Eur J Appl Physiol (2014) 114:2625–2634
DOI 10.1007/s00421-014-2986-7

ORIGINAL ARTICLE

# Variants within the *MMP3* gene and patellar tendon properties in vivo in an asymptomatic population

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Received: 24 April 2014 / Accepted: 20 August 2014 / Published online: 29 August 2014


**Abstract**
*Background/aim* Gene variants encoding for proteins involved in homeostatic processes within tendons may influence its material and mechanical properties in humans. The purpose of this study was to examine the association between three polymorphisms of the *MMP3* gene, (rs679620, rs591058 and rs650108) and patellar tendon dimensional and mechanical properties in vivo.
*Methods* One hundred and sixty, healthy, recreationally-active, Caucasian men and women, aged 18–39 were recruited. *MMP3* genotype determined using real-time PCR was used to select 84 participants showing greatest genetic differences to complete phenotype measurements. Patellar tendon dimensions (volume) and functional (elastic modulus) properties were assessed in vivo using geometric modelling, isokinetic dynamometry, electromyography and ultrasonography.
*Results* No significant associations were evident between the completely linked *MMP3* rs591058 and rs679620 gene variants, and closely linked rs650108 gene variant, and either patellar tendon volume (rs679620, $P = 0.845$; rs650108, $P = 0.984$) or elastic modulus (rs679620, $P = 0.226$; rs650108, $P = 0.088$). Similarly, there were no associations with the *Z*-score that combined those dimension and functional properties into a composite value (rs679620, $P = 0.654$; rs650108, $P = 0.390$). Similarly, no association was evident when comparing individuals with/without the rarer alleles ($P > 0.01$ in all cases).
*Conclusions* Patellar tendon properties do not seem to be influenced by the *MMP3* gene variants measured. Although these *MMP3* gene variants have previously been associated with the risk of tendon pathology, that association is unlikely to be mediated via underlying tendon dimensional and functional properties.

**Keywords** Genetic association studies · *MMP3* gene variants · Patellar tendon properties · Asymptomatic · In vivo

**Abbreviations**

| | |
|---|---|
| AT | Achilles tendinopathies |
| BF | Biceps femoris |
| CcT | Co-contraction torque |
| *COL5A1* | Gene encoding collagen type V alpha 1 chain |
| CSA | Cross-sectional area |
| DEXA | Dual X-ray absorptiometry |
| *E* | Elastic modulus |
| EDTA | Ethylenediaminotetraacetic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| EMG | Electromyography |
| *E*2 | Oestradiol |
| $F_{Max}$ | Maximal force |

Communicated by Olivier Seynnes.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00421-014-2986-7) contains supplementary material, which is available to authorized users.

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| | |
|---|---|
| GPa | Gigapascals |
| HWE | Hardy–Weinberg equilibrium |
| *K* | Stiffness |
| *MMP3* | Gene encoding matrix metalloproteinase-3 protein |
| MRI | Magnetic resonance imaging |
| MVC | Maximal voluntary contraction |
| PCR | Polymerase chain reaction |
| PTL | Patellar tendon length |
| PTMA | Patellar tendon moment arm |
| RMS | Root mean square |
| ROM | Range of motion |

## Introduction

Knowledge of the mechanical properties of musculoskeletal tissues including tendon, can assist in understanding the aetiologies of injury (Ker et al. 1988; Seynnes et al. 2009) as well as physical performance potential (Fletcher et al. 2010; Lichtwark and Barclay 2010; Wilson et al. 1994). The primary parameter describing tendon material and mechanical properties is tendon stiffness, which describes the change in length in relation to the force applied (force–displacement relation). It is dependent on the volume of the tendon and tendon modulus, which describes the relation between tendon stress and strain (McGinnis 2005). Therefore, the mechanical properties of tendon are influenced by the volume of tendon as well as the tissue material properties.

Recently, a genetic component has been associated with tendinopathies, particularly that of the *COL5A1* rs12722 gene variant (Collins and Posthumus 2011). One previous study was able to associate this gene variant with mechanical properties of the muscle fascicles and aponeurosis of the vastus lateralis muscle (Kubo et al. 2013), although another was not when examining the patellar tendon (Foster et al. 2014). Therefore, we do not know that the behaviour of the two structures is necessarily comparable. In addition, gene variants within the *MMP3* gene which encodes for matrix metalloproteinase-3 protein, a key regulatory enzyme of the extracellular matrix (ECM) capable of degrading multiple structural components of the ECM such as the collagens (Matrisian 1990), have also been associated with tendinopathies (Raleigh et al. 2009). Specifically, three gene variants within the *MMP3* gene (rs679620, rs591058, rs650108; Fig. 1) have independently been associated with chronic Achilles tendinopathies. Furthermore, the rs679620 gene variant is non-synonymous in that it causes a change in the amino acid sequence, and consequently an altered protein function.

Even though the above gene variants have not been associated with tendon dimensional and mechanical properties per se, it is however important to state that all genes that contain sequence variants shown to be associated with tendon injury to date, encode for proteins that serve essential structural and functional roles within tendon (Collins and Raleigh 2009). Indeed, down-regulated expression levels of MMP3 mRNA and its associated proteins have been found in injured or ruptured tendons compared to non-injured tendon groups (de Mos et al. 2007; Ireland et al. 2001; Jones et al. 2006; Lo et al. 2004; Riley et al. 2002). Therefore, it could be postulated that relatively small changes in MMP3

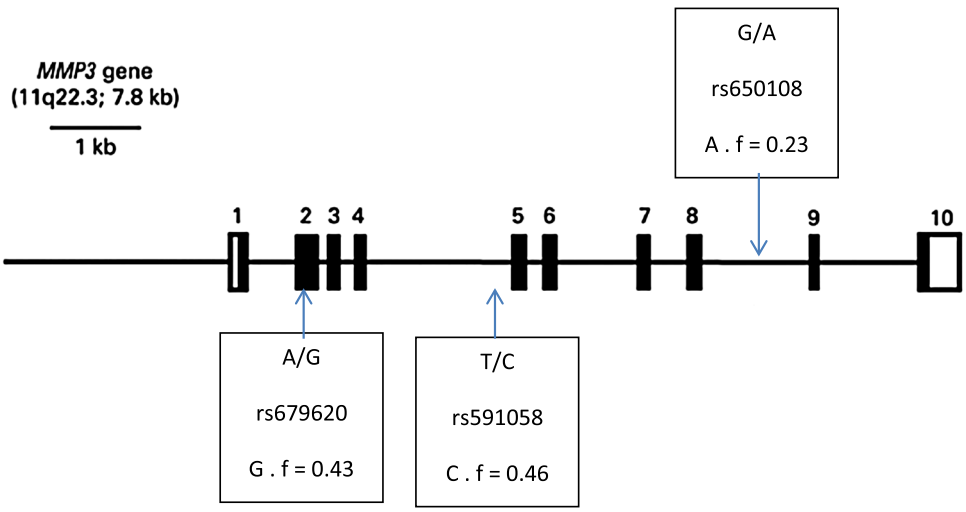


**Fig. 1** A simplified representation of the *MMP3* gene in the negative orientation on chromosome 11. Both exons (*black rectangles*) and introns (*horizontal lines*) are shown, with exons being indicated by *numbers*. Untranslated regions (*solid white rectangles*) are also shown. All three gene variants are separated by similar distances; rs679620 in exon 2 is 2,282 base pairs from rs5901058 in intron 4, whereas rs591058 is 2,551 base pairs from rs650108 in intron 8. This figure is modified from Raleigh et al. (2009) with information on minor allele frequency and loci derived from Ensembl (http://www.ensembl.org/index.html)

expression within non-pathological ranges, as a result of the function of these gene variants, could result in interindividual variation in the degree of fibrillar collagen degradation, and ultimately the mechanical properties of tendon.

The aim of this study was therefore to investigate whether the *MMP3* rs679620, rs591058 and rs650108 gene variants influence dimensional and mechanical properties within the patellar tendon, through volume and modulus measures in vivo, in an asymptomatic population.

## Method

### Participants

From an initial screening phase, 160 healthy and recreationally active Caucasian individuals were recruited (100 males and 60 females). In an effort to increase the ability to detect genotype-phenotype associations, a 'stress the genotype' approach was implemented (Montgomery et al. 2002). Eighty-four participants (45 males and 39 females) with a high proportion of homozygosity across the three *MMP3* gene variants completed the tendon phenotype assessments. [mean, (range), (SD) age 23 (18–39), (4) years, height 173 (153–191), (10) cm, body mass 71.6 (47.4–98.9), (12.4) kg, and BMI 24 (18.75–29.8), (2.8) kg/m$^2$]. This 'stress the genotype' approach should increase the ability to detect a polymorphic influence on patellar tendon properties.

To minimise confounding factors, potential participants were not recruited if they were non-Caucasian, were very sedentary or very active (completed less than 2.5 h/week or more than 5 h/week of moderate exercise (Tucker et al. 2011) as determined by a questionnaire), had any current or recent lower limb injuries in the past year before testing, including tendinopathies of the patellar tendon, were aged under 18 or over 40 years, were diabetic, smoked regularly, were regular users of medication, or had a body mass index (BMI) outside the range 18.5–30 kg/m$^2$. Female volunteers were not recruited if pregnant or using hormone-based contraception. The investigation was approved by the local ethics committee at Manchester Metropolitan University, and all participants gave their written informed consent to participate. The study conformed to the latest revision of the Declaration of Helsinki.

## Measurement of tendon dimensional and mechanical properties

### Maximal patellar tendon isometric force

All measurements of torque were carried out on an isokinetic dynamometer (Cybex, Phoenix Healthcare, UK) on the left leg. This instrument has been previously assessed for test–retest reliability for measures of peak torque of the knee extensors (Maffiuletti et al. 2007; McCleary and Andersen 1992). The knee was fixed at 90° flexion (full extension = 0°) and hip angle at 85° (supine position = 0°) and in order to minimise any extraneous movement of the hip joint or the trunk, participants were strapped over the shoulders, pelvis and thighs. Settings of chair height and positioning relative to the dynamometer were adjusted individually [See papers by (Pearson et al. 2007) and (O'Brien et al. 2010) for pictures of set-up] gravity corrections were then made following the manufacturers' own procedure, having adjusted the attachment of the lever arm cuff relative to the length of the participant's shank. Previous work from within our laboratory has utilised similar methods of measurement, finding them to be both valid and reliable (Onambele-Pearson et al. 2010; Onambele et al. 2007). Participants were instructed to perform ramp isometric knee extensions to maximum over a 5–7 s period. Maximal tendon force was calculated as described previously (Eq. 1), (Onambele-Pearson and Pearson 2007; Pearson and Onambele 2006).

$$F_{\mathrm{Max}} = (\mathrm{MVC}_{\mathrm{KE}} + \mathrm{CcT}) / \mathrm{PTMA} \quad (1)$$

where $F_{\mathrm{Max}}$ is the maximal patella tendon force, $\mathrm{MVC}_{\mathrm{KE}}$ the maximal isometric knee-extensor torque (i.e., the measured torque during testing), CcT the knee flexion torque of the hamstrings during knee extension (antagonist co-contraction torque—see next section for calculation), and PTMA the patellar tendon moment arm (see sections below).

### Estimation of antagonist co-contraction using electromyography

Electromyographic (EMG) activity was assessed using a pair of self-adhesive Ag–AgCl electrodes (Ambu Neuroline 72000-S/25, Ballerup, Denmark) placed in a bi-polar configuration (Zipp 1982) at a site corresponding to the distal one-third on the long head of the biceps femoris (BF) (representative muscle of the knee flexors). The raw EMG signal was collected at a frequency of 2,000 Hz, pre-amplified (×2,000), band pass filtered between 500 and 10 Hz by the same system that processed the torque data (Acknowledge, Biopac Systems, Santa Barbara, CA, USA), and displayed in real-time on the same output graph (iMac, Apple, California). The root mean square (RMS) EMG activity corresponding to the peak torque period was analysed and averaged for a 500 ms period during the plateau of peak torque (i.e., 250 ms either side of the instantaneous peak torque). These EMG data were used as a measure of antagonist co-contraction (i.e., CcT) during isometric knee extensions (Reeves et al. 2003), calculated as the product of BF EMG

activity during $MVC_{KE}$, divided by BF peak flexor EMG at 90° knee flexion MVC, multiplied by hamstrings maximal flexion torque.

### Patellar tendon displacement

Patellar tendon displacement was determined using real-time *B*-mode ultrasonography (AU5, Esaote, Biomedica, Italy) as described previously (Onambele et al. 2007). Briefly, during a ramp isometric knee extension performed over 5–7 s with the knee fixed at 90º flexion, the ultrasound probe (7.5 MHz linear array probe, 40 mm wide) was positioned in a sagittal plane over the patellar tendon alternatively over; (a) patella proximal (inferior pole of the patella) or (b) tibia-distal (tibial tuberosity) excursions, so that the sum of tibial and patellar displacements would be computed as total displacement. Total displacements were determined at 10 % PT force level intervals (from 10 to 100 %) using digitising software (Kinovea, version 0.8.15, Joan Charmant and Contributors, France), consistent with others (Onambele et al. 2007). This method is widely used and has high reliability [e.g. (Kubo et al. 2001; Pearson and Onambele 2006; Reeves et al. 2003)]. Measurements were taken after five preconditioning knee extension contractions to ensure reproducibility (Maganaris 2003), and in the early afternoon (between 1200 and 1500 hours), to minimise variability in tendon stiffness related to time of day (Pearson and Onambele 2006).

#### *Patellar tendon moment arm length*

Patellar tendon moment arm length (PTMA) was measured from an 11 s sagittal plane scan of the left leg of each participant at rest, using a single, low-energy X-ray beam (0.9 μSv) protocol on a DEXA (dual X-ray absorptiometry) scan (Hologic QDR, Vertec, Reading, UK). For the imaging limb, the participant lay on their right-hand side with the left hip and knee flexed at 90° so that the source detector probes could pass across the knee within a 20 cm scanning window. The PTMA was defined as the perpendicular distance from the patellar tendon to the midpoint of the distance between the estimated tibio-femoral contact points in the lateral and medial femoral condyles (Baltzopoulos 1995; Tsaopoulos et al. 2006). The measurement of 2D PTMA using DEXA shows very strong agreement with MRI (Erskine et al. 2014).

### Calculation of patellar tendon stiffness

Patellar tendon stiffness (*K,* N $mm^{-1}$), was calculated from the slope of the tangents of the force–displacement relations (at 10 % force intervals), which were fitted with a second-order polynomial function forced through zero. The 10 % force intervals derive from the average maximum force ($F_{Max}$) experienced by the tendon during three separate ramp KE MVC (Eq. 1). The displacement of the patella tendon was measured as described previously. In addition, to allow for stiffness comparisons at an absolute load across populations, tendon stiffness was also calculated at a standardised force level which corresponded to just under the maximum baseline value of the weakest person (male = 1,067 N; female = 1,034 N).

### Calculation of elastic modulus

Patellar tendon cross-sectional area (PTCSA) and patellar tendon length (PTL) were measured in the resting state at a knee joint angle of 90°. PTCSA was determined from the mean of transverse-plane ultrasound images taken at 25, 50 and 75 % of patellar tendon length, and processed using digitising software (Image J, National Institute of Health, Bethesda, MD, USA). PTL was assessed from sagittal-plane ultrasound images and measured from the inferior pole of the patella to the superior aspect of the tibial tuberosity. Elastic Modulus (*E*) in GPa was calculated by multiplying *K* by the ratio of PTL to PTCSA ($E = K \times (\text{PTL} \div \text{PTCSA})$).

### Calculation of tendon volume

Patellar tendon volume (PTV) was calculated by geometric principles assuming a uniformly tapering truncated cone between measurement positions (i.e., the product of PTCSA at the three sections of the tendon, 25, 50, 75 %, and PTL). However, these sections represent the 'central' volume so it is possible that the normalisation is over-estimating the stress in the tendon, since not all the tissue is taken into account in the central 50 % portion. Muscle and tendon geometry have previously been modelled using similar methods (Fuller et al. 1999; Jones and Pearson 1969; Tothill and Stewart 2002).

#### *Z-score analyses*

To provide a stable measure of the overall association of genotype with patellar tendon properties, composites were formed with unit-weighted *Z*-scores of constituent tests (Ackerman and Cianciolo 2000), i.e. Elastic Modulus and tendon volume. Hence, the dimensional (volume) and functional (Elastic Modulus) properties of tendon could be scaled and analysed simultaneously. Thus, the raw test scores of *E* (GPa) and volume ($mm^3$) were converted to *Z*-scores using Eq. 2;

$$Z\text{-score} = (\text{variable score} - \text{mean})/\text{standard deviation}$$
$$Z\text{-score}_{\text{Composite}} = Z\text{-score}_{\text{E}} + Z\text{-score}_{\text{PTV}} \quad (2)$$

## Genetic analysis

### Sample collection

Buccal cells were collected using mouth swabs (Whatman Sterile OmniSwab, GE Healthcare, USA). Samples were immediately stored at −20 °C until DNA extraction.

### DNA extraction

Standard procedures for genomic DNA isolation from buccal swabs were carried out using the Qiagen QIAcube spin column protocol and buffers in the Qiagen DNA Blood Mini kit (Qiagen, West Sussex, UK).

### DNA quantification

The concentration and purity of the sample was calculated using a biophotometer (WPA UV1101, Biochrom, Cambridge, UK). Briefly, 12 µL of the DNA sample was pipetted into a glass cuvette, the absorbance readings of ultraviolet light at wavelengths of 260 and 280 nm were performed and the 260/280 nm ratio was determined. Good quality DNA will have a ratio of 1.7–2.0 (Glasel 1995), and all samples fell within these ratios. Eluted DNA concentrations were in the region of 10–30 ng/µL.

### MMP3 genotyping

*MMP3* rs679620, rs591058 and rs650108 genotypes were determined using fluorescence-based TaqMan real-time polymerase chain reaction (PCR). Predesigned primers and allele-specific probes specific to the 'G' allele (VIC) and 'A' allele (FAM) of the *MMP3* rs679620 gene variant, 'C' allele (VIC) and 'T' allele (FAM) of the *MMP3* rs591058 gene variant, and 'A' allele (VIC) and 'G' (FAM) of the *MMP3* rs650108 gene variant, were used (Applied Biosystems, Foster City, CA, USA). The assay volume within each well of a 96-well PCR plate (Bio-Rad Laboratories Ltd, Herts, UK) was 10 µL, which included 1 µL of purified DNA, 5 µL of 2× TaqMan genotyping master mix (Applied Biosystems), 0.5 µL of 20× genotyping assay (Applied Biosystems) and 3.5 µL nuclease-free $H_2O$ (Qiagen). Each PCR run included positive controls for each genotype and negative controls (sterile distilled water). The PCR plate was sealed using MicroSeal 'B' Adhesive seals (Bio-Rad) and ran on a Chromo4 Real-Time PCR Detection System (Bio-Rad) for 10 min at 95 °C. This was followed by 40 cycles of denaturing at 92 °C for 15 s, primer annealing and extension at 60 °C for 60 s, and plate read. Genotypes were determined by endpoint fluorescence of VIC and FAM signals using the Chromo4 PCR machine, and results were analysed using Opticon Monitor Software version 3.1.32 (Bio-Rad). All analyses were run in duplicate, there was 100 % agreement between duplicate wells and genotyping was completed in all samples.

### Hardy–Weinberg equilibrium

Genotype data were tested for Hardy–Weinberg Equilibrium (HWE) using a freely available software package (Rodriguez et al. 2009). This test was conducted on the initial cohort before selecting individuals for the phenotype tests (based on a higher degree of homozygosity), in order to establish whether the genotype and allele frequencies were constant between the initial cohort and the general population. Indeed, the genotype data for this cohort was in HWE [$P > 0.05$ with 1df (one degree of freedom)].

### Oestradiol measures

It has been reported that oestradiol levels are associated with tendon mechanical properties in vivo (Burgess et al. 2009). Therefore, following the measures of patellar tendon properties, female participants reported to the biochemistry laboratory where whole blood (5 mL) was drawn from a superficial forearm vein into serum separator tubes containing anti-coagulant (EDTA) (Sarstedt Monovette-Red cap, Numbrecht, Germany). After storage on ice for ~30 min, the blood was centrifuged at 2–5 °C for 10 min at 4,100 rpm, with the supernatant extracted (~2 mL) and stored at −20 °C for later analysis. Serum 17ß-oestradiol (E2) was quantitatively determined using standard enzyme-linked immunosorbent assay (ELISA) procedures (Alpha Diagnostic International, San Antonio, USA; minimal detectable concentration of ~10 pg/mL, intra-assay precision of 9.9 %, inter-assay precision of 10.1 %). E2 concentration at day 1 of the menstrual cycle was extrapolated using data from women with similar characteristics to those in the current study (i.e., age range 20–36 years, no use of contraceptives) (Stricker et al. 2006). Extrapolated serum oestradiol in the 39 female participants was therefore 34.0 (30.0) pg/mL.

### Statistical power to detect genotype-phenotype associations

Statistical power calculations were performed using G*Power 3.1.6 software (Franz Faul, Universitat Kiel, Germany) (Faul et al. 2007). With alpha set at 0.05 and beta at 0.80, using mean and standard deviation data of patellar tendon properties obtained in our lab, it was estimated that approximately 80 participants would be required to detect differences in the order of ~1–2 % for tendon volume and ~10–15 % for tendon modulus between two genotype groups. Two genotype groups were used in this calculation because the intention, a priori, was to 'stress the genotype'

**Table 1** Age, physical characteristics and genotype at *MMP3* rs591058 and rs679620 loci

| | All | CC GG | CT GA | TT AA | *P* value (3 group comparison) | CC & CT GG and GA | *P* value (2 group comparison) |
|---|---|---|---|---|---|---|---|
| *n* | 84 | 29 | 6 | 49 | – | 35 | – |
| Age (year) | 22.7 (4.0) | 21.5 (2.3) | 21.7 (0.8) | 23.5 (4.8) | **0.247** | 21.5 (2.2) | **0.120** |
| Sex (% male) | 53.6 | 41.4 | 0 | 67.4 | – | 34.3 | – |
| Height (cm) | 172.7 (9.5) | 171.9 (10.4) | 167.8 (6.2) | 173.7 (9.2) | **0.196** | 171.2 (9.9) | **0.179** |
| Mass (kg) | 71.6 (12.4) | 70.5 (12.9) | 62.7 (9.4) | 73.3 (12.0) | **0.114** | 69.2 (12.6) | **0.129** |
| BMI (kg $m^{-2}$) | 24.0 (2.8) | 24.1 (3.1) | 24.7 (3.3) | 23.8 (2.5) | **0.690** | 24.2 (3.1) | **0.477** |

*P* values for the comparisons of three genotype groups, and two genotype groups (when heterozygotes were pooled with one homozygote) were derived from a one-way ANOVA, and independent *t* test, respectively

**Table 2** Age, physical characteristics and genotype at the *MMP3* rs650108 loci

| | All | GG | GA | AA | *P* value (3 group comparison) | GA and AA | *P* value (2 group comparison) |
|---|---|---|---|---|---|---|---|
| *n* | 84 | 61 | 15 | 8 | – | 23 | – |
| Age (year) | 22.7 (4.0) | 23.0 (4.5) | 21.1 (2.3) | 23.0 (2.5) | **0.143** | 21.7 (2.5) | **0.404** |
| Sex (% male) | 53.6 | 62.3 | 23.1 | 50 | – | 34.3 | – |
| Height (cm) | 172.7 (9.5) | 173.5 (9.4) | 169.4 (10.1) | 172.7 (8.7) | **0.340** | 170.6 (9.6) | **0.216** |
| Mass (kg) | 71.6 (12.4) | 72.9 (12.5) | 66.8 (12.2) | 70.6 (10.4) | **0.218** | 68.1 (11.5) | **0.111** |
| BMI (kg $m^{-2}$) | 24.0 (2.8) | 24.1 (2.6) | 23.2 (2.8) | 24.7 (3.8) | **0.408** | 23.7 (3.2) | **0.628** |

The *P* values of the GG vs. GA vs. AA, and GG vs. GA/AA combined genotype groups are derived from a one-way ANOVA, and independent *t* test, respectively

(see first paragraph of "Method") by conducting detailed phenotype testing on individuals homozygous for the polymorphisms under study where possible, while combining any small number of heterozygotes with one homozygote group. This was largely successful for rs591058 and rs679620 (cf. top row of Table 1 in Results) but less successful for rs650108 (cf. top row of Table 2) where statistical power therefore did not reach the target value.

### Statistical analyses

Reliability of patellar tendon CSA, length, maximal displacement, and maximal strain were evaluated using ratio limits of agreement (Nevill and Atkinson 1997) to quantify the absolute reliability or 'agreement' between measurements on separate occasions. All data were analysed with SPSS version 19.0.0. One-way analysis of variance (ANOVA) was performed on all three genotype groups and the measures of PTV and *Z*-scores. In addition, independent *t* tests were performed on volume and *Z*-scores when combining heterozygotes with the smallest homozygote group. The Kruskal–Wallis non-parametric equivalent statistical test was performed on *E* and its association with the three genotype groups, while the Mann–Whitney *U* test was used to compare E between one homozygote group and the other combined genotype group. PTV differed between sexes and BMI was correlated with both PTV and Z-score, so sex and BMI were used as covariates accordingly. Age and oestradiol concentration showed no correlations with any phenotype and so were not used as covariates. Alpha was set at 0.05. Unless otherwise stated, data are presented as mean (standard deviation).

## Results

There were no significant differences in age, height, mass and BMI between the three genotype groups, nor between the combined genotype groups of the *MMP3* rs591058/679620 gene variants (Table 1) and *MMP3* rs650108 gene variant (Table 2). The *MMP3* rs591058 and rs679620 gene variants produced identical results and are therefore presented simultaneously, because they were in perfect linkage disequilibrium (LD) (allele C of rs591058 corresponded to allele G of rs679620).

Between-day repeated measurements of tendon CSA, length, maximal displacement, and maximal strain in a subgroup of participants were examined using ratio limits of agreement. Tendon volume showed no bias and excellent agreement (×/÷ 1.019) and *E* showed no bias and very good agreement (×/÷ 1.144).

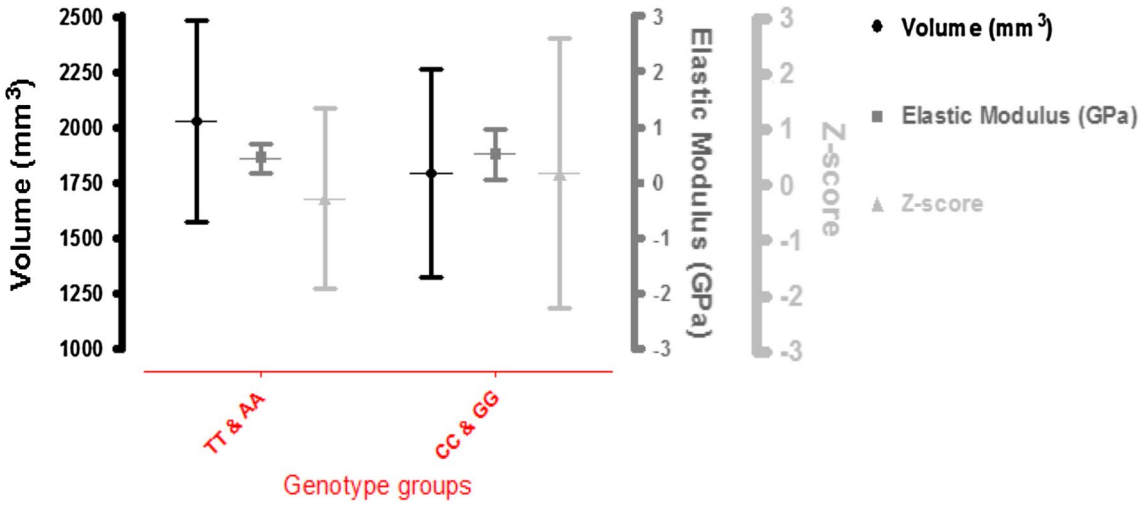


**Fig. 2** Patellar tendon properties for *MMP3* rs591058 and rs679620 genotype groups. Elastic modulus and *Z*-scores are expressed as median (range)

As circulating oestradiol levels showed no significant correlation with patellar tendon properties (CSA, $r = 0.155$, $P = 0.345$; Tendon length, $r = -0.243$, $P = 0.137$; Maximal displacement, $r = -0.258$, $P = 0.113$; Maximal strain, $r = -0.171$, $P = 0.298$; Maximal stiffness, $r = 0.148$, $P = 0.368$; Standardised stiffness, $r = 0.267$, $P = 0.100$; Volume, $r = 0.010$, $P = 0.950$; *E*, $r = 0.146$, $P = 0.375$; *Z*-scores, $r = 0.055$, $P = 0.740$), and having adjusted for non-genetic factors such as sex and BMI, male and female participants data were therefore pooled into a single population. There were no significant differences in patellar tendon CSA, length, maximal displacement, maximal strain, stiffness at a common force level (Online Resource 1), or indeed tendon volume ($P = 0.845$) and *E* ($P = 0.226$) or *Z*-scores ($P = 0.654$) (Fig. 2), between the three genotype groups of the *MMP3* rs591058/679620 gene variants. Nor, were there any significant differences when comparing the TT/AA genotype groups to the combined CT and CC/GA and GG genotype groups (Volume, $P = 0.859$; *E*, $P = 0.125$; *Z*-scores, $P = 0.357$). Both the CT and CC/GA and GG genotype groups were combined due to the small CC and GG groups of the rs590158 and rs679620 gene variants, respectively.

In addition, there were no significant differences between the three genotype groups of the *MMP3* rs650108 gene variant in patellar tendon CSA, length, maximal displacement, maximal strain, and stiffness (Online Resource 2), or for measures of volume ($P = 0.984$), *E* ($P = 0.088$) or *Z*-scores ($P = 0.390$) (Fig. 3). Or indeed, when comparing the GG genotype group to the combined GA and AA genotype groups (Volume, $P = 0.881$; *E*, $P = 0.112$; *Z*-scores, $P = 0.483$). The GA and AA genotype groups were combined due to the small AA group size.

## Discussion

This study finds no association between the three variants of the *MMP3* gene investigated, and patellar tendon properties. Specifically, the *MMP3* gene variants investigated do not associate with dimensional (volume) and functional (*E*) properties of the patellar tendon, or indeed as a composite (*Z*-scores), in an asymptomatic population. In this study, patellar tendon modulus was comparable with other data for this phenotype in healthy, young, male and female combined subpopulations, between 0.44 and 0.72 GPa compared with 0.55–0.60 GPa (O'Brien et al. 2010) and 0.75 GPa (Hsin-Yi and Paul 2010). The larger sample size used in the present study ($n = 84$) compared to those aforementioned studies ($n = 10$–$20$) (Hsin-Yi and Paul 2010; O'Brien et al. 2010) gives us increasing confidence that the modulus data are representative of the wider population. No previous study has reported PTV.

Although none of the variants investigated in this study were found to be associated with patellar tendon properties, it does not exclude the possibility that other variants within genes clustered in close proximity to the *MMP3* gene on chromosome 11q22, such as *MMP10*, *MMP1*, and *MMP12,* are associated with patellar tendon properties. Like *MMP3*, the protein products of these genes are capable of degrading a diverse array of extracellular matrix (ECM) proteins (Pasternak and Aspenberg 2009; Somerville et al. 2003), so one might assume that because *MMP3* variants have been associated with tendinopathies (Raleigh et al. 2009), associations may be observed between these other *MMP* gene variants and tendon properties because the intrinsic regulatory proteins associated with tendon pathologies are also directly involved in non-pathological maintenance processes within tendon.

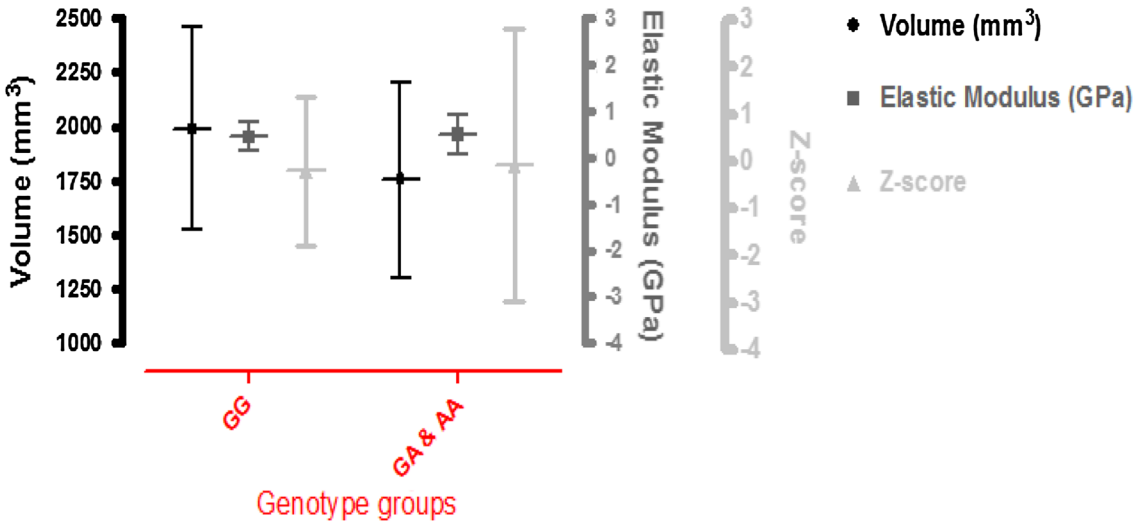


**Fig. 3** Patellar tendon properties for *MMP3* rs650108 genotype groups. Elastic modulus and *Z*-scores are expressed as median (range)

Consistency of results when considering each of the three gene variants and measures of patellar tendon properties was expected, given that they are known to be in high linkage disequilibrium with one another. In particular, the rs591058 and rs679620 variants are in perfect linkage disequilibrium, with the 'C' allele of rs591058 corresponding with the 'G' allele of rs679620. In addition, the rs650108 variant displays high linkage disequilibrium with the rs679620 and rs591058 variants ($r = 0.673$, $P = 0.01$).

A promoter polymorphism within the *MMP3* gene (rs3025058) is also believed to be tightly linked to the rs679620 variant (Beyzade et al. 2003; Chen et al. 2012; Raleigh et al. 2009). Both markers are associated with MMP3 expression levels, notably the rs3025058 5A allele and the rs679620 'A' allele are associated with relatively lower levels of MMP3, and the highest level of pathological activity related to rheumatoid arthritis. In addition, lower MMP3 protein expression levels have been reported in human tendon displaying pathological characteristics (de Mos et al. 2007; Ireland et al. 2001; Jones et al. 2006; Parkinson et al. 2010; Riley et al. 2002). These observations may represent a failure of the normal matrix remodelling process (Riley et al. 2002). However within a non-pathological range, lower MMP3 levels may favour a state of imbalance with greater synthesis relative to degradation, thus, substrates involved in cross-linking and stabilisation of intact fibrillar collagen may be relatively less affected by degradation processes.

Ultimately, the AA genotype of the rs679620 variant may associate with a higher matrix stiffness and higher *E*, when considering the above rationale, yet this assumption was not evident in this study as E was not significantly different between the AA and GG genotype groups ($P = 0.226$; 0.44 GPa vs. 0.48 GPa, respectively). Nor, was it evident in the only other previous genetic association study attempting to link this gene variant with tendon mechanical stiffness of the knee extensors and plantar flexors (Kubo et al. 2013). Correspondingly, the TT and CC genotype groups of the rs591058 variant do not differ significantly. However, the functional significance of the rs650108 variant has yet to be determined, most likely due to the fact that it resides in an intron, which makes it more difficult to explain why as part of a haplotype, it associates with pathological states (Koch et al. 2010; Raleigh et al. 2009). It may be that within normal physiological ranges, the activity of MMP3 is tightly controlled by tissue inhibitors of MMPs (TIMPs) (Riley 2005), thus inhibiting the degeneration of the ECM and loss of material properties. This has been reported in stress-deprived tendons in vitro, subjected to inhibitors to prevent the activation of MMP activity (Arnoczky et al. 2007). Therefore, variations in MMP3 activity related to differences in the ability to turn over the matrix may only become apparent following damage and the onset of pathological states.

We believe a valuable approach undertaken in this study was to increase the ability to detect genotype-phenotype associations. By recruiting a high proportion of participants from an original cohort who were homozygous for the gene variants under investigation, we were able to target our efforts during a detailed phenotype assessment. Hence, the genotype was being 'stressed'(Montgomery et al. 2002). Despite this approach, we observed no associations, although it is precisely because of our approach that we have confidence in our conclusions. Future researchers investigating gene variants and their association with tendon properties are encouraged to adopt this approach in

synergy with a larger sample size where possible (a limitation of this study), to further increase the ability to detect associations of small effect size and provide even greater confidence in findings. Arguably, using a large sample size becomes even more important when considering that whole tendon mechanical properties are likely to be a product of the structure and function, and thus mechanical properties of the ECM at a sub-structural level, i.e., the inter-fascicular matrix. A recent study's findings in vivo (Pearson et al. 2014) suggest there may be regional strain differences between the deep, middle and surface structures at the proximal and distal aspects of the patellar tendon. However, this might not be of particular concern for gene association studies because the whole tendon property is a composite of all tendon regions and the whole tendon remains the functional in vivo structure.

Future research should also attempt to determine whether there is a differential expression of *MMP3* genes in tendon displaying relatively high and low tendon modulus, to assist in determining whether these genes are causally implicated in modifying mechanical properties of tendon, through mechanisms which remodel its microstructure. Additionally, associations could be established between protein expression levels and the genotypes of *MMP3* sequence variants.

In conclusion, this study finds no evidence of an association between the three variants of the *MMP3* gene investigated and dimensional and mechanical properties of the patellar tendon. However, our observations need replication and/or new study using other tendons. In addition, the relationship between pathological tendon conditions and tendon mechanical properties should be investigated to improve injury prevention models.

**Conflict of interest** None.

**Ethical standard** Ethics approval was provided by Human Research Ethics Committee of Manchester Metropolitan University.

**Patient consent** Obtained.

## References


Ackerman PL, Cianciolo AT (2000) Cognitive, perceptual-speed, and psychomotor determinants of individual differences during skill acquisition. J Exp Psychol Appl 6:259–290

Arnoczky SP, Lavagnino M, Egerbacher M, Caballero O, Gardner K (2007) Matrix metalloproteinase inhibitors prevent a decrease in the mechanical properties of stress-deprived tendons: an in vitro experimental study. Am J Sports Med 35:763–769. doi:10.1177/0363546506296043

Baltzopoulos V (1995) A videofluoroscopy method for optical distortion correction and measurement of knee-joint kinematics. Clin Biomech Bristol Avon 10:85–92. (pii: 026800339592044M)

Beyzade S, Zhang S, Wong YK, Day IN, Eriksson P, Ye S (2003) Influences of matrix metalloproteinase-3 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction. J Am Coll Cardiol 41:2130–2137. (pii: S0735109703004820)

Burgess KE, Pearson SJ, Onambele GL (2009) Patellar tendon properties with fluctuating menstrual cycle hormones. J Strength Cond Res. doi:10.1519/JSC.0b013e3181aeb12b

Chen Y, Nixon NB, Dawes PT, Mattey DL (2012) Influence of variations across the MMP-1 and -3 genes on the serum levels of MMP-1 and -3 and disease activity in rheumatoid arthritis. Genes Immun 13:29–37. doi:10.1038/gene.2011.46

Collins M, Posthumus M (2011) Type V collagen genotype and exercise-related phenotype relationships: a novel hypothesis. Exerc Sport Sci Rev 39:191–198. doi:10.1097/JES.0b013e318224e853

Collins M, Raleigh SM (2009) Genetic risk factors for musculoskeletal soft tissue injuries. Med Sport Sci 54:136–149. doi:10.1159/000235701

de Mos M et al (2007) Achilles tendinosis. Am J Sports Med 35:1549–1556. doi:10.1177/0363546507301885

Erskine RM, Morse CI, Day SH, Williams AG, Onambele-Pearson GL (2014) The human patellar tendon moment arm assessed in vivo using dual-energy X-ray absorptiometry. J Biomech 47:1294–1298. doi:10.1016/j.jbiomech.2014.02.016

Faul F, Erdfelder E, Lang AG, Buchner A (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39:175–191

Fletcher JR, Esau SP, MacIntosh BR (2010) Changes in tendon stiffness and running economy in highly trained distance runners. Eur J Appl Physiol 110:1037–1046. doi:10.1007/s00421-010-1582-8

Foster BP, Morse CI, Onambele GL, Williams AG (2014) Human COL5A1 rs12722 gene polymorphism and tendon properties in vivo in an asymptomatic population. Eur J Appl Physiol. doi:10.1007/s00421-014-2868-z

Fuller NJ, Hardingham CR, Graves M, Screaton N, Dixon AK, Ward LC, Elia M (1999) Predicting composition of leg sections with anthropometry and bioelectrical impedance analysis, using magnetic resonance imaging as reference. Clin Sci Lond 96:647–657

Glasel JA (1995) Validity of nucleic acid purities monitored by 260 nm/280 nm absorbance ratios. Biotechniques 18:62–63

Hsin-Yi L, Paul SW (2010) Reliability of a two-scan ultrasonography method for evaluating patellar tendon stiffness. Bull Appl Mech 6:41–43

Ireland D, Harrall R, Curry V, Holloway G, Hackney R, Hazleman B, Riley G (2001) Multiple changes in gene expression in chronic human Achilles tendinopathy. Matrix Biol 20:159–169

Jones PR, Pearson J (1969) Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. J Physiol 204:63P–66P

Jones GC et al (2006) Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human achilles tendon. Arthritis Rheum 54:832–842. doi:10.1002/art.21672

Ker RF, Alexander RM, Bennett MB (1988) Why are mammalian tendons so thick? J Zool 216:309–324. doi:10.1111/j.1469-7998.1988.tb02432.x

Koch W, de Waha A, Hoppmann P, Schomig A, Kastrati A (2010) Haplotypes and 5A/6A polymorphism of the matrix metalloproteinase-3 gene in coronary disease: case-control study and a meta-analysis. Atherosclerosis 208:171–176. doi:10.1016/j.atherosclerosis.2009.08.021

Kubo K, Kanehisa H, Kawakami Y, Fukunaga T (2001) Influence of static stretching on viscoelastic properties of human tendon structures in vivo. J Appl Physiol 90:520–527

Kubo K, Yata H, Tsunoda N (2013) Effect of gene polymorphisms on the mechanical properties of human tendon structures. SpringerPlus 2:343. doi:10.1186/2193-1801-2-343

Lichtwark GA, Barclay CJ (2010) The influence of tendon compliance on muscle power output and efficiency during cyclic contractions. J Exp Biol 213:707–714. doi:10.1242/jeb.038026

Lo IK, Marchuk LL, Leatherbarrow KE, Frank CB, Hart DA (2004) Collagen fibrillogenesis and mRNA levels in the maturing rabbit medial collateral ligament and patellar tendon. Connect Tissue Res 45:11–22. doi:10.1080/03008200490278070

Maffiuletti NA, Bizzini M, Desbrosses K, Babault N, Munzinger U (2007) Reliability of knee extension and flexion measurements using the Con-Trex isokinetic dynamometer. Clin Physiol Funct Imaging 27:346–353. doi:10.1111/j.1475-097X.2007.00758.x

Maganaris CN (2003) Tendon conditioning: artefact or property? Proc Biol Sci 270(Suppl 1):S39–S42. doi:10.1098/rsbl.2003.0004

Matrisian LM (1990) Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet 6:121–125

McCleary RW, Andersen JC (1992) Test-retest reliability of reciprocal isokinetic knee extension and flexion peak torque measurements. J Athl Train 27:362–365

McGinnis PM (2005) Biomechanics of sport and exercise, 2nd edn. Human Kinetics Champaign, IL, London

Montgomery H, Brull D, Humphries SE (2002) Analysis of gene-environment interactions by "stressing-the-genotype" studies: the angiotensin converting enzyme and exercise-induced left ventricular hypertrophy as an example. Ital Heart J 3:10–14

Nevill AM, Atkinson G (1997) Assessing agreement between measurements recorded on a ratio scale in sports medicine and sports science. Br J Sports Med 31:314–318

O'Brien TD, Reeves ND, Baltzopoulos V, Jones DA, Maganaris CN (2010) Mechanical properties of the patellar tendon in adults and children. J Biomech 43:1190–1195. doi:10.1016/j.jbiomech.2009.11.028

Onambele GN, Burgess K, Pearson SJ (2007) Gender-specific in vivo measurement of the structural and mechanical properties of the human patellar tendon. J Orthop Res 25:1635–1642. doi:10.1002/jor.20404

Onambele-Pearson NL, Pearson SJ (2007) Time-of-day effect on patella tendon stiffness alters vastus lateralis fascicle length but not the quadriceps force-angle relationship. J Biomech 40:1031–1037. (pii: S0021-9290(06)00125-4)

Onambele-Pearson GL, Breen L, Stewart CE (2010) Influence of exercise intensity in older persons with unchanged habitual nutritional intake: skeletal muscle and endocrine adaptations. Age 32:139–153. doi:10.1007/s11357-010-9141-0

Parkinson J, Samiric T, Ilic MZ, Cook J, Feller JA, Handley CJ (2010) Changes in proteoglycan metabolism is a characteristic of human patellar tendinopathy. Arthritis Rheum 62:3028–3035. doi:10.1002/art.27587

Pasternak B, Aspenberg P (2009) Metalloproteinases and their inhibitors-diagnostic and therapeutic opportunities in orthopedics. Acta Orthop 80:693–703. doi:10.3109/17453670903448257

Pearson SJ, Onambele GN (2006) Influence of time of day on tendon compliance and estimations of voluntary activation levels. Muscle Nerve 33:792–800. doi:10.1002/mus.20529

Pearson SJ, Burgess K, Onambele GN (2007) Creep and the in vivo assessment of human patellar tendon mechanical properties. Clin Biomech Bristol Avon 22:712–717. doi:10.1016/j.clinbiomech.2007.02.006

Pearson SJ, Ritchings T, Mohamed AS (2014) Regional strain variations in the human patellar tendon. Med Sci Sports Exerc 46:1343–1351. doi:10.1249/MSS.0000000000000247

Raleigh SM, van der Merwe L, Ribbans WJ, Smith RKW, Schwellnus MP, Collins M (2009) Variants within the MMP3 gene are associated with Achilles tendinopathy: possible interaction with the COL5A1 gene. Br J Sports Med 43:514–520. doi:10.1136/bjsm.2008.053892

Reeves ND, Maganaris CN, Narici MV (2003) Effect of strength training on human patella tendon mechanical properties of older individuals. J Physiol 548(971–981):2002. doi:10.1113/jphysiol.2002.035576.035576

Riley GP (2005) Gene expression and matrix turnover in overused and damaged tendons. Scand J Med Sci Sports 15:241–251. doi:10.1111/j.1600-0838.2005.00456.x

Riley GP, Curry V, DeGroot J, van El B, Verzijl N, Hazleman BL, Bank RA (2002) Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. Matrix Biol 21:185–195. (pii: S0945053X01001962)

Rodriguez S, Gaunt TR, Day IN (2009) Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 169:505–514. doi:10.1093/aje/kwn359

Seynnes OR, Erskine RM, Maganaris CN, Longo S, Simoneau EM, Grosset JF, Narici MV (2009) Training-induced changes in structural and mechanical properties of the patellar tendon are related to muscle hypertrophy but not to strength gains. J Appl Physiol 107:523–530. doi:10.1152/japplphysiol.00213.2009

Somerville RP, Oblander SA, Apte SS (2003) Matrix metalloproteinases: old dogs with new tricks. Genome Biol 4:216. doi:10.1186/gb-2003-4-6-216

Stricker R, Eberhart R, Chevailler MC, Quinn FA, Bischof P (2006) Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyzer. Clin Chem Lab Med 44:883–887. doi:10.1515/CCLM.2006.160

Tothill P, Stewart AD (2002) Estimation of thigh muscle and adipose tissue volume using magnetic resonance imaging and anthropometry. J Sports Sci 20:563–576. doi:10.1080/026404102760000062

Tsaopoulos DE, Baltzopoulos V, Maganaris CN (2006) Human patellar tendon moment arm length: measurement considerations and clinical implications for joint loading assessment. Clin Biomech Bristol Avon 21:657–667. doi:10.1016/j.clinbiomech.2006.02.009

Tucker JM, Welk GJ, Beyler NK (2011) Physical activity in US: adults compliance with the physical activity guidelines for Americans. Am J Prev Med 40:454–461. doi:10.1016/j.amepre.2010.12.016

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

Zipp P (1982) Recommendations for the standardization of lead positions in surface electromyography Europ. J Appl Physiol 50:41–54. doi:10.1007/bf00952243