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Endurance and Resistance Exercise Induce Muscle Fiber Type Specific Responses in Androgen Binding Capacity

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This study examined the effects of different exercise training programs on androgen receptor content and receptor affinity to dihydrotestosterone in fast glycolytic (FG) and slow oxidative (SO) skeletal muscle fibers in rats. Twenty-four male Sprague–Dawley rats were equally divided into three groups: control, endurance exercise trained and resistance exercise trained. After the exercise programs were completed, the extensor digitorum longus (EDL), predominantly a FG muscle, and the soleus, predominantly a SO muscle, were isolated, weighed and both androgen receptor content and affinity to dihydrotestosterone were determined. Resistance training evoked a significant ($P < 0.05$) hypertrophic response in the soleus but not the EDL. Endurance training was not associated with any significant hypertrophy in either the soleus or the EDL. Neither the endurance nor the resistance training program resulted in changes in androgen receptor affinity to dihydrotestosterone. However, alterations in androgen receptor content were noted. The endurance training program resulted in a significant increase in androgen receptor content in the soleus, but no significant difference in the EDL. The resistance training program elicited a significant decrease in androgen receptor content in the soleus, and a significant increase in the EDL. These results indicate that different exercise stimuli induce changes in androgen receptor content that are specific to skeletal muscle fiber type.

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INTRODUCTION

Androgenic hormones possess muscle growth promoting effects that include increased rates of amino acid uptake and protein synthesis [1]. Androgens, like other steroid hormones, stimulate the expression of specific proteins encoded in the DNA of the cell via their receptor proteins. Presently, there is ambiguity concerning the location of androgen receptors when they are in the unbound state. Some reports indicate that unbound androgen receptors are found in the cytosol of the cell [2, 3], while others suggest that they are nuclear proteins [4, 5]. Regardless of the location of the unbound receptor, when steroid binding occurs the receptor then becomes activated and the steroid–receptor complex is translocated to the genetic material within the nucleus [6]. The binding of this complex to the steroid responsive element (SRE) in the 5′upstream region of selective genes increases rates of transcription [6, 7]. It has also been suggested that the binding of steroids to their receptors may enhance translational effects [7].

It has been frequently proposed that a major mechanism by which cells regulate their responses to endocrine factors is by up- and down-regulating their capacities to bind specific hormones circulating in the bloodstream [1]. It has been found that different stimuli can alter the ability of skeletal muscle to maximally bind androgens [8]. The effects of endurance training on the androgen binding capacity of muscle have been previously investigated [9, 10]. However, no data have been reported concerning the effects of different forms of exercise training on androgen receptor content in the different fiber types of skeletal muscle.

The aim of the present study was 2-fold: (1) to

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investigate whether different exercise training programs (i.e. endurance vs resistance training) would evoke changes in the androgen binding characteristics in skeletal muscle; and (2), if so, to determine if these exercise induced alterations in androgen binding capacity were muscle fiber type specific.

EXPERIMENTAL

Training protocols

Twenty-four young male Sprague–Dawley rats (Holtzman Laboratories, Madison, WI) weighing ~250 g were randomly assigned to one of three groups. The first group ($n = 8$) served as unexercised controls. Animals assigned to the second group ($n = 8$) participated in an endurance training program (ET) for a period of 11 weeks and the third group ($n = 8$) participated in an 11 week resistance training (RT) program. To minimize diurnal effects on endogenous hormone secretion, all training sessions were conducted between 08:00–11:00 h. The ET program included 5 training sessions per week and consisted of continuous swimming in tepid water (35°C) for up to 60 min. The water was kept slightly turbulent to ensure that the rats remained swimming. Previously, it was found that a similar swim training program elicited training adaptations in rats [11]. In addition, it has been demonstrated that rats recruit both the soleus [12] and the extensor digitorum longus muscles [13] while swimming.

The RT program consisted of climbing a ladder that was 1 meter long, set at an 80° angle with a weight attached to the animal's tail. A cool water spray was used when necessary to induce the animals to climb the full length of the ladder. Each exercise session included 8 repetitions of climbing the ladder, repetitions were performed at 2 min intervals. The resistance applied to the rats was progressively increased by 50 g every other week so that at the end of the program the resistance carried by the animals was 250 g, in addition to body weight. The RT program included 3 sessions per week with 48–72 h rest between sessions. Ladder climbing has been commonly used as a resistance exercise training model with rats and has been shown to evoke a hypertrophic response in the hindquarter muscles of these animals [14, 15].

All animals were individually housed in suspended cages on a 12 h light–dark cycle. They were provided with rat chow and water *ad libitum*. At the end of the 11 week study all animals were sacrificed by carbon dioxide inhalation and weighed. All ET and RT rats were sacrificed 24–48 h following their last exercise session. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Connecticut.

Androgen receptor binding studies

The extensor digitorum longus (EDL) and soleus muscles were selected for study because of their relative fiber type homogeneity and because previously published research has employed these muscles as representative of fast and slow twitch muscle, respectively [16, 17]. The EDL is composed primarily of fast twitch fibers; 56% fast glycolytic and 42% fast oxidative glycolytic [18]. However, when expressed as a percentage of total muscle mass, fast glycolytic fibers account for 79% and fast oxidative fibers 20% of the EDL's weight [18]. The soleus consists mainly of slow twitch or slow oxidative fibers (87%) and, to a smaller degree (13%), fast oxidative glycolytic fibers. When expressed relative to muscle mass, slow oxidative fibers make up 89% of the weight of soleus while fast oxidative glycolytic fibers account for 11% [18].

Following sacrifice of the animals, the EDL and soleus muscles were quickly exposed. These muscles were then surgically removed, dissected free of fat and connective tissue, and weighed. The muscles were frozen in isopentane chilled with liquid nitrogen and stored in liquid nitrogen until analysis.

To determine androgen binding, a modification of the technique described by Hickson *et al.* [10] was used. Muscle tissue was minced with scissors and homogenized on ice with two 45 s passes with a Polytron (Brinkmann Instruments) at a setting of 8 in 4 vol of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.4) and 10 mM molybdate. The homogenate was transferred to centrifuge tubes and spun at 105,000 *g* for 60 min at 4°C (Beckmann Tl-100). The supernatant was transferred to 1.5 ml microfuge tubes along with 0.5 vol of dextran coated charcoal. Samples were incubated overnight at 4°C with shaking and then spun at 13,000 *g* for 5 min. The supernatant was then transferred to chilled microfuge tubes and used for both androgen binding experiments and protein determinations.

To a series of microfuge tubes containing 0.2 ml of muscle extract, 5α-dihydro{1,2,4,5,6,7-^{3}H}-testosterone (sp. act. 106 Ci/mmol, Amersham Co., Bucks, U.K.) was added at increasing concentrations ranging from 0.2 to 200 pmol to determine total binding. A 500-fold excess of unlabeled steroid was then added to one-half of the tubes to determine nonspecific binding. Samples were shaken overnight at 4°C to measure total receptor sites. To each sample, 0.1 ml of dextran coated charcoal was added and samples were incubated for an additional 60 min at 4°C with shaking prior to centrifugation at 13,000 *g* for 5 min to separate bound vs free steroid. Radioactivity in the supernatant was determined by liquid scintillation spectrophotometry. All binding capacity and affinity analyses were performed in triplicate. Dihydrotesterone was used as the ligand because, of the natural androgens, it has the greatest affinity for the androgen receptor and because

methyltrienolone, a synthetic ligand often used in androgen binding assays, has been found to extensively cross-react with the glucocorticoid receptor when it is present in high concentrations [9]. Cytosolic protein was determined by using a modified Lowry assay [19] with bovine serum albumin as standard.

Statistical analysis

Animal body weight, muscle wet weight and muscle wet weight to body weight ratios were analyzed by one-way ANOVA and Scheffe *post-hoc* procedures. To perform statistical assessments of androgen binding, third order polynomial regression analyses were employed to generate steroid binding curves and one-way ANOVA procedures were used to compare the binding curves. In all analyses, statistical significance was set at the 95% confidence interval.

RESULTS

Neither the resistance exercise trained group nor the endurance exercise trained group experienced a significant change in body weight compared to the control group (Table 1). Resistance training caused a significant hypertrophic response in the soleus without a concomitant response in the EDL (Table 1). The ET group demonstrated no significant changes in soleus or EDL wet weights (Table 1). When muscle wet weight to body weight ratios were compared, the only training induced adaptation was found in the soleus muscles of the RT group (Table 1).

The effects of exercise training on androgen binding in the soleus and EDL muscles are presented in Figs 1 and 2, respectively. Statistical analysis of the saturation binding curves indicate that in the EDL muscles, resistance training elicited a significant increase in androgen binding capacity while endurance training did not significantly alter androgen binding. The soleus muscles of the RT group demonstrated a significant decrement in androgen binding capacity. In contrast, the soleus muscles of the ET group were found to have a significantly greater androgen binding capacity.

Both receptor content and affinity (K_d) were determined from these saturation curves. The data from the saturation curves were replotted as Scatchard plots to

Table 1. Effects of exercise training programs on body weight, muscle wet weight and muscle wet weight to body weight ratios

	Control	Endurance exercise	Resistance exercise
Body weight (g)	478.0 ± 12.9	467.5 ± 12.3	487.4 ± 19.2
Soleus wet weight (mg)	150.9 ± 3.6	157.6 ± 3.2	175.8 ± 5.8*
EDL wet weight (mg)	202.1 ± 5.7	209.5 ± 5.6	204.9 ± 10.3
Soleus/body weight†	3.2 ± 0.05	3.4 ± 0.08	3.6 ± 0.09‡
EDL/body weight†	4.2 ± 0.1	4.5 ± 0.1	4.2 ± 0.2

Values are means ± SE. $n = 8$ in each group for body weight values. $n = 16$ in each group for muscle wet weight and muscle wet weight to body weight values.

*Indicates significant difference ($P < 0.001$) from control and endurance training values.

†Muscle wet weight to body weight ratios are expressed as muscle weight/body weight × 10^4.

‡Indicates significant difference ($P < 0.001$) from control value.

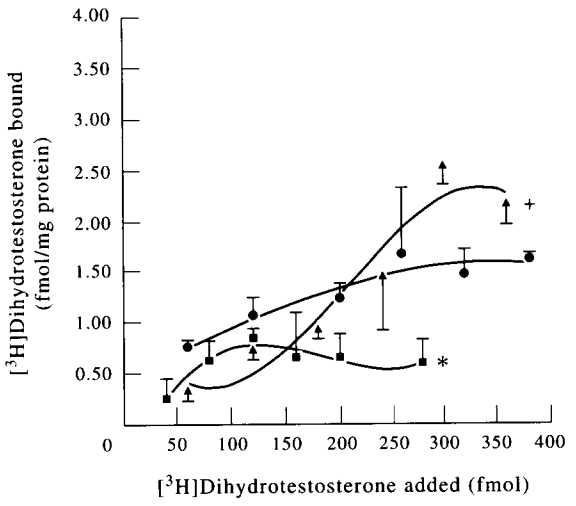

Fig. 1. Androgen binding saturation curves of soleus muscles from control, resistance trained and endurance trained groups. Data points represent specific binding. ●, control (means ± SE); ■, resistance trained (mean ± SE); ▲, endurance trained (mean ± SE). *Indicates significant difference ($P < 0.05$) from control and endurance trained groups. †Indicates significant difference ($P < 0.05$) from control and resistance trained groups. Mean percentages of total androgen binding comprised by specific androgen binding are 39.0, 20.8 and 24.7 for control, resistance trained and endurance trained groups, respectively.

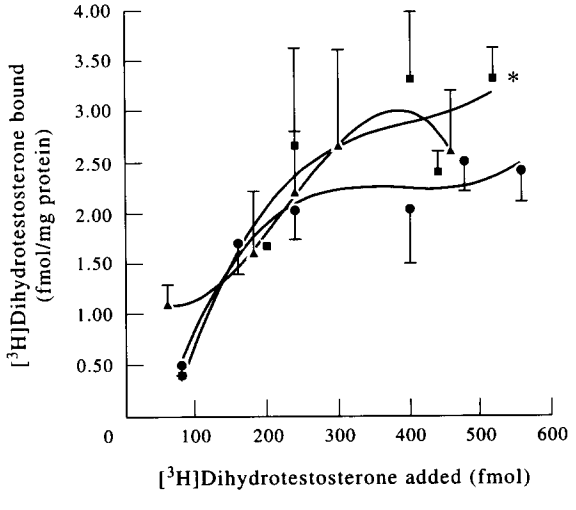

Fig. 2. Androgen binding saturation curves of EDL muscles from control, resistance trained and endurance trained groups. Data points represent specific binding. ●, control (mean ± SE); ■, resistance trained (mean ± SE); ▲, endurance trained (mean ± SE). *Indicates significant difference ($P < 0.05$) from control and endurance trained groups. Mean percentages of total androgen binding comprised by specific androgen binding are 37.9, 27.9 and 27.6 for control, resistance trained and endurance trained groups, respectively.

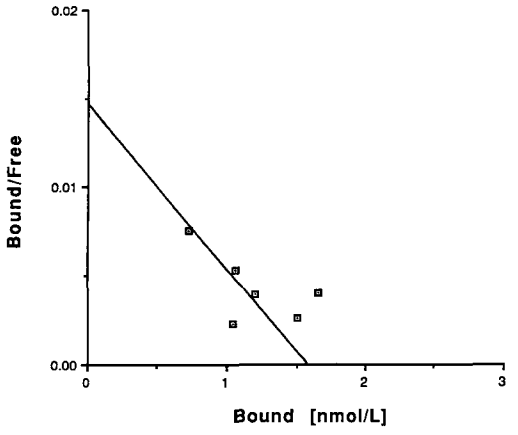

Fig. 3. Scatchard plot of specific androgen binding for control soleus.

confirm our results. As an example, the Scatchard plot for control soleus muscles is presented in Fig. 3. Neither the soleus nor the EDL muscles demonstrated any exercise induced changes in androgen receptor affinity from the control values of 1.6 nM and 2.3 nM, respectively. These results are consistent with those of Tchaikovsky *et al.* [20], who also found that exercise training did not alter androgen receptor affinity in skeletal muscle. This suggests that the changes in androgen binding capacity associated with exercise training are primarily due to alterations in receptor content. It was found that resistance training elicited a 33% increase in maximal androgen binding in the EDL, but a 50% decrease in the soleus. However, endurance training was found to increase maximal androgen binding, and thus receptor content, by 44% in the soleus. The relationship between exercise induced alterations in androgen binding and muscle wet weight can be found in Table 2.

DISCUSSION

Our data are consistent with previous studies suggesting that neither resistance training [15, 21] nor swim training [17] significantly alter body weight in rats. The present study also shows an absence of hypertrophy in the EDL following ET and RT, compared to control animals. Similarly, Tamaki *et al.* [21] found no hypertrophy in the EDL muscles of resistance trained rats. However, the soleus of the RT group did experience a significant hypertrophic response, suggesting that the form of resistance training utilized in the present study substantially recruited the soleus. These results are consistent with Wong and Booth [22], who also found significant soleus muscle hypertrophy in resistance trained rats. Also consistent with the present results, Flavier *et al.* [17] found that swim training did not affect the size of the soleus muscle in rats.

Table 2. Relationship between exercise induced changes in androgen binding capacity and wet weight of soleus and EDL muscles

	Androgen binding capacity	Wet weight
Soleus		
Resistance exercise	Decreased	Increased
Endurance exercise	Increased	No difference
EDL		
Resistance exercise	Increased	No difference
Endurance exercise	No difference	No difference

The exercise training programs employed did elicit alterations in the androgen receptor content of both fast twitch and slow twitch muscle. Interestingly, endurance exercise and resistance exercise brought about different alterations in androgen binding capacity and these exercise induced responses were dependent upon muscle fiber type. These observations may be related to differences in the availability of blood-borne androgens. While the concentration of serum androgens is similar in all muscle fiber types, differences exist in the blood flow [23] and thus the total availability of circulating androgens to slow oxidative and fast glycolytic muscle tissue. In response to this difference in androgen availability, specific muscle fiber types may uniquely alter their androgen receptor contents in order to cope with the stress imparted by exercise.

Androgen binding in soleus and EDL muscles was investigated in the hope of providing insight into the mechanisms by which resistance and endurance exercise differentially affect slow and fast twitch muscle. It was interesting to note that in untrained animals, the EDL had a significantly greater mass than the soleus and that this difference was paralleled by significant differences in androgen binding capacity. That is, the larger EDL also had a greater concentration of androgen receptors than the smaller soleus. However, the relationship between muscle size and androgen receptor content appeared to be altered by exercise training. This perturbation of the relationship between muscle size and androgen binding depends both on the type of exercise training and muscle fiber type. For example, while soleus muscles of the ET group experienced no hypertrophy, they were found to have a significantly greater androgen receptor content than control soleus muscles. Conversely, RT soleus demonstrated significant hypertrophy with a concomitant significant decrement in androgen receptor content. In contrast, there was no evidence that either resistance or endurance training caused any alterations in EDL muscle mass. Yet despite the absence of hypertrophy, the EDL muscles of the RT group demonstrated a significant increase in androgen receptor content. Again, the positive relationship between muscle size and androgen binding seen in untrained animals appears to be altered with exercise.

In the present study, androgen binding was deter-

mined with labeled dihydrotesterone rather than the synthetic ligand methyltrienolone. While this synthetic ligand has the advantage of not reacting with serum binding proteins that may be trapped within the muscle, it is known to significantly cross-react with glucocorticoid receptors. On the other hand, dihydrotesterone is a natural androgen that has a high affinity for the androgen receptor, but does not interact with the glucocorticoid receptor. Although dihydrotesterone does have some affinity for serum binding proteins, the exercise induced relative changes in androgen binding could not be accounted for by interactions of dihydrotesterone with serum proteins. Since it has been found that exercise training affects neither the concentration of serum binding proteins [24] nor blood flow to resting muscle [23], the total availability of blood-borne binding proteins would be no different between trained and untrained muscle. Similarly, serum binding proteins would not account for the difference in androgen binding between untrained soleus and EDL muscles. The present data indicate that compared to the untrained soleus, the untrained EDL has a significantly greater androgen binding capacity even though it has been shown [23] that blood flow, and thus serum binding protein availability, is markedly less in fast glycolytic muscle.

In summary, the data presented here indicate that the stimulus of exercise is sufficiently potent to elicit alterations in the androgen receptor content of skeletal muscle, and that these responses are specific both to the form of exercise and to muscle fiber type. Further, while there appears to be a relationship between relative androgen binding capacity and muscle mass in untrained animals, exercise training disturbs this relationship. Thus, the data reported here reveal no obvious relationship between exercise induced changes in skeletal muscle mass and exercise related alterations in androgen binding capacity. Clearly, further research is needed to help us understand the complex nature of the relationship between exercise induced adaptations of muscle androgen binding properties and their impact on muscle mass and morphology.

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