In Vivo Assessment of Both Active and Passive Parts of the Plantarflexors Series Elastic Component Stiffness Using the Alpha Method: A Reliability Study

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- biomechanics
- muscle
- tendon
- ankle joint
- compliance
- human

Abstract

The aim of this study was to investigate the reliability of an *in vivo* adaptation of the short range stiffness experiment associated with the application of a mathematical model to determine the stiffness of both torque dependent and independent components of the plantarflexors series elastic component. Fourteen subjects participated in this study. The experimental protocol consisted of quickly moving the ankle joint in dorsiflexion during constant voluntary isometric plantarflexion at 7 submaximal torque levels. Relationships between joint stiffness and torque were established and the stiffness of both torque dependent and independent components were determined using the alpha method. The day-to-day reliability was assessed for joint stiffness and stiffness of both torque dependent and independent components (ICC higher than 0.88 and CVs lower than 6.0%). This method could then be used to better understand adaptive subjacent mechanisms to assess the effects of training protocols, and the rehabilitation of neuromuscular pathologies or traumatisms.

Introduction

Since the mechanical properties of both muscle and tendon are highly involved in the process of energy storage and recovery and in muscle tension transmission [9] these properties play an important role in daily activities and sport practices. Stiffness is the most commonly used parameter to characterize muscle-tendon complex (MTC) mechanical properties. Several methods, such as sinusoidal perturbations [1,19,34] and the short range stiffness experiment [35,36] were developed to determine non-invasively joint stiffness *in vivo* [7,13,20,31]. From a physical point of view, the joint stiffness identified *in vivo* should be called "quasi-stiffness", characterizing a system able to resist externally imposed displacements [29]. In the present study, this quasi-stiffness will be regarded as stiffness as in previous studies [7,31,38]. Furthermore, joint external torque is generated by synergistic muscle contraction (i.e. involving more than one muscle). *In vivo* measurement of the joint stiffness takes into account the synergy of the MTCs behaviors of the considered muscle group [14]. Then, considering the Hill-model type [23] adapted for *in vivo* experiments [10,12,18,20], this joint stiffness could be linked to the stiffness of the series elastic component (SEC), composed of a passive part (i.e. mainly tendons-aponeurosis structures) and an active part (i.e. contractile elements)[25].

In 1977, Morgan developed the alpha method in order to dissociate muscle and tendon stiffness from a global measurement [32]. This method assumes that the SEC of MTC is modelled as two springs connected in series, where each spring could represent either muscular or tendinous structures. Ettema and Huijing [17] corrected this interpretation of the alpha method considering that, in fact, it allows one to distinguish the force dependent and the force independent components of the SEC. In that way, the same mathematical model is used (i.e. in the studies of Morgan, and Ettema and Huijing) but its interpretation is different.

The adaptation of the alpha method for an *in vivo* measurement received little attention in the literature. In fact, this method has only been applied with electrostimulation on human first dorsal inter-osseous and plantarflexors muscles [11,39]. Nevertheless, electrostimulation has some disadvantages, including pain, inaccurate stimulations or a low intensity of contraction. The low inten-

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sity of contraction is a real problem to assess the behaviour of muscle and tendon stiffness considering assumption of the alpha method, as it was evoked in a study assessing the tendon stiffness using ultrasonography during voluntary isometric contraction [30]. In addition, the reliability of this method has never been demonstrated for *in vivo* measurements.
The purpose of this investigation was two-fold. Firstly, the method of assessing ankle joint stiffness was implemented *in vivo*. Secondly, the reliability of MTC stiffness measurements in plantarflexion, and characterization of both the torque dependent and independent components of the SEC stiffness obtained by using the alpha method was assessed.

Materials and Methods

Subjects

Eight healthy males (24. 1 (2.2) years, 179.6 (9.1) cm, 74.3 (10.8) kg) and six healthy females (20.7 (1.6) years, 166.2 (7.5) cm, 58.0 (8.6) kg) volunteered to participate in the present study. This study was conducted according to the Helsinki Statement (last modified in 2004) and has been approved by the local ethics committee.

Measurement techniques

A Biodex dynamometer (Biodex medical systems, Shirley, NY, USA) and the Biodex research toolkit were used to measure the external torque produced at the ankle joint (T), the ankle angle (θ) and the ankle angular velocity (ω). Subjects were sat with the hip joint angle of 70° flexed (full extension = 0°) and measurements were performed on the right leg which was fully extended (knee flexion angle = 0°). The left leg was flexed as in the sitting position.
Surface electromyographic (sEMG) signals of the *gastrocnemius medialis*, *gastrocnemius lateralis*, *soleus* and *tibialis anterior* muscles were recorded using active surface electrodes with an inter-electrode distance of 10 mm (DE-2.1, Delsys Inc, Boston, MA, USA) placed on the belly of muscles according to SENIAM recommendations [22]. sEMG and mechanical (T, θ and ω) signals were recorded simultaneously and sampled at 1 000 Hz using an A/D converter (National Instrument, Delsys Inc, Boston, MA, USA), and saved on a computer hard drive using EMGWorks 3.1 software (Delsys Inc, Boston, MA, USA).

Experimental protocol

Each subject completed two test sessions with two days of rest in between. The protocol performed during each session included: *i*) A warm-up composed of three min of submaximal

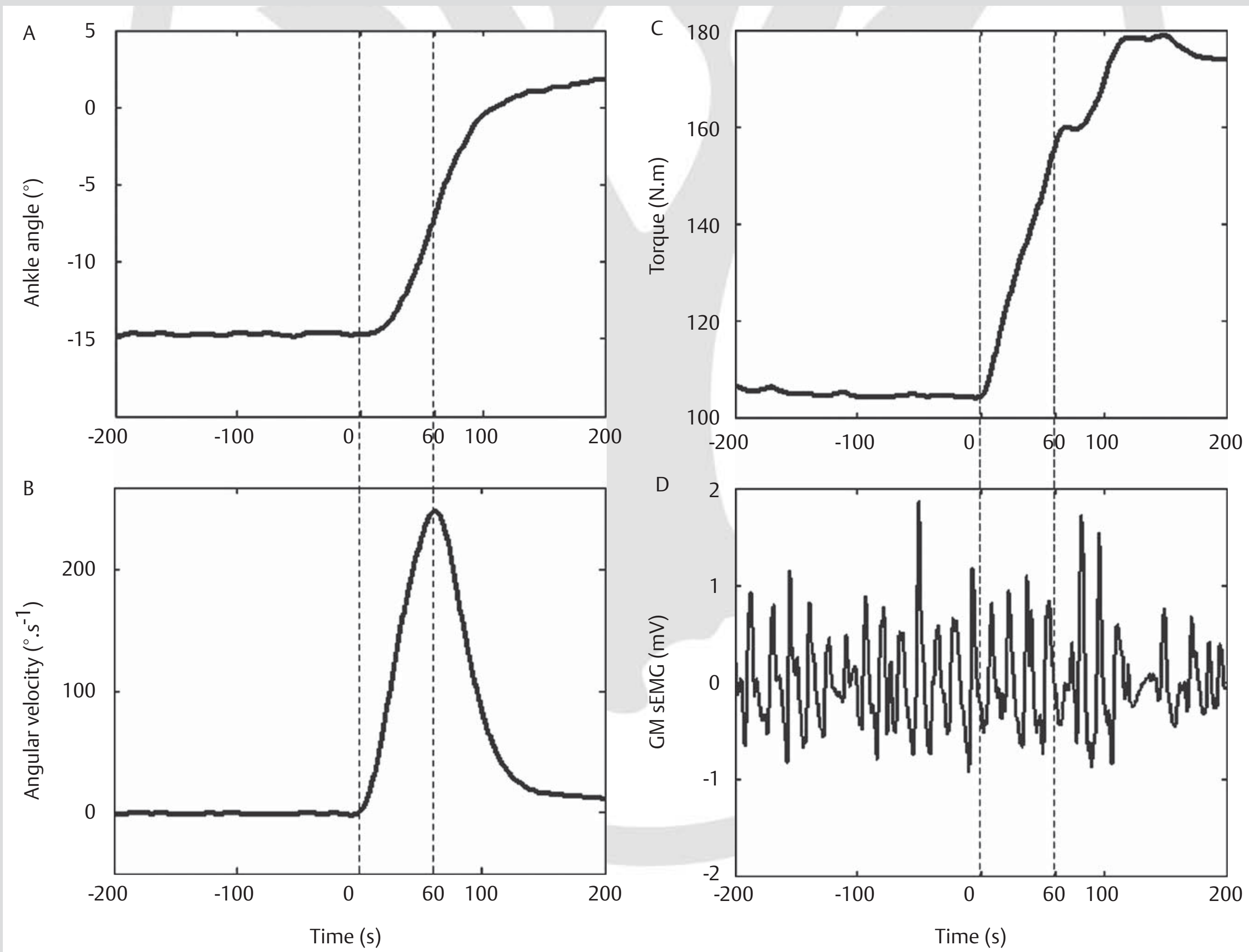

Fig. 1 Typical raw data obtained during a trial of the short range stiffness experiment: A- ankle angle, B- angular velocity, C- external torque and D- surface electromyographic (sEMG) signals of gastrocnemius medialis (GM sEMG) (gastrocnemius lateralis, soleus and tibialis anterior which are not shown on the figure). After the isometric contraction, triceps surae was quickly stretched. The first 60 ms of the stretch were used to determine the joint stiffness.

plantarflexions (PF) in isometric conditions. *ii)* After two min of rest, two trials of maximal voluntary contraction (MVC) in PF were performed at 75° (full PF=0°) with two min of rest after each trial. *iii)* Subjects were familiarized with a short-range stiffness experiment. They had to sustain two submaximal torques of 40% and 80% of MVC. A stretch was then applied as fast as possible using the dynamometer in dorsiflexion (DF) on a range of motion of 20° (i.e. from 75° to 95°). The first 60 ms were considered for the data processing. Sixty ms after the beginning of the stretch, a range of motion of about 7° was performed, the angular velocity reached about 250°/s and the acceleration was null (to avoid inertial effects on torque measurements). Two min of rest were observed after each trial. *iv)* Short range stiffness experiments (14 trials) were performed at 7 levels of torque in a random order (two trials each 10% of MVC from 30–90% of MVC) similarly to the protocol described in *iii)*. Between each trial, two min of rest were observed.

Data processing

Mechanical and sEMG signals were respectively filtered using a low pass (100 Hz) and a band pass (6–400 Hz) zero-phase second order Butterworth filters. A preliminary study had shown that this filter induces negligible distortion of mechanical signals. The torque measured by the dynamometer was corrected from inertia and the weight of the dynamometer tool to obtain the external torque at the ankle joint. The external torque and the ankle angle were determined when the joint started being moved (*i.e.* when $\omega > 0$) and 60 ms after (▶ **Fig. 1**). This time period was chosen to avoid any potential neural effects that would occur with a longer duration stretch [7,8]. The joint stiffness (S) was calculated as the ratio between external torque and ankle joint angle changes [2,7,31] for each isometric torque level. Furthermore, parallel elastic component was ignored considering passive forces at ankle angle used in the present study. In the same way, any viscous behaviour of the SEC was ignored as the literature suggests that the viscous effects are small [15].

The joint compliance (C) (i.e. inverse of joint stiffness) was considered as the compliance of two springs placed in series, one representing compliance of a torque dependent and the other a torque independent component (*i.e.* C_{SEC1} and C_{SEC2} respectively):

$$C = C_{SEC1} + C_{SEC2} \qquad (1)$$

C_{SEC1} is assumed to be inversely proportional to the torque [32]:

$$C_{SEC1} = \frac{\alpha_0}{T} \qquad (2)$$

where α_0/T represents the torque dependent component compliance of the SEC. The C_{SEC2} is assumed to be constant (i.e. torque independent component).

Eq. 1 could then be written as follows:

$$\alpha = C \times T = \alpha_0 + C_{SEC2} \times T \qquad (3)$$

where α is calculated as the product between torque and compliance, and α_0 represents the elastic extension with the torque dependent component of the SEC. α_0 is assumed to be constant for all isometric torques [16,17,32].

Thus, a linear regression was applied to the relationship alpha (α) – torque (T). The correlation coefficient of this linear fit was calculated. Then, α_0 and C_{SEC2} were extracted as the Y-intercept, and the slope, respectively (▶ **Fig. 2-A**). These two parameters (i.e. α_0 and C_{SEC2}) were used to calculate the joint stiffness using Eq.**4**.

$$S = \frac{T}{T \times C_{SEC2} + \alpha_0} \qquad (4)$$

A joint stiffness (S) – torque (T) relationship (▶ **Fig. 2-B**) for each test session was fitted using Eq.**4** on full range of torque (i.e. from 0 to 100% of MVC). A correlation coefficient (R^2) between model (i.e. joint stiffness calculated using Eq.**4**) and experimental data (i.e. calculation of joint stiffness as the ration of torque and ankle angle changes) was calculated. The minimal (S_{min}) and maximal (S_{max}) joint stiffness on the considered range of torque were assessed from model for each subject and each session. A stiffness index of torque dependent components (SI_{SEC1}) and a stiffness of torque independent components (S_{SEC2}) were also characterised as the inverse of α_0 and C_{SEC2} respectively.

In addition, sEMG activities were visually checked to ensure that no stretch reflex was present in the first 60 ms of the stretch, otherwise the trial was excluded from further analysis. The trial was also excluded if the external torque was not kept constant by the subject before the stretch. Day-to-day reliability of the five parameters of interest, MVC, S_{min}, S_{max}, C_{SEC2}, S_{SEC2}, α_0 and

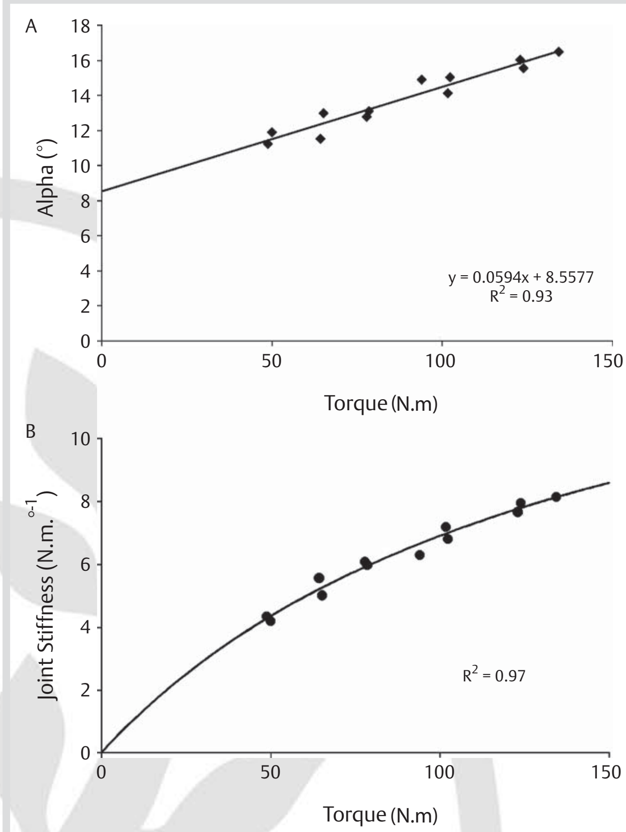

Fig. 2 Typical example of relationships obtained on a range of torque from 30 to 90% of MVC for one subject during short range stiffness experiment to determine joint stiffness (S) and alpha method application to distinguish behaviour of series elastic components. **A**- Alpha-Torque relationship plotted with the stiffness index of active structures (S_{ISEC1}) defined as the inverse of the Y-axis intercept and the stiffness of passive structures (S_{SEC2}) as the inverse of the slope of the relationship, **B**- Joint Stiffness-Torque relationship.

Table 1 Determination of the day to day reliability for the stiffness parameters.

	Test 1		Test 2					
	Mean	SD	Mean	SD	SEM	CV (%)	ICC	Lower and upper CL
MVC (N.m)	127	23	134	21	6.7	5.4	0.91	(0.74–0.97)
α_0 (°)	6.16	1.58	5.88	1.63	0.34	6.0	0.96	(0.86–0.99)
C_{SEC2} (°.N^{-1}.m^{-1})	0.087	0.022	0.093	0.026	0.006	5.9	0.94	(0.80–0.98)
SI_{SEC1} (°$^{-1}$)	0.17	0.04	0.18	0.05	0.01	6.0	0.94	(0.83–0.98)
S_{SEC2} (N.m.°$^{-1}$)	12.19	3.10	11.54	3.11	0.65	5.9	0.96	(0.86–0.99)
S_{min} (N.m.°$^{-1}$)	4.04	0.43	4.21	0.46	0.16	3.8	0.88	(0.64–0.96)
S_{max} (N.m.°$^{-1}$)	7.17	0.81	7.15	0.84	0.20	3.1	0.94	(0.83–0.98)

SD: standard deviation, SEM: standard error of measurement and CV: coefficient of variation, ICC: intra-class correlation, CL: confidence limits
MVC: maximal voluntary contraction, α_0: elastic extension with the torque dependent component of the SEC
C_{SEC2}: passive part of the SEC compliance, SI_{SEC1}: active part of the SEC stiffness index, S_{SEC2}: passive part of the SEC stiffness, S_{min}: ankle joint stiffness for 30% of MVC and S_{max}: ankle joint stiffness for 90% of MVC

$SI_{SEC1}$was assessed using intraclass correlation coefficient (ICC) assessed with a 2, *k* formula [37], associated standard error of measurement (SEM) and coefficient of variation (CV) for each parameter [24]. Bland-Altman plots were performed to determine the relation between error and size of measured values for joint stiffness, SI_{SEC1} and S_{SEC2} as previously described in the literature [3,4,6]. Bias, limits of agreement and interval confidence of the limits of agreement for those parameters were then determined.

Results

Linear regression applied on alpha-torque relationship for each subject and test session showed a very good determination coefficient (mean R^2=0. 89±0.05, range: 0.72–0.95). A high correlation was found between joint stiffness experimental data and model fit (mean R^2=0.87 ±0.09, range: 0.66–0.97). The mean results obtained on 14 subjects in MVC, S_{min}, S_{max}, C_{SEC2}, S_{SEC2}, α_0, SI_{SEC1} and the associated ICC, SEM and CV for each parameter are reported in **Table 1**. These results (**Table 1**) show an excellent reliability for MVC, S_{max}, C_{SEC2}, S_{SEC2}, α_0, SI_{SEC1} (ICC of 0.90, 0.94, 0.96, 0.94 and 0.96, respectively) and a very good reliability for S_{min} (ICC of 0.88). For all parameters CVs were lower than 6.0%. Considering the Bland-Altman plots, (**Fig. 3**), the bias and the limits of agreement (±confidence interval) were 0.078 (±0.101) N.m.°$^{-1}$ and −0.437–0.592 (±0.176) N.m.°$^{-1}$ for measurement of joint stiffness; 0.010 (±0.09) °$^{-1}$ and −0.019–0.040 (±0.015) °$^{-1}$ for SI_{SEC1} measurements; −0.65 (±0.53) N.m.°$^{-1}$ and −2.45–1.15 (±0.93) N.m.°$^{-1}$ for measurement of S_{SEC2}.

Discussion

Many different methods have been developed to assess ankle joint and MTC stiffness. However, the majority of the previous methods are not capable of simultaneously determining the stiffness of the different structures, including structures of passive and active parts of the SEC. In the present study, the alpha method was adapted to determine the stiffness of both force dependent and force independent components of the plantarflexors SEC, *in vivo*, during voluntary contractions and to assess the reliability of measurements.

Joint stiffness has been measured *in vivo* during voluntary contractions of the plantarflexors [7,8], dorsiflexors [38] and knee extensors [31] using techniques similar to that of the present study. The joint stiffness values in the present study ranged from an average of 4.13 N.m.°$^{-1}$ at 30% and 7.16 N.m.°$^{-1}$ at 90% of MVC. These values are within the range of those presented by Blanpied and Smidt [7], who also examined stiffness in plantarflexors. At a maximal stretch velocity of 170°.s^{-1}, these authors found that joint stiffness was between 3.61 and 5.75 N.m.°$^{-1}$ for contractions ranging from 30–60% of MVC. The minimal and maximal joint stiffness extracted from the modelled joint stiffness-torque relationship were found to be reliable (ICC of 0.88 and 0.94, respectively). In addition, Bland-Altman plots showed uniform random error with no systematic error (**Fig. 3**). Considering the narrow limits of agreement and relative changes in joint stiffness values observed after chronic interventions described in previous studies [e.g. 21, 28], the statistical error found in the present study is within an acceptable range.

The mechanical loads applied to the muscle in the present *in vivo* study were similar to those reported by previous investigators using the alpha method on isolated muscle. The stretch velocity was set as fast as possible using our isokinetic dynamometer. The MTC stretching velocity, estimated from published anthropometric data [19], was about 90–110 mm.s^{-1}. This is similar to the velocity of muscle stretch reported by Morgan et al. [33] on isolated muscle, which was between 100 and 200 mm.s^{-1}.The stretching velocity used in the present study was greater than other *in vivo* studies. For instance, Blanpied and Smidt [7] used a maximal stretching velocity that was about 105°/.s^{-1} compared to maximal stretching velocity of 250°/.s^{-1} in the study. The mean stretching velocity used by McHugh and Hogan [31] was about 30°/.s^{-1}, notably slower than 120°/.s^{-1} used in the present study. Using methodology similar to that of the present study, the influence of stretching velocity on stiffness-torque relationship was shown to be negligible for maximal stretching velocities greater than 70°/.s^{-1} [7]. Therefore, it can be assumed that the stretching velocity in the present study was sufficient to prevent contributions from the contractile component into the stiffness measurement.

The moment arm length of the plantar flexor muscles was not constant throughout the range of stretch used in the present study. This may affect the stiffness-torque relationship. Using the model of Grieve et al. [19], we estimated that the change in moment arm length was only about 4.4%. This slight error has negligible influences in determining effect of chronic interventions on the same subject. Similarly, this would also not likely affect stiffness comparisons across different populations. Using the alpha method for a range of forces from 30 to 90% of MVC

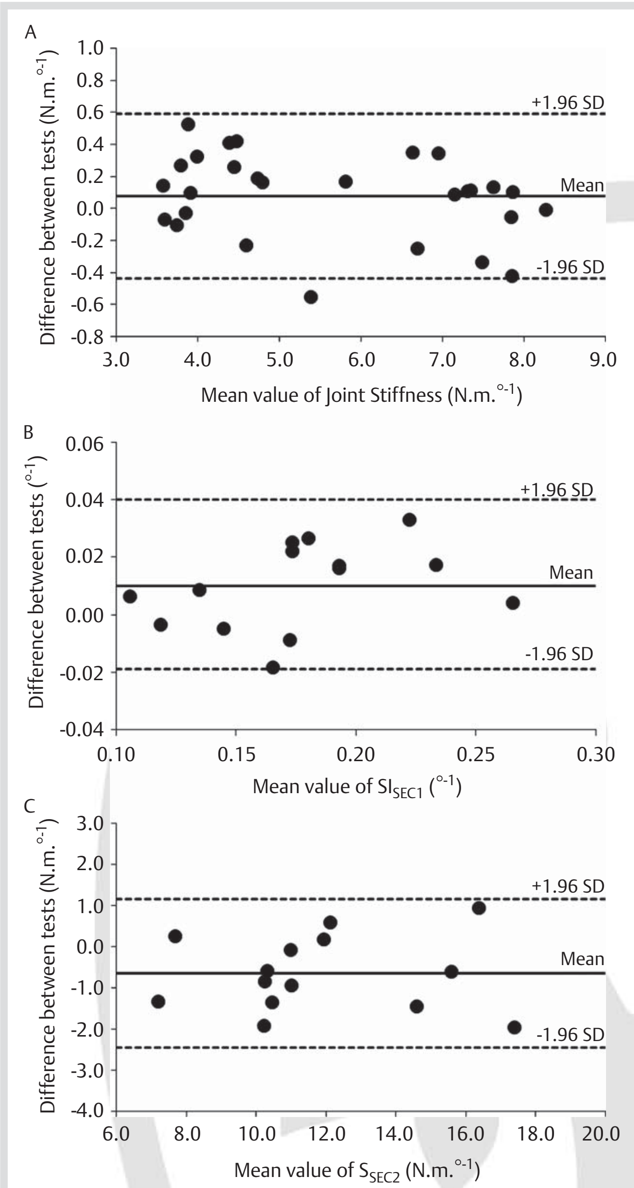

Fig. 3 Bland-Altman analysis for the inter-test agreement for each of the mechanical properties assessed. The parameters measured were: **A**- joint stiffness, **B**- stiffness index of the active part of the series elastic component, **C**- stiffness of the passive part of the series elastic component.

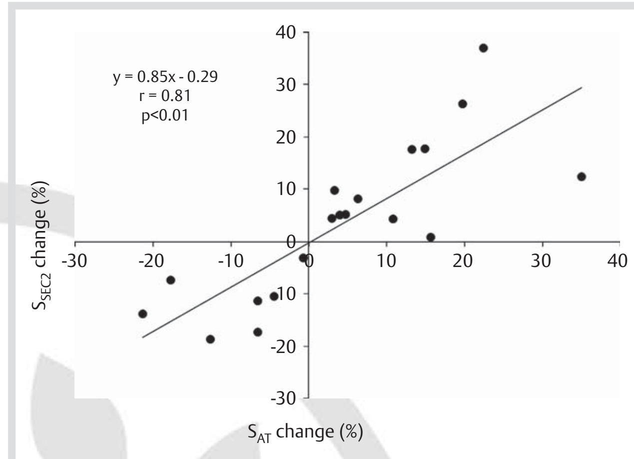

Fig. 4 Relationship between changes in the Achilles tendon stiffness (S_{AT}) determined using ultrasonography during isometric contraction and the stiffness of passive structures of the series elastic component (S_{SEC2}) determined using the alpha method after 14 weeks of plyometric training.

dissociates the joint stiffness in a torque dependent and a torque independent component [17]. Original results of the present study showed that S_{SEC2} and SI_{SEC1} are reliable (ICC of 0.96 and 0.94 for S_{SEC2} and SI_{SEC1}, respectively). Initially, the alpha method was developed to separate both muscle and tendon stiffness [32,33]. However, this method assumes that tendon stiffness remains constant throughout the range of force and this assumption could be discussed within this framework. Proske and Morgan [33] considered that tendon stiffness was constant for muscle forces higher than 20–30% of the maximal isometric force. More recent *in vivo* studies using ultrasonography have shown that tendon stiffness in Achilles tendon could be considered as constant for torque greater than 50% of MVC [26,27]. Therefore, a preliminary study was performed on the range of force from 50 to 90% of MVC. Moderate reliability was observed for S_{SEC2} and SI_{SEC1} (ICC of 0.77 and 0.88, respectively) which can be mainly explained by the small range of forces between 50 and 90% of MVC. Nevertheless, stiffness index values obtained in the present study for 30–90% and 50–90% MVC ranges were compared. No significant difference ($p > 0.05$) was found between values of SI_{SEC1} and S_{SEC2} in these two ranges. Considering these preliminary results and the high reliability of measurements obtained from 30–90% MVC range led us to consider the SI_{SEC1} and S_{SEC2} calculated over this entire range of 30–90% MVC. It can be assumed that the torque dependent component could be mainly considered as the active part of the SEC (i.e. muscular structures), and, the torque independent component as the passive part of the SEC (i.e. tendinous structures).

There is, to our knowledge, no *in vivo* method to simultaneously determine the stiffness of each component of the SEC. Therefore, stiffness values obtained in the present study using the alpha method are quite difficult to validate. Nevertheless, since the Achilles tendon is a structure included in the passive part of the SEC [5], it is possible to compare the passive part of SEC stiffness (S_{SEC2}) obtained using the alpha method to Achilles tendon stiffness (S_{AT}) as determined using ultrasonography during an isometric contraction [26,27]. A pilot study showed no significant correlation ($n = 32$, $p > 0.05$) between these two parameters. This can be explained by differences in experimental conditions. For instance, there was a difference in the assessed structures (i.e. distal tendon of the GM *versus* passive structures of the SEC) and stretching velocity (i.e. a "low" velocity of 3–5 $mm.s^{-1}$ during the isometric contraction *versus* a "high" velocity of about 90–110 $mm.s^{-1}$ in the present study). Despite the differences, changes induced by a chronic intervention (e.g. bed rest or training) should be similar for the passive part of the SEC stiffness and Achilles tendon stiffness. To determine the effect of 14 weeks of plyometric training, S_{AT} and S_{SEC2} were assessed in two groups, a trained group ($n = 9$) and a control group ($n = 10$). The preliminary results showed a significant increase of S_{SEC2} and S_{AT} for the trained group after the 14 week period. Furthermore, the Achilles tendon stiffness (using ultrasonography) and the passive part of SEC stiffness (using the alpha method) demonstrated a mean increase of 10.0 and 13.2% respectively. A significant correlation was found ($r = 0.81$, $p < 0.01$) between changes in these two parameters after 14 weeks of plyometric training (**Fig. 4**).

Therefore, it can be reasonably considered that the stiffness of the passive part of the SEC determined using the alpha method is representative of the real behaviour of the SEC passive structures.

The linear relationship between S_{SEC1} and torque that is assumed in the alpha method cannot be evaluated without using invasive *in vivo* techniques. Nevertheless, a linear relationship between stiffness of the SEC and torque has been determined in previous *in vivo* studies [7,8,13,21,28]. In addition, previous studies [16,17,33] have considered the relationship between muscular stiffness and torque to be linear, assuming that if the short-range stiffness was due to deformation of existing bridges without significant breakdown or reformation, then the stiffness would be proportional to the number of bridges. Hence, the stiffness of the cross-bridge array should be directly proportional to the tension, as both are proportional to the number of bridges [32].

This study non-invasively implemented the alpha method during submaximal voluntary contractions using *in vivo* techniques. Two previous studies adapted the alpha method in humans [11,39], however, using electrostimulation. While these studies used the alpha method to dissociate muscle and tendon stiffness, the results may not be as valid for higher force magnitudes. Electrostimulation produces force values between 15–30% of MVC [39], which may be too low for the assumption concerning the range of force where the tendon stiffness remains constant.

The alpha method provides reliable measurement of joint stiffness (i.e. S_{min} and S_{max}), and stiffness of both torque dependent and independent components of plantarflexors (i.e. S_{SEC1} and S_{SEC2}) which could be linked to both active and passive parts of the SEC. Simultaneously determining SI_{SEC1} and S_{SEC2} allows for a better understanding of the mechanical behaviours and interactions between muscle and tendon structures. This method could also be used to assess changes in active and passive parts of SEC stiffness for different populations (i.e. age, gender, sport activity, pathologies) and/or after different kinds of interventions (e.g. training, pathological processes, rehabilitation).

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