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EXERCISE MYOPATHY: CHANGES IN MYOFIBRILS OF FAST-TWITCH MUSCLE FIBRES

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ABSTRACT: The purpose of the present study was to determine the relationships between the changes of myofibrils in fast-twitch oxidative-glycolytic (type IIA) fibres and fast-twitch glycolytic (type IIB) muscle fibres, protein synthesis and degradation rate in exercise-induced myopathic skeletal muscle. Exhaustive exercise was used to induce myopathy in Wistar rats. Intensity of glycogenolysis in muscle fibres during exercise, protein synthesis rate, degradation rate and structural changes of myofibrils were measured using morphological and biochemical methods. Myofibril cross sectional area (CSA) in type IIA fibres decreased 33% and type IIB fibres 44%. Protein degradation rate increased in both type IIA and IIB fibres, 63% and 69% respectively in comparison with the control group. According to the intensity of glycogenolysis, fast oxidative-glycolytic fibres are recruited more frequently during overtraining. Myofibrils in both types of fast-twitch myopathic muscle fibres are significantly thinner as the result of more intensive protein degradation. Regeneration capacity according to the presence of satellite cells is higher in type IIA fibres than in type IIB fibres in myopathic muscle.

KEY WORDS: exhaustive exercise, myofibril structure, protein synthesis and degradation rate, intensity of glycogenolysis

INTRODUCTION

Inappropriate volume or intensity of exercise training may cause a maladaptive cellular or tissue response due to an imbalance between load and recovery [3]. This leads to performance decrements and overtraining syndrome [7]. The overtraining syndrome is not completely understood, but accumulating evidence indicates that disruptions in cellular homeostasis appear to be key factors in the process [5,30]. Tissue effects arise from these cellular disruptions. Overtraining can be defined as stress-recovery imbalance, i.e. too much stress combined with too little time for regeneration [4,9,11].

Due to the destruction of the myofibrillar apparatus and atrophy of muscle fibres, exercise myopathy develops as a result of exhaustive exercise [20,25]. This functional state is also known as the overtraining syndrome [3]. Long-lasting exhaustive exercise may lead to depletion of the energy system and neuromuscular fatigue [1]. In striated muscles with high oxidative potential, intracellular phosphotransfer systems constitute a major mechanism linking the mitochondria and intracellular energetic units [14,28]. The effectiveness of metabolic signalling strongly depends on the structural-functional relationship of the interaction between mitochondria and sarcomeres [29]. Endurance exercise activates the connection between mitochondria and myofibrils, but the disruption of linking of mitochondria to contractile, regulatory and minor protein structures exhibits impaired oxidative phosphorylation in atrophic muscle [23,26].

The adaptation of fast-twitch (FT) skeletal muscles to endurance training shows coordination between an increase in oxidative capacity and faster turnover rate of myosin heavy chain (MyHC) isoforms in the myofibrillar apparatus [17]. In exhausted skeletal muscle, due to the destruction of myofibrils and other cell organelles, the spaces between myofibrils increase and contain fragments of T-tubules, sarcoplasmic reticulum, mitochondria and glycogen granules [18]. The destruction of muscle fibre organelles is accompanied by the activation of lysosomal structures [26]. This is proof of muscular disease – myopathy resulting in muscular weakness and proof against neuropathy.

Slow-twitch (ST) muscles are more resistant than FT muscles to exhaustive endurance exercise, the reason for which has been proposed to be related to MyHC composition [20]. Muscle fibres with predominantly MyHC I and IIa isoforms have relatively high oxidative

capacity and are recruited more during endurance type of exercise [17]. The multidirectional changes in the given MyHC isoforms in ST and FT muscles with an increase in exercise training volume not only support the different resistance of ST and FT muscles to exhaustion, but also show the sensitivity of MyHC isoforms to proteolytic enzymes in different types of muscles [18].

This raises the question of how substantial the changes are in skeletal muscle fibres' glycogen content, muscle protein synthesis and degradation rate, and myofibril structure. We hypothesized that in exercise-induced myopathic FT muscle fibres the myofibrils are thinner as the protein synthesis rate decreases and the protein degradation rate increases. The purpose of the present study was to reveal the relationships between structural changes of myofibrils in oxidative-glycolytic or type IIA fibres and glycolytic or type IIB muscle fibres, protein fractional synthesis and degradation rate in exercise-induced myopathic muscle.

MATERIALS AND METHODS

The use of animals was in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes and all procedures used in this study were approved by the Animal Experiment Committee of the Estonian Ministry of Agriculture, Tallinn, Estonia.

Animals, exercise, anaesthesia, and labelled amino acid infusion.
The animals involved were 16-17-week-old male Wistar rats. All the animals were housed in identical conditions in polycarbonated type III cages at 21°C. They received diet [SDS-RM-1(C) 3/8, Witham, Essex, UK] and water *ad libitum*. The rats were assigned to control (n=8) and exhausted exercise (overtraining) induced myopathic groups (n=8).

After a brief 5-day acclimation that consisted of treadmill running for 5-10 min, rats were subjected to running with the speed of 35 $m \cdot min^{-1}$. The overtraining group ran 7 days per week and the training volume reached 2 h 20 min per day in the 4th week of training [20].

L-[4.5-3H] leucine (170 $Ci \cdot mmol^{-1}$) was infused intraperitoneally once per hour for 6 h, 250 $mCi \cdot 100\ g^{-1}$ bw.

Separation of fast oxidative-glycolytic and fast glycolytic muscle fibres.
For studies of the fast glycolytic (FG) fibres or type IIB fibres and fast oxidative-glycolytic (FOG) fibres or type IIA fibres, the quadriceps femoris muscle was dissected, liberated from fat and connective tissue, and separated into a superficial white portion and deep red portion. In FG fibres, myosin heavy chain (MyHC) IIb isoform relative content was 97.0±5.0%, MyHC IId isoform relative content 3.0±0.2%; cytochrome aa_3 concentration was 9.20±0.80 $ng \cdot g^{-1}$ muscle wet weight, and myoglobin concentration was 0.85± 0.09 $mg \cdot g^{-1}$ muscle wet weight. In FOG fibres, MyHC IIb isoform relative content was 22.5±1.1%, MyHC IId isoform relative content was 25.0±1.2%, MyHC IIa isoform relative content was 44.5±2.3% and MyHC I isoform relative content was 8%±0.8%; cytochromes aa_3 concentration was 31.8±2.9 $nm \cdot g^{-1}$ and myoglobin concentration 3.4±0.3 $mg \cdot g^{-1}$. Cytochrome aa_3 and myoglobin were measured as described previously [16].

Ultrastructural studies
Muscle samples for ultrastructural studies were fixed in 2.5% glutaraldehyde, post-fixed in 1% sodium tetroxide, dehydrated in graded alcohol and embedded in Epon-812. Ultra-thin sections were cut from longitudinally and transversely oriented blocks, stained with uranyl acetate and lead hydroxide, using 8 blocks from each animal. For analysis of myofibril CSA, imaging and analysis software (Cell* Soft Imaging System GmbH, Münster, Germany) was used. The number of satellite cells containing a nucleus per 1000 nuclei was calculated under an electron microscope. The satellite cell frequency was determined as the ratio of the nucleus-containing satellite cells divided by the total number of myonuclei including satellite cells' nuclei.

Separation of total muscle protein
The minced muscle samples were homogenized in a buffer containing 50 mM KCl, 10 mM K_2HPO_4, 1 mM EGTA, 1 mM $MgCl_2$, and 1 mM dithiothreitol at pH=7.0 and analyzed as total protein fraction. The total muscle homogenate was dissolved in 0.3 M NaOH and was analyzed for radioactivity and protein.

Separation of myofibrillar protein
Frozen muscles were thawed on ice, cut into small pieces, and washed with five volumes of 20 mM NaCl, 5 mM sodium phosphate, and 1 mM EGTA (pH=6.5). Myosin was extracted with three volumes of 100 mM sodium hydrophosphate, 5 mM EGTA, and 1 mM dithiothreitol (pH=8.5), and after 30 min of gentle shaking, the myofibrillar fraction was diluted with one volume of glycerol and stored at -20°C.

Estimation of 3-methylhistidine (3-MeHis) in skeletal muscle and urine
24 h urine was collected in a metabolic cage. The 3-MeHis in skeletal muscle and urine was used as an indicator for myofibrillar protein degradation. The determination was performed as described previously [24]. Briefly, total muscle protein was hydrolyzed in 6M HCl for 20 h at 110°C in vacuum sealed flasks. HCl was removed by evaporation and the hydrolysate was dissolved in 0.2 M pyridine to achieve a concentration of 10-20 $mg \cdot ml^{-1}$. 3-MeHis in the urine and muscle tissue was estimated with HPLC [24].

Fractional degradation of muscle protein
The degradation rate of myofibrillar protein was calculated as follows: 3-MeHis excretion ($mmol \cdot day^{-1}$ x 0.75 x 100) divided by selected muscle myofibrillar protein (g) x 3-MeHis ($mmol \cdot g^{-1} \cdot muscle^{-1}$) and expressed as % per day [18].

MyHC isoform separation
MyHC isoforms were separated by 7.2% SDS-PAGE using 0.75 mm

thick gels. Myofibrils containing 0.5 μg of protein were loaded on the gel after being incubated for 10 min at 65°C in sample buffer containing 62.5 mM Tris-HCl, pH=6.8, 20% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 2.0% SDS, 0.05% bromophenol blue. Electrophoresis lasted for 24 h at 120 V [6]. Gels were silver-stained by the method of Oakley et al. [12]. Protein isoform bands were analyzed densitometrically by the Image Master® 1D program, Version 3.0 (Amersham Pharmacia Biotech, USA) and the percentage distribution of the various isoforms was evaluated.

Protein assay

Total muscle protein and myofibrillar protein were assayed using the technique described by Bradford [2].

Estimation of glycogen content and intensity of glycogenolysis

Glycogen was determined in small muscle samples according to Lo et al. [10]. Intensity of glycogenolysis was expressed as μmol glucose per min per g of muscle wet weight as follows:

$(Gb - Ga) \cdot min^{-1}$

Gb – content of glycogen in muscle tissue before exercise
Ga – content of glycogen in muscle tissue after exercise
min – exercise time in min

Statistics

Means and standard errors of means were calculated from individual values using standard procedures of Excel. The data were analyzed by R 2.12.2. [13]. Pearson correlation coefficients were used to describe relationships between variables. Differences between groups were analyzed by the Wilcoxon rank sum (Mann-Whitney U) test. Probability distributions were compared using the Kolmogorov-Smirnov test. Differences were considered significant at $p<0.05$.

RESULTS

Exhaustive endurance type of exercise training which leads to myopathy caused 2.2 times more intensive glycogenolysis in FOG muscle fibres (0.076±0.006 μM glucose$\cdot g^{-1}$ tissue$\cdot min^{-1}$) than in G fibres (0.034±0.003 μM glucose$\cdot g^{-1}$ tissue$\cdot min^{-1}$; $p<0.001$). As shown in Fig. 1A, the fractional protein synthesis rate in FOG fibres decreased significantly 24 h after the last overtraining session, while in FG fibres there was only a tendency to decrease. The fractional protein degradation rate increased at the same time in both FOG and FG fibres, 63% and 69%, respectively (Fig. 1B). Myofibril CSA in FOG fibres decreased by about 33% and in FG fibres by about 44% (Fig. 2). Myofibrils in FT fibres are thinner in comparison with the control group (Fig. 3A,B,C), which is mainly due to the intensive protein degradation rate. The regenerative capacity of myopathic fibres has been preserved, as is shown by the occurrence of satellite cells in these muscle fibres (Fig. 3D). There is a correlation between CSA of myofibrils and fractional protein synthesis rate in FOG fibres ($r=0.485$; $p<0.01$) and a negative correlation between CSA of myofibrils and fractional protein degradation rate ($r=-0.535$; $p<0.004$). In myopathic FG fibres, a negative correlation was found between CSA of myofibrils and fractional protein degradation rate ($r=-0.671$; $p<0.001$). Myofibrils of both FOG and FG fibres are thinner in myopathic muscle fibres because of the high protein degradation rate. According to the intensity of glycogenolysis, there are grounds to believe that FOG fibres are recruited more during exhaustive exercise

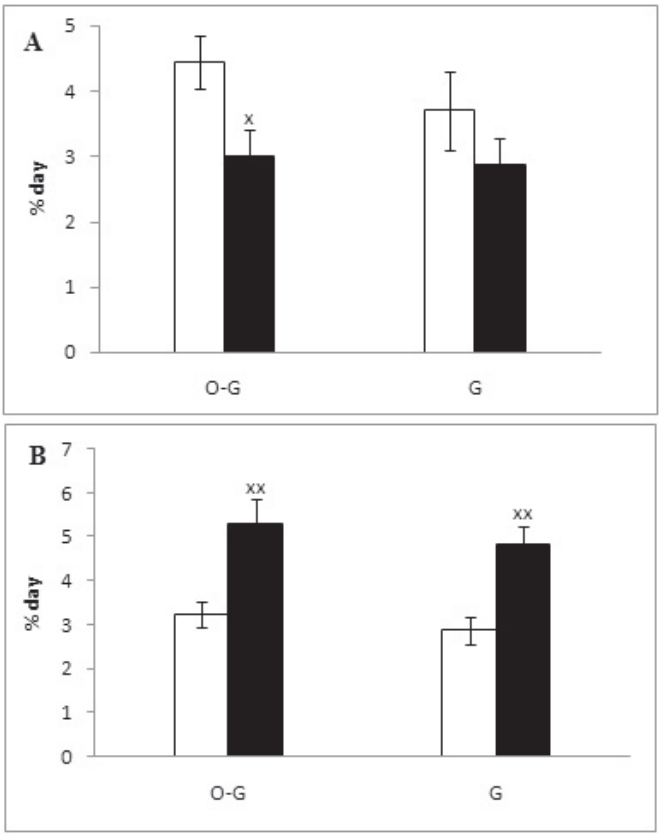

FIG. 1. FRACTIONAL SYNTHESIS (A) AND DEGRADATION (B) RATE OF MUSCLE PROTEIN IN MYOPATHIC OXIDATIVE-GLYCOLYTIC AND GLYCOLYTIC MUSCLE FIBRES 24 H AFTER EXERCISE SESSION
Note: O-G – oxidative-glycolytic (type IIA) muscle fibres, G – glycolytic (type IIB) muscle fibres, x – $p<0.01$, xx – $p<0.001$

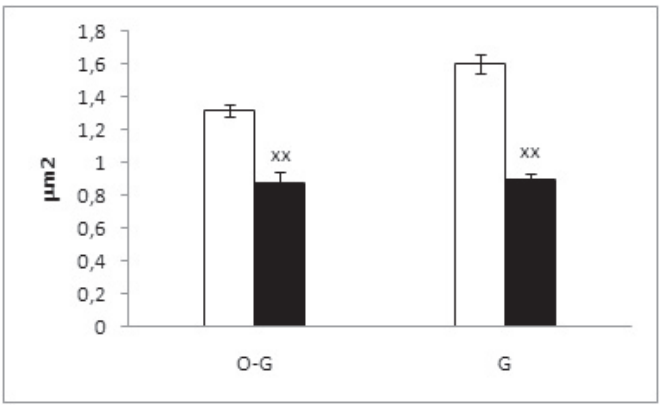

FIG. 2. CHANGES IN MYOFIBRIL CSA IN MYOPATHIC OXIDATIVE-GLYCOLYTIC AND GLYCOLYTIC MUSCLE FIBRES
Note: O-G – oxidative-glycolytic (type IIA) muscle fibres, G – glycolytic (type IIB) muscle fibres, xx – $p<0.001$

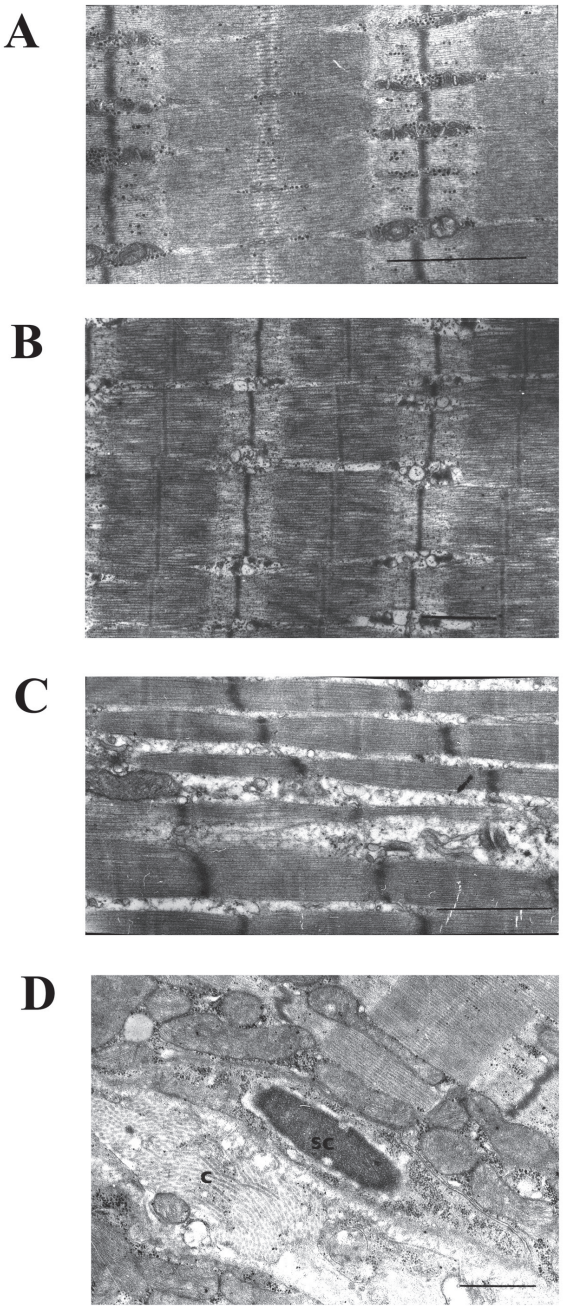

FIG. 3. CHANGES IN MYOFIBRIL CSA AND REGENERATIVE CAPACITY IN FAST-TWITCH EXERCISE INDUCED MYOPATHIC MUSCLE FIBRES
Note: A – myofibrils of control animals' glycolytic muscle fibre, B – myofibrils of control animals' oxidative-glycolytic muscle fibre, C – myofibrils of exercise-induced myopathic oxidative-glycolytic muscle fibre, D – satellite cell under the basal lamina of exercise-induced myopathic oxidative-glycolytic muscle fibre
Bar: 1 μm

training than FG fibres. The CSA of myofibrils in both fibre types decreases significantly in myopathic fibres in comparison with control fibres.

DISCUSSION

It has previously been shown that due to the atrophy of muscle fibres and destruction of myofibrils, exercise-induced myopathy develops as a result of overtraining [7,26]. The most sensitive fibres to overtraining are FT muscle fibres [25]. The present study shows that there are some similarities between structural and biochemical changes in exercise-induced myopathic FT OG and G muscle fibres. Contrary to the widespread opinion that FG muscle fibres are not recruited actively during low-intensity exercise, increased intensity of glycogenolysis in these fibres and degradation rate of muscle protein, as shown in the present study, confirm the participation of these fibres in the process of development of the overtraining syndrome. In comparison with FOG fibres, the destruction of myofibrils in FG fibres seems to be of smaller scope and these results are supported by the earlier findings about destructive changes of neuromuscular junctions during development of overtraining syndrome [25]. Similar changes in CSA of myofibrils have been observed in glucocorticoid-induced muscle myopathy [8]. As FT muscle fibres are more sensitive to the action of corticosteroid than ST fibres [15,21], it is likely that the increase of endogenous corticosterone level during overtraining may be a factor in the pathogeneses of exercise-induced myopathy. Muscle weakness in case of glucocorticoid myopathy is caused by lesions in the neuromuscular synapses [25].

Similarities in functional and structural changes in skeletal muscle of glucocorticoid and exercise myopathies have provided a basis for speculation that overtraining-induced myopathy may be a mild form of corticosteroid myopathy [9]. In spite of similarities in the destruction of myofibrillar apparatus, development of myopathy in both cases and in the regeneration capacity, the present study and some previous studies [19,21,26] do not support this theory. Therefore, the high corticosterone level during exhaustive exercise training lasts only during a relatively short period, and in the recovery period this hormone level decreases [27]. The main destruction of myofibrils has been registered in glucocorticoid-induced myopathic FG muscle fibres [22,25] and in exercise-induced myopathy in FOG fibres [7,25,26]. This is the real difference between these two types of myopathies at the level of muscle fibres. Similarities are visible in the process of destruction of myofibrils in both types of myopathies, such as the disarray of myosin filaments from the periphery of myofibrils [25]. These results show that in both types of myopathies the defect is primarily within the muscle, not in the nerves. Muscle fibres with higher oxidative capacity are also more susceptible to oxidative damage by reactive oxygen species than fibres with low oxidative capacity and predominantly with MyHC IIb and IId isoforms.

Higher oxidative capacity of muscle fibres makes them more resistant to the degradation of muscle proteins also in myopathic muscle. As type IIA muscle fibres are recruited more frequently during overtraining, there is also notable structural destruction. Due to the relatively high regenerative capacity of type IIA fibres, these fibres are able to maintain low intensity muscle contraction in myopathic muscle.

The gaps preventing a more comprehensive understanding of the pathogenic mechanism of exercise-induced myopathy may provide novel markers for diagnosis of this myopathy in future studies.

CONCLUSIONS

In conclusion, according to the intensity of glycogenolysis, FOG fibres are recruited more during overtraining but the CSA of myofibrils in both FT myopathic muscle fibres decreases significantly. In FOG fibres, the protein synthesis rate decreased significantly 24 h after the last overtraining session, while FG fibres only had a tendency to decrease. The protein degradation rate increased more than 60% in both fibre types in myopathic muscle. Regeneration capacity, as shown by the presence of satellite cells, is higher in FOG fibres than in FG fibres in myopathic muscle. Destruction of myofibrils is somewhat less and the regeneration capacity is higher in FT myopathic fibres with higher oxidative capacity.

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Conflict of interest: The authors declare that they have no conflict of interests.

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