Vascular Sensitivity to Phenylephrine in Rats Submitted to Anaerobic Training and Nandrolone Treatment

Tatiana Sousa Cunha, Maria José Costa Sampaio Moura, Celene Fernandes Bernardes, Ana Paula Tanno, Fernanda Klein Marcondes

Abstract—The effect of anaerobic physical training and nandrolone treatment on the sensitivity to phenylephrine in thoracic aorta and lipoprotein plasma levels of rats was studied. Sedentary and trained male Wistar rats were treated with vehicle or nandrolone (5 mg/kg IM; twice per week) for 6 weeks. Training was performed by jumping into water (4 sets, 10 repetitions, 30-second rest, 50% to 70% body weight load, 5 days/week, 6 weeks). Two days after the last training session, the animals were killed and blood samples for lipoprotein dosage were obtained. Thoracic aorta was isolated and concentration–effect curves of phenylephrine were performed in intact endothelium and endothelium-denuded aortic rings in the absence or presence of N\textsuperscript{G}-L-arginine-methyl ester. No changes were observed in endothelium-denuded aortic rings. However, in endothelium-intact thoracic aorta, anaerobic physical training induced subsensitivity to phenylephrine (pD\textsubscript{2} = 7.11 ± 0.07) compared with sedentary group (7.55 ± 1.74), and this effect was canceled by the inhibition of nitric oxide synthesis. No difference was observed between trained (7.22 ± 0.07) and sedentary (7.28 ± 0.09) groups treated with nandrolone. Anaerobic training induced an increase in high-density lipoprotein levels in vehicle-treated rats, but there were no changes in nandrolone-treated groups. Training associated with nandrolone induced an increase in low-density lipoprotein levels but no change in the other groups. If altering endothelium-dependent vasodilation is considered to be a beneficial adaptation to anaerobic physical training, it is concluded that nandrolone treatment worsens animals’ endothelial function, and this effect may be related to lipoprotein blood levels.

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Key Words: aorta ■ endothelium ■ exercise ■ lipoproteins ■ nitric oxide ■ phenylephrine ■ rats

Anaerobic androgenic steroids (AAS) are formed from testosterone or one of its derivatives and present both anaerobic and androgenic effects. Although the therapeutic indications of AAS are hypogonadism and protein metabolism deficiency, high doses of AAS are frequently used by persons in good health to improve physical performance and appearance, and this practice results in serious health risks. With regard to the cardiovascular system, it is well known that AAS can cause stroke, ischemic heart disease, myocardial infarction, electrocardiographic alterations, and sudden cardiac death. However, there are few studies about their effects on vascular reactivity. Ferra et al observed an inhibition of endothelium-dependent and endothelium-independent relaxation in the aortic rings of rabbits treated with nandrolone. Vasoconstrictor responses to angiotensin and tyramine, but not to noradrenaline, and vasodepressor responses to acetylcholine are reduced in testosterone-treated dogs.

Although it is well documented that postexercise hypotension results from a decrease in systemic vascular resistance after aerobic exercise, both in humans and animals, there are no data about the vascular effects of anaerobic training. Because the use of supraphysiological doses of AAS is frequently associated with anaerobic exercise, the aim of this study was to determine its influence, as well as that of nandrolone treatment, on the sensitivity to phenylephrine in the thoracic aorta of rats.

Because vascular wall properties are influenced by high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels, and because androgenic steroids have different effects on blood concentration of HDL and LDL, the serum levels of these lipoproteins were also evaluated.

Methods

Animals

Fifty-three male Wistar rats (60 days old) were housed in a temperature-controlled room with a 12:12-hour light–dark cycle. Water and rodent chow were provided ad libitum. After approval by the institutional Committee for Ethics in Animal Research (protocol number 391-1), the rats were randomized into 4 groups: sedentary...
Training Protocol
The anaerobic training protocol has been described previously. Briefly, after 1 week of water adaptation the rats were exercised by jumping into the water (30±2°C), once per day for 5 days, for 5 weeks, carrying a load of 50% body weight strapped to the chest, with 30 seconds of rest between each set of jumps. In the third and fourth training weeks, the animals performed the same exercise carrying a load of 60% body weight, and in the last week, this load was adjusted to 70% of body weight. The weights were attached to the animals’ chests not only to avoid flotation but also to make them exercise against an overload, simulating the squat exercise in water.

Blood Sampling, Tissue Collection, and Analytic Methods
Forty-eight hours after the last exercise session, the rats were anesthetized under halothane, and blood was immediately collected from left renal vein and centrifuged to separate the serum. Seric total cholesterol, HDL, and LDL were determined using commercially available kits (Labortal). The animals were killed by pneumothorax and the thoracic aorta was excised and dissected free of fatty and connective tissue before being cut into 2 3-mm-long to 5-mm-long rings.

Concentration–Effect Curve
Two matched rings, taken from the same animal, were obtained from the middle portion of each aorta and used for functional assays as previously described. Briefly, cumulative concentration–effect curves for phenylephrine (PE) were obtained from one ring with intact endothelium and the other without endothelium. To evaluate the role of nitric oxide (NO) on the modulation of thoracic aorta sensitivity to PE, rings with intact endothelium obtained from other animals were incubated for 40 minutes with the NO synthesis inhibitor, Nω-ω-arginine-methyl-ester (L-NAME) (10−5 M) before concentration–effect curves. Changes in thoracic aorta sensitivity to PE were evaluated by determining the concentration that produced 50% of the maximum response (EC50) and were expressed as the mean negative logarithm (pD2).

Statistical Analysis
Statistical differences were determined by 2-way analysis of variance (ANOVA) followed by the Tukey test. Differences were considered significant at P<0.05. The results are presented as means±SEM.

Results
Neither physical training nor nandrolone treatment altered the maximum response of isolated thoracic aorta to PE (Table). There was subsensitivity to PE in endothelium-intact thoracic aorta rings isolated from trained rats treated with vehicle when compared with the respective sedentary group also treated with vehicle (Table), with 2.8-fold shift to the right in the concentration–effect curve (Figure 1) (P<0.05). However, physical training did not induce any changes in the sensitivity to PE in endothelium-intact thoracic aorta rings isolated from AAS treated animals. There was no difference among the groups in the sensitivity to PE of endothelium-denuded aortic rings (Table and Figure 1) (P>0.05). In the presence of the NO synthesis inhibitor, L-NAME, no alterations in the sensitivity to PE were observed in endothelium-intact thoracic aorta (Figure 2) (P>0.05). No changes were observed in the serum concentration of total cholesterol among the groups (Figure 3) (P>0.05). Rats treated with vehicle and submitted to anaerobic physical training presented higher levels of HDL compared with the respective vehicle sedentary group and trained AAS-treated group (Figure 3) (P<0.05), with no changes between AAS-treated sedentary and trained rats (Figure 3) (P>0.05). Although anaerobic training did not change LDL seric levels in vehicle-treated animals, trained rats treated with nandrolone presented higher levels of LDL compared with the respective sedentary group (Figure 3) (P<0.05).

Discussion
According to clinical, epidemiological, and pathological studies, aerobic physical activity appears to play an important role in the treatment and prevention of several cardiovascular diseases. Previous studies demonstrated that regular exercise could reduce sympathetic tone,13 depress the pressor response to norepinephrine,14 attenuate baroreflex,15 and improve the bioavailability of endogenous NO in rodents and humans.16,17 Despite some well-documented adaptations with regard to vascular responsiveness after aerobic training, there are no data about the effect of anaerobic training on vascular reactivity.

The anaerobic nature of the high-resistance exercise protocol used in this study was confirmed by a previous study,18 because blood lactate concentration after the second set of jumps was >5.5 mmol/L, the maximal lactate steady-state for rats.19 Moreover, it was demonstrated that this protocol was also efficient for inducing skeletal muscle hypertrophy in rats.19

Because supra-physiological AAS dose users are frequently anaerobic exercise practitioners, it was of interest to evaluate the effects of weight-lifting and AAS treatment on vascular responsiveness. Therefore, the aorta sensitivity to PE was analyzed in rats. Anaerobic training induced subsensitivity to PE in thoracic aorta isolated from vehicle - treated rats compared with the respective sedentary group, with no changes between the 2 groups (trained and sedentary) treated with AAS.
It has been hypothesized that the decrease in arterial pressure, induced by physical exercise, is related to an increase in the vasodilator and a decrease in the vasoconstrictor responsiveness of blood vessels. Support for this hypothesis has been shown in response to different physical exercise programs. Abdominal aorta obtained from treadmill-trained rabbits presented higher endothelium-dependent vasorelaxation induced by acetylcholine (ACh). Thoracic aorta from rats submitted to swimming during 6 weeks showed a decrease in response to PE, but not to potassium chloride, as also reported in porcine coronary arteries. The aortic sub-sensitivity to PE observed after anaerobic training in the present study suggests that even in response to the metabolic demands of different physical exercises (aerobic versus anaerobic), vascular adaptations are established to promote a decrease in arterial blood pressure. Moreover, this response can be related to endothelial adaptations to the higher blood flow induced by aerobic and anaerobic exercise training.

This subsensitivity to PE was only observed in endothelium-intact aortic rings, with no changes in endothelium-denuded aorta, indicating that this effect is endothelium-dependent. Moreover, this effect was canceled by aorta pre-incubation with the nitric oxide synthase (NOS) inhibitor L-NAME.

In the presence of NOS inhibitors, the exercise-induced enhancement of endothelium-dependent vasorelaxation is abolished. Thoracic aortas isolated from swimming trained rats were also subsensitive to PE, and this effect was caused by an increase in NO production by endothelial cells. Delp and Laughin reported an upregulation of the expression of endothelial cell NOS mRNA in thoracic aorta from rats submitted to 4 to 10 weeks of training. These results are consistent with others who have reported an increase in NOS mRNA levels in aorta of exercise-trained dogs, and in coronary resistance vessels of exercise-trained pigs. The expression of inducible NOS and endothelial NOS mRNA were increased in endothelial cells by chronic treadmill training, and chronic exercise blunted PE-induced vascular responses, probably by increasing NO release via inducible NOS, in rat thoracic aorta.

In this context, the high-intensity training protocol used here may also cause greater release of NO, which diminishes or antagonizes the vasoconstrictor effects of PE. This could explain the subsensitivity observed in thoracic aorta isolated from trained rats compared with the sedentary group. In support of this hypothesis, the lower sensitivity to the vasoconstrictor effect of PE in the trained group was canceled by the inhibition of NO production by L-NAME.
However, when anaerobic training was associated with AAS treatment, no changes in aorta responsiveness to PE were observed, suggesting that this hormone interferes in NO production or in some other mechanisms involved in the vascular effects of PE.

Different results have been reported concerning the effects of AAS in vascular reactivity. Perfused canine hind limb vasoconstrictor responses to angiotensin and tyramine and vasodilator response to ACh were reduced in testosterone-treated animals, whereas the vasoconstrictor effect of noradrenaline was not altered. However, the pressor response elicited by noradrenaline was reduced by testosterone in spinal cats.31

Moreover, the testosterone vascular effect mechanisms have not also been clearly identified. It has been demonstrated that in rat aorta, testosterone causes endothelium-dependent vasorelaxation, which is likely to be mediated by activation of NO activity.32 However, NOS inhibition had no effect on vasorelaxation by testosterone in rabbit aorta.33 Other studies reported that endothelium- and NO-independent vasorelaxation induced by testosterone may involve activation of potassium channels in vascular smooth muscle cells.32,33

There appears to be no studies analyzing the combined effects of anaerobic training and AAS treatment on vascular reactivity. There is one report about the effect of nandrolone decanoate on the vascular reactivity of sedentary male rabbits.6 Nandrolone reduced endothelium-dependent relaxation induced by ACh and endothelium-independent relaxation induced by NO, sodium nitroprusside, and 8-bromo-cGMP in thoracic aortas from these animals. The authors also demonstrated that this effect was mediated by diminished production of ACh and NO vasorelaxation mediator, cGMP. It was concluded that nandrolone decanoate seems to decrease NO-induced vasodilatation by decreasing guanylate cyclase activity in the vascular smooth cells.6

This effect could also explain the data presented here concerning the association of AAS treatment and anaerobic training. In the present study, nandrolone decanoate canceled the subresponsiveness of PE observed in thoracic aorta isolated from trained rats and this subsensitivity seems to be related to major NO synthesis induced by chronic exercise. According to Ferrer et al,6 nandrolone decanoate decreased the smooth muscle cell response when anaerobic training was associated with AAS treatment in the present study, and it is suggested that nandrolone seems to block the vasodilatory response to the higher NO synthesis induced by physical training in the thoracic aorta isolated from rats.

However, in contrast to observations by Ferrer et al,6 in the present study nandrolone had no effect on vascular response to PE in sedentary rats. Animal species, AAS treatment duration, and dose administration regimens may explain this difference; in the present study, rats were treated with 5 mg/kg of nandrolone decanoate, twice per week for 6 weeks, whereas in the Ferrer et al6 study, rabbits were treated with one administration of the same AAS, 10 mg/kg once per week for 4, 8, or 12 weeks.

In the present study, it was also observed that anaerobic training induced an increase in HDL seric concentration in rats. Its association with nandrolone treatment canceled this effect and resulted in higher LDL levels compared with the respective AAS sedentary group. According to previous studies, these changes in lipoprotein profile may be related to AAS treatment, and these alterations are able to modify vascular reactivity. It has been reported that the incubation of isolated rabbit aorta with LDL reduced endothelium-dependent relaxation34,35 and guanylate cyclase activation induced by vasodilators.36 This effect is an early marker of atherosclerosis and is antagonized by HDL.36 According to these reports, it may be suggested that the increase in LDL levels observed in trained rats treated with nandrolone in the present study may explain why thoracic aorta isolated from

Figure 3. Seric levels of lipoproteins from sedentary (S) or trained (T) rats, treated with vehicle (V) or nandrolone (N). *P<0.05 compared with SV and TN groups. **P<0.05 compared with SN group (2-way ANOVA + Tukey; P<0.05). N=9 to 10.
these animals did not present subsensitivity to PE. Higher LDL levels induced by nandrolone could decrease endothelium-dependent relaxation and guanylate cyclase activation, and thus show opposite effects to those induced by training. Moreover, the higher HDL levels observed in trained rats could prevent the effects of LDL in the thoracic aorta.

However, in opposition to some reported effects of AAS on lipoprotein profiles, no changes were observed in sedentary rats treated with nandrolone decanoate. In rabbits, decreased HDL was observed after intramuscular administration of nandrolone decanoate at 4, 8, and 12 weeks, whereas LDL was reduced, unaltered, or increased at 4, 8, and 12 weeks, respectively.\(^6\) Differences between rat and rabbit hepatic AAS metabolizing enzymes could account for the diverse effects observed in lipoprotein blood levels in these species.

In humans, the effects of anabolic steroids on lipoprotein levels are not clear. Some studies have shown that AAS causes a reduction in the plasmatic concentration of vascular protective HDL\(^2\) and an increase in vascular aggressive LDL.\(^3\) However, the effects of physiological levels of testosterone were not consistent: testosterone replacement in men is reported to be associated with decreased LDL,\(^3\) no change in HDL levels,\(^4\) and a favorable effect on HDL levels.\(^4\) In addition, the administration of nandrolone decanoate (100 mg/kg, intramuscularly), once per week for 6 weeks did not change HDL or LDL levels of 24 volunteers.\(^9\) These discrepancies can be related to the chemical structure of AAS and administration route. Oral administration of 17-alkylated AAS seems to be related to increased HDL and decreased LDL levels, not to oral or parenteral administration of 17-beta sterified AAS, like nandrolone decanoate, \(^9\) for example. Some reports showed that there is a greater induction of HDL catabolic enzyme hepatic triglyceride lipase by oral versus parenteral AAS administration.\(^2,4\)

In reports on subjects who self-administered AAS, it is difficult to determine the steroid doses that have in fact been used, considering that there is no consistency among such users. Those who consume AAS without any therapeutic indications generally combine at least one oral and several parenteral AAS simultaneously.\(^1,1^1\) Therefore, when evaluating AAS effects on lipoprotein levels, these factors should be taken into account and may explain the disparate results.

In summary, for the first time to our knowledge, it was demonstrated that chronic anaerobic physical training causes a decrease in aorta vascular sensitivity to PE, a beneficial adaptation that seems to be related to the enhancement of NO production and to higher blood concentration of HDL. However, nandrolone treatment blocks this subsensitivity to PE promoted by physical training, probably because this hormone decreases NO vasodilating effect and enhances blood concentration of LDL, damaging the endothelial function of trained animals.

**Perspectives**

The present study provides evidence that supra-physiological doses of AAS not only induce side effects but also avoid the development of adaptations promoted by physical training. Although the underlying physiological mechanisms of these findings warrant further investigations, it is possible to say that the findings presented here could be helpful in prevention programs for AAS abuse.

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**References**

37. Webb OL, Laskarzewski PM, Glueck CJ. Severe depression of high-density lipoprotein cholesterol levels in weight lifters and body builders by self-administered exogenous testosterone and anabolic-androgenic steroids. *Metabolism.* 1984;33:971–975.
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