# Evaluation of the ability to make non-invasive estimation of muscle contractile properties on the basis of the muscle belly response

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**Abstract**—*The histochemical and biomechanical relationships of limb muscles are examined in two groups of 15 men aged between 17 and 40 years. Seven muscles are chosen: biceps brachii, triceps brachii (TB), flexor digitorum superficialis, extensor digitorum, biceps femoris, tibialis anterior and gastrocnemius caput mediale (GCM). The aim of the preliminary study is to evaluate an alternative method based on a tensiomyographic (TMG) non-invasive measurement technique. The percentage of type I muscle fibres obtained with the histochemical method is 2.2 times higher for the slowest measured muscle (GCM) than for the fastest (TB). The contraction time of a muscle belly twitch response measured by TMG is 1.9 times higher for GCM than for TB. Statistical analysis of the data obtained by tensiomyographic and histochemical techniques shows a significant correlation between the contraction time of muscle response measured by TMG and the percentage of type I muscle fibres (correlation coefficient equals 0.93). Results of the study suggest using the TMG measuring technique as a basis for the estimation of the percentage of type I muscle fibres.*

**Keywords**—*Human skeletal muscle, Muscle fibre types, Histochemistry, Tensiomyography, Biomechanics*

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## 1 Introduction

MEASUREMENTS of muscle belly responses to electrical stimuli have revealed differences among muscles. From our preliminary studies (VALENČIČ and KNEZ, 1997), it was concluded that measuring the muscle belly response with a displacement sensor provides valuable information about muscle contraction characteristics.

In VALENČIČ and KNEZ (1997), it has been found that the analysed normalised velocity parameter was four times greater for the vastus lateralis and brachioradialis muscles than for the soleus muscle. The vastus lateralis and brachioradialis muscles are histochemically known as fast muscles. On the other hand, the soleus muscle is a slow muscle (POLGAR *et al.*, 1973; EDGERTON *et al.*, 1974; LEXELL *et al.*, 1984). The values of the analysed parameters of the gastrocnemius and the tibialis anterior muscles are between the values of the parameters for fast and slow muscles. The differences between muscle responses are due to their structure. This paper shows that the proposed method (TMG) gives valuable information about muscle structure.

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## 2 Material, methods and procedures

Two groups of subjects were measured using one of the two measuring methods. For the purpose of statistical analysis, both groups were homogenous concerning age, sex and health condition (there was no evidence of previous neuromuscular disease).

### 2.1 *Muscle necropsies*

Samples of muscles were obtained at autopsy from 15 male subjects, aged between 17 and 40 years. All 15 subjects suffered from sudden death. Post-mortem examination revealed no significant pathological changes other than those related to the immediate cause of death. There was no evidence of previous neuromuscular disease in any of these cases.

Muscle samples from biceps brachii (BB), triceps brachii (TB), flexor digitorum superficialis (FDS), extensor digitorum (ED), biceps femoris (BF), tibialis anterior (TA) and gastrocnemius caput mediale (GCM) were removed from the right extremities, 5–24 h after death. Blocks measuring approximately 1 $cm^3$ were frozen in liquid nitrogen and cooled to $-196\,^{\circ}C$. Cryostat sections were cut at a thickness of 10 μm, and myofibrillar adenosinetriphosphatase activity was demonstrated using the calcium histochemical method at pH 9.4 (PADYKULA and HERMAN, 1955) and, after pre-incubation, at pH 4.6 and at pH 4.3 (GUTH and SAMAHA, 1970).

In each muscle sample, one area was selected at random and was photographed by an Opton photomicroscope with a constant magnification of 116×, so as to include at least two fascicles and

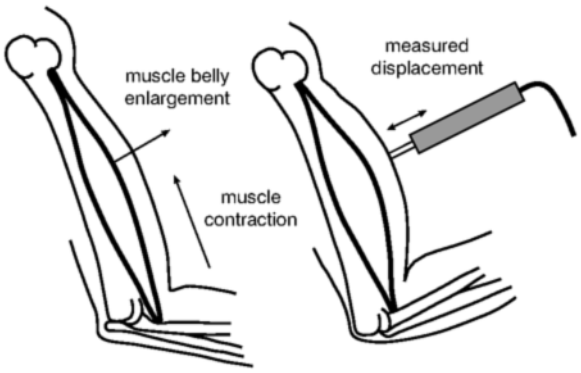


**Fig. 1** *Principle of TMG measuring method: when muscle contracts, its belly enlarges. Radial enlargements can be measured by displacement sensor*

a total of at least 100 fibres. The contours of the fibres within the selected fascicles were digitised with a Cherry graphic tablet coupled to an IBM PC/AT–compatible computer. The percentage of muscle fibre types was determined using the computer-aided method of PERNUŠ *et al.* (1986).

### 2.2 *TMG recordings*

Tensiomyography is a measuring method for detection of skeletal muscles' contractile properties. It is based on a muscle-contraction characteristic: when muscle contracts, its belly enlarges. With a displacement sensor, the radial enlargement of the muscle belly can be measured (Fig. 1).

During measurement, the displacement-measuring sensor is pressed to the skin above the measured muscle belly radial to the muscle surface. For measurements, an inductive sensor incorporating a spring of 0.17 N mm$^{-1}$ was used. It provides an initial pressure of approximately $1.5 \times 10^{-2}$ N mm$^{-2}$ on a tip area of 113 mm$^2$. The responses of seven different muscles of the right side of the body were compared in 15 healthy male subjects, aged between 17 and 40 years.

The measured subject was sitting on a measuring chair or lying on the front on a measuring bed, depending on the measured muscle. The measured leg or arm was fastened to the frame with one or two bands to achieve the isometric condition during the measurement. The measuring point for each muscle was anatomically determined on the basis of the anatomic guide for electromyographers (DELAGI *et al.*, 1975) described in Table 1.

*Table 1 Anatomical position of measuring point for seven measured muscles based on DELAGI et al. (1975)*

| | |
|---|---|
| Biceps brachii (BB) | midpoint of line between lateral head of clavicula and head of radius |
| Triceps brachii (TB) | posterior face of mid-arm on TB caput laterale just above tendon of TB |
| Flexor digitorum superficialis (FDS) | junction of upper and middle third of forearm (10 cm caudal from medial humeral epicondyle) |
| Extensor digitorum (ED) | junction of upper and middle third of forearm (10 cm caudal from lateral humeral epicondyle) |
| Biceps femoris (BF) | midpoint of line between fibula head and ischial tuberosity |
| Tibialis anterior (TA) | four fingerbreadths below tibial tuberosity and one fingerbreath lateral to tibial crest |
| Gastrocnemius caput mediale (GCM) | one hand breadth below popliteal crease on medial mass of calf |

Muscle was stimulated with single twitch stimuli using two self-adhesive electrodes placed symmetrically to the sensor. The anode was placed distally, and the cathode was placed proximally, 20–50 mm from the measuring point. The bipolar electrical stimulation used consisted of a single DC pulse of 1 ms duration and 10–40 V above the threshold amplitude. The stimulator used was a Grass 8800 stimulator, with voltage output through an insulation unit. The measured muscle responses were stored and analysed in a PC.

TMG signals were analysed to determine the parameters: delay time, contraction time, sustain time and relaxation time. The most significant parameter for this study is the contraction time of the muscle response. The contraction time $\Delta t_c$ is the time between 10 % and 90 % of the maximum value of the muscle response (VALENČIČ and KNEZ, 1997).

### 2.3 *Statistics*

The significance of the correlation between the percentage of type I muscle fibres, determined histochemically, and the contraction time was established by the Pearson's correlation coefficient.

The null hypothesis was: percentage of muscle fibre type I (data obtained by histochemical analysis) is correlated with the contraction time (data obtained by non-invasive biomechanical measurement technique TMG).

## 3 Results and discussion

The TMG measuring method is non-invasive, and, from the response of the whole muscle, its functional properties can be determined, in contrast to invasive measuring methods, for the evaluation of muscle properties presented by many authors (BUCHTAL and SCHMALBRUCH, 1970; SICA and MCCOMAS, 1971; STEIN *et al.*, 1972; PARKER *et al.*, 1984).

Many such methods involve the response of only one motor unit that is not truly representative of the whole muscle. Another group of methods consists of non-invasive methods, yielding muscle force as their final result; however, these methods have several disadvantages: they usually involve the measurement of torque in the joint, which is influenced by joint characteristics and the contribution of other muscles producing torque in the same joint. If the measuring method for muscle force measurements is able to eliminate the influences of other muscles or of the joint itself, it is usually rather complex and can be used for measurement of only a limited number of muscles or muscles moving one joint (GYDIKOV *et al.*, 1976).

The TMG method does not measure the muscle force directly, but measures muscle belly displacement instead. However, displacement of the muscle belly during contraction is correlated to muscle force. The method enables measurements of single muscle responses, and the same set of equipment is suitable for measurement of all surface skeletal muscles' responses.

Most muscle belly responses measured by radial displacement sensors are somewhat saturated, especially when compared with measured force. In our opinion, this specific shape is characteristic of muscle belly response for several possible reasons: i.e. the influence of muscle fascia (probably of second order) and plastic deformation of muscle shape due to sensor pressure.

Refering to KNAFLITZ *et al.* (1990), the recruitment order for surface electrical stimulation is not determined as it is in voluntary contraction, where slow muscle fibres are recruited first, or in nerve stimulation, where fast muscle fibres are recruited first. Therefore we cannot determine the percentage of recruited fast or slow muscle fibres exactly (in the present study, supra-maximum stimulation was never used).

Wrist extensors and flexors are, respectively, located close to the extensor digitorum and flexor digitorum superficialis, but, at the measuring point (determined by DELAGI *et al.*, 1975), the muscle belly of the measured muscle is distinctly apart from other muscles and their bellies.

The TMG method provides selective measurements of the muscle belly response to electrical stimulation. With surface electrical stimulation, neighbouring muscles are also activated, and yet the response of different muscles can be easily distinguished with a small displacement sensor. With a high-amplitude stimulation, underlying muscles can also be activated. We usually use low-amplitude bipolar stimulation, which mostly activates muscle fibres at the muscle surface.

In the literature (BUCHTAL and SCHMALBRUCH, 1970; SICA and MCCOMAS, 1971; STEIN *et al.*, 1972), the histochemical methods used are based on demonstration of metabolic-enzyme activity, which correlates with the fatigability of the muscle. Our histochemical method, based on the demonstration of the adenosinetriphosphatase activity, is correlated with the velocity of the muscle contraction.

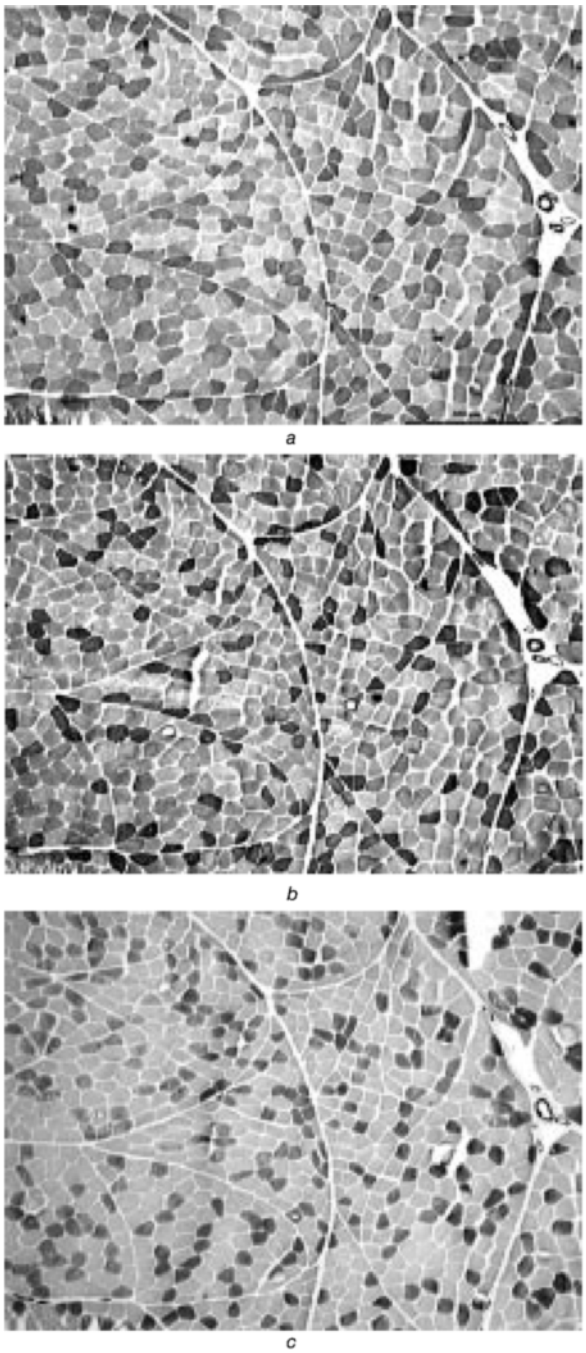


**Fig. 2** *Histochemical appearance of different fibre types in ED (fast muscle of upper limb). (a) Demonstration of myofibrillar adenosinetriphosphatase activity with calcium method at pH 9.4. (b) Demonstration of myofibrillar adenosinetriphosphatase activity after pre-incubation at pH 4.6. (c) Demonstration of myofibrillar adenosinetriphosphatase activity after pre-incubation at pH 4.3*

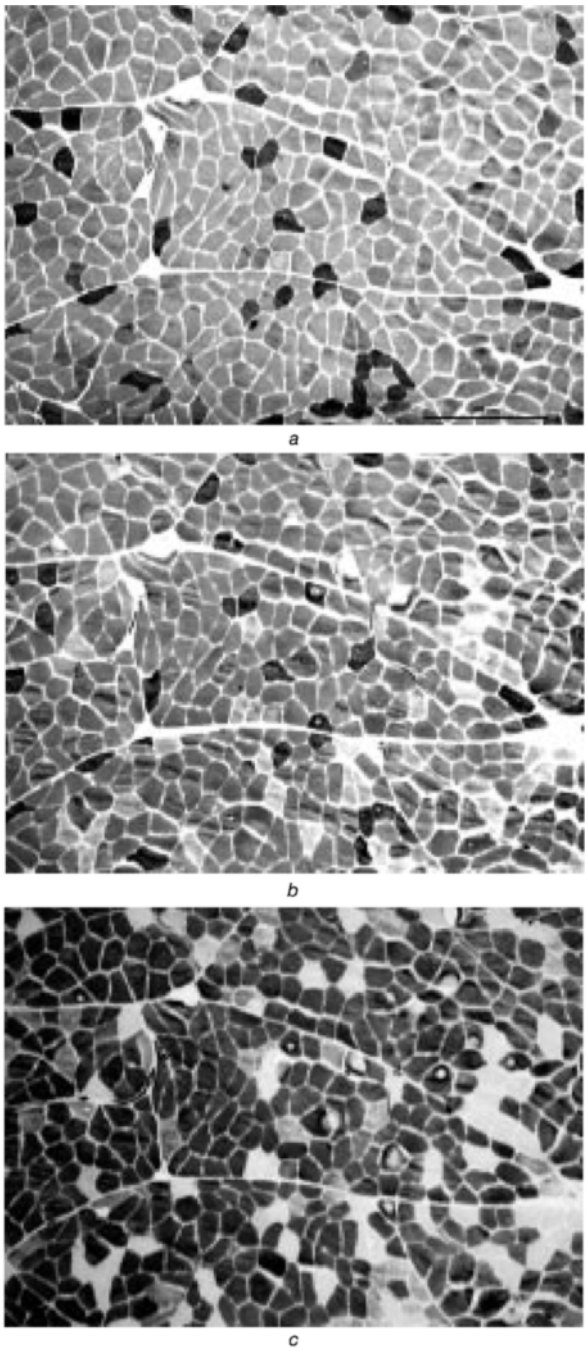


**Fig. 3** *Histochemical appearance of different fibre types in GCM (slow muscle of lower limb). (a) Demonstration of myofibrillar adenosinetriphosphatase activity with calcium method at pH 9.4. (b) Demonstration of myofibrillar adenosinetriphosphatase activity after pre-incubation at pH 4.6. (c) Demonstration of myofibrillar adenosinetriphosphatase activity after pre-incubation at pH 4.3*

*Table 2 Contraction time of muscle belly twitch response and percentage of type I muscle fibres for seven measured muscles in two groups of subjects (average values and standard deviations)*

| Muscle | Contraction time, ms | Percentage of type I muscle fibres |
|---|---|---|
| Biceps brachii | 34 ± 4 | 52 ± 8 |
| Triceps brachii | 30 ± 6 | 35 ± 8 |
| Flexor digitorum superficialis | 30 ± 10 | 43 ± 6 |
| Extensor digitorum | 29 ± 5 | 51 ± 6 |
| Tibialis anterior | 50 ± 9 | 77 ± 8 |
| Gastrocnemius caput mediale | 55 ± 11 | 78 ± 7 |
| Biceps femoris | 36 ± 9 | 43 ± 10 |

Sections of the ED and GCM muscles stained to demonstrate the activity of myofibrillar adenosinetriphosphatase with the calcium method, at pH 9.4 and, after pre-incubation, at pH 4.6 and at pH 4.3, showed the well-known mosaic distribution (Figs. 2 and 3).

Table 2 shows the numerical results of both histochemical and TMG analysis for seven measured muscles. The percentage of type I muscle fibres obtained with the histochemical methods was 2.24 times higher for the slowest measured muscle (GCM) than for the fastest (TB). The contraction time of the muscle belly twitch response measured by TMG was 1.86 times higher for GCM than for TB.

The Pearson's correlation coefficient between the percentage of type I muscle fibres measured by the histochemical technique and the biomechanical parameter contraction time obtained by TMG was 0.93. The null hypothesis is accepted with a significance level of two-sides alpha error = 0.05.

The percentage of type I muscle fibres and the contraction time of the muscle belly response to twitch stimulation are highly correlated (Figs. 4 and 5).

Information on skeletal muscle structure is very important when observing muscular diseases or other changes in muscles. In athletes, it is important for improving the training process. The most reliable methods for muscle structure determination are invasive.

In this study, results of a non-invasive measuring method, tensiomyography, were compared with results of invasive histochemical analyses of skeletal muscles. Statistical analyses of the parameter of muscle belly response to a twitch stimulus contraction time and percentage of type I muscle fibres show a strong correlation between the two parameters. The study uses a non-invasive measuring method, TMG, to obtain reliable estimated information on muscle structure. The method is selective, the same equipment can be used for measuring all surface skeletal muscles, and results are available immediately after the measurement. The authors propose the TMG measuring method as a non-invasive alternative to an invasive histochemical analysis.

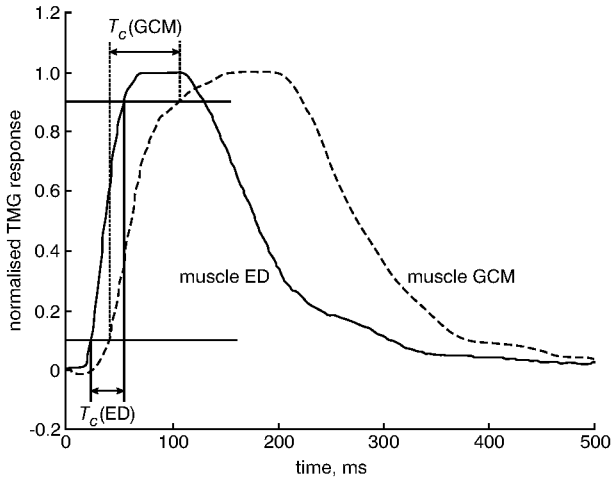


**Fig. 4** *Contraction time of muscle belly response to twitch stimulation for ED (fast muscle) and GCM (slow muscle) measured by TMG. Contraction time is greater in slow muscles than in fast muscles*

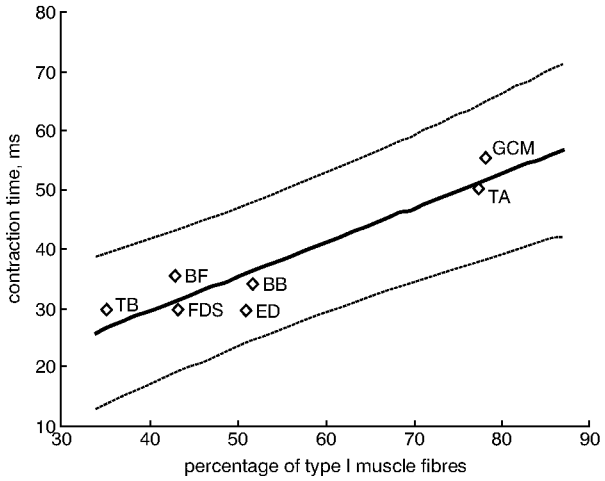


**Fig. 5** *Mean value and both confidential borders of percentage of type I muscle fibres and contraction time of muscle belly response to twitch stimulation in muscles biceps brachii (BB), triceps brachii (TB), flexor digitorum superficialis (FDS), extensor digitorum (ED), biceps femoris (BF), tibialis anterior (TA) and gastrocnemius caput mediale (GCM)*

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