# Histochemical and mechanomyographical evaluation of the adaptive potential of human biceps femoris muscle

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*Abstract* - **The goal of this study was to estimate the ability of biceps femoris (BF) muscle, a hamstring muscle crucial for biarticulate movement, to adapt to changed functional demands. For this purpose and due to ethical reasons, in a group of healthy sedentary men and of 15 sprinters, a non-invasive mechanomyography (MMG) method was used to measure the muscle twitch contraction times (Tc). These correlate with the proportions of slow and fast fibers in the muscle. To further elucidate the data obtained by MMG method and to obtain reference data for the muscle, the fiber type proportions in autoptic samples of BF in sedentary young men were determined according to histochemical reaction for myofibrillar adenosine triphosphatase (mATPase). With MMG we indirectly demonstrated that biceps femoris muscle has a strong potential to transform into a faster contracting muscle after sprint training, since the average Tc in sprinters was much lower (19.5 ± 2.3 ms) than in the sedentary group (30.25 ± 3.5 ms). The results of the histochemical analysis of BF muscle also imply a high adapting potential of this muscle. Beside type 1, 2a and 2× (2b) fibers a relatively high proportion of intermediate type 2c fibers (5.7% ± 0.7), which co-expressed MyHC-1 and -2a, was found. Therefore, type 2c might represent a potential pool of fibers, capable of transformation either to slow type 1 or to fast type 2a in order to tune the functional response of BF muscle according to the actual functional demands of the organism.**

## I. Introduction

The functional characteristics of skeletal muscles depend on the muscle fiber properties. The fiber type proportions differ among different muscles and depend not only on their functional role, but on other factors, such as innervations and the pattern of mechanical stimuli as well (1). In response to different stimuli, the muscle fibers are able to adapt their properties with a change in the MyHC isoform expression, the process known as fiber type transition. Such a dynamic nature of muscles has been known as muscle plasticity (2).

There are very few reports on the histochemical structure and on the effect of short-term high-intensity training on the contractile properties of the biceps femoris muscle (BF). As one of the posterior femoral or hamstrings muscles (semitendinosus, semimembranosus and biceps femoris), BF is frequently injured during sports activities, sports injuries most commonly appear in the running section of athletics, especially in the first 10-20 m of a sprint (3). BF crosses the posterior aspect of both the hip and the knee joints. The flexion of the knee and the stabilizing effect are very important function of this muscle. The extension of the hip joint, when the thigh is the moving part, is another important function, particularly when the trunk is bent forwards and is to be raised to the erect position.

Since it has been demonstrated that a strong correlation exists between the whole-muscle twitch contraction time, measured by tensiomyography (TMG) and the histochemically determined percentage of slow twitch or type 1 muscle fibers, i.e. the higher type 1 proportion the higher the contraction time, the TMG measurement method was proposed as a non-invasive alternative to an invasive histochemical analysis (4,5). TMG can be classified as mechanomyography (MMG) which is investigating the mechanisms of muscle contractile properties with a particular reference to the mechanical nature of the measurements. This method is based on the assumption that radial belly displacement detected by an optomagnetic sensor is proportional to the muscle force. The procedure has been evaluated in healthy young subjects (4, 5) and by subjects after above knee amputation (6). With the proposed method the response of a single muscle within a given muscle group can be measured. The aim of this study was to estimate the extent of fiber type transitions in BF muscle after at least five years of specific sprint training. Since the histochemical classification of fiber types could not be performed on byoptic samples due to ethical reasons, the non-invasive mechanomyography method was used to determine the contractile properties (contraction time) of BF muscle, as a reflection of the ratio between the slow and fast fibers in group of sedentary and of sprint trained young men. To further elucidate the data obtained by MMG method and to obtain a basic reference data for the muscle, the fiber type proportions in autoptic samples of BF of sedentary young men were determined histochemically.

## II. Material And Methods

*Subjects*

A non-invasive MMG method was used for the measurement of skeletal muscle contraction, expressed as contraction time (Tc), in a group of 15 healthy sedentary male subjects (17-40 years old), who volunteered for the investigation and in a group of 15 male sprinters (23.2 ± 3.1 years old, height 1.79 ± 0.05 m and body mass 77.6 ± 7.7 kg), who were recruited for this study.



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*Muscle samples*

Biceps femoris muscle samples were taken at autopsy from a group of 15 male subjects, aged between 17 - 40 years, who had died suddenly (suicide, traffic accident). The autopsies were performed within 5 to 24 hours after death. The local ethical committee approved the muscle sampling. The background of the autopsied subjects is known from the legal medicine documentation. The post-mortem examination revealed no significant pathological changes other than those related to the immediate cause of death.

Muscle blocks (approximately 1 cm3) were frozen rapidly in liquid nitrogen cooled to –196 °C. Serial sections (10 μm) were cut in a cryostat at –20°C and processed for the histochemical demonstration of mATPase activity.

To compare the data obtained from BF muscle, the fiber type proportions assessed by the histochemical reaction for mATPase and the data obtained by mechanomyography in other lower and upper limb muscles (biceps brachii, triceps brachii, brachioradialis, flexor digitorum superficialis, extensor digitorum, biceps femoris, tibialis anterior, gastrocnemius – caput mediale and soleus), analyzed in our parallel study, are presented in this paper as well.

*MATPase histochemistry*

Type 1, 2a, 2x (2b) and 2c fibers were determined according to the activity of mATPase reaction using the calcium histochemical method with alkaline preincubation at pH 9.4 and acid preincubation at pH 4.6 and 4.3. The fast fibers display a high mATPase activity under alkaline conditions and low activity under acid conditions, whereas slow fibers exhibit the inverse. Type 2a fibers stain deeply after alkaline preincubation (pH 9.4) and lightly after preincubations at pH 4.6 and pH 4.3, type 2x (2b) stain deeply after alkaline preincubation, moderately at pH 4.6 and lightly after preincubation at pH 4.3. Type 2c fibers are stable to various degrees throughout the pH range from 4.3 to 9.4 (7).

In each muscle sample an area was selected at random and was photographed by Opton photomicroscope with a constant magnification of 116×, so as to include at least 2 fascicles and a total of at least 100 fibers. The contours of the fibers within the selected fascicles were digitized with the aid of a Cherry graphic tablet coupled to an IBM PC/AT - compatible computer. The percentage of muscle fiber types was determined using the computer - aided method of reference (8).

*Mechanomyography (MMG)*

The contraction times (Tc), defined as the time between 10% and 90% of the maximum value of the muscle response, of the right BF muscles were measured. The measured subject was laying on his front on the measuring bed. The sensor location was determined anatomically according to reference (9). Maximal amplitude/response was used as an additional criterion for an optimal sensor position. The BF muscle was measured at the midpoint of the line between the fibula head and the ischial tuberosity. The muscle was stimulated with a single twitch stimulus using two self-adhesive electrodes placed symmetrically to the sensor. The anode was placed distally and the cathode proximally, 20-50 mm from the measuring point. The bipolar electrical stimulation used, consisted of a single DC pulse of 1 ms duration and supramaximal intensity (the current was increased gradually from the threshold to the maximal displacement amplitude/response of the muscle).

For the measurements an inductive sensor incorporating a spring of 0.17 N/mm was used. It provides an initial pressure of approximately 1.5 × 10-2 N/mm2 on a tip area of 113 mm2. The measured leg was fastened to the frame with one or two bands to achieve the isometric condition during the measurement.

The measured muscle responses were stored and analyzed with a software package (TMG-BMC) running on PC. The MMG signals were analyzed to determine the contraction time of the muscle response.

*Statistical analysis*

All three experimental groups of subjects were homogenous concerning the age, sex, and health condition. The data obtained with the histochemical methods were statistically analyzed with the SYSTAT (1991) packet. The results were expressed as mean values and were given plus or minus one standard error. They were compared by analysis of variance and Bonferroni test. The data obtained with the MMG method were statistically analyzed with the MATLAB STATISTICAL TOOLBOX. The results were expressed as mean values and were given plus or minus one standard deviation. The relationship between the maximum running velocity data and the contraction time of muscle belly was tested using the Pearson's correlation coefficient. $P<0.05$ was taken as the limit of significance in all statistical tests.

For the test retest reliability, we used the protocol suggested by reference (10)