



Contents lists available at ScienceDirect

## Journal of Electromyography and Kinesiology

journal homepage: [www.elsevier.com/locate/jelekin](http://www.elsevier.com/locate/jelekin)

## Influence of muscle activity on musculotendinous stiffness quantification in stunted, prepubertal children

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### ARTICLE INFO

#### Article history:

Received 30 January 2013

Received in revised form 10 June 2013

Accepted 9 July 2013

#### Keywords:

Musculotendinous stiffness

Electromyogram

Stunting

Children

### ABSTRACT

The quick-release technique to estimate musculotendinous (MT) stiffness has been extensively used over the last years, in both animals and humans, to gain insights in the adaptive process of the series elastic component (SEC). Recently, MT stiffness quantification, i.e., SEC behavior, has been revisited for subjects not able to fully activate their muscles (effects of long-term spaceflight or non-mature muscles). Such a phenomenon can also be encountered in stunted children. So, the aim of the present study was to analyze the effect of stunting on MT stiffness taking into account possible defect in muscle activation. For this study, 20 eutrophic children (EU) with an average age of 9 years  $\pm$  4 months were compared to 11 age matched stunted children (S) evaluated by the height-to-age index. The MT stiffness index was obtained with regard to stiffness–torque and stiffness–soleus EMG relationships. The children of the S group presented a significantly lower Maximal Voluntary Contraction (MVC) in plantar flexion in comparison with children of the EU group ( $-37.8\%$ ). The significantly lower MT stiffness index for S children ( $-42.6\%$ ) was evidenced only when quantified with regard to the stiffness–soleus EMG relationship ( $66.5 \pm 42.8$  vs.  $38.2 \pm 19.9$  Nm rad<sup>-1</sup>sec<sup>-1</sup>). Possible delay in fiber type differentiation or tendinous structure maturation can account for the lower MT stiffness index in S children. In conclusion, stunting during early childhood delays the differentiation and maturation processes of musculotendinous structures as shown by the lower MT stiffness quantified with regards to muscle activity, also altered for stunted prepubertal children.

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### 1. Introduction

In humans, musculotendinous (MT) stiffness can be measured in active conditions using the quick-release technique transposed from animal testing (Goubel and Pertuzon, 1973). The quick-release technique requires the subject to maintain a submaximal torque in isometric conditions, while quick-release movements are induced from a sudden and fast release of the support. The angular MT stiffness is then estimated from the angular displacement and the angular acceleration measured over the first milliseconds of the elastic recoil. When different angular MT stiffness values are related to the initial torque values, the slope of the linear relationship so obtained gives the index of MT stiffness, which is independent of the torque.

Changes in MT stiffness with regard to a period of hyperactivity or maturation have been investigated previously using this technique. For instance, Grosset et al. (2009) showed that plyometric training lead the MT stiffness index to decrease, while endurance training had the opposite effect. Knowing that fast type fibers are more compliant than slow type fibers (Goubel and Marini, 1987), these changes in MT stiffness index can be then ascribed in part to a fiber-type transition phenomenon of skeletal muscle. With regard to the maturation process in prepubertal children, it was shown that immature activation capacities of the plantar flexors (Grosset et al., 2008) can mask intrinsic MT stiffness adaptation and evolution (Lambertz et al., 2003). Indeed, for subjects presenting immature activation capacities, the maintenance of a given torque leads to increased activity of agonist and antagonist muscles, therefore leading to a lower resultant external torque, despite a higher number of active cross-bridges in the agonist muscles. Knowing that MT stiffness is directly related to the number of active cross-bridges (Hill, 1938; Huxley and Simmons, 1971), MT stiffness will be higher in this case and consequently

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overestimated (Lambertz et al., 2001, 2003). Thus, changes in MT stiffness of the triceps surae with age in prepubertal children were analyzed and the indexes of stiffness were quantified either with regard to the external torque, or with regard to the triceps surae surface electromyogram (EMG). The expected increase in MT stiffness index with age notably due to maturation of elastic structures (see Nakagawa et al., 1996; Kubo et al., 2001) was only observed when the stiffness indexes were evaluated with respect to EMG, while MT stiffness index quantified with respect to torque showed opposite changes with age (Lambertz et al., 2003).

In the present study, we hypothesized that stunted prepubertal children may present a delay in maturation, not only in musculo-tendinous structures, but also in muscle activation. Thus, EMG data analyses are included in order to highlight (1) differences in muscle activity thanks to EMG–torque relationships and (2) intrinsic differences in musculo-tendinous stiffness despite possible differences in muscle activity between stunted prepubertal children and eutrophic children thanks to MT stiffness–EMG relationships. The discrepancy between evolutions in stiffness indexes calculated either with regard to the torque or to the EMG will confirm the necessity of evaluating the MT stiffness index with respect to EMG each time when altered muscle activation capacities are suspected.

## 2. Materials and methods

### 2.1. Subjects

Thirty-one healthy prepubertal children, without pre, peri or postnatal interferences (neurologic, orthopedic or infectious diseases), with a chronological age of 9 years ( $\pm 4$  months) were identified to participate in this study. These children were of both genders and come from two villages of the Sertão region of the state of Pernambuco, in the northeast of Brazil. The experimenter and the legal guardians determined their pubertal status. Based on breast development, pubic hair and no apparent changes in the voice and skin, all children were classified as prepubescent.

Stunting due to early mal- or under-nutrition was determined by means of the height-for-age index, as recommended by the World Health Organization (WHO, 1995). In this study, stunting was considered as  $< -2$  SD of the WHO reference Z-score (De Onis et al., 2007), so that 11 children composed the stunted group (S,  $108 \pm 4$  months, WHO  $-2$  SD Z-score =  $120.4 \pm 1.1$  cm) and 20 children composed the eutrophic group (EU,  $108 \pm 4$  months, WHO  $-1$  SD Z-score to  $+1$  SD Z-score =  $132.4 \pm 6.3$  cm). Furthermore, erect sitting height was used as additional measure to indicate stunting due to malnutrition (Schooling et al., 2008, 2011). Anthropometric data also included the measurement of body mass, lower leg length and calf circumference, and the calculation of the body mass index (BMI).

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Committee of Ethics in Research of the Federal University of Pernambuco (Process No. 015/2005), according to the Regulatory Research Standards Involving Human Beings, Resolution 196/96 of the Brazilian National Health Council. The legal guardians were fully instructed about the experimental procedure and written informed consent was obtained from all subjects. The guardians, as well as the child, were free to withdraw from the study at any time.

### 2.2. Biomechanical and EMG measurements

The biomechanical device consists of a transportable ankle ergometer device (Bio2M, France) as described by Lambertz et al. (2008). Briefly, the transportable ergometer device is composed

of an adjustable seat and an adjustable rotational footplate so that subjects with different anthropometric characteristics can be evaluated. Isometric force is measured by an S-type load cell (maximal force 500 N) that is connected to an electro solenoid (maximal holding force 790 N) ensuring the static contraction condition of the muscles. For anisometric testing, angular displacement ( $\theta$ ) is measured by an optical absolute encoder. In its dynamic mode, musculotendinous stiffness is assessed by means of the quick-release technique (Goubel and Pertuzon, 1973).

Surface electromyograms (EMG) were detected on the soleus (Sol) using self-adhesive, active Ag/AgCl surface electrodes (10 mm in diameter, 3 M, USA, amplification 20 times). The electrode impedance was reduced to below 5 k $\Omega$  by exfoliating and cleaning the skin areas. The voluntary EMG was recorded differentially, DC amplified (amplification 600 times) and band-pass filtered (20–500 Hz) (EMG System do Brazil, Brazil). Post-acquisition EMG filtering included band-pass filtering (80–300 Hz). These unusual filter characteristics were necessary, since slight peak frequencies at 60 Hz were observed due to unavoidable environmental noise.

### 2.3. Experimental protocol and data processing

The child was comfortably placed on the adjustable seat of the ergometer device without a back support, and the thigh and the knee were maintained by a restraint system to keep them immobilized. The right foot was rigidly attached to the adjustable footplate so that the lateral malleolus coincided with the axis of rotation of the footplate. Therefore, the experiments were performed around this assumed axis of joint rotation. The knee was extended to 120° (180° is full extension) and the ankle was flexed to 90°, i.e., the neutral position. It can be considered that with regard to the anatomical position of the knee (120°) and the ankle (90°) during mechanical testing, that Sol is the main contributor to the plantar flexor torque (Huijing et al., 1987), although the literature reports that deeper plantar flexor muscles also contribute in adult subjects (Finni et al., 2006).

A full test session including rest periods lasted approximately 1 h and comprised: (i) explanation of the test, (ii) preparation of the child, (iii) familiarization with the test where children were trained to perform the correct contraction during which it was ensured by observation that children executed a plantarflexion and (iv) the actual test. The rest periods were standardized in terms of intratest (30 s) and intertest (1 min). The experimental protocol was always performed by the same two investigators. The cumulative time of contraction for all the mechanical tests never exceeded 90 s per subject. Given the resting time after each voluntary contraction (each lasting a maximum of 5 s), it can be assumed that there was no muscular fatigue.

The maximal motor direct response of the Sol (Sol  $M_{max}$ , 10 kHz sampling frequency, amplification 100 times) was elicited by applying a percutaneous supramaximal electrical stimulation to the posterior tibial nerve (the cathode in the popliteal fossa and the anode over the thigh, proximal to the patella) using an isolated constant current stimulator. The Sol  $M_{max}$  peak-to-peak amplitude was calculated as the mean of five Sol  $M_{max}$  responses. This  $M_{max}$  evaluation was necessary to normalize EMG data (Sol EMG/ $M_{max}$ ). Two children from the EU group and three children from the S group did not take part in this part of the experimental protocol.

The absolute force was determined under isometric conditions from a maximal voluntary contraction of the triceps surae (MVC, 1 kHz sampling frequency) in plantarflexion. The MVC was defined as the greatest force maintained for at least 500 ms over three attempts. The absolute MVC force was converted to a torque measurement.

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