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

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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.

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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

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.

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.
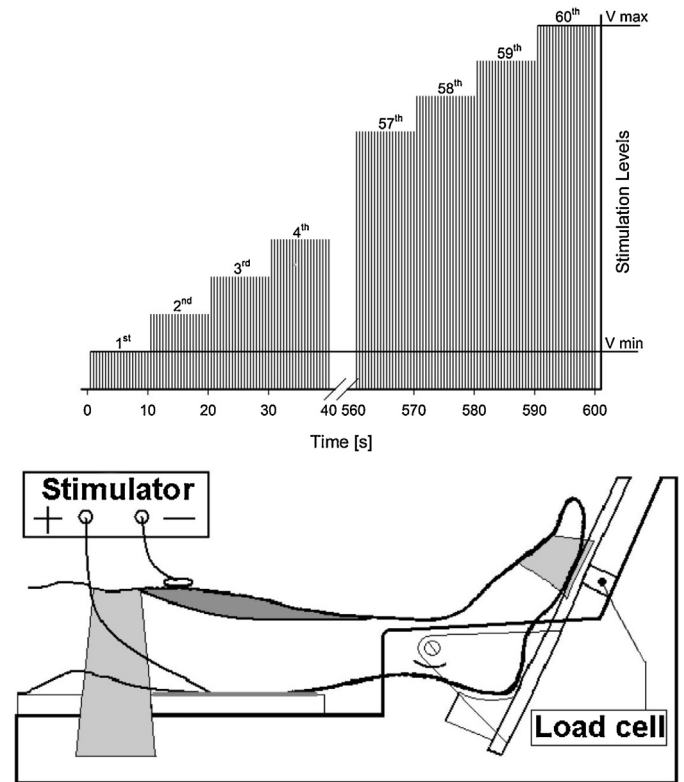

Fig. 1. The upper panel shows the 2 Hz stimulation train. The amplitude changes between Vmin and Vmax every 10 s in 60 steps. Vmin and Vmax were the stimuli amplitudes eliciting the least and the maximal electrical response, respectively. In the bottom panel a schematic drawing of the ergometer for TA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

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.

10 s. The smaller modelled twitch was subtracted from the larger one to extract the contribution of the recruited SBF passing from the lower to the higher LS. The final result of the procedure was a sequence of SBFs modelled STs which summation provided the STmax.

In order to check the possible presence of fatigue throughout the experimental session in five age-matched young subjects, who have more fast fatigable MUs, STmax was elicited for 600 s. In none of the five subjects the STmax parameters, see Section 2.4, changed from the first to the last minute of stimulation. The test was made on subjects other than those of the Y group studied.

2.4. ST analysis

For STmax and for each SBF contribution to the STmax building-up, a number of parameters were calculated: the peak twitch (PT, in mN), the contraction time (CT, in ms), from the onset of the force (0,5% of the PT) to the PT, and the ratio between the area under the twitch and the twitch amplitude (A/PT, in mN ms/mN) as a shape factor.

D50 parameter. This parameter was formerly proposed by Sleutjes et al. (2014) as a tool to quantify the summation pattern of the individual evoked MU action potentials (MUAPs) to the compound muscle action potential (CMAP). Specifically D50 identifies the number of the largest consecutive differences that are needed to build-up 50% of the maximum CMAP. It reduces in case of motor units loss and/or larger MUs due to re-innervation. In the present study D50 parameter is the number of the largest SBF STs that are needed to build-up 50% of the STmax.

2.5. Justification of the method

During maximal stimulation of common peroneal nerve, Moglia et al. (1995) using the same ergometer as used in this study, recorded static torques of ankle flexors of about 5 N m. According to Hasson, Kent-Braun, and Caldwell (2011) the TA occupies the 30% of the transverse section of these muscles. Hence its expected contribution to the dorsi-flexors torque can be about 1.5 N m. Given the average STmax we recorded in young was about 0.8 N m (converted value from Table 1) it can be concluded that a significant sample of TA was studied.

CT and A/PT were used to distinguish between fast or slow twitch responses. A/PT shows the "influence of twitch time on the analysed area" and has been suggested by Celichowski et al. (1998). We obtained A/PT values nearly 3 times higher than in rats (Celichowski, Grottel, & Bichler, 1998). The explanation may be that once the area has been scaled to the twitch peak, A/PT depends much on the duration of the mechanical event. The latter, in our recordings from humans SBF, is much longer than in rat single MUs.

2.6. Statistical analysis

A t-test was used to compare the above cited parameters obtained in young and elderly groups. Significance was set at $p < 0.05$. When the parameter values distribution of the groups did not pass the normality test the non-parametric test of Mann–Whitney Rank Sum Test was used.

3. Results

3.1. STmax

Table 1 contains the parameters of the maximal twitches evoked in the two groups.

3.2. Extracted SBF contributions to STmax

The number of extracted SBF twitches contributing to the STmax were 143 and 151 for young and old subjects, respectively. In Fig. 2 the normalized components from STmax scanning of two representative subjects (one young and one old) are reported. The components classified as fast, according to the criteria indicated in Section 3.3, are present only in the young subject and are reported in white.

Differences were found between the number of the individual twitches summating in the STmax [15.89 ± 4.25 and 12.45 ± 4.65 for young and old, respectively] and D50 [5.67 ± 1.23 and 3.91 ± 1.87 for young and old, respectively]. The difference between the two groups was statistically significant only for D50.

In Fig. 3 (top panel) the distribution of the PT is reported. No significant difference was found between the two groups. The average value of the recorded tension on the load cell was 227 ± 101 mN in young and $231 \pm 136,08$ mN in old.

In Fig. 3 (middle panel) the distribution of the contraction time (CT) is reported. The average value of the contraction time was 69 ± 9 ms in young and 85 ± 12 ms in old subjects. The difference was statistically significant.

The distribution of the ratio between the area and the twitch amplitude (A/PT) is reported in Fig. 3 (bottom panel). The average values of the A/PT were 108 ± 19 in young and 160 ± 32 in old subjects. The difference was statistically significant.

Table 1
STmax parameters.

	Young (n = 9)	Old (n = 11)	
PT(mN)	3545 ± 1233,5	2815 ± 1388	
CT(ms)	71 ± 5	87 ± 4	*
A/PT(mN·ms/mN)	109,5 ± 10	160,8 ± 12,4	*

STmax = single twitch obtained during maximal stimulation at the motor point, PT = amplitude, CT = contraction time, A/PT = ratio between the area below STmax and its amplitude. The asterisk indicates a statistical significant difference between the two groups.
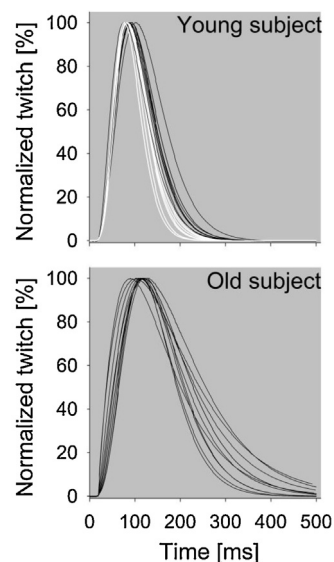

Fig. 2. Maximal single twitch (STmax) decomposition in a young and an old representative subject. The twitches are scaled to their maximum. The white twitches are the fast STmax components identified according with the criteria indicated in Section 3.3.

Fig. 4. Contraction time (CT) and area/twitch peak (A/PT) relationship for STs obtained in young subjects. The two parameters are linearly related according to the following equation: CT = 31.947 + 0.338 A/PT (R = 0.745, p < 0.001). Two examples, from a representative subject, illustrating the difference between the fast twitch and slow twitch (below and beyond the thresholds values, respectively) are reported in the insets.
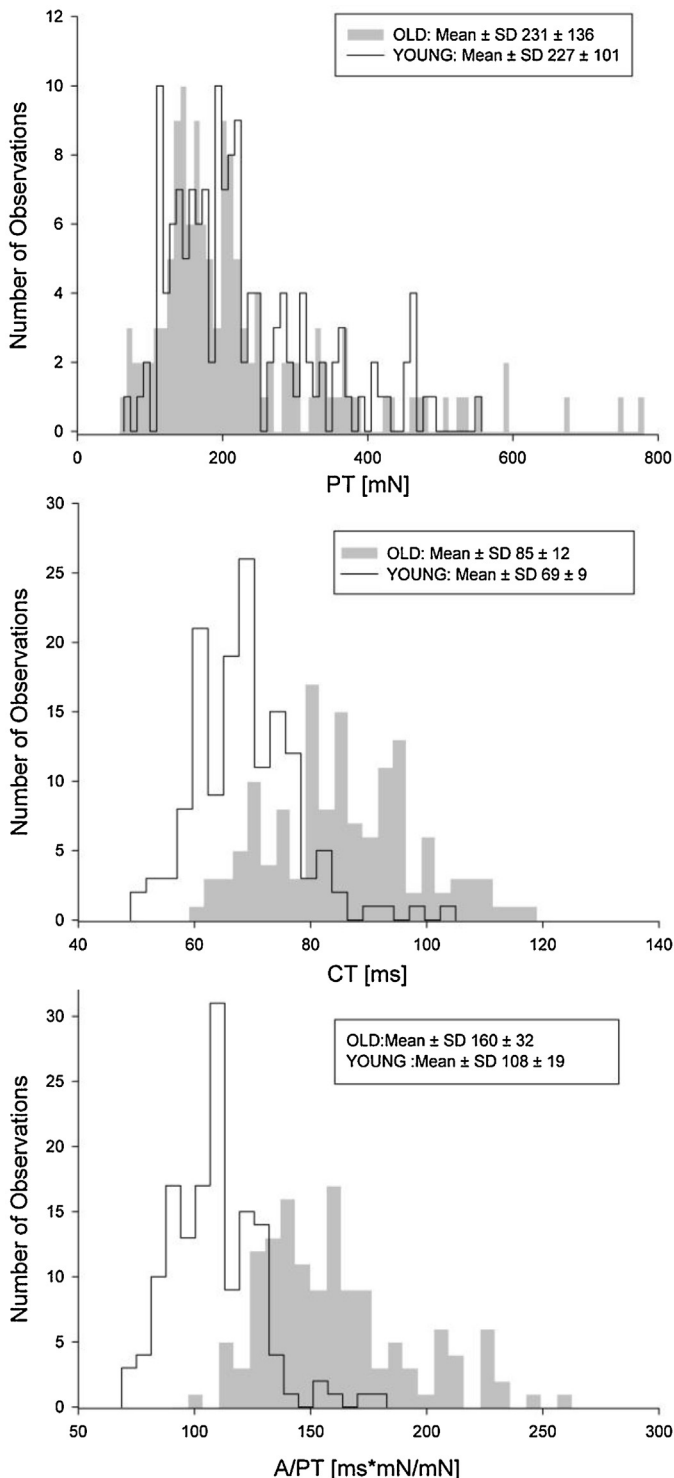
Fig. 3. Histograms of the contractile parameters of the extracted single twitches. The average values of the parameters of the two groups are statistically different for contraction time and area/peak twitch ratio.

3.3. Classification of extracted SBF twitches

The classification of the extracted mechanical contribution of a small bundle of muscle fibres to the maximal single twitch as fast or slow can be done on the basis of the parameters which describe the velocity of the tension production, such as the CT, or a shape parameter, such as the A/PT ratio calculated on the normalized SBF ST.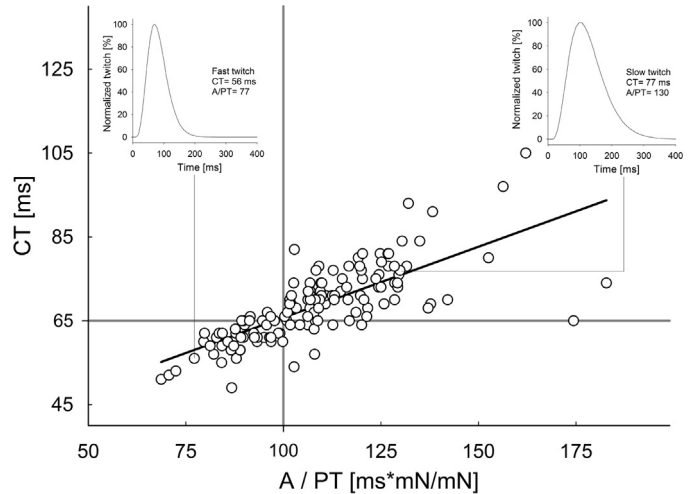 Indeed, these two parameters, among others, have been reported to be strictly related to the slow or fast MU type (Celichowski et al., 1998) and are linearly correlated according to the following equation: CT = 31.947 + 0.338 A/PT (R = 0.745, p < 0.001). In the two insets of Fig. 4, data from the same young representative subject, the relationship between slow and fast twitch tension dynamics in time and the specific values of the two parameters is clearly shown. On this basis we sought a CT and A/PT threshold values for slow and fast responses definition. This was made according to the method used by Buchthal and Schmalbruch (1970). These authors suggested that within the overall CT values array, obtained from several SBFs from the whole investigated group and ordered from the smaller to the larger, the boundary between slow and fast responses could be found considering the known relative proportion of slow and fast fibres in the muscle under study. For example if in a muscle there are 34% of fast fibres (as reported for young subjects TA in the work of Henriksson-Larsén, Fridén, & Wretling, 1985) the 34% of shorter CT values can be attributed to SBF with a prevalence of these fibres whereas the remaining 66% of the responses can be attributed to slow responses. After ordering the 143 SBF CTs from young subjects from the lowest to the highest value the border between the 34% fast and the 66% slow responses was found at 65 ms. A similar procedure was adopted for the identification of the threshold for A/PT parameter which was established as 100 mN ms/mN. The distribution of the CT–A/PT coupled values and the two thresholds are reported in Fig. 4. To decide which of these two parameters was most accurate in the identification of a response as slow or fast we proceeded as follow: below and beyond each threshold, 65 ms for CT or 100 mN ms/mN for A/PT, a set of slow or fast normalized STs were defined. The slowest and the fastest ST of each set were sample by sample differentiated and the sum of the obtained differences was calculated. The values obtained from the slow and fast sets were summated and the result from A/PT threshold was about 20% smaller than the one from CT threshold. This suggests that A/PT = 100 allowed to identify more homogenous sets of normalized SBF STs contributing to STmax. As a consequence we used this boundary to categorize the extracted twitches. Applying this threshold to each young subject responses we found that in the group 71.05 ± 24.16% were slow responses accounting on the

average for $67.33 \pm 26.95\%$ of the STmax amplitude. In old subjects all the extracted twitches were classified as slow because their A/PT was always beyond 100.

4. Discussion

This study provided a new method for the decomposition of the ST obtained at the maximal stimulation amplitude into fast and slow components.

4.1. Maximal single twitches in young and old subjects

Two out of the three parameters used to characterise the maximal single twitch (STmax) were different in the two groups. Only the amplitude of the twitch did not reach the statistical significance. The reason will be discussed later when the characteristics of the individual contributions to STmax will be considered. A/PT was the parameter mostly influenced by the ageing process showing more than 50% difference between the two investigated groups. This means that age does not influence the possibility to evoke an adequate amount of tension when stimulating at the motor point but, changing the time behaviour of the tension transient, contributes to explain the slowing of the muscle mechanical response with age (Hicks, Cupido, Martin, & Dent, 1991). These results do not help to understand the aspects of the underlying process: how the STmax was composed in young subjects and how the summation pattern changed with age? Was this process only due to an alteration in the fast/slow fibres proportion or also the changes in their relative efficiency of contraction may play a role? To provide evidence based answers to the above questions we applied the method reported in Section 2.3 identifying the mechanical contribution to STmax of the SBFs sequentially recruited during small increments of the stimulation amplitude from Vmin to Vmax.

4.2. Features of the extracted contributions to maximal ST

4.2.1. D50

The "processes of MU loss and reinnervation are reflected in CMAP scan discontinuities" (Sleutjes et al., 2014). In the same paper the authors demonstrated that such changes in CMAP scan shape are well monitored by D50 value reduction and strongly related to the decrease of the number of available MUs in patients with amyotrophic lateral sclerosis (ALS) or progressive muscular dystrophy (PMD). The ageing process also determines, in the skeletal muscle, loss of motoneurons and reinnervation of the orphan muscle fibres. The process outcome is that the number of functioning MUs decreases while their individual tension generation capacity increases (Aagaard, Suetta, Caserotti, Magnusson, & Kjaer, 2010). On this basis, in this work, D50 was calculated with the aim to disclose the effect of reinnervation on the STmax build up. The number of consecutive largest individual twitches summated to reach 50% of STmax was significantly reduced in old subjects. The similar behaviour of D50 calculated from STmax scan in the old subjects of this work and from CMAP scan in patients with SLA and PMD (Sleutjes et al., 2014) suggests that the reduction of functioning MUs is similarly affecting the build-up of the electrical and mechanical output of skeletal muscle during graded evoked activity.

4.2.2. Peak twitch amplitude

We did not find any significant difference in the two groups. This is in line with McNeil, Doherty, Stashuk, and Rice (2005) who found that in the TA the decrease in the MU number does not limit the function until beyond 80 years. This can be attributed to the so called age related MU remodelling (McNeil et al., 2005) that creates

larger and slower MUs but counteracts the cross-sectional area reduction up to a 50% loss of motoneurons took place (Aagaard et al., 2010). On the average amplitudes of our individual twitches were higher than the values reported by Van Cutsem, Feiereisen, Duchateau, and Hainaut (1997) in their study on single motor units. This may suggest that, as stated by Buchthal and Schmalbruch (1970) the neuromuscular stimulation can activate SBF containing more than one MU. McNeil et al. (2005) in young man observed about 150 MUs in tibialis anterior, whereas in the present study we were able to evoke less than 20 increases in the evoked muscle twitch. Therefore, assuming that with the applied stimulation at the motor point we were able to activate about a half of a muscle (see Section 2.5) each SBF ST reflects the mechanical contribution of 2–3 MUs.

4.2.3. Contraction time

Considering the young subjects it is evident that the STmax decomposition provided different types of mechanical contribution from the different fibres bundles recruited at the specific levels of stimulation. Our data are well in line with the CT ranges of MU twitches i.e. 30–80 ms, reported for TA in humans by Buchthal and Schmalbruch (1970). The CT is one of the mechanical parameters used to classify the motor units as fast or slow. In cats (Burke, Levine, Tsairis, & Zajac, 1973) and rats (Celichowski et al., 1998) the MUs are identified as fast or slow when CT is below or above 45 and 19 ms, respectively. This suggests that the threshold may reflect specie specific features of the contractile and viscoelastic elements interaction (Mrówczyński, Celichowski, & Krutki, 2006). In humans the application of invasive methods of single MU activation is not possible and different methods such as the spike triggered averaging during voluntary contraction can be used to extract the single MU mechanical response. In TA McComas and Thomas (1968),Sica and McComas (1971) and Van Cutsem et al. (1997) reported CT spectra for pulled slow and fast MU ranging from 20 to 86 ms. These data concerning human CT values stress that the larger is the muscle the longer is the CT of their MUs. Moreover when the CTs of SBFs show values compatible with those of fast or slow human TA MUs we may hypothesize that in any case, in a given extracted SBF ST, a functional prevalence of fast or slow fibres can be present.

4.2.4. Twitch shape parameter (A/PT)

A tool to track muscle contractile changes with age. In the young group the use of the A/PT threshold value (100) classified the 71% of the SBF mechanical contributions as slow. On the average these slow twitches contributed for 67% of the cumulative twitch. The smaller functional significance of the 71% slow SBFs mechanical output may be due to the fact that fast contributions are related with the activation of fast fibres having larger area and, as a consequence, more tension generation capacity (Henriksson-Larsén et al., 1985). On the other hand the functional weight of 33% of the fast components well fits with the muscle area, 34%, occupied by the fast twitch in the young subjects TA (Henriksson-Larsén et al., 1985). According to Brunner et al. (2007) the quantitative changes in fast and slow fibres with age is not enough to explain the functional changes of the skeletal muscle. In their review Brunner et al. (2007) underline that in aged muscles the fibres classified as type II present small and slow contribution to the force generation process. As a consequence our finding that 100% of the STmax components are slow in our aged subjects can be regarded as a functional information independent from histochemical classification of the fibres included in the recruited SBFs. This biomechanical change in TA action can be considered as the mechanical counterpart of the electrical one described by the Yamada et al. (2000). In this last EMG study the not significant changes in the time and frequency domain parameters extracted

from the signal were attributed to a substantial lack of fast twitch fibres functional role in aged subjects. This phenomenon was interpreted as the crucial aspect of the "neuromuscular deterioration" that "potentially cause inadequacy in the clearance and placement of the foot during walking, which in turn may lead to stumbling and falling in the elderly" (Yamada et al., 2000).

4.3. Conclusions

The data of our study validate this non-invasive method for the identification of the SBFs contributions to STmax as slow or fast. In this view the method can be considered as a non-invasive functional biopsy of the muscle. When applied to aged subjects it was able to disclose the outcome of the ageing process on the proportion of slow/fast mechanical contributions to the muscle mechanical output.

Future studies will test the reliability of this methods as a tool to follow changes in the MU number and properties due to different training regimens, disuse or neuromuscular diseases specifically affecting the spinal motoneurons or the muscles fibres efficiency.

Disclosure

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