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Fibre type related changes in the metabolic profile and fibre diameter of human vastus medialis muscle after anterior cruciate ligament rupture

Christoph Stockmar[a], Helmut Lill[b], Almut Trapp[a], Christoph Josten[a], Karla Punkt[c,*]

[a]*Department of Trauma, Reconstructive and Plastic Surgery, University of Leipzig, Liebigstraße 20, D-04103 Leipzig, Germany*
[b]*Department of Trauma and Reconstructive Surgery, Friederikenstift Hannover, Humboldtstraße 5, D-30169 Hannover, Germany*
[c]*Institute of Anatomy, University of Leipzig, Liebigstraße 13, D-04103 Leipzig, Germany*

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Atrophy

Summary

Vastus medialis muscles of patients with chronic anterior instability of the knee after anterior cruciate ligament rupture were analysed to investigate changes in defined muscle fibres of the diseased leg in comparison to the healthy leg of the same patient. Metabolic and morphological parameters were obtained by cytophotometrical measurements of the activities of succinate dehydrogenase (a marker of oxidative metabolism) and glycerol-3-phosphate dehydrogenase (a marker of glycolytic metabolism) of slow-oxidative (SO), fast-oxidative glycolytic (FOG) and fast-glycolytic (FG) fibre types in serial sections and by measuring the minimal fibre diameters of type I (slow) and type II (fast) fibres. We found decreased glycolytic activity and a shift to more oxidative metabolism in each fibre type suggesting diminished fast force and shift to endurance force development. The latter was interpreted as a sign of active compensation for the knee instability. Significantly decreased minimal fibre diameters to 85.9% in type I fibres, and to 88.7% in type II fibres of the diseased muscle were measured, indicating the fibre atrophy. Our findings suggest that the atrophied muscle fibres of the affected vastus medialis muscle adapt to the altered conditions by changing their metabolic profile. Muscle fibres of different types were found to be affected similarly.

*Corresponding author. Tel.: +49 341 97 22007; fax: +49 341 97 22009.
E-mail address: punktk@medizin.uni-leipzig.de (K. Punkt).

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Introduction

Muscular weakness of the quadriceps muscle is a typical and well known post-traumatic change after anterior cruciate ligament (ACL) rupture (Okada, 1989; Nyland et al., 2001, 2003; Rose et al., 2004). The vastus medialis muscle is the accepted key muscle for strength deficits of the lower extremity (Nyland et al., 2003; Freiwald et al., 1993; Okada, 1989). Changes in the metabolic activity of vastus medialis muscle in connection with fibre analysis should be meaningful for interpretation of the clinical symptoms after ACL rupture. However, such data have not been published and it is not known whether there are changes in metabolic and morphologic muscle fibre properties of muscles from the affected leg, or if such changes are fibre type specific. The metabolic properties of a muscle fibre can be determined by cytophotometrically measured activities of succinate dehydrogenase (SDH, a marker of oxidative activity) and glycerol-3-phosphate dehydrogenase (GPDH, a marker of glycolytic activity) in serial cross sections of the same fibre (Punkt, 2002). Activities of these enzymes characterize the metabolic profile of muscle fibres; metabolic shifts can be quantified by changes in the activity quotient GPDH/SDH (Punkt, 2002; Punkt et al., 2002a, b). On the basis of physiological-metabolic fibre typing it is possible to investigate changes in muscles related to a certain fibre type under different conditions, e.g. altered muscle activity in comparison to the normal situation. Previously, we found significant changes in enzyme activities related to defined fibre types of rat, hamster and human muscles under different conditions, including ageing, hypoxia, myopathy and diabetes (Oberbach et al., 2006; Punkt et al., 1993, 1996, 1998, 2002b). These studies revealed that muscle fibres of different physiological-metabolic types were differently affected by muscle diseases.

The purpose of the present study was to investigate fibre type related changes in human vastus medialis muscle after ACL rupture. Vastus medialis muscle biopsies otained during the reconstruction operation of ACL rupture from the affected leg were analysed in comparison to the healthy leg from the same patient. The muscle fibres were typed using immunohistochemistry, enzyme histochemistry and cytophotometry. Metabolic and morphological parameters of defined fibre types were obtained by cytophotometrical measurements of the activities of GPDH and SDH in serial sections and calculating the GPDH/SDH activity quotients and by measuring the minimal fibre diameters. The fibre related results were compared with enzyme activities of the muscle homogenate.

Material and methods

Patients

Six male patients aged from 18 to 38 years with chronic anterior instability of the knee who had diagnosis of ACL confirmed by arthroscopy were studied. Chronic anterior instability of the knee was defined as being of more than 6 weeks duration. All patients underwent surgery between 2 and 12 months after ACL rupture. The investigation was approved by the ethics commission of the University of Leipzig (Reg. no.: 095/2002) and complies with European guidelines. Every patient signed an informed consent form before participating in the study.

Muscle biopsies

Samples of vastus medialis muscles were taken during arthroscopy assisted ACL reconstruction with semitendinosus/gracilis tendon autograft. Biopsies of the injured and healthy sides were harvested at a defined location: 10 cm above joint gap, at the lateral edge of the vastus medialis muscle.

Processing of muscle samples

The samples were coated in talcum powder and frozen immediately in liquid nitrogen. Samples of normal and injured muscles were mounted together on a cryostat chuck, and 10 μm-thick cross sections were cut using a cryostat 1800 (Reichert Jung, Vienna, Austria) and then used for immunohistochemical, enzyme histochemical and cytophotometrical analyses. By mounting the samples in this way, variations caused by differences in section thickness and incubation conditions were avoided, at least for those sections on the same glass slide. The muscle pieces that remained after cryosectioning were removed from the cryostat chuck and used for biochemical measurements.

Biochemistry

After cryosectioning, the remaining muscle samples were very small and, therefore, samples from each group were pooled to obtain enough protein for biochemical measurements. The frozen muscle samples were homogenized in 50 mM Tris-HCl buffer, pH 7.4 containing 1 mM EDTA, 1 mM