ANDROGEN PARTICIPATION IN THE ADAPTATION OF MYOFIBRES AND EXTRACELLULAR MATRIX OF RAT SKELETAL MUSCLES TO ENDURANCE TRAINING

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ABSTRACT
The PURPOSE of the present study was to investigate the changes in the size of skeletal muscle fibres and extracellular matrix (ECM) after submaximal training and androgen receptor blocker (ARB) treatment. METHODS: Two groups of animals were used: trained (T) and non-trained (NT). Half of the trained (T+F) and untrained (NT+F) rats were treated with the ARB Flutamide. RESULTS: The analysis of the muscle fibre cross-sectional areas (CSA) of soleus (Sol), extensor digitorum longus (EDL) and gastrocnemius (Gas) showed that trained rats had larger CSA than non-trained. ARB application significantly reduced CSA of EDL and Gas in the trained animals. ARB had no effect on the amount of ECM in Sol and EDL after training, while in Gas the amount was greater than in the trained controls. CONCLUSIONS: The reduction in the CSA of EDL and Gas muscle fibres is probably due to the higher content of type II fibres, which are more sensitive to androgens. The unchanged amount of ECM in Sol and EDL of the ARB-treated and trained rats suggested a lack of androgen effect. The ECM remodelling in Gas following endurance training is androgen-dependent.

Key words: androgen receptor blocker, muscle weight, cross-sectional area, muscle fibres

INTRODUCTION
The type of physical training is the main factor determining presence of muscular hypertrophy. Adaptation to regular exercise also includes hypertrophy of the slow-twitch, oxidative-type muscle fibres, which can be established on their cross-sectional areas (CSA) (1). This process occurs by influencing the equilibrium between protein synthesis and protein degradation. The synthesis of different protein fractions - myofibrillar, sarcoplasmic or mitochondrial is activated depending on the type of physical load (2). The molecular mechanisms responsible for the adaptation of skeletal muscles to endurance training are still uncertain, as well as the role of androgens in these processes (3).

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week training on a treadmill (EXER-3R-Treadmill, Columbus Instruments, Columbus, USA) with submaximal loading (70-75% \( VO_{2\max} \)) 5 days a week. The experimental protocol was approved by the Ethical Committee on Human and Animal Experimentation of the Medical University - Plovdiv, and the Committee on Ethical Treatment of Animals of the Bulgarian Food Safety Agency. All experimental procedures were performed according to the recommendations of the European Commission on Protection and Humane Treatment of Laboratory Animals. The duration of the training increased gradually during the first week, at the end of the second week it reached 40 minutes a day and was of that duration until the end of the experiment. Half of the trained (T+F) and non-trained (NT+F) rats were treated with the ARB Flutamide (Sigma Aldrich, Munich, Germany) (15 mg∙kg\(^{-1}\)) dissolved in sesame oil and administered subcutaneously for 8 weeks, whereas the other half received sesame oil as a vehicle once a day, for the same period of time. Two days after the last training the rats were decapitated under thiopental anaesthesia (30 mg∙kg\(^{-1}\)). The weights of soleus m. (Sol), extensor digitorum longus m. (EDL) and gastrocnemius m. (Gas) were measured within 3 minutes after decapitation (laboratory scale TP512A). Parts of the muscles were fixed in Bouin’s fixative for 24 hours and embedded in paraffin. Azan staining was applied to microtome cross sections (6 \( \mu \)m) of Sol (85% fibres type I), EDL (96% fibres type II) and medial head of Gas (51% fibres type I and 49% fibres type II), (8).

The cross-sectional areas of myofibres and the relative distribution of connective tissue in the muscles of 6 animals from each experimental group were determined by the specialized software DP-Soft 3.2 (Olympus, Japan). The data obtained were analysed using two-way factorial analysis of variance, Tuckey and Games-Howell post hoc tests were applied to determine intergroup differences. Differences at \( P<0.05 \) were accepted as statistically significant. The results are presented as \( x \pm SEM \).

RESULTS AND DISCUSSION
Sol muscle mass tended to decrease in the rats treated with Flutamide (NT+F and T+F), as compared with those treated with placebo (0.103±0.005 g v/s 0.117±0.005 g; \( P=0.078 \)). EDL weight also showed a tendency to decrease after ARB administration (NT+F and T+F), as compared with placebo administration (0.131±0.006 g v/s 0.148±0.006 g; \( P=0.056 \)). Training had no significant main effect on the muscle mass of all tree muscles (\( P>0.05 \)). No interaction between the two factors was found in the experimental groups (\( P>0.05 \)) (Figure 1).

The analysis of CSA of Sol, EDL and Gas muscle fibres showed that trained rats had larger CSA than non-trained. ARB application to the trained animals significantly reduced CSA of EDL and Gas (Figures 2-7). ARB had no effect on the amount of ECM in Sol and EDL of the trained rats, whereas in Gas its amount was greater than in the trained controls (Figure 8).

![Figure 1](image-url)
Figure 2. A-D. Soleus cross sections of animals from the experimental groups. Azan staining (magn. x200).

Figure 3. Cross-sectional area of soleus muscle fibres (µm²).
*P<0.001 trained vs non-trained.

Figure 4. A-D. EDL cross sections of animals from the experimental groups. Azan staining (magn. x200).
Figure 5. Cross-sectional area of EDL muscle fibres (µm²).
*P<0.001 compared with NT; **P<0.01 compared with T; #P<0.001 compared with NT+F.

Figure 6. A-D. Gastrocnemius cross sections of animals from the experimental groups. Azan staining (magn. x200).

Figure 7. Cross-sectional area of gastrocnemius muscle fibres (µm²).
*P<0.01 compared with NT; **P<0.001 compared with NT+F; #P<0.001 compared with NT.
The tendency to reduce Sol and EDL muscle mass that we observed after a period of an 8-week treatment with ARB has been observed by other authors who have reported depletion of lean muscle mass following Flutamide treatment. Our findings support the results obtained by other investigations showing decreased muscle mass of EDL (9, 10) and Sol (10) in people and animals with low levels of endogenous testosterone (Ts) after orchiectomy. These data suggest that both types of muscle fibres (fast and slow) are characterized by androgen-dependent muscle mass and the effect of the androgens occurs mainly through a genomic mechanism via androgen receptors (AR).

The results obtained by us showed that the submaximal load resulted in a significant increase in CSA of all three muscles examined. Skeletal muscles are highly plastic tissues that adapt to the increased motor and metabolic needs during exercise. A number of signaling pathways and myogenic regulatory factors are activated which trigger protein synthesis (myofibrillar, sarcoplasmic, mitochondrial). These different types of muscle protein synthesis are the basis of the adaptation response to physical exercise (2). As a result of the increased protein production, the myofibre bulk increases, which is observed in transverse sections of the muscle.

ARB application to the trained animals significantly reduced the cross-sectional area of EDL muscle fibres. Decrease in CSA was also found in Gas, but the differences did not reach significance. Our findings can be explained by the different sensitivity of the fibre types to Ts and more specifically to the AR localized therein. These results support the concept of a stronger response of type II muscle fibres to androgens (11). On the other hand, the muscle fibre CSA of the Flutamide-treated and trained animals (T+F) was greater than that of non-trained Flutamide-treated (NT+F) animals. This fact implies that the endurance training could have beneficial effect on symptoms of sarcopenia in patients undergoing androgen-deprivation therapy and ARB administration.

The data did not show any change in the amount of ECM in Sol and EDL of the trained animals, no matter whether treated or not with Flutamide. This is likely to be due to the type of physical exercise used in our experiment. The mechanical loading of skeletal muscles during an 8-week submaximal treadmill training was probably not sufficient to cause any changes in the amount of ECM. Other researchers have reported strength training or muscle injuries due to overload as factors stimulating connective tissue formation (12). In Gas (composed of muscle fibres type I and II) of the trained controls (T) a reduction in ECM percentage was detected, which is indicative of the directions in the development of the processes involving the amount of connective tissue. These adaptive changes to submaximal training are related to the need for enhanced gas and nutrient exchange between capillaries and muscle fibres during and after exercise. The process of ECM remodelling in Gas after chronic exercise was influenced by androgens which was demonstrated by an
increased amount of connective tissue in the animals of the T+F group.

CONCLUSIONS
The reduction in CSA of EDL and Gas muscle fibres in the trained animals after ARB application is probably due to the higher content of type II fibres, which are considered to be more sensitive to androgens. The unchanged amount of ECM in Sol and EDL of the ARB-treated and trained rats showed a lack of androgen effect but the ECM remodelling in Gas resulting from the submaximal training was androgen-dependent.

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