The influence of patellar tendon and muscle-tendon unit stiffness on quadriceps explosive strength in man

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Running Title: rate of torque development and tissue stiffness

Abstract: The influence of musculotendinous tissue stiffness on contractile rate of torque development (RTD) remains opaque. This study examined the relationships between both patellar tendon (PT) and vastus lateralis (VL) muscle-tendon unit (MTU) stiffness, and voluntary and evoked knee extension RTD. Fifty-two healthy untrained males completed duplicate laboratory ses-sions. Absolute and relative RTD was measured at 50 Nm/25%MVT increments from onset and sequentially during explosive voluntary and evoked octet (supramaximal stimulation: [8 pulses at 300Hz]) isometric contractions. Isometric maximum voluntary torque (MVT) was also assessed. PT and MTU stiffness were derived from simultaneous force and ultrasound recordings of the PT and VL aponeurosis during constant RTD ramp contractions. Absolute and relative (to MVT and resting tissue length) stiffness (k) was measured over identical torque increments as RTD. Pearson's correlations tested relationships between stiffness and RTD measurements over matching absolute/relative torque increments. Absolute and relative PT k was unrelated to

equivalent voluntary or evoked RTD (r=0.020.255, P=0.069-0.891). Absolute MTU k was unrelated to voluntary or evoked RTD (r{less than or equal to}0.191, P{greater than or equal to}0.184), however some measures of relative MTU k were related to relative voluntary/evoked RTD (e.g. RTD25-50%MVT r=0.374/0.353, P=0.007/0.014). In conclusion, relative MTU k explained a small proportion of the variance in relative voluntary and evoked RTD (both {less than or equal to}19%), despite no association of absolute MTU k or absolute/relative PT k with equivalent RTD measures. Therefore the muscle-aponeurosis component, but not free tendon was associated with rela-tive RTD, although it seems an overriding influence of MVT negated any relationship of ab-solute MTU k and absolute RTD.

New Findings: What is the central question of this study? • Do tendon and/or muscle-tendon unit stiffness influence rate of torque development. What is the main finding and its importance? • Under our experimental conditions, some measures of relative (to maximum voluntary torque and tissue length) muscle-tendon unit stiffness had small correlations to volun-tary/evoked rate of torque development over matching torque increments. However, abso-lute and relative tendon stiffness were unrelated to voluntary and evoked rate of torque development. Therefore, the muscle-aponeurosis, but not free-tendon influences relative rate of torque development. Other factors more strongly determine rate of torque devel-opment than tissue

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New Findings (limit 100 words)

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ABSTRACT

The influence of musculotendinous tissue stiffness on contractile rate of torque development (RTD) remains opaque. This study examined the relationships between both patellar tendon (PT) and vastus lateralis (VL) muscle-tendon unit (MTU) stiffness, and voluntary and evoked knee extension RTD. Fifty-two healthy untrained males completed duplicate laboratory sessions. Absolute and relative RTD was measured at 50 Nm/25%MVT increments from onset and sequentially during explosive voluntary and evoked octet (supramaximal stimulation: [8 pulses at 300Hz]) isometric contractions. Isometric maximum voluntary torque (MVT) was also assessed. PT and MTU stiffness were derived from simultaneous force and ultrasound recordings of the PT and VL aponeurosis during constant RTD ramp contractions. Absolute and relative (to MVT and resting tissue length) stiffness (k) was measured over identical torque increments as RTD. Pearson's correlations tested relationships between stiffness and RTD measurements over matching absolute/relative torque increments. Absolute and relative PT k was unrelated to equivalent voluntary or evoked RTD (r=0.020.255, P=0.069-0.891). Absolute MTU k was unrelated to voluntary or evoked RTD ($r \leq 0.191$, $P \geq 0.184$), however some measures of relative MTU k were related to relative voluntary/evoked RTD (e.g. $RTD_{25\text{-}50\%MVT}$ r=0.374/0.353, P=0.007/0.014). In conclusion, relative MTU k explained a small proportion of the variance in relative voluntary and evoked RTD (both ≤19%), despite no association of absolute MTU k or absolute/relative PT k with equivalent RTD measures. Therefore the muscle-aponeurosis component, but not free tendon was associated with relative RTD, although it seems an overriding influence of MVT negated any relationship of absolute MTU k and absolute RTD.

INTRODUCTION

Explosive strength is the ability to increase torque from low or resting levels as quickly as possible (Maffiuletti et al. 2016). It is commonly examined under isometric conditions and expressed as the rate of torque development (RTD) derived from the rising phase (i.e. slope) of the contractile torque-time curve. Explosive strength is considered important in situations where the time to develop torque is limited: for instance in athletic activities such as sprinting and jumping (Tillin et al. 2013a); and in injury-related situations such as maintaining balance (Izquierdo et al. 1999, Robinovitch et al. 2002) or stabilizing joints (e.g. anterior cruciate ligament tears [ACL] in ≤50ms: Krosshaug et al. 2007; Koga et al. 2010) following mechanical perturbation. Further, RTD deficits have a deleterious impact on physical function in musculoskeletal patients (e.g. osteoarthritis: Maffiuletti et al. 2010; Winters & Rudolph, 2014). Developing a greater understanding of the determinants of RTD could therefore have potentially widespread functional and clinical implications.

During isometric contractions, the rate of skeletal muscle contractile force production is slowed by the necessity of the muscle to shorten in order to stretch the elastic components that transmit muscle force (Hill, 1951; Edman & Josephson, 2007). The mechanical stiffness (resistance to elongation) of the muscle-tendon unit (MTU) and particularly its tendinous tissue components (external 'free' tendon and aponeurosis) are therefore widely hypothesised to influence *in vivo* RTD (Wilson et al. 1994; Kubo et al. 2001; Reeves et al. 2003). Stiffer tissues are thought to provide greater mechanical resistance that can constrain muscle shortening during the initial stages of contraction thereby permitting muscle fibers to operate in the higher force region of the force-velocity relationship (Wilson et al. 1994). Moreover, the force transmission time of stiffer tissues is theoretically shorter (Waugh et al. 2013) and thus stiffer tissues may exert a substantial influence on explosive strength. In contrast, tissue elongation during the rising torque-time curve maybe sufficiently negligible and the duration of force transmission through connective tissues of such brevity (Nordez et al. 2009; DeWall et al. 2014), that the inter-individual differences in tendon/MTU stiffness could be practically irrelevant to the inter-individual variation in RTD.

There is currently no empirical evidence on the relationship of tendon stiffness and *in vivo* RTD and therefore this question needs to be investigated. The relationship between MTU stiffness and RTD has received some attention with mixed findings, ranging from no association to moderate positive correlations (Bojsen-Møller et al. 2005; Wang et al. 2012; Waugh et

al. 2013; Hannah & Folland, 2015), and a recent review highlighted the need for more rigorous investigations (Maffiuletti et al. 2016). Studies of musculotendinous tissue stiffness are mired by methodological shortcomings (Seynnes et al. 2015). In particular, the loading-rate sensitivity of stiffness measurements (i.e. faster rate yields greater stiffness: Lieber et al. 2000; Pearson et al. 2007; Theis et al. 2012; Kösters et al. 2014) necessitates a constant RTD during the ramp contractions used to measure stiffness. Nonetheless previous studies have invariably standardised contraction duration (Bojsen-Møller et al. 2005; Wang et al. 2012; Waugh et al. 2013; Hannah & Folland, 2015), which leads to different loading rates according to each individual's maximum voluntary torque (MVT) and may bias stiffness measurements to stronger individuals contracting at higher loading rates (Bojsen-Møller et al. 2005; Wang et al. 2012; Waugh et al. 2013; Hannah & Folland, 2015). This may explain why the relationship of MTU stiffness and explosive strength has been found to be dependent on the influence of MVT on both variables, with no independent relationship of RTD and MTU stiffness (Hannah & Folland, 2015). However the relationship between constant loading rate measurements of stiffness, either MTU or tendon, and RTD has not been investigated.

Tissue stiffness is also known to increase with torque (increasing gradient of the curvilinear torque-elongation relationship; Maganaris & Paul, 1999; Maganaris & Paul, 2002; Reeves et al. 2003). Yet MTU stiffness has typically been measured over a high torque increment (e.g. 50-90% MVT [Bojsen-Møller et al. 2005]; 50-100%MVT [Kubo et al. 2001; Wang et al. 2012]) even though RTD is usually measured from the lowest possible torque - rest (e.g. 0-50% MVT). Thus the relevance of high torque measures of stiffness to functional measurements starting from rest or low levels of torque, that are known to involve markedly lower stiffness properties, appears questionable. To avoid this dissociation between measured and functionally relevant stiffness, both variables could be measured over the same torque increment. Previous studies of MTU stiffness and RTD have also tended to incorporate diverse subgroups (e.g. tendinopathic and healthy limbs [Wang et al. 2012], children of different ages [Waugh et al. 2013], divergent athletic groups [Bojsen-Møller et al. 2005] adult males and females [Waugh et al. 2013; Hannah & Folland, 2015]) that are known to exhibit discrete characteristics that influence RTD (Maffiuletti et al. 2016) (e.g. pain, neuromuscular activation, maximum strength and muscle fiber type composition) and likely confound the relationship between tissue stiffness and RTD.

Any influence of tissue stiffness on explosive strength might be expected to be more pronounced for evoked contractions that drive the muscle at its maximum possible RTD (Deutekom et al. 2000; de Ruiter et al. 2004; Folland et al. 2014) and depend entirely on the characteristics of the MTU rather than the voluntary nervous system. The influence of tendon stiffness on evoked RTD has not been investigated and only Hannah & Folland (2015) have examined the relationship between MTU stiffness and evoked explosive strength, finding a stronger correlation for evoked than voluntary RTD.

The present study aimed to comprehensively examine the relationship between both tendon and MTU stiffness, with voluntary and evoked RTD measurements of explosive strength. All relationships between stiffness and RTD variables were examined over the same torque increment for both variables and in a large cohort of healthy young men, with duplicate measurement sessions that assessed stiffness with constant loading rate measurements. In addition to evaluating the relationship of absolute measures of stiffness and RTD, the association of relative measures was examined to remove any influence of maximum strength.

METHODS

Ethical Approval

The experimental testing procedures were explained to each participant and all participants provided written informed consent before their involvement in this study, which was approved by the Loughborough University Ethical advisory committee, and was conducted in accordance with the principles of the Declaration of Helsinki.

Participants

Fifty-two young men (age 25 ± 2 years, height 176 ± 7 cm, weight 72 ± 9 kg) who were healthy, free from musculoskeletal injury, and recreationally active (2160 ± 1309 MET minutes per week, International Physical Activity Questionnaire short format), but not involved in any form of systematic training in the prior 18 months were included in this study.

Experimental Design

Participants completed a familiarisation session, involving practice of all voluntary contractions performed during subsequent measurement sessions and habituation with evoked (electrically stimulated) contractions, followed by two duplicate measurement sessions separated by 7-10 days. Measurement sessions involved a series of unilateral isometric contractions of

the knee extensors of the dominant (preferred kicking) leg in the following order: maximum voluntary (MVCs) and explosive voluntary contractions, electrically evoked octet contractions (second measurement session only) and voluntary ramp contractions. Finally, knee flexor MVCs were also completed. Knee joint torque was recorded throughout contractions, and knee flexor surface electromyography (EMG) was recorded during knee flexor MVCs and knee extensor ramp contractions (to enable correction of knee extensor torque for antagonist activation). MVT was determined from MVCs, while voluntary and evoked RTD measurements of explosive strength were determined from explosive voluntary and evoked octet contractions, respectively. Ramp contractions were performed to permit tissue stiffness measurements from simultaneous torque and elongation, via ultrasound imaging, recordings. Measurement sessions with each individual were performed at a consistent time of the day, and all sessions started between 12:00-19:00 hours. Participants were instructed not to participate in strenuous physical activity or consume alcohol for 36 hours, and refrain from caffeine consumption for 6 hours, before measurement sessions. On a separate occasion, sagittal plane MRI images of the knee joint were acquired to measure patellar tendon (PT) moment arm in order to convert external torques to tendon force.

Torque Measurement

Participants were positioned in an isometric strength-testing chair with knee and hip angles of 115° and 126° (180° = full extension), respectively. Adjustable straps were tightly fastened across the pelvis and shoulders to prevent extraneous movement. An ankle strap (35 mm width reinforced canvas webbing) was placed ~15% of tibial length (distance from lateral malleolus to knee joint space) above the medial malleolus, and positioned perpendicular to the tibia and in series with a calibrated S-Beam strain gauge (Force Logic, Berkshire, UK). The analogue force signal was amplified (x370; A50 amplifier, Force Logic UK) and sampled at 2,000 Hz using an A/D converter (Micro 1401; CED, Cambridge, UK) and recorded with Spike 2 computer software (CED). In offline analysis, force signals were low-pass filtered at 500 Hz using a fourth order zero-lag Butterworth filter, 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.

Knee Flexor Electromyography (EMG)

Surface EMG recordings over the biceps femoris (BF) and semitendinosus (ST) were made with a wireless EMG system (Trigno; Delsys Inc, Boston, MA) during knee flexor MVCs and

knee extensor ramp contractions. Following preparation of the skin (shaving, abrading and cleansing with alcohol) single differential Trigno standard EMG sensors (1 cm inter-electrode distance; Delsys Inc, Boston, MA) were attached over each muscle using adhesive interfaces. Sensors were positioned parallel to the presumed frontal plane orientation of the underlying muscle fibers at 45% of thigh length (distance from the greater trochanter to the lateral knee joint space) measured from the popliteal crease. EMG signals were amplified at source (x300; 20-450 Hz bandwidth) before further amplification (overall effective gain x909) and sampled at 2000 Hz via the same A/D converter and computer software as the force signal, to enable data synchronization. In offline analysis, EMG signals were corrected for the 48 ms delay inherent to the Trigno EMG system.

Knee Extension and Flexion Maximum Voluntary Contractions

Following a brief warm-up (3 s contractions at 50% [x3], 75% [x3] and 90% [x1] of perceived maximum), participants performed 3-4 MVCs and were instructed to either 'push as hard as possible' (knee extension) or 'pull as hard as possible' (knee flexion) for 3-5 s and rest ≥ 30 s. A horizontal cursor indicating the greatest torque obtained within the session was displayed for biofeedback and verbal encouragement was provided during all MVCs. The highest instantaneous torque recorded during any MVC was defined as MVT. During knee flexor MVCs EMG amplitude was calculated as the root mean square (RMS) of the filtered EMG signal of the BF and ST over a 500 ms epoch at knee flexion MVT (250 ms either side) and averaged across the two muscles to give EMG_{MAX}.

Explosive Voluntary Contractions

Participants performed a series of 10 explosive voluntary contractions each separated by 15 s. Participants were instructed to extend their knee 'as fast and as hard as possible'; with the emphasis on 'fast', for 1 s from a relaxed state upon hearing an auditory signal. Contractions involving a visible countermovement or pre-tension were discarded and another attempt made. To indicate if a countermovement or pre-tension had occurred, resting torque was displayed on a sensitive scale. During each explosive contractions participants were required to exceed 80%MVT, which was depicted by an on-screen marker. To provide performance feedback the time taken to reach 80%MVT was shown after each contraction and the slope of the rising torque-time curve (10 ms time constant) was displayed throughout these contractions with the peak slope of their best attempt indicated with an on-screen cursor. The three best explosive contractions (highest torque at 100 ms and no discernible countermovement or

pre-tension, change in baseline force <0.34 Nm in the preceding 300 ms) were analysed in detail. Contraction torque onset was defined as the last trough before the torque signal permanently deflected away from the envelope of the baseline noise; identified via manual inspection using a systematic standard method by the same trained investigator, in accordance with previously published methods (Tillin et al. 2010). Manual onset detection is considered to provide greater accuracy and reliability than an automatic approach (Tillin et al. 2013b). The torque signal was initially viewed with y and x-axis scales of 0.68 Nm and 300 ms respectively and a vertical cursor placed on torque onset. Accurate placement of the cursor was verified by viewing the signal with a higher resolution. RTD (ΔTorque or Δ%MVT /ΔTime) measurements of explosive strength were calculated from the time taken between contraction onset and absolute (50, 100 and 150 Nm [Vol $RTD_{0\text{-}50Nm}$, Vol $RTD_{0\text{-}100Nm}$, Vol $RTD_{0\text{-}150Nm}$) and relative (25, 50 and 75%MVT [Vol $RTD_{0\text{-}25\%MVT}$, Vol $RTD_{0\text{-}50\%MVT}$, Vol $RTD_{0\text{-}75\%MVT}$]) torques, as well as RTD between sequential torque levels (absolute 50-100 and 100-150 Nm [Vol $RTD_{50\text{-}100Nm}$ and Vol $RTD_{100\text{-}150Nm}$]; relative 25-50 and 50-75%MVT [Vol $RTD_{25\text{-}50\%MVT}$, Vol $RTD_{50\text{-}75\%MVT}$]). Values recorded from each of the three analysed (best) contractions were averaged.

Evoked Octet Contractions

The femoral nerve was electrically stimulated (constant current, variable voltage stimulator; DS7AH, Digitimer Ltd., UK) with square-wave pulses (0.2 ms duration) to elicit involuntary contractions of the knee extensors whilst the participant was voluntarily passive. Electrical stimuli were applied via a cathode probe (1 cm diameter; Electro Medical Supplies, Wantage, UK) protruding 2 cm perpendicular from the center of a plastic base (4 x 5 cm). The cathode and an anode (carbon rubber electrode, 7 x 10 cm; Electro Medical Supplies, Wantage, UK) were coated with electrode gel and securely taped to the skin over the femoral nerve in the femoral triangle and the greater trochanter respectively. The precise location of the cathode was determined as the position that evoked the greatest twitch response to a submaximal electrical current. Twitch contractions were elicited at incremental currents (~15 s apart) until a simultaneous plateau in peak torque and the peak slope of the rising twitch torque was observed. Thereafter, the electrical current was lowered and octet stimulation (8 pulses at 300 Hz) was delivered in step-wise increments until the stimulation intensity that elicited twitch force plateau (defined as the maximal stimulation intensity/ current) was reached. Real-time inspection of octet peak torque and peak rate of torque development (10 ms epoch) confirmed a plateau in both variables with incremental stimulation. Subsequently, three supramaximal

(120% maximal current) octet contractions were elicited. Absolute and relative RTD (ΔTorque or Δ%MVT/ΔTime) measurements of evoked explosive strength were calculated from the time taken between contraction onset and absolute (50 and 100 Nm [Oct $RTD_{0\text{-}50Nm}$ and Oct $RTD_{0\text{-}100Nm}$) and relative (25 and 50%MVT [Oct $RTD_{0\text{-}25\%MVT}$ and Oct $RTD_{0\text{-}50\%MVT}$]) torques, as well as RTD between sequential torques (absolute 50-100Nm [Oct $RTD_{50\text{-}100Nm}$]; relative 25-50%MVT [Oct $RTD_{25\text{-}50\%MVT}$]). Values recorded from each of the three supra-maximal contractions were averaged. Evoked measures were not acquired for three participants who did not tolerate the discomfort associated with the octet stimulation.

Ramp Contractions for Determination of Tissue Stiffness

Tissue stiffness was derived from synchronous recordings of torque and tissue elongation (corrected for passive tissue displacement via video recording of knee joint changes) during isometric knee extension ramp contractions. Participants completed two sub-maximum practice ramp contractions prior to five (obtaining reliable measures of tissue stiffness requires numerous efforts [Schulze et al. 2012]) maximum attempts with 90 s rest between contractions. Prior to each ramp contraction participants were shown a target torque-time trace on a computer monitor that increased at a constant gradient (50 $Nm.s^{-1}$ loading rate) from zero up to MVT. They were instructed to match the target trace as closely as possible for as long as possible (i.e. up to MVT), and real-time torque was displayed over the target torque-time trace for feedback. The preceding knee extensor MVCs and sub-maximum contractions were considered sufficient to elicit tissue preconditioning, The three most suitable ramp contractions, according to highest peak torque, the closeness to the target loading rate and ultrasound image clarity, were analysed and measurements averaged across these three contractions.

Measurement of Tissue Elongation

Video images from two ultrasound machines and one video camera were captured to obtain tissue and knee joint displacements during ramp contractions. An ultrasound probe (7.5 MHz linear array transducer, B-mode, scanning width 60 mm and depth 50 mm; Toshiba Power Vision 6000, SSA-370A: Otawara-Shi, Japan) was fitted into a custom made high-density foam cast that was strapped to the lateral aspect of the thigh with the mid-point of the probe positioned at ~50% thigh length. The probe was aligned so the fascicles inserting into the vastus lateralis (VL) muscle deep aponeurosis could be visualized at rest and during contraction. An echo-absorptive marker (multiple layers of transpore medical tape) was placed beneath the ultrasound probe to provide a reference for any probe movement over the skin. An-

other ultrasound probe (5-10 MHz linear array transducer, B-mode, scanning width 92 mm and depth 65 mm, EUP-L53L; Hitachi EUB-8500) was fitted into a custom made high-density foam cast that was held firmly over the anterior aspect of the knee with the probe aligned longitudinal to the patellar tendon such that the patellar apex and insertion of the posterior tendon fibers at the tibia could be visualized at rest and throughout the contraction. The ultrasound machines were interfaced with the computer collecting torque data in Spike 2 and the video feeds were recorded synchronously with torque using Spike 2 video capture at 25 Hz. To circumvent the difficulty of judging the consistency of grey-scale ultrasound patterns for the points of interest and possible experimenter bias in tissue elongation measurements, some degree of automated analysis is recommended (Seynnes et al. 2015). During off-line analysis tissue elongation was tracked frame-by-frame using public-domain semi-automatic video analysis software: Tracker, version 4.86 (www.cabrillo.edu/~dbrown/tracker). VL fascicle deep aponeurosis cross point displacement relative to the skin marker provided a measure of muscle-tendon unit (MTU) elongation. Patellar tendon elongation was determined by the longitudinal displacement of the patella apex and the tendon tibial insertion. To enable correction of tissue displacement due to joint angle changes during ramp contractions individual ratios of tissue displacement relative to joint angular displacement (mm/°) were obtained from passive movements (i.e. plotting the tissue displacement-knee joint angle relationship). This ratio was used to determine tissue displacement resulting from knee angle change during ramp contractions, which was subsequently subtracted from total measured displacement. Corrections were only applied to aponeurosis displacement. Tendon elongation under passive conditions was deemed negligible. Passive movements were conducted prior to the ramp contractions. Participants were instructed to completely relax as their knee was moved through 90 to 130°. During passive movements and ramp contractions, knee joint angle (angle between visible markers placed on the greater trochanter, lateral knee joint space and lateral malleolus) was derived from sagittal plane video recorded using a camera mounted on a tripod positioned (1.5 m) perpendicular to the strength-testing chair. The video camera was interfaced with a computer and recorded using spike 2 video-capture at 25 Hz (simultaneously with force, EMG, and ultrasound images during the ramp contractions) and analysed via Tracker software.

Calculation of Tendon Force

PT force was calculated by dividing external absolute knee extensor torque by the patellar tendon moment arm length. The latter was measured from sagittal plane T1-weighted MR

(1.5T Signa HDxt, GE) images (2 mm thickness, 0 mm gap) as the perpendicular distance from the PT line of action to the tibio-femoral contact point, which was the midpoint of the contact distance between the tibia and femur. Due to constraints in the size of the knee coil, sagittal images were acquired in an extended knee position (~163°). Moment arm length for any specific knee angle measured at rest or during ramp contraction was estimated from previously published data fitted with a quadratic function (Kellis and Baltzopoulos, 1999), scaled to each participant's measured moment arm length at 163°. Absolute internal knee extensor torque was given by summing net knee extension torque and the estimated knee flexor co-contraction torque. Antagonist knee flexor torque was estimated by expressing the average knee flexor EMG amplitude (RMS 50 ms moving window) during ramp contractions relative to the knee flexor EMG_{MAX} and multiplying by the knee flexor MVT (assuming a linear relationship between EMG amplitude and torque). During analysis, torque and EMG amplitude were down-sampled to 25 Hz to match the ultrasound video frequency.

Calculation of Muscle-Tendon Unit and Patellar Tendon Stiffness

MTU (corrected for passive tissue displacement) and PT elongation were plotted (for each ramp contraction analysed) against tendon force. Tendon force-elongation plots were fitted with a second-order polynomial forced through zero. Using the associated quadratic equations MTU and PT elongation was determined at specific absolute (50, 100 and 150 Nm) and individual relative knee extension torques (25, 50 and 75% MVT). Absolute stiffness (Δ tendon force [N]/Δ elongation [mm]; $N.mm^{-1}$) was calculated over 0-50, 0-100 and 0-150 Nm torque increments (MTU/PT $k_{0\text{-}50Nm}$, MTU/PT $k_{0\text{-}100Nm}$, MTU/PT $k_{0\text{-}150Nm}$) and sequential torque increments of 50-100 and 100-150 Nm (MTU/PT $k_{50\text{-}100Nm}$ and MTU/PT $k_{100\text{-}150Nm}$). MTU and PT elongation at relative torques were converted to strain (ε, %; ratio of Δ tissue length to resting tissue length). Relative stiffness ($\Delta\%MVT/\Delta\varepsilon$ [$\%MVT.\varepsilon^{-1}$]) was calculated over relative 0-25, 0-50, 0-75%MVT increments (MTU/PT $k_{0\text{-}25\%MVT}$, MTU/PT $k_{0\text{-}50\%MVT}$, MTU/PT $k_{0\text{-}75\%MVT}$) and sequential increments 25-50 and 50-75%MVT (MTU/PT $k_{25\text{-}50\%MVT}$ and MTU/PT $k_{25\text{-}75\%}$MVT). The stiffness measures derived from each of the three ramp contractions analysed was averaged to give each individual's representative values. MTU resting length was assessed with a tape measure over the surface of the skin from the tibial tuberosity to center of the measurement site over VL. PT length was taken as the distance between the patellar apex and the insertion of the posterior fibers of the tendon on the tibia, measured from ultrasound images acquired at rest prior to the ramp knee extensions.

Statistical Analysis

MVT, RTD and tissue stiffness (k) measures from duplicate measurement sessions were averaged for criterion measures used in statistical tests. Using SPSS Version 20.0 (IBM Corp., Armonk, NY), Pearson's product moment bivariate correlations were performed to examine the relationships between absolute or relative RTD (voluntary or evoked) vs. tissue stiffness variables (MTU or PT) measured over equivalent torque increments; e.g. absolute PT $k_{0\text{-}100Nm}$ vs. Vol $RTD_{0\text{-}100Nm}$, relative MTU $k_{0\text{-}50\%MVT}$ vs. Vol $RTD_{0\text{-}50\%MVT}$ (Table 1). Absolute stiffness measures were also correlated against MVT. Additional (a posteriori) correlations were performed between matched relative torque increment voluntary/evoked octet RTD and MTU stiffness over 5% increments from contraction onset (e.g. Vol $RTD_{50\text{-}55\%MVT}$ [$\%MVT.s^{-1}$] vs. MTU $k_{50\text{-}55\%MVT}$ [$\%MVT.\varepsilon^{-1}$]), to more specifically characterise the relationships found between the relative RTD and relative MTU stiffness. Statistical significance level was $P<0.05$. Descriptive data are presented as mean ± standard deviation (SD). To provide an index of measurement reliability average within participant coefficient of variation (CVw, %) was calculated between the two measurement sessions, although it is worth noting that the criterion values (averaged across two sessions) will have higher reliability than each individual session. Inter-individual variability is reported as between participant coefficient of variation (CVb, %) of criterion measures.

RESULTS

Measurement Reliability

Within-participant test-retest reliability was excellent for MVT (CVw 3.0%), and good for voluntary RTD (CVw ≤ 8.6% for absolute and relative measures). Matched MTU and PT k measures were not as reliable, but improved at higher torque increments: MTU k and relative MTU k, CVw 15.4 to 9.7% and 14.1 to 11.0%; PT k and relative PT k, CVw 13.9 to 8.8% and 13.6 to 8.1%.

Inter-individual Variability

Knee extension MVT was 245.0 ± 41.8 Nm (CVb 17.1%, 2.3-fold range). Voluntary torque-time curves (Figure 1 A and C) exhibited similar between participant variability in absolute and relative RTD measures CVb of 14.4 to 20.5% (1.8- to 2.9-fold range). Voluntary sequential RTD was more variable (CVb 32.0-33.0%) as was relative sequential RTD (CVb 24.0-25.0%). Evoked octet torque-time curves (Figure 1 B and D) showed octet RTD and relative

octet RTD varied much less than voluntary $RTD_{0\text{-}50Nm}$ and $_{0\text{-}25\%MVT}$. Octet sequential RTD/relative RTD was highly variable (CVb was 37.4 and 45.3%).

There was large inter-individual variability in external torque-elongation relationships for both the MTU and PT as shown in Figure 2 A and B. The variability in elongation was greatest at the initial torque increment (50 Nm: MTU 7.6-fold range; PT 3.7-fold range) and progressively reduced at higher torque increments and sequential torques (e.g. 100-150 Nm: MTU 3.2-fold range; PT 2.4-fold range). Similarly, relative knee extensor torque-MTU/PT strain curves (Figure 2 C/D) showed tissue strain to be most variable at the initial relative torque level (25%MVT: MTU 6.6-fold range; PT 3.5-fold range), with less inter-individual variability at higher and sequential relative torques (e.g. 50-75%MVT: MTU 3.2-fold range; PT 2.4-fold range). PT elongation was ~20% of MTU elongation at all torque increments. Alternatively, PT strain was 1.5-fold greater than MTU strain. For clarity, whilst the external torque-elongation/strain relationships are shown for illustrative purposes, individual stiffness values were derived from tendon force-elongation/strain relationships.

Bivariate Correlations of PT Stiffness and Explosive Strength

Voluntary and evoked RTD were unrelated to PT k measured over matching torque increment (r = 0.02 to 0.242, $P \geq 0.094$ [Figure 3 A]; e.g. scatterplots in Figure 3 B). Likewise, relative voluntary and evoked RTD were also unrelated to relative PT k (r = 0.048 to 0.255, P = 0.069 to 0.736; Figure 4 A). PT k measures were also unrelated to MVT (r = 0.094 to 0.127, all $P \geq 0.371$).

Bivariate Correlations of MTU Stiffness and Explosive Strength

Voluntary and evoked RTD were unrelated to MTU k measured over the same torque increments (r = 0.038 to 0.191, $P \geq 0.184$ [Figure 3 A]; e.g. scatterplot in Figure 3 C). In contrast, some voluntary and evoked relative RTD measures were positively associated with relative MTU k (Figure 4 A) e.g. relative Vol $RTD_{25\text{-}50\%MVT}$ r = 0.374, P = 0.007 (Figure 4 B) and Oct $RTD_{25\text{-}50\%MVT}$ r = 0.353, P = 0.014 (Figure 4 C). Following these associations a more detailed secondary analysis using 5%MVT increments (relative MTU k and relative RTD again measured over the same increments) showed that relative MTU k was positively related to relative voluntary RTD for the increments from 35-55%MVT (r = 0.312 to 0.434, $P \leq 0.026$; Figure 5). Relative evoked RTD was also positively related to relative MTU k from 5-

45%MVT (r = 0.315 to 0.461, $P \leq 0.029$; Figure 5). Finally MTU k measures were unrelated to MVT (MTU, r = -0.124 to -0.09, all $P \geq 0.388$).

DISCUSSION

The present study carefully investigated whether both PT and MTU stiffness (k) were related to voluntary and evoked explosive muscle strength *in vivo*, with both variables assessed over the same torque increment, and expressed in absolute and relative terms. Bivariate correlations revealed no relationships between absolute PT and MTU k and voluntary and evoked RTD. Likewise relative PT k was unrelated to relative voluntary or evoked RTD. However, relative MTU k had modest positive relationships to some measures of relative RTD during explosive voluntary (Vol $RTD_{0\text{-}75\%MVT}$, R^2 = 0.101; Vol $RTD_{25\text{-}50\%MVT}$, R^2 = 0.140), and evoked octet (Oct $RTD_{0\text{-}50\%MVT}$, R^2 = 0.081; Oct $RTD_{25\text{-}50\%MVT}$, R^2 = 0.125) contractions. Subsequent correlations between relative RTD and MTU k in 5%MVT increments showed relative MTU k contributes to explaining voluntary relative RTD between 35-55%MVT (R^2 = 0.097 – 0.188), and relative RTD throughout evoked octet contraction (5-45%MVT; R^2 = 0.099 – 0.194).

Our finding of no relationships between absolute MTU k and RTD measures is contrary to earlier work that generally supported weak-moderate positive relationships between MTU k and RTD (Bojsen-Møller et al. 2005; Waugh et al. 2013; Hannah & Folland, 2015). However these studies used loading rates that were dependent upon and thus may have been confounded by MVT, did not match the torque increments of stiffness and RTD measurement (Bojsen-Møller et al. 2005; Waugh et al. 2013), and included heterogeneous sub-groups exhibiting differences in potentially confounding variables (Bojsen-Møller et al. 2005; Waugh et al. 2013; Hannah & Folland, 2015). These methodological issues may have skewed previous stiffness measurements in favour of stronger individuals who also tend to have higher RTD values (Andersen & Aagaard, 2006; Folland et al. 2014). For example, calculating stiffness over a tendon force increment that is relative to maximum strength (e.g. 50-90%MVT [Bojsen-Møller]; 50-100%MVT [Wang et al. 2012]; 10-80% MVT [Waugh et al. 2013]) means the force increment for stiffness determination is higher for stronger individuals. As there is a well-documented force-stiffness relationship this method creates a methodological artifact whereby stronger individuals will inherently be measured to have a greater stiffness. In addition, use of inconsistent loading rates, as a consequence of standardized duration ramp contractions to different force increments for each individual, would produce higher stiffness

values for the higher loading rates of stronger individuals (Pearson et al. 2007; Theis et al. 2012; Kösters et al. 2014).

In contrast, stiffness measurements in the present study were more thorough: duplicate measurement sessions each involving multiple, standardised loading ramp contractions, measurements of stiffness and RTD over the same torque increment for all individuals, and use of a large cohort of exclusively young males with similar physical activity status. Nevertheless this approach revealed wide inter-individual variability in MTU k, yet such differences did not manifest into a noticeable association with absolute RTD. Seemingly the lack of relationship between absolute MTU k and RTD could be ascribed to our avoidance of a specious association mediated by the confounding influence of maximum strength.

Some measures of relative MTU k and relative RTD were significantly associated. Specifically, during the initial analysis relative MTU k was related to Vol $RTD_{0\text{-}75\%MVT}$ and relative Vol $RTD_{25\text{-}50\%MVT}$, and during the secondary analysis of 5% torque increments relative MTU k and relative RTD were associated during the between 35-55%MVT. This contrasts with the results of a previous study that found no relationship between relative MTU k and relative RTD (Hannah & Folland, 2015). However, this previous study involved ramp contractions with a constant duration and thus variable loading rates that may introduce a bias as well as males and females that exhibit a number of distinct differences that might confound the relationship. In the current study the consistent significant relationship between relative MTU k and voluntary relative RTD from 35-55%MVT suggest a genuine systematic relationship, although the explained variance was small (≤18.8%). The logical explanation for these volitional relationships was via an effect of relative MTU k on the contractile capability for relative RTD as shown by the finding that relative MTU k was also significantly related to octet relative RTD; specifically octet $RTD_{0\text{-}50\%MVT}$, octet $RTD_{25\text{-}50\%MVT}$ and subsequently from the 5% torque increments RTD from 5-50%MVT. Furthermore the torque increment over which voluntary relative RTD was associated with relative MTU k (25-55%MVT) was on average 52-93 ms into the explosive contraction, which is consistent with the steepest phase of the voluntary contractions (50-100 ms) where voluntary RTD is primarily determined by the contractile capacity for RTD (Folland et al. 2014). The finding that relative RTD was in part explained by relative MTU k, despite no corresponding relationships for absolute measures suggests an overwhelming influence of maximum strength on absolute RTD that seemingly negated any influence of MTU k. Finally, the rather limited explained variance of relative

octet RTD by relative MTU k (≤19.4%) indicates that contractile RTD is largely determined by other factors; such as activation kinetics (Edman and Josephson, 2007), contractile protein composition (Harridge et al. 1996) and muscle fascicle length (Blazevich et al. 2009).

The present study was the first attempt to investigate if there is a relationship between *in vivo* free tendon k and RTD. We found PT k was not related to voluntary or evoked knee extensor RTD. The PT exhibited minimal elongation (~3 mm) that seems unlikely to appreciably influence muscle length changes and thus force-generating potential. Also, the rate of force transmission through tendons is exceptionally rapid (Nordez et al. 2009; DeWall et al. 2014) especially for short tendons such as the PT (length ~45-50 mm), and likely explains the lack of a relationship between PT k and RTD. Furthermore PT k was unrelated to MVT and it is notable that we found no relation between relative PT k and voluntary/evoked relative RTD measures. Research with isolated muscles has found the force rise time under isometric conditions of fixed sarcomere length was not appreciably faster than during muscle-tendon unit fixed-end contractions, indicating a negligible impact of tendon compliance on isometric rate of force development (Haugen & Sten-Knudsen, 1987). Similarly, Kawakami and Lieber (2000) showed that the internal sarcomere shortening during fixed-end contractions of an isolated MTU was unchanged once the proximal and distal ends of the aponeurosis where clamped, indicating that the tendon did not impact muscle internal shortening during isometric force production. Whether our results can be generalised to other MTU's where the tendon may contribute more significantly to the overall MTU stiffness is uncertain and requires further research.

Our results imply the relationships we found between relative MTU k and relative RTD are due to the contribution of elastic tissues proximal to the tendon. Conceptually our method reflects the elongation of distal (to the ultrasound measurement site) tendinous tissues (aponeurosis-tendon). Thus our findings regarding relative MTU k and relative RTD are presumably consequent to aponeurosis force-length characteristics. Indeed, modeling studies demonstrate that greater aponeurosis stiffness results in a reduction in its stretch that decreases muscle fibre strain (Rehorn & Blemker, 2010; Rahemi et al. 2014). Lesser muscle fibre strain permits slower fibre shortening. More favourable fibre contractile conditions for force production permitted by a stiffer aponeurosis could account for our evidence of greater relative RTD (both voluntary and evoked) being associated with a stiffer relative MTU.

In the current study stiffness was measured during constant-RTD ramp contractions that were necessarily performed at a lower RTD than the explosive contractions; in order to capture the tissue elongation with fixed frame rate of 25 Hz ultrasound. An underlying assumption of the study was that the measured stiffness values, during the ramp contractions, are relevant to higher strain rates of explosive contractions. However we know that stiffness exhibits strain rate sensitivity (Lieber et al. 2000; Pearson et al. 2007; Theis et al. 2012; Kösters et al. 2014) and thus tendinous tissues will behave in a stiffer manner during the explosive contractions than the ramp contractions, and that this discrepancy will be most pronounced for individuals with a high RTD (upto 3-fold higher than participants with the lowest RTD). Although the measures of stiffness in the current study discriminated between individuals with extensive inter-individual variability (2 to 8-fold variability in PT/MTU elongation at the same torque), it is conceivable that our measures of stiffness did not fully reflect stiffness during the explosive contractile conditions relevant to RTD, and could potentially have underestimated the strength of the relationships between stiffness and RTD. Future work could employ high frame rate ultrasound (4000 Hz, Nordez et al. 2009) to evaluate stiffness during explosive contractions i.e. simultaneous to RTD measures, to investigate if this changes the nature of the relationships we have documented.

In conclusion, absolute MTU and PT k were not associated with voluntary or evoked RTD, and this was also the case for relative PT k and relative RTD. However greater relative MTU k was related to higher relative voluntary and evoked RTD. These results suggest a differential influence of MTU tissue components (muscle-aponeurosis vs. tendon) on relative RTD. An overriding influence of maximum strength is presumed to negate any relationship between absolute MTU k and RTD.

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COMPETING INTEREST

None declared.

AUHTOR CONTRIBUTIONS

All experiments for this study was conducted in the muscle function laboratory of the School of Sport, Exercise and Health Sciences at Loughborough University, UK.

Conception and design of the experiments: G.J.M, T.G.B, T.M.M-W, N.A.T, J.P.F.

Collection, analysis and interpretation of data: G.J.M, T.G.B, T.M.M-W, N.A.T, J.P.F.

Drafting the article or revising it critically for important intellectual content: G.J.M, T.G.B, T.M.M-W, N.A.T, J.P.F.

All authors approved the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed

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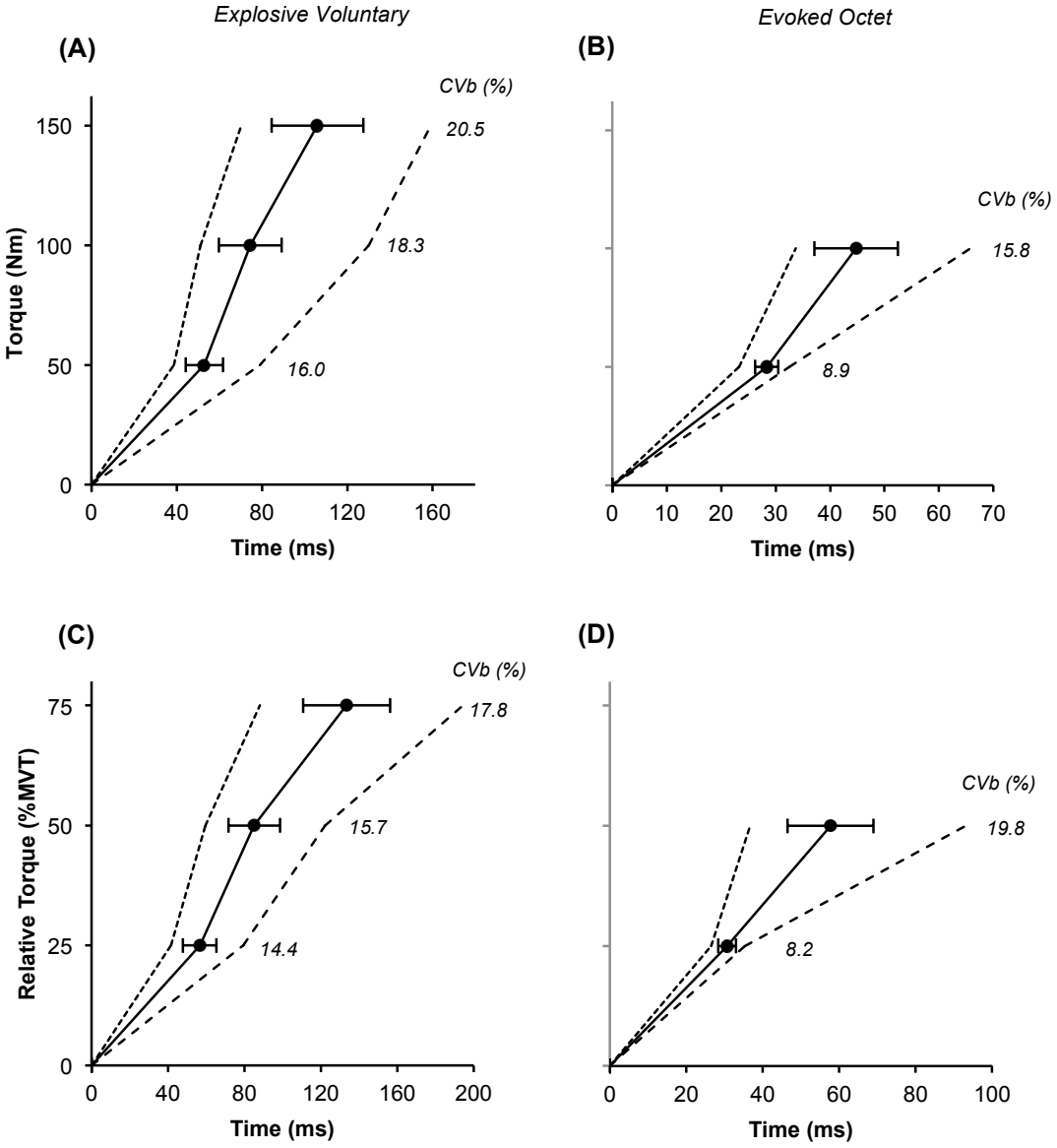
TABLES

Table 1. Matched explosive strength and tissue stiffness variables measured over the same absolute or relative torque increments

	Explosive Strength: Voluntary (Vol) or Evoked (Oct) Rate of Torque Development (RTD)	Vs.	**Stiffness of the Muscle-Tendon Unit (MTU) or Patellar Tendon (PT)**
Absolute measures:	Vol/Oct $RTD_{0\text{-}50Nm}$		MTU/PT $k_{0\text{-}50Nm}$
	Vol/Oct $RTD_{0\text{-}100Nm}$		MTU/PT $k_{0\text{-}100Nm}$
	$Vol_{0\text{-}150Nm}$		MTU/PT $k_{0\text{-}150Nm}$
	Vol/Oct $RTD_{50\text{-}100Nm}$		MTU/PT $k_{50\text{-}100Nm}$
	$Vol_{100\text{-}150Nm}$		MTU/PT $k_{100\text{-}150Nm}$
Relative measures:	Vol/Oct $RTD_{0\text{-}25\%MVT}$		MTU/PT $k_{0\text{-}25\%MVT}$
	Vol/Oct $RTD_{0\text{-}50\%MVT}$		MTU/PT $k_{0\text{-}50\%MVT}$
	$Vol_{0\text{-}75\%MVT}$		MTU/PT $k_{0\text{-}75\%MVT}$
	Vol/Oct $RTD_{25\text{-}50\%MVT}$		MTU/PT $k_{25\text{-}50\%MVT}$
	$Vol_{50\text{-}75\%MVT}$		MTU/PT $k_{50\text{-}75\%MVT}$

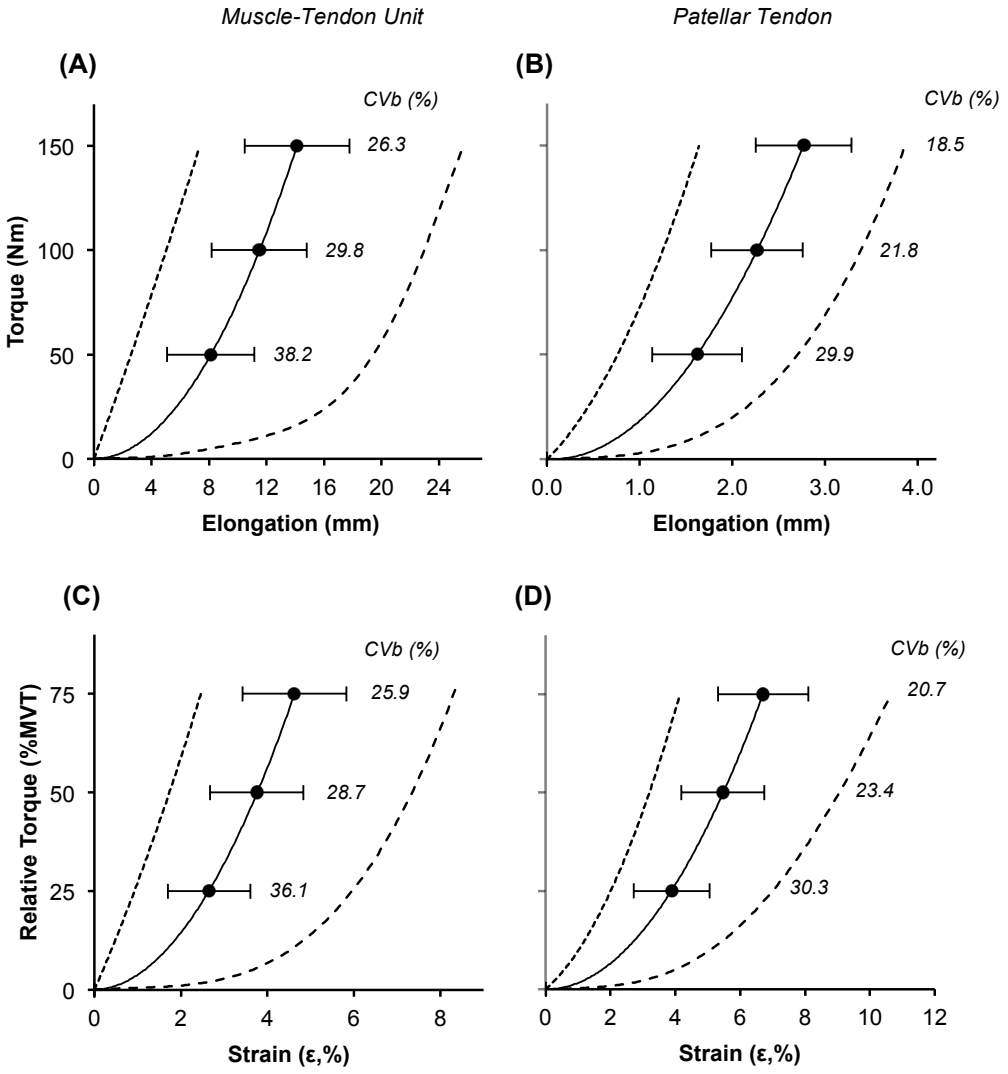
Rate of Torque Development Variability

Figure. 1. Inter-individual variability of torque-time curves during explosive voluntary (A, C; n=52) and evoked octet (B, D, n=49) contractions of the knee extensors expressed in absolute (Nm; A, B) and relative (% maximum voluntary torque, MVT; C, D) terms. Black line and circles (bars) are mean (SD) and the dotted and dashed lines depict the minimum and maximum torque values respectively. *Italic* numbers give the between participant coefficient of variation (CVb %) for the rate of torque development (absolute, ΔTorque/ΔTime [A and B]; relative, Δ%MVT/ΔTime [C and D]) calculated from 0 to the specified torque increment.



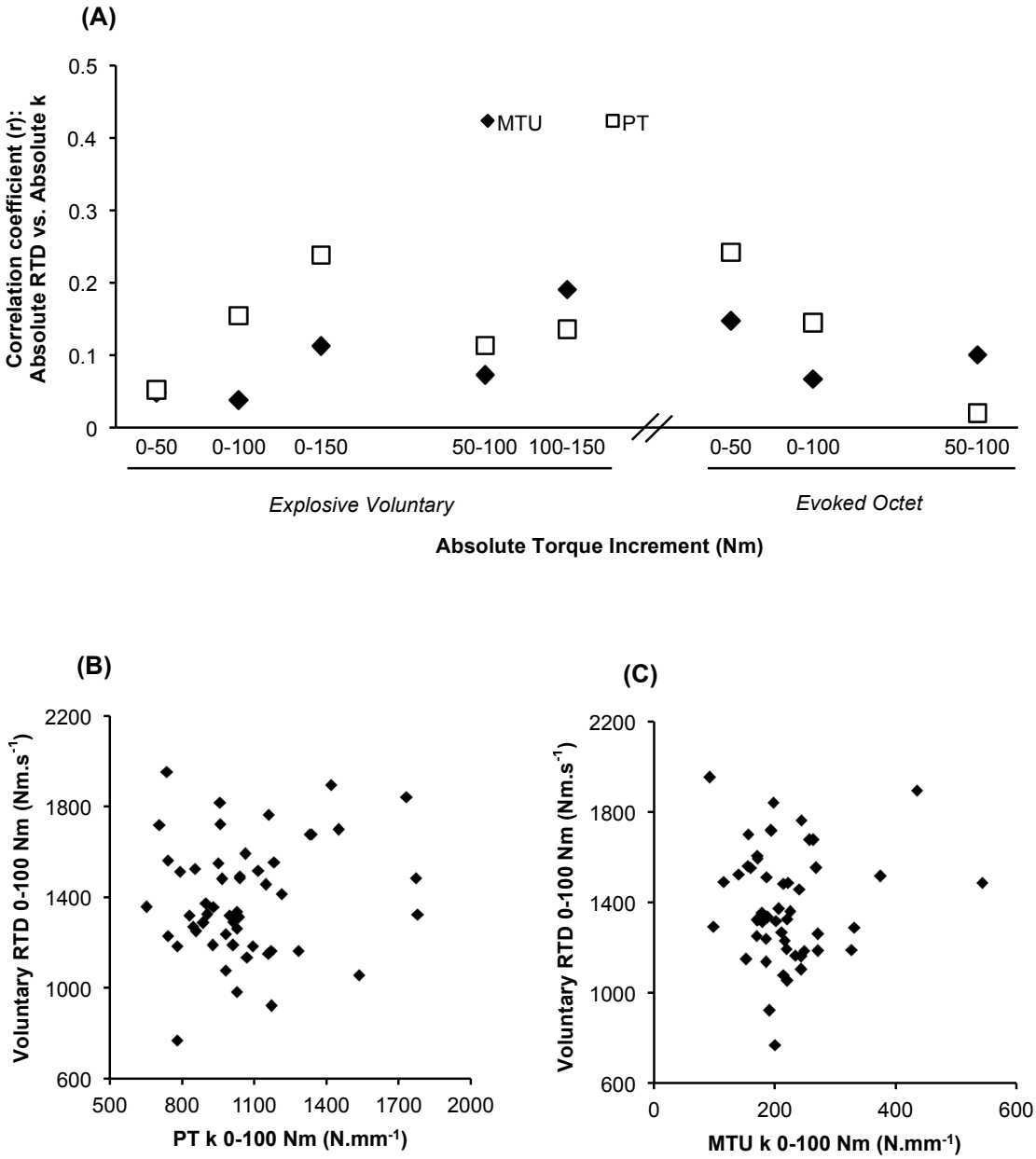
Tissue Stiffness Variability

Figure 2. Inter-individual variability in absolute torque-tissue elongation and relative torque (%MVT)-tissue strain curves for the muscle-tendon unit (MTU; A and C) and patellar tendon (PT; B and D). Data acquired during isometric ramp knee extensor contractions. Black line and circles (bars) are mean (SD) torque-elongation/strain curve, while dotted and dashed lines depict individuals with the minimum and maximum values of elongation/strain respectively. *Italic* numbers give between participant coefficient of variation (CVb %) for elongation and strain measured from 0 to the specified torque level. Stiffness measurements were subsequently derived from individual tendon force-elongation/strain relationships.



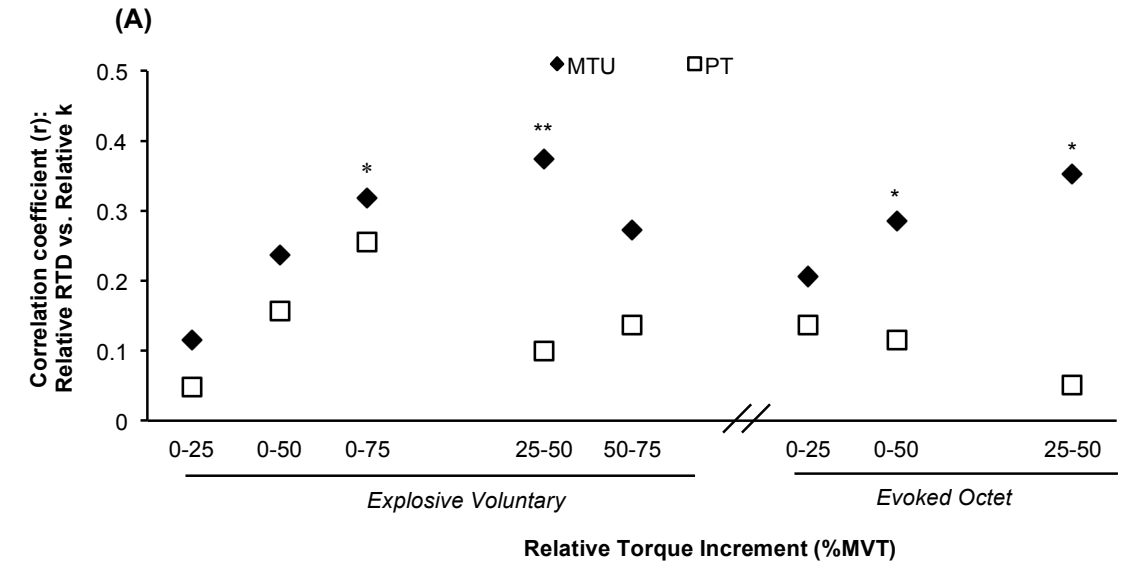
Relationships between Absolute Rate of Torque Development and Absolute Stiffness

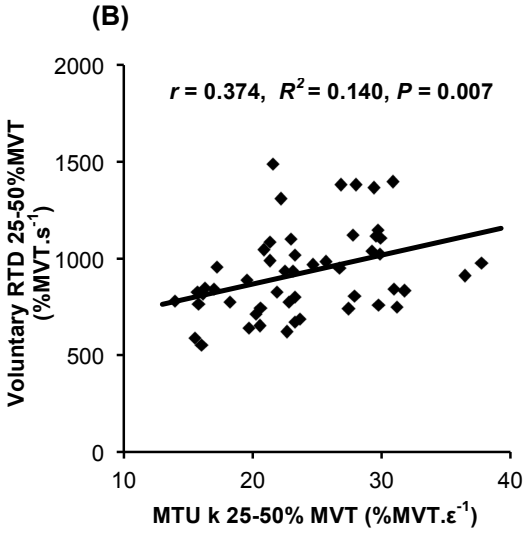
Figure 3. (A) Pearson's product moment correlation coefficients between absolute measures of rate of torque development (RTD, $Nm.s^{-1}$) during explosive voluntary or evoked octet contractions and stiffness (k; $N.mm^{-1}$) of the muscle-tendon unit (MTU, black diamonds) or patellar tendon (PT, white squares), both measured over the same torque increment. (B & C) Example scatterplots of the bivariate relationships between RTD during explosive voluntary (n=51) contractions and patellar tendon (PT, B) and muscle-tendon unit (MTU, C) k measured over 0-100 Nm.

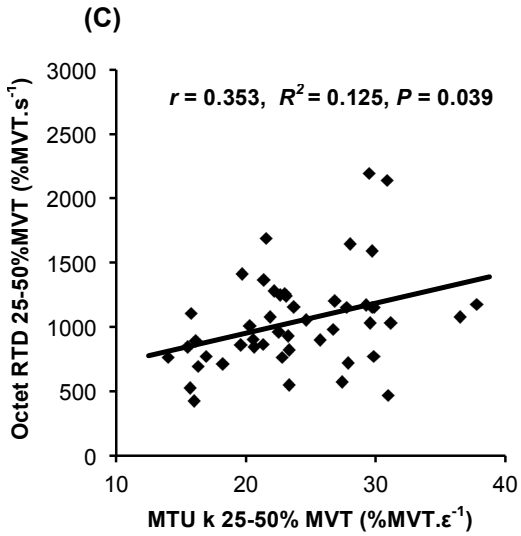


Relationships between Relative Rate of Torque Development and Relative Stiffness

Figure. 4. (A) Pearson's product moment correlation coefficients between the relative rate of torque development (RTD, %MVT.s^{-1}) during explosive voluntary and evoked octet contractions and the muscle-tendon unit (MTU) and patellar tendon (PT) relative stiffness (k; %MVT.ε^{-1}) determined for the same relative torque increment. **P<0.01, *P<0.05. (B & C) Example scatterplots of the bivariate relationships between relative RTD during explosive voluntary (Vol; B [n=51]) and evoked octet (Oct; C [n=48]) contractions and relative MTU k measured over 25-50%MVT.







Relationships between Relative Rate of Torque Development and Relative Muscle-Tendon Unit Stiffness

Figure. 5. Bivariate correlations between relative RTD (%MVT.s^{-1}) and relative MTU k (%MVT/ε^{-1}) for the same torque increment. Correlations performed with n=51 for voluntary and n=48 for evoked contractions. Statistical significance level: *P<0.05, **P<0.01,***P<0.001.