

The effect of nandrolone treatment with and without enforced swimming on histological and biochemical changes in the heart and coronary artery of male rats

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ABSTRACT

Objective: Chronic anabolic androgenic steroid (AAS) consumption increases incidence of cardiovascular abnormalities in athletes and mechanisms underlying those abnormalities continue to be investigated. This study examines whether nandrolone consumption induced cardiac and coronary artery wall abnormalities via oxidative stress. It was also designed to determine whether enforced swimming augmented possible cardiotoxic effects of nandrolone in rat heart.

Methods: Twenty-four male Wistar rats were divided into 3 groups: control, nandrolone, and nandrolone with enforced swimming. Nandrolone group received 10 mg/kg body weight nandrolone 3 times a week for 6 weeks. Nandrolone group with enforced swimming received the same amount of nandrolone and was forced to swim with excess weight of 20% body weight.

Results: After 6 weeks of treatment, results indicated proliferation of heart muscle and coronary smooth muscle cells and lipid peroxidation; significant rise in levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), nicotinamide adenine dinucleotide phosphate oxidase, homocysteine (Hcy), apolipoprotein B, low-density lipoprotein, and cholesterol, as well as severe fibrosis in heart tissue and around coronary arteries of nandrolone and nandrolone with enforced swimming groups compared with control group.

Conclusion: These findings strongly support idea that nandrolone intake by sedentary rats and exercised rats induced heart abnormality mediated by oxidative stress, which was manifest in increased lipid peroxidation, Hcy, and 8-OHdG in heart tissue. (*Anatol J Cardiol* 2017; 17: 176-83)

Keywords: exercise, heart fibrosis, nandrolone, oxidative stress, rat

Introduction

Though they have a variety of clinical applications, testosterone-derived anabolic-androgenic steroids (AAS) are often misused by competitive athletes and bodybuilders, as well as by non-athletes for aesthetic purposes rather than enhancement of sports performance (1, 2). Investigations conducted in the previous 5 decades have documented various toxic effects of AAS in different organs, such as decreased levels of luteinizing hormones and follicle-stimulating hormones, decreased spermatogenesis, testicular atrophy, elevated levels of liver enzymes, and hepatic dysfunction, as well as liver tumors and other malignancies (3, 4). In addition, abuse of AAS by children/adolescents causes premature epiphyseal closure, resulting in decreased height in adulthood and increased risk of musculotendinous injuries (5, 6). Furthermore, endocrine disorders such as accelerated maturation, changes in physique and development of secondary sexual

characteristics, glucose tolerance alteration, increase in insulin resistance, decreased thyroid hormones, and masculinization in women have also been reported to be associated with AAS use by athletes (7, 8). Recently, cardiovascular system abnormalities induced by AAS have attracted researchers' attention. Previous studies indicated that AAS exposure creates adverse cardiovascular effects such as hypertension, left ventricular (LV) hypertrophy, and impaired diastolic filling (9). Furthermore, it contributes to arrhythmia, erythrocytosis, lipoprotein profile alteration, and thrombosis (10). Some abnormalities in vascular reactivity and cardiovascular reflex control of cardiovascular system following nandrolone decanoate exposure have also been reported (11, 12). While research on early AAS exposure focused on identifying specific abnormalities in the cardiovascular system, much of the recent attention has been directed to underlying mechanisms through which AAS consumption in combination with exercise or sedentary status contribute to cardiovascular

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system deficits. Although some research studies have identified different abnormalities in the cardiovascular system as result of AAS exposure, precise mechanism underlying AAS-induced cardiovascular damage has not yet been completely clarified. Several studies have recently examined role of oxidative stress in development of AAS-induced organ abnormalities, possibly via formation of free radicals (13, 14). Hence, in the current study, we evaluated possible adverse effects of nandrolone decanoate on cardiac tissue of male rats at both histological and molecular level, and under sedentary and physical training conditions. Proliferation cell nuclear antibody (PCNA) and Masson's trichrome staining were applied in evaluation of histopathological alteration and deoxyribonucleic acid (DNA) damage to heart tissue. Based on levels of oxidized low-density lipoprotein (Ox-LDL), nicotinamide adenine dinucleotide oxidase (NADPH oxidase), and homocysteine (Hcy), it was determined that oxidative stress had been induced in rats' hearts through long-term monitored administration of nandrolone. In addition, recent studies have demonstrated that enforced swimming, as an example of extensive exercise, leads to oxidative stress and subsequent organ damage (15). It has previously been shown that enforced swimming caused rise in lipid peroxidation and decreased catalase and superoxide dismutase (SOD) levels in animal model (15, 16). Based on these observations, current study was also designed to determine whether enforced swimming augmented possible cardiotoxic effects of nandrolone in rats.

Methods

Twenty-four adult male Wistar rats weighing 220 ± 20 g were assigned to 3 groups ($n=8$ in each group): control, nandrolone, and nandrolone with forced swimming (NFS). Dose of 10 mg/kg body weight nandrolone (nandrolone ampoules) was administered by single injection in femoral muscle, 3 times per week for 6 weeks (Saturdays, Mondays, and Wednesdays). This dosage was selected based on research of Pope and Katz and corresponded to inappropriate dose administered by athletes, namely, 10 to 100 times higher than therapeutic dose (17). Swimming tests were performed in a metal cylinder tank (60 cm height $\times$ 100 cm diameter) filled with clean water of 40 cm depth and temperature of $25 \pm 2^\circ\text{C}$. Animals were placed in tank individually to swim for 20 minutes 3 times per week (Saturdays, Mondays, and Wednesdays) for 6 weeks. Piece of metal (20% body weight) was attached to tail to prompt rats to continue swimming.

After 6 weeks of treatment, rats were anesthetized with 10% chloral hydrate (0.5 mL/kg body weight, intraperitoneally). Depth of anesthesia was assessed by pinching a hind paw.

After weighing each rat, thoracic cavity was opened and blood samples were collected directly from heart. Samples were then mixed with ethylenediaminetetraacetic acid as anticoagulant substance. Blood samples were centrifuged at $4000 \times g$ for 20 minutes within 30 minutes of collection. Without performing repeated freeze-thaw cycles, plasma samples were stored at

-80°C . Next, heart was dissected. Excised heart was freed from fat, blood clots, and adventitial tissue and weighed. Then, entire LV wall (without septum) was excised from heart and weighed.

Ventricle was divided into 2 parts for analysis. First portion was fixed in buffered formalin for histopathological investigation. After taking standard dehydration steps, it was embedded in paraffin. To conduct biochemical analysis, the other part of ventricle was washed with ice-cold physiological saline and then dried on filter papers. An ice-cold extraction buffer (10% wt/vol), containing 50mM phosphate buffer (pH 7.4) was added and homogenized using Ultra Turrax (T10B; IKA-Werke GmbH & Co., Staufen, Germany). Homogenates were centrifuged at $10\,000 \times g$ at 4°C for 20 minutes. Supernatant sample was obtained and stored at -80°C until time of analysis.

Biochemical assay

Quantity of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured using quantitative sandwich enzyme-linked immune assay (ELISA) method and commercial rat 8-OHdG ELISA kit (Cusabio, Wuhan, China) according to manufacturer's recommended protocol. Hcy level was measured using ELISA kit (Axis-Shield, Dundee, Scotland) according to manufacturer's guidelines. Assessment of level of NADPH oxidase (NOX1) in heart supernatant was carried out using rat NADPH Oxidase 1 (NOX1) ELISA Kit (Cusabio, Wuhan, China) according to manufacturer's recommendations. Paraoxonase level in plasma samples was measured using paraoxonase assay kit, following protocol provided by manufacturer (Cusabio, Wuhan, China). Ox-LDL level of heart tissue was measured using sandwich ELISA kit (Merco-dia AB, Uppsala, Sweden). Quantities of apolipoprotein (Apo) A and B were measured using nephelometric method and Mono Binding Kit (The Binding Site Group, Birmingham, England), as instructed by the manufacturer. The serum triglyceride and total cholesterol levels were assayed adopting colorimetric and enzymatic methods. Serum LDL-C and high-density lipoprotein-cholesterol (HDL-C) were measured by applying the Biosystems method directly (Biosystems S.A., Barcelona, Spain).

Histopathological examinations

For the purpose of histopathological staining, 5- μm thick histological sections from paraffin-embedded heart tissue were used. PCNA anti-body staining protocol was used to assay heart and coronary vessel proliferation rates. In brief, after taking tissue-processing steps such as deparaffinization, rehydration, and gradual ethanol passage, sections of cardiac tissue with 5- μm thickness were stained using monoclonal anti-PCNA antibody (Dako A/S, Glostrup, Denmark). Optimal results were obtained with EnVision visualization system (Dako A/S, Glostrup Denmark). In addition, hematoxylin was adopted as counterstain. Assessment included proper negative controls. Moreover, 2 expert pathologists independently inspected all slides. PCNA-positive indices were regarded as indicators of muscle cell proliferation.

In order to assess percentage of PCNA-positive indices, coronary artery and all cells in each cross section of the heart were scored. Criteria for quality of PCNA-positive indices were as follows (18): normal (PCNA-positive indices present in less than 5% of muscle cells), mild (PCNA-positive indices present in less than 25% of muscle cells), mild to moderate (PCNA-positive indices present in 25% to 50% of muscle cells), moderate to severe (PCNA-positive indices present in 50% to 75% of muscle cells), and severe (PCNA-positive indices present in 75% to 100% of muscle cells). To evaluate heart and coronary vessel fibrosis, 5 μ m heart tissue sections were stained using Masson's trichrome in accordance with manufacturer's instructions (Trichrome Stain Kit; Asia Pajohesh, Amol, Iran). Severity of tissue fibrosis was estimated adopting semi-quantitative method developed by Ashcroft et al. (19). A score was assigned ranging from 0 (normal heart) to 8 (total fibrosis) using the following criteria: grade 0=normal heart, grade 1=minimal fibrosis thickening of coronary vessel walls or myocardial, grades 2 and 3=moderate thickening of coronary vessels walls or myocardial without obvious damage to structure of heart tissue, grades 4 and 5=increased fibrosis with definite damage to architecture of heart and formation of bands or small masses of fibrosis, grades 6 and 7=severe distortion of structure and large areas of fibrosis, and grade 8=total fibrotic obliteration.

Statistical analyses

Normal distribution of data within each group was verified with Kolmogorov-Smirnov test using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). By conducting a one-way analysis of variance and then Tukey's post-hoc test, statistical differences between groups were tested (SPSS version 17.0). Data obtained from each test were expressed as mean $\pm$ SE, and $p<0.05$ was considered statistically significant.

Results

Serum lipid profile

Nandrolone treatment had no significant effect on plasma Apo A level ($p<0.2$) (Table 1). Plasma Apo A level significantly decreased in NFS group compared with nandrolone and control groups ($p<0.05$). Plasma Apo B levels in nandrolone and NFS

groups were significantly higher than those of control group ($p<0.05$). In NFS group, Apo B level showed greater increase compared with that in nandrolone group ($p<0.05$). Ratio of Apo B/Apo A in nandrolone and NFS groups showed significant increase compared with that of control group ($p<0.05$), and this increase was more pronounced in NFS group compared with that of nandrolone group ($p<0.5$). Plasma HDL level was lower in both nandrolone and NFS groups compared with that of control group ($p<0.05$). Decrease of plasma HDL level was severe in NFS group compared with that of nandrolone group ($p<0.05$). Nandrolone and NFS group animals demonstrated significantly ($p<0.05$) higher plasma LDL levels compared with control animals ($p<0.05$). Additionally, NFS rats showed significantly higher plasma LDL level compared with nandrolone rats. Plasma cholesterol levels were significantly higher in nandrolone and NFS groups compared with control group ($p<0.05$). Cholesterol level in forced swimming group along with nandrolone group increased compared with that of nandrolone group ($p<0.01$). Triglyceride level showed no significant difference among different groups.

Heart tissue parameters

Quantity of Ox-LDL and NADPH oxidase in heart tissue increased significantly in nandrolone and NFS groups compared with that of control group ($p<0.05$) (Table 2). Amount of NADPH oxidase was significantly higher in NFS group compared with nandrolone group ($p<0.02$). Level of 8-OHdG in heart tissue was higher in both nandrolone and NFS groups compared with that of control group ($p<0.05$). No significant difference was found between nandrolone group and NFS group regarding 8-OHdG in heart tissue. Heart tissue Hcy level showed significant increases in both nandrolone and NFS groups compared with control group ($p<0.05$). Elevation of Hcy level in NFS group was more pronounced than that of nandrolone group. Neither nandrolone nor induced exercise group had change in plasma paraoxonase level ($p<0.05$).

After 6-week experimental period, ratio of LV weight (mg) to body weight (g) in control, nandrolone, and NFS groups were 1.1 ± 0.02 , 1.38 ± 0.05 , and 1.4 ± 0.05 , respectively. Ratio of LV weight to body weight, as an indicator of ventricular hypertrophy, showed significant increase in nandrolone and NFS groups compared with control group ($p<0.05$).

Table 1. Serum lipid profile changes in different groups

	Control	Nandrolone	Nandrolone+swim	F (2, 15)	P
Apo A, mg/L	0.99 ± 0.009	0.98 ± 0.02	$0.88\pm0.01^{*†}$	6.38	0.001
Apo B, mg/L	0.41 ± 0.003	$0.44\pm0.01^{*}$	$0.58\pm0.01^{*†}$	57.33	0.01
Apo B/Apo A	0.41 ± 0.006	$0.45\pm0.05^{*}$	$0.64\pm0.018^{*†}$	12.93	0.001
HDL, mg/dL	41.83 ± 0.47	$38.16\pm0.7^{*}$	$34.33\pm0.84^{*†}$	39.9	0.001
LDL, mg/dL	89 ± 0.6	$96\pm0.3^{*}$	$103\pm0.8^{*†}$	10.83	0.002
Cholesterol, mg/dL	99.8 ± 0.6	$109\pm2.8^{*}$	$113\pm2.7^{*†}$	4.93	0.05
Triglyceride, mg/dL	90.5 ± 7.28	84.16 ± 5.7	86.83 ± 6.03	1.8	0.19

Apo - apolipoprotein, HDL - high-density lipoprotein, LDL - low-density lipoprotein

Values are mean $\pm$ SE for 8 rats per group. *Denotes significant difference ($P<0.05$) compared to control. †Denotes significant difference ($P<0.05$) compared to nandrolone group

Table 2. Effect of nandrolone and nandrolone+forced swimming on changes to heart tissue oxidized low-density lipoprotein, nicotinamide adenine dinucleotide oxidase, 8-hydroxy-2'-deoxyguanosine, homocysteine, and serum paraoxonase

	Control	Nandrolone	Nandrolone+swim	F (2, 15)	P
Ox-LDL, ng/L	56.8±1	64.9±1.5*	65.48±1.4*	12.91	0.001
NADPH oxidase, pg/mL	8306±14	9157±22*	11250±21*†	59.49	0.001
8-OHdG, ng/mL	8±0.3	8.88±0.19*	8.89±0.12*	15.9	0.001
Hcy, µmol/L	4.46±0.2	5.7±0.3*	6.7±0.4*†	10	0.002
Paraoxonase, ng/mL	97.5±1.32	96.3±1.8	99±1.4	0.78	0.4

Hcy - homocysteine; NADPH - nicotinamide adenine dinucleotide; Ox-LDL - oxidized low-density lipoprotein; 8-OHdG - 8-hydroxy-2'-deoxyguanosine
Values are mean ±SE for 8 rats per group. *Denotes significant difference ($P<0.05$) compared to control. †Denotes significant difference ($P<0.05$) compared to nandrolone group

Histopathological changes

Figure 1 shows percentage of cardiac muscle cell and coronary artery smooth muscle cell proliferation (PCNA-positive indices) in experimental group. Percentage of PCNA-positive indices significantly increased in coronary smooth muscle cell of rats treated with nandrolone (50.3±3.6) and rats in NFS group (50.2±3) compared with control group (4.8±3).

Ratio of proliferated cardiac myocytes (PCNA-positive indices) to heart tissue of control, nandrolone, and NFS groups was 8±0.8%, 46.6±0.6%, and 56±4%, respectively (Fig. 1). Nandrolone administration led to mild to moderate cardiac muscle cell proliferation. In addition, enforced swimming along with nandrolone induced moderate to severe muscle cell proliferation (Fig. 1). Figure 2 shows microscopic fibrosis scores for the study groups. There was thin line of fibrous bonds in heart tissue obtained from control group. Microscopic lesion score in heart tissue and around coronary vessels was 6 to 7, indicating severe structure distortion and large areas of fibrosis in nandrolone and NFS groups compared with control group.

Discussion

In the present study, we evaluated effect of nandrolone on a large number of cardiovascular risk factors and histological changes to the heart in rats. Moreover, we compared deleterious effects of nandrolone on heart in both sedentary and resistance training conditions. Primary findings of the present study may be summarized as follows: administration of nandrolone, with or without training, led to increase in Ox-LDL, NADPH oxidase, and 8-OHdG levels in heart tissue of rats. Lipid profile changes such as increase in plasma level of LDL, cholesterol, and Apo B, and decrease in HDL were also seen in both experimental groups compared with control group. Structural changes, such as severe distortion, diffuse areas of fibrosis, and mild to moderate cardiac and coronary muscle cell proliferation were also present in the hearts of rats from nandrolone and NFS groups compared with control group. All biochemical changes were severe in NFS group compared with nandrolone-treated group.

Differential effect of AAS on cardiovascular system in human and animal studies has been reported before. Mechanism underlying hazardous effect of AAS is not yet fully understood. In the

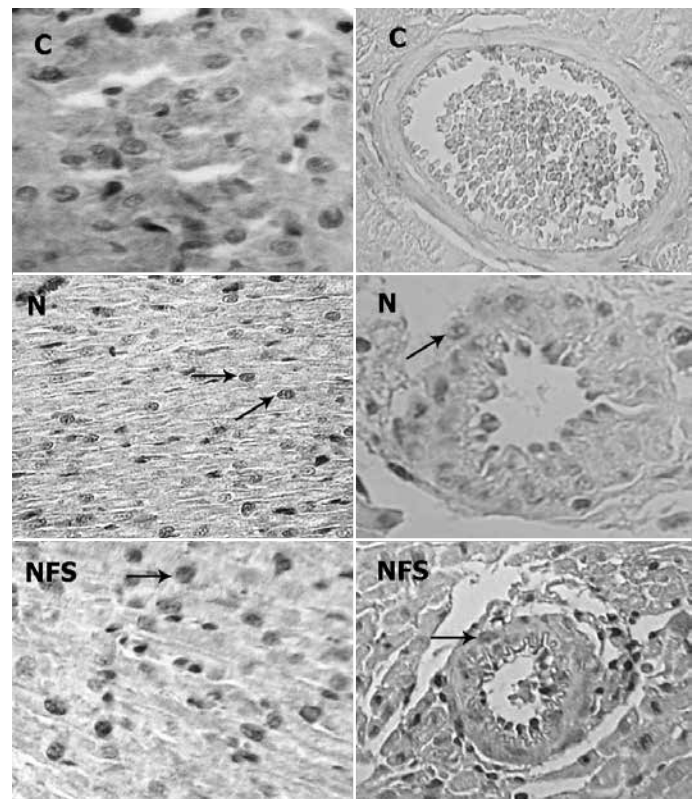


Figure 1. Immunohistochemical staining of heart tissue and coronary vessels by proliferating cell nuclear antigen (PCNA) anti-body showed mild to moderate heart muscle cell proliferation (left column), and moderate to severe coronary muscle cell proliferation in nandrolone and nandrolone-swim (right column) groups.

(Original magnification×200). PCNA positive indices (→). C - control, N - nandrolone, NFS - nandrolone-swim

current study, levels of LDL, cholesterol, and Apo-B were significantly increased in nandrolone and NFS rats compared with levels of control rats. These simple markers are widely accepted as risk factor for cardiovascular system disease (20). Based on previous studies, there is contrary correlation between AAS consumption and lipid profile changes. Johanson et al. (21) reported that nandrolone had no marked effect on lipid profile, but as in several other studies, our results indicated that nandrolone has harmful effect on lipid profile, mainly by increasing LDL and cholesterol levels, as well as decreasing HDL levels (22, 23). As independent and well-known risk factors, high level of LDL and a low

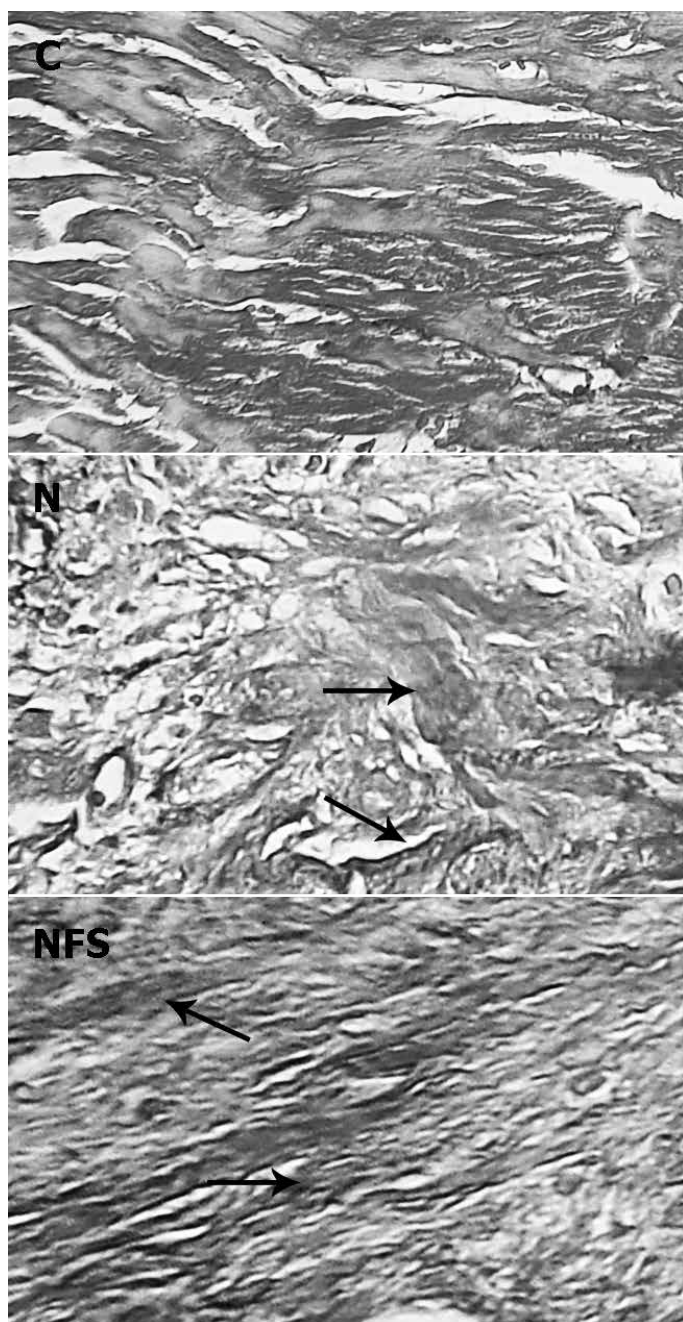


Figure 2. Photomicrograph of heart tissue of rats (Masson trichrome staining). In (C), sample obtained from the control group; in (N), sample obtained from the nandrolone group; in (NFS), sample obtained from the nandrolone-swim group

(Original magnification×200). Fibrosis bond (→)

level of HDL induce cardiovascular abnormality through several mechanisms involving oxidative stress, endothelial cell dysfunction, and foam cell formation (24, 25). Interestingly, aside from dyslipidemia, in this study, a parallel increase in oxidative stress indicators such as Ox-LDL and NADPH oxidase, along with cardiac and coronary muscle cell proliferation were seen in nandrolone and NFS groups compared with control group. It has been well established that LDL alone increases risk of heart disease

threefold (26). Harmful effects of LDL on cardiovascular system depend on composition and size (27). Small LDL particles easily penetrate arterial wall and undergo oxidation process because of its larger compartments (27, 28). In addition, high affinity of LDL for arterial wall proteoglycans prolongs LDL residence in subendothelial spaces (29). After being trapped in subendothelial spaces, LDL undergoes oxidation reaction and forms Ox-LDL. Ox-LDL affects vascular smooth muscle cell growth, inducing proliferation or apoptosis. It stimulates growth via an oxidative mechanism that causes release of fibroblast growth factor-2, strengthens mitogenic effect of angiotensin II, and stimulates mitogen-activated protein kinase (MAPK) activation (25). In addition, it has been demonstrated in previous studies that Ox-LDL induces expression of proteins known as cell cycle regulatory proteins (25). Plasma HDL levels, in this study, were also reduced in animals treated with nandrolone and subjected to exercise. Reduced plasma HDL level has been established as major risk factor of heart disease (26, 27). In addition, studies in animal and human models have indicated that high plasma level of HDL is generally related to protection against atherosclerosis (26, 27). Protective effects of HDL against heart disease in general arise from involvement of HDL in reverse cholesterol transport (28). Reverse cholesterol transport, as a primary pathway, removes excess cholesterol from peripheral cells to selected extracellular acceptors such as HDL and apolipoproteins (29). HDL also has a protective effect against LDL oxidation, and consequently against heart disease. This valuable effect of HDL preventing oxidation of LDL is due to 2-enzyme system of paraoxonase and acetylhydrolase associated with normal HDL levels (30). Therefore, when HDL level is reduced, protective effect of these enzymes against LDL oxidation decreases as well, and it makes LDL oxidation and its damaging effect on cardiovascular system more likely. Interestingly, in the current study, despite decrease in HDL level and increase in Ox-LDL, no significant change in paraoxonase level was observed in nandrolone or NFS groups, compared with control group. Accordingly, it was possible for reduced plasma level of HDL to exert its effect on LDL oxidation through acetylhydrolase or reverse cholesterol transport. We also observed that treatment with nandrolone increased concentration of Apo B and Apo B/Apo A ratio without producing significant changes in plasma Apo A level. Nandrolone treatment combined with swimming decreased Apo A concentration and increased Apo B and Apo B/Apo A ratio simultaneously. Apo A and Apo B contain proteins from a spectrum of lipoproteins including LDL, very low-density lipoprotein, and DHL. Moreover, metabolic fate of Apo A and Apo B is determined by these lipoproteins (31). In general, Apo B carries lipids from the liver and gut to tissues that use lipids, whereas Apo A-containing particles facilitate reverse lipid transport and carry excess lipids from peripheral tissues to the liver (31). In terms of management of cardiovascular diseases, there are advantages associated with measuring Apo A and Apo B concentrations (32). Level of concentration reflects number of particles from respective lipoprotein classes and thus the opposite aspect of cardiovascular risk (32).

High Apo B/Apo A ratio designates a high number of atherogenic lipoprotein particles that are likely to be deposited in the arterial wall (31). Results of the current study showed a dramatic increase in the Apo B level and Apo B/Apo A ratio, along with coronary and cardiac muscle cell proliferation in nandrolone and NFS groups, changes that may favor deposit of lipids in the arteries and consequent complications such as proliferation. In this study, we observed that with increase in unfavorable lipid profile, level of Hcy increased significantly as well. According to the literature, few studies have investigated effect of AAS use on Hcy production. Consistent with our study, Zmuda et al. (33) reported that AAS administration increased serum level of Hcy, but in contrast to our results, Graham et al. (34) demonstrated that abuse of AAS decreases plasma Hcy level. A large number of studies have indicated that sulfur-containing amino acid Hcy is an atherogenic determinant, and that a strong association exists between Hcy level and risk of cardiovascular disease or severity of atherosclerosis (35). The molecular mechanism underlying Hcy-mediated cardiovascular system abnormality is not fully understood. A recent study by Sharma et al. (35) indicated that Hcy increased activity of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, a rate-limiting enzyme in cholesterol biosynthesis. As a risk factor in the current study, cholesterol concentration showed parallel increase with Hcy. In addition, Hcy increased oxidative degradation of nitric oxide (NO) through oxidation of its sulfhydryl group, as well as level of hydrogen peroxide and superoxide anion (36). Reduced NO impaired endothelial vasodilator function, a predictor of vascular morbidity and mortality (37). Moreover, Hcy incubation with vascular smooth muscle cells resulted in significant vascular smooth muscle cell proliferation and increase in expression of proteins such as glycolytic metabolism proteins and cytoskeletal proteins like lamin C (38). In the present study, pro-oxidant properties of Hcy were confirmed by increased concentration of Ox-LDL and NADPH oxides in nandrolone and NFS groups. Significant cardiac and coronary smooth muscle cell proliferation along with unfavorable lipid profile and increase in oxidative stress parameters in the current study may have originated from nandrolone-induced increase in Hcy. Another important finding of this study was significant increase in NADPH oxidase levels in nandrolone and NFS groups compared with levels in control group. Although NADPH oxidase proteins were first described through their important role in function of phagocytic cells (39), recently, a growing body of evidence has indicated that these proteins play a crucial role in cardiovascular system abnormality through redox signaling mediators such as endothelial activation, angiogenesis, atherosclerosis, cardiac hypertrophy, and vascular and cardiac remodeling (40). Many studies have revealed that reactive oxygen species (ROS) derived from NADPH oxidase are involved in surface expression of intercellular and vascular adhesion molecules on endothelial cells, tumor necrosis factor- α , renin-angiotensin system, and hypercholesterolemia (41). NADPH oxidase-derived ROS has been shown to act through activation of MAPK or nuclear factor- κ B (41). This specific

signaling pathway dependent on NADPH oxidase-derived ROS has a central role in development of chronic pressure overload cardiac hypertrophy (42).

In the current study, unfavorable lipid profile and enhanced Hcy and NADPH oxidase levels were observed, along with cardiac tissue fibrosis and cardiac muscle cell proliferation. The mechanism through which nandrolone induces cell proliferation and fibrosis is not fully understood, but it may be result of oxidative stress and inflammatory reactions. A recent study investigating effect of intramuscular administration of nandrolone decanoate on rabbits found that it caused fibrosis in heart tissue and was mediated by oxidative stress (43). In addition, accumulating evidence suggests that ROS resulting from oxidative stress and inflammatory cytokines play key role in heart tissue proliferation and fibrosis (44). Based on results of the current study and previous reports, we suggest that nandrolone induces oxidative stress, and that inflammatory cytokines may trigger fibrotic and proliferative signaling pathways and contribute to proliferation and fibrosis of the heart and cardiomyopathy.

Another important finding of current study was the significant increase in LV weight/body weight ratio (as an indicator of heart hypertrophy) in nandrolone and NFS groups. To our knowledge, this is the first *in vivo* study to show that nandrolone exposure with or without exercise increased NADPH oxidase level along with cardiac hypertrophy in rats. In the present study, administration of nandrolone caused DNA damage to heart tissue, indicated by an increased 8-OHdG level in heart tissue. Similarly, Ahmed et al. (42) reported enhanced testicular DNA damage following administration of nandrolone to rats based on results of comet assay. One of the predominant forms of free radical-induced lesion to DNA is 8-OHdG. It is result of oxidation, hydroxyl group radical is added to eighth position of guanine molecule (45). Oxidative stress observed in the current study, indicated by increased Ox-LDL and NADPH oxidase levels on the one hand, and oxidative DNA damage along with tissue fibrosis and cell proliferation on the other hand, led us to speculate that nandrolone exerts its hazardous effects on heart tissue through oxidative stress.

Finally, study results showed all biochemical changes were severe in the NFS group compared with nandrolone-treated group. Previous studies have shown that physical training alone has various effects (increased, decreased, or no change) on oxidative stress damage markers (46–48). As in present study, previous reports have indicated that combination of nandrolone plus exercise led to change in myocardial enzymes and increase in renin-angiotensin system activity, a plausible mechanism of heart hypertrophy (49, 50). Conversely, another study indicated that endurance exercises can improve redox system balance through enhancing activity of some antioxidant enzymes such as SOD and catalase (51). Further research is required in comprehensive detail to determine whether exercise training alongside nandrolone consumption augments or reduces cardiotoxic effects of nandrolone.

Study limitations

Our study had a few limitations. First, as a molecular underlying for heart failure, activation of fetal gene expressions, including myosin heavy chain isoforms, was not analyzed in the current study. We did not study alterations of calcium ion homeostasis or norepinephrine, important hallmarks of molecular alteration in heart failure. Another limitation was not assessing acute phase inflammatory protein changes such as alpha and beta globulins in plasma of the animals after treatment.

Conclusion

In conclusion, we found that 6 weeks of nandrolone treatment with or without accompanying physical training increased oxidative stress damage markers, unfavorable lipid profile, cardiac hypertrophy, fibrosis, and cardiac and coronary vessel proliferation in rats. Our results offer a new perspective on nandrolone-induced cardiac damage. That is, large number of heart risk factors and oxidative stress should be considered as underlying factors. However, all these factors contributed to development of nandrolone-induced adverse effect on cardiac and coronary tissue and will keep the field of nandrolone heart research busy for a very long time. Further studies are necessary to confirm the role of increased oxidative stress damage markers with sudden cardiac death induced by AAS.

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