude. Lower VL activation (RMS) was observed in older compared to young participants ($F_{(1, 41)} = 6.9$, $\eta^2 = 0.14$; $p < 0.05$) and in females compared to males ($F_{(1, 41)} = 6.4$, $\eta^2 = 0.14$; $p < 0.01$) (Table 2). The ANCOVA analysis performed adopting subcutaneous fat of VL (covering the area where the electrodes were attached) as a covariate was not significant. Whereas, concerning the co-activation of antagonist muscles, the BF co-activation was higher in females than males ($F_{(1, 41)} = 4.46$, $\eta^2 = 0.10$; $p < 0.05$) but with no difference between young and older participants. Nevertheless, in this analysis after adopting the subcutaneous fat covering the BF area as a covariate ($F_{(1, 40)} = 9.0$, $\eta^2 = 0.18$; $p < 0.01$), the BF co-activation was shown to be similar for both males and females and for young and older participants. A main effect for age but not for sex was detected in relation to MDF. Indeed, in the older participants, the MDF was lower in comparison with their younger counterparts ($F_{(1, 41)} = 7.09$, $\eta^2 = 0.15$; $p < 0.05$) (Table 2). Interestingly, after adopting subcutaneous fat of VL as a covariate ($F_{(1, 40)} = 6.2$, $\eta^2 = 0.14$; $p < 0.05$), this age effect was retained ($F_{(1, 40)} = 10.3$, $\eta^2 = 0.20$; $p < 0.01$).

Table 2 Effects of age and sex on mechanical and neural factors

		Young		Older		Main effect		Interaction effect
		Male (n = 11)	Female (n = 13)	Male (n = 11)	Female (n = 9)	Age Diff %, p value	Sex Diff %, p value	Age × sex p value
Muscle Strength and RTD	KE MVIC (Nm)	317.9 ± 73.3	179.6 ± 52.2	167.2 ± 38.7	124.7 ± 31.8	39.1, <0.001	35.2, <0.001	<0.01
	KF MVIC (Nm)	176.0 ± 53.0	96.0 ± 28.0	82.6 ± 20.9	55.1 ± 11.1	47.1, <0.001	38.7, <0.001	<0.05
	RTD (Nm/s)	1044.7 ± 252.0	592.6 ± 185.5	413.2 ± 190.0	391.1 ± 136.3	49.6, <0.001	30.1, <0.001	<0.01
Tissue stiffness	MAS (N/m)	1281.7 ± 403.4	796.4 ± 609.4	605.2 ± 213.8	485.5 ± 144.3	45.9, <0.001	29.1, <0.05	0.150
	MS (N/m)	488.7 ± 50.2	416.7 ± 52.4	466.6 ± 39.7	394.2 ± 37.4	3.5, 0.115	14.7, <0.001	0.989
MA of VL	Muscle thickness (mm)	24.6 ± 3.4	21.6 ± 2.7	20.3 ± 2.1	18.6 ± 1.5	15.1, <0.001	9.1, <0.01	0.397
	Lf (mm)	105.1 ± 10.8	104.2 ± 7.1	88.1 ± 10.1	91.9 ± 11.1	14.1, <0.001	−2.6, 0.683	0.533
	(°)	13.5 ± 2.9	11.8 ± 1.9	13.1 ± 1.7	11.1 ± 1.4	3.0, 0.392	13.7, <0.01	0.847
Surface EMG	VL activation (mV)	0.214 ± 0.063	0.160 ± 0.049	0.158 ± 0.073	0.119 ± 0.063	24.1, <0.05	22.8, <0.05	0.677
	BF co-activation (%)	18.0 ± 10.2	25.9 ± 9.4	16.2 ± 10.7	20.4 ± 8.5	19.0, 0.223	−38.3, < 0.05	0.567
	MDF (Hz)	65.6 ± 10.3	62.4 ± 9.0	58.6 ± 10.9	54.1 ± 7.2	11.4, <0.05	5.0, 0.193	0.815

Values are mean ± SD

KE knee extensors, *KF* knee flexors, *MVIC* maximal voluntary isometric contraction, *RTD* rate of torque development, *MAS* musculo-articular stiffness, *MS* muscle stiffness, *MA* muscle architecture, *Lf* fascicle length, pennation angle, *VL* vastus lateralis, *BF* biceps femoris, *MDF* median frequency

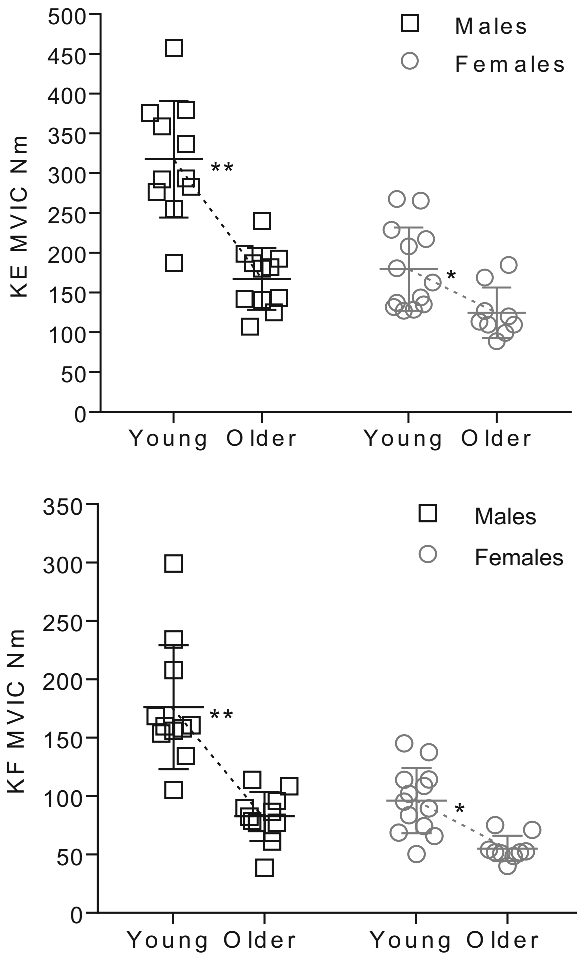

Fig. 2 Maximal voluntary isometric contraction (*MVIC*) of knee extensors (*KE*) and flexors (*KF*) for young and older males and females. *$p < 0.05$ and **$p < 0.001$ significant difference between young and older males and females, respectively. Values are mean ± SD

Test-retest reliability

Acceptable to excellent reliabilities between the two testing sessions were observed for all measurements (Table 3) with no significant differences in any variable between the two testing sessions.

Discussion

In the present study, a comprehensive analysis of the age-related alterations in both mechanical and neuromuscular factors was conducted for the first time in both sexes with acceptable to excellent reliability for the variables of interest. The study revealed that both

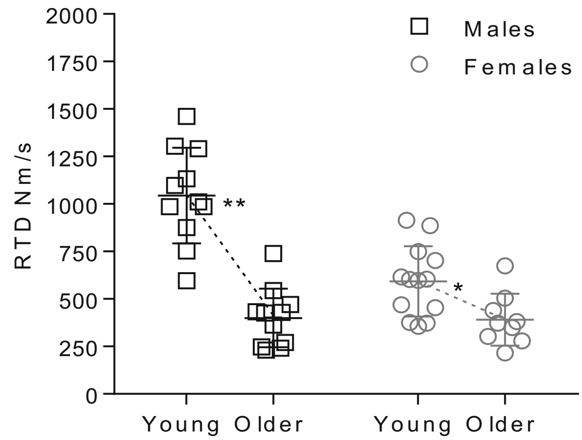

Fig. 3 Rate of torque development (*RTD*) for young and older males and females. *$p < 0.05$ and **$p < 0.001$ significant difference between young and older males and females, respectively. Values are mean ± SD

mechanical and neuromuscular properties were reduced with age, although with some peculiar sex-related differences especially in relation to muscle strength.

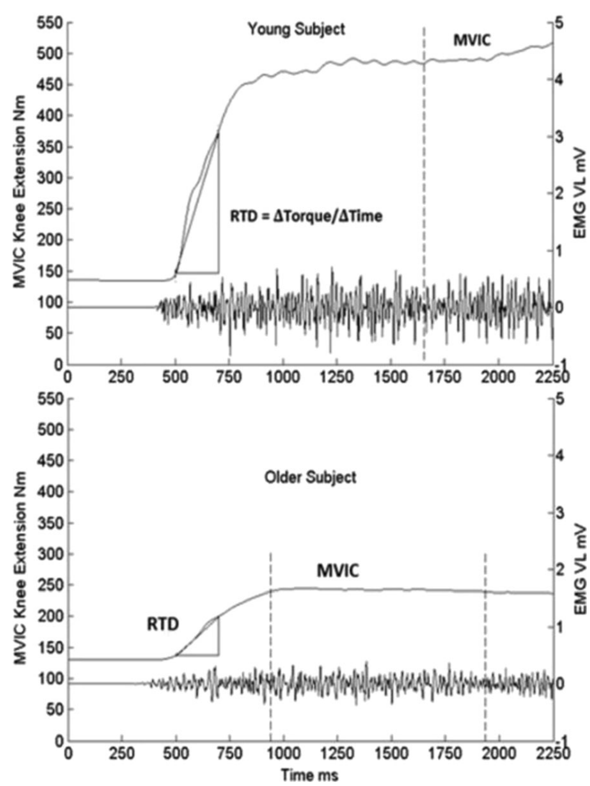

Fig. 4 Force and surface electromyogram (*EMG*) obtained from vastus lateralis (*VL*) muscle during maximal voluntary isometric contraction (*MVIC*) for knee extension in one of young and older participants, respectively

Table 3 Intraclass correlation coefficients (ICC) with 95 % confident intervals (CI) and coefficients of variation (CV) for all variables of interest

	ICC (95 % CI)	CV
KE MVIC (Nm)	0.98 (0.95–0.99)	3.12
KF MVIC (Nm)	0.96 (0.93–0.98)	4.71
RTD (Nm/s)	0.90 (0.82–0.94)	7.44
VL activation (mV)	0.72 (0.54–0.84)	10.26
BF co-activation (%)	0.71 (0.52–0.83)	12.09
MDF (Hz)	0.52 (0.27–0.71)	4.60
Muscle thickness (mm)	0.90 (0.82–0.94)	2.04
Lf (mm)	0.80 (0.67–0.89)	3.31
(°)	0.84 (0.73–0.91)	2.65
MAS (N/m)	0.85 (0.73–0.91)	9.82
MS (N/m)	0.75 (0.58–0.85)	3.22

KE knee extensors, *KF* knee flexors, *MVIC* maximal voluntary isometric contraction, *RTD* rate of torque development, *VL* vastus lateralis, *BF* biceps femoris, *MDF* median frequency, *Lf* fascicle length, pennation angle, *MAS* musculo-articular stiffness, *MS* muscle stiffness

MVIC and RTD

As expected, young participants were stronger than older participants both for knee extension and flexion. In accord with previous studies (Lindle et al. 1997; Akima et al. 2001; Ditroilo et al. 2010), a lower maximal strength during knee extension and flexion was observed in the elderly people with a more evident age-related decline detected in males compared with females. Ageing has been also reported to be associated with decrease in the explosive power output (De Vito et al. 1998; Macaluso and De Vito 2003). When considering the same muscle group, a diminution in the ability to rapidly produce force seems to occur to a greater extent than a diminution in muscle strength. Indeed, in the present study, older participants exerted a slower contractile RTD for KE in comparison to young participants with a more pronounced decline in males than females. Loss of muscle mass is likely the most significant factor contributing to the different age-related declines for MVIC and RTD observed in OM compared to OF. Janssen et al. (2000) have shown that males are characterized with greater muscle mass than females with greater loss of muscle mass with ageing. It is well known that contractile RTD is strongly related with MVIC (Klass et al. 2008; Andersen et al. 2010); hence, presumably the different declines in males and females with advanced age are due to the greater absolute forces exerted by the YM than YF. The results of the present study have shown when RTD was expressed as %MVIC/s, there was no difference between young and older males and females, which would support previously published findings (Kent-Braun and Ng 1999; Thompson et al. 2014). Moreover, our results are comparable with the results of previous studies which have examined the age-related decline in maximal (i.e. MVIC) and rapid (i.e. RTD/rate of force development RFD) force generation capacity of different lower limb muscles: knee extensors (Ditroilo et al. 2010; Ditroilo et al. 2012), knee flexors (Thompson et al. 2013), ankle dorsi flexors (Klass et al. 2008) and ankle plantar flexors (Thompson et al. 2014). In absolute terms, a reduction in RTD is functionally relevant given that the ability to exert rapid force plays an important role in explosive muscle performance, which is essential for the proper execution of daily living activities, such as maintaining balance and walking ability (Häkkinen et al. 1998b; Aagaard et al. 2002).

Neural activation (surface EMG)

Loss of maximal and explosive force capability in older adults could also be ascribable to an age-related reduction in the firing frequency of motor units (MUs) and/or the number of recruited MUs (Brown et al. 1988; Doherty et al. 1993). It is clear that the surface EMG analysis does not allow for discrimination among the different factors potentially causing these neuromuscular alterations. However, it is reasonable to suggest an age-related decrement in neural activation based on observed relatively lower RMS and MDF of the surface EMG measured in the older compared to young participants which is in agreement with previous studies (Tracy and Enoka 2002; Bazzucchi et al. 2004; Billot et al. 2014). In particular, the lower MDF found during KE MVIC, in our older participants, is likely due to a decrement in the muscle fibre conduction velocity (Kupa et al. 1995; Merletti et al. 2002; Bazzucchi et al. 2004). This may potentially reflect a change in muscle fibre type towards a greater proportion of muscle fibre I types (Merletti et al. 2002). Another factor that can result in reduction in muscle strength relates to the possibility that OM and OF were not able to fully voluntarily activate their MU pool (Yue et al. 1999). On the other hand, when proper methodology is adopted and visual feedback is provided (as in the present study), healthy older individuals in their 6th and 7th decades should be able to fully activate their musculature as previously detailed (Macaluso and De Vito 2004). In addition, our study also showed that age has an equal effect in male and female participants for MDF and EMG RMS VL activation suggesting that age-related deteriorations in MUs activation were not sex dependent.

In the present study, we found that the co-activation level of antagonist muscle (i.e. KF) was not significantly different between young and older males and females during the execution of a maximal isometric knee extension test (i.e. KE MVIC). Similarly, Billot et al. (2014) with populations comparable to the present study and examining the same muscle groups did not find any age effect on co-activation level of the hamstring muscles. However, several previous studies investigating the same joint have shown contrasting results with the EMG antagonist co-activation level reported to be higher in older individuals when compared to their younger counterparts (Izquierdo et al. 1999; Macaluso et al. 2002). It has been suggested that, at least for the knee joint musculature, agonist-antagonist co-activation may serve to protect and stabilize the joint during forceful contraction (Baratta et al. 1988). Indeed, a greater level of antagonist co-activation can reduce the agonist muscle performance through the opposing mechanical movement of the antagonist muscles (Carolan and Cafarelli 1992) and also by reciprocal inhibition (Crone and Nielsen 1989). This discrepancy between results could be partly explained by the presence of adipose tissue. Since the co-activation is usually assessed using surface EMG amplitude measurements, it is important to assess the potential impact that adipose tissue covering the muscles of interest can have on the measure because of its well-known effect on the level of cross-talk among neighbouring muscles and the muscle under consideration (Solomonow et al. 1994; Kellis 1998). In our study when the effect of subcutaneous fat was not considered, a sex effect was observed indicating a higher level of co-activation in females than males. However, when the subcutaneous fat was included in the analysis (ANCOVA), the previously observed sex difference disappeared. Therefore, we suggest that the effect of subcutaneous fat should be taken into account in order to minimize/remove the aforementioned risk of cross-talk when using surface EMG amplitude as a measure of activation across different populations (Solomonow et al. 1994; Kellis 1998).

MA and tissue stiffness

Our results relative to MA are in agreement with some of previous studies that have observed age-associated changes in VL (Kubo et al. 2003b; Strasser et al. 2013). Muscle thickness and L*f* of older participants were observed to be 15.1 and 14.1 % smaller than those of young participants, respectively. On the other hand, we did not observe a difference in and this result is supported by the findings of previous studies (Karamanidis and Arampatzis 2005, 2006) but contradicts several other studies (Kubo et al. 2003a, 2003b). An explanation for the above inconsistencies may be due to the difference in the population considered in terms of age and sample size. Studying participants up to the 8th decade with a large sample size (121 participants) revealed an age-related decline in for VL (Kubo et al. 2003b), but a similar was observed by analysing participants up to the 6th decade with a smaller sample size (49 participants) (Karamanidis and Arampatzis 2006). Greater muscle thickness and can lead a given area of tendon or aponeurosis to link to more contractile tissues, which depends on the number of sarcomeres placed in parallel, hence generating higher relative force (Powell et al. 1984). In turn, longer fascicle length can exert faster shortening velocities and extend the length range of force development, which depends on the number of sarcomeres placed in series (Stafilidis and Arampatzis 2007; Strasser et al. 2013). Hence, our study would suggest that with age more sarcomeres are lost in series than in parallel. The more consistent drop with ageing observed for RTD with respect to KE MVIC found in the present study could bring consistency to the observed MA alterations (i.e. reduction in L*f*). Indeed, it has been often demonstrated that explosive force characteristics (i.e. RTD/RFD) may decrease during ageing to a greater magnitude than maximal isometric strength (Ditroilo et al. 2010; Ditroilo et al. 2012; Thompson et al. 2013). Therefore, the observed shorter L*f* in old muscle, by decreasing the shortening velocity, could represent an important factor contributing to the reduced RTD.

Other than MA, additional mechanical factors that may explain the present findings are the changes in tissue stiffness because of elastic deformation of a connective material/tissue during contraction (Ditroilo et al. 2011b). In agreement with the present study, a lower level of MAS at the knee joint has been reported in the older adults when compared to young adults (Ditroilo et al. 2012) and in young females when compared to males (Wang et al. 2015). Certainly, during the free-oscillation test when a brief perturbation is applied, a higher compliance of the musculo-articular system likely results in a greater amplitude of the ensuing oscillation which must be absorbed over a larger distance and/or time. The reduced stiffness implies a longer time necessary to take up the slack residing in the tendon (Watsford et al. 2010), which translates into a lower RTD (Wilson et al. 1994; Watsford et al. 2010). This may affect several actions of everyday living in the elderly populations (Ditroilo et al. 2012) and eventually increase the risk of soft tissue injuries (Butler et al. 2003). In line with previous studies, MS was also lower in older compared to the young participants (Ikezoe et al. 2012) and in young females compared to young males (Wang et al. 2015). During muscle contraction, the number of bonds between actin and myosin increases, making the muscle stiffer (Ford et al. 1981). Therefore, one factor to explain the age-related reduction in MS may be the loss of the sarcomeres in series due to the reduction of fibre length observed in older participants (Narici and Maganaris 2006). In functional terms, these MAS and MS decrement in older adults may have detrimental impacts on the ability to rapidly regain balance during sudden postural perturbations, as a more compliant tissue system may fail to resist sudden joint displacements quickly and effectively, hence, potentially increasing the risk of falls and more in general muscle performance deficits.

Conclusion

There are a number of limitations to this investigation. Firstly, it was a cross-sectional study and involved a limited number of participants. However, the large number of parameters considered made difficult to expand the number of participants without loading in excess the individuals. In addition, we believe that the observed reliability in the variables of interest was a good safeguard in respect to this sample size limitation. The second limitation was related to the fact that we did not assess muscle mass directly, which could influence the capacity of strength production. However, the muscle thickness was measured from the quadriceps muscle by ultrasonography, and this has been previously correlated with muscle strength (Ikezoe et al. 2012; Strasser et al. 2013) and cross-sectional area (Abe et al. 1997). Lastly, physical activity and functional assessment were not evaluated, although the history of strength/heavy exercise was determined by a questionnaire. But it is acknowledged that young females may tend to be more sedentary than males. As such, future study should include these relevant assessments. In summary, this study provided comprehensive information on the age-related neuromuscular and mechanical modifications affecting the neuromuscular system by comparing young and older adults of both sexes. All variables of interest displayed acceptable to excellent reliabilities. The present results showed that ageing led to a great deficit in the ability to perform maximal and rapid forceful contraction on the knee joint musculature with a more evident deficit observed in males. These findings are sustained by marked alterations both in mechanical and neuromuscular characteristics as evidenced by changes in neural activation of agonist muscles, MA and tissue stiffness, which may potentially impair the execution of motor tasks of older adults. Such information may assist in planning exercise interventions to counteract the age-associated reduction in muscle function of older healthy populations.

Acknowledgments The authors would like to acknowledge Dr. Lorena Nappa for her assistance with data collection. Rui Wu wishes to thank the UCD-CSC scheme supported by University College Dublin and Chinese Scholarship Council.

Compliance with ethical standards The study was approved by the University Human Research Ethic Committee, and all participants provided written informed consent to the procedures and aims of the study.

References

Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P (2002) Increased rate of force development and neural drive of human skeletal muscle following resistance training. J Appl Physiol 93:1318–1326. doi:10.1152/japplphysiol.00283.2002

Abe T, Kawakami Y, Suzuki Y, Gunji A, Fukunaga T (1997) Effects of 20 days bed rest on muscle morphology. J Gravit Physiol 4:S10–S14

Akima H, Kano Y, Enomoto Y, Ishizu M, Okada M, Oishi Y, Katsuta S, Kuno S (2001) Muscle function in 164 men and women aged 20–84 yr. Med Sci Sports Exerc 33:220–226

Andersen LL, Andersen JL, Zebis MK, Aagaard P (2010) Early and late rate of force development: differential adaptive responses to resistance training? Scand J Med Sci Sports 20: e162–e169. doi:10.1111/j.1600-0838.2009.00933.x

Baratta R, Solomonow M, Zhou BH, Letson D, Chuinard R, D'Ambrosia R (1988) Muscular coactivation. The role of the antagonist musculature in maintaining knee stability. Am J Sports Med 16:113–122

Bazzucchi I, Felici F, Macaluso A, De Vito G (2004) Differences between young and older women in maximal force, force fluctuations, and surface EMG during isometric knee extension and elbow flexion. Muscle Nerve 30:626–635. doi:10.1002/mus.20151

Billot M, Duclay J, Simoneau-Buessinger EM, Ballay Y, Martin A (2014) Is co-contraction responsible for the decline in maximal knee joint torque in older males? Age (Dordr) 36:899–910. doi:10.1007/s11357-014-9616-5

Bojsen-Moller J, Magnusson SP, Rasmussen LR, Kjaer M, Aagaard P (2005) Muscle performance during maximal isometric and dynamic contractions is influenced by the stiffness of the tendinous structures. J Appl Physiol 99:986–994. doi:10.1152/japplphysiol.01305.2004

Brown WF, Strong MJ, Snow R (1988) Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. Muscle Nerve 11:423–432. doi:10.1002/mus.880110503

Butler RJ, Crowell HP 3rd, Davis IM (2003) Lower extremity stiffness: implications for performance and injury. Clin Biomech (Bristol, Avon) 18:511–517

Carolan B, Cafarelli E (1992) Adaptations in coactivation after isometric resistance training. J Appl Physiol 73:911–917

Cohen J (1988) Statistical power analysis for the behavioral sciences. Lawrence Erlbaum, Hillsdale, NJ

Crone C, Nielsen J (1989) Spinal mechanisms in man contributing to reciprocal inhibition during voluntary dorsiflexion of the foot. J Physiol 416:255–272

D'Antona G, Pellegrino MA, Adami R, Rossi R, Carlizzi CN, Canepari M, Saltin B, Bottinelli R (2003) The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. J Physiol 552:499–511. doi:10.1113/jphysiol.2003.046276

De Vito G, Bernardi M, Forte R, Pulejo C, Macaluso A, Figura F (1998) Determinants of maximal instantaneous muscle power in women aged 50-75 years. Eur J Appl Physiol Occup Physiol 78:59–64

De Vito G, McHugh D, Macaluso A, Riches PE (2003) Is the coactivation of biceps femoris during isometric knee extension affected by adiposity in healthy young humans? J Electromyogr Kinesiol 13:425–431. doi:10.1016/S1050-6411(03)00061-0

Ditroilo M, Cully L, Boreham CA, De Vito G (2012) Assessment of musculo-articular and muscle stiffness in young and older men. Muscle Nerve 46:559–565. doi:10.1002/mus.23354

Ditroilo M, Forte R, Benelli P, Gambarara D, De Vito G (2010) Effects of age and limb dominance on upper and lower limb muscle function in healthy males and females aged 40-80 years. J Sports Sci 28:667–677. doi:10.1080/02640411003642098

Ditroilo M, Watsford M, De Vito G (2011a) Validity and inter-day reliability of a free-oscillation test to measure knee extensor and knee flexor musculo-articular stiffness. J Electromyogr Kinesiol 21:492–498. doi:10.1016/j.jelekin.2010.11.004

Ditroilo M, Watsford M, Murphy A, De Vito G (2011b) Assessing musculo-articular stiffness using free oscillations: theory, measurement and analysis. Sports Med 41:1019–1032. doi:10.2165/11591470-000000000-00000

Doherty TJ, Vandervoort AA, Taylor AW, Brown WF (1993) Effects of motor unit losses on strength in older men and women. J Appl Physiol 74:868–874

Ford LE, Huxley AF, Simmons RM (1981) The relation between stiffness and filament overlap in stimulated frog muscle fibres. J Physiol 311:219–249

Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R (2000) Aging of skeletal muscle: a 12-yr longitudinal study. J Appl Physiol 88:1321–1326

Greig CA, Young A, Skelton DA, Pippet E, Butler FM, Mahmud SM (1994) Exercise studies with elderly volunteers. Age Ageing 23:185–189

Häkkinen K, Alen M, Kallinen M, Izquierdo M, Jokelainen K, Lassila H, Mälkiä E, Kraemer WJ, Newton RU (1998a) Muscle CSA, force production, and activation of leg extensors during isometric and dynamic actions in middle-aged and elderly men and women. J Aging Phys Act 6:232–247

Häkkinen K, Kallinen M, Izquierdo M, Jokelainen K, Lassila H, Malkia E, Kraemer WJ, Newton RU, Alen M (1998b) Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people. J Appl Physiol 84:1341–1349

Hermens H, Freriks B, Merletti R, Stegeman D, Blok J, Rau G, Disselhorst-Klug C, Hagg G (1999) SENIAM European recommendations for surface electromyography: results of the SENIAM project. Roessingh Research and Development, Enschede, Netherlands

Ikezoe T, Asakawa Y, Fukumoto Y, Tsukagoshi R, Ichihashi N (2012) Associations of muscle stiffness and thickness with muscle strength and muscle power in elderly women. Geriatr Gerontol Int 12:86–92. doi:10.1111/j.1447-0594.2011.00735.x

Izquierdo M, Ibanez J, Gorostiaga E, Garrues M, Zuniga A, Anton A, Larrion JL, Häkkinen K (1999) Maximal strength and power characteristics in isometric and dynamic actions of the upper and lower extremities in middle-aged and older men. Acta Physiol Scand 167:57–68. doi:10.1046/j.1365-201x.1999.00590.x

Janssen I, Heymsfield SB, Wang ZM, Ross R (2000) Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. J Appl Physiol 89:81–88

Jubrias SA, Odderson IR, Esselman PC, Conley KE (1997) Decline in isokinetic force with age: muscle cross-sectional area and specific force. Pflugers Arch 434:246–253

Karamanidis K, Arampatzis A (2005) Mechanical and morphological properties of different muscle-tendon units in the lower extremity and running mechanics: effect of aging and physical activity. J Exp Biol 208:3907–3923. doi:10.1242/jeb.01830

Karamanidis K, Arampatzis A (2006) Mechanical and morphological properties of human quadriceps femoris and triceps surae muscle-tendon unit in relation to aging and running. J Biomech 39:406–417. doi:10.1016/j.jbiomech.2004.12.017

Kellis E (1998) Quantification of quadriceps and hamstring antagonist activity. Sports Med 25:37–62

Kent-Braun JA, Ng AV (1999) Specific strength and voluntary muscle activation in young and elderly women and men. J Appl Physiol 87:22–29

Klass M, Baudry S, Duchateau J (2008) Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. J Appl Physiol 104:739–746. doi:10.1152/japplphysiol.00550.2007

Kubo K, Kanehisa H, Azuma K, Ishizu M, Kuno SY, Okada M, Fukunaga T (2003a) Muscle architectural characteristics in women aged 20-79 years. Med Sci Sports Exerc 35:39–44. doi:10.1249/01.mss.0000043385.66520.09

Kubo K, Kanehisa H, Azuma K, Ishizu M, Kuno SY, Okada M, Fukunaga T (2003b) Muscle architectural characteristics in young and elderly men and women. Int J Sports Med 24: 125–130. doi:10.1055/s-2003-38204

Kupa EJ, Roy SH, Kandarian SC, De Luca CJ (1995) Effects of muscle fiber type and size on EMG median frequency and conduction velocity. J Appl Physiol 79:23–32

Langlois JA, Visser M, Davidovic LS, Maggi S, Li G, Harris TB (1998) Hip fracture risk in older white men is associated with change in body weight from age 50 years to old age. Arch Intern Med 158:990–996

Larsson L, Grimby G, Karlsson J (1979) Muscle strength and speed of movement in relation to age and muscle morphology. J Appl Physiol Respir Environ Exerc Physiol 46:451–456

Lindle RS, Metter EJ, Lynch NA, Fleg JL, Fozard JL, Tobin J, Roy TA, Hurley BF (1997) Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. J Appl Physiol 83:1581–1587

Macaluso A, De Vito G (2003) Comparison between young and older women in explosive power output and its determinants during a single leg-press action after optimisation of load. Eur J Appl Physiol 90:458–463. doi:10.1007/s00421-003-0866-7

Macaluso A, De Vito G (2004) Muscle strength, power and adaptations to resistance training in older people. Eur J Appl Physiol 91:450–472

Macaluso A, Nimmo MA, Foster JE, Cockburn M, McMillan NC, De Vito G (2002) Contractile muscle volume and agonist-antagonist coactivation account for differences in torque between young and older women. Muscle Nerve 25:858–863. doi:10.1002/mus.10113

Maden-Wilkinson TM, Degens H, Jones DA, McPhee JS (2013) Comparison of MRI and DXA to measure muscle size and age-related atrophy in thigh muscles. J Musculoskelet Nueronal Interact 13:282–290

Merletti R, Farina D, Gazzoni M, Schieroni MP (2002) Effect of age on muscle functions investigated with surface electromyography. Muscle Nerve 25:65–76

Narici MV, Maganaris CN (2006) Adaptability of elderly human muscles and tendons to increased loading. J Anat 208:433–443. doi:10.1111/j.1469-7580.2006.00548.x

Narici MV, Maganaris CN, Reeves ND, Capodaglio P (2003) Effect of aging on human muscle architecture. J Appl Physiol 95:2229–2234. doi:10.1152/japplphysiol.00433.2003

Nordander C, Willner J, Hansson GA, Larsson B, Unge J, Granquist L, Skerfving S (2003) Influence of the subcutaneous fat layer, as measured by ultrasound, skinfold calipers and BMI, on the EMG amplitude. Eur J Appl Physiol 89: 514–519. doi:10.1007/s00421-003-0819-1

Powell PL, Roy RR, Kanim P, Bello MA, Edgerton VR (1984) Predictability of skeletal muscle tension from architectural determinations in guinea pig hindlimbs. J Appl Physiol Respir Environ Exerc Physiol 57:1715–1721

Reeves ND, Maganaris CN, Narici MV (2003) Effect of strength training on human patella tendon mechanical properties of older individuals. J Physiol 548:971–981. doi:10.1113/jphysiol.2002.035576

Sinaki M, McPhee MC, Hodgson SF, Merritt JM, Offord KP (1986) Relationship between bone mineral density of spine and strength of back extensors in healthy postmenopausal women. Mayo Clin Proc 61:116–122

Solomonow M, Baratta R, Bernardi M, Zhou B, Lu Y, Zhu M, Acierno S (1994) Surface and wire EMG crosstalk in neighbouring muscles. J Electromyogr Kinesiol 4:131–142. doi:10.1016/1050-6411(94)90014-0

Stafilidis S, Arampatzis A (2007) Muscle-tendon unit mechanical and morphological properties and sprint performance. J Sports Sci 25:1035–1046. doi:10.1080/02640410600951589

Strasser EM, Draskovits T, Praschak M, Quittan M, Graf A (2013) Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. Age (Dordr) 35:2377–2388. doi:10.1007/s11357-013-9517-z

Thompson BJ, Ryan ED, Herda TJ, Costa PB, Herda AA, Cramer JT (2014) Age-related changes in the rate of muscle activation and rapid force characteristics. Age (Dordr) 36:839–849. doi:10.1007/s11357-013-9605-0

Thompson BJ, Ryan ED, Sobolewski EJ, Conchola EC, Cramer JT (2013) Age related differences in maximal and rapid torque characteristics of the leg extensors and flexors in young, middle-aged and old men. Exp Gerontol 48:277–282. doi:10.1016/j.exger.2012.10.009

Tracy BL, Enoka RM (2002) Older adults are less steady during submaximal isometric contractions with the knee extensor muscles. J Appl Physiol 92:1004–1012. doi:10.1152/japplphysiol.00954.2001

Wang D, De Vito G, Ditroilo M, Fong DT, Delahunt E (2015) A comparison of muscle stiffness and musculoarticular stiffness of the knee joint in young athletic males and females. J Electromyogr Kinesiol 25:495–500. doi:10.1016/j.jelekin.2015.03.003

Watsford M, Ditroilo M, Fernandez-Pena E, D'Amen G, Lucertini F (2010) Muscle stiffness and rate of torque development during sprint cycling. Med Sci Sports Exerc 42:1324–1332. doi:10.1249/MSS.0b013e3181ce509d

Wilson GJ, Murphy AJ, Pryor JF (1994) Musculotendinous stiffness: its relationship to eccentric, isometric, and concentric performance. J Appl Physiol 76:2714–2719

Wolfson L, Judge J, Whipple R, King M (1995) Strength is a major factor in balance, gait, and the occurrence of falls. J Gerontol A Biol Sci Med Sci 50:64–67

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

Yue GH, Ranganathan VK, Siemionow V, Liu JZ, Sahgal V (1999) Older adults exhibit a reduced ability to fully activate their biceps brachii muscle. J Gerontol A Biol Sci Med Sci 54:M249–M253