Molecular adaptations of voltage-gated sodium channel (NaCh), α-syntrophin and dystrophin after fatiguing stretch-shortening cycle (SSC) exercise

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Introduction
A reduced sarcolemmal density of NaChs has been found in dystrophin-deficient mouse (mdx) muscle (Ribaux et al. 2001). NaCh α-subunit is known to interact with dystrophin associated protein, α-syntrophin (Gee et al. 1998), which density is also highly decreased in mdx muscle. In addition, Komulainen et al. (1998) have found a loss of dystrophin labeling after eccentric muscle action in rat. Thus dystrophin may be sensitive for high loads during SSC exercise, and consequently the function of the NaChs in the sarcolemma might be affected. Ribaux et al. (2001) argued that NaCh deficit could lead to impairment of action potential spreading in the sarcolemma during sustained muscle activity conditions. This could further explain the proposed excitation-contraction coupling (ECC) disturbance during progression of muscle damage, which is related to disturbance of Ca²⁺ kinetics in the sarcoplasmic reticulum (SR), and thus impairs the muscles ability to produce force (Warren et al. 1993). In mice, it is also noted that during muscle damage the sarcolemma itself may be damaged (Friden & Lieber 1998). In humans, however, Yu et al. (2002) did not recognize sarcolemmal disturbance and muscle fiber degeneration after eccentric muscle actions. The aim of the present study was to asses whether SSC exercise modulates the expression and/or immunohistochemical staining (IHC) of the dystrophin, α-syntrophin and NaChs in humans.

Methods
8 healthy male subjects were measured before, immediately after (IA) and 2 days (2D) after the SSC exercise, which consisted of unilateral jumps on the sledge apparatus: 100 maximal drop jumps followed by submaximal jumping (mean number of jumps was 876) until complete exhaustion. In the measurements, the subjects performed 2-3 times unilateral (the right one was the experimental leg and the left one was the control leg) maximal knee extensions (MVC). The muscle biopsies were taken IA (<30 min) and 2D after the fatiguing exercises from the VL muscle for molecular adaptations of voltage-gated sodium channel (NaCh), α-syntrophin, dystrophin and α-syntrophin. For real-time PCR, the extracted total RNA was transcribed to cDNA. The ABI Prism 7700 Detection System was applied to perform TaqMan probe-based (Assays-on Demand) RT-PCR reactions (Applied Biosystems, Foster City, USA). IHC stainings were visualized by light- and laser scanning microscopy. Blood samples were drawn for analyzing creatine kinase (CK) activity.

Results
Isometric MVC decreased (±SD) 31±9 % IA (p<0.001) and 14±16 % 2D (p<0.01) as compared to the initial value (Fig.1). CK activity increased from the initial level of 350±244 IU/l to 824±843 IU/l (p<0.05) on 2D. No changes were observed in the IHC stainings and routine HE-staining (Fig.2). Due to high inter-individual difference, there was no change (p>0.05) in the mRNA levels in any time-point of the studied proteins. However, the proportional changes of NaCh and α-syntrophin mRNA levels were positively correlated (r=0.93, p<0.001) on 2D.

Discussion/Conclusion
Despite the fact that the muscles were very sore, no morphological damage was observed in any subject. This is well in line with the previous studies in humans (Yu, et al 2002). Although there were high inter-individual variation in the mRNA levels, a consistent pattern of NaCh and α-syntrophin mRNA regulation (either up- or downregulation) was observed on 2D. This could imply a functional relationship between these two proteins, as previously suggested (Gee et al. 1998). It also seems that the regulation of the presently studied proteins occur at later stage as compared to the observations of Chen et al. (2003), who found an early induction of various stress response and growth promotion genes after eccentric exercise. Future studies are needed to confirm the wide range time dependent patterns (before and beyond 2D) of NaChs, α-syntrophin and dystrophin adaptations after fatiguing exercise in humans.

References