Chronic Alcohol Intake, Resistance Training, and Muscle Androgen Receptor Content

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ABSTRACT


Introduction: Chronic alcohol intake and resistance training (RT) have opposite effects on muscle physiology. Purpose: This study examined the effect of chronic alcohol intake on androgen receptor (AR) content in skeletal muscle to determine whether this effect was influenced by RT.

Methods: A total of 48 male Sprague Dawley® rats (mass = 456 ± 1 g; mean ± SE) were divided into five groups: baseline (N = 8), sedentary + alcohol (Sed-Al) (N = 8), sedentary + normal diet (Sed-Nml) (N = 8), exercise + alcohol (Ex-Al) (N = 12), and exercise + normal diet (Ex-Nml) (N = 12). Exercise groups completed a 6 1/3–wk “squat” RT protocol; alcohol groups received an ethanol-rich (35% caloric content of alcohol) diet throughout the 6 1/3–wk period. Baseline animals were killed at the onset of the 6 1/3–wk training period.

Results: Western blot analysis showed no effect of alcohol or RT on the AR of the extensor digitorum longus. Alcohol significantly reduced AR content of the rectus femoris (P < 0.05) and prevented RT-induced increases in AR content of the soleus.

Conclusion: Chronic alcohol intake appeared to reduce the AR content of the type IIB fiber-predominant rectus femoris, and this reduction was not affected by RT. In the type I-predominant soleus, chronic alcohol intake alone had no effect but seemed to prevent RT-induced increases in AR content.

Key Words: ANABOLIC, ATROPHY, ENDOCRINE, ETHANOL, EXERCISE, TESTOSTERONE

Skeletal muscle atrophy (i.e., myopathy) afflicts one third to two thirds of chronic alcohol abusers (22,32). This chronic alcohol abuse myopathy (CAAM) is highly selective for type II muscle fibers and can decrease skeletal muscle mass by approximately 30% (22,25,29,32). The decrease in skeletal muscle mass may be caused by a reduction in protein synthesis following both acute (18,24) and chronic (25) alcohol intake.

The interaction of chronic alcohol intake and AR has not been previously studied in skeletal muscle. Muscle protein synthesis (14) and intramuscular uptake of amino acids (2) in men are thought to be stimulated by testosterone. Testosterone-induced cellular regulation is modulated by the binding of testosterone to the androgen receptor (AR) in the cytosol. This testosterone–AR complex stimulates protein synthesis via numerous pathways and mechanisms. Little research exists describing the effect of chronic alcohol intake on AR expression; however, in rat nonmuscle tissue (5,10,11), chronic alcohol intake decreases AR, potentially in a dose-dependent manner (11).

In contrast to alcohol that causes myopathy, resistance training (RT) induces muscle hypertrophy in men (1), which can have an anabolic or anticatabolic effect in populations with various myopathies (12,13,16). The anabolic effect of RT may be enhanced by a resistance exercise–induced increase in several androgenic endocrine responses (17). This response includes an acute increase in circulating testosterone, which is a stimulus for AR production (8). Thus, AR is a candidate androgenic endocrine parameter of interest, as 7–21 d of functional ablation overload increased the AR content of the soleus (21) and plantaris (20) in rats. Acute bouts of intense swimming exercise in rats (31) and resistance exercise in humans (34) also promote AR upregulation. Thus, it appears that the AR content is upregulated following RT, providing a possible mechanism by which RT promotes muscle anabolism.

The combined effects of exercise and alcohol intake have not been well described. The acute androgenic response...
after post resistance-exercise alcohol intake (0.83 g·kg\(^{-1}\) body mass) includes elevated concentrations of serum-free testosterone 60–300 min after exercise (33), but no changes in serum concentrations of total testosterone, luteinizing hormone, serum sex hormone–binding globulin, or the free androgen index (19,33). This acute elevation in serum-free testosterone concentration following postexercise alcohol intake was speculated to be caused by reduced testosterone uptake by the AR owing to a reduction in AR content (33). We could not find any published studies that examined the effect of exercise on AR adaptation to chronic alcohol intake. Thus, this study was conducted to 1) examine the effect of chronic alcohol intake on the AR content in skeletal muscle, and 2) determine whether this effect was influenced by RT.

METHODS

Experimental approach to the problem. To investigate the effect of chronic alcohol intake and RT on AR content, 48 male Sprague Dawley® rats were assigned to one of five treatments: baseline, exercise + alcohol (Ex-Al), sedentary + alcohol (Sed-Al), exercise + normal diet (Ex-Nml), or sedentary + normal diet (Sed-Nml). Alcohol groups were fed a diet in which 35% of total calories were provided by alcohol; the normal groups were feed the same diet, except maltose dextrin was substituted for alcohol. Both diets were administered for 6 1/3 wk. During this 6 1/3–wk period, exercise groups performed a weighted squat training protocol; whereas sedentary groups performed no exercise. Following treatment, muscle samples were homogenized, separated by SDS-PAGE, and then analyzed for AR content using Western blot analysis.

Animals. A total of 48 male 6-month-old Sprague Dawley® rats (Harlan, Indianapolis, IN) were used in this study. All animal-related procedures were approved by The AMC Cancer Research Center institutional animal care and use committee before the rats were acquired and conducted in accordance with the principles of the American College of Sports Medicine. Animals were individually housed (allowing for measurement of dietary intake) in a humidity- and temperature-controlled facility on a 12-h light–dark cycle. Before the treatment period, rats were familiarized with human handling and the RT program over a 3-wk period. Animals were then stratified by body mass (456 ± 1 g; mean ± SE) and randomly assigned to one of five groups: baseline, Ex-Al, Sed-Al, Ex-Nml, or Sed-Nml. The Ex-Al and Ex-Nml groups contained 12 animals and the remaining three groups each contained eight animals. The baseline group was included to elucidate potential effects of maturation on AR and muscle weight.

Resistance training and diet intervention. Resistance training was conducted using the method previously described by Fluckey et al. (15). Briefly, all animals completed a 3-wk familiarization during which no external resistance was used. Electroshock provided the negative feedback stimulus throughout familiarization and actual RT. Because a high occurrence of electrical shocks induces neuroendocrine responses (3), care was taken to minimize the number of shocks used. After operant conditioning, animals required little or no shock to perform the desired training protocol.

The rectangular training cage (210 × 210 × 350 mm) was equipped with an electrifiable metal grid in the floor. A luminescent lever was positioned within the training cage on the wall 220 mm above the floor. The lever would illuminate and 3 s later, a mild shock was administered for 5 s. Rats were operantly conditioned to push the illuminated lever, simultaneously turning off the illumination and the shock. When animals learned to successfully complete the task, the lever would illuminate, forcing rats to perform lengthening and shortening muscle actions that simulated a whole-body standing and a squatting movement (Fig. 1.). At 1 wk into familiarization, unweighted Velcro and denim vests were strapped on animals during training sessions; these vests were used to attach weights to the back and front of the Ex animals during the training period. When animals successfully responded as desired to 90% of light stimuli, the 6 1/3–wk training program was initiated.

All exercise groups performed three resistance exercise sessions per week for a total of 19 sessions. Each training session consisted of 30 repetitions with a 1-min rest interval between repetitions. A minimum of 48 h separated training sessions. The initial training load, including the vest, was set to 93 g (~20% of the animal’s average body mass). The load was gradually increased with training to a final load of 602 g (~120% of the average body mass). Sedentary groups performed no exercise, allowing them to serve as controls. Compliance to the training protocol was considered when animals completed 26 of 30 repetitions in 80% of the sessions.

Baseline animals were killed the day the training program commenced, whereas treatment animals were killed 24 h after the conclusion of the 6 1/3–wk training program. Animals were euthanized by carbon dioxide overdose. Rectus femoris, soleus, and extensor digitorum longus (EDL)
muscles were surgically excised, cleaned of fat and connective tissue, weighed, flash frozen in liquid N2, and stored at −80°C. Muscles were selected based on their involvement during the squat exercise and their fiber-type composition. The soleus and rectus femoris are prime movers in the “squat” exercise used in this study, whereas the EDL is a minor synergist, only stabilizing the back feet. The soleus of Sprague Dawley® rats is composed of approximately 86% type I by mass, whereas the rectus femoris and EDL are composed almost exclusively of type II fibers (~97% and ~98% by mass, respectively) (6). The use of these muscles allows for comparison of muscle fiber type–specific effects of chronic alcohol intake, RT, and their interaction.

Rats were fed Bio-Serv Liquid Rat Diet LD’82 (Bio-Serv, Frenchtown, NJ). The alcohol diet was administered as previously described (27). Briefly, rats were acclimated to the control diet during the first week of the familiarization period. For Ex-Al and Sed-Al, alcohol (ethanol, AAPER, Alcohol and Chemical Co., Shelbyville, KY) was gradually introduced into the diet, increasing from 0 to 35% of caloric intake during the first week of training. A caloric equivalent of maltodextrin was added to the Ex-Nml and Sed-Nml diets in place of alcohol, per manufacturer’s instruction. The alcohol diet was administered ad libitum through the 6 1/3–wk RT period. To ensure equal caloric intake among groups, the alcohol-fed groups’ dietary intake was measured daily, and nonalcohol-fed rats were pair-fed to their respective alcohol-fed counterparts. The weight of the animals was recorded twice per week throughout the entire study. A graphical representation of the study design is presented in Figure 2.

**Total protein.** Total muscle protein concentration was quantified as described previously (4). An approximately 25- to 50-mg midbelly section of frozen tissue was cut on dry ice from one soleus, one rectus femoris, and one EDL muscle from each animal. Each specimen was homogenized on ice for 30–60 s using a 3-mL Pyrex ground-glass homogenizer attached to an overhead stirrer with a variable-speed motor (Wheaton Science Products, Millville, NJ), as a 3.5% (w/v) solution in Mueller buffer (50 HEPES, pH 7.4, 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 mM Na2HPO4·12H2O, 100 mM β-glycerophosphate, 25 mM NaF, 1 mM Na3VO4, 0.5 μg·mL−1 leupeptin, 0.5 μg·mL−1 pepstatin, and 0.3 μg·mL−1 aprotinin). The homogenate was then diluted 1:5 in Mueller buffer, aliquoted, flash frozen, and stored at −80°C until analysis, at which point the homogenate was centrifuged in a microcentrifuge (Biofuge A, Heraeus Sepatech, Germany) for 15 min at 13,000 rpm and 4°C. Protein concentration of the supernatant was determined using the Lowry-based detergent–compatible method (Bio-Rad, Hercules, CA).

**Western blot analysis.** Western blot analysis was performed as previously described (4,20,21). A supernatant volume containing 40 μg of crude protein, calculated from the Lowry assay, was incubated for 15 min at 65°C in an equal volume of protein sample buffer (20% glycerol, 6% SDS, 0.125 M Tris, pH 6.8, and 0.5% bromophenol blue). The protein was then fractionated on an 8% polyacrylamide gel (Novex Pre-Cast Gel, Invitrogen Inc., Carlsbad, CA) at 18 V and approximately 40-50 mA overnight at 4°C. To control for interassay variance, samples from each of the five treatments were included on each gel. A colored molecular weight standard (SeeBlue Plus2 Pre-Stained Standard, Invitrogen Inc., Carlsbad, CA) was run on each gel along with the unknowns. Protein transfer to the membranes was verified by Ponceau staining. Membranes were then washed in 1X TBS (pH 7.6), and subsequently soaked in blocking buffer (1% BSA, 1% normal goat serum, 0.2% sodium azide, and 0.05% Tween 20) for 1 h at 25°C. The AR primary polyclonal rabbit antibody (N-20, Santa Cruz Biotechnology, CA) (1:600 dilution) was added to the membranes in blocking buffer and incubated at 25°C for an additional 1 h. Membranes were washed five times for 5 min each in TBS, and then incubated at 25°C for 1 h with goat antirabbit secondary antibody conjugated with alkaline phosphatase (1:2000 dilution) in blocking buffer. Finally, membranes were equilibrated in Tris buffer (0.1M, pH 9.6), and the secondary antibody was visualized using a chromogenic substrate (0.05 g·L−1 BCIP, 1% DFM, 0.1g·L−1 NBT, 0.1% DMSO, 0.004M MgCl2) allowing for AR quantification by densitometry scanning (ScanJet 6300C, Hewlett-Packard Company, Palo Alto, CA) and the ImageJ software (NIH).

**Statistical analysis.** Data are presented as mean ± SE. Androgen receptor content, muscle mass, muscle mass corrected for body mass, and body mass were examined using two-way ANOVA (training status × diet type). In the event of a significant F-ratio pairwise comparisons were further evaluated using alpha corrected independent t-tests. One-way ANOVA followed by Fisher’s LSD post hoc analysis compared pretraining body mass, androgen receptor content, muscle mass, muscle mass corrected for posttraining (or postsacrifice) body mass for the baseline, Sed-Al,
Sed-Nml, Ex-Al, and Ex-Nml groups. Independent t-tests were used to compare average caloric intake between the exercise and the sedentary groups and training volume and average repetitions between Ex-Nml and Ex-Al. All statistical analyses were performed using SPSS 11.0 for Windows software package (SPSS Inc, Chicago, IL). Using nQuery Advisor software (Statistical Solutions, Saugus, MA), the statistical power for the N sizes used ranged from 0.80 to 0.90. Significance in this investigation was set at $P \leq 0.05$.

RESULTS

**Body and muscle mass.** Two Ex-Al animals and four Ex-Nml animals did not comply with the training protocol and were excluded from the analysis. Body mass, wet muscle mass, and wet muscle mass corrected for body mass are presented in Table 1. Pretraining body mass was similar between groups ($P = 0.95$); however, RT rats had significantly lower posttraining body mass than sedentary rats ($P < 0.05$). No significant effects existed for wet muscle mass between the four experimental groups; however, EDL wet mass was significantly higher ($P < 0.05$) for Ex-Nml than for baseline. For wet muscle mass corrected for body mass, EDL was significantly lower ($P < 0.05$) for Sed-Nml compared with Ex-Nml and baseline. Soleus wet muscle mass corrected for body mass was significantly lower ($P < 0.05$) for Sed-Nml compared with baseline. Soleus wet muscle mass corrected for body mass was significantly lower for both the soleus and the rectus femoris ($P < 0.05$). No significant effects existed for AR content of the EDL.

**Dietary intake and training volume.** The average daily caloric intake during the training period was $85 \pm 1$ kcal for the exercise groups and $86 \pm 1$ kcal for the sedentary groups. The average daily alcohol intake during the training period was $20 \pm 0.3$ g for both Ex-Al and Sed-Al. There was no significant difference in average daily caloric intake or average alcohol intake between the exercise and the sedentary groups ($P = 0.544$). The daily caloric intake for the exercise and sedentary groups are presented in Figure 3. There was no significant difference between Ex-Nml and Ex-Al for average training session repetitions ($29.9 \pm 0.05$ and $29.9 \pm 0.07$, respectively) or total volume lifted during the training period ($184,480 \pm 332$ and $184,567 \pm 332$ g, respectively).

**Androgen receptor content.** Densitometry measurements of the AR content per 40 µg of muscle protein from the soleus, rectus femoris, and EDL are presented in Figure 4. Soleus AR content was significantly greater for Ex-Nml than Ex-Al ($P < 0.05$), and although AR content was not significantly different ($P = 0.09$), there was a large effect size between Ex-Nml and Sed-Nml ($P = 0.09$, effect size$_{pooled SD} = 0.94$, effect size$_{control SD} = 1.50$) and Ex-Al and Sed-Al ($P = 0.09$, effect size$_{pooled SD} = 0.96$). Rectus femoris AR content was significantly lower in rats consuming alcohol than nonalcohol-fed rats ($P < 0.05$). When compared with baseline, Ex-Nml had significantly higher AR content for both the soleus and the rectus femoris ($P < 0.05$). No significant effects existed for AR content of the EDL.

**DISCUSSION**

Chronic alcohol intake decreased AR content of the type IIB fiber-predominant rectus femoris, and appeared to prevent RT-induced increases in AR content of type I fiber-predominant soleus. This is the first study to report, in selected muscles, that alcohol decreases AR content of skeletal muscle and attenuates possible RT-induced upregulation of AR content.
myosin heavy-chain isoforms (28). It appears that type IIB and IIX/D fibers are more susceptible to alcohol-induced adaptations compared with type IIA.

Androgen receptors may be involved in the adaptation to RT, especially through an effect on protein synthesis. In the present study, no main effect was seen of RT on AR content of the soleus, rectus femoris, or EDL. For the soleus, however, large effect sizes were noted between Ex-Nml and both Sed-Nml (effect size = 0.94–1.50) and Sed-Al (effect size = 0.96), and Ex-Nml was significantly greater than baseline. Although no significant differences were found between Ex-Nml and the two sedentary groups, this suggests that RT with a normal diet may elevate soleus AR content. Based on previous findings (7,20,21), we expected that RT would increase AR content of rectus femoris and soleus because these muscles were actively involved in the training protocol. The RT protocol of the present study lasted 6 wk, the weights lifted were low to moderate (~20–120% of body mass), and the repetitions were high (30 per session). The relatively low to moderate weight lifted during the training protocol may not be sufficient stimuli to induce significant increases in AR content. Future studies should examine the effect of heavier loads on AR content. The ablation overload used by Lee et al. (20,21) may provide a greater stimuli than the “squat” protocol used in the present study; this could explain the difference in AR results between the studies. Because a minimal intensity and load stimuli are required for acute increases in testosterone, which has been shown to stimulate AR production (8), the present exercise protocol may not have produced this minimal stimuli during the initial part of the training period.

Although testosterone may be a likely candidate for exercise-induced upregulation of AR, other mechanisms may also play an important role in the AR regulation. In the present study, AR appeared to be upregulated in the soleus but not in the rectus femoris. Thus, the exercise-induced stimuli for AR upregulation seem to not be limited to testosterone. Future investigation should further elucidate potential additional mechanisms for AR regulation and the meaning of such regulation. A direct exercise-induced effect may help explain the difference in AR response in the present study between the soleus and the rectus femoris. Because of recruitment patterns, the type IIB predominant rectus femoris would be less involved in the exercise than the type I predominate soleus, during the initial weeks of light training. The short duration of the training period may also partially explain the absence of increases in muscle mass in exercise groups; when training concluded, animals were likely in the early stage of strength training adaptations (i.e., primarily neural and quality of protein adaptations) (9,23,30).

A statistical significant interaction effect between chronic alcohol intake and RT existed only for the soleus. Alcohol had no effect on soleus AR content of sedentary animals. This finding is consistent with Slavin et al. (29), who found type I muscle fibers resistant to alcohol-induced myopathy. AR content of the soleus was approximately 38% lower for Ex-Al than Ex-Nml, suggesting that chronic alcohol intake eliminates the normal AR adaptation to RT in the soleus. If
similar results occur in humans, our findings indicate that chronic intake of alcohol (35% of caloric intake) can prevent some desirable adaptations to RT.

We expected that RT would attenuate alcohol-induced reductions in AR content of the type II fiber–predominant rectus femoris; however, our results did not support this hypothesis. Compared with Nml animals, Ex-Al and Sed-Al animals had similar reductions in AR content, indicating that RT had no effect on AR content in the rectus femoris. It is possible that greater RT stimuli are required to attenuate the alcohol-induced reduction in AR content. Future research should examine the effect of greater exercise stimuli on AR content. In addition, the effect of exercise and chronic alcohol intake on AR content in additional muscles involved in the squat exercise should be examined.

In conclusion, chronic alcohol intake appears to reduce the AR content of type IIB fiber–predominant rectus femoris, and this reduction is not attenuated by resistance training. In type I fiber–predominant soleus muscles, chronic alcohol intake alone has no effect, but seems to prevent RT-induced increases in AR content.

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