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# INFLUENCE OF LOADING RATE ON PATELLAR TENDON MECHANICS *IN VIVO.*

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## Abstract

***Background***. Rate-dependent properties of tendons have consistently been observed *in vitro* but *in vivo* studies comparing the effects of loading duration on this phenomenon remain conflicting. The main purpose of the present study was to evaluate whether tendon loading rate *per se* would affect *in vivo* tendon mechanical properties.

***Methods***. Twenty-two physically active male subjects were recruited and patellar tendon deformation was recorded with ultrasonography under voluntary isometric contractions at rates of 50, 80 and 110 Nm/s, controlled via visual feedback.

***Findings***. Subjects were able to accurately generate all three loading rates (2% to 15%), with a greater steadiness at 50 (CV=12.4%) and 110 Nm/s (CV=13.1%) than at 80 Nm/s (CV=22.9%). Loading rate did not appreciably affect strain or stress. However, mechanical ($\eta p^2$ = 0.555) and material ($\eta p^2$ = 0.670) properties were significantly higher at 80 Nm/s (21.4 % and 21.6 %, respectively) and at 110 Nm/s (32.5 % and 32.0 %, respectively) than at 50 Nm/s. Similarly, stiffness and Young's modulus were 9.9 % and 8.8 % important methodological implications for the assessment of this tendon and possibly other human tendons.

higher, respectively, at 110 Nm/s than at 80 Nm/s.

***Interpretation***. These results indicate that *in vivo* measurements of patellar tendon mechanics are influenced by loading rate. Moreover, they bear

## Introduction

Tendons are anisotropic connective tissues exhibiting nonlinear and viscoelastic mechanical properties (Butler et al., 1978; Connizzo et al., 2013; Wang, 2006). Numerous *in vitro* studies, yet not all (Blevins et al., 1994), showed a strain rate sensitivity of various tendon fascicles and tendon proper in human (Cheng et al., 2009; Haut and Haut, 1997; Svensson et al., 2012) and animal specimen (Buckley et al., 2013; Clemmer et al., 2010; Danto and Woo, 1993; Davis and De Vita, 2012; Lynch et al., 2003; Robinson et al., 2004). This mechanical behaviour is always taken into consideration during *in vitro* testing of tendon mechanics, mostly via the standardisation of strain rate.

In the past two decades, the estimation of tendon mechanical properties *in vivo* has been developed around non-invasive ultrasonographic imaging of tendon elongation. Tests are usually performed by asking the subject to perform a maximal isometric ramp contraction over a pre-defined time window (Carroll et al., 2008; Couppé et al., 2013; Maganaris et al., 2004; O'Brien et al., 2010; Pearson et al., 2007; Reeves et al., 2003; Seynnes et al., 2009; Waugh et al., 2012). Owing to the rate-dependent mechanics described above, the validity and reliability of this technique is limited by the variability of self-paced torque development and by inter-individual differences in maximal torque *in vivo*.

A few authors have attempted to control the influence of tendon viscoelasticity by studying tendon deformation over a fixed time window, with (Fouré et al., 2010; Gerus et al., 2011; Peltonen et al., 2013) or without (Kubo et al., 2002a; Pearson et al., 2007) a direct visual feedback. However, these studies relied essentially on contraction duration, rather than loading rate *per se*, and rate-dependent properties of tendons have never been assessed with a thorough control of loading rate independently from the maximum force level. In addition, results from these reports are conflicting, showing an effect of contraction duration on patellar tendon stiffness (Pearson et al., 2007), whilst this effect was inconsistently observed in the Achilles tendon (Kubo et al., 2002b; Peltonen et al., 2013; Theis et al., 2012). Therefore, the purpose of the present study was i) to assess the feasibility of loading rate control during voluntary contractions and ii), to investigate rate-dependent mechanical behaviour of the human patellar tendon *in vivo*. We hypothesized that with a thorough control of loading rate, the patellar tendon would display rate sensitive mechanics, as evidenced by a dose-response relationship between its mechanical properties and loading rate.

## Materials and methods

### Subjects

Twenty-two physically active male students (age, 23.6 (SD 3.1) years; height, 180.3 (SD 6.5) cm; mass 76.2 (SD 8.1) kg) from the University of Salzburg were recruited amongst participants to a previous study on the reliability of our dynamometer (Dirnberger et al., 2012). However, the data from two subjects were discarded because of the insufficient quality of ultrasound captures (see below, *Tendon Mechanical Properties* section). Therefore, the results were obtained from the remaining twenty subjects (age 23.7 (SD 3.2) years; height 180.1 (SD 6.5) cm; mass 75.8 (SD 8.3) kg). They were free of physical disabilities or orthopaedic problems limiting testing of the right limb and recreationally active. Other exclusion criteria included untreated hypertension, medical history of diabetes, thrombosis or known cardiovascular disorders. All subjects signed a written declaration of consent before testing. The volunteers were asked to maintain their regular physical activity levels during the study, but to refrain from resistance training or vigorous physical activity of the lower extremities. The study protocol was approved by the Ethics Committee of the University of Salzburg in Austria and conformed to the requirements of the Declaration of Helsinki.

### Tendon force

Measurements were preceded by a warm up of six minutes of cycling on a stationary ergometer (Heinz Kettler GmbH and Co. KG, Ense-Parsit, Germany) at a sub-maximal intensity of 1.5 W/KgBW and a pedal rate of 70 rpm.

Participants were seated and fastened on a rigid dynamometer chair (IsoMed 2000 D&R Ferstl GmbH, Hemau, Germany). The backrest of the dynamometer was adjusted to set the hip angle at 75° (0° corresponding to supine position). The knee joint was fixed at 90° flexion (0° corresponding to full extension) and the lateral femoral epicondyle served as a reference to align the main joint axis with the axis of rotation of the dynamometer. Gravity correction of the torque was calculated online with the integrated dynamometer software.

Subjects were instructed to perform isometric ramp contractions to maximal exertion at three predefined loading rates: 50, 80, and 110 Nm/s. The order of loading rate was randomly selected. Rest periods were set at 60 s between trials at each loading rate and at 3 min between the loading rates (Figure 1).

**Insert Figure 1**: Experimental design

During contractions, subjects were given a visual feedback of the joint moment vs. time (Figure 2). Three to five practice trials were conducted at each loading rate to familiarise the subjects with the task. A three-minute rest period was allowed between familiarization and the testing protocol to minimize fatigue. Three short preconditioning contractions were completed immediately prior to the measurements.

**Insert Figure 2**: Tendon testing protocol

An echo-absorptive wire was fixed on the skin, across the tendon, to indicate possible motion artefacts and the ultrasound transducer was positioned sagittally over the tendon. Video clips were acquired during the ramp contraction at a frame rate of 43 fps, and synchronised with torque and sEMG signals for offline calculation of tendon force.

In order to account for antagonist co-activation during knee extension, surface electromyographic (sEMG) activity of the biceps femoris muscle was recorded during isometric knee extensions and flexions. Skin preparation and placement of two self-adhesive electrodes (Ag/AgCL; 120 dB, Input impedance: 1200 GOhm; 10 mm diameter, 22 mm spacing, Biovision, Wehrheim, Germany) on the biceps femoris muscle were carried out according to SENIAM guidelines (Hermens et al., 1999). To record the maximal sEMG activity of the biceps femoris muscle during agonist contractions, participants were instructed to exert two maximal knee flexion contractions. Raw signals were amplified (bandwidth 30-300 Hz, 3 dB; Biovision) and digitized (sampling frequency of 1 kHz, Daqcard-700, National Instruments, Austin, Texas, USA) with a custom made software (IKE-Software Solutions, Salzburg, Austria) and sEMG root mean square was calculated over a period of 1 s around the MVC peak torque for the knee flexion trials. By assuming a linear relationship between sEMG activity and moment, antagonist co-activation moment during knee extension trials was estimated and added to the measured moment to obtain the net extension moment.

To calculate tendon force, the net knee extension moment was divided by the tendon moment arm length. The latter was obtained from anthropometric measurements (Visser et al., 1990).

**Tendon geometry**

Sonographic images of the patellar tendon were taken by using a linear array transducer (LA523, 10- to 15-MHz transducer, MyLab25, Esaote, Genoa, Italy). Patellar tendon length was measured externally, as the distance between the tibial enthesis and the apex of the patella. Tendon cross-sectional area was imaged using transversal scans at the proximal insertion of the patellar tendon (CSAp), the mid portion (CSAm) and just above the insertion at the tibial enthesis (CSAd). An average of the CSAs measured at these three scan positions was used for further analysis. All tendon CSA scans were evaluated with ImageJ (version 1.41, NIH, Bethesda, USA) by the same investigator.

**Tendon mechanical properties**

Total tendon elongation was computed offline as the sum of tibial and patella positional alterations during each trial. Ultrasonographic sequences of images were first inspected visually to identify trials where the shape of the patella apex and/or that of the tibial plateau were not consistent throughout. Subjects were excluded if less than two ultrasound recordings per testing condition were deemed fit for analysis. The patella apex and the tibial plateau were tracked frame-by-frame using a semi-automatic analysis software (Tracker 4.8 (Cabrillo.edu/-dbrown/tracker)).

The tendon force-elongation relationship was plotted for each individual and fitted with a second-order polynomial function. Any data set with a coefficient of determination $R^2 < 0.90$ was excluded from further analyses. For standardisation purposes, the maximal force of the weakest subject was defined as the common force level. Therefore, tendon stiffness (k) and Young's modulus (E) were calculated over force and stress ranges corresponding to the highest 10% of these variables common to all participants, using Eq. (1) and Eq. (2). Stress was calculated by dividing tendon force by the mean CSA and strain was obtained as the ratio of tendon length relative to initial resting length. The common force range was 4474 N to 4971 N and the common stress range was XX MPa to XX MPa.

$$k = \frac{\partial F}{\partial l} \qquad \text{Eq. 1}$$

$$E = k \times \frac{l_0}{CSA} \qquad \text{Eq. 2}$$

Where $\partial F$ is the change in force and $\partial l$ describes the tendon deformation over the force interval. CSA is the mean tendon cross sectional area and $l_0$ the resting tendon length.

**Assessment of loading rate accuracy**

To verify if the subject could accurately follow the predefined loading rates, accuracy and steadiness were calculated. A Matlab (version R2012b, The MathWorks) routine was used to analyze the torque values of each ramp contraction between a threshold of 8 Nm up to maximum of the weakest subject. Accuracy was estimated by calculating the mean differences between target- and real loading rate. To evaluate the steadiness of torque development, the coefficient of variation (CV) was used.

## Measurement reliability

Inter-day reliability was determined for measurements of tendon length, CSA and elongation by a test-retest design in a subgroup of eight subjects (age 22.8 (SD 3.4) years; height 182.0 (SD 6.9) cm; mass 75.6 (SD 7.1) kg). Two testing sessions were conducted at the same time of the day (± 2 h), 4 days in apart, and by the same investigator. Relative reproducibility was determined using the intraclass correlation coefficient (ICC 2.1), coefficient of variation (CV) and mean differences (table 1). In line with Vincent (1995), an ICC over 0.9 was considered as high, between 0.8 and 0.9 as moderate, and below 0.8 as low.

**Insert Table 1**: Reliability of patellar tendon measurements of deformation and morphology

## Statistics

All statistics were performed using Statistical Package for Social Sciences (SPSS Inc. V.17., Chicago, Illinois, USA) and Microsoft Excel 2007 (Microsoft Corp., Redmont, Washington, USA). Data were analysed using repeated measures analyses of variance (ANOVA) with a Bonferroni post hoc test to determine whether there were any significant effects of the loading rates. Level of significance was set at $P < 0.05$. Data are presented as means, standard deviation (SD) and partial eta squared ($\eta_p^2$) was calculated.

## Results

Tendon mean CSA and length were 97 (SD 14) $mm^2$ and 50 (SD 5 mm), respectively. No significant difference was found in elongation, strain or stress measured at different loading rates (Table 2). Mechanical and material properties were significantly higher at 80 Nm/s ( 21.4 % and 21.6 %, respectively) and at 110 Nm/s (32.5 % and 32.0 %, respectively) than at 50 Nm/s. Similarly, stiffness and Young’s modulus were 9.9 % and 8.8 % higher, respectively, at 110 Nm/s than at 80 Nm/s (Figure 3 and 4b).

**Insert Table 2**: Patellar tendon load deformation characteristics as a function of loading rate

**Insert Figure 3**: Patellar tendon stiffness and Young´s modulus as a function of loading rate

The accuracy with which subject could follow loading rate targets ranged from 0.9 to 12.2 Nm/s, with no significant difference between loading rates. However, the variability of exerted loading rates about the target, as expressed by steadiness, was found to be lesser at 50 Nm/s (12.4 %) and higher at 80 Nm/s (22.9 %) (Figure 4a).

**Insert Figure 4a**: Accuracy and steadiness of torque development as a function of loading rate

**Insert Figure 4b**: Patellar tendon stress-strain relationship as a function of loading rate

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## Discussion

The primary objective of the study was to explore the effect of loading rate on the mechanical properties of human patellar tendon when measured *in vivo*. Our results clearly indicate incremental changes in stiffness and Young's modulus across loading rates of 50, 80 and 110 Nm/s. These findings are in line with most of *in vitro* reports (Cheng et al., 2009; Clemmer et al., 2010; Danto and Woo, 1993; Svensson et al., 2012) and with one *in vivo* study showing the influence of contraction duration on the patellar tendon properties (Pearson et al., 2007). However, they contrast with the monotonous properties measured in the Achilles tendon over various contraction durations (Gerus et al., 2011; Kubo et al., 2002a; Peltonen et al., 2013).

In the recent years, some authors have improved *in vivo* testing procedures to address the possible influence of tendon viscosity, including online visual feedback in their protocol to approach loading rate consistency (Fouré et al., 2010; Gerus et al., 2011; Maganaris, 2003; Nordez et al., 2010; Peltonen et al., 2013). However, to our knowledge, inter-individual differences in MVIC accounting for possible differences in loading rates over fixed time windows have never been addressed. To illustrate the relevance of this methodological point, one can calculate that two individuals with a 20% difference in force performing 2-s ramp contractions to 300 and 360 Nm, respectively, would exert loading rates differing by 30 Nm/s. Our findings demonstrate that such differences in the loading rate of the patellar tendon can affect measurements of stiffness and modulus. Clearly, the impact of this phenomenon would probably be reduced in some testing conditions, for it is inversely proportional to torque/force magnitude and contraction duration. Nevertheless, our results indicate the necessity to control loading rate during testing of the patellar tendon in healthy adults with an expected inter-individual variability in force. This necessity may even be increased in studies including subgroups of different age, sex or MVIC (Carroll et al., 2008; Couppé et al., 2009; O'Brien et al., 2010; Stenroth et al., 2012), or in intervention studies on the effect of training or disuse (Couppé et al., 2012; Kubo et al., 2009; Kubo et al., 2001; Matschke et al., 2013; Reeves, 2005), where larger differences in strength and in loading rate are expectable. In any case, these results suggests that the validity and dispersion of tendon properties measured *in vivo* are affected by inadequate control of loading rate.

Most in vitro studies have long established the influence of stress and strain rates upon the mechanical properties of soft connective tissue like tendons and ligaments (Butler et al., 1978). The exact mechanisms of this influence remain elusive but are attributable to the relative viscosity of these tissues. Viscosity can arise from various structural or fluid interactions processes at different hierarchical levels (Gupta et al., 2010; Kannus, 2000; Screen et al., 2005; Screen and Tanner, 2012). One of the main theories is the high affinity for water with some constituents of the extracellular matrix and the reduced fluid dispersion time at higher strain rate (Ciarletta and Ben Amar, 2009; Elliott et al., 2003). This theoretical mechanism probably explains discrepant results between *in vitro* studies of tendon properties, in which the hydration level of specimen may differ (Haut and Haut, 1997). Furthermore, considering the link between tendon mechanical properties and their functional requirements (Shadwick, 1990), tendon microstructure and relative viscosity could differ in function of their functional requirements (Screen et al., 2013). The elastic fraction of viscoelastic materials, ascribed to collagen fibre type and tissue geometry, defines the proportion of strain energy that can be retrieved without any loss (Dunn and Silver, 1983). Consistently, the elastic fraction of tendons with an important role in storage and release of elastic energy (e.g. Achilles tendon) seems particularly high, relative to the viscous fraction, than in other tendons (Stenroth et al., 2012; Tian et al., 2011). These differences would partly explain the discrepancy between the present findings on the patellar tendon and *in vivo* studies on Achilles tendon (Gerus et al., 2011; Kubo et al., 2002a; Peltonen et al., 2013). Peltonen et al.

(2013) formulated similar conclusions based on their observations of similar Achilles tendon stiffness measured during brief or long contractions. Nevertheless, further studies controlling Achilles tendon loading rate *per se* would ascertain this hypothesis.

In addition to the relevance of loading rate control for *in vivo* testing of the patella tendon, this study demonstrates the feasibility of this approach and suggests some limitations. Mean accuracy and steadiness measurements indicate loading rate targets can be met with a satisfactory precision, ranging from 2% to 15%, for a mean coefficient of variation ranging 12% to 23%. These data also show that accuracy and steadiness are greatest in the higher force region, in which stiffness and modulus are measured (Figure 4a). Despite this agreement between the force generated by the subjects and the visual targets, statistical analyses also indicate that slower (50 Nm/s) and faster (110 Nm/s) loading rates can be achieved more reliably than the intermediate one (80 Nm/s) (Figure 4a). This trend should be confirmed over a broader range of time windows corresponding to current research practice (i.e. >1s to 10s). However, these results suggest that loading rate variability is reduced in contractions in which the influence of motor control is reduced, or in contractions slow enough to enable effective motor control.

## Conclusion

The present study demonstrated the feasibility of loading rate control during *in vivo* testing of the patellar tendon mechanical properties, indicating that this task is best achieved at fast and slow rates of isometric force exertion. The comparison of three loading rates showed that measurements of patellar tendon properties *in vivo* are affected by differences in loading rate. These findings and previous studies on the Achilles tendon suggests that, unlike the latter, the viscous fraction of the patellar tendon properties is sufficient to influence *in vivo* testing outcomes.

## Conflict of interest statement

The authors disclose that there is no potential conflict of interest, including no financial and personal relationships with other people or organizations, patent applications or consultancies that could inappropriately influence this work.

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**Figure 1**: Experimental design

**Figure 2**: Tendon testing protocol

(modified from Bojsen-Moller et al., 2005)

**Figure 3**: Patellar tendon stiffness and Young´s modulus as a function of loading rate

+ $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

**Figure 4a**: Accuracy and steadiness of torque development as a function of loading rate

Dashed lines denote targeted torque development; Plain white lines denote mean torque development; Shaded areas denote steadiness. Mean accuracy and steadiness values are reported for each loading rates. * $P < 0.05$, ** $P < 0.01$

**Figure 4b**: Patellar tendon stress-strain relationship as a function of loading rate

Figure 1

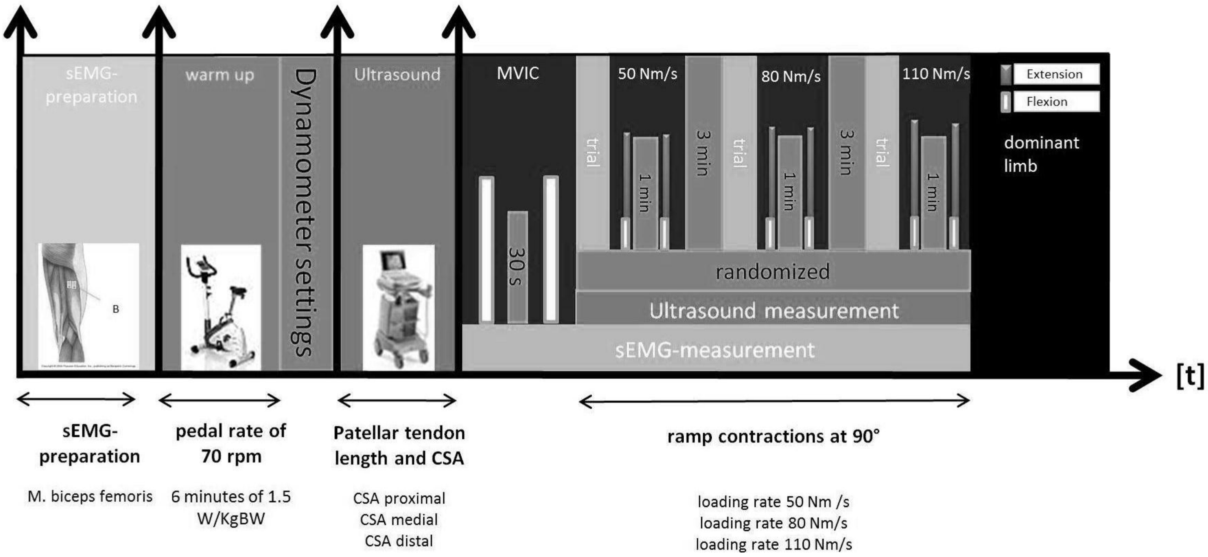

sEMG-preparation
warm up
Dynamometer settings
Ultrasound
MVIC
30 s
50 Nm/s
80 Nm/s
110 Nm/s
trial
3 min
1 min
Extension
Flexion
dominant limb
randomized
Ultrasound measurement
sEMG-measurement
[t]
sEMG-preparation
M. biceps femoris
pedal rate of 70 rpm
6 minutes of 1.5 W/KgBW
Patellar tendon length and CSA
CSA proximal
CSA medial
CSA distal
ramp contractions at 90°
loading rate 50 Nm /s
loading rate 80 Nm/s
loading rate 110 Nm/s

Figure 2

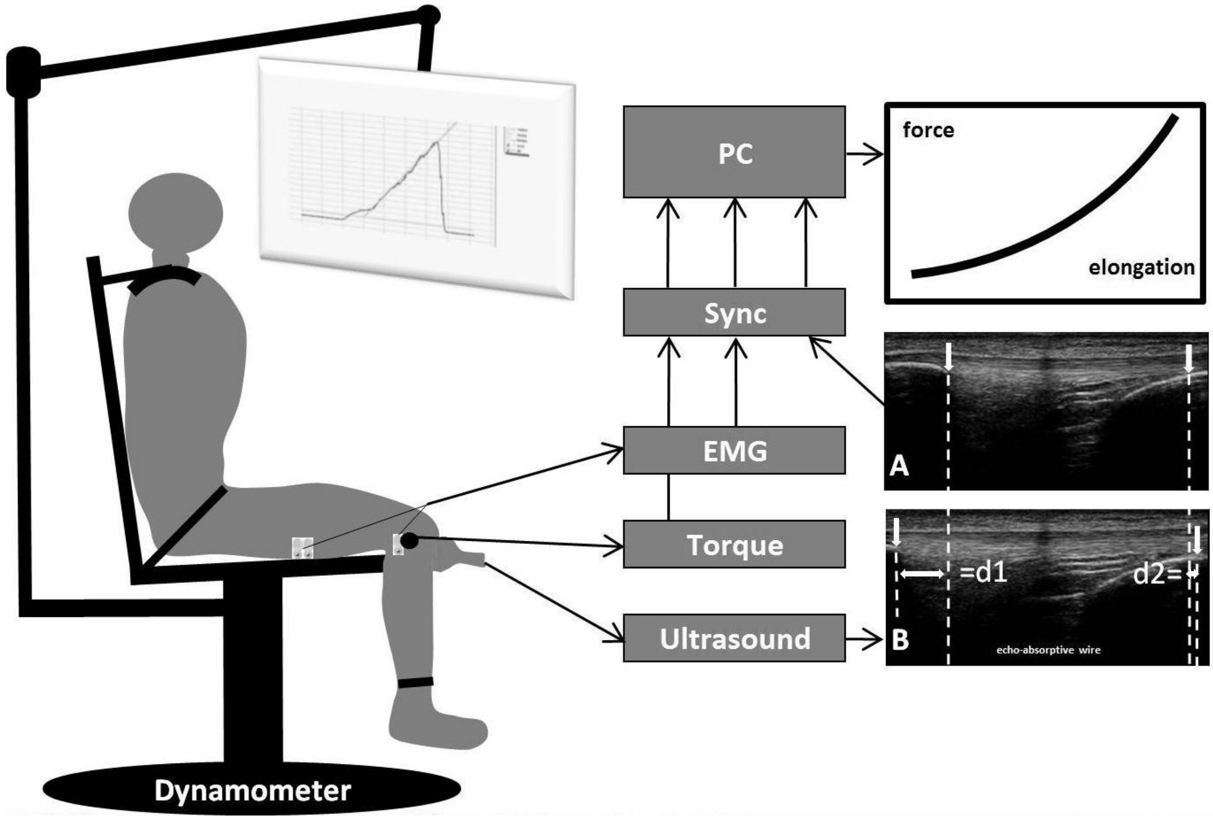
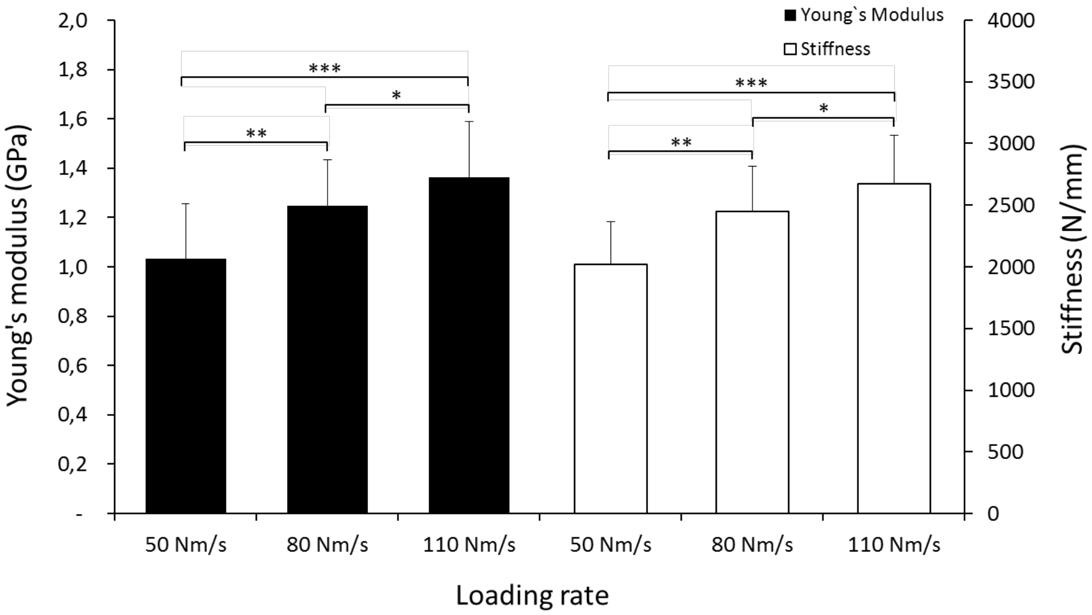
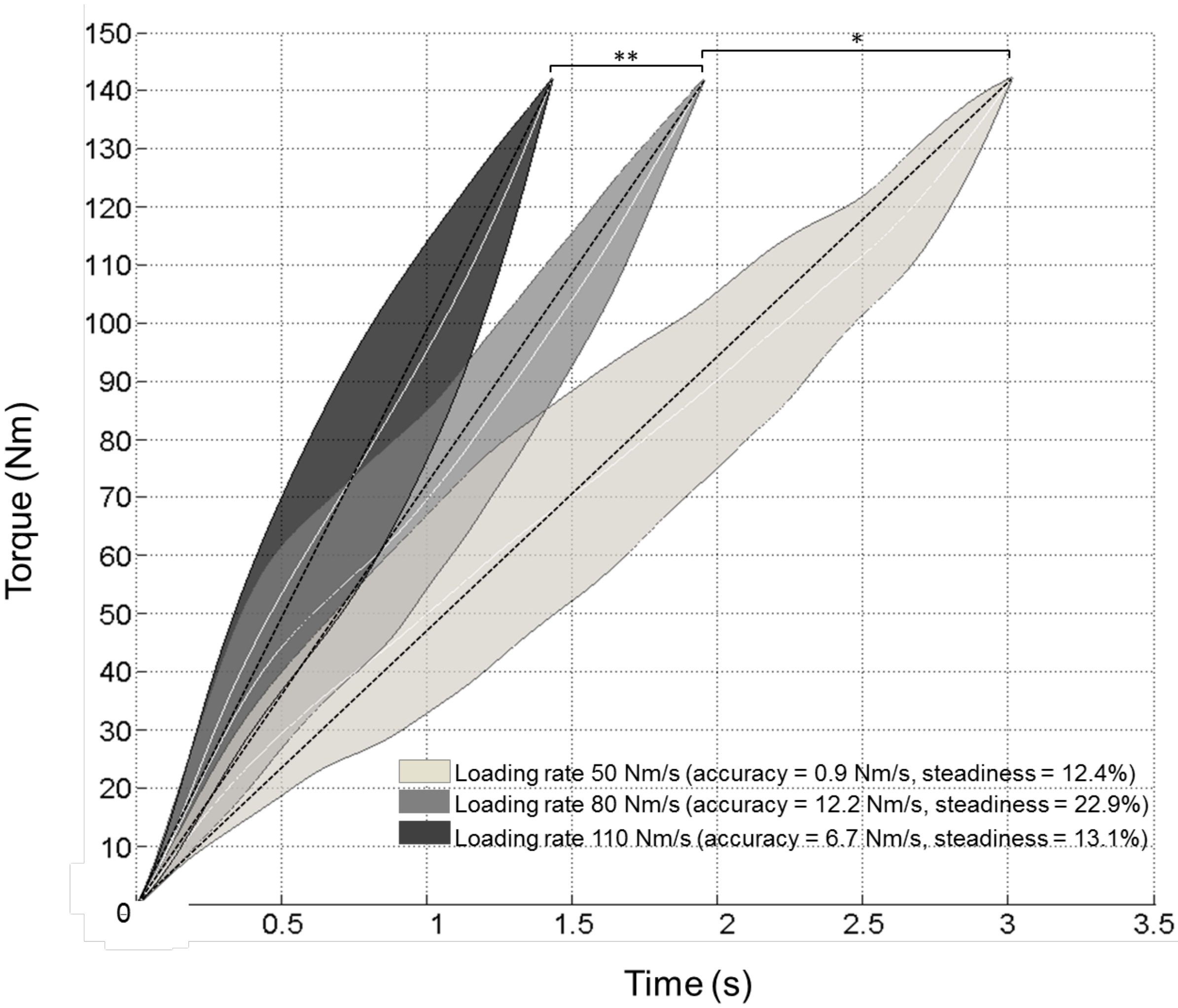

PC
force
elongation
Sync
EMG
Torque
Ultrasound
A
B
=d1
d2=
echo-absorptive wire
Dynamometer

Figure 3



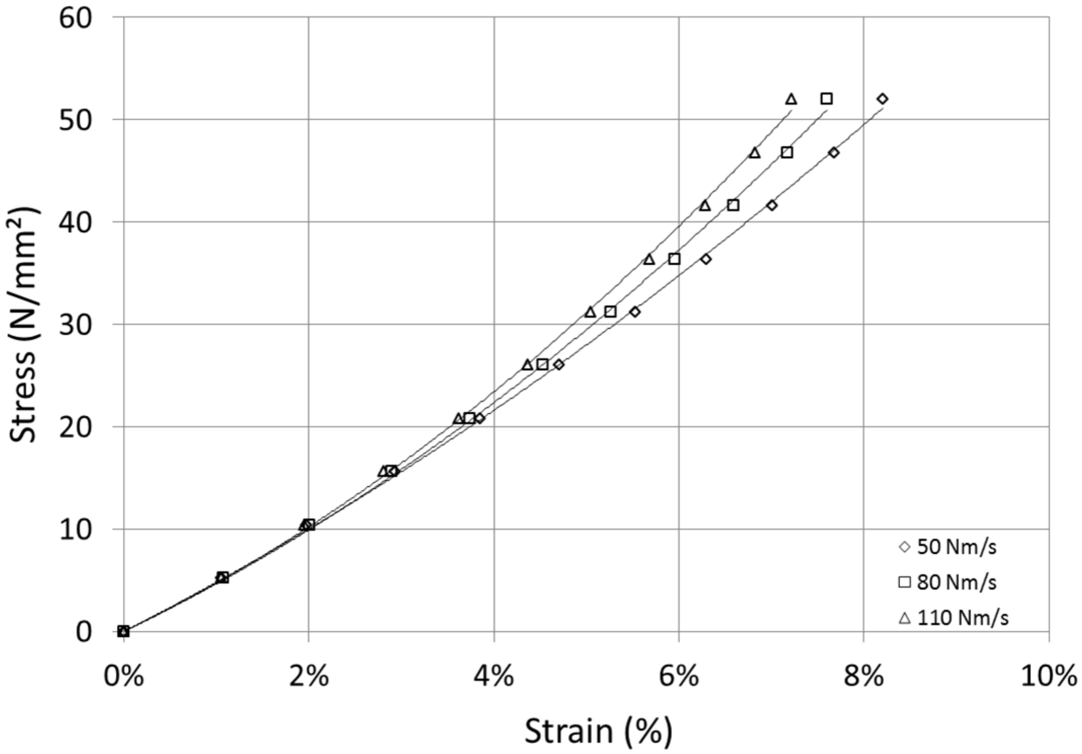
Young`s Modulus
Stiffness
Young's modulus (GPa)
Stiffness (N/mm)
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
-
4000
3500
3000
2500
2000
1500
1000
500
0
***
*
**
50 Nm/s
80 Nm/s
110 Nm/s
Loading rate

Figure 4a



Torque (Nm)
Time (s)
**
*
Loading rate 50 Nm/s (accuracy = 0.9 Nm/s, steadiness = 12.4%)
Loading rate 80 Nm/s (accuracy = 12.2 Nm/s, steadiness = 22.9%)
Loading rate 110 Nm/s (accuracy = 6.7 Nm/s, steadiness = 13.1%)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
0.5
1
1.5
2
2.5
3
3.5

1 Figure 4b



Stress (N/mm²)
60
50
40
30
20
10
0
0%
2%
4%
6%
8%
10%
Strain (%)
◇ 50 Nm/s
□ 80 Nm/s
△ 110 Nm/s


2

**Table 1**: Reliability of patellar tendon measurements of deformation and morphology

| | ICC (2.1) | CV (%) |
|---|---|---|
| Elongation (mm) | | |
| 50 Nm/s | 0.812 | 7.5 |
| 80 Nm/s | 0.959 | 4.7 |
| 110 Nm/s | 0.916 | 11.6 |
| CSA (mm2) | | |
| proximal | 0.975 | 1.6 |
| medial | 0.924 | 2.3 |
| distal | 0.952 | 2.4 |
| mean | 0.959 | 1.8 |
| Length (mm) | 0.942 | 2.0 |

CSA: cross-sectional area

**Table 2**: Patellar tendon load deformation characteristics as a function of loading rate

| | 50 Nm/s | 80 Nm/s | 110 Nm/s |
|---|---|---|---|
| Elongation (mm) | 3.9 (1.0) | 3.7 (1.2) | 3.5 (1.1) |
| Strain (%) | 8.1 (2.3) | 7.5 (2.5) | 7.1 (2.4) |
| Stress (MPa) | 73.3 (11.9) | 73.5 (10.5) | 74.3 (10.0) |

Values are reported as mean and standard deviation (SD).