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Measurement of maximal isometric torque and muscle quality of the knee extensors and flexors in healthy 50 – 70y women.

Peter Francis<sup>1,2</sup>, Clodagh Toomey<sup>3,2</sup>, William Mc Cormack<sup>2</sup>, Mark Lyons<sup>2</sup> and Philip Jakeman<sup>2</sup>

<sup>1</sup>Department of Rehabilitation and Health Science, Leeds Beckett University, Leeds, United Kingdom

<sup>2</sup>Department of Physical Education and Sport Sciences, Faculty of Education and Health Sciences, University of Limerick, Limerick, Republic of Ireland.

<sup>3</sup>Faculty of Kinesiology, University of Calgary, Alberta, Calgary, Canada.

Correspondence

Peter Francis, School of Rehabilitation and Health Sciences, Leeds Beckett University, LS13HE, Leeds, United Kingdom.

E-mail: peter.francis@leedsbeckett.ac.uk

## Summary

Muscle quality is defined as strength per unit muscle mass. The aim of this study was to measure the maximal voluntary isometric torque of the knee extensor and flexor muscle groups in healthy older women and to develop an index of muscle quality based on the combined knee extensor and flexor torque per unit LTM of the upper leg. One hundred and thirty six healthy 50 – 70y women completed an initial measurement of isometric peak torque of the knee extensors and flexors (Con-Trex MJ; CMV AG, Dubendorf, Switzerland) that was repeated 7 days later. Subsequently, 131 women returned for whole and regional body composition analysis (iDXA™; GE Healthcare, Chalfont St Giles, Bucks., UK). Isometric peak torque demonstrated excellent within-assessment reliability for both the knee extensors and flexors (ICC range: 0.991 – 1.000). Test-retest reliability was lower (ICC range: 0.777 – 0.828) with an observed mean increase of 5% in peak torque (6.2 (17.2) N·m) on the second day of assessment ( $p < .001$ ). The relative mean decrease in combined isometric peak torque (-12.2%;  $p = .001$ ) was double that of the relative, non-significant, median difference in upper leg LTM (-5.3%;  $p = .102$ ) between those in the 5<sup>th</sup> and 6<sup>th</sup> decade. The majority of difference in peak isometric torque came from the knee extensors (15.1N·m,  $p < .001$  vs. 2.4N·m,  $p = 0.234$ ). Isometric peak torque normalised for upper leg LTM (muscle quality) was 8% lower between decades ( $p = .029$ ). These findings suggest strength per unit tissue may provide a better indication of age-related differences in muscle quality prior to change in LTM.

Keywords: peak torque, lean tissue mass, ageing, sarcopenia, reliability.

## Introduction

Muscle quality or specific torque is defined as strength per unit muscle mass (Lynch et al., 1999; Delmonico et al., 2007). Indices of muscle quality can contribute to the diagnosis of sarcopenia (Cruz-Jentoft et al., 2010; Fielding et al., 2011) and be used in the evaluation of therapeutic intervention to reduce physical frailty (Kukuljan et al., 2009, Rabelo et al., 2011). Magnetic resonance imaging (MRI) and Computed Tomography (CT) are considered gold standard imaging methods to estimate skeletal muscle because they can provide volume (and therefore mass) measures of skin, skeletal muscle, adipose tissue and attendant non-fat components (Mitsiopoulos et al., 1998). However, high financial and time costs, limited availability to equipment at some sites, concerns over radiation exposure and the specialised training required to use MRI and CT limits its

widespread use in research on large samples. Dual energy x-ray absorptiometry (DXA) is an attractive alternative imaging method which can distinguish fat, bone mineral and lean tissue. The main drawback is that DXA overestimates fat free mass when compared to MRI and CT (Delmonico et al., 2007; Maden-Wilkinson et al., 2013). This is mainly due to DXA's inability to differentiate between skeletal muscle and non-skeletal muscle fat free tissue components (Wang et al., 1996), such as interstitial adipose tissue and connective tissue, which tend to increase with age. Despite this, Delmonico et al. (2007) have reported a strong correlation between CT and DXA for the estimation of skeletal muscle tissue ( $r=0.88$ ,  $p<.001$ ). Furthermore, whole body scans require 7– 14 minutes, less operator training and expose the participant to a minimal dose of radiation.

The measurement of LTM requires participants to follow standardised pre-test procedures, an operator that has been trained in scan positioning and operation and as such, the measurement error in the assessment of LTM, resides primarily within the measurement device and the biological variability of the individual. In contrast, the measurement of a maximal voluntary contraction (MVC) to represent a given LTM can prove more challenging. Unlike a DXA scan, the measurement of volitional strength can be affected by factors extrinsic to the measurement device such as participant motivation, a learning effect during testing and verbal instruction by the tester.

Despite strength per unit muscle mass being the proposed definition of muscle quality, research groups have varied in the LTM and strength measures included in their indices of quality. Lynch et al. (1999) expressed knee extensor and flexor peak torque ( $30^\circ/s$ ) per kilogram total leg LTM, thus including the LTM of the triceps surae which does not contribute to the force being generated. Newman et al. (2003), Goodpaster et al. (2006) and Delmonico et al. (2009) have expressed knee extensor peak torque ( $60^\circ/s$ ) per kilogram total leg LTM, which means knee extensor torque is being divided by a LTM which includes the knee flexors and triceps surae. The identification of the most appropriate index of muscle quality when using MVC and LTM as indices of strength and muscle mass is required.

It has been suggested that older women have greater muscular dysfunction than older men, particularly in the leg muscle (Lynch et al., 1999). Recent statistics show healthy life expectancy lags two decades behind life expectancy (61.3 – 62.5y vs. 81.5 – 82.6y) for women in the EU (Rechel et al., 2013). The first aim of this study was to investigate test-retest reliability of knee extensor and knee flexor peak torque in the dominant limb as

measured from a MVC during repeated assessments separated by 7 days. A secondary aim was to express strength per unit LTM by dividing upper leg voluntary strength by upper leg LTM and furthermore, to assess its appropriateness as an index of muscle quality compared to existing literature.

## Methods

### Subjects and experimental procedures

#### *Isokinetic Assessment*

A convenience sample of 136 women aged 50 – 70y (mean age (SD): 60.2 (5.2) years, median height (IQR): 161.0 (7.5) cm, median mass (IQR): 67.1 (14.7) kg) were included in this study. Participants were recruited via email and word of mouth from the University of Limerick campus community and greater Limerick community. Participants received a full medical screening and physical examination prior to the assessment of a MVC. Those with clinical cardiovascular or musculoskeletal disease such as diabetes or severe osteoarthritis were excluded. After receiving a complete explanation of the procedures, benefits and risks of the study, all participants gave their written informed consent. This study was approved by the Research Ethics Committee of the University of Limerick (EHSREC 10-RA04).

#### *Body Composition*

From the convenience sample above (n=136), 131 women subsequently reported for a body composition assessment using DXA. This aspect of the study was approved by the Research Ethics Committee of the University of Limerick (EHSREC 09/18).

#### Assessment of MVC

The participants were tested during two identical sessions held 7 days apart. Laboratory temperature was between 22 - 24°C for all testing sessions. All measurements were recorded by the same exercise scientist to

avoid intertester variability. Warm up consisted of 5 minutes on a bicycle ergometer (Monark Ergomedic; 828E), at a workload of 40 watts in accordance with the methodology of Lindle et al. (1997). The knee extensors and flexors of the dominant limb (the limb used to kick a ball) were tested using a commercially available dynamometer (Con-Trex MJ; CMV AG, Dübendorf, Switzerland), which allows instantaneous isometric torque assessment. Participants were seated with a hip flexion angle of 110°. The back of the knee joint was on the edge of the seat with a knee angle of -60° from anatomical zero (180°), which has been demonstrated to be the angle of maximal isometric force generation (Thorstensson et al., 1976). The distal shin pad of the dynamometer was attached 4-5cm proximal to the medial malleolus using a velcro strap. Lever length (22.7 (2.3) cm) was recorded as in Lynch et al. (1999). Two seatbelts were applied across the chest and pelvis while velcro straps were applied to the mid-thigh to reduce extraneous movement during contractions. The dynamometer rotational axis was aligned with the lateral femoral condyle (knee joint axis of rotation). The absolute zero (point at which gravity is acting vertically) was recorded prior to each trial. Participants were instructed to perform 2 submaximal voluntary isometric contractions (50 and 75% of perceived maximum) prior to each test series as in Maffiuletti et al. (2007), with a 1 minute rest period in between. The participant then performed 3 MVC's of the knee extensors separated by 2 minutes of stationary rest. The participant was instructed to consistently produce their maximal force rapidly (as hard and as fast as possible in the sagittal plane) and to maintain that force for 3-4 seconds. Participants received a 5 second count down with a distinct emphasis on "Go". No overt verbal encouragement was provided due to the difficulty in standardising it for all participants (Perrin and Costill, 1993). Visual feedback of the instantaneous dynamometer torque was provided to the participants on a computer screen. This was used to show the participant the type of contraction required and also to provide encouragement to reach their maximum as in Bazzucchi et al. (2004). The participant then received 2-3 minutes of stationary rest to allow set up of the protocol to measure MVC of the knee flexors. To assess the knee flexors, the distal shin pad was removed and replaced along the posterior portion of the triceps surae; 4 -5cm above the medial malleolus. The participant was then instructed to repeat the procedure used for extension above whilst kicking back (flexion) in the sagittal plane.

#### Criterion Measures for Acceptance of MVC

An MVC produced a measure of isometric peak torque in a single effort which required >200ms and was sustained for ~250ms. Attempts not sustained for MVC (identified by an impact spike prior to 300ms), containing

an initial countermovement (identified by a visible drop/rise in the torque signal)  $>5$  N·m or with a non-linear time-torque trace (identified by a double movement) were disqualified and excluded from further analysis. Torque, position and angular velocity data were recorded at a sampling rate of 256 Hz. Maximal contractions which satisfied the inclusion criteria above were analysed for peak torque using the Con-Trex analysis software. Using the analysis tool the cursor was placed at the beginning of the first visible rise in the torque time trace and moved across until it met a horizontal phase of contraction. The software then displays peak torque in newton meters (N·m) and time to peak torque in seconds (s). The decision to analyse these files through the software and not through an exported excel file was made after a comparison of 20 files generated from 6 participants demonstrated an average coefficient of variance (CV) between methods of 0.3%. A paired samples t-test ( $t_{19} = -1.028$ ,  $p=0.317$ ) confirmed there was no statistically significant difference between the two methods.

#### Measurement of Lean Tissue Mass

To standardise test conditions and tissue hydration, participants were instructed to refrain from strenuous exercise in the 12 hour period before testing and to attend after an overnight fast. Participants consumed 500ml of water one hour prior to the scan and were instructed to void and defecate, if required, immediately prior to measurement. Height was measured to the nearest 0.1cm (SECA stadiometer) and body mass to the nearest 0.1kg (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.). Whole body and regional body composition were estimated by using DXA (iDXA™; GE Healthcare, Chalfont St Giles, Bucks., UK). Daily calibration of the scanner employed a phantom spine containing composites of bone, fat and lean tissue mass. Participants wore light clothing and removed all metal objects such as belts and jewellery. The scan measurements and analyses were taken using standard procedures recommended by the manufacture as in Leahy et al. (2013). Participants were instructed to remain as still as possible for the duration of the scan. In the event that participants were larger than the boundary of the scan, the right hand side of the body was scanned and results doubled. The ULBC research group was responsible for quality control, including DXA calibration, operator, scan position and database control.

## Segmentation of Upper Leg Lean Tissue Mass

The enCORE system software produced estimates of lean soft tissue, fat, and bone mineral content and density for both the whole body and specific regions. 12 appendicular sites were isolated from the head and trunk using DXA regional computer generated default lines, with manual adjustment, on the anterior view. Specific anatomical landmarks were used to segment each section of the appendicular lean tissue mass. The thigh, representing upper leg lean tissue mass was measured from the inferior side of the lesser trochanter until the tibio-femoral joint. The root mean square co-efficient of variance is 0.6% for repeated measures of whole body composition using iDXA (Leahy et al., 2013). The root mean square coefficient for the older adults (n=22) in this study was 0.7% and 2.3% for whole body and upper leg lean tissue mass respectively.

## Statistical Analysis

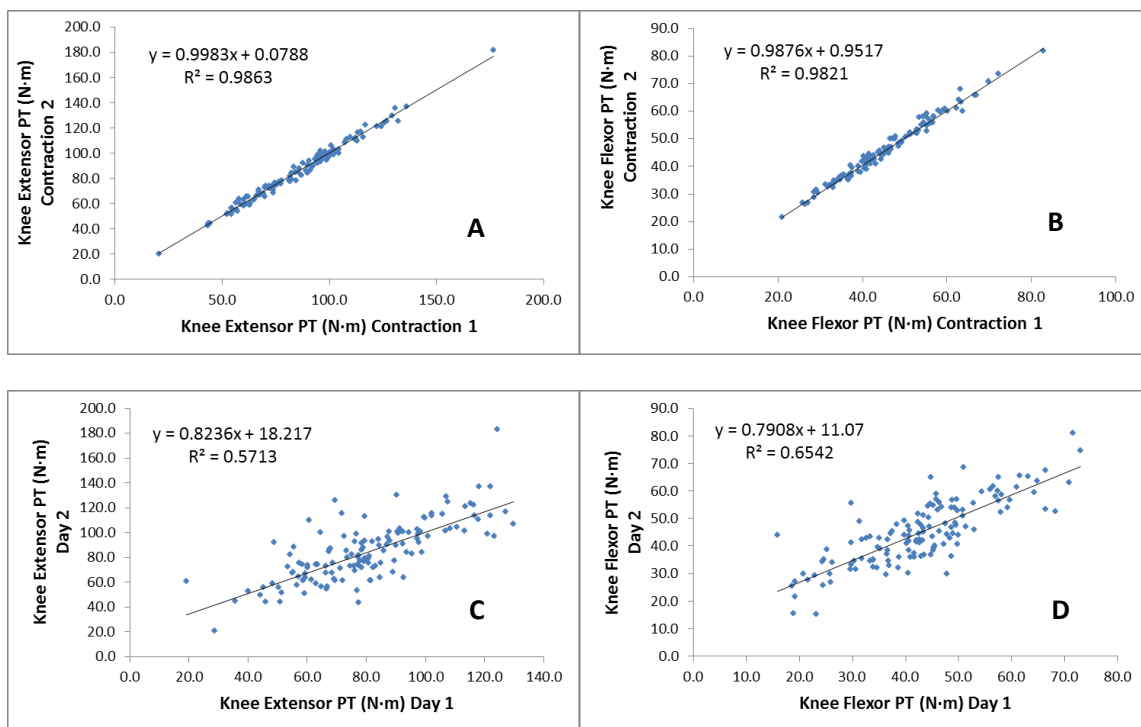
For peak torque, within and between assessment reliability were calculated. The coefficient of variance (CV) was used to assess the intra-subject variation between two measurements. For each subject, CV was calculated as:  $(SD \text{ of repeated measures} / \text{mean of repeated measures}) * 100$ . The intra class correlation coefficient (ICC) was used to assess relative reliability as it indicates the error in measurements as a proportion of total variance in measures. To assess differences in peak torque between days a Kolmogorov-Smirnov test was firstly conducted to assess whether variables were normally distributed. Mean and standard deviation (SD), median and interquartile range (IQR) and ranges are reported. Differences in peak torque between testing sessions were assessed using a paired sample t-test. The standard error of measurement (SEM) was calculated between days for the entire sample. This was used to calculate the smallest real difference (SRD):  $SRD = 1.96 * SEM * \sqrt{2}$  (Lexell et al., 2005). Linear regression was used to assess the variance in knee extensor and flexor peak torque explained by upper leg LTM and the variance in combined torque explained the knee extensors and flexors. Cross-sectional change in upper leg lean tissue mass, voluntary muscle strength and muscle quality were analysed using an independent sample t-test or a Wilcoxon signed rank test for normal and non-normal data respectively. Statistical analyses were performed using IBM SPSS statistics 19.0 for windows (SPSS, Inc., Chicago, IL).



## Results

### Within Session Reliability for Isometric Peak Torque

The reliability of estimate for peak torque of the knee extensors and flexors on day 1 of assessment is based on 121 participants who produced at least two contractions which satisfied the criteria for MVC. The CV for the knee extensors and flexors was 3 % in both cases with a range of 0-9%. There was an increase in the number of participants with repeated measures which satisfied the criteria for a MVC (knee extensors (n=9) and flexors (n=7)) on day 2 (CV 3%; range 0-6%). The intra-class correlations (ICC) on day 1 and day 2 for the knee extensors (0.993 and 1.006 respectively) and flexors (0.991 and 0.993 respectively) demonstrate excellent within day reliability. The intra-class correlations between testing sessions for knee extensors and flexors were 0.777 and 0.828 respectively. The within and between session reliability is graphically illustrated using X-Y scatter plots in Figure 1.



**Figure 1: Within session reliability of knee extensor (A) and knee flexor (B) peak torque and between session reliability of knee extensor (C) and knee flexor (D) peak torque.**

### Between Session Learning Effect in Isometric Peak Torque

Peak isometric torque as measured from a MVC performed on two occasions separated by 7 days are displayed in Table 1. There was a 5.1% (4.1N·m; CI 1.4 – 6.8) increase in knee extensor peak torque (79.9 (21.8) vs. 84.0 (23.8) N·m) between test days ( $t_{135} = 3.000$ ,  $p = 0.003$ ). A greater proportion of the sample ( $n= 79$ , 58%) had a higher peak torque (0.5 – 58 N·m) on day 2; the remainder of the sample ( $n=57$ , 42%) had lower peak torque (0.2 – 35 N·m). There was a 4.9% (2.1N·m; CI 0.9 – 3.4) increase in knee flexor peak torque (42.9 (11.7) vs. 45.0 (11.9) N·m) between test days ( $t_{135} = 3.4$ ,  $p<.001$ ). A greater proportion of the sample ( $n= 86$ , 63%) had a higher peak torque (0.2 – 28 N·m) on day 2; the remainder of the sample ( $n=50$ , 37%) had lower peak torque (0.1 – 18 N·m). The standard error of measurement for the sample ( $n=136$ ) between day 1 and day 2 was 1.4 N·m and 0.6 N·m for the knee extensors and flexors respectively. The smallest real difference was calculated as 3.9 N·m (4.7%) and 1.8 N·m (4.0%) for the knee extensors and flexors respectively. The 4.1N·m and 2.1N·m increase in knee extensor and flexor peak torque between days is therefore above the smallest real difference.

**Table 1: Peak torque, rate of torque development and time to peak torque in 50 – 70y women.**

	Session 1	Session 2	$\Delta$ (S2 – S1)
<b>Peak Torque (N·m)</b>			
<b>Knee Extensors (N·m)</b>	79.9 (21.8)	84.0 (23.8)	4.1 (16) ( $p=.003$ )
Min-Max	19.0 – 129.7	20.4 – 182.5	CI: 1.4 – 6.8
<b>Knee Flexors (N·m)</b>	42.9 (11.7)	45.0 (11.9)	2.1 (7.3) ( $p=.001$ )
Min-Max	15.9 – 73.0	15.1 – 81.0	CI: 0.9 – 3.4

Values are reported as Mean (SD), Median (IQR), Min-Max and CI (95% Confidence Interval). \*—non-normal data.

### Age-related Difference in Muscle Quality

Physical characteristics of those included in the cross-sectional analysis are displayed in Table 2. Age-related difference in upper leg LTM, peak isometric and muscle quality are displayed in Table 3. There was a median difference of 5.3% in upper leg lean tissue mass (3.8 (0.8) kg vs. 3.6 (0.2) kg) between those in the 5<sup>th</sup> and 6<sup>th</sup> decade, although this was not of statistical significance ( $p=0.102$ ). Peak torque of the knee extensors and flexors combined was 12.2% (17.5N·m;  $p=.001$ ) lower for those in the 6<sup>th</sup> decade. The majority of the difference in upper

leg peak torque was accounted for by the knee extensors (15.1 (3.8) vs. 2.4 (2.0) N·m); there was no statistically significant difference in the knee flexors (p=0.234). The knee extensors explained a greater proportion of the variance in combined peak torque than the knee flexors (90% vs. 60%, p<.001). Upper leg LTM accounted for ~15% (p<.001) of the variance in upper leg strength (Figure 2). Expressed as strength per unit tissue (N·m per kg upper leg LTM), muscle quality was 8% (3.0 N·m/kg; p=0.029) lower between decades. Expressed as knee extensor torque per kg upper leg LTM, muscle quality was 11.6% (2.9 N·m/kg; p=.004) lower for women in the 6<sup>th</sup> decade.

**Table 2: Physical characteristics of 50 – 70y Irish women.**

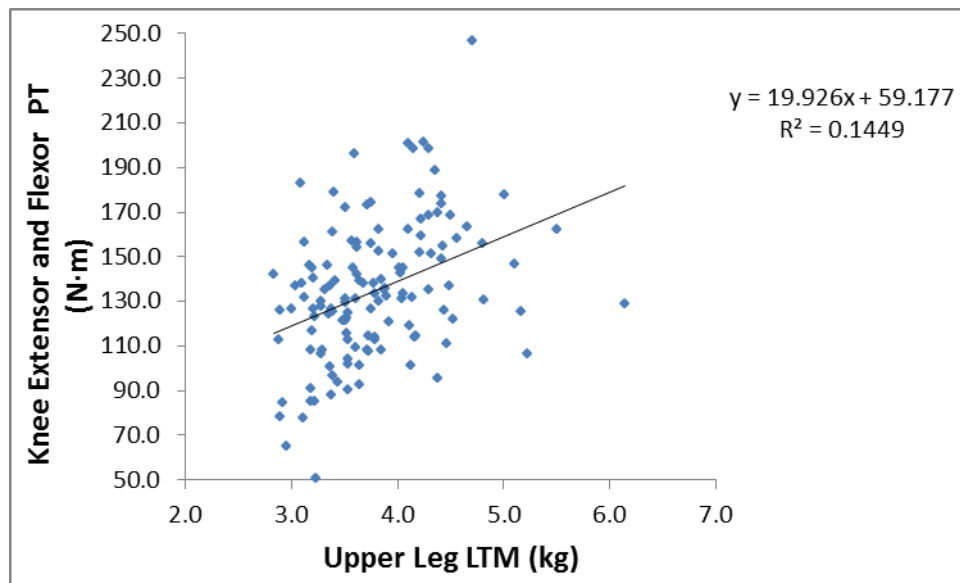
<b>Age Range (y)</b>	<b>50 – 70y</b>	<b>50 – 59</b>	<b>60 – 70y</b>
<b>Participants (n)</b>	n=131	n=62	n=69
<b>Age</b>	60.2 (5.2)	55.7 (2.8)	64.3 (5.2)
Median (IQR)	60.3 (8.5)	55.6 (4.8)	64.3 (4.4)
Min-Max	50.8 – 70.6	50.8 – 59.9	60.0 – 70.6
<b>Height</b>	162.7 (5.6)	163.0 (6.2)	162.5 (5.6)*
Median (IQR)	162.0 (6.9)	162.1 (7.2)	162.0 (6.6)
Min-Max	147.0 – 178.0	147 - 178	149.7 – 174.2
<b>Mass (kg)*</b>	69.5 (13.0)	71.0 (15.3)*	68.2 (13.0)*
Median (IQR)	66.6 (13.6)	66.8 (13.8)	66.6 (12.7)
Min-Max	46.7 – 125.7	51.3 – 125.7	46.7 – 102.0
<b>BMI (kg/m<sup>2</sup>)</b>	26.2 (4.8)	26.5 (5.9)*	25.8 (4.8)
Median (IQR)	25.3 (4.4)	25.6 (4.7)	25.3 (4.3)
Min-Max	18.9 – 50.4	18.9 – 50.4	20.2 – 39.8
<b>% Body Fat</b>	37.7 (7.0)	37.5 (8.1)	37.8 (7.0)
Median (IQR)	37.9 (8.2)	38.5 (9.7)	36.9 (8.1)
Min-Max	19.0 – 58.2	19.0 – 58.2	26.2 – 51.2

Values reported as Mean (SD), Median (IQR) and Min-Max. \*=non-normal distribution.

**Table 3: Age-related change in upper leg LTM, strength and muscle quality in healthy women (50 – 70y).**

	50-70y (n=131)	50-59y (n=62)	60-70y (n=69)	Δ 8.6y
LTM (kg)	39.8 (5.7) 32.6 – 58.3	40.0 (7.1)* 33.1 – 58.3	39.3 (5.3) 32.6 – 47.9	0.7; p=.152
Upper Leg LTM (kg)	3.7 (0.8) 2.8 – 6.1	3.8 (0.8) 2.9 – 6.1	3.6 (0.8)* 2.8 – 4.8	0.2; p=.102
Knee Extensors (N·m)	88.3 (23.1) 29.0 – 182.5	96.2 (24.3) 51.0 – 182.5	81.2 (23.1) 29.0 – 127.0	15.1 (3.8); p<.001 CI: 7.5 – 22.7
Knee Flexors (N·m)	46.5 (11.4) 19.0 – 81.0	47.8 (11.6) 23.0 – 81.0	45.4 (11.4) 19.0 – 74.8	2.4 (2.0); p=.234 CI: -1.6 – 6.3
Combined Torque (N·m)	134.8 (30.8) 50.7 – 246.5	144.0 (31.3) 77.9 - 246.5	126.6 (30.8) 50.7 – 198.7	17.5 (5.2); p=.001 CI: 7.2 – 27.7
Muscle Quality (Combined Torque N·m/kg)	35.8 (7.8) 15.7 – 59.3	37.4 (8.3) 20.3 – 59.3	34.4 (7.8) 15.7 – 50.1	3.0 (1.3); p=.029 CI: 0.3 – 5.6
Muscle Quality (Knee Extensor N·m/kg)	23.5 (6.0) 9.0 – 40.5	25.0 (6.5) 11.9 – 40.5	22.1 (5.1) 9.0 – 36.2	2.9 (1.0); p=.004 CI: 0.9 – 4.9

Values are reported as Mean (SD), Median (IQR), Min-Max and CI (95% Confidence Interval). \*=non-normal data.



**Figure 2: The relationship between upper leg LTM and upper leg isometric peak torque.**

## Discussion

Muscle quality has been defined as strength per unit muscle mass. The aim of this study was to measure the maximal voluntary isometric torque of the knee extensor and flexor muscle groups in healthy older women and to develop an index of muscle quality based on the combined knee extensor and flexor torque per unit LTM of the upper leg. A secondary aim was to identify the relative contributions of the knee extensors and flexors to age-related differences in muscle quality in a cross-sectional analysis between the 5<sup>th</sup> and 6<sup>th</sup> decade in healthy older women.

Test-retest reliability for the assessment of peak torque in young cohorts is well established using Biodex (Feiring et al., 1990, Lund et al., 2005), Cybex (Li et al., 1996), Kin Com (Arnold et al., 1993) and Contrex (Maffiuletti et al., 2007). However, dynamometers have been widely used in the assessment of age-related difference in muscle function during both cross sectional (Frontera et al., 1991, Lindle et al., 1997, Lanza et al., 2003, Bazzucchi et al., 2004, Pojednic et al., 2012), and longitudinal observations (Hughes et al., 2001, Newman et al., 2006). Despite this, considerably less work has been done in the assessment of the reliability of dynamometers relative to the older adults under investigation. In the present study, within assessment reliability for maximal voluntary isometric peak torque using the Con-Trex MJ dynamometer was high for both the knee extensor (0.993 and 1.006) and knee flexor (0.991 and 0.993) muscle groups. There was a statistically significant learning effect (5%) in the assessment of a maximal voluntary contraction between test day 1 and 2 which was equivalent to 50% of the observed age-related difference in peak isometric torque (12.2%) between the 5<sup>th</sup> and 6<sup>th</sup> decade. These findings highlight the importance of reducing potential learning effects prior to generating reference data in older adults and/or when evaluating therapeutic intervention where 5% may be a clinically significant change in strength. Researchers which have attended to test-retest reliability previously have done so with varying sample size and outcomes. Frontera et al. (1991) reported a learning effect in 200 older (45 – 78y) men and women, whereas Lindle et al. (1997) (n=10) and Tracy et al. (1999) (n=23) reported no difference in knee extensor and flexor peak torque in older men and women between test sessions held 5 – 10 days apart. Lanza et al. (2003) reported a learning effect in older (n=12) but not younger (n=12) dorsi-flexor peak torque. The contrasting findings between studies may in part be due to the variance in dynamometers used, sample size assessed and protocols employed. To the authors knowledge, this is the first study to report test-retest reliability on a robust

sample (n=136) of healthy older women (50 -70y) prior to accepting a measure of voluntary isometric peak torque.

Upper leg LTM accounted for ~15% of the variance in combined knee extensor and flexor peak torque providing further support for the hypothesis that muscle size and strength are not as closely linked as previously assumed (Goodpaster et al., 2001). In fact, the combined index of knee extensor and flexor peak torque declined at double the rate of the non-significant median decline in its representative upper leg LTM (12.2%,  $p=.001$  vs. 5.3%,  $p=.102$ ). These findings are consistent with the cross-sectional work of Kyle et al. (2001) who reported LTM (DXA) to remain stable in women up until ~60y and the longitudinal of Hughes et al. (2001) who found FFM (hydrodensitometry) to remain stable in women during a 9.4y follow up of men (n=53) and women (n=78) aged 46 – 80y at baseline. The decline in LTM, although not of statistical significance, was greater at the upper leg compared to the whole body (5.3% vs. 1.8%). The greater decline in limb-specific LTM is supported by Lynch et al. (1999) and Janssen et al. (2000) who reported a 4.9% and 5.7% per decade decline in total leg LTM and muscle mass between the 3<sup>rd</sup> and 6<sup>th</sup> decade using DXA and MRI respectively. The relatively static LTM at the whole body level in this study, combined with the findings of Kyle et al. (2001) and Hughes et al. (2001) suggest methods other than MRI and CT, may mask age-related decline in muscle mass. Furthermore, while the current study may have revealed the greatest age-related difference in LTM to occur at the upper leg; Delmonico et al. (2007) reported that compared to CT, DXA overestimated upper leg skeletal muscle by 1.8kg (5.1kg vs. 3.3kg) in older (60 (7.5) y) men at baseline and underestimated skeletal mass gains (2.9% vs. 3.9%) during 10 weeks of unilateral strength training of the knee extensors. These findings have since been confirmed in the examination of young and older thigh lean mass comparing MRI to DXA (Maden-Wilkinson et al., 2013). The consequence of the over-estimation of skeletal muscle mass by DXA is that LTM may remain constant when age-related reductions in strength are becoming apparent due to a reduction in contractile mass and an increase non-contractile mass with aging (Taffee et al., 2009). As muscle quality is represented as strength per unit mass, the age-related difference in muscle quality may be exaggerated by a constant LTM and falling muscle strength. MRI and CT are capable of identifying a reduction in contractile muscle mass and density and therefore the strength per unit mass difference may be less than observed with DXA.

The difference between the age-related difference in upper leg LTM and peak isometric torque suggests that muscle quality (strength per unit LTM) may provide an earlier insight into the development of sarcopenia and perhaps is more relevant to declining physical function than LTM alone, particularly when measured by DXA. Muscle quality expressed as strength per unit tissue was 8% lower for women in the 6<sup>th</sup> decade compared with those in the 5<sup>th</sup> decade. When compared to our data, Madsen et al. (1997) reported a similar age-related difference in combined knee extensor and flexor torque (12.2% vs. 13.6%) and muscle quality (8.0% vs. 10.3%). Similarly, based on an algorithm developed from 502 men and women (19 – 93y), Lynch et al. (1999) reported a 7.8% decline in muscle quality using total leg LTM to represent the combined index of concentric knee extensor and flexor peak torque in 50 – 70y women. One of the unique aspects of our study is the use of upper leg LTM as it was deemed to be more representative of the muscles assessed on the dynamometer. Many authors have chosen to express muscle quality using knee extensor peak torque per kilogram total leg LTM, which strictly speaking is not strength per unit tissue. The reasons for this may be explained by our results which appear to demonstrate greater variability in the muscle quality index when the knee flexors are included. The knee extensors account for a greater percentage of the variance in the combined torque measure (90% vs. 60%,  $p < .001$ ). Furthermore, the age-related difference in muscle strength between the 5<sup>th</sup> and 6<sup>th</sup> decade is driven by the knee extensors (15.1N·m,  $p < .001$  vs. 2.4N·m,  $p = 0.234$ ). Our results are in agreement with Frontera et al. (2008) who reported a 26% ( $p < .001$ ) decline in knee extensor torque compared to a non-significant decline in the knee flexors (6.8%,  $p < .38$ ) during the 7<sup>th</sup> decade. Furthermore, using MRI the authors shed light on the relative compartment changes. The anterior compartment (extensors) declined by 5.7% ( $p = .005$ ) while the posterior compartment (flexors) had a non-statistically significant 3.2% decline ( $p = 0.20$ ). Preferential atrophy in the anterior compartment of the upper leg has since been supported by studies using larger cohorts of older adults (Ogawa et al., 2012, Maden-Wilkinson et al., 2013) and is likely a contributing factor to preferential decline in knee extensor torque in the current investigation.

In light of the preferential difference in knee extensor torque, muscle quality expressed as knee extensor per kg upper leg LTM is 11.6% lower for the women in the 6<sup>th</sup> decade. When comparing our work to those using knee extensor strength only, our results are in agreement with Metter et al. (1999) who in pooling data (Lindle et al., 1997; Lynch et al., 1999) from the Baltimore Longitudinal Study of Aging (BLSA) reported a cross sectional difference in knee extensor strength (15%) and muscle quality (knee extensor strength per kilogram total leg

LTM) (11.2%) between women in the 5th and 6th decade. The women in the present study demonstrated a 15.7% and 11.6% difference between decades in knee extensor strength and muscle quality, respectively. The discrepancy in reporting the rate of change in muscle quality amongst existing literature is perhaps due to the segment of LTM chosen, the protocol for assessment of MVC and the care taken to reduce the learning effect in order to ensure a valid measure of maximum voluntary strength is obtained.

The limited data and mixed results from older cohorts, in addition to the significant learning effect (5%) witnessed in the present sample (n=136) of older women suggest caution in using a younger cohort to substantiate reliability of the Con-Trex dynamometer for use with healthy older women. The reliability of strength assessment procedures relative to the population under investigation must be substantiated before dynamometers can be used to legitimately contribute to the diagnosis of sarcopenia or assess the efficacy of therapeutic interventions. Future research should consider conducting a similar study with the addition of a third test day in order to determine whether the learning effect continues or is eradicated after two testing sessions. The finding of a greater difference in muscle quality (strength per unit tissue) than absolute LTM between the 5<sup>th</sup> and 6<sup>th</sup> decade provides further evidence that the loss of strength is somewhat greater than the loss of muscle mass with aging (Hughes et al 2001; Delmonico et al., 2009). However, the magnitude of difference must be interpreted cognisant of the cross-sectional study design and the fact that DXA overestimates skeletal muscle tissue compared to gold standard imaging techniques such as CT and MRI, which may inflate the muscle quality decline reported in our study. There is considerable variability in the indices of muscle or lean tissue mass and torque used to report muscle quality in older adults. Our results suggest that knee extensor torque is a more appropriate estimate of upper leg force generating capabilities and appears to account for the majority of age-related difference in muscle quality expressed as combined torque per kg upper leg LTM. The use of upper leg LTM to represent the force generating capacity of the upper leg appeared to be the most valid approach to the authors based on the definition of muscle quality but it does not appear to affect age-related differences compared to other studies using total leg LTM.

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## Conflicts of Interest

The authors have no conflict of interest.

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