



Enhanced vasoconstriction and reduced vasorelaxation induced by testosterone and nandrolone in hypercholesterolemic rabbits

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Abstract

Androgenic-anabolic steroids (AAS) are widely abused by athletes and this abuse has been associated with many serious circulatory events including sudden cardiac death, myocardial infarction and cardiac hypertrophy. The effect of chronic treatment for 16 weeks with testosterone ($25 \text{ mg}^{-1} \text{ kg}^{-1} \text{ week}^{-1}$) and nandrolone ($50 \text{ mg}^{-1} \text{ kg}^{-1} \text{ week}^{-1}$) on serum lipids of male hypercholesterolemic New Zealand rabbits was investigated. The responses of isolated rabbit aortic rings to some vasoconstrictors (epinephrine, serotonin and endothelin-1) and vasodilators (adenosine and sodium nitroprusside) were also measured after treatment. Testosterone and nandrolone significantly reduced HDL-cholesterol levels, potentiated vasoconstriction responses to epinephrine, serotonin and endothelin-1, and attenuated vasorelaxant responses to sodium nitroprusside in rabbits. Nandrolone also caused a significant increase in LDL-cholesterol levels. No significant changes in adenosine relaxation were found in rabbits. The results of the present study showed that the abuse of AAS in presence of hypercholesterolemia can enhance atherogenicity and vasospasm as well as attenuation of vasorelaxation. Therefore the abuse of AAS is harmful to the vascular system and should be prohibited.

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1. Introduction

Androgenic-anabolic steroids (AAS) are synthetic derivatives of testosterone, the main natural androgen. The primary indication for androgens is as replacement therapy in male hypogonadal disorders caused by either pituitary or testicular disorders [1]. The abuse of androgens by athletes in the belief that their athletic performance will be improved constitutes a widespread form of drug abuse [2]. Athletes not only take higher doses than are indicated for therapeutic purposes, but also commonly use several anabolic steroids at once, popularly known as “Staking” [1] and “Pyramid” their doses in cycles of 6–12 weeks [3].

The abuse of AAS has been associated with many serious circulatory events. As example, acute myocardial infarction [4–6] and sudden cardiac death (SCD) resulting mainly from hypertrophic cardiomegaly [7,8] were reported in athletes abusing AAS. Ventricular tachycardia was presented in a young body builder abusing the anabolic steroid, stanozolol [9]. Also, a young man developed a stroke after taking

AAS to increase his muscle mass [10]. Severe circulatory events were also reported in patients receiving steroids therapeutically, including transient ischaemic attack in a patient with congenital protein C deficiency during treatment with stanozolol [11] and acute myocardial infarction in a patient with human immunodeficiency virus (HIV) associating his use of anabolic steroid [12]. This study was undertaken to investigate some of the possible causes of the adverse vascular disorders associated with AAS abuse in presence of hypercholesterolemia.

2. Materials and methods

2.1. Drugs and chemicals

Nandrolone decanoate as Deca-Durabolin ampoules, $50 \text{ mg}/1 \text{ ml}$ (Nile Co., Egypt, under license from N.V. Organon, Oss, Holland). Testosterone enanthate as Primo-teston-Depot ampoules, $250 \text{ mg}/1 \text{ ml}$ (CID Co., Egypt, under license from Schering AG, Germany). Endothelin-1 (ET-1), 5-hydroxytryptamine (5-HT), creatinine sulphate and adenosine were purchased from Sigma Chemical Co., St. Louis, MO, USA. Epinephrine, as ampoules, $250 \text{ } \mu\text{g}/1 \text{ ml}$

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(Misr Co., Egypt). Sodium nitroprusside and cholesterol were obtained from WinLab, Leicestershire, UK.

2.2. Animals

Fifteen male New Zealand white rabbits weighing 2 ± 0.3 kg, purchased from local source, were used in this study. All rabbits were fed moderately atherogenic diet consisting of normal rabbit pellets supplemented with 0.3% w/w cholesterol [13,14]. Rabbits were divided into three groups of five animals each.

Group (1): Nandrolone-treated group, received ($25 \text{ mg}^{-1} \text{ kg}^{-1} \text{ week}^{-1}$) intramuscular injections of nandrolone decanoate [15].

Group (2): Testosterone-treated group, received ($50 \text{ mg}^{-1} \text{ kg}^{-1} \text{ week}^{-1}$) intramuscular injections of testosterone enanthate [16].

Group (3): Control group, received weekly intramuscular injections of the vehicle (arachis oil).

The pharmacological treatment and the moderately atherogenic diet were continued for 16 weeks.

2.3. Experimental protocol

2.3.1. Effect of testosterone or nandrolone on serum lipids of rabbits

Blood samples were collected after fasting for 12–16 h from rabbit's marginal ear vein, before and after pharmacological treatment and diet. Clear serum was used for determination of total cholesterol, HDL-cholesterol, triglycerides and LDL-cholesterol using commercial kits from Stanbio Laboratory, TX, USA.

2.3.2. Effect of testosterone or nandrolone on the responses of isolated rabbit aortic rings to different vasoconstrictors and vasodilators

2.3.2.1. Tissue preparation. After completion of pharmacological treatment, rabbits were sacrificed, the chest was opened and the descending thoracic aortae were separated and placed in a petri dish containing cold physiological solution (Kreb's Henseleit) composed of (mM/l): NaCl 118, KCl 4.7, CaCl_2 2.5, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, NaHCO_3 25 and glucose 11.1. The aortae were dissected free of fat and connective tissue, and aortic rings (4–6 mm) were prepared. Each ring was suspended horizontally between two stainless steel parallel hooks, one of which was fixed and the other attached to isometric transducer for the measurement of isometric tension in an organ bath filled with 20 ml of the physiological salt solution at a temperature of 37°C and bubbled with carbogen mixture.

The rings were allowed to equilibrate under 1 g resting tension for 90 min [17]; during which the bath solution was replaced periodically every 15 min. Isometric tension generated by the vascular smooth muscle was measured using

a displacement transducer (model 50-7905, Harvard Apparatus Ltd., South Natick, MA, USA) and recorded on a two-channel oscillograph (model 50-8622, Harvard Apparatus Ltd.).

2.3.2.2. Responses to vasoconstrictors. Isolated rabbit aortic rings were contracted by the addition of KCl at a final bath concentration of 30 mM then washed till reaching the base line. This procedure was repeated till consistent responses were obtained, then dose–response curves to epinephrine (0.2, 0.5 and $1 \mu\text{M}$), 5-HT (0.1, 0.2 and $0.5 \mu\text{M}$) and ET-1 (1, 5 and 10 nM) were constructed. Because of the long lasting and slowly reversible contractions characteristic of ET-1, only one concentration of ET-1 was studied on the same ring. The responses were calculated as percentage of the contraction induced by KCl (30 mM).

2.3.2.3. Responses to vasodilators. Isolated rabbit aortic rings were precontracted by the addition of KCl (30 mM; final bath concentration) and after reaching a steady-state contraction, cumulative dose–response curves were constructed by the addition of increasing concentrations of adenosine (0.1, 1 and $10 \mu\text{M}$) and sodium nitroprusside (0.1, 1 and $10 \mu\text{M}$) to the bath. The responses (relaxations) were calculated as percentage of the contraction induced by KCl (30 mM).

2.4. Statistical analysis

Data are expressed as mean \pm S.E.M. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. Also, paired Student's *t*-test was used to compare after-treatment values with their respective before-treatment values.

3. Results

3.1. Effect of testosterone or nandrolone on serum lipids of rabbits

The effect of chronic treatment with testosterone and nandrolone on the serum lipids of rabbits is illustrated in Fig. 1. The moderately atherogenic diet significantly increased serum total cholesterol, triglycerides, LDL- and HDL-cholesterol levels in all groups when compared to their respective initial (before treatment) values. Chronic treatment with testosterone and nandrolone resulted in a significantly lower HDL-cholesterol level when compared to control after treatment. Nandrolone also caused a significantly higher LDL-cholesterol level when compared to control after treatment. Both testosterone and nandrolone caused no significant changes in serum total cholesterol and triglycerides levels when compared to control after treatment.

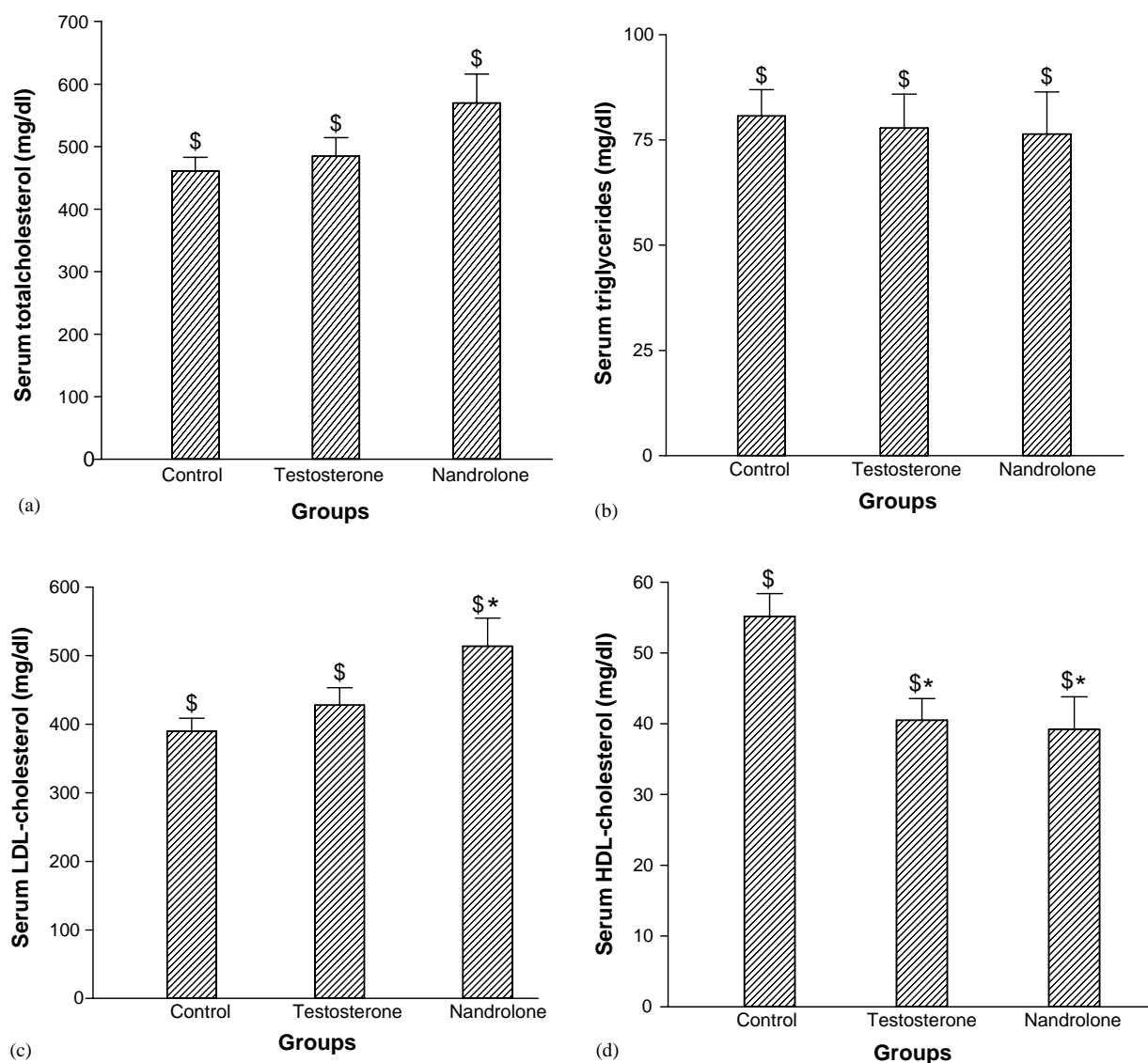


Fig. 1. Effect of chronic treatment with testosterone and nandrolone on the serum (a) total cholesterol, (b) triglycerides, (c) LDL-cholesterol and (d) HDL-cholesterol of rabbits. $^{\$}P < 0.01$ compared with their respective initial (pretreatment) values using paired Student's *t*-test. $^{*}P < 0.05$ compared to control group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test (▨ : before treatment; ▨ : after treatment).

3.2. Effect of testosterone or nandrolone on the responses of isolated rabbit aortic rings to different vasoconstrictors and vasodilators

3.2.1. Effect on epinephrine-induced contraction

Chronic treatment with testosterone and nandrolone significantly enhanced contractile response of rabbit aortic rings to epinephrine ($1\ \mu\text{M}$) when compared to control (Fig. 2a).

3.2.2. Effect on 5-hydroxytryptamine (5-HT)-induced contraction

Chronic treatment with testosterone significantly enhanced contractile response of rabbit aortic rings to 5-HT (0.2 and $0.5\ \mu\text{M}$) when compared to control. Chronic treatment with nandrolone significantly enhanced the contractile

response of rabbit aortic rings to 5-HT ($0.5\ \mu\text{M}$) when compared to control. The increase in 5-HT-induced contractions in case of testosterone (39.1, 65.6 and 50.6%) was higher than that of nandrolone (21.6, 38.7 and 38.1%) compared to control group at 5-HT concentrations of 0.1 , 0.2 and $0.5\ \mu\text{M}$, respectively (Fig. 2b).

3.2.3. Effect on endothelin-1 (ET-1)-induced contraction

Chronic treatment with testosterone and nandrolone significantly enhanced contractile response of isolated rabbit aortic rings to ET-1 (5 and $10\ \text{nM}$) when compared to control. The increase in ET-1-induced contractions in case of nandrolone (23.2, 50.5 and 39.3%) was higher than that of testosterone (8.8, 36.2 and 23.7%) compared to control group at ET-1 concentrations of 1 , 5 and $10\ \text{nM}$, respectively (Fig. 2c).

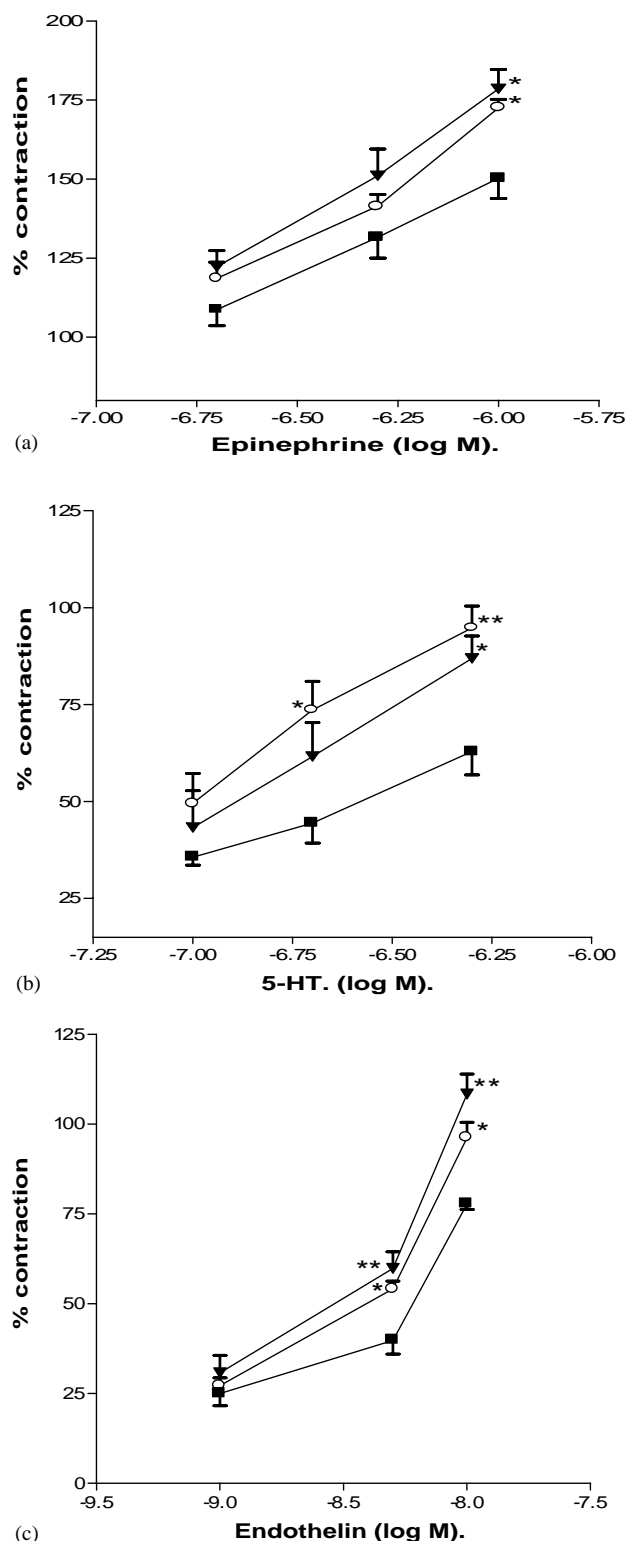


Fig. 2. Effect of chronic treatment with testosterone and nandrolone on the contraction of isolated rabbit aortic rings induced by (a) epinephrine, (b) 5-HT and (c) ET-1. Data are expressed as mean \pm S.E.M., $n = 5$, where the response is calculated as percentage of the contraction induced by KCl (30 mM). * $P < 0.05$ and ** $P < 0.01$ significantly different compared with control using one-way ANOVA followed by Tukey–Kramer multiple comparisons test (■: control group; ○: testosterone group; ▼: nandrolone group).

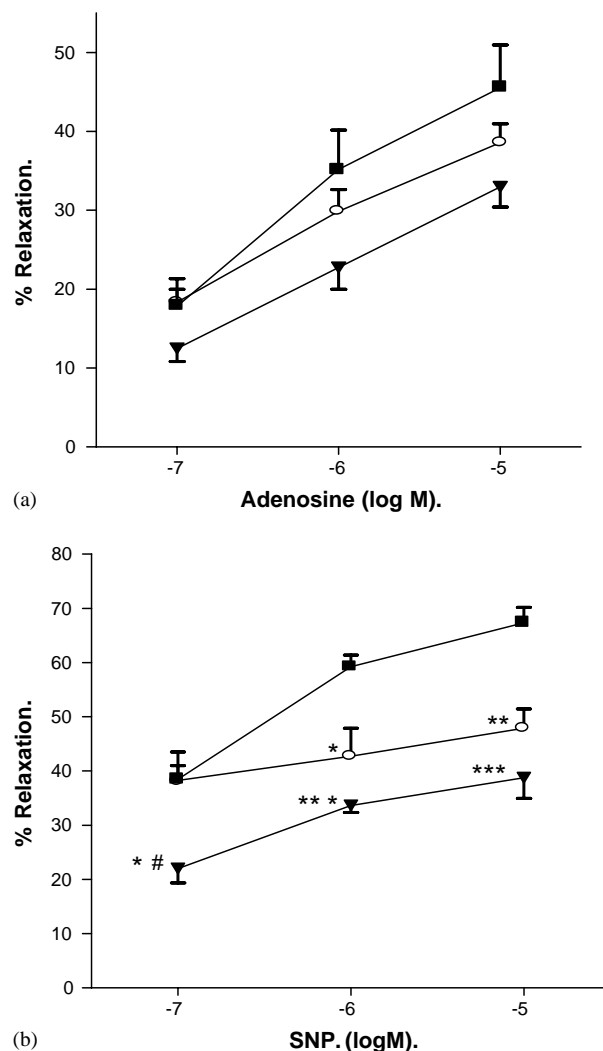


Fig. 3. Effect of chronic treatment with testosterone and nandrolone on (a) adenosine-induced relaxation and (b) SNP-induced relaxation of isolated rabbit aortic rings precontracted by KCl (30 mM). Data are expressed as mean \pm S.E.M., $n = 5$, where the response is calculated as percentage relaxation of the contraction induced by KCl (30 mM). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significantly different compared with control. # $P < 0.05$ significantly different compared with testosterone using one-way ANOVA followed by Tukey–Kramer multiple comparisons test (■: control group; ○: testosterone group; ▼: nandrolone group).

3.2.4. Effect on adenosine-induced relaxation

Chronic treatment with testosterone and nandrolone caused no significant changes in adenosine-induced relaxation when compared to control (Fig. 3a).

3.2.5. Effect on sodium nitroprusside (SNP)-induced relaxation

Chronic treatment with nandrolone resulted in a significant reduction in SNP-induced relaxation at all SNP concentrations, while chronic treatment with testosterone significantly reduced SNP-induced relaxation (1 and 10 μ M) when compared to control. Also, nandrolone effect at low

SNP concentration (0.1 μM) was significant than that of testosterone. The reduction of SNP-induced relaxation in case of nandrolone (42.9, 43.2 and 42.3%) was higher than that of testosterone (5.2, 27.9 and 28.8%) compared to control group at SNP concentrations of 0.1, 1 and 10 μM , respectively (Fig. 3b).

4. Discussion

In the present study, both testosterone and nandrolone treatment produced a significant decrease in HDL-cholesterol levels, and only nandrolone treatment produced a significant increase in LDL-cholesterol.

Ferrer et al. [15] found that nandrolone reduced HDL-cholesterol levels and increased LDL-cholesterol levels in rabbits treated for 12 weeks (10 mg kg⁻¹ week⁻¹). In human, it was also found that AAS can decrease HDL-cholesterol and increase LDL-cholesterol levels [18]. On the other hand, Fogelberg et al. [19] found no significant influence of the anabolic steroid stanozolol either on the extent of atherosclerotic involvement or on HDL- or LDL-cholesterol levels in rabbits.

Anabolic steroids cause induction of post-heparin plasma hepatic triglyceride lipase (HTGL) which causes selective hydrolysis of HDL₂ subfraction greater than HDL₃ and thus the overall HDL level decrease [20,21]. Also, HTGL catabolism of VLDL-cholesterol increases serum LDL-cholesterol concentrations [22]. On the other hand, estrogens suppress HTGL and increase HDL-cholesterol (especially HDL₂) levels [23]. Since nandrolone is a poor substrate for aromatase [24], thus nandrolone is less metabolized to estrogen than testosterone. Consequently, nandrolone has higher ability to induce HTGL which may explain our results in which nandrolone has higher atherogenic effect on plasma lipids than testosterone.

The present study also found that chronic treatment with testosterone or nandrolone enhanced the vasoconstrictor responses of isolated rabbit aortic rings to epinephrine, 5-HT and ET-1. Furthermore, the effect of nandrolone was higher than that of testosterone except in 5-HT contractions, where testosterone effect was higher than nandrolone.

Anabolic steroids have been associated with coronary artery vasospasm and myocardial infarction in the absence of both atherosclerosis and thrombosis [25]. The effect of AAS on blood vessels reactivity is still contradictory. Enhanced reactivity to norepinephrine has been shown in several species following administration of testosterone or methyltestosterone [26,27]. Also, Teoh et al. [28] found that acute exposure to low levels of testosterone, in vitro, significantly potentiated vasoconstriction to ET-1 and 5-HT. On the other hand, Ferrer et al. [29] found that chronic treatment with nandrolone reduced the vasoconstrictor responses in rabbit arteries to norepinephrine and 5-HT, and the thoracic aorta was the most affected by treatment.

Testosterone can selectively inhibit extraneuronal uptake of neuroamines [26,27] which may enhance the vasoconstrictor response to epinephrine and 5-HT. Also, AAS are associated with increased serum LDL levels, which may be oxidized by monocytes/macrophages in arterial wall [30]. Oxidized-LDL can enhance vasoconstriction by increasing the activity of protein kinase C [31] which is involved in the vasoconstriction mechanism induced by epinephrine, 5-HT and ET-1, thus may enhance vascular responses to these agents. AAS together with the hypercholesterolemia can also impair endothelial release and function of the vasodilators NO and PGI₂ [15,32,33] which may also enhance vasoconstrictor responses to epinephrine, 5-HT and ET-1.

Teoh et al. [28] found that acute exposure to 17 β -estradiol significantly attenuated vasoconstriction to ET-1 and 5-HT. Estrogens also can increase the release and function of NO and decrease that of ET-1, which facilitates vasodilation and decrease vasoconstriction [34]. Since nandrolone is less converted to estrogen than testosterone [24] and also nandrolone is associated with higher levels of LDL (as in our results), thus the higher effect of nandrolone than that of testosterone may be explained.

In case of 5-HT, the present study showed that the effect of testosterone was higher than that of nandrolone, which suggests that testosterone, more than nandrolone, may up-regulate 5-HT receptors, rather than affecting the signal transduction pathways involved in 5-HT contractions. Also, Teoh et al. [28] have found that the acute effect of testosterone on contractile responses of porcine coronary arteries may involve unidentified membrane steroid receptors rather than the classical cytosolic steroid receptor which may also play a part in this response.

The results of the present study demonstrated that chronic treatment with testosterone and nandrolone reduced the vasorelaxant response to SNP in isolated rabbit aortic rings precontracted with KCl. The reduction of SNP-induced vasodilation in case of nandrolone was higher than that of testosterone. On the other hand, the vasorelaxant responses to adenosine were not significantly changed.

Ferrer et al. [15] demonstrated that chronic treatment of rabbits with the anabolic steroid, nandrolone reduces NO-mediated relaxation only in thoracic aorta. Also, Hutchison et al. [14] found that physiologic levels of testosterone impair, in vitro, endothelium-dependent relaxation and augment the endothelial dysfunction associated with hypercholesterolemia and environmental tobacco smoking. Green et al. [35] also found that both endothelium-dependent relaxation (caused by methacholine) and endothelium-independent relaxation (caused by SNP) were significantly inhibited in men who were self-administering nandrolone.

AAS can inhibit guanylate cyclase [15], which may explain the attenuated vasorelaxant response to nitric oxide (NO) spontaneously released from SNP. In case of adenosine, we suggest that AAS may have little effect on the

cAMP-dependent pathway and/or adenosine receptors, thus slightly affecting adenosine relaxation.

Generally, the results of the present study demonstrated that AAS in presence of hypercholesterolemia can enhance vasoconstriction and attenuate vasodilation. In contrast, acute exposure to supraphysiologic levels of testosterone can produce endothelium-dependent relaxation in isolated rabbit coronary arteries and aorta [17], in rat aorta [36] and in porcine coronary arteries [37], and these relaxations may occur by opening Ca^{2+} - and voltage-activated K^{+} -channels [37]. However, these studies used supraphysiological and very large doses, as example, 50–100 times greater than those found in normal male volunteers, and approximately 10 times greater than those found in New Zealand white rabbits [17]. Also, these studies did not involve the presence of hypercholesterolemia, and tested only acute, but not chronic effects of AAS. Interestingly, Teoh et al. [28] demonstrated that acute exposure to physiological levels of testosterone enhanced vasoconstriction responses. Thus, dose levels, duration of treatment and presence of hypercholesterolemia are important factors affecting the vascular responses to AAS.

In conclusion, the abuse of AAS in presence of hypercholesterolemic can enhance atherogenicity and vasospasm and attenuate vasorelaxation. Thus, the abuse of AAS is harmful to the vascular system and should be prohibited.

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