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Markers of Metabolic Adaptation in Gastrocnemius Muscle after Administration of Antiandrogen in Endurance Trained Rats

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We studied the effect of Flutamide, an androgen receptor (AR) blocker, on glycogen content, the glycogen synthase (GS) and irisin expression in gastrocnemius muscles of rats undergoing endurance training for 8 weeks. Trained animals were found to have a higher glycogen content and stronger expression of GS and irisin than untrained animals. The higher glycogen content in gastrocnemius corresponds to the increased expression of GS in trained rats, which indicates that this enzyme takes part in the adaptation processes. Flutamide treatment increased the serum testosterone levels and decreased glycogen and irisin expressions. Glycogen and irisin in the muscle decreased when training was combined with administration of Flutamide, without any significant effect on GS, compared to those in untrained animals, but their levels were higher than those in Flutamide-treated untrained animals. These results suggest that endurance training can be used as a non-drug therapeutic modality to lessen the negative effects of antiandrogen therapy on skeletal muscles.

Kew words: endurance training, Flutamide, glycogen, glycogen synthase, irisin.

Introduction

Endurance training induces adaptations in skeletal muscles and increases the aerobic physical performance. Testosterone plays a key physiologic role in maintaining the proper function of skeletal muscle [9, 11] and the glycogen metabolism [5]. Depletion of muscle glycogen is an accurate marker of the onset of exhaustion in aerobic training exercises. Glycogen synthase (GS) is a key enzyme in the synthesis of glycogen. Its activity has been demonstrated to be dependent on the initial glycogen depots content and the muscle contraction itself [6, 7, 10]. Myocytes have been shown to release irisin when stimulated in physical exercise – the peptide increases the thermogenesis in white adipose cells and the total energy expenditure [2].

AR blockers are used in the treatment of prostate cancer [8]. Their long-term use has been associated with reduced capacity of physical performance [3]. It is not clear whether this side effect is associated with changes in the glycogen depots of skeletal muscles. There are no data in the available literature what effect anti-androgens have on glycogen content, the expression of GS and irisin in the muscles during endurance training.

The aim of the present study was to investigate the effect of androgen receptor blockers on the glycogen content, the expression of glycogen synthase and irisin in gastrocnemius muscle in endurance trained rats.

Materials and Methods

Male Wistar rats were allocated into two groups (n=12): a group of trained animals (T), and a group of sedentary animals (NT). Trained rats underwent 8-week submaximal treadmill training at 70-75% of VO_{2max} for 5 days per week. Half of the trained and untrained rats were treated with the androgen receptor blocker Flutamide (15 mg·kg^{-1}) dissolved in sesame oil and administered subcutaneously (T+F and NT+F); the remaining animals were given only sesame oil. Mixed blood and gastrocnemius muscle tissue samples were obtained from each rat at the end of experiment: a part of the muscle samples was frozen in liquid nitrogen, and another part – in the Bouin's fixative. We measured the levels of total testosterone in serum (Testosterone rat ELISA kit, Biotrend Chemikalien, Gmbh, Germany). The cryostatic sections were tested for glycogen using the PAS reaction. The paraffin sections (5 μm) were studied immunohistochemically (ABC Staining System, ImmunoCruz, Santa Cruz Biotechnology, USA) using the following antibodies: anti-glycogen synthase CT (04-357, Chemicon Millipore, USA), dilution 1:100; irisin rat antibody (42-112, Phoenix Pharmaceuticals, USA), dilution 1:100. The preparations were analysed using a special software program (DP-Soft 3.2, Olympus, Japan). We measured the intensity of the reactions (in relative units, RU) of 50 fibers of a muscle. The results were analysed statistically using two-way ANOVA. The data are presented as mean ± SEM.

Results

Administration of AR blocker affected the serum levels of testosterone. The Flutamide treated animals had higher concentrations of testosterone than the rats receiving placebo (8.48 ± 0.91 ng·ml^{-1} vs 1.83 ± 0.97 ng·ml^{-1}; $p < 0.001$). These findings were the result of the blocking of the hypothalamus-hypophysis-gonadal axis and show the efficiency of the drug dose we used in the experiment (**Fig. 1**).

We found no effect both of the training in comparison with the untrained rats (1.86 ± 0.26 g vs 1.92 ± 0.47 g; $p > 0.05$), and of the treating with AR blocker in com-

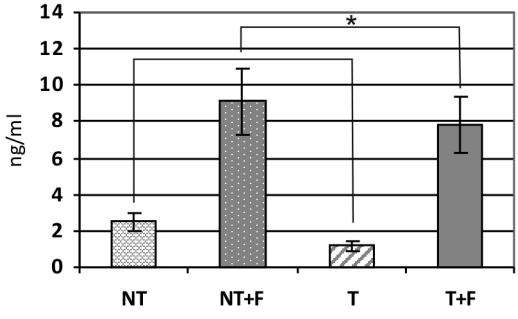

Fig. 1. Serum testosterone levels (ng·ml^{-1}) at the end of the experiment. *$p < 0.001$ in comparison with the placebo treated animals

parison with the placebo treated animals (1.90 ± 0.24 g vs 1.89 ± 0.46 g; $p > 0.05$) on the muscle mass of gastrocnemius muscle.

Aerobic training had a significant main effect on glycogen content in gastrocnemius muscle (**Fig. 2**). Trained animals were found to have a higher content of glycogen than that in the untrained rats (33.03 ± 1.33 RU vs 22.44 ± 1.33 RU, $p < 0.001$). Glycogen tended to decrease in the Flutamide treated rats ($p = 0.069$). We found a significant interaction of training and Flutamide treatment ($p = 0.001$). The highest glycogen content was found in the gastrocnemius of the endurance trained animals that received placebo. The AR blocker reduced the glycogen content in the trained animals ($p = 0.057$) in comparison with the single effect of endurance training.

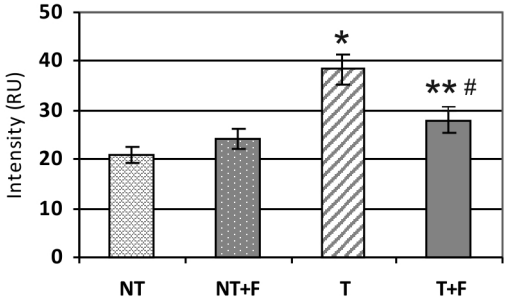

Fig. 2. Glycogen content (RU) in gastrocnemius m. *$p < 0.01$ in comparison with NT; **$p < 0.05$ in comparison with NT; #$p = 0.057$ in comparison with T

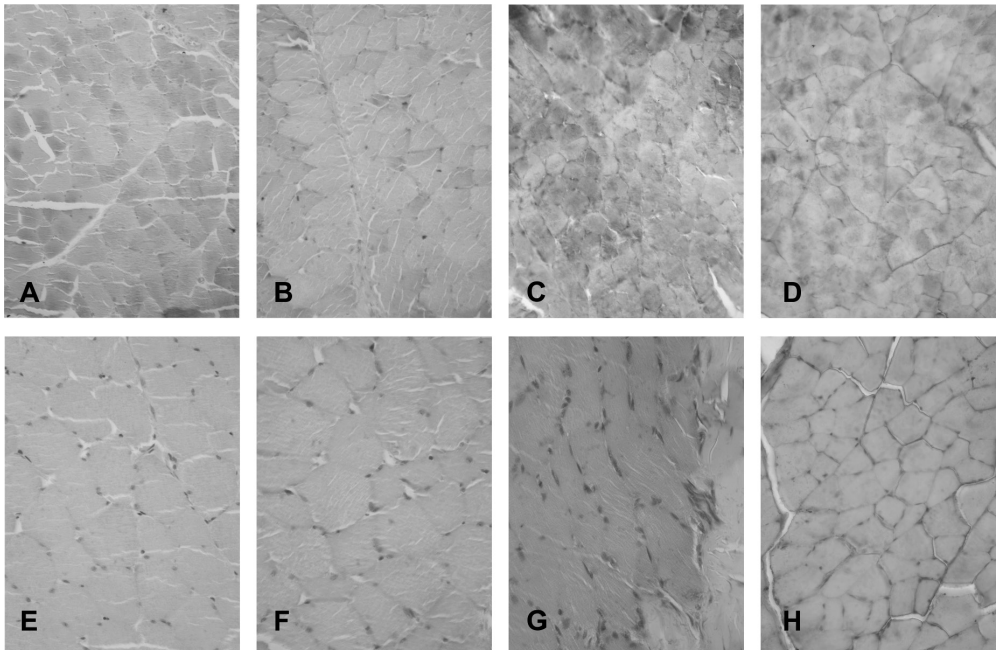

Fig. 3. Immunohistochemical reactions for glycogen synthase (A-D, ×200) and irisin (E-H, ×400) in gastrocnemius m. of animals of the experimental groups: NT (A, E), NT+F (B, F), T (C, G), T+F (D, H)

Analysis of the immunoreactions showed that the trained animals had stronger expression of glycogen synthase than the untrained rats (91.88 ± 1.57 RU vs 86.30 ± 1.57 RU; $p < 0.05$). Treatment with AR blocker induced no statistically significant main effect on this parameter ($p > 0.05$), (**Fig. 3**).

Endurance training had a significant effect on the expression of irisin. The trained animals showed a stronger immune expression in comparison with the untrained rats (81.49 ± 0.84 RU vs 75.69 ± 0.84 RU; $p < 0.001$) (**Fig. 3**). Administration of AR blocker also exerted an effect: the irisin expression was lower in the Flutamide treated animals than it was in the animals receiving placebo. We also found a significant two-way interaction ($p < 0.01$). The irisin reaction was strongest in the gastrocnemius of trained control animals. The combining effect of training and Flutamide treatment lead to a decrease of irisin expression compared with the effect of the training itself ($p < 0.001$).

Discussion

Our results showed that glycogen content in muscles at rest was higher in trained animals than it was in the untrained rats, a finding confirmed by other researchers as well [4]. GS activation after a single bout training session is important for so called glycogen overcompensation. The increased expression of GS in trained rats we found in the study shows that this enzyme takes part in the adaptation processes in endurance training. Blocking the androgen receptors in trained rats reduces the glycogen content in the gastrocnemius, which proves that the androgen receptors and androgens are involved in these processes of adaptation. The fact that Flutamide has no effect on GS expression shows that the effect of training on this enzyme is AR independent.

Irisin has been demonstrated to have a very low expression in the skeletal muscle of untrained animals [2], a finding which is corroborated by the results in the present study concerning the gastrocnemius muscle. Endurance training upregulated the irisin expression in the gastrocnemius which is consistent with the data provided by other researchers [1]. Administration of an AR blocker into trained rats lead to reduction of irisin expression in comparison with that in trained controls (T) which shows for the first time participation of AR in the processes of increased synthesis of irisin during training.

Conclusions

AR blocking reduces the glycogen content and irisin when aerobic training is combined with an AR blocker administration, which is one of the possible mechanism by which physical capacity is decreased. The glycogen content and irisin expression remain high in comparison with these in untrained animals treated with an AR blocker. On the basis of these results we can conclude that endurance training can be used as a non-drug therapeutic modality to lessen the negative effects of anti-androgen therapy on skeletal muscles. Our results show for the first time the role of androgens in irisin production during aerobic training.

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