Archives of Gerontology and Geriatrics 66 (2016) 1–6

Contents lists available at ScienceDirect

Archives of Gerontology and Geriatrics

journal homepage: www.elsevier.com/locate/archger

The age related slow and fast contributions to the overall changes in tibialis anterior contractile features disclosed by maximal single twitch scan

Claudio Orizio[a,*], Marta Cogliati[a], Luciano Bissolotti[b], Bertrand Diemont[a], Massimiliano Gobbo[a], Jan Celichowski[c]

[a] *Department of Clinical and Experimental Sciences, University of Brescia, Viale Europa 11; 25123 Brescia, Italy*
[b] *Rehabilitation Service, Fondazione Teresa Camplani-Casa di Cura Domus Salutis, Via Lazzaretto, 3, 25123 Brescia, Italy*
[c] *Department of Neurobiology, University School of Physical Education, 27/39 Królowej Jadwigi St., 61-871 Poznan, Poland*

ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 29 April 2016
Accepted 2 May 2016
Available online 3 May 2016

Keywords:
Neuromuscular electrical stimulation
Single twitch modelling
Skeletal muscle
Single twitch
Ageing

ABSTRACT

This work aimed to verify if maximal electrically evoked single twitch (STmax) scan discloses the relative functional weight of fast and slow small bundles of fibres (SBF) in determining the contractile features of tibialis anterior (TA) with ageing. SBFs were recruited by TA main motor point stimulation through 60 increasing levels of stimulation (LS): 20 stimuli at 2 Hz for each LS. The lowest and highest LS provided the least ST and STmax, respectively. The scanned STmax was decomposed into individual SBF STs. They were identified when twitches from adjacent LS were significantly different and then subtracted from each other. Nine young (Y) and eleven old (O) subjects were investigated. Contraction time (CT) and STarea/STpeak (A/PT) were calculated per each SBF ST. 143 and 155 SBF STs were obtained in Y and O, respectively. Y: CT and A/PT range: 45–105 ms and 67–183 mN s/mN, respectively. Literature data set TA fast fibres at 34% so, from the arrays of CT and A/PT, 65 ms and 100 mN s/mN were identified as the upper limit for SBF fast ST classification. O: no SBF ST could be classified as fast. Conclusions: STmax scan reveals age-related changes in the relative contribution of fast and slow SBFs to the overall muscle mechanics.

1. Introduction

The anatomical changes that take place during the ageing process in the skeletal muscle are well described by using muscular biopsy. Indeed, when contractile elements are considered, both changes in the relative proportion of slow and fast fibres (Ryall, Schertzer, & Lynch, 2008) and changes in the myosin heavy chain isoforms (D'Antona et al., 2003) have been described and may contribute to explain the reduction of the twitch amplitude and velocity (Ryall et al., 2008; Łochyński, Kaczmarek, Krutki, & Celichowski, 2010). More generally the data obtained by muscular biopsy provide the ratio between the area occupied by the type II and type I fibres without identification of their functional contribution to the tension at the tendon.

This goal could be achieved using the invasive method suggested by Buchthal and Schmalbruch (1970). It was designed to characterise the single twitch (ST) of the small bundles of fibres (SBF) in intact human muscle. According to the authors SBF can be considered as the muscle quantum that can be activated by stimulation and should be attributed to "more than one motor unit". This is in line with the experimental evidence that the very and stable stimulation of the single motor unit in human intact muscles presents several difficulties even when the current injection is made at "a peripheral nerve at an accessible position" (McComas, 1998). The Buchthal and Schmalbruch (1970) invasive stimulation procedure was based on both intramuscular and intraneural needle electrical stimulation (at different sites of the muscle innervation zone and at different levels of stimulation amplitudes). The authors demonstrated that the relative number of slow or fast SBF contraction time (CT) range mirrored the bioptic histochemical results for the relative slow/fast twitch fibre area.

Intramuscular or intraneural electrical stimulation are not the only stimulation procedures that can be used to evoke muscle

Abbreviations: A/PT, ratio between the area and the peak of the evoked twitch; CT, contraction time; LS, level of stimulation; MU, motor unit; NMES, neuromuscular electrical stimulation; PT, peak twitch; SBF, small bundle of fibres; ST, single twitch; STmax, single twitch obtained at Vmax; TA, muscle tibialis anterior; Vmin/Vmax, stimulation amplitude eliciting the least/maximal detectable twitch.

* Corresponding author.
E-mail address: claudio.orizio@unibs.it (C. Orizio).

http://dx.doi.org/10.1016/j.archger.2016.05.003
0167-4943/

activity. Indeed, transcutaneous neuromuscular electrical stimulation (NMES) is a non-invasive technique that can be used to evoke well graded and controlled muscle action (Orizio, Gobbo, Diemont, & Solomonow, 2007; Orizio et al., 2013). In fact, according to Gobbo, Maffiuletti, Orizio, & Minetto (2014) the stimulation with variable amplitude of stimuli at the muscle motor point (i.e. “the skin area above the muscle in which an electrical pulse applied transcutaneously evokes a muscle twitch with the least injected current”), allows to accurately control the level of motor units spatial recruitment. This method is often used to study muscle electromechanical features and performance (Gobbo et al., 2014; Gobbo, Gaffurini, Bissolotti, Esposito, & Orizio, 2011; Orizio et al., 2007, 2013; Botter et al., 2011). The NMES maximal stimulation amplitude activates all the motor neuron axons or intramuscular axonal branches at the chosen motor point and, when low stimulation rate is administered, a sequence of maximal single twitches (STmax) is detectable from force transducer applied to bone segment of the involved joint.

With all this in mind the present work aimed to:

- Develop a non-invasive method, based on NMES, to classify as slow or fast the contribution to the STmax of the SBFs identified at specific submaximal levels of stimulation amplitude,
- Validate the method as a tool able to disclose the age related changes in slow/fast functional contribution to muscle mechanical output.

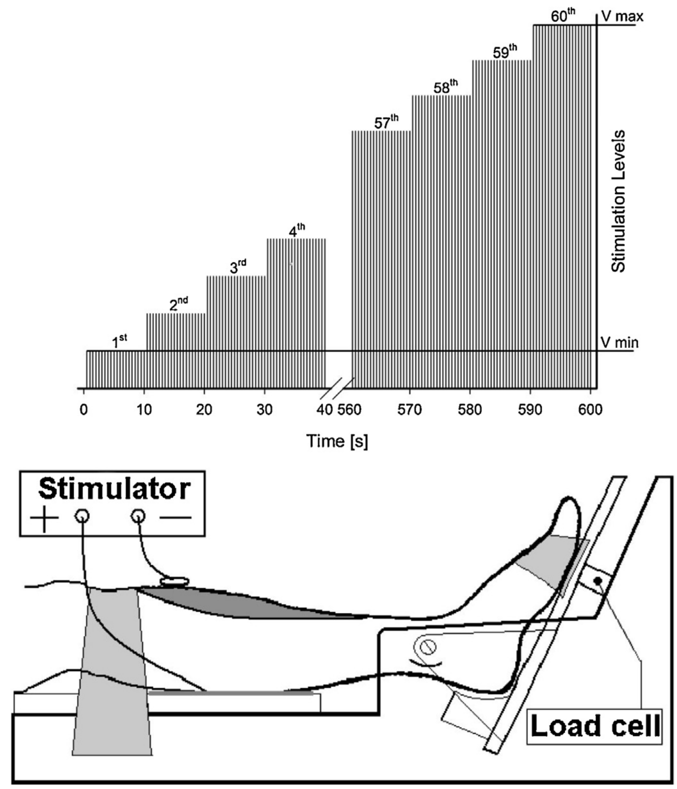

Fig. 1. The upper panel shows the 2 Hz stimulation train. The amplitude changes between Vmin and Vmax every 10 s in 60 steps. Vmin and Vmax were the stimuli amplitudes eliciting the least and the maximal electrical response, respectively. In the bottom panel a schematic drawing of the ergometer for TA isometric torque measurement and of the stimulation set-up are reported.

2. Materials and methods

2.1. Subjects

Eleven old female subjects (age 65–80 years old) and nine young female subjects (age 20–30 years old), without neurological or orthopaedic diseases, gave their informed consent to participate in the study after being given a full explanation of the experimental procedure according to the Declaration of Helsinki (1964) and its amends. The local Ethical Research Committee approved the proposed experimental design.

2.2. Measurements

For each subject, the tibialis anterior (TA) of the dominant leg, was investigated. The leg was fixed in a specifically designed dynamometer (see Fig. 1) equipped with a load cell to measure the applied tension during static TA contraction (Orizio et al., 2007). The ankle was placed at 20 deg of plantar-flexion. This angle allowed the subjects the most comfortable position without any over-stretching occurring. According to Maganaris (2001) this angle also provided a muscle length producing the maximal tension during contraction. The foot was strapped to a wooden plate connected to a load cell (model SM–100 N, Interface Inc, Scottsdale, US-AZ) having a linear response between 0 and 100 N and sensing the tension produced by the portion of TA stimulated at the most proximal motor point. The load cell signal filtering bandwidth was DC–128 Hz.

The load cell signal was A/D converted (CED-1401, Cambridge Electronic Design, Cambridge, UK) and stored in a PC at the sampling rate of 1024 Hz.

2.3. Procedure

According to Gobbo et al. (2014), the adhesive stimulating cathode electrode (5 × 5 cm) was placed on the main motor point of TA. The anode electrode (15 × 10 cm) was positioned on the gastrocnemius muscle.

By increasing the amplitude of a 1 Hz stimulation train (10 pulses per each 0.1 V amplitude level) in the range 0.5–5 V, Vmin and Vmax were identified as the stimulus amplitude eliciting the least appreciable and the largest single twitch. The latter was identified when the mechanical response did not increase with further increments of the stimulation amplitude, meaning that all MUs the chosen motor point could recruit were activated. In this case the single twitch was defined as STmax.

The Vmax-Vmin interval was divided in 60 levels of stimulation (LS). At each LS a 2 Hz train was delivered for 10 s. The total time of stimulation was 600 s. A schematic representation of the stimulation pattern is reported in Fig. 1 upper panel.

A *t*-test was used to verify the presence of statistically significant difference between the amplitude of STs of two adjacent LS. On the basis of Bonferroni correction for multiple comparisons the level of significance was set at $p < 0.0008$. If this was the case the 20 twitches from both the lower and higher LS were separately averaged and modelled as suggested by Raikova, Krutki, Aladjov, and Celichowski (2007) according to the following equation:

$$F(t) = \sum_{i=1}^{N} F_i\left(t, T_{imp}^{(i)}, T_{lead}^{(i)}, F_{max}^{(i)}, T_c^{(i)}, T_{hr}^{(i)}\right)$$

where: $T_{imp}^{(i)}$ is the moment of the *i*th stimulus; $T_{lead}^{(i)}$ is the time between the stimulus and the start of force development; $F_{max}^{(i)}$ is the maximal force of the *i*th twitch; $T_c^{(i)}$ is the contraction time, the time from the start of the SBF mechanical activity to$F_{max}^{(i)}$; $T_{hr}^{(i)}$ is the half relaxation time, the time from $F_{max}^{(i)}$ to $F_{max}^{(i)}/2$; $N = 20$; *t* is the time position of a stimulus within the given stimulation period, i.e.