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Fiber Type Distribution in Soleus Muscle of Trained Rats with and without Anabolic Steroid Supplementaion

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The aim of the study was to investigate the combined effect of anabolic androgenic steroid (AAS) treatment and endurance training on fiber type distribution in soleus muscle of male rats. Wistar rats were allocated into three groups: one was sedentary and two groups were trained on treadmill for 8 weeks. One of the trained groups received weekly 10 mg.kg^{-1} Nandrolone Decanoate (T+ND), and the sedentary (S) and the other trained group (T) received placebo for the last 6 weeks of the trail. At the end of experiment, on serial cryostat cross sections from soleus muscle, reactions for myofibrillar ATPase activity after alkaline and acid preincubations were carried out. Muscle fiber types were determined as I, IIc, IIa и IId/x. The results didn't show significant difference in relative percentage of the fiber types between groups.

Key words: rat, soleus muscle, submaximal training, Nandrolone Decanoate, fiber type.

Endurance training induces a variety of physiological and morphological adaptations in skeletal muscles and increases aerobic working capacity [5]. Endurance training promotes fast-to-slow shift in fiber type distribution and myosin heavy chains (MHC) expression (4, 8). The extend of the fiber type transition depends on of the muscle type and the duration of the training [3].

Anabolic androgenic steroids (AAS), synthetic derivatives of testosterone, are used by participants in endurance sports but little is known about their effects on endurance performance and fiber type distribution [9]. Data about the effects of AAS treatment on fiber type distribution (MHC expression) in untrained rats are contradictory [1, 6]. To our knowledge only one study investigates these effects on treadmill trained rats, but because of the design of the experiment it is not clear whether the reported changes in m. soleus are due to the combined influences of training and AAS treatment or to the training alone [6]. The interaction between submaximal training and AAS treatment on fiber type distribution in typically red muscle as soleus is not well defined.

The aim of our experiment was to determine the effects of AAS treatment on muscle fiber type distribution in soleus muscle of submaximal trained male rats.

Material and Methods

Male Wistar rats were distributed into 3 groups (n=7). One was sedentary (S) and two groups were trained on treadmill with submaximal loading (about 70-75% VO_{2max}) 5 day.wk^{-1} for 8 weeks. After 2nd week, when the training session reached 40 min.d^{-1}, one of the trained groups received weekly 10 mg.kg^{-1} Nandrolone Decanoate (ND; Retabolil, Gedeon Richter, Hungary) and the other trained group and sedentary rats received placebo (Pl) i.m. for the last 6 weeks of the trial. The experiment was approved by the Ethic Committee at the Medical University of Plovdiv.

All the groups: sedentary + Pl (S); trained + Pl (T) and trained + ND (T+ND) were subjected on running endurance test at the beginning and the end of the experiment. At the end of the trial each animal was decapitated and entire soleus muscles were collected in liquid nitrogen and stored at —70°C until analysis. On serial cryostat cross sections (10 µm) enzymohistochemical reactions for myofibrillar ATPase activity after alkaline (10.55 pH) and acid preincubations (4.5, 4.6, 4.7 pH) were carried out according the technique of Sant'ana Pereira et al. [7]. Muscle fibers were classified as type I, IIa, IId/x or IIb on the base of histochemical staining. In addition to the four "pure" fiber types intermediate hybrid fibers (type IIc) were also evident in all groups. Type IIc fibers contain both MHC type I and type IIa [2]. Fiber typing was done using computer image-processing system. The proportion of fiber types was determined from a sample of 200-300 fibers across the entire section of each muscle. Data were expressed as mean ± SEM and were analyzed by one-way ANOVA.

Results and Discussion

Soleus muscle is slow-twitch skeletal muscle and predominantly contains slow type I fibers (about 75-85%). In rat soleus muscle there is a lack of the fastest fiber type IIb (2). Histochemical reactions in rat soleus muscle of the experimental groups are presented in Fig. 1.

Mean fiber type percentage values for soleus muscle are presented in Fig. 2.

Our results about fiber type distribution in the soleus muscle of the control group correspond to those reported by other anthors [2, 3].

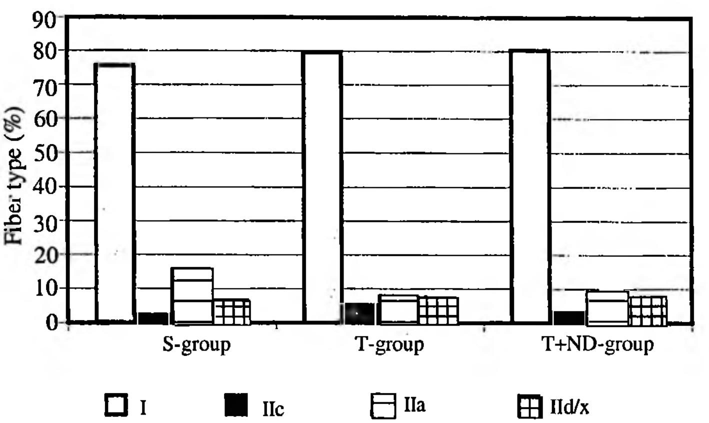


Fig. 2. Fiber type distribution (%) in soleus muscle of the studied groups at the end of the experiment

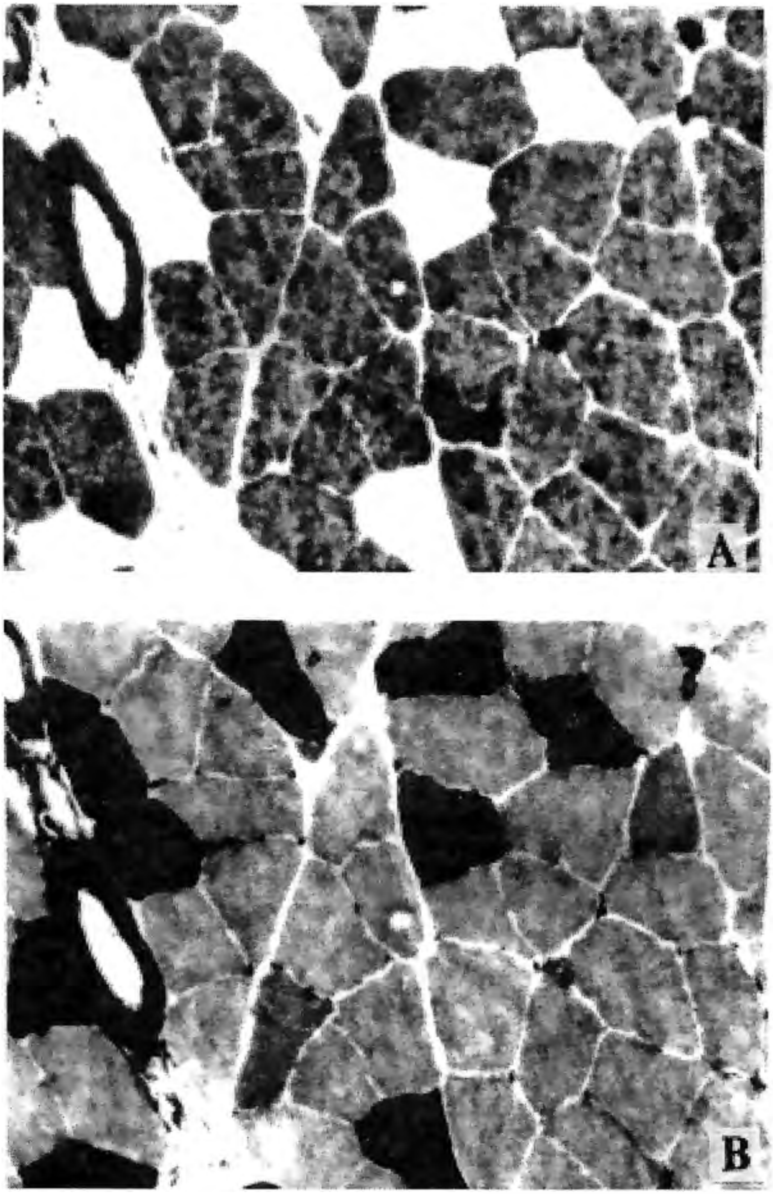
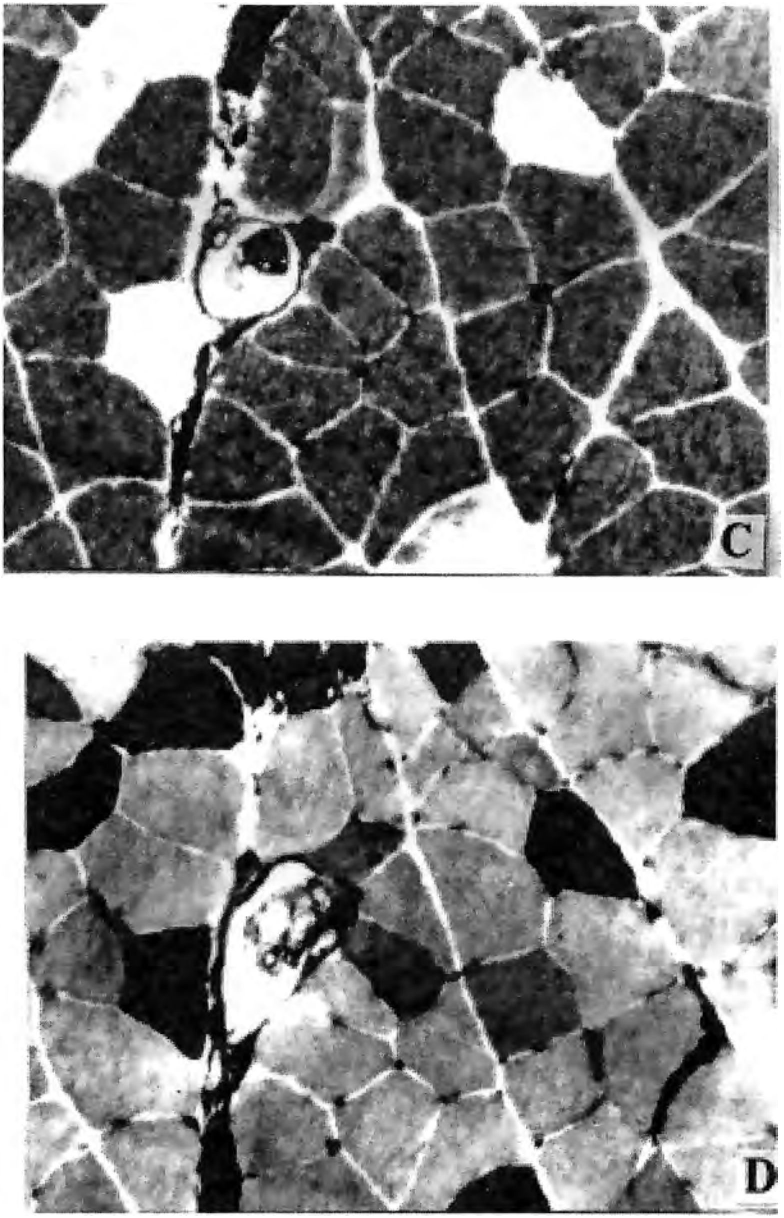


Fig. 1. Myofibrillar ATPase histochemistry of serial cross-sections of rat soleus muscle from group S (**A**, **B**), from group T (**C**, **D**) and from group T+ND (**E**, **F**), after acid preincubation at pH 4.6 (**A**, **C**, **E**) and alkaline preincubation at pH 10.55 (**B**, **D**, **F**) (× 200)



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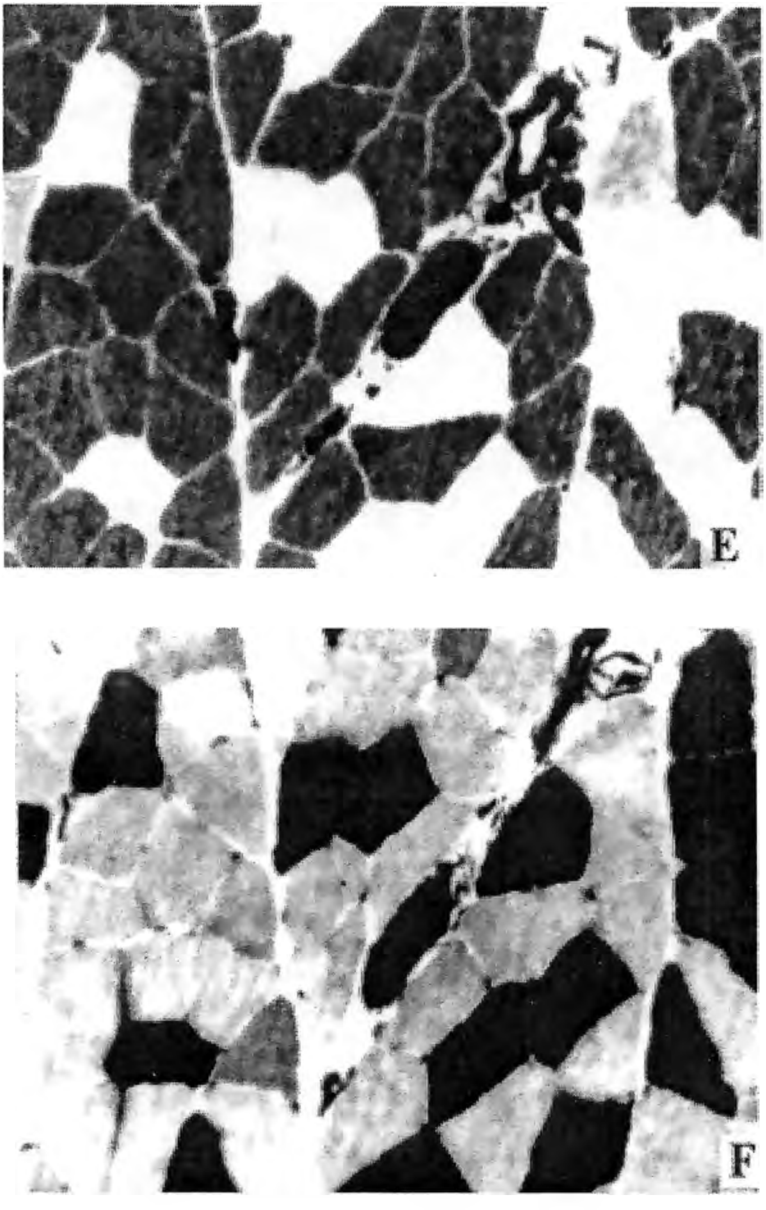

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The statistical analysis of the data didn't show significant difference in relative percentage of I, IIc, IIa and IId/x fiber types between the studied groups ($P>0.05$). In both trained groups (with or without Nandrolone Decanoate) there was a tendency endurance training to increase the percentage of I type and to decrease type IIa myofibers in comparison with the control group, but the differences did not reach statistical significance.

The fiber type distribution in the different muscles is predominantly genetically determined. MHC isoforms differ in their ATPase activity — MHC-IIb has highest and MHC-I has lowest one — and this is resulted in the differences of the contracting velocity. It is clear from many experimental studies that fibers are capable to change their phenotype characteristics. Physical activity (training or detraining) and some hormones (thyroid) lead to changes in fiber type distribution. The transformation of the myofiber types has an obligatory pathway in this order: I ⇔ IIc ⇔ IIa ⇔ IId/x ⇔ IIb [3, 8]. In rats endurance training promotes fast-to-slow shift in fast fiber subtypes (IIa, IId/x and IIb) in fast and mixed muscles but have no effect in slow-twitch m. soleus [3, 4, 8].

Our results demonstrate that the combined influence of submaximal training and AAS treatment have no effect on the fiber type distribution in slow-twitch m. soleus compared with sedentary and endurance trained male rats. In contrast with these results, our previous study on mixed gastrocnemius muscle of the same animals showed well-expressed fast-to-slow shift (i.e., from IIb to type IId/x to type IIa) in T+ND compared with the S and T rats [10]. The summarized data of these studies showed that probably the combine effect of submaximal training and AAS treatment on fiber type distribution depends on the type of the muscle.

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