The Human Muscle Size and Strength Relationship. Effects of Architecture, Muscle Force and Measurement Location.

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Title page

Title:
THE HUMAN MUSCLE SIZE AND STRENGTH RELATIONSHIP: EFFECTS OF ARCHITECTURE, MUSCLE FORCE AND MEASUREMENT LOCATION

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Abbreviated title for running head:
MUSCLE SIZE AND STRENGTH RELATIONSHIP

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Key words
Magnetic resonance imaging
Quadriceps femoris
Muscle volume
Physiological cross-sectional area

Abstract

Purpose: This study aimed to determine the best muscle size index of muscle strength by establishing if incorporating muscle architecture measurements improved the human muscle size-strength relationship. The influence of calculating muscle force, and the location of anatomical cross-sectional area (ACSA) measurements on this relationship were also examined.

Methods: Fifty-two recreationally active males completed unilateral isometric knee extension strength assessments and MRI scans of the dominant thigh and knee to determine quadriceps femoris (QF) size variables (ACSA along the length of the femur, maximum ACSA [$ACSA_{MAX}$] and volume [VOL]) and patellar tendon moment arm. Ultrasound images (2 sites per constituent muscle) were analyzed to quantify muscle architecture (fascicle length, pennation angle), and when combined with VOL (from MRI), facilitated calculation of QF effective PCSA ($_{EFF}$PCSA) as potentially the best muscle size determinant of strength. Muscle force was calculated by dividing maximum voluntary torque (MVT) by the moment arm and addition of antagonist torque (derived from hamstring EMG).

Results: The associations of $_{EFF}$PCSA (r=0.685), $ACSA_{MAX}$ (r=0.697), or VOL (r=0.773) with strength did not differ, although qualitatively VOL explained 59.8% of the variance in strength, ~11-13% greater than $_{EFF}$PCSA or $ACSA_{MAX}$. All muscle size variables had weaker associations with muscle force than MVT. The association of strength-ACSA at 65% of femur length (r=0.719) was greater than for ACSA measured between 10-55% and 75-90% (r=-0.042-0.633) of femur length.

Conclusions: In conclusion, using contemporary methods to assess muscle architecture and calculate $_{EFF}$PCSA did not enhance the muscle strength-size association. For understanding/monitoring muscle size, the major determinant of strength, these findings

support the assessment of muscle volume, that is independent of architecture measurements, and was most highly correlated to strength.

Introduction

Muscular strength, the maximum voluntary torque (MVT) a muscle group can produce, influences the performance of athletic events (1) and functional activities of daily life (2), is a risk factor for muscle injury (3), and is implicated in the development and progression of joint degeneration (i.e. osteoarthritis; (4, 5)). Knowledge of the factors underpinning strength and strength change are important in order to understand, assess/monitor and potentially modify the most important determinants. Whilst it is well known that muscle size is a key determinant of maximum strength (6–8), the most important muscle size determinant of strength is unclear. In fact the relationship of muscle size and strength may be influenced by both muscle size and strength measurements, with little investigation of the inclusion of muscle architecture assessment in the measurement of muscle size, the effect of calculating muscle force as the index of strength, which accounts for joint-level confounders (moment arm and antagonist co-activation), or the influence of muscle size measurement location (relative to segment length).

Theoretically, physiological cross-sectional area (PCSA) most accurately reflects the number of sarcomeres/myosin-actin cross-bridges arranged in parallel and thus able to generate tension between the tendons (9), and would be expected to be the strongest determinant of maximum strength (7, 10). However, studies comparing three common measures of muscle size have reported either maximum anatomical cross-sectional area ($ACSA_{MAX}$; (7)), muscle volume (VOL; (11)- elbow extensors), or PCSA ((11)- elbow flexors) to be the best correlate of strength. This inconsistency might be explained by the methodological limitations in the measurement of architecture and thus PCSA within these studies. Whilst $ACSA_{MAX}$ and VOL can be accurately assessed with T1-weighted magnetic resonance imaging (MRI), considered the gold standard technique (12), PCSA cannot be assessed directly by MRI alone. PCSA is typically calculated by dividing VOL (from MRI) by fascicle length (L_F), and further corrected

52 for the loss of force transmission to the aponeurosis/tendon in pennate muscle (i.e. pennation
53 angle, θ_P), sometimes referred to as effective PCSA ($_{EFF}$PCSA). Therefore, PCSA relies on
54 both the determination of VOL (via MRI) and the precise measurements of muscle architecture
55 (L_F and θ_P, via ultrasound).
56
57 Previous studies comparing associations between strength and different muscle size
58 indices (i.e. $ACSA_{MAX}$ vs. PCSA vs. VOL), have used the following muscle architecture (L_F
59 and θ_P) approximations for the calculation of PCSA: estimation from cadaveric data (7);
60 estimation based on muscle length ((11)– elbow flexors); or ultrasonographic measurements at
61 one site in one constituent muscle ((11)– elbow extensors). It is known that muscle architecture
62 varies both between and along constituent muscles of a muscle group (13, 14), therefore
63 performing multiple ultrasonographic measurements along the length of each constituent
64 muscle may better account for such variations. Furthermore, L_F has commonly been assessed,
65 and hence PCSA calculated, with relatively short ultrasound arrays that visualise ~30-50% of
66 L_F (40 mm array (15, 16); 45 mm (17); 60 mm (18)) and consequently, require prediction of
67 the remainder of the fascicular path in order to estimate L_F. This limitation can be minimized
68 by use of a longer ultrasound array, thereby reducing the proportion of overall fascicle length
69 that is extrapolated. Moreover, these issues in the assessment of L_F and θ_P, may provide a
70 methodological explanation for the variable findings as to the best muscle size index of
71 strength. It is conceivable that more careful architecture, and thus also PCSA, measurements,
72 could result in PCSA being the superior determinant of muscle strength and demonstrate the
73 utility of incorporating muscle architecture measurements within the assessment of human
74 muscle size. Finally, muscle thickness assessed with ultrasound imaging is often considered a
75 convenient, but somewhat crude index of muscle size. Perhaps surprisingly the relationship

between muscle thickness and strength has not been compared to that of MRI-derived measures of muscle size (ACSA, PCSA and VOL) and strength.

The theoretical basis for a close association of cross-sectional area measurements (ACSA and PCSA) and muscular strength, as opposed to VOL, is based on the assumption of longitudinal transmission of force along the muscle from the cross-section of greatest area and thus the largest amount of contractile material aligned in parallel. However, lateral force transmission may provide an alternative means of transferring force from intermediate points along muscle fibres (19), allowing a single muscle fibre to act as a series of independent force generators each able to transmit force to the aponeuroses/tendons via radial structural proteins, costameres, the sarcolemma and extra-cellular matrix (20); see Fig. 11 of reference (21). Lateral force transmission might favor a stronger relationship between VOL, which incorporates length, and strength compared to cross-sectional areas.

The assumption of predominantly longitudinal force transmission also underpins the notion that the best measure of ACSA in relation to strength is the cross-section with the greatest amount of contractile material in parallel (i.e. $ACSA_{MAX}$, usually calculated as the sum of maximum ACSAs from each constituent muscle, typically occurring at different points along the limb (7, 22, 23)). However, it has been suggested based on ultrasound measurements that for the quadriceps femoris (QF) muscle group relatively proximal ACSA, may be more strongly associated with strength (24) and strength gains after resistance training (25) than mid-muscle measurements. To date there has been no MRI study of ACSA at set intervals along the length of the muscle/bone in relation to strength or how location/length specific ACSA measurements compare to $ACSA_{MAX}$.

The association between muscle size variables (i.e. $_{EFF}$PCSA, $ACSA_{MAX}$ and VOL) and joint-level function (i.e. strength/MVT) may be somewhat diluted/confounded by joint neuromechanical factors such as the leverage (moment arm) of the agonist muscles, as well as co-activation and thus opposing torque from the antagonist muscles. It is possible that muscle size variables could be very highly correlated with muscle force, as is the case for isolated animal muscles (r=0.99; (8)), and thus explain a greater proportion of the variance in muscle force than is the case for joint-level MVT. However, this has not been investigated.

Therefore, the aim of this study was to determine the effect of incorporating muscle architecture measurements, calculating muscle force, and ACSA measurement location on the human muscle size-strength relationship. Specifically by comparing: (i) the relationships of four distinct QF muscle size measures ($ACSA_{MAX}$ and VOL from MRI, $_{EFF}$PCSA from the combination of MRI with ultrasound measurements of architecture, and muscle thickness from ultrasound) with knee extensor strength in a large cohort of healthy young men; (ii) the association of these muscle size measures with muscle force as opposed to joint-level MVT; and (iii) the relationship of strength to ACSA, according to the site of ACSA measurements along the femur. We hypothesised that: rigorous muscle architecture measurements (L_F and/or θ_P), at eight sites throughout the QF, in combination with high resolution MRI, would facilitate $_{EFF}$PCSA, rather than $ACSA_{MAX}$ or VOL, being the most powerful determinant of knee extension MVT; that higher associations would be found between muscle size indices with muscle force, than with MVT, once joint-level confounders were accounted for; and that the MVT-ACSA relationship would be influenced by the location of the ACSA measurement along the femur length.

Materials and Methods

Participants

Fifty-two young, healthy men (age 25 ± 2 years, height 1.76 ± 0.07 m, body mass 72 ± 9 kg) free from musculoskeletal injury provided written informed consent prior to participation in this study that was approved by the Loughborough University Ethical Advisory Committee. All participants were recreationally active (2200 ± 1355 metabolic equivalent min/wk; assessed with the short format International Physical Activity Questionnaire; (26)), were not completing any form of systematic physical training, and had not completed lower-body strength training for >18 months. Only male participants were included in order to prevent the potential confounding influence of sex differences in specific tension (force per muscle area: in vivo- (27, 28); and in vitro [i.e. single muscle fibre specific tension]- (29)) on the muscle strength and size relationships investigated in this study.

Overview

Participants completed a familiarisation session and two neuromuscular function measurement sessions of their dominant leg, 7-10 days apart at a consistent time of day (starting between 1200 and 1900), and an imaging session (within ±7 days of the second function measurement session). Familiarisation involved participants completing knee extension and flexion maximum voluntary contractions (MVCs) to become accustomed to these assessments. Function measurement sessions involved assessment of unilateral isometric knee extension strength (i.e. MVT) and antagonist co-activation (hamstrings EMG), as well as knee flexion MVCs for EMG normalization. Musculoskeletal imaging of the dominant limb involved acquisition of magnetic resonance T1-weighted axial plane images (1.5 T) of the QF to assess: ACSA along the length of each constituent muscle, VOL, and in combination with ultrasound-derived muscle architecture measurements, $_{EFF}$PCSA. Ultrasonographic images were recorded

at two locations along the length of each constituent muscle of the QF to assess L_F, θ_P, and muscle thickness using a 92-mm wide transducer that was typically able to visualise ~80-90% of total L_F (see Fig. 1A). Sagittal plane MRI scans of the knee were also acquired and analyzed to determine patellar tendon moment arm (PT_{MA}) which was used (along with antagonist EMG during knee extension) to calculate muscle force.

Torque recording

Knee extension and flexion torque was recorded whilst participants were seated on a rigid custom-made isometric dynamometer (see Fig. 6B of reference (30)) with knee and hip angles of 115° and 126° (180° = full extension), respectively. This knee joint angle was selected as the angle of peak torque (31). Extraneous bodily movement was minimized by fastening adjustable straps across the pelvis and shoulders. An S-beam strain gauge with a low baseline noise range (<0.1%MVT; Force Logic, Swallowfield, UK) mounted to the dynamometer was positioned posterior and perpendicular to the tibia and then secured around the participant's leg at ~15% of tibial length (distance from lateral malleolus to knee joint space) above the medial malleolus with an ankle strap (35 mm width reinforced canvas webbing). The analog force signal from the strain gauge was amplified (x370) and sampled at 2,000 Hz using an external analog-to-digital (A/D) converter (Micro 1401; CED, Cambridge, UK) and recorded with Spike 2 computer software (CED, Cambridge, UK). In offline analysis, force data were low-pass filtered at 500 Hz using a fourth-order, zero-lag Butterworth filter (32), gravity corrected by subtracting baseline force, and multiplied by lever length, the distance from the knee joint space to the center of the ankle strap, to calculate torque values.

176 *EMG recording*

177 Surface EMG was recorded from the medial and lateral hamstring muscles using a
178 wireless EMG system (Trigno; Delsys Inc., Boston, MA). Hamstring EMG measurements were
179 performed to allow estimation of muscle force during knee extension MVT (see "Moment arm
180 and calculation of muscle force" section of methods below). Prior to sensor placement skin
181 preparation (shaving, abrading, and cleansing with 70% ethanol) was conducted. Single
182 differential Trigno Standard EMG sensors (Delsys Inc., Boston, MA; fixed 1-cm interelectrode
183 distance) were then positioned on the medial (semitendinosus and semimembranosus) and
184 lateral (biceps femoris long head) hamstring muscles using adhesive interfaces at 45% of thigh
185 length (above the popliteal fossa). The location of the sensors was determined by palpating the
186 borders of the biceps femoris long head and the medial hamstrings (semitendinosus and
187 semimembranosus) respectively. Each sensor was then placed at 50% of the mediolateral
188 muscle width and parallel to the presumed orientation of the underlying fibres. The isometric
189 dynamometer had a section of the seat removed to accommodate the placement of Hamstring
190 EMG sensors on the skin without compression. EMG signals were amplified at source (x300;
191 20- to 450-Hz bandwidth) before further amplification (overall effective gain, x909), and
192 sampled at 2,000 Hz via the same A/D converter and computer software as the force signal, to
193 enable data synchronization. In offline analysis, EMG signals were corrected for the 48-ms
194 delay inherent to the Trigno EMG system.

195

196 *Knee extension maximum voluntary contractions.*

197 Following a brief warm-up of the dominant leg knee extensors [3 s contractions at 50%
198 (x3), 75% (x3), and 90% (x1) of perceived maximum] participants performed 3-4 MVCs with
199 the instruction to "push as hard as possible" for 3-5 s. MVCs were separated by ≥30 s of rest.
200 Biofeedback was provided by placing a horizontal cursor on the torque-time curve with a

horizontal cursor indicating the greatest torque obtained within that session, and verbal encouragement was provided during all MVCs. Knee extensor MVT was defined as the greatest instantaneous torque achieved during any MVC during that measurement session. RMS EMG from each of the hamstring sites during knee extension MVT (i.e. over a 500-ms time period, 250-ms either side of instantaneous knee extension MVT) was normalized to that measured during knee flexion MVT (knee flexion EMG_{MAX}; see below) and then averaged across the two hamstring sites (normalized antagonist HEMG). Knee extension MVT had a between-session within-participant coefficient of variation (calculated as: [SD ÷ mean] x 100) value of 3.0%.

Knee flexion maximum voluntary contractions

Knee flexion MVCs were then performed in the same manner as knee extension MVCs, following prior warm-up contractions. Knee flexion MVT was defined as the greatest instantaneous torque achieved during any MVC during that measurement session. RMS hamstring EMG for a 500-ms epoch at knee flexion MVT (250-ms either side) was analyzed for each site (knee flexion EMG_{MAX}); this approach was intended to capture muscle activity measures during the 500-ms period with the highest mean torque during the plateau phase of the 3-5 s MVC where torque is relatively stable.

Fascicle length, pennation angle and muscle thickness

$L_{F,}$ θ_P and muscle thickness of the constituent QF muscles (vastus lateralis (VL), vastus intermedius (VI) vastus medialis (VM), and rectus femoris (RF)) were measured using a B-mode ultrasonography machine (EUB-8500, Hitachi Medical Systems UK Ltd, Northamptonshire, UK) and a 92-mm, 5-10 MHz linear-array transducer (EUP-L53L) coated with water soluble transmission gel. To match the knee joint angle during MVCs, these images

were collected whilst participants sat at rest in the same isometric dynamometer and joint configuration used for maximum strength testing. Images were captured at rest at two sites along the length of each constituent muscle of the QF. Specifically, ultrasound images were recorded at 50% of superficial medio-lateral muscle width at the following locations along the length of the femur from the knee joint space: 30% and 50% of femur length (VI), 50 and 70% of femur length (VL); 20% and 40% of femur length (VM); 55% and 75% of femur length (RF). The locations of these ultrasound recordings were largely adopted from prior research incorporating multiple ultrasound measurements along the length of each constituent muscle of the QF (33). The transducer was positioned parallel to the long axis of the thigh and perpendicular to the skin, the position of the transducer was then subtly adjusted to align with the plane of the fascicles at each site so that an image with the deep and superficial aponeuroses and the trajectory of several fascicles was clearly identifiable with minimal pressure applied to the dermal surface. Video output from the ultrasound machine was transferred to a computer (via an S-video to USB converter) and images recorded using ezcap video capture software. Images were subsequently imported into public domain software and analyzed (Image J, v1.48, National Institutes of Health, Bethesda, USA).

θ_P at each individual recording site was measured as the angle of insertion of the muscle fascicles into the deep aponeurosis (Fig. 1A), except for the VI where θ_P was measured as the angle between the proximal end of each fascicle and the femur. L_F was measured as the length of the fascicular path between the insertions into the superficial and deep aponeurosis (Fig. 1A). When the fascicular path extended beyond the acquired image the missing portion of the fascicle was estimated by extrapolating linearly the fascicular path and the aponeurosis (18, 34). L_F and θ_P at each measurement site were taken as the mean of three individual fascicles. L_F and θ_P of each constituent muscle was averaged across the two measurement sites of that

muscle. Overall QF L_F and θ_P were calculated as the mean of the four constituent muscles. Muscle thickness at each measurement site (i.e. 2 per muscle) was quantified as the mean of the distance between the deep and superficial aponeurosis at each end, and the middle of the image before being averaged across sites within each muscle (Fig. 1A). Finally, the muscle thickness for each constituent muscle was summed to quantify overall QF muscle thickness.

MRI scan and analysis procedures for muscle size and patellar tendon moment arm

Muscle size variables. Participants reported for their MRI scan (1.5 T Signa HDxt, GE) having not completed any strenuous physical activity in ≥36 h, and had received prior instruction to arrive in a relaxed state having eaten and drunk normally. Upon arrival participants sat quietly for 15 min prior to their MRI scan. Participants lay resting supine at a knee joint angle of 163° (180° = full extension) whilst MR imaging was conducted. A receiver 8-channel whole body coil allowed axial T1-weighted images (time of repetition/time to echo 550/14, image matrix 512 x 512, field of view 260 x 260 mm, pixel size 0.508 x 0.508 mm, slice thickness 5 mm, inter-slice gap 0 mm) of the dominant leg to be acquired from the anterior superior iliac spine to the knee joint space in two overlapping blocks. Oil filled capsules placed on the lateral side of the thigh allowed alignment of the blocks during analysis (OsiriX software, Version 6.0, Pixmeo, Geneva, Switzerland). The constituent muscles (VM, VL, VI, and RF) were manually segmented in every third image (i.e. every 15 mm; Fig. 1B) starting from the most proximal image in which the muscle appeared. The number of images (slices) manually segmented along the length of each constituent muscle was (mean ± SD): VL, 24 ± 2 (range 21-28); VI, 24 ± 1 (range 21-28); VM, 23 ± 2 (range 20-26); RF, 22 ± 1 (range 20-25).

The ACSA values measured along the length of each muscle were expressed relative to femur length (defined by the number of axial slices between the proximal greater trochanter (100% femur length) and the knee joint space (0% femur length)). Cubic spline interpolation (1000 point; GraphPad Prism 6; GraphPad Software) was then used to quantify ACSAs for each constituent muscle at 5% intervals along the length of the femur, and overall QF ACSA at each 5% interval calculated by summation. QF $ACSA_{MAX}$ was calculated as the summation of the maximal measured ACSA from each constituent muscle and the location relative to femur length was also recorded.

The volume of each constituent QF muscle was calculated as the area under the ACSA-femur length curve following cubic spline interpolation, and the constituent muscle volumes summed for overall QF VOL. The $_{EFF}$PCSA of each individual QF muscle was calculated as: muscle volume (cm^3) divided by L_F multiplied by the cosine of θ_P. L_F and θ_P were mean values from two ultrasound measurement sites along the length of each individual QF muscle. Overall QF $_{EFF}$PCSA was calculated via the summation of the individual QF muscle $_{EFF}$PCSAs.

Moment arm and calculation of muscle force. Immediately after thigh imaging, a lower extremity knee coil was used to acquire sagittal images (time of repetition/time to echo 480/14, image matrix 512 x 512,field of view 160 x 160 mm, pixel size 0.313 x 0.313, slice thickness 2 mm, inter-slice gap 0 mm) of the knee joint. Sagittal plane images were used to determine PT_{MA}, the perpendicular distance from the patellar tendon line of action to the tibio-femoral contact point, which was approximately the mid-point of the distance between the tibiofemoral contact points of the medial and lateral femoral condyles (Fig. 1C). The tibio-femoral contact point was used as an approximation of the joint centre of rotation (35). Due to constraints in the size of the knee coil, sagittal images were acquired in an extended knee

300 position (~163°). PT_{MA} length was then corrected to that during MVC by estimating moment
301 arm at 115° from previously published data fitted with a quadratic function (36) scaled to each
302 participant's measured moment arm length at 163°. Internal muscle force was subsequently
303 calculated as follows: (external knee extensor MVT + estimated antagonist knee flexor torque)
304 ÷ corrected PT_{MA}. Antagonist knee flexor torque at knee extension MVT was estimated by
305 expressing the antagonist HEMG amplitude during knee extensor MVT relative to the knee
306 flexor EMG_{MAX} (normalized antagonist HEMG) and multiplying by the knee flexor MVT
307 (assuming a linear relationship between EMG amplitude and torque).
308
309 *Statistics*
310 All statistical analyses were conducted using SPSS Version 26.0 (IBM Corp., Armonk,
311 NY, USA), unless stated. Significance was defined as $P < 0.05$. MVT (knee extension and
312 flexion) and normalized antagonist HEMG from each of the duplicate test sessions was
313 averaged for each participant to produce criterion values for the calculation of muscle force
314 and statistical analysis. Knee extension MVT, muscle force, QF size (VOL, $ACSA_{MAX}$,
315 $_{EFF}$PCSA, and muscle thickness), PT_{MA}, QF L_F and QF θ_P were tested for outliers using the
316 Grubbs' test, also referred to as the ESD method (extreme studentized deviate;
317 https://www.graphpad.com/quickcalcs/grubbs1/ (36)), and no outliers were detected. Values
318 presented for group level results are Mean ± SD. Data normality was assessed using the Shapiro
319 Wilk test. MVT was the only variable found to not be normally distributed and was transformed
320 (log 10) to meet parametric statistical testing requirements of normality prior to further
321 statistical analysis. Pearson's product moment bivariate correlations were conducted between
322 MVT / muscle force and the different measures of overall QF size (VOL, $ACSA_{MAX,\ EFF}$PCSA,
323 and muscle thickness). To statistically assess differences between Pearson's product moment
324 bivariate correlations (e.g. MVT vs. QF VOL compared to MVT vs. QF $ACSA_{MAX}$, MVT vs.

QF VOL compared to muscle force vs. QF VOL, or MVT vs. ACSA at 50% of femur length compared to MVT vs ACSA at 70% of femur length) an online resource (http://comparingcorrelations.org; (37) was used to implement Meng et al.'s (38) z test (two dependent groups [i.e., same group], overlapping [i.e. one variable in common], two-tailed test, alpha level= 0.05, confidence value= 0.95, null value= 0). Pearson's product moment bivariate correlations were also calculated between MVT and location specific QF ACSA measurement at 5% intervals between 10 and 90% of femur length. Between-participant coefficient of variation (CV_B) for all variables was calculated as follows: [cohort SD ÷ cohort mean] x 100. L_F and θ_P were compared between muscles (i.e. once mean values had been derived across two measurement sites within each muscle) using a one-way ANOVA with stepwise multiple comparison corrected least significant difference (LSD) post-hoc testing.

Results

Group level muscle strength, size and architecture

Knee extension MVT was 246 ± 42 Nm (range: 173-396 Nm; CV_B 17.3%) and QF muscle force was 5874 ± 960 N (range: 3886-8681 N; CV_B 16.3%), respectively. Whole QF $_{EFF}$PCSA, VOL, and $ACSA_{MAX}$ were 167 ± 19 cm^2 (range: 124-206 cm^2; CV_B 11.4%), 1838 ± 263 cm^3 (range: 1254-2573 cm^3; CV_B 14.3%), and 90 ± 12 cm^2 (range: 68-125 cm^2; CV_B 13.8%), respectively. QF muscle thickness was 92 ± 11 mm (range: 74-123 mm; CV_B 11.5%). Constituent QF muscle size variables are reported in Table 1.

Overall QF L_F was 106.6 ± 8.9 mm (range: 87.8-125.5 mm; CV_B 8.4%), and QF θ_P was 15.5 ± 1.8° (range: 12.2-19.0°; CV_B 11.5%). L_F (i.e. mean of 2 sites) differed between constituent QF muscles (ANOVA P<0.001). Specifically, VI L_F (98.7 ± 9.7 mm) was shorter than that of the VM (105.8 ± 15.2 mm), VL (113.1 ± 11.7 mm) and RF (108.9 ± 14.7 mm; LSD

350 0.001≤P<0.019) and VM L_F was shorter than that of VL (LSD P=0.019). L_F did not differ
351 between any other individual QF muscles (LSD 0.211≤P≤0.221). θ_P differed between
352 constituent QF muscles (One-way ANOVA P<0.001). Post-hoc testing revealed that θ_P
353 differed between all individual QF muscles (LSD [all] P<0.001; VL 16.0 ± 3.2°; VI 13.4 ±
354 3.3°; VM 19.2 ± 3.9°; and RF 13.5 ± 2.6°) except between VI and RF (LSD P=0.814). PT_{MA}
355 was 4.41 ± 0.30 cm (range: 3.65-5.16 mm; CV_B 6.7%).
356
357 *Correlation of muscle size variables with maximum voluntary torque and muscle force.*
358 MVT was correlated with all four QF size variables with the bivariate correlation
359 between MVT and VOL producing the highest r-value (r=0.773, P<0.001), followed by
360 $ACSA_{MAX}$ (r=0.697, P<0.001), $_{EFF}$PCSA (r=0.685, P<0.001) and muscle thickness (r=0.406,
361 P=0.003; Fig. 2A-D). Statistical comparisons revealed no differences between the bivariate
362 correlation coefficients of MVT and MRI-derived measures of muscle size: MVT with VOL
363 or $ACSA_{MAX}$ (z=1.614, P=0.107), MVT with VOL or $_{EFF}$PCSA (z=1.555 , P=0.120), MVT
364 with $ACSA_{MAX}$ or $_{EFF}$PCSA (z=0.151, P=0.880). However, correlation coefficients between
365 MVT with VOL (z=3.699, P<0.001), $ACSA_{MAX}$ (z=2.417, P=0.015), or $_{EFF}$PCSA (z=2.393,
366 P=0.017) were each greater than the correlation coefficient between MVT and muscle
367 thickness.
368 Muscle force was correlated with, or had a tendency to be correlated with, all four QF
369 muscle size variables but with lower correlation coefficients than for MVT (VOL r=0.627,
370 P<0.001; $ACSA_{MAX}$ r=0.598, P<0.001; $_{EFF}$PCSA r=0.575, P<0.001; and muscle thickness
371 r=0.269, P=0.054; Fig. 3A-D). The correlation coefficients produced for each muscle size
372 variable with muscle force were lower than for with MVT: VOL with muscle force or MVT
373 (z=3.306, P<0.001), $ACSA_{MAX}$ with muscle force or MVT (z=2.069 , P=0.039), $_{EFF}$PCSA with
374 muscle force or MVT (z=2.252, P=0.024), and muscle thickness with muscle force or MVT

(z= 2.275, P=0.023). In summary, muscle size variables explained 9.2 to 20.4% more of the variance in MVT than in muscle force. For context we also calculated the correlation between PT_{MA} and MVT finding a weak, significant relationship (r=0.285; P=0.041).

Anatomical cross-sectional area along the length of the femur

The $ACSA_{MAX}$ of each of the four constituent muscles occurred at 28.4 ± 3.6% (VM), 57.1 ± 5.1% (VL), 57.9 ± 4.3% (VI) and 68.2 ± 5.7% femur length (RF; Fig. 4A). Considering location/femur length specific ACSA of the whole QF (i.e. sum of all 4 constituents at each femur length), the highest ACSA occurred at 55% femur length (71 ± 10 cm^2; Fig. 4B). As the association between muscle force and muscle size measures were weaker than that for MVT (see previous paragraph), only bivariate correlations between location specific ACSA measures and MVT were conducted. Significant correlations were observed between MVT and QF ACSA measurements at 25-85% of femur length, with the highest correlation (r= 0.719, P<0.001) occurring at 65% of femur length (i.e. more proximal than the position of highest location specific ACSA; Fig. 4C). In fact, ACSA measured at 65% femur length and two adjacent locations (60 and 70%) had marginally, but not significantly ($0.0688 \leq z \leq 0.4744$, $0.6352 \leq P \leq 0.9452$), higher correlation coefficients with MVT (r=0.701 to 0.719) than $ACSA_{MAX}$ (r=0.697). Statistical comparisons between correlation coefficients revealed that the association between MVT and ACSA at 65% of femur length was: greater than the association between MVT and ACSA at 10-45% of femur length ($1.979 \leq z \leq 4.457$, $0.001 \leq P \leq 0.048$) and 75-90% of femur length ($2.093 \leq z \leq 3.493$, $0.001 \leq P \leq 0.036$); and had a tendency to be greater than 50-55% of femur length (z= 1.711-1.843, P=0.065-0.087). The association of MVT-ACSA at 65% of femur length did not differ compared to the that between MVT-ACSA at 60% or 70% of femur length ($0.556 \leq z \leq 0.656$, $0.512 \leq P \leq 0.579$).

Discussion

This study examined the relationship between QF size measures (VOL, $_{EFF}$PCSA, $ACSA_{MAX}$ and muscle thickness) and both knee extensor strength (MVT) and internal muscle force in a large cohort of healthy young men, as well as the influence of ACSA location along the femur on the relationship with strength. Our first two hypotheses were refuted as incorporating thorough muscle architecture measurements when determining $_{EFF}$PCSA did not result in this index of QF size becoming the pre-eminent muscle size determinant of MVT, and muscle force was not more strongly associated with muscle size variables than joint-level MVT. In agreement with our final hypothesis the MVT-ACSA relationship was influenced by ACSA measurement location with stronger correlations at specific locations of 60-70% of femur length than for other locations.

Knowledge of the muscle size measurements underpinning strength and strength change are important in order to understand, assess/monitor and potentially modify the most important determinants. Surprisingly, in the current study determination of $_{EFF}$PCSA involving comprehensive MRI scanning along the length of the constituent QF muscles, and the incorporation of two ultrasonographic muscle architecture measurements per muscle did not result in $_{EFF}$PCSA being the highest correlate of MVT. In fact, comparisons of correlation coefficients revealed no difference between the association of $_{EFF}$PCSA, $ACSA_{MAX}$, or VOL with MVT, although qualitatively VOL explained 59.8% of the variance in MVT, which was ~11-13% greater than either $_{EFF}$PCSA (46.9%) or $ACSA_{MAX}$ (48.6%). As far as we are aware the current study involved the most thorough in vivo investigation to date with: (i) a large cohort (*n*=52 vs. *n*=19 (17), *n*=26 (11), *n*=39 (7)); (ii) a relatively homogenous cohort (low-moderate physically active males, as opposed to mixed sex and training status groups that might introduce other variables); (iii) duplicate measurements of knee extension strength on

two occasions with a highly reliable dynamometer and protocol (between-session within-participant coefficient of variation of 3.0% in the current experiment). In addition, specific to the $_{EFF}$PCSA measurements we took multiple ultrasonographic measurements at two sites for each constituent muscle with a long ultrasound array to provide a rigorous assessment of muscle architecture, as opposed to estimation from cadaveric data (7) or muscle length ((11)– elbow flexors), or single site ultrasound measurements ((11)– elbow extensors). In keeping with previous literature (39) this revealed that muscle architecture (θ_P and. L_F) differed between the constituents QF muscles indicating that all four muscles should be assessed to accurately determine $_{EFF}$PCSA of each muscle and thus also of the whole muscle group. Despite these attempts to improve the $_{EFF}$PCSA measurement, the current study provides robust evidence that with these techniques $_{EFF}$PCSA is not more strongly associated with strength than VOL or $ACSA_{MAX}$, and qualitatively VOL actually explained ~13% more variance than $_{EFF}$PCSA. Our finding that VOL was qualitatively the greatest correlate of muscular strength over cross-sectional area measurements ($_{EFF}$PCSA and $ACSA_{MAX}$) was consistent with one previous report ((11)– elbow extensors), but not others that found $_{EFF}$PCSA ((11)– elbow flexors) or $ACSA_{MAX}$ (7) to be pre-eminent, but these studies did not complete statistical comparisons of these associations. Only one previous report did a statistical comparison, also finding VOL to be a superior, but not statistically greater, determinant of muscular strength than ACSA (17). Overall, the differences between these three indices of muscle size ($_{EFF}$PCSA, $ACSA_{MAX}$, VOL) in predicting strength appear relatively subtle, but with 3 of 5 datasets indicating VOL maybe marginally superior.

The statistically equivalent but qualitatively weaker association of $_{EFF}$PCSA with strength, compared to VOL, appears contrary to classic physiological theory that $_{EFF}$PCSA best represents the number of actin-myosin cross bridges in parallel and able to generate tension

between tendons. There seem to be four possible explanations for this finding. Firstly, our ability to assess complex muscle architecture, and thus accurately determine $_{EFF}$PCSA, may be limited with the use of two-dimensional ultrasonography. Employing more sophisticated three-dimensional muscle architecture imaging techniques (i.e. diffusion tensor MRI) for the determination of $_{EFF}$PCSA seems a logical progression for future research. Secondly, it seems likely that VOL can be measured with less error than $_{EFF}$PCSA, given the calculation of PCSA involves the combination of multiple variables with the potential for accumulated measurement error that might reduce the association with MVT. Thirdly, classic physiology assumes purely longitudinal force transmission. However, evidence for the existence and importance of lateral force transmission has been documented in both amphibian myofibers (19) and mammalian whole muscle (40, 41). Therefore, lateral force transmission, potentially between fibres, fascicles and even constituent muscles (20, 42, 43) could mean that $_{EFF}$PCSA is not the only geometric determinant of contractile force production. Specifically, due to lateral force transmission the length and/or shape of a muscle/muscle group may also influence force production and explain why VOL qualitatively explained the largest amount of variance in muscular strength in the current study. Finally, whilst PT_{MA} was a weaker predictor of isometric strength in this study (r=0.285) than in some previous reports (17, 24, 44–46; r= 0.400 to 0.790), it is possible that VOL, which incorporates muscle length as well as cross-sectional area, may provide a (better) proxy for body size and PT_{MA}, potentially explaining the higher correlation of VOL with MVT than cross-sectional areas.

Muscle thickness measured with ultrasound was a statistically weaker correlate of MVT (r=0.406) than the MRI measures of muscle size (i.e. VOL, $ACSA_{MAX}$, or $_{EFF}$PCSA; r= 0.685-0.773). Similar (r= 0.411-0.470; (47, 48), and greater (r= 0.550-0.700 (49, 50)) associations between QF muscle thickness and muscular strength have been reported in the research

literature. However, these prior studies measured only one site per muscle and several measured only two of the four constituent QF muscles (47, 48, 50). Overall, the measurement of muscle thickness with ultrasound appears to be a substantially weaker determinant of isometric strength than MRI-based measures of muscle size, in this study explaining >30% less variance in strength (explained variance: 16.5% for MT vs 46.9-59.8% for MRI measures). Thus, while ultrasound measures of muscle thickness have the advantages of convenience and low cost they are significantly less informative about the functional capacity of the quadriceps and thus may have limited utility in comparing or monitoring individuals (i.e. during training, disuse or aging).

We had anticipated that the joint-level relationship between muscle size and strength may be limited by the influence of other neuromechanical factors (i.e. moment arm and antagonist activation/torque) that might dilute/confound this relationship, and thus removal of these factors would lead to a stronger relationship between muscle force and size variables. However, the relationships between muscle force and muscle size variables were consistently and statistically lower than for joint-level MVT (i.e. explaining 9.2 to 20.4% less variance). In essence contrary to our hypothesis removal of the confounding factors (moment arm and antagonist torque) weakened rather than strengthened the relationship of muscle size with force/torque. Given the convincing evidence for a very high correlation between $_{EFF}$PCSA and muscle force in isolated muscles (r=0.99; (8)), it seems our attempt to remove joint-level neuromechanical factors may have further confounded rather than distilled the relationship between muscle size and force/torque. This finding might question the validity of the moment arm and antagonist measurements used in the current study, or suggest accumulated measurement error when estimating muscle force via a calculation involving multiple variables. Furthermore, the calculation of muscle force has been used as an intermediate step

for the assessment of specific tension (which subsequently involves dividing muscle force by cross-sectional area; (51, 52)), and these results also query the validity of estimating specific tension in this way.

It is possible that the contemporary methods that we employed are not sufficient to accurately assess antagonist torque. Whilst we assessed activation of the largest of the knee flexors (medial and lateral hamstrings), only two/three of nine knee flexor muscles were measured; and antagonist torque was estimated based on an assumed linear relationship with EMG (22), and this relationship may also be confounded by cross-talk from other muscles (53). Improvements in the assessment of antagonist torque during knee extension seem warranted and this may require more comprehensive assessment of antagonist co-activation. For example, with the measurement of surface EMG of additional knee flexor muscles (i.e. the medial and lateral gastrocnemius) and more careful determination of the torque-EMG relationship for these muscles. In practice, however, antagonist torque was estimated as 5.3% of knee extension MVT, and thus appeared to be a relatively modest correction within the calculation of quadriceps muscle force. Given this modest influence of correcting for antagonist EMG on the muscle force calculation, this may place particular concern on the functional validity of the PT_{MA} measurement also used in the calculation of muscle force. Most contemporary studies, including the current investigation, typically measure PT_{MA} in resting conditions to ensure good image quality (i.e. no movement artefact), and at a relatively extended knee joint angle due to space constraints within the bore of an MRI scanner. However, it is known that PT_{MA} changes with contraction vs. rest (54) and joint angle (36). Although we corrected PT_{MA} values according to the differences in knee joint angle between the imaging and the strength measurements (52), it is possible that these confounding factors in the assessment of PT_{MA} may have compromised the precision of this measurement.

The relationship between MVT and ACSA in the present investigation was found to be systematically influenced by the location of the ACSA measurement relative to femur length, with r-values progressively increasing from 10% up to a peak at 65% of femur length (r=0.719) before gradually declining between 65% and 90% of femur length. Thus the location showing the largest association between MVT and ACSA (65% of femur length) was proximal of the location with the largest location specific ACSA of the whole quadriceps, which had a marginally lower correlation (55% of femur length, r=0.633). The correlation coefficient between MVT and ACSA measured at 65% of femur length was found to be greater than, or have a tendency to be greater than, all other ACSA measures along the femur other than those directly adjacent (i.e. ACSA at 60 and 70% of femur length). Additionally, the r-value for the association between MVT and ACSA at 65% femur length was also marginally superior, but statistically equivalent to that of $ACSA_{MAX}$ (i.e. sum of maximum ACSA from each constituent QF muscle, irrespective of location) and MVT (r=0.697). Therefore, this rigorous MRI assessment at locations all along the femur supports the earlier suggestion based on ultrasound measurements that proximal QF ACSA may be particularly important for the strength of this muscle group (24). One practical implication of our observation that ACSA at 65% of femur length had a similar association with MVT as $ACSA_{MAX}$ is that a single slice image at this one location, which is a quicker, cheaper and analytically less laborious than the imaging of the whole muscle needed to determine $ACSA_{MAX}$, may be as effective at predicting function.

It was notable that the location specific ACSA (65% of femur length) showing the highest correlation with MVT was also proximal to the $ACSA_{MAX}$ of all three vastii muscles (VM 28.4%; VL 57.1%; VI 57.9%) collectively comprising 87% of QF volume, with only the RF having a more proximal $ACSA_{MAX}$ (68.2%). We are not aware of any reason that the RF would have a disproportionate influence on knee extension strength. Alternative explanations

for this observation of proximal QF ACSA being most strongly related to strength may include the differences between the imaging and strength measurements, in terms of both the state of muscle contraction (resting for MRI vs. maximum contraction for MVT) and joint configurations (hip and knee close to the anatomical position for MRI scanning vs. flexed hip and knee at MVT for the isometric dynamometry). For example with the hip and knee flexed in the dynamometer it might be expected that all four constituents of the QF would move distally compared to the anatomical position in the MRI scanner (i.e. proximal muscle locations [~ 65% of femur length] in the scanner may be closer to mid-thigh when seated on the dynamometer). On the other hand, as the muscle contracts, even during an isometric contraction the fibres and fascicles shorten considerably. We have previously found fascicle length within the QF to shorten by 24% between rest and MVT, and as the muscle remains isovolumetric, cross-sectional area shows a corresponding increase ($_{EFF}$PCSA +27% from rest to MVT; (18)). In the QF this shortening of the muscle belly occurs in a non-symmetrical manner primarily due to lengthening of the distal connective tissues, as for example the deep VL aponeurosis at mid-thigh (50% femur length) has been shown to move ~17 mm proximally from rest to 80%MVT (55). Therefore, the substantial increase in cross-sectional area, from rest to MVT is likely accompanied by a proximal shift in the location of $ACSA_{MAX}$. In summary the way in which these potentially competing effects of contraction state and joint configuration combine to explain the apparent importance of proximal ACSA for isometric strength measurements is unclear.

The current study was not without its limitations and it is important to acknowledge them. The highly specific nature of the strength assessment (isometric contraction of the knee extensors at a knee joint angle of 115°) used in the current investigation means that the results presented cannot necessarily be generalized or assumed to be the same for other muscle groups,

575 modes of contraction (i.e. concentric or eccentric) or joint angles. Theoretically, as muscle
576 architecture is known to change substantially with contractile force (18) the most relevant
577 muscle architecture measurements for maximum contractile function would seem be those
578 measured at MVT. In practice, however, obtaining high quality architecture measurements at
579 MVT is highly challenging, and would not have been feasible for two sites of each of the four
580 individual muscles within the current study. Furthermore, based on our prior work the use of
581 architecture measurements made during MVCs, compared to resting measurements, did not
582 enhance the relationship between knee extension MVT and QF $_{EFF}$PCSA (18). Finally, the aim
583 of this study was to examine human muscle size-strength relationships and the influence of
584 some specific methodological considerations. We are conscious that other factors, beyond the
585 scope of this study, have also been suggested to influence strength (e.g. fibre type composition
586 (56) and agonist neuromuscular activation (57, 58)), and future work could examine
587 multifactorial determinants of strength.
588
589 In conclusion, despite incorporating comprehensive muscle architecture measurements
590 to enhance the determination of $_{EFF}$PCSA, statistical comparisons of correlation coefficients
591 revealed no differences between the association of $_{EFF}$PCSA, ACSA or VOL with MVT; and
592 VOL explained the highest variance in isometric knee extension MVT (~60%). This suggests
593 that with contemporary methods of muscle architecture measurements, $_{EFF}$PCSA offers no
594 advantage over purely MRI-derived indices of muscle size, and researchers interested in
595 understanding/explaining muscular strength may wish to use muscle volume as it does not
596 require additional architecture measurements and appears to explain marginally more variance
597 in strength. The location of ACSA measurements substantially effected the strength of the
598 association with MVT, with the highest association for ACSA measured at the relatively
599 proximal position of 65% of femur length. ACSA measured at this location was as strongly

associated with MVT as $ACSA_{MAX}$ despite requiring only a single slice/image in contrast to scanning the whole muscle for $ACSA_{MAX}$.

Grants

This study was supported financially by the Versus Arthritis Centre for Sport, Exercise, and Osteoarthritis (Grant reference 20194).

Acknowledgements

The authors thank the participants for their time in taking part in the study.

The authors declare no conflict of interest (financial or otherwise) and that the results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of this study do not constitute endorsement by the American College of Sports Medicine.

AUTHOR CONTRIBUTIONS

T.G.B., G.J.M., T.M.M.-W., and J.P.F. conception and design of research;

T.G.B., G.J.M., and T.M.M.-W. performed experiments;

T.G.B. and T.M.M.-W. analyzed data;

T.G.B. and J.P.F. interpreted results of experiments;

T.G.B. prepared figures;

T.G.B., and J.P.F. drafted manuscript;

T.G.B., G.J.M., T.M.M.-W., and J.P.F. edited and revised manuscript;

T.G.B., G.J.M., T.M.M.-W., and J.P.F. approved final version of manuscript.

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777

FIGURE CAPTIONS

Fig. 1 Representative: (A) ultrasound images of vastus lateralis (VL; 50% of femur length), vastus intermedius (VI; 50% of femur length), rectus femoris (75% of femur length), and vastus medialis (VM; 40% of femur length; 0% is knee joint space); (B) axial magnetic resonance image of the thigh; and (C) sagittal magnetic resonance image of the knee joint. TFCP, tibio-femoral contact point.

Fig. 2 Scatterplots showing the relationships between knee extension maximum voluntary torque (MVT) and quadriceps femoris size measures: (A) muscle volume [VOL]; (B) maximum anatomical cross-sectional area [$ACSA_{MAX}$]; (C) effective physiological cross-sectional area [$_{EFF}PCSA$]; and (D) muscle thickness. Solid lines indicate the trend of the relationship between variables.

Fig. 3 Scatterplots showing the relationships between quadriceps femoris muscle force and quadriceps femoris size measures: (A) muscle volume [VOL]; (B) maximum anatomical cross-sectional area [$ACSA_{MAX}$]; (C) effective physiological cross-sectional area [$_{EFF}PCSA$]; and (D) muscle thickness. Solid lines indicate the trend of the relationship between variables.

Fig. 4 Location specific anatomical cross-sectional area (ACSA) at 5% intervals along the length of the femur for (A) the constituent quadriceps femoris (QF) muscles and (B) overall QF muscle group. (C) Bivariate correlations between QF ACSA at 5% intervals along femur length and knee extension isometric maximum voluntary torque; 0%= distal, 100%= proximal. Significance of bivariate correlations are indicated as follows: * $P<0.05$, ** $P<0.01$, ***$P<0.001$. Data displayed in A and B are mean ± SD.

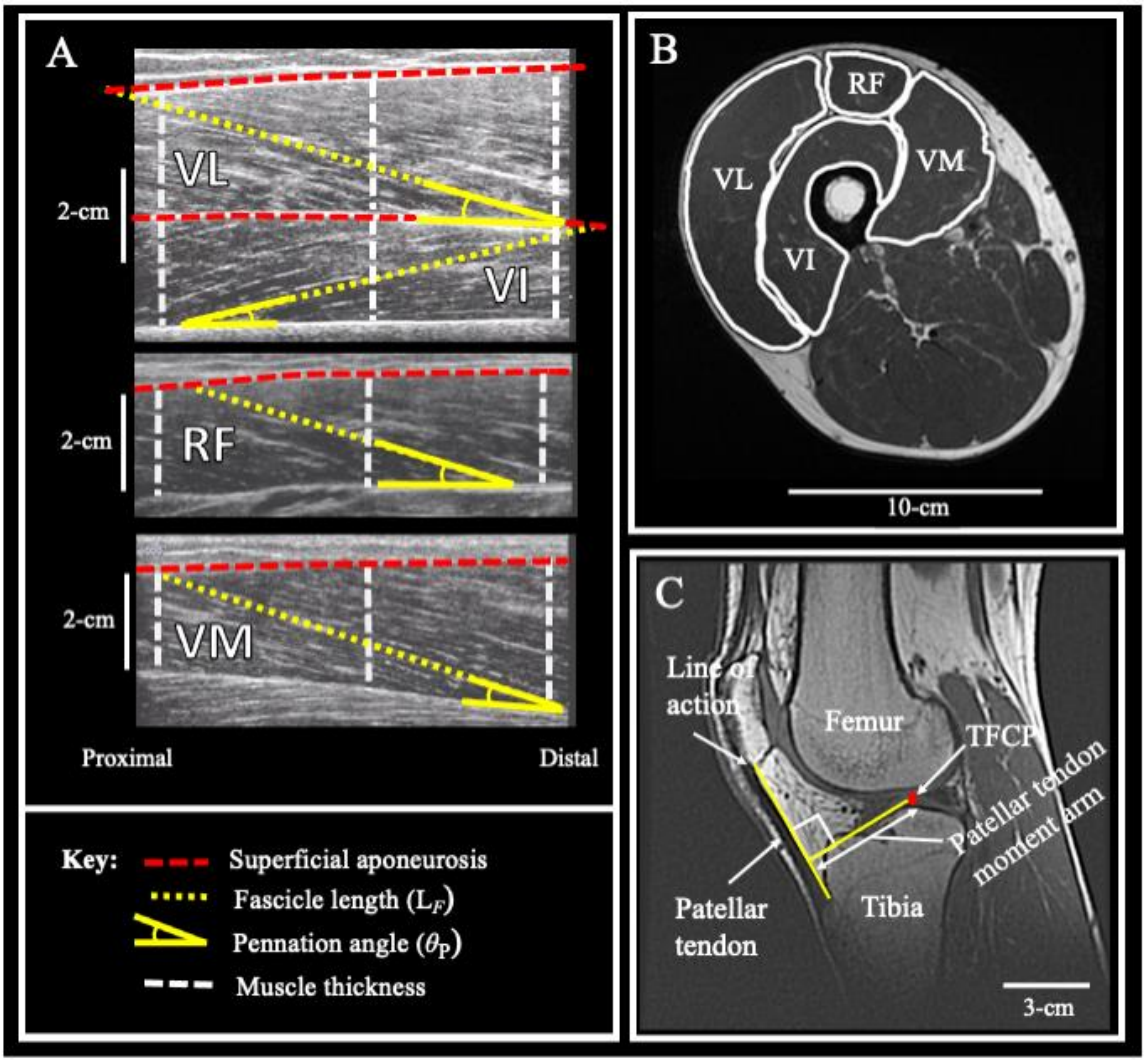
A
VL
VI
2-cm
RF
2-cm
VM
2-cm
Proximal
Distal
Key:
Superficial aponeurosis
Fascicle length (L_F)
Pennation angle (θ_P)
Muscle thickness
B
RF
VL
VM
VI
10-cm
C
Line of action
Femur
TFCP
Patellar tendon moment arm
Patellar tendon
Tibia
3-cm

Fig. 1

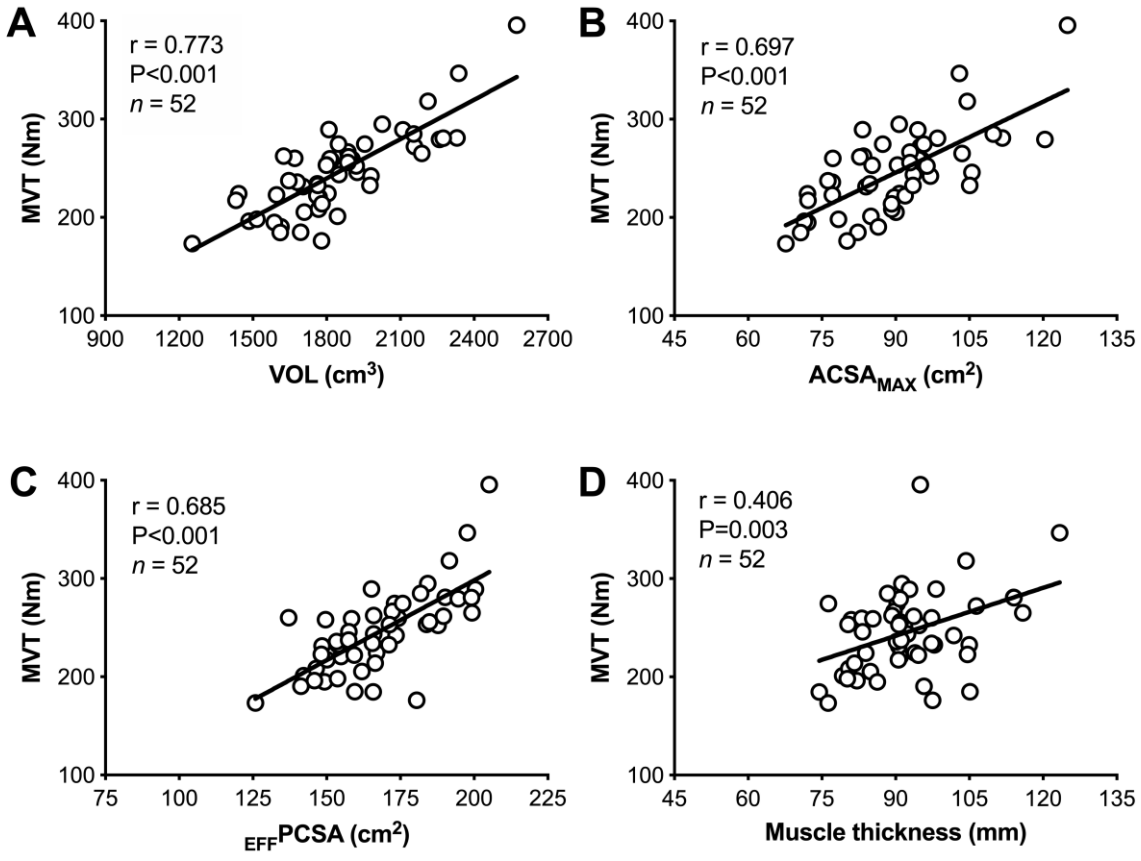
A
r = 0.773
P<0.001
n = 52
MVT (Nm)
VOL (cm3)
B
r = 0.697
P<0.001
n = 52
MVT (Nm)
ACSAMAX (cm2)
C
r = 0.685
P<0.001
n = 52
MVT (Nm)
EFFPCSA (cm2)
D
r = 0.406
P=0.003
n = 52
MVT (Nm)
Muscle thickness (mm)

Fig. 2

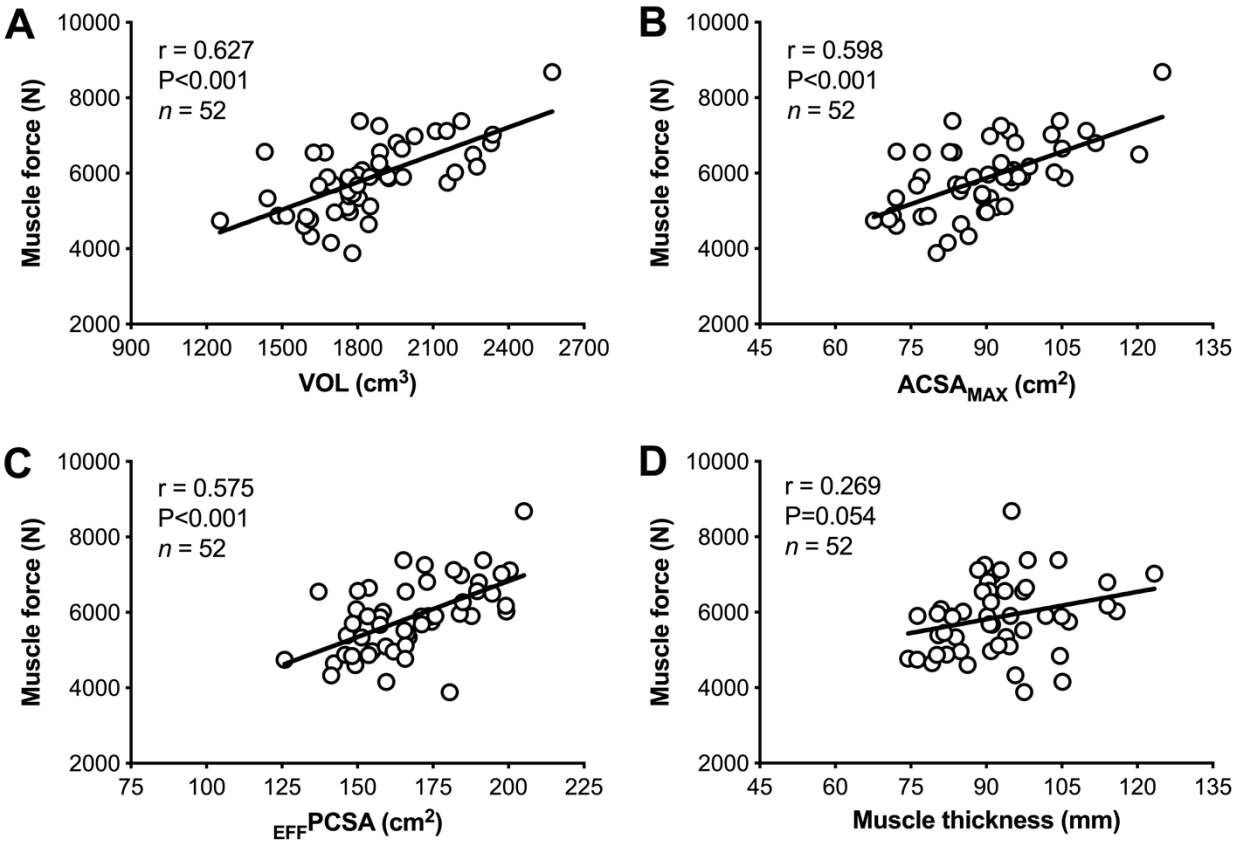
A
r = 0.627
P<0.001
n = 52
Muscle force (N)
VOL (cm3)
B
r = 0.598
P<0.001
n = 52
Muscle force (N)
ACSAMAX (cm2)
C
r = 0.575
P<0.001
n = 52
Muscle force (N)
EFFPCSA (cm2)
D
r = 0.269
P=0.054
n = 52
Muscle force (N)
Muscle thickness (mm)

Fig. 3

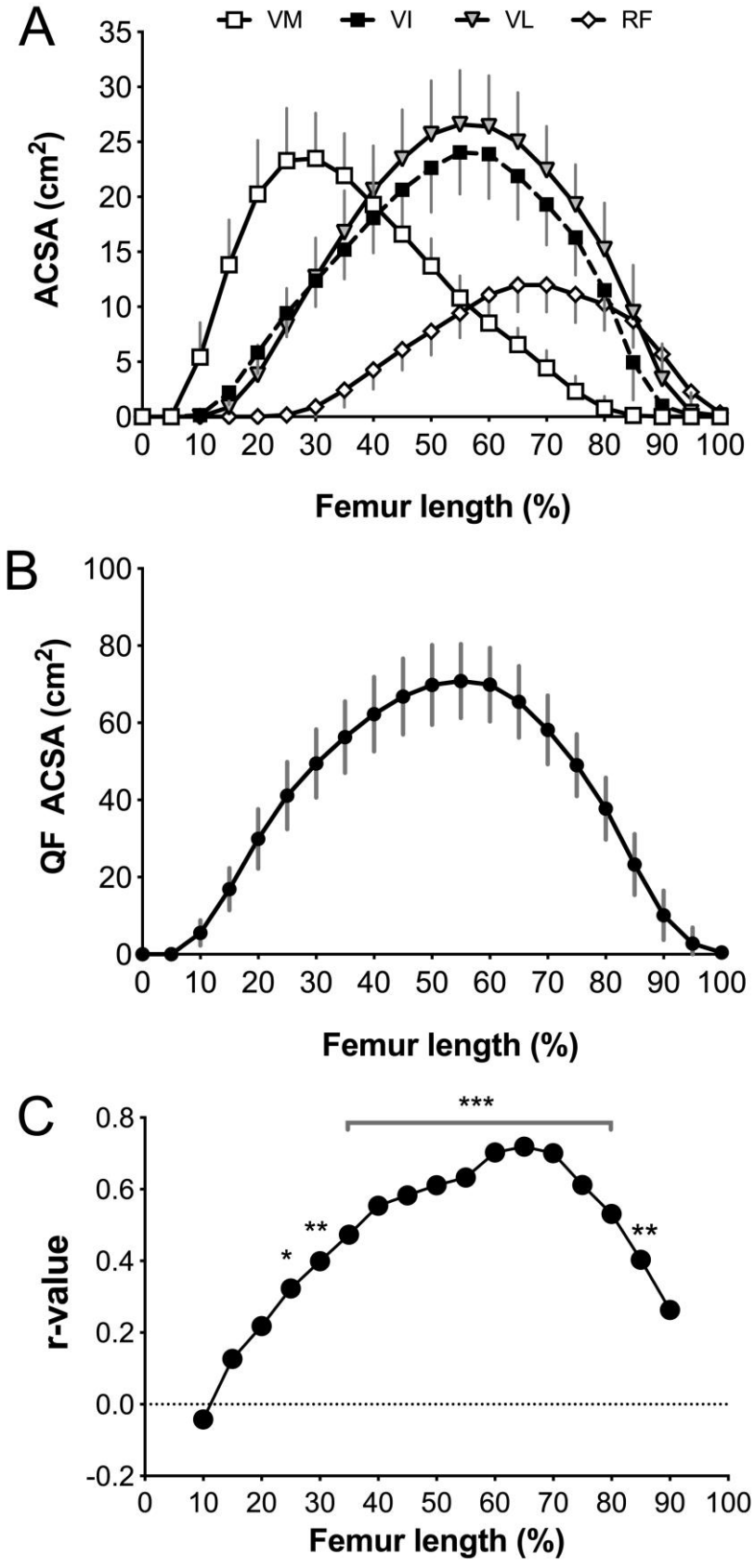
A
VM
VI
VL
RF
ACSA (cm²)
Femur length (%)
B
QF ACSA (cm²)
Femur length (%)
C
r-value
***
**
*
**
Femur length (%)

Fig. 4

TABLES

Table 1. Muscle size of overall and constituent quadriceps femoris (QF) muscles

	Muscle volume (cm^3)	$ACSA_{MAX}$ (cm^2)	$_{EFF}PCSA$ (cm^2)	Muscle thickness (mm)
VM	441 ± 68	25 ± 4	40 ± 7	26 ± 5
VI	547 ± 104	25 ± 4	54 ± 10	20 ± 4
VL	610 ± 98	28 ± 5	52 ± 8	25 ± 3
RF	240 ± 47	13 ± 2	21 ± 3	22 ± 3
QF	1838 ± 263	90 ± 12	167 ± 19	92 ± 11

Data are Mean ± SD. VM, vastus medialis. VI, vastus intermedius. VL, vastus lateralis. RF, rectus femoris.