Resistance training associated with the administration of anabolic-androgenic steroids improves insulin sensitivity in ovariectomized rats

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Abstract: The aim of the present study was to investigate the effects of anabolic-androgenic steroids and resistance training (RT) on insulin sensitivity in ovariectomized rats. Adult female Wistar rats were divided into ten experimental groups (n = 5 animals per group): (1) sedentary (Sed-Intact); (2) sedentary ovariectomized (Sed-Ovx); (3) sedentary nandrolone (Sed-Intact-ND); (4) sedentary ovariectomized plus nandrolone (Sed-Ovx-ND); (5) trained (TR-Intact); (6) trained nandrolone (TR-Intact-ND); (7) trained ovariectomized (TR-Ovx); (8) trained ovariectomized plus nandrolone; (9) trained sham; and (10) trained ovariectomized plus sham. Four sessions of RT were used, during which the animals climbed a 1.1 m vertical ladder with weights attached to their tails. The sessions were performed once every 3 days, with between four and nine climbs and with eight to twelve dynamic movements per climb. To test the sensitivity of insulin in the pancreas, glucose and insulin tolerance tests were performed. For insulin sensitivity, there was a statistically significant interaction for the TR-Ovx group, which presented higher sensitivity than the Sed-Intact, Sed-Ovx, and TR-Intact groups. Sed-Intact-ND and TR-Intact-ND groups exhibited higher values of insulin sensitivity than the Sed-Intact group. Except for the TR-Intact group, sensitivity was greater in trained groups than in the Sed-Intact group. There was higher insulin sensitivity in the TR-Intact-ND group than in the Sed-Intact and Sed-Intact-ND groups (P < 0.05). In conclusion, ovariectomy and short-term RT alone induced no change on insulin action. Administration of nandrolone decanoate improved insulin action, mainly when it was associated with RT.

Keywords: ovariectomy, glucose, pancreas, nandrolone decanoate

Introduction

Menopause has been associated with an increased risk of coronary artery disease, diabetes, skeletal muscle wasting (sarcopenia), bone mineral density loss (osteopenia), changes in body composition, lipid profile, fat deposition, and increased inflammatory markers. Ovariectomy is an experimental model used to mimic menopause in animals, inducing an increase in food intake and body weight, insulin resistance, sarcopenia, and muscle force generation. It has been shown that these deleterious effects of ovariectomy can be partially reversed by exercise training and steroid hormone replacement.

Physical training, such as resistance, jumping, or swimming exercise, and wheel or treadmill running by rats has repeatedly been reported to be associated with enhanced insulin sensitivity, glucose transportation, increased bone density, improved immune system, and positive adaptations in cardiac muscle, skeletal muscle, and adipose tissue. However, these positive adaptations in ovariectomized rats have mainly been observed with long-term exercise training.
Anabolic-androgenic steroid (AAS) compounds are synthetic androgens commonly used by athletes to increase physical strength, endurance, and performance\textsuperscript{13} and to modify vascular function.\textsuperscript{14} Although many studies have examined physiological, morphological, and psychological responses to AAS use in males, less is known about the incidence, patterns, and consequences of AAS use in females.\textsuperscript{15–17} AAS compounds may be used therapeutically in women, usually in small doses, to treat aplastic anemia and may also be used as antitumor agents in breast cancer and osteoporosis. In this regard, AASs such as nandrolone decanoate (ND) modulate transcription and translation of pancreatic islets cells and improve insulin action and glucose uptake.\textsuperscript{15,16} However, it is important to note that the World Anti-Doping Agency\textsuperscript{18} lists ND as a prohibited substance, with use of ND considered as doping.

Testosterone increases insulin mRNA levels in vitro as well as in vivo. Additionally, the stimulating effect of testosterone is also observed on insulin promoter activity, content, and release in male rats.\textsuperscript{19} In this regard, nandrolone and other anabolic steroids have been used by athletes to build muscle mass and to enhance weight-lifting performance. A recent placebo-controlled study showed that supraphysiologic doses of testosterone resulted in an increase in muscle mass and strength in humans.\textsuperscript{20}

To the best of the authors’ knowledge, this is the first study to investigate the effects of ovarian hormone absence, short-term resistance training (RT), and AAS therapy on glucose tolerance and glucose-stimulated insulin response. The authors’ initial hypothesis was that resistance exercise associated with AAS would improve tissue response.

Methods

Animals

Fifty adult female Wistar rats (Rattus norvegicus var. albinus, Rodentia, Mammalia) from the breeding colony of the Methodist University of Piracicaba, Brazil, were used in this study. They were approximately 90 days old and had an initial weight of approximately 250 g, plus or minus 30 g. The animals were kept in collective cages (five rats per cage) and they received commercial rodent chow (Labina-Purina, Descalvado, São Paulo, Brazil) and water ad libitum. The room temperature was kept constant at 23°C, plus or minus 2°C, and the room had a cycle of 12 hours of light and 12 hours of dark, with lights on from 0600 to 1800 hours each day.

The Federal University of São Carlos Committee of Experimental Animals approved the research (protocol No. 048/2007). All animal procedures were conducted in accordance with the guide for care and use of laboratory animals from the National Research Council, 1996.

Experimental groups

The experimental design is presented in Table 1. Fifty rats were randomly distributed into the following ten experimental groups (n = 5 animals per group): (1) sedentary (Sed-Intact); (2) sedentary ovariecetomized (Sed-Ovx); (3) sedentary nandrolone (Sed-Intact-ND); (4) sedentary ovariecetomized plus nandrolone (Sed-OVX-ND); (5) trained (TR-Intact); (6) trained nandrolone (TR-Intact-ND); (7) trained ovariecetomized (TR-Ovx); (8) trained ovariecetomized plus nandrolone (TR-OVX-ND); (9) trained sham (TR-Intact-sham); and (10) trained ovariecetomized plus sham (TR-OVX-sham).

The Sed-Intact and Sed-Ovx animals were kept in their cages for 4 days without any type of exercise. The Sed-Ovx animals had their ovaries removed. Animals in the ND groups received an intramuscular injection of ND (Organon do Brasil, São Paulo, Brazil) into the hind limb every training day. Animals in the RT groups underwent four sessions of the proposed RT protocol. Sham groups received an intramuscular injection of the vehicle only (propylene glycol; Galena Química e Farmacêutica Ltda, Campinas, SP, Brazil).

Ovariectomy

Rats were ovariecetomized at 90 days of age, according to the technique described by Kaluo.\textsuperscript{21} Ethyl ether was used as anesthetic in all animals undergoing ovariecetomy. All animals were given 1 week of recovery after the surgical procedure.

RT exercise

The four sessions of RT included climbing exercise, and the sessions were performed once every 3 days. Initially, the rats were adapted to the RT protocol, which required the animals to climb a vertical ladder (1.1 × 0.18 m, 2 cm grid, 80° incline) with weights secured to their tails. The size of the ladder induced the animals to perform eight to twelve dynamic movements per climb. The load apparatus was secured to the tail by wrapping the proximal portion of the tail with a self-adhesive foam strip. A Velcro strap was wrapped around the foam strip and fastened. With the load apparatus secured to the tail, each rat was placed at the bottom of the ladder and familiarized with climbing. If necessary, a stimulus with tweezers was applied to the animal’s tail to initiate movement. At the top of the ladder, the rats reached a housing chamber (20 × 20 × 20 cm), where they were allowed to rest for 120 seconds. This procedure was repeated until the rats voluntarily climbed the ladder three consecutive times, without stimulus.
Three days after this familiarization, the first training session took place. The session consisted of four to eight ladder climbs while carrying progressively heavier loads. For the initial climb, the load carried was 75% of the animal’s body mass. After this, an additional 30 g weight was added, until a load was reached with which the rat could not climb the entire length of the ladder. Failure was determined when the animal could not progress up the ladder after three successive stimuli to the tail. The highest load successfully carried the entire length of the ladder was considered the rat’s maximal carrying capacity for that training session. The next training session consisted of four ladder climbs with 50%, 75%, 90%, and 100% of the rat’s previous maximal carrying capacity, determined in the previous session. During subsequent ladder climbs, an additional 30 g load was added until a new maximal carrying capacity was determined. The RT protocol was adapted from Hornberger and Faccar, according to the needs of the present research.

### AAS treatment

Nonphysiologic doses of ND (0.1 mg/kg) diluted in propylene glycol were injected subcutaneously at the triceps surae on both posterior paws and in an alternating fashion. The drug was injected after every RT session. This dose is comparable with the dose reported as being frequently used for hormone replacement therapy.

The control groups that did not receive AAS treatment (Sed-Intact, TR-Intact-sham, and TR-OVX-sham) received the vehicle propylene glycol.

### Insulin sensitivity tests

#### Glucose tolerance test

Animals were anesthetized with sodium thiopental (40 mg/kg) (Abbott Laboratorios do Brasil, São Paulo, Brazil), and glucose solution (1 g/kg), was administered intraperitoneally after a 4-hour fast for the glucose tolerance test (GTT). Blood samples were collected before (0 minutes) and at 5, 10, 20, 30, 40, 60, and 90 minutes after glucose loading, and glycemia was determined using a glucometer (Advantage®; Boehringer Mannheim, Indianapolis, IN).

Insulin sensitivity was determined by calculating the area above the curve of the GTT. Lower values of area indicate higher insulin sensitivity, while higher values of area indicate lower insulin sensitivity. For animals in the training groups, the GTT was performed 24 hours after the last RT training session.

#### Insulin tolerance test

Twenty-four hours after the GTT, all animals were submitted to an insulin tolerance test (ITT). Briefly, animals were anesthetized with sodium thiopental (40 mg/kg) and a 1 U/kg (1 U/mL) dose of fast insulin (Biobrás, São Paulo, Brazil) was administered intraperitoneally. Blood samples were collected before (0 minutes) and at 2.5, 5, 10, 15, and 20 minutes after insulin loading, and glucose was determined by glucometer.

Tissue sensitivity was assayed by calculating the area above the curve of ITT. Lower values of area indicate higher tissue sensitivity, while higher values of area indicate lower tissue sensitivity.

<table>
<thead>
<tr>
<th>Experimental design</th>
<th>Sedentary groups</th>
<th>Trained groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact</strong></td>
<td>Animals were kept in their cages for 4 days and performed no exercise</td>
<td>Animals underwent four sessions of the proposed RT protocol</td>
</tr>
<tr>
<td><strong>Ovariectomized</strong></td>
<td>Animals had their ovaries removed and performed no exercise</td>
<td>Animals had their ovaries removed and underwent four sessions of the proposed RT protocol</td>
</tr>
<tr>
<td><strong>Nandrolone</strong></td>
<td>Animals received injections of ND into the hind limb, had their ovaries removed, and performed no exercise</td>
<td>Animals received injections of ND into the hind limb every training day and underwent four sessions of the proposed RT protocol</td>
</tr>
<tr>
<td><strong>Ovariectomized plus nandrolone</strong></td>
<td>Animals received injections of ND into the hind limb, had their ovaries removed, and performed no exercise</td>
<td>Animals received injections of ND into the hind limb every training day, had their ovaries removed, and underwent four sessions of the proposed RT protocol</td>
</tr>
<tr>
<td><strong>Sham</strong></td>
<td>Animals received injections of a vehicle every training day and underwent four sessions of the proposed RT protocol</td>
<td>Animals received injections of a vehicle every training day and underwent four sessions of the proposed RT protocol</td>
</tr>
<tr>
<td><strong>Ovariectomized plus sham</strong></td>
<td>Animals received injections of a vehicle, and underwent four sessions of the proposed RT protocol</td>
<td>Animals had their ovaries removed, received injections of a vehicle, and underwent four sessions of the proposed RT protocol</td>
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</table>

**Abbreviations:** ND, nandrolone decanoate; RT, resistance training.
Statistical analysis

All data are presented as mean plus or minus the standard deviation. The statistical analysis was performed initially using the Kolmogorov-Smirnov normality test and the homoscedasticity test (Bartlett criterion). All variables analyzed in the study presented normal distribution and homoscedasticity, which allowed the application of the analysis of variance test (variables: RT + ND + sham × different moments) and F test. Tukey’s post hoc test was applied in the event of a significant ($P < 0.05$) F ratio. The software package Statistica (version 6.1; StatSoft Inc, Tulsa, OK) was used, with a significance level of 0.05.

Results

Effects of ovariectomy and RT on insulin and tissue sensitivity

There was a statistically significant interaction among groups for insulin sensitivity. The TR-Ovx group presented a lower value for area above the curve, indicating higher insulin sensitivity than the Sed-Intact (45.2%), Sed-Ovx (52.4%), and TR-Intact (32.1%) groups (Figure 1A). For the tissue sensitivity, there was a statistically significant difference only between TR-Ovx and Sed-Ovx groups, indicated by the higher rate of glucose removed during the GTT in the TR-Ovx group (Figure 1B).

Effects of ovariectomy and ND

The Sed-Intact-ND group presented higher insulin sensitivity than the other sedentary groups: Sed-Intact, Sed-Ovx, and Sed-OVX-ND groups being 61.6%, 66.6%, and 66.4%, respectively (Figure 2A). Similarly, for the tissue sensitivity, the Sed-OVX-ND group presented improved values compared with the Sed-Intact, Sed-Ovx, and Sed-Intact-ND groups (Figure 2B).

Effects of RT and ND

There was an interaction between the interventions (RT and ND) in insulin sensitivity. The Sed-Intact-ND and TR-Intact-ND groups exhibited higher values of insulin sensitivity than the Sed-Intact group, as shown in Figure 3A. Also, the TR-Intact group showed reduced insulin sensitivity compared with the Sed-Intact-ND and TR-Intact-ND groups (Figure 3A). The TR-Intact-ND group exhibited higher tissue sensitivity than the Sed-Intact and Sed-Intact-ND groups (Figure 3B).

Effects of ovariectomy, RT, and ND

Except for the TR-Intact group, the trained groups (TR-Intact-sham, TR-OVX-sham, TR-Ovx, and TR-OVX-ND) showed greater tissue sensitivity than the Sed-Intact group (Figure 4A). Moreover, the trained groups TR-Intact-sham, TR-OVX-sham, TR-Ovx, and TR-OVX-ND exhibited greater tissue sensitivity than the TR-Intact group (Figure 4A).

The TR-OVX-ND group showed greater tissue sensitivity than the Sed-Intact, TR-Ovx, and TR-Intact-sham groups. Furthermore, the TR-OVX-ND group exhibited higher tissue sensitivity than the TR-OVX-sham group (Figure 4B).

Discussion

The purpose of the present study was to analyze the influence of ovariectomy, RT, and AASs on insulin and tissue sensitivity. The authors’ initial hypothesis was partially confirmed, in that ovariectomy and AASs changed tissue responsivity. Additionally, AASs improved insulin sensitivity, mainly when associated with RT, short-term RT alone caused minor effects on glucose homeostasis, and AASs appeared to modulate insulin action.

Studies on the effects of exogenous AASs on tissue and insulin sensitivity have shown conflicting results. For example,
AAS administration has been reported to increase the transcription and translation of insulin from pancreatic islet cells and to improve insulin action, modulating glucose levels.\textsuperscript{15,16} Marin et al\textsuperscript{25} reported that anabolic steroids alter insulin sensitivity, and therefore a single supraphysiologic dose of ND (250 mg) improved insulin sensitivity, while a higher dose (500 mg) showed no effects on insulin sensitivity.

Regarding the effects of RT in intact animals without the use of exogenous ND, there was no increase in insulin and tissue sensitivity, which might have been related with the short-term exercise training (Figure 1). This can be partly explained by a study by Hernandez et al\textsuperscript{26} in which animals showed a temporal pattern for changes in rate of glucose uptake after resistance exercise: glucose uptake initially decreased after a period of 3 hours and then markedly increased 6 hours later, remaining elevated 12 hours post exercise in male intact rats. Furthermore, chronic exercise training has been reported to promote an increase in insulin receptors associated with increased activity of the PI3-kinase intracellular pathway and the transportation of GLUT4 protein to the plasma membrane, which enhances insulin signaling in tissue.\textsuperscript{27,28} On the other hand, Christ et al\textsuperscript{29} showed that adaptations occurred during training could lead to improved insulin-stimulated muscle glucose uptake without affecting insulin receptor signaling through the PI3-kinase pathway.

Regarding the effects of ND and RT, the present study revealed that, independently of the training status (sedentary or trained), the animals that received ND showed higher sensitivity response, with lower values for area above the curve. This response could be associated with the capacity of AAS administration in modifying influx of calcium related to insulin secretion.\textsuperscript{30} In addition, Hernandez et al\textsuperscript{26} showed that arterial plasma insulin concentrations are not different between trained and sedentary groups when measured during an isotope infusion.

**Figure 2** (A) Insulin sensitivity evaluated in the glucose tolerance test and (B) glucose sensitivity evaluated in the insulin tolerance test for experimental groups of sedentary (Sed-Intact), sedentary ovariectomized (Sed-Ovx), sedentary ovariectomized plus nandrolone (Sed-OVX-ND), and sedentary nandrolone (Sed-Intact-ND) rats (n = 5 animals per group).  
**Notes:** Values are presented by mean ± standard deviation; P ≤ 0.05. *Statistically significant difference compared with Sed-Intact; †compared with Sed-Ovx; ‡compared with Sed-OVX-ND.

**Figure 3** (A) Insulin sensitivity evaluated in the glucose tolerance test and (B) glucose sensitivity evaluated in the insulin tolerance test for experimental groups of sedentary (Sed-Intact), sedentary nandrolone (Sed-intact-ND), trained nandrolone (Tr-intact-ND), and trained (Tr-intact) rats (n = 5 animals per group).  
**Notes:** Values are presented by mean ± standard deviation; P ≤ 0.05. *Statistically significant difference compared with Sed-Intact; †compared with Sed-intact-ND; ‡compared with Tr-intact-ND.
Ovarian hormones, especially estrogen, participate in the regulation of the pancreatic secretion of insulin, insulin sensitivity, and carbohydrate metabolism. The absence of estrogen hormone is associated with several metabolic disorders such as insulin resistance and altered glucose metabolism.\textsuperscript{31–34} The results from the present study revealed no alteration in insulin tissue sensitivity in ovariectomized rats. These results might have been associated with short-term ovariectomy, since a period of 14–21 days seems to be necessary for postoperative hormonal re-adaptation, as for the manifestation of the deleterious effects of estrogen absence.\textsuperscript{26} Furthermore, studies with long-term ovariectomy showed negative effects of it on the morphology of pancreatic islets, insulin secretion, and glucose oxidation.\textsuperscript{35–37} Ovariectomized rats submitted to short-term resistance exercise exhibited an improvement in insulin sensitivity, with lower effects on peripheral sensitivity.

In this study, administration of ND improved insulin action and, as a consequence, increased glucose uptake. Hobbs et al.\textsuperscript{38} showed that acute higher doses of ND in humans enhanced insulin secretion, and glucose oxidation.\textsuperscript{35–37} Ovariectomized rats that received ND showed a moderate rise in blood glucose concentrations, which may result in a moderate rise in blood glucose concentrations and a wide range of other metabolic changes.\textsuperscript{39}

**Conclusion**

In conclusion, the presented study data provide new insights into some aspects of the regulation of insulin and tissue sensitivity. Ovariectomy and short-term RT induced minor change in insulin tissue sensitivity. Acute administration of ND improved insulin action. When associated with short-term RT, ND also improved insulin and tissue sensitivity.

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**Disclosure**

The authors report no conflicts of interest in this work.

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