The effect of the magnitude of the impulse applied during the free oscillation technique in muscular and tendon stiffness around the ankle

Versão definitiva após defesa

Márcio Lopes Soares

Dissertação para obtenção do Grau de Mestre em

Ciência do Desporto: Treino Desportivo

Orientador:
Universidade da Beira Interior
Prof. Doutor José Aurélio Marques Faria
Co-Orientador:
University College Dublin
Prof. Doutor Massimiliano Ditroilo

dezembro de 2021

Acknowledgements

To my mother Dulce Maria who always supported me in my choices and encouraged me to pursue my dreams and goals.

To my wife Karina for being my life mate, friend, advisor and, above all, supporting me in everything. I love you!

To the memory of my father José Soares who ever since I decided to come to Portugal supported me and who, I'm sure, is looking out for me.

To my supervisor Aurélio Faria for all the dedication, patience, knowledge and help that he gave me in these 3 years.

To my co-supervisor Massimiliano Ditroilo for all knowledge and help.

To teacher Dina Miragaia for her help and availability in the beginning of this journey.

To my friend Inês Santos for all support and for listening when I needed.

Resumo

Foi sugerido que a magnitude do impulso aplicado, durante a técnica de oscilação livre, pode influenciar a rigidez. No entanto, desconhece-se se a rigidez do músculo e do tendão podem ser afetadas de forma similar ou não. Como tal, o objetivo deste estudo foi investigar se impulsos de diferentes magnitudes podem afetar a rigidez muscular e/ou tendinosa.

A rigidez muscular e tendinosa foram avaliadas com a técnica de oscilação livre utilizando três impulsos diferentes (impulso 1, 2 e 3). Sendo que cada um destes impulsos apresentava, respetivamente, os seguintes picos de força de 100, 150 e 200 N. Vinte e sete estudantes universitários do sexo masculino (idade 20,7 ± 1,3 anos; altura 1,73 ± 0,05 m; massa 74,7 ± 8,8 kg) voluntariaram-se para este estudo. Tendo-se verificado uma diminuição significativa na rigidez "verdadeira" e "aparente" (p <0,0005) do impulso 1 (k_m = 630 ± 247 KN / m; K_m = 296 ± 102 KN / m) para o impulso 2 (k_m = 518 ± 208 KN / m; K_m = 245 ± 89 KN / m) e do impulso 1 (k_m = 630 ± 247 KN / m; K_m = 296 ± 102 KN / m) para o impulso 3 (k_m = 566 ± 384 KN / m); K_m = 265 ± 167 KN / m), respetivamente. No entanto, não foram encontradas diferenças significativas em relação à rigidez do tendão "verdadeira" e "aparente". Os resultados do presente estudo sugerem que a rigidez muscular ("verdadeira" e "aparente") é afetada pela magnitude do impulso, mas não a rigidez do tendão. A magnitude do impulso pode afetar a co-contração e a atividade reflexa que, por sua vez, pode afetar o nível de rigidez muscular.

Palavras-chave

Rigidez tornozelo; técnica oscilação livre; magnitude impulso; rigidez muscular; rigidez tendinosa

Abstract

It has been reported that the impulse magnitude, during the free oscillation technique, can influence stiffness. However, is not clear whether both muscle and tendon stiffness can be affected similarly. Therefore, the aim of this study was to investigate if impulses of different magnitudes can affect the stiffness of muscle and/or tendon.

Muscle and tendon stiffness were assessed using the free oscillation technique with three different impulse magnitudes (impulse 1, 2 and 3) associated respectively with the peak forces of 100, 150 and 200 N. Twenty-seven male college students (age 20.7 ± 1.3 years; height 1.73 ± 0.05 m; mass 74.7 ± 8.8 kg) volunteered for this study. It was found a significant decrease in "true" and "apparent" stiffness (p<0.0005) from impulse 1 (k_m = 630 ± 247 KN/m; K_m = 296 ± 102 KN/m) to impulse 2 (k_m = 518 ± 208 KN/m; K_m = 245 ± 89 KN/m) and from impulse 1 (k_m = 630 ± 247 KN/m; K_m = 296 ± 102 KN/m) to impulse 3 (k_m = 566 ± 384 KN/m; K_m = 265 ± 167 KN/m), respectively. However, no significant differences were found regarding "true" and "apparent" tendon stiffness. The present study results suggest that muscle stiffness ("true" and "apparent") is affected by the impulse magnitude, but not tendon stiffness. The impulse magnitude may impact co-contraction and stretch reflex which in turn might affect the level of muscle stiffness.

Keywords

Ankle stiffness; free oscillation technique; impulse magnitude; muscle stiffness; tendon stiffness

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1. Introduction

The importance of the muscle-tendon complex (MTC) in human movement is widely recognized whether it is associated with everyday activities or sports. The elastic behaviour of the MTC is usually studied using spring-mass models (Shorten, 1987). One of the most studied biomechanical properties in this context is stiffness (Faria et al., 2018; Kubo, Ikebukuro, & Yata, 2020; Ludvig, Plocharski, Plocharski, & Perreault, 2017; Paris-Garcia, Barroso, & Paris, 2018; Weir, Willwacher, Trudeau, Wyatt, & Hamill, 2020). In simple terms, stiffness is defined as the resistance to deformation of an elastic structure by the action of a force (Butler, Crowell, & Davis, 2003). The relevance of stiffness has been reported in several domains, among which its association with sports performance (Ditroilo, Watsford, Fernandez-Pena, et al., 2011; Faria, Gabriel, Abrantes, Wood, & Moreira, 2013; Kuitunen, Komi, & Kyrolainen, 2002; Wang, De Vito, Ditroilo, Fong, & Delahunt, 2015; Woods, Watsford, Cavanagh, & Pruyn, 2015), the risk of injuries (Butler et al., 2003; Pruyn et al., 2012; Rodriguez, Watsford, Bower, & Murphy, 2017; Sporri et al., 2019), as well as stability and postural control (Edwards, 2007; Winter, Patla, Rietdyk, & Ishac, 2001). Various methods have been used to assess stiffness. At microscopic level, the analysis of muscle or tendon fibers is common (Kubo et al., 2007; Nakamura, Ikezoe, Takeno, & Ichihashi, 2012) while at the macroscopic level, the stiffness analysis around one or more joints is usually performed (Ditroilo, Cully, Boreham, & De Vito, 2012; Faria et al., 2018; Kuitunen et al., 2002). The assessment of stiffness through the free oscillation technique is one of the most used methods in the literature that aims to assess stiffness around a particular joint. The knee and ankle joints are those that have received the most attention in the literature, with special emphasis on the ankle joint (Babic & Lenarcic, 2004; Faria et al., 2013; Faria, Gabriel, Moreira, Bras, & Ditroilo, 2016; Faria et al., 2018; Paris-Garcia, Barroso, Canas, Ribas, & Paris, 2013; Paris-Garcia et al., 2018).

The ankle joint plays an important role in the generation of propulsive force, shock absorption and body stability (Perry, 1992; Whittle, 2007). Naturally, the ankle MTC is of paramount importance in these functions, which explains the extensive scientific literature generally dedicated to its understanding, and particularly to stiffness.

In the free oscillation technique, the ankle MTC must momentarily support an isometric load and, during this period, an impulse is applied with the objective of destabilizing the ankle joint and causing the leg to oscillate in the sagittal plane (Faria, Gabriel, Abrantes, Bras, & Moreira, 2009). This oscillation is then measured and stiffness estimated (Blackburn, Padua, & Guskiewicz, 2008; Faria et al., 2010; Faria et al., 2013; Faria et al., 2016; Faria et al., 2018; Paris-Garcia et al., 2013; Paris-Garcia, Barroso, Doblare, Canas, & Paris, 2015; Paris-Garcia et al., 2018). Based on the assumption that an elastic system oscillates at its natural or resonant frequency regardless of the magnitude of the applied impulse (Ditroilo, Watsford, Murphy, & De Vito, 2011), the vast majority of studies apply this impulse manually (Faria et al., 2016). However, there are indications that the magnitude of the impulse has the potential to affect the co-activation (Nielsen, Sinkjaer, Toft, & Kagamihara, 1994) and/or the stretch reflex (Faria et al., 2009; Hunter & Spriggs, 2000), which in turn can influence the stiffness. Based on these considerations, a study was carried out to assess the effect of three impulses of different magnitudes (100, 150 and 200 N) on stiffness (Faria et al., 2016). It was found that with the increase of the applied impulse the stiffness decreased significantly. However, it was not possible to determine whether the magnitude of the impulse could affect stiffness of both the muscular and the tendon structures or only one of these structures.

As such, the aim of the present study was to assess whether the magnitude of the applied impulse affects only the muscle stiffness and/or tendon stiffness.

2 – Methods

2.1 - Participants

Twenty-seven male university students (age 20.7 ± 1.3 years; height 1.73 ± 0.05 m; body mass 74.7 ± 8.8 kg), recruited through advertising from at the University of Beira Interior community, healthy and with no history of ankle injuries volunteered for the study. Participants were informed of the scope of the study, and written informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki.

2.2 - Impulse Calibration

Impulse calibration was performed as illustrated in Figure 1. Lever 1 was placed on top of the force plate at 10 cm from its edge (lever 1 also rests 10 cm on the knee during stiffness assessment). The horizontal levelling of lever 1 was performed using a spirit level and adjusting the vertical position of the mechanism I. The calibration of the impulses (1, 2 and 3) associated with the peak forces of 100, 150 and 200 N were carried out as follows: 1) adjusting the initial vertical position of the mechanism F and 2) the horizontal position of the weight G. Then the lever 2 was raised to the highest position allowed by the mechanism F and then released. After hitting lever 1, lever 2 rebounded and was immediately grabbed by the evaluator to avoid a second impact. The vertical force obtained by the Kistler force plate (Kistler 9281B; Kistler Instruments, Amherst, NY, USA) was filtered using a low pass filter with a cut-off frequency of 75 Hz. The peak force was measured from the baseline to the first oscillation peak illustrated in Figure 2. From this process the following results were obtained: Peak force for impulse 1 = 100 ± 0.85 N, peak force for impulse 2 = 150 ± 0.95 N and peak force for impulse 3 = 200 ± 0.86 N. The mean and standard deviation for each of the 3 pulses was obtained from 13 trials. Using numerical integration, from the area illustrated in Figure 2, the value of each impulse was estimated. The mean of the tests for each impulse was as follows: Impulse 1 = 1.5 ± 0.00925 N.s, Impulse 2 = 2.3 ± 0.01306 N.s and Impulse 3 = 3.1 ± 0.01442 N.s.

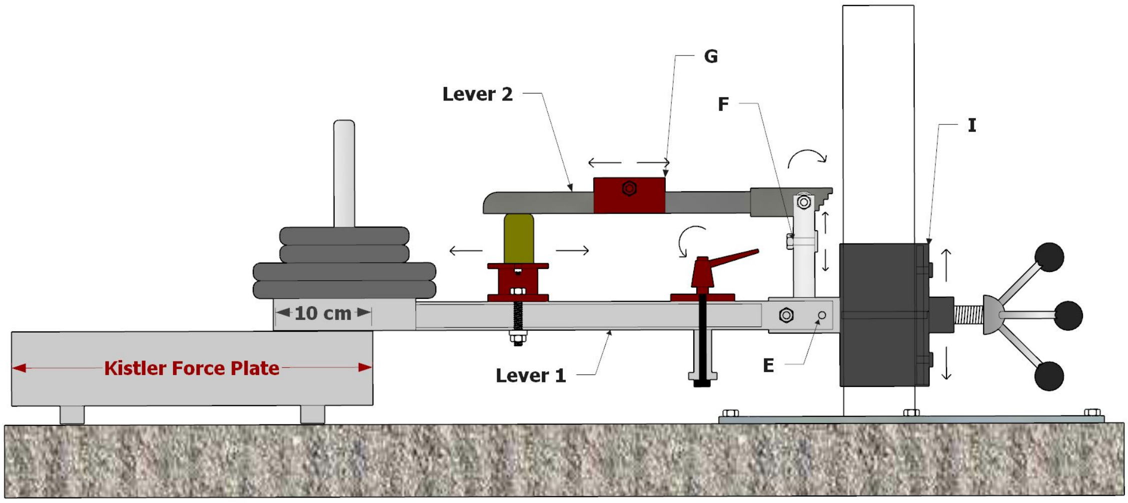

Figure 1 - Equipment and position used to calibrate the impulses: (E) mechanism to lock and unlock lever 1; both (F) and (G) are mechanisms used to calibrate the impulses: (F) to set the initial position of the lever 2; (G) is a weight that can be moved to left and right of lever 2; (I) to adjust lever 1 height; (Lever 2) the calibrated lever that apply the impulse into lever 1.

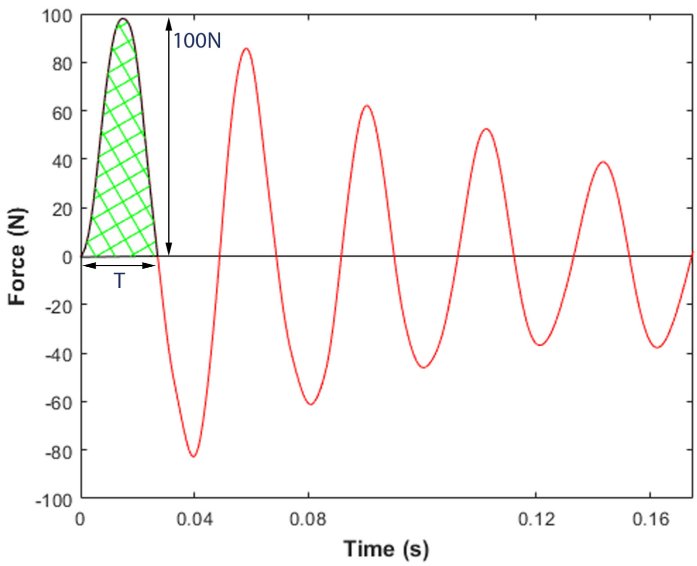

Figure 2 - Free oscillation showing the period (Ts), the peak force (100 N) and the area used to calculate the impulses.

2.3 - Stiffness Assessment

Stiffness was evaluated using the free oscillation technique. In this technique, the participants sat in the position illustrated in Figure 3 (a), with their arms crossed over their chests and without their thighs supported. The position of everyone was adjusted so that the foot-leg, leg-thigh and thigh-trunk angles were 90º, while maintaining and metatarsophalangeal joint of the right foot (barefoot) aligned with the edge of the metal block (C) placed on top of the force plate (Kistler 9281B; Kistler Instruments, Amherst, NY, USA). The calcaneus was also supported on the metal block (D), to minimize the effects of fatigue, until the setup was fully prepared for stiffness evaluation. Only the stiffness of the right lower limb was evaluated because published studies suggest that there are no significant stiffness differences between lower limbs (Murphy, Watsford, Coutts, & Pine, 2003). The maintenance of the foot position between measurements was ensured through the mechanism (A) that allows measuring the distance between the end of the toes and the end of the metal block (C). Then, lever 1 was supported at 10 cm of the knee, and this position was controlled through mechanism (B) for all stiffness evaluations. A spirit level was used to horizontally level lever 1. To assess the stiffness seven external loads (i.e., 10, 15, 20, 25, 30, 35 and 40 kg) were placed individually on top of lever 1 randomly. For each load the stiffness was evaluated using each of the three calibrated impulses. Before starting the measurements, lever 1 was unlocked through mechanism (E) and the metal block (D) was removed to allow the calcaneus to oscillate in the sagittal plane during stiffness measurement. Participants were instructed to perform an isometric contraction keeping the ankle at 90º and sustain lever 1 with the load horizontally for a period of ~10s. Each one of the three calibrated impulses (1, 2 and 3) were randomly applied by dropping the lever 2 onto lever 1 and generating a peak force of 100, 150 and 200N respectively. After each impact, lever 2 rebounded and the investigator grabbed it before it touched again the lever 1. Besides being blindfolded participants were instructed to not to react to any stimulus during assessments (Blackburn et al., 2008; Faria et al., 2018; Hunter & Spriggs, 2000). The ankle oscillations produced by the impulse were recorded and used to assess stiffness. Five trials were performed for each impulse, while rests periods of 2-5 minutes were taken between measurements to avoid fatigue.

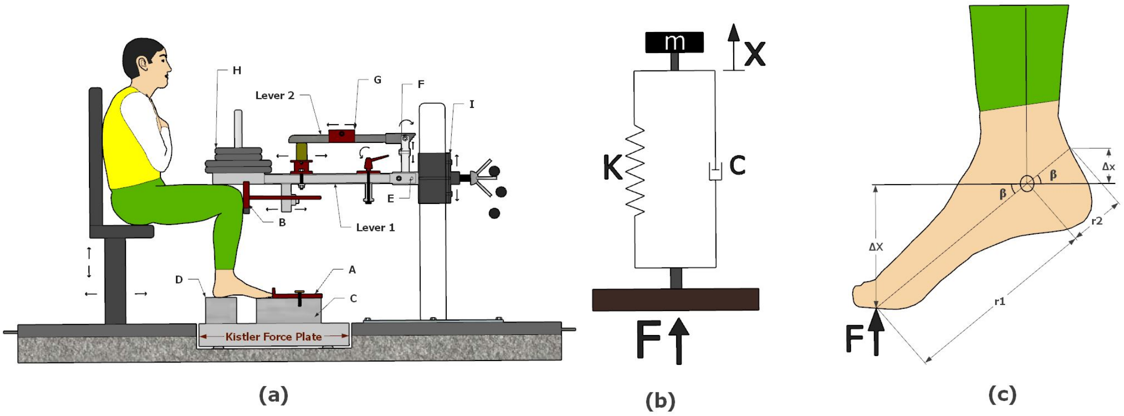

Figure 3 - (a) Equipment and position used to assess stiffness of the ankle joint: (A) to lock the foot in position, (B) to lock the knee in position, (C and D) metal blocks used to support the foot, (E) to lock lever 1, (F) to set the initial position of lever 2, (G) weight to calibrate lever 2 that can be moved to left and right, (H) standard weights (loads), (I) to adjust lever 1 height; **(b)** the mass-spring model of the system: (F) reaction force, (m) mass of the system, (K) "apparent" stiffness, (C) "apparent" damped coefficient and (X) displacement of the mass; **(c)** the diagram of the moment arms: the distance between ankle joint and forefoot (r_1) and the distance between ankle joint and rearfoot (r_2).

2.4 - Data analysis

Based on the model illustrated in Figure 3b, the stiffness K and the damping coefficient C around the ankle joint were evaluated. These variables calculated from the ground reaction forces are generally referred to as "apparent" stiffness, as they are related to the vertical displacement of the center of mass of the body considered into the analysis. However, if we consider the force arms r_1 and r_2 (Figure 3c), we can obtain the "true" stiffness and the damping coefficient that will be described from now on in lowercase and in italics as *k* and *c*.

2.4.1 - "Apparent" stiffness

The equation of motion used to model the damped mass-spring system, illustrated in Figure 3b, was Eq. 1,

$$(F =)\ ma = -K\mathrm{x} - Cv \quad (1)$$

which reorganized into Eq. 2.

$$\mathrm{m}\ddot{\mathrm{x}} + \mathrm{Kx} + \mathrm{C}\dot{\mathrm{x}} = 0 \quad (2)$$

where C represents the "apparent" damping coefficient (Ns/m), $\dot{X}$ the velocity (m/s), K the "apparent" stiffness (N/m), X the displacement (m), *m* the total mass (kg) of the system (foot + leg + thigh + lever + standard weights) and $\ddot{X}$ acceleration (m/s2). For an underdamped system, the solution to this equation is Eq. 3:

$$X(t) = e^{-\gamma t}(A_x \cos \omega_d t + B_x \sin \omega_d t) \quad (3)$$

where A_x and B_x are integration constants.

Eq. 4 to 6 were used to evaluate the parameter γ, the damped angular frequency of oscillation ω_d and the undamped angular frequency of oscillation ω_o.

$$\gamma = \frac{C}{2m} \quad (4) \qquad \omega_d = \sqrt{\omega_0^2 - \gamma^2} \quad (5) \qquad \omega_0 = \sqrt{\frac{K}{m}} \quad (6)$$

Assuming the mass of the system is represented by the block *m* in Figure 3b, the relationship between X and the ground reaction force F is:

$$m\ddot{x} = F - mg \quad (7)$$

where the acceleration due to gravity is represented by g. By replacing Eq. 3 into Eq. 7 we obtain Eq. 8.

$$F(t) = e^{-\gamma t}(A_F \cos \omega_d t + B_F \sin \omega_d t) + mg \quad (8)$$

where A_F and B_F are integration constants.

Is possible to estimate the mass of the system before the application of the impulse or after the attenuation of the oscillation. Though, it is difficult to accurate determine the mass of the system due to some micro-movements and oscillations that take place. Furthermore, can occur some base line drift during the trials, therefore the parameter d*t* was added to Eq. 8 originating Eq. 9. This equation was then used as a model function to estimate through the nonlinear least squares method γ, ω_d, A_F, B_F, d*t* and M (Figure 4). Dividing M by gravity it was then possible to estimate the mass *m*.

$$F(t) = e^{-\gamma t}(A_F \cos \omega_d t + B_F \sin \omega_d t) + dt + M \quad (9)$$

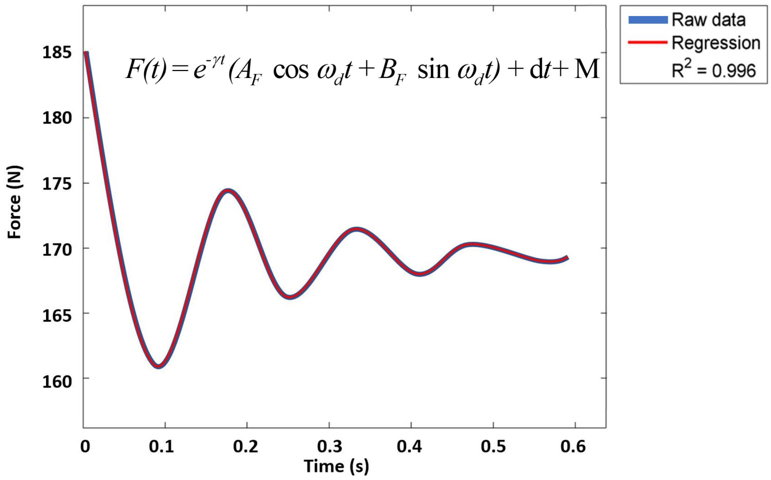

Figure 4 - Representation of raw experimental data and regression curve used to estimate damping coefficient and "apparent" stiffness.

Subsequently the "apparent" stiffness K and the damping coefficient C were obtained from Eq. 10 and 11.

$$K = m(\omega_d^2 + \gamma^2) \quad (10) \qquad C = 2m\gamma \quad (11)$$

2.4.2 - "True" stiffness

The foot displacements (Δx and ΔX) illustrated in Figure 3c are related as follows (Eq. 12):

$$\Delta X = \frac{r_1}{r_2} \Delta x \quad (12)$$

By considering the moment arms (r_1 and r_2) illustrated in Figure 3c, the "true" stiffness k and the damping coefficient c can be determined. From the condition of dynamic equilibrium of forces in relation to ankle joint, the equation of motion is:

$$\left(I_{\text{foot}} + m_{\text{foot}} r_{gravity}^2\right)\frac{d^2\theta}{dt^2} = F r_1 \cos\theta - f r_2 \cos\theta \quad (13)$$

where I_{foot} is the moment of inertia of the foot in relation to the center of gravity, m_{foot} the mass of the foot, $r_{gravity}$ is the distance between the center of rotation and the center of gravity of the foot, θ the instantaneous angular position of the foot, F is the ground reaction force and f the tension of the tendon. Considering that the force that represents the total mass of the system is almost aligned with the axis of rotation its moment is negligible in absolute terms. Since the summands of the left-hand side of Eq. 13 are too small when compared to the ones of the right-hand side, Eq. 13 can be approximated to:

$$\mathrm{F}r_1 = f r_2 \quad (14)$$

In this way it is possible to estimate f after measuring the ground reaction force F and determining the distances r_1 and r_2.

The same rational used to determine the "apparent" parameters (K and C) can be used to determine the "true" MTC parameters. Considering the condition of dynamic equilibrium, we obtain Eq. 15. Replacing Eq. 12 and 14 in Eq. 2 we obtain Eq. 16.

$$f = -kx - c\dot{x} \quad (15) \qquad f = -\left(\frac{r_1}{r_2}\right)^2 \mathrm{K}x - \left(\frac{r_1}{r_2}\right)^2 \mathrm{C}\dot{x} \quad (16)$$

Considering that Eq. 15 and 16 are equivalent, the terms of both equations must match, therefore, Eq. 17 can be established:

$$k = \left(\frac{r_1}{r_2}\right)^2 \mathrm{K} \quad \text{and} \quad c = \left(\frac{r_1}{r_2}\right)^2 \mathrm{C} \quad (17)$$

Once muscle-tendon complex stiffness k has been calculated is possible to determine tendon (k_t) and muscle (k_m) stiffness based on Hill model. As illustrated in Figure 5, k is an increasing function of f and, therefore, it is assumed that the active stiffness of the series elastic component (SEC) is proportional to the muscle tension $k_m f$. The resulting stiffness k is given by passive k_t and active k_m stiffness as in Eq. 18.

$$k = \frac{k_t k_m f}{k_t + k_m f} \quad (18)$$

Most of the passive stiffness (k_t) lies in tendon, additionally it is assumed to result from a small elongation and therefore can be considered constant (Fukashiro, Noda, & Shibayama, 2001; Shorten, 1987). Muscle force and stiffness depend on the number of cross-bridges attached and, both muscle force and stiffness increase with increasing cross-bridges attached. Therefore, muscle stiffness can be considered an increasing function of muscle force (Babic & Lenarcic, 2004). Thus, Eq. 18 can be used as a model function to estimate by the nonlinear least squares method (k_t) and (k_m).

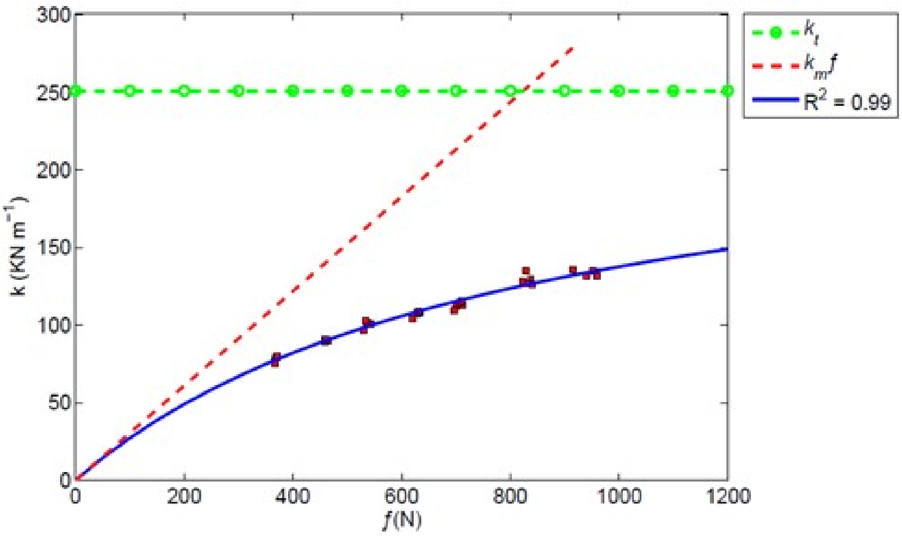

Figure 5 - Illustration of the k-*f* curve obtained from the experimental data (i.e. red squares). The muscle stiffness (k_m) was estimated by the first regression line while (k_t) was estimated by the last.

2.4.3 - Transformation of "true" into "apparent" parameters

Some studies report only "apparent" stiffness K or stiffness calculated from the movement of the center of mass. Therefore, it is important to transform the "true" parameters of the MTC (k_t and k_m) into "apparent" parameters (K_t and K_m). This can be done using Eq. 19. A more detailed description of this procedure can be found in the literature (Fukashiro et al., 2001).

$$a = \frac{r_1}{r_2}, \quad K_t = \frac{k_t}{a^2}, \quad \text{and} \quad K_m = \frac{k_m}{a} \quad (19)$$

2.5 - Statistical analysis

The Statistical Package for the Social Sciences (IBM SPSS Statistics 21.0, Chicago, IL, USA) was used to detect whether impulses of different magnitudes can influence the measurement of biomechanical properties using the free oscillation technique. A p value ≤ 0.05 was considered statistically significant. After evaluating the statistical assumptions (i.e., outliers, normality and sphericity), the Friedman test or the analysis of variance (ANOVA) for repeated measures were conducted to determine the existence of statistically significant differences in the biomechanical properties (ie stiffness, baseline ground reaction force and frequency of oscillation) for each perturbation applied. When statistically significant differences were found between groups, pairwise comparisons were performed with Bonferroni correction for multiple comparisons and statistical significance was accepted at the level of $p < 0.0167$ (i.e., $p = 0.05 / 3$ groups). ANOVA for repeated measures is quite robust to deviations from normality. Whenever this assumption was violated, both the Friedman test and the ANOVA for repeated measures were performed. When using the Friedman test, it is more appropriate to report the median, while for repeated measures ANOVA, the mean and standard deviation. As such, considering that most published studies report mean values, whenever possible (i.e., when Friedman's tests and repeated measures ANOVA gave the same results) only ANOVA results were reported.

3 – Results

The data presented in this study are complementary data from the study by Faria et al. (2016). For each of the three applied impulse magnitudes, the “true” stiffness of the muscle (k_m) and tendon (k_t) was determined, followed by the respective “apparent” parameters (K_m) and (K_t).

Statistical analysis of total “true” and “apparent” stiffness showed that as the magnitude of the applied impulse increased, stiffness decreased. Post-hoc comparisons also revealed that this decrease was significant (p<0.0005) across all applied impulses (Figure 6 - A and B). For each one of the three impulse magnitudes applied it was determined “true” muscle (k_m) and tendon (k_t) stiffness and then the respective “apparent” parameters (K_m) and (K_t). The “true” muscle stiffness (k_m) had a significant decrease (p<0.0005) between impulses 1 (630 ± 247 KN/m) and 2 (518 ± 208 KN/m) and between impulses 1 (630 ± 247 KN/m) and 3 (566 ± 384 KN/m) as illustrated in Figure 6 - C. The “apparent” muscle stiffness (K_m) also had a significant reduction (p<0.0005) between impulses 1 (296 ± 102 KN/m) and 2 (245 ± 89 KN/m) and between impulses 1 (296 ± 102 KN/m) and 3 (265 ± 167 KN/m) (Figure 6 – E). However, there were no significant differences for “true” and “apparent” tendon stiffness (Figure 6 – D and F).

As the magnitude of the impulse applied increased, the natural frequency also decreased significantly (p<0,0005) with mean value of 4.9 ± 0.5 Hz for impulse 1, 4.8 ± 0.47 Hz for impulse 2 and 4.7 ± 0.45 Hz for impulse 3 (p<0,0005) as illustrated in Figure 7A. Friedman tests also revealed no significant differences p = 0.680 in the baseline ground reaction force (BGRF) before apply impulse 1 (Mdn = 305 N), impulse 2 (Mdn = 301 N) and impulse 3 (Mdn = 306 N) as illustrated in Figure 7B.

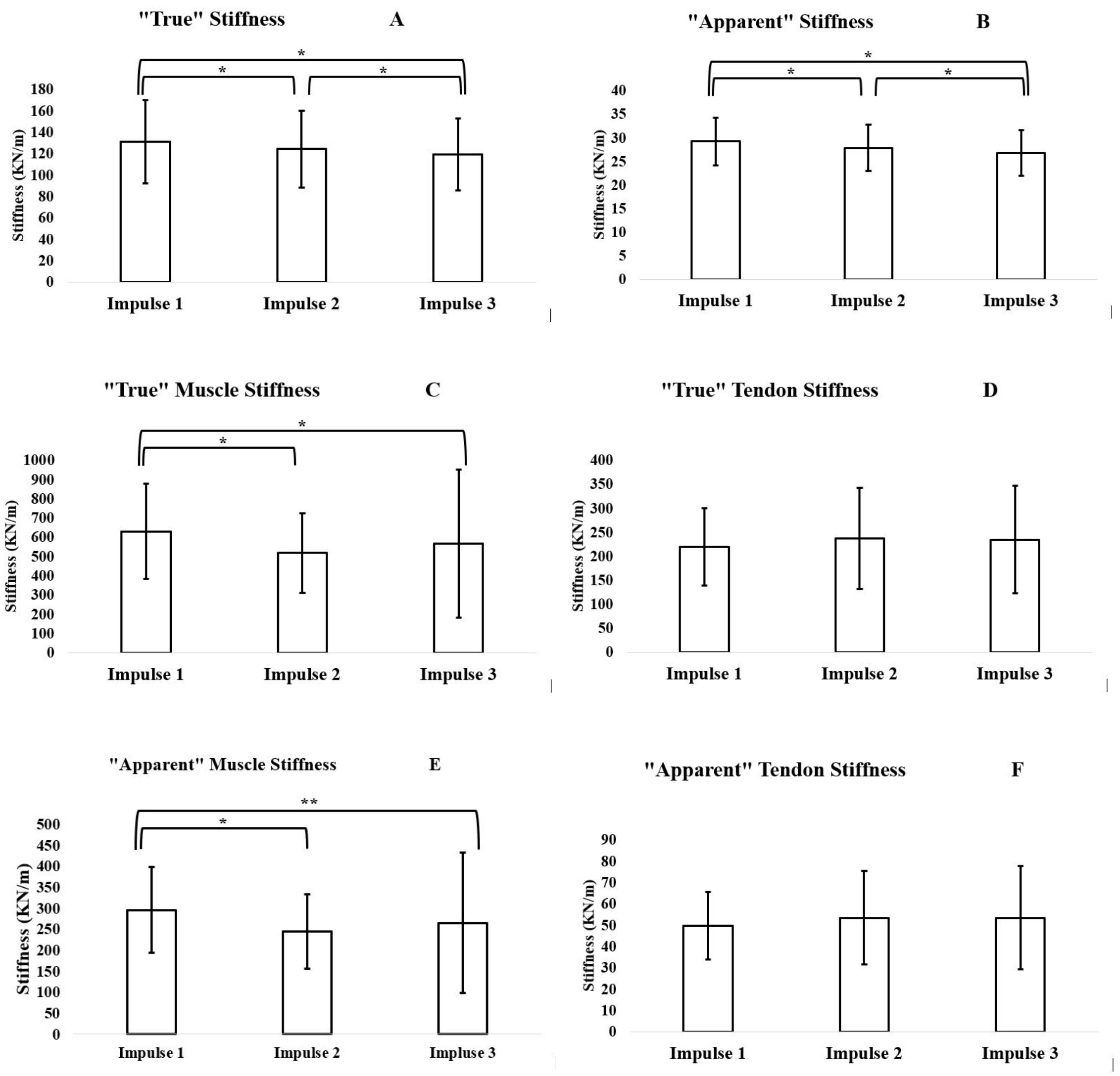

Figure 6 – Mean and standard deviation for impulses 1, 2 and 3 (KN/m): A = "True" Stiffness; B = "Apparent" Stiffness; C = "True" Muscle Stiffness; D = "True" Tendon Stiffness; E = "Apparent" Muscle Stiffness and F = "Apparent" Tendon Stiffness; **p*<0,0005 ***p*<0,01.

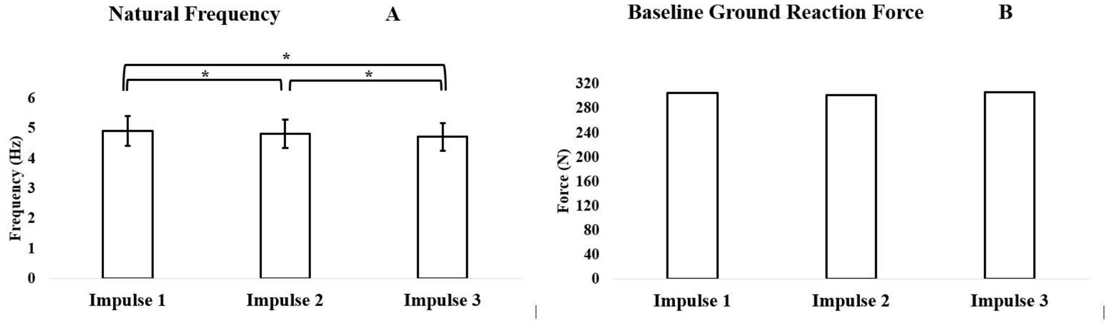

Figure 7 – A = Mean and standard deviation for natural frequency of oscillation (Hz) **p*<0,0005 and B = Median values for baseline ground reaction force (BGRF)

4 – Discussion and conclusion

The aim of the present study was to investigate whether impulses of different magnitudes, applied using the free oscillation technique, could independently influence muscle or tendon stiffness.

In the present study there were variations in "true" muscle stiffness (k_m) between 630 KN/m (impulse 1) and 566 KN/m (impulse 3) and tendon stiffness (k_t) between 219 kN/m (impulse 1) and 235 kN/m (impulse 3). These results for muscle and tendon are similar to those found in other studies that also used the free leg oscillation technique. Fukashiro et al. (2001) reported values of 611 KN/m and 364 KN/m; Paris-Garcia et al. (2015) of 605.2 KN/m and 312.8 KN/m and Babic and Lenarcic (2004) values of 665.2 KN/m and 408.6 KN/m for muscle and tendon, respectively. The tendon stiffness of the present study was lower than the values reported in previous studies. However, Fukashiro et al. (2001), when comparing their results with those of other studies, found that human values can range from 165 to 440 KN/m for tendon stiffness. Additionally, in another study, Fukashiro, Abe, Shibayama, and Brechue (2002) reported mean tendon stiffness values of 272.5 KN/m for white men.

There were two major findings in this study. The first was that muscle stiffness was affected by the magnitude of the applied impulse with a significant decrease (p<0.0005) from impulse 1 (k_m = 630 ± 247 KN/m; K_m = 296 ± 102 KN/m) to impulse 2 (k_m = 518 ± 208 KN/m; K_m = 245 ± 89 KN/m) and from impulse 1 (k_m = 630 ± 247 KN/m; K_m = 296 ± 102 KN/m) to impulse 3 (k_m = 566 ± 384 KN/m; K_m = 265 ± 167 KN/m). Even though the force-velocity relationship indicates that the eccentric force tend to be greater than the isometric force it has been suggested that when an active muscle is quickly stretched by an external force from an isometric situation (like when an impulse is applied), the number of cross-bridges attached can decrease, leading to a decrease in force production and stiffness (Fukashiro et al., 2001; Kirsch, Boskov, & Rymer, 1994). If this holds true, it may help explain why in the present study stiffness decreased with increasing impulse magnitude. Noteworthy, however is that the increase in stiffness between impulse 2 (k_m = 518 ± 208 KN/m; K_m = 245 ± 89 KN/m) and 3 (k_m = 566 ± 384 KN/m; K_m = 265 ± 167 KN/m) can't be explained by the previous rational and is not easy to obtain a plausible explanation for this result. One possibility may be related to high reflex-mediated stiffness that may occur at very small stretches produced by impulse 1 which will tend to decrease thereafter, particularly for impulse 2 and 3 (Faria et al., 2016).

Another factor that may have caused a decrease in muscle stiffness across impulse conditions was a decrease in co-activation. Studies have shown that as anterior tibial co-activation increases, ankle stiffness also increases (Kuitunen et al., 2002; Muller, Siebert, & Blickhan, 2012; Nielsen et al., 1994). The initial level of muscle activation was controlled thru BGRF which was assessed before the application of each of the three impulse conditions analysed. However, no significant differences in BGRF were found between impulse conditions, which indicates a similar level of muscle activation across experiments. A previous pilot EMG study also suggested negligible co-contraction effects during the stiffness evaluation. The measurement position was also similar for the 3 conditions before applying each impulse and was guaranteed by blocking the chair to ensure the same location and orientation of the segments (i.e., trunk and thigh) and by controlling the position of the knee and foot thru the mechanisms illustrated in Figure 3a – A, B and C. This strict control of the segments ensured that the angle of the ankle joint was always the same for all the assessments. Considering that the same position was used to assess stiffness for each one of the 3 impulses applied (that were randomly but consecutively applied) if any residual co-contraction effects existed, they should have been reflected similarly in all the impulses applied. Thus, beyond the impulse magnitude variation, the operating state was assumed to be invariant between the 3 impulses evaluated. Another possible explanation for reducing muscle stiffness with increasing impulse magnitude is stretch reflex. According to Mrachacz-Kersting and Sinkjaer (2003), stretch reflex is responsible for up to 52% of the torque produced by the knee joint after a disturbance. Ludvig et al. (2017) found a reduction in stretch reflex close to 25% and a reduction in stiffness in response to a disturbance applied to the knee joint. In the study by Van Der Helm, Schouten, de Vlugt, and Brouwn (2002), a perturbation of different frequencies were applied in the hand and it was found that as the frequency of the perturbation increased, the stretch reflex decreased. Furthermore, it has been reported that an increased frequency of motion with added external stiffness, derived from steel springs in parallel with the plantarflexion musculature, can cause an invariant phase delay in reflex response (Granata, Wilson, Massimini, & Gabriel, 2004). These phase relation between motion and reflex response has been reported to influence joint stiffness (Neilson & Neilson, 1978), leading Granata et al. (2004) to suggested that the invariant delay associated with stretch reflex create a phase-interference with system behaviour to reduce stiffness. Rack, Ross, Thilmann, and Walters (1983) also observed this phase-interference behaviour by driving the ankle joint at specific frequencies. The authors reported that the reflex response was almost in-phase with motion at low frequencies but shifted out-of-phase as the frequency of oscillation increased. The explanation was that with increasing frequencies the reflex delay remains constant but the period of oscillation declines leading the two signals to be in-phase and out-of-phase at different frequencies.

In the present study the natural frequency of oscillation was greater in the impulse 1 followed by impulse 2 and 3, as reported in results (Figure 7A). Therefore, if the applied impulses of different magnitudes created an invariant delay associated with stretch reflex and produced a phase-interference with system behaviour the outcome can consist in lower stiffness values due higher impulse magnitudes. However, since the stretch reflex was not assessed in the present study, further research is needed to address this issue.

The second finding was that tendon stiffness did not change significantly with increasing impulse magnitude with values being (k_t = 219,4 ± 80,7 KN/m; K_t = 49,6 ± 15,9 KN/m), (k_t = 236,5 ± 105,3 KN/m; K_t = 53,4 ± 21,9 KN/m) and (k_t = 234,6 ± 112,5 KN/m; K_t = 53,4 ± 24,3 KN/m) for impulse 1, 2 and3 respectively. It seems that tendons tend to maintain fairly invariants, with some studies showing that an increase in tendon stiffness is usually associated to an increase in tendon cross-section area due to training programs (Bohm, Mersmann, & Arampatzis, 2015; Couppe et al., 2008; Malliaras et al., 2013; Wiesinger, Kosters, Muller, & Seynnes, 2015) which doesn't occurred in the present study.

In summary, the present study results suggest that muscle stiffness is affected by the impulse magnitude applied, but not tendon stiffness. The impulse magnitude may impact co-contraction and stretch reflex which in turn can affect the level of muscle stiffness. It should be recognized however that the present study is not without limitations, particularly the contribution of co-activation and stretch reflex were not measured in the present study, furthermore only a population of males were analysed when it is known that females' stiffness might vary, specifically due to the influence hormones.

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Appendix A

Table 1 - Comparison of stiffness assessed using three impulses of different magnitudes

Variables		Impulse 1 Mean ± SD	Impulse 2 Mean ± SD	Impulse 3 Mean ± SD	Repeated Measures Anova	Post-hoc with Bonferroni Correction
Total Stiffness (N=189) (Loads 10-40 kg)	K (N/m)	29224 ± 5087	27839 ± 4914	26835 ± 4880	$\chi2(2) = 17.879$, $p < 0.0005$M ; $F(1.850, 347.804) = 87.756$, $p < 0.0005$, $\eta2 = 0.318$H	1-2, $p < 0.0005$ 1-3, $p < 0.0005$ 2-3, $p < 0.0005$
	k (KN/m)	130939 ± 39044	124228 ± 35956	119071 ± 33531	$\chi2(2) = 29.384$, $p < 0.0005$M; $F(1.761, 331.088) = 101.508$, $p < 0.0005$, $\eta2 = 0.351$H	1-2, $p < 0.0005$ 1-3, $p < 0.0005$ 2-3, $p < 0.0005$
Load 10 kg (N=27)	K (N/m)	24001 ± 4117	22403 ± 3436	21296 ± 4022	$\chi2(2) = 3.766$, $p = 0.152$M ; $F(2, 52) = 25.216$, $p < 0.0005$, $\eta2 = 0.492$	1-2, $p < 0.0005$ 1-3, $p < 0.0005$
	k (kN/m)	106919 ± 31554	99955 ± 28468	91778 ± 23524	$\chi2(2) = 3.149$, $p = 0.207$M ; $F(2, 52) = 30.879$, $p < 0.0005$, $\eta2 = 0.543$	1-2, $p < 0.0005$ 1-3, $p < 0.0005$
Load 15 kg (N=27)	K (N/m)	25962 ± 3890	24622 ± 3499	23878 ± 4045	$\chi2(2) = 6.668$, $p < 0.036$M ; $F(1.713, 44.532) = 10.437$, $p < 0.0005$, $\eta2 = 0.286$H	1-3, $p < 0 .01$
	k (kN/m)	116742 ± 34300	109972 ± 30326	105058 ± 27823	$\chi2(2) = 8.142$, $p < 0.017$M ; $F(1.647, 42.833) = 13.444$, $p < 0.0005$, $\eta2 = 0.341$H	1-3, $p < 0.01$ 1-2, $p = 0 .01$
Load 20 kg (N=27)	K (N/m)	28329 ± 3754	26123 ± 2575	25664 ± 2903	$\chi2(2) = 10.110$, $p = 0.006$M; $F(1.501, 39.021) = 19.865$, $p = 0.0005$, $\eta2 = 0.433$G	1-2, $p = 0.0005$ 1-3, $p = 0.0005$
	k (kN/m)	126411 ± 34260	114319 ± 23794	109841 ± 18539	$\chi2(2) = 23.580$, $p < 0.0005$M; $F(1.242, 33.109) = 18.419$, $p < 0.0005$, $\eta2 = 0.415$G	1-2, $p = 0.0005$ 1-3, $p = 0.0005$
Load 25 kg (N=27)	K (N/m)	29680 ± 3311	28078 ± 3603	27192 ± 3333	$\chi2(2) = 5.092$, $p = 0.078$M; $F(2, 52) = 18.401$, $p < 0.0005$, $\eta2 = 0.414$	1-2, $p < 0.016$ 1-3, $p < 0.0005$
	k (kN/m)	133364 ± 35890	123861 ± 30140	120293 ± 29527	$\chi2(2) = 5.364$, $p = 0.068$M; $F(2, 52) = 21.582$, $p < 0.0005$, $\eta2 = 0.454$	1-2, $p < 0.01$ 1-3, $p < 0.0005$
Load 30 kg (N=27)	K (N/m)	31031 ± 4141	29522 ± 4172	28549 ± 3144	$\chi2(2) = 6.020$, $p = 0.049$M; $F(1.744, 45.355) = 10.086$, $p < 0.0005$, $\eta2 = 0.280$H	1-2, $p < 0.01$ 1-3, $p < 0.01$
	k (kN/m)	139086 ± 37958	131658 ± 35780	123459 ± 22384	$\chi2(2) = 14.869$, $p < 0.01$M ; $F(1.381, 35.904) = 11.653$, $p < 0.01$, $\eta2 = 0.309$G	1-2, $p < 0.01$ 1-3, $p < 0.01$
Load 35 kg (N=27)	K (N/m)	31957 ± 3585	31605 ± 3614	29768 ± 3994	$\chi2(2) = 7.679$, $p < 0.05$M ; $F(1.667, 43.343) = 13.388$, $p < 0.0005$, $\eta2 = 0.340$H	1-3, $p < 0 .01$ 2-3, $p < 0.0005$
	k (kN/m)	143869 ± 40489	139815 ± 32607	132190 ± 32985	$\chi2(2) = 10.831$, $p < 0.01$M ; $F(1.480, 38.473) = 12.603$, $p < 0.0005$, $\eta2 = 0.326$G	1-3, $p < 0 .01$ 2-3, $p < 0.0005$
Load 40 kg (N=27)	K (N/m)	32885 ± 4659	32111 ± 4200	30977 ± 4353	$\chi2(2) = 8.682$, $p < 0.05$M ; $F(1.626, 42.264) = 7.330$, $p < 0.01$, $\eta2 = 0.220$H	1-3, $p = 0 .013$

	k (kN/m)	145826 ± 36482	141080 ± 32934	136090 ± 31065	χ2(2) = 16.656, p < 0.0005M ; F(1.346, 34.985) = 9.116, p < 0.01, η2 = 0.260G	1-3, p < 0 .01
"True" k (N=27) (Loads 10-40 kg)	kt (KN/m)	219.4 ± 80.7	236.5 ± 105.3	234.6 ± 112.5	-----------------------------------	------------------
	km (N.m-1.N-1)	630 ± 247	518 ± 208	566 ± 384	χ2(2) = 1.385, p = 0.5M ; F(2, 52) = 15.811, p < 0.0005, η2 = 0.378	1-2, p < 0.0005 1-3, p < 0.0005
"Apparent" K (N=27) (Loads 10-40 kg)	Kt (KN/m)	49.6 ± 15.9	53.4 ± 21.9	53.4 ± 24.3	-----------------------------------	------------------
	Km (N.m-1.N-1)	296 ± 102	245 ± 89	265 ± 167	χ2(2) = 2.349, p = 0.31M ; F(2, 52) = 14.451, p < 0.0005, η2 = 0.357	1-2, p < 0.0005 1-3, p < 0.01

K = "apparent stiffness", k = "true stiffness", M the Mauchly's Test of Sphericity, G and H Repeated Measures Anova with Greenhouse-Geisser and Huynd-Feldt corrections respectively.