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Long-term treatment with Nandrolone Decanoate impairs mesenteric vascular relaxation in both sedentary and exercised female rats

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ABSTRACT

Nandrolone Decanoate (ND) is an Anabolic Androgenic Steroid (AAS) that under abusive regimen can lead to multiple physiological adverse effects. Studies of AAS-mediated cardiovascular (CV) alterations were mostly taken from male subjects, even though women are also susceptible to the effects of AAS and gender-specific differences in susceptibility to vascular diseases exist. Here we investigate ND-induced vascular reactivity alterations in both sedentary and exercised female rats and whether these alterations depend on endothelium-derived factors. We show that chronic exposure of female Wistar rats to ND (20 mg/Kg/week for 4 weeks) impaired the vascular mesenteric bed (MVB) reactivity to vasodilator (acetylcholine) agonist. The endothelium-dependent Nitric Oxide (NO) component was reduced in ND-treated rats, whereas neither the endothelium-derived hyperpolarizing factor (EDHF) component nor prostanoids were altered in the MVBs. Endothelial dysfunction observed in ND-treated rats was associated with decreased eNOS (Ser^{1177}) and Akt (Ser^{473}) phosphorylation sites and upregulation of iNOS and NADPH oxidase expression. Exercise training by weight lifting in water did not improve the vascular alterations induced by ND treatment. ND treatment also significantly reduced the serum levels of estradiol in females, overriding its CV protective effect. These results help uncover the role of ND modulating endothelial function in the setting of CV disease caused by the abuse of AAS in females. If this translates to humans, young women abusing AAS can potentially lose the cardio protective effect rendered by estrogen and be more susceptible to CV alterations.

1. Introduction

Originally developed for clinical purposes [1], Anabolic Androgenic Steroids (AAS) have been illicitly used for enhancement of physical appearance and performance and became a health concern [2]. Regardless of strong evidences supporting the physical, psychological, and behavioral negative effects due both acute and chronic AAS abuse [3,4], the AAS self-administration by professional athletes has spread into the general population in the past decades [3], being particularly more common within adolescents and young adults [5,6]. Although AAS abusers are greater in men rather than in women, studies point out that a few hundreds of thousands of current AAS users are girls and young women [6–8].

The great majority of AAS adverse effects are permanent [3,9] and the undesirable consequences of steroids abuse have a particular detrimental effect in women and adolescents [4,10]. For instance, the virilization and premature epiphyseal closure are likely irreversible in those groups [11]. Additional relevance stands for the disruption of reproductive and neuroendocrine function in females [12,13], engendering additional concerns of the effects of exposure to high levels of synthetic steroids in women.

Generally, doses of abuse of AAS appear to produce a range of negative cardiovascular effects, such as cardiomegaly, arrhythmias, myocardium infarction, hypertrophy, hypertension, dysfunction in tonic cardiac autonomic regulation, and increased muscle sympathetic nerve activity [14–16]. Additionally, AAS nandrolone, one of the most frequently consumed AAS among abusers [17], has also

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been associated with the development of a cardiac pro-inflammatory state by increasing both inflammatory cytokines and local angiotensin-converting enzyme [14,18].

It is well known that the endothelium plays a crucial role in the control of vascular function by the release of several potent vasoactive factors that control vascular tone, such as Nitric Oxide (NO), endothelium derived hyperpolarizing factors (EDHFs) and Prostaglandins (PGs) [19]. Alterations in vascular reactivity are frequently a consequence of a dysfunctional endothelium, and these changes may contribute to the beginning and development of cardiovascular diseases [20].

Even though the increased cardiovascular risks associated with AAS abuse are well recognized, the effect of supra-physiological doses of androgens in the vasculature remains poorly understood. Prior studies suggest that AAS can cause an impairment of endothelial function and vascular reactivity in both humans and animals [21–23]. Mechanistically, the disruption of the endothelial function seems likely to be involved with increased levels of androgens and their impact on the function of NO, PGI_2, and EDHF [16,24–26]. Furthermore, some reports have shown that AAS nandrolone attenuates the physiological vascular adaptations promoted by aerobic physical exercise [25,27]. Interestingly, however, most of the AAS users are practitioners of resistance exercise [28], a moderate to high intensity physical strength activity that relies more on the anaerobic metabolic pathway [29]. Despite of some reports showing aerobic exercises leading to vascular adaptations that ultimately improve the arterial function [30,31], resistance training has been reported to impair, improve or not affect vascular function [32–34]. Therefore, determining possible resistance exercise-induced alterations in the vascular reactivity of ND-treated animals is particularly important.

Evidences reported in the literature showing the effects of AAS on the vasculature were mostly conducted in males, yet the results remain elusive [25,35,36]. In addition to that, sex differences in the regulation of vascular homeostasis have been reported by several studies [37,38] and support the idea that AAS vascular effects could be sex-specific, suggesting that conclusions drawn from studies conducted in males should not be extrapolated to females. Despite of comprehensive evidences indicating the cardio protective effects of estrogen on vascular endothelial function and vascular reactivity [39,40], only a few studies describing AAS effects on vessels and vascular function have been performed in females [41,42]. Moreover, the role of endothelium and of the different components of endothelium-dependent dilatation were not investigated in those studies and it remains unclear whether AAS can cause vascular dysfunction in females.

Therefore, we performed this study to investigate both the isolated and combined effects of high-dose AAS-nandrolone and exercise training on vascular reactivity in mesenteric beds from female rats and the involvement of some key endothelial factors in the vascular response. Because we observed that AAS-nandrolone treatment reduced estrogen levels, we also sought to compare the vascular reactivity profiles between ovariectomized (an estrogen deficiency model) and ND treated female rats. We further hypothesize that high doses of AAS-nandrolone can lead to vascular dysfunction induced by alterations in the endothelium-derived vasorelaxant factors pathways in female rats.

2. Materials and methods

2.1. General procedures

2.1.1. Animals

All experimental procedures were performed in Wistar female rats weighting from 180 to 200 g provided by the university facility. The animals were housed individually in plastic cages and maintained under standard laboratory conditions (22 °C; 12/12 light–dark cycle; lights on at 7:00AM). Water and food were available *ad libitum*. Animal care procedures were conducted in accordance with the biomedical research guidelines for the care and use of laboratory animals, as stated by the Brazilian College of Animal Experimentation (COBEA). The experimental protocol was reviewed and approved by the Ethics Committee for the Use of Animals of the Federal University of Espırito Santo (protocol number 031/2012).

2.1.2. Measurement of food intake, weight gain and adiposity index

During the study, daily food intake and body weight were measured every morning. For each rat, the amount of daily food intake was determined by subtracting the amount of food remaining from the previous measurement. At the end of the experimental protocol, the animals were sacrificed and the adipose tissue fat pads were then dissected and weighed. Total body fat was measured as the sum of the following individual fat pad weights: gonadal fat + retroperitoneal fat + visceral fat. The adiposity index was calculated as (total body fat/final BW) × 100 [43].

2.1.3. Estrous cycle determination

Daily vaginal smears were taken from each female rat as previously described [44] to confirm whether their estrous cycles were proceeding normally [(i) estrus, (ii) metaestrus, (iii) diestrus, and (iv) proestrus]. The vaginal epithelial cells were examined via a microscope for at least 7 consecutive days before the experiment. The swabs were performed between 8:00 and 10:00 A.M. to maintain consistency. We standardized the experimental protocols when the animals that did not receive ND were in proestrus phase to avoid any influences caused by the hormonal variation at different phases of the estrous cycle [45] in the parameters studied bellow.

2.1.4. Hormone levels

Blood samples were collected on the day of the vascular reactivity experiments by arterial puncture before isolating the Mesenteric Vascular Beds. Estradiol and testosterone levels were determined in the serum using commercially available ELISA kits (DRG International Inc., Springfield, NJ, USA), according to the manufacturer's guidelines. All the samples were measured in duplicate.

2.1.5. Vascular reactivity studies in the perfused isolated mesenteric vascular bed (MVB)

The mesenteric vascular beds (MVBs) were isolated as described by us previously [44,46]. Briefly, the rats were anesthetized with ketamine and xylazine (90 and 10 mg/kg, i.p., respectively), the abdominal cavity was exposed, and the superior mesenteric artery was dissected and cannulated with a polyethylene catheter. The MVB was excised from the intestinal loops, placed in a chamber and the preparation was perfused with oxygenated (95% O_2 – 5% CO_2) Krebs' solution [(in mM) 130 NaCl, 4.7 KCl, 1.6 $CaCl_2{\cdot}2H_2O$, 1.17 $MgSO_4{\cdot}6H_2O$, 14.9 $NaHCO_3$, 1.18 KH_2PO_4, 0.026 EDTA, and 11.1 glucose, pH 7.4] at a constant flow rate of 4 mL/min and maintained at 37 °C. The MVBs were perfused for 30 min for the stabilization of the basal perfusion pressure. Changes in the perfusion pressure, which reflect peripheral resistance, were measured with a pressure transducer (Spectramed P23XL) connected to an acquisition system (MP100A, BIOPAC System, Inc., Santa Barbara, USA). After the stabilization period, the MVB was constricted by Krebs' solution containing NE capable of increasing the basal perfusion pressure by 90–120 mmHg. After establishing a steady level of contraction, dose-response curves were obtained by bolus injections (0.1 ml) of acetylcholine (Ach) (1.68×10^{-10} to 1.68×10^{-2} M) into the perfusion solutions of