Life Sciences 89 (2011) 631–637

Contents lists available at SciVerse ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Nandrolone and resistance training induce heart remodeling: Role of fetal genes and implications for cardiac pathophysiology

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ARTICLE INFO

Article history:
Received 25 February 2011
Accepted 27 July 2011

Keywords:
Cardiac hypertrophy
Genes
Training
Collagen
Echocardiography
Nandrolone

ABSTRACT

Aims: This study was conducted to assess the isolated and combined effects of nandrolone and resistance training on cardiac morphology, function, and mRNA expression of pathological cardiac hypertrophy markers.
Main methods: Wistar rats were randomly divided into four groups and submitted to 6 weeks of treatment with nandrolone and/or resistance training. Cardiac parameters were determined by echocardiography. Heart was analyzed for collagen infiltration. Real-time RT-PCR was used to assess the pathological cardiac hypertrophy markers.
Key findings: Both resistance training and nandrolone induced cardiac hypertrophy. Nandrolone increased the cardiac collagen content, and reduced the cardiac index in non-trained and trained groups, when compared with the respective vehicle-treated groups. Nandrolone reduced the ratio of maximum early to late transmitral flow velocity in non-trained and trained groups, when compared with the respective vehicle-treated groups. Nandrolone reduced the alpha-myosin heavy chain gene expression in both non-trained and trained groups, when compared with the respective vehicle-treated groups. Training reduced the beta-myosin heavy chain gene expression in the groups treated with vehicle and nandrolone. Only the association between training and nandrolone increased the expression of the skeletal alpha-actin gene and atrial natriuretic peptide in the left ventricle.
Significance: This study indicated that nandrolone, whether associated with resistance training or not, induces cardiac hypertrophy, which is associated with enhanced collagen content, re-expression of fetal genes the in left ventricle, and impaired diastolic and systolic function.

Introduction

Anabolic androgenic steroids (AAS) comprise a group of drugs commonly used among athletes, frequently in combination with resistance training (Kuhn, 2002). Although the increase in muscle mass and power induced by AAS (Bhasin et al., 1996) is still questionable (Parkinson and Evans, 2006), it has been well established that AAS abuse is associated with detrimental side effects (Sullivan et al., 1998). Several researchers have observed that AAS may induce pathological left ventricular hypertrophy (Pärssinen et al., 2000a; Nottin et al., 2006), with disproportionate extracellular collagen accumulation and interstitial fibrosis (Nieminen et al., 1996; Palatini et al., 1996; Rocha et al., 2007). On the other hand, some reports have shown no differences in left ventricular morphofunction between AAS users and non-users (Thompson et al., 1992; Pärssinen et al., 2000b). These conflicting data may be related to the difficulty of finding athletes who admit the exact pattern of AAS administration. Moreover, not only the type and regimen of AAS administration, but the additional influence of dietary intake and patterns and/or training intensity may have different effects on individual subjects (Parkinson and Evans, 2006). Although there have been conflicting results and uncertainty as regards the underlying

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0024-3205
doi:10.1016/j.lfs.2011.08.004

mechanisms of AAS-induced left ventricular hypertrophy, it is known that inappropriate increase in left ventricular mass itself may compromise cardiac function (Collins et al., 2001).

In this context, animal models are very useful when the aim of the study is to evaluate cause-and-effect relationships, such as the effect of a specific type or dose of AAS on the heart, for example. One of the major advantages of using animal models is the ability to have precise control of environmental variables, quality and pattern of food intake, as well as AAS administration regimen and dosages.

Considering that many young people, who practice intense physical exercises, particularly resistance training performed in a vigorous manner, frequently consume high doses of AAS, usually in complete disregard for the guidance provided by their physical educators, it would be interesting to evaluate the effect of the association of AAS and intense physical exercise.

Resistance training by weight lifting in water (Cunha et al., 2005a,b) is an experimental protocol that mimics this reality. It was observed that subsensitivity to phenylephrine induced by this protocol in the rat thoracic aorta was blocked by nandrolone decanoate. It was suggested that AAS seemed to damage endothelial function by increasing low density cholesterol levels (Cunha et al., 2005a). In order to evaluate the cardiovascular effects of AAS, it would also be interesting to evaluate the cardiac effects of nandrolone administration and resistance training in the same experimental protocol.

Therefore, the aim of this study was to investigate under highly controlled conditions the isolated and combined effects of high-dose nandrolone administration and resistance training on cardiac function, and the mechanisms underlying the functional adaptations. For this purpose, we evaluated the left ventricular morphology and function and the expression of pathological cardiac hypertrophy markers.

Materials and methods

General procedures

Two-month-old male Wistar rats (CEMIB/UNICAMP, Campinas, SP), were housed in a temperature-controlled room (22 ± 2 °C) with lights on from 6 a.m. to 6 p.m., and received commercial rodent chow (Moinho Primor®) and filtered water ad libitum. The rats were randomized into four groups: Non-trained + Vehicle (NTV) or Nandrolone (NTN), Trained + vehicle (TV) or nandrolone (TN). An intramuscular injection of either vehicle (0.2 mL/kg) or nandrolone (5 mg/kg) was administered to Vehicle-treated (vehicle = propylene glycol) or Nandrolone Decanoate-treated rats (Deca-Durabolin®, Organon, São Paulo, Brazil), twice a week, for 6 weeks. The dose of nandrolone is comparable with that frequently used by athletes in doping processes (600 mg/week or approximately 8 mg/kg/week) (Pope and Katz, 1988; Norton et al., 2000). All procedures were performed in compliance with The Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996), and the Institutional Committee for Ethics in Animal Research approved all experiments (Protocol Number 944-1).

Training protocol

The training protocol was conducted as previously described (Cunha et al., 2005a, b). Briefly, during the first (pre-training) week, trained animals were adapted to water. Adaptation consisted of sessions of weight lifting (once a day for five days) in water at 30 °C ± 2 °C with an incremental number of sets (two to four) and repetitions (five to ten), 30 s rest between each set, carrying a load of 50% body weight strapped to the chest. On the last day of the adaptation period, animals were performing four sets of ten repetitions (lifts). Subsequently, trained rats were submitted to a high-intensity exercise training for 5 weeks. During the first 2 weeks of training, animals performed 4 sets of 10 jumps (lifts) per set carrying a load of 50% body weight strapped to the chest, with 30 s rest between each set. In the third and fourth training weeks, animals performed the same exercise carrying a load of 60% body weight, and in the last week, this load was adjusted to 70% body weight.

Echocardiography studies

Echocardiographic features were obtained using the recommendations of the American Society of Echocardiography. The exams were performed in anesthetized rats (ketamine, 50 mg/kg, xylazine 10 mg/kg, i.p), 48 h after the last exercise session. All the transthoracic echocardiograms were performed by a single, blinded observer with the use of a Sequoia 512 (ACUSON Corporation, Mountain View, CA) appliance, which offers a 10- to 14-MHz multifrequential linear transducer, as described in detail elsewhere (Santos et al., 2006).

Collagen content

Other animals, submitted to the same treatment, were sacrificed by decapitation and the heart was removed from the thoracic cavity, trimmed of excess non-cardiac tissue and weighed. The left ventricle was dissected, weighed, sliced into three sections perpendicular to the long axis and fixed in 10% neutral buffered formalin for 24 h. After embedding the material in paraffin, 6 μm sections were taken from the midventricular level and stained with Picrosirius Red, which allows the collagen molecules to be studied (Junqueira et al., 1979; Montes, 1996). Slides were analyzed in an Axioscope (Zeiss, Oberkochen, DE), using KS400 image analysis software.

Testosterone

Blood was collected from the trunk in a heparin-coated tube, centrifuged (700 g for 20 min at 4 °C), and plasma was frozen and stored at −20 °C for total testosterone quantification as previously described (Castor et al., 2009).

Cardiac hypertrophy markers

Other animals, submitted to the same treatment, were sacrificed by decapitation and the heart was removed from the thoracic cavity. The relative messenger ribonucleic acid (mRNA) levels of Atrial Natriuretic Peptide (ANP), skeletal α-actin, alfa-Myosin Heavy Chain (α-MHC) and beta-Myosin Heavy Chain (β-MHC) in the left ventricle were analyzed by Real-Time Polymerase Chain Reaction (Real-time RT-PCR).

Frozen tissue samples (100 mg) were homogenized in Trizol and ribonucleic acid (RNA) was isolated according to the manufacturer's instructions (Invitrogen Life Technologies). RNA concentration was determined by spectrophotometric measurement of the absorbance at 260 nm and checked for integrity by EtBr-agarose gel electrophoresis. RNA was primed with 0.5 μg/μl oligo dT (12–18) to generate the first strand desoxyribonucleic acid (DNA). Reverse transcription (RT) was performed using SuperScript™ II Reverse Transcriptase.

Before the samples were analyzed, a standard curve for each amplicon was obtained using serial dilutions of cDNA to determine amplification primer efficiency and the amount of material for each reaction. Primers were designed using Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). A DNA sequence was obtained from GenBank and primers were made for separate exons to distinguish the PCR products derived from cDNA from those derived from genomic DNA contaminants by their size. The mRNA expression of pathological left ventricle hypertrophy markers was assessed by oligonucleotide primers as follows: for *α-MHC* (sense: 5′-CGA GTC CCA GGT CAA CAA G-3′, antisense: 5′-AGG CTC TTT CTG CTG GAC C-3′); for *β-MHC* (sense: 5′-CAT CCC CAA TGA GAC GAA G-3′, antisense: 5′-AGG CTC TTT CTG CTG GAC A-3′); for *ANP* (sense: 5′-CTT CGG GGG TAG

GAT TGA C-3′, antisense: 5′-CTT GGG ATC TTT TGC GAT CT-3′); for *Skeletal α-actin* (sense: 5′-ACC ACA GGC ATT GTT CTG GA-3′, antisense: 5′-TAA GGT AGT CAG TGA GGT CC-3′). Real-time quantification of the target genes was performed with a *SYBR Green PCR Master Mix*, (Applied Biosystem, PE) using the ABI PRISM 7700 Sequence Detection System. The expression of *cyclophilin* (sense: 5′-AAT GCT GGA CCA AAC ACA AA-3′, antisense: 5′-CCT TCT TTC ACC TTC CCA AA-3′) was measured as an internal control for sample variation in RT reaction. An aliquot of the RT reaction was used for 50 cycle PCR amplification in the presence of SYBR green fluorescent dye according to a protocol provided by the manufacturer. Relative quantities of target gene expression were compared after normalization to the values of cyclophilin (ΔCT). Fold change in mRNA expression was calculated using the differences in ΔCT values between the two samples (ΔΔCT) and equation $2^{-\Delta\Delta CT}$. Results were expressed as % of control.

Statistical analysis

Results are expressed as means ± SEM. Data were compared using two-way analysis of variance (ANOVA) followed by the Tukey test. Differences were considered statistically significant when $p<0.05$.

Results

Body weight and testosterone levels

When the protocol ended, the trained animals treated with either vehicle (TV: 337 ± 11 g) or nandrolone (TN: 298 ± 10 g) presented lower body weight than the non-trained rats (NTV: 374 ± 12 and NTN: 333 ± 5 g; $p<0.05$). Furthermore, both NTN and TN groups presented lower body weight than the respective NTV and TV groups at the end of the experimental protocol ($p<0.05$). Testosterone was significantly diminished in the NTN (55.95 ± 11.40 ng/dL) and TN (42.39 ± 6.81 ng/dL) groups, compared with the NTV (157.10 ± 18.40 ng/dL) and TV (104.50 ± 26.46 ng/dL) groups, respectively ($p<0.05$).

Left ventricular morphology

No significant differences in left ventricle mass (LVMe) evaluated by echocardiography, or by wet left ventricle weight (Wet LV weight) were observed among the groups (Table 1; $p>0.05$). However, LVMe corrected by body weight (LVMe/BW), and Wet LV weight corrected by body weight (Wet LV weight/BW) were found to be significantly higher in the trained groups, whether treated with nandrolone or not, when compared with the respective non-trained groups (Table 1; $p<0.05$). In the NTN group, nandrolone promoted higher LVMe/BW, and higher Wet LV weight/BW, in comparison with the NTV group (Table 1; $p<0.05$). Relative left ventricle wall thickness (RWT) was increased in response to high-intensity physical training in rats treated with vehicle or nandrolone, when compared with the respective non-trained groups (Table 1; $p<0.05$). Moreover, the NTN group also presented higher RWT in comparison with the NTV group (Table 1; $p<0.05$). The interventricular septum thickness in the end-diastole (IVSDia) was increased in both the NTN and TV groups, when compared with the NTV group. The association between nandrolone and training potentiated the increase in IVSDia in comparison with all groups (Table 1; $p<0.05$).

Systolic function

Nandrolone treatment reduced the cardiac output/body weight ratio (cardiac index), in non-trained and trained groups, when compared with the respective vehicle-treated groups (Table 1; $p<0.05$). There was no difference in left ventricular fractional shortening or in heart rate among the groups (Table 1; $p>0.05$).

Diastolic function

Nandrolone treatment reduced early transmitral flow velocity (E-wave) in both non-trained and trained groups, when compared with the respective vehicle-treated groups (Table 1; $p<0.05$). Late transmitral flow velocity (A-wave) was significantly higher in the TV and TN groups, compared with the NTV and NTN groups, respectively (Table 1; $p<0.05$). The NTN group also presented a higher A-wave, when compared with the NTV group (Table 1; $p<0.05$). Ratio of maximum early to late transmitral flow velocity (E/A ratio) was significantly lower in the trained groups, whether they were treated with nandrolone or not, respectively, when compared with the non-trained groups (Table 1; $p<0.05$). Nandrolone treatment induced a decrease in the E/A ratio both in the NTN and TN groups, compared with the respective NTV and TV groups (Table 1; $p<0.05$). Isovolumic relaxation time corrected by the square root of the RR interval was increased in the NTN and TN groups when compared with the respective NTV and TV Groups (Table 1; $p<0.05$).

Table 1
Cardiac parameters determined by the echocardiographic system in rats submitted to resistance training and treatment with nandrolone or vehicle.

Parameters	NTV	TV	NTN	TN
Wet LV weight (g)	0.66 ± 0.01	0.66 ± 0.01	0.69 ± 0.02	0.67 ± 0.01
LVMe (g)	0.65 ± 0.02	0.66 ± 0.03	0.67 ± 0.02	0.67 ± 0.04
Wet LV weight(g)/BW (kg)	1.78 ± 0.06[a]	1.98 ± 0.06[b]	2.07 ± 0.05[b]	2.27 ± 0.05[c]
LVMe (g)/BW (kg)	1.71 ± 0.07[a]	1.95 ± 0.13[b]	1.92 ± 0.06[b]	2.24 ± 0.09[c]
RWT (mm)	0.38 ± 0.01[a]	0.46 ± 0.01[b]	0.51 ± 0.02[b]	0.56 ± 0.02[c]
IVSDia (mm)	0.38 ± 0.02[a]	0.45 ± 0.01[b]	0.48 ± 0.01[b]	0.56 ± 0.03[c]
CI (mL/min/g)	213.5 ± 11[a]	204.3 ± 19[a]	184.1 ± 18[b]	150.6 ± 14[b]
HR (bpm)	219 ± 6.2	224 ± 12.4	251 ± 14.3	257 ± 36.0
FS (%)	0.42 ± 0.01	0.43 ± 0.02	0.45 ± 0.02	0.47 ± 0.03
E-wave (cm/s)	0.54 ± 0.03[a]	0.51 ± 0.03[a]	0.44 ± 0.02[b]	0.44 ± 0.03[b]
A-wave (cm/s)	0.27 ± 0.02[a]	0.32 ± 0.02[b]	0.32 ± 0.02[b]	0.39 ± 0.03[c]
E/A ratio	1.91 ± 0.10[a]	1.50 ± 0.05[b]	1.46 ± 0.08[b]	1.26 ± 0.10[c]
IVRTcor	1.80 ± 0.02[a]	1.98 ± 0.08[a]	2.34 ± 0.08[b]	2.24 ± 0.07[b]

NTV = Non-trained + vehicle; NTN = Non-trained + nandrolone; TV = Trained + vehicle; TN = Trained + nandrolone. Wet LV weight = Wet left ventricle weight; LVMe = Left ventricle mass; Wet LV/BW = Wet left ventricle weight corrected by body weight; LVMe/BW = Left ventricle mass corrected by body weight; RWT = Relative wall thickness; IVSDia = Interventricular septum in the end-diastole; CI = Cardiac index; HR = Heart rate; FS = Fractional shortening; E-wave = Early transmitral flow velocity; A-wave = Late transmitral flow velocity; E/A ratio = Ratio of maximum early to late transmitral flow velocity; IVRTcor = Isovolumic relaxation time corrected by RR interval. Different letters indicate statistically different groups (Two-way ANOVA + Tukey test; $p<0.05$). N = 8 animals/group.

Left ventricular collagen content

Nandrolone treatment increased left ventricular collagen content, in both non-trained and trained groups, compared with respective non-trained and trained vehicle-treated groups (Fig. 1; $p<0.05$). Resistance training associated with nandrolone also induced an increase in collagen content in comparison with nandrolone non-trained and vehicle trained animals (Fig. 1; $p<0.05$).

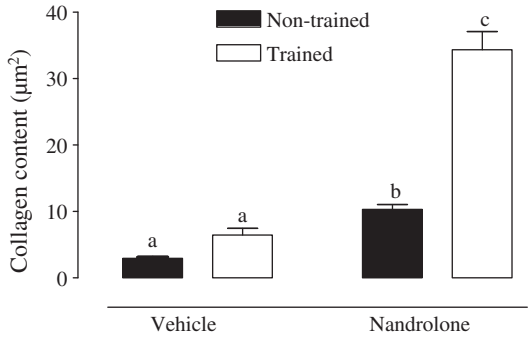

Fig. 1. Left ventricular collagen content, evaluated by polarization microscopy, in rats submitted to resistance training and treatment with nandrolone or vehicle. Different letters indicate statistically different groups (Two-way ANOVA + Tukey test; $p<0.05$). N = 5 animals/group. Data are reported as means ± SEM.

Cardiac hypertrophy markers

Fig. 2 shows the effects of resistance training and treatment with nandrolone on the gene expression of molecular markers of pathological cardiac hypertrophy. Nandrolone significantly reduced the expression of α-MHC mRNA in the left ventricle of the NTN and TN groups, in comparison with the respective NTV and TV groups (Fig. 2A; $p<0.05$), with no change induced by resistance training (Fig. 2A; $p>0.05$).

Resistance training significantly reduced the expression of β-MHC mRNA in the left ventricle of the TV and TN groups, when compared with the NTV and NTN groups, respectively (Fig. 2B; $p<0.05$). Nandrolone promoted an increase of 32% in the expression of β-MHC in the NTN group in comparison with the NTV control group (Fig. 2B). Although this percentage was not statistically significant, it showed a tendency to promote the up-regulation of β-MHC during cardiac hypertrophy produced by nandrolone ($p=0.056$). Resistance training *per se* promoted a 60% reduction in β-MHC expression in the TV group when compared with the control group NTV (Fig. 2B; $p<0.05$), and resistance training associated with nandrolone (TN group) promoted a reduction of only 23% in β-MHC expression when compared with the control group NTV (Fig. 2B; $p<0.05$). These results also indicated that nandrolone tended to impair the effect of the resistance training by promoting the down-regulation of β-MHC (Fig. 2B; $p=0.056$).

Skeletal α-actin gene expression was significantly increased only in the TN group in comparison with all groups (Fig. 2C; $p<0.05$).

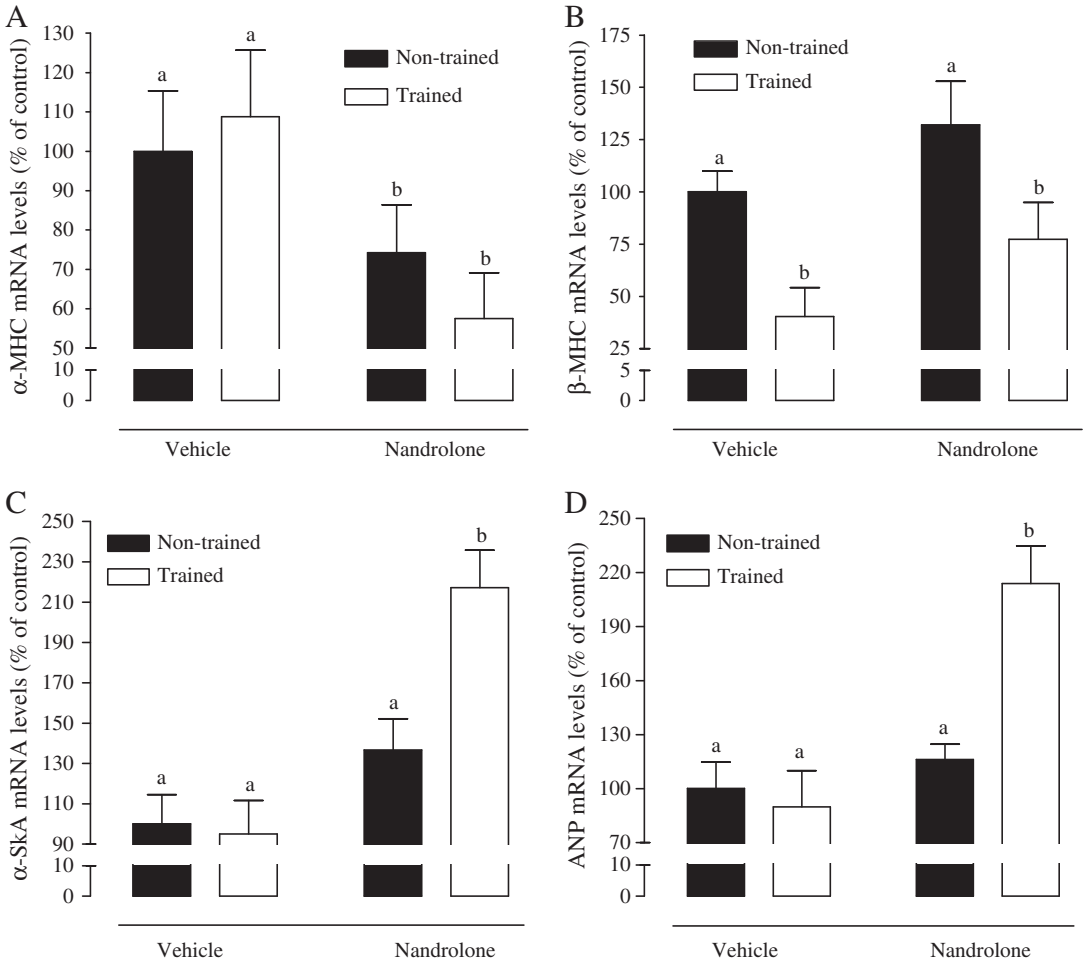

Fig. 2. Molecular markers of pathological left ventricle hypertrophy. Rats were either submitted to resistance training, or not, and treatment with Nandrolone or Vehicle. Messenger ribonucleic acid (mRNA) levels: (A) α-MHC (alpha-myosin heavy chain); (B) β-MHC (beta-myosin heavy chain); (C) α-SkA (Skeletal alpha-actin); (D) ANP (Atrial Natriuretic Peptide). N = 9–10 animals/group. Different letters indicate statistically different groups (Two-way ANOVA + Tukey test; $p<0.05$). Data are reported as means ± SEM.

Atrial Natriuretic Peptide gene expression was also significantly increased only in the TN group in comparison with all groups (Fig. 2D; $p<0.05$).

Discussion

The key findings of the present study were that resistance training and nandrolone treatment induced left ventricular hypertrophy associated with changes in left ventricle geometry, impaired systolic and diastolic function, and stimulated the re-expression of molecular markers of pathological cardiac hypertrophy, evidencing the malefic impact of the association between nandrolone and resistance training on the cardiovascular system.

The effectiveness of supra-physiological nandrolone doses administered by intramuscular injections was confirmed by the significant decrease in plasma total testosterone levels in trained or non-trained rats of the nandrolone-treated groups, when compared with the respective vehicle-treated groups. This result is in agreement with those showing that nandrolone treatment results in a decrease in plasma testosterone (Trifunovic et al., 1995; Castor et al., 2009), and indicates that a supra-physiological dose of the anabolic androgenic steroids (AAS) was administered, and that the hypothalamic-pituitary-gonadal axis was depressed by exogenous AAS (McGinnis, 2004; Castor et al., 2009).

Concentric left ventricular hypertrophy was evidenced by the increase in both the left ventricle mass/body weight ratio and relative wall thickness index in the TV, TN, and NTN groups. It is important to emphasize that these effects were found to be pronounced in the TN group.

It is well known that inappropriate left ventricular mass plays a negative prognostic role in the development and progression of cardiovascular abnormalities (Devereux et al., 1986; Collins et al., 2001). Therefore, to assess the influence of concentric hypertrophy on myocardial performance, systolic and diastolic functions were evaluated using Doppler echocardiography.

The echocardiographic results showed that nandrolone by itself or combined with resistance training, induced pathological concentric hypertrophy, evidenced by a decrease in the cardiac output index, and by increased isovolumic relaxation time in both trained and non-trained groups, when compared with the respective vehicle-treated groups. This is a malefic adaptation that may reduce the blood supply to organs and muscles. In addition, nandrolone depressed left ventricle diastolic function, decreasing the contribution to left ventricle passive filling and increasing active filling, resulting in a lower E/A ratio, which was paralleled by an increase in ventricular collagen content.

The decrease in E/A ratio in the TV group in comparison with the NTV group was not paralleled by a change in the left ventricular collagen content or isovolumic relaxation time in this group. Similar findings were obtained in humans by Karhunen et al. (1988), who observed that supra-physiological doses of nandrolone induced cardiac hypertrophy and depressed myocardial contractility. Urhausen et al. (1989) reported that AAS in highly trained bodybuilders induced an increase in left ventricular isovolumic relaxation time, and De Piccoli et al. (1991) showed that AAS abuse is associated with pathological left ventricle hypertrophy and altered diastolic filling. The discrepancies between the results (Hartgens et al., 2003) obtained in some studies conducted in humans could be attributed to the duration and/or the intensity of training programs and AAS abuse patterns, and to the difficulty of finding athletes, who will admit to having taken AAS and are willing to participate in scientific investigations (Kuhn, 2002).

According to D'Andrea et al. (2002), the dynamics of left ventricle passive filling is influenced by the time course of active relaxation and the passive deformation properties of the myocardium, including the thickness of the wall and its composition, particularly collagen deposition. In the present study, it was observed that nandrolone treatment, whether associated with resistance training or not, induced an increase in myocardial collagen content, and consequently pathological left ventricle hypertrophy. Thus, the left ventricular dysfunction observed is related to the structural remodeling of the myocardium, and particularly to the development of myocardial fibrosis. It is important to mention that other myocardial structural alterations, such as destroyed mitochondria, aberrant myofibrils, and necrotic cells in the myocardium may also account for the dysfunction (Melchert et al., 1992).

Exercise is known to be a potent cardiac hypertrophic stimulus, but the magnitude and pattern of left ventricular hypertrophy is dependent on the nature, duration, and intensity of exercise (Pluim et al., 2000; Haykowsky et al., 2002). In the heart, there are different morphological adaptations to resistance and aerobic training. Aerobically trained athletes mainly develop increased left ventricular chamber size and mass, while resistance trained athletes develop predominantly increased left ventricular wall thickness without a significant left ventricular chamber enlargement (Suman et al., 2000; D'Andrea et al., 2006). In both cases left ventricular hypertrophy is called “athlete's heart”. This physiological adaptation helps the myocardium support the increased demands of exercise while maintaining or enhancing normal function (Iemitsu et al., 2001) and is not related to collagen accumulation in the extracellular matrix (Thomas et al., 2000). In the present study, the resistance training *per se* promoted the cardiac hypertrophy, but without an increase in myocardial collagen content. However, resistance training associated with nandrolone promoted cardiac hypertrophy associated with increased myocardial collagen content, and systolic and diastolic dysfunction, configuring a pathological left ventricle hypertrophy. Therefore the interaction between nandrolone and resistance training seems to have additive or even synergistic effects on myocardial growth contributing to functional derangements.

Changes in cardiac physiology and structure, such as those observed in the present study, induced by the administration of supra-physiological doses of nandrolone, either associated with resistance training, or not, could be involved in higher risk of death (for example, in powerlifters who are AAS users) (Thompson et al., 1992). Although the association between AAS and exercise may change exercise-induced physiological cardiac hypertrophy into pathophysiological hypertrophy, the physiological mechanisms of this change are still poorly understood (Riezzo et al., 2011a).

Riezzo et al. (2011a) reported increased heart weight, and cardiac hypertrophy in trained mice treated with high doses of nandrolone, and attributed this effect to an enhanced protein synthesis in response to the nandrolone administration. It has been also suggested that β-adrenoceptor-mediated mechanisms are involved in the development of cardiac hypertrophy (Hannan et al., 2003; Sethi et al., 2007; Riezzo et al., 2011b), depending on the type and stage of hypertrophy (Sethi et al., 2007). At the early stage of hypertrophy, unaltered (Sethi et al., 2007) and increased β_1-adrenoceptor density has been observed due to pressure overload, which in turn induces concentric cardiac hypertrophy (Karliner et al., 1980; Ganguly et al., 1989). In addition volume overload that is known to induce excentric cardiac hypertrophy, increased β_1-adrenoceptor density (Sethi et al., 2007). On the other hand, in the late stage of both types of hypertrophy, the β_1-adrenoceptor density is attenuated (Sethi et al., 2007; Riezzo et al., 2011b).

In addition to the possible β-adrenoceptor-mediated mechanisms mentioned above, the cardiac hypertrophy can also lead to changes in contractile protein gene expression, altering the distribution of α- and β-MHC (Myosin Heavy Chain) isoforms, both in physiological and especially in pathological conditions, in which we can observe re-expression of skeletal α-actin, and the genes of non-contractile proteins such as Atrial Natriuretic Peptide (ANP) (Oliveira and Krieger, 2005; Soci et al., 2011), whose expression is rarely increased in normal adult rats. In the present study, resistance training promoted cardiac hypertrophy in the TV and TN groups. In both groups, training was capable of reducing β-MHC gene expression, which is predominant in the left ventricle of rats in fetal life, without

changes in α-MHC gene expression, predominantly expressed in the adult rats (Mahdavi et al., 1984).

It is important to point out that the resistance training protocol used in the present study is efficient in promoting the down-regulation of β-MHC mRNA expression, but is not efficient in promoting the up-regulation of α-MHC mRNA. These results seem to contradict the condition that occurs in pathological cardiac hypertrophy, which is an up-regulation of β-MHC mRNA. In addition, the resistance training was not able to promote significant re-expression of skeletal α-actin, and/or ANP genes. Thus, the molecular analysis of this study shows that the cardiac hypertrophy resulting from resistance training alone is not of a pathological nature.

The nandrolone by itself produced cardiac hypertrophy. In fact, a previous study reported the presence of androgen receptors in cardiac cells in both humans and animals (Marsh et al., 1998). Moreover, there is evidence of the presence of endogenous pathways of androgen actions in the development of cardiac hypertrophy, and a higher expression of androgen receptors in hypertrophied hearts in humans and rats (Liu et al., 2003; Rocha et al., 2007). AAS appear to act directly on the heart through the action of nuclear receptors, increasing the expression of mRNA and stimulating cardiac protein synthesis, resulting in myocardial hypertrophy (Melchert and Welder, 1995).

Generally, pathological left ventricular hypertrophy is characterized by an increase in myocyte size, and gene reprogramming, such as enhanced expression of β-MHC, skeletal α-actin, and/or ANP genes (Kim and Iwao, 2000). In the present study, nandrolone showed a trend towards promoting the up-regulation of β-MHC in the NTN group when compared with the control group NTV, and simultaneously it was capable of minimizing the beneficial effect of resistance training. The ventricular β-MHC isoform has lower ATPase activity, therefore, the increase in proportion of this myocardial isoform is associated with slower shortening velocity of the cardiac fibers, and pathological cardiac hypertrophy (Kim and Iwao, 2000).

If the relative increase in the proportion of α-MHC isoform in comparison with β-MHC is indicative of high ATPase activity, which is associated with improvement in systolic function (Scheinowitz et al., 2003), an increase in the proportion of β-MHC could obviously indicate the opposite effect.

Nandrolone treatment associated with resistance training potentiated the cardiac hypertrophy, which may result from the high intensity of the training protocol used in this study.

The association of nandrolone plus training promoted significant re-expression of skeletal α-actin and ANP genes in the TN group when compared with all the other groups. The significant re-expression of ANP, associated with other markers, such as skeletal α-actin, can be considered one among several criteria that indicate pathological cardiac hypertrophy (Klein et al., 1995; Barauna et al., 2008). These results show that when nandrolone treatment is associated with resistance training it promotes not only an increase in myocyte size (quantitative change) but also significant myocyte gene reprogramming (qualitative change).

Conclusions

Considering the increased ventricle collagen content, diastolic and systolic function impairment, and the re-expression of the fetal genes observed in the left ventricle of rats, we concluded that nandrolone by itself or associated with high-intensity resistance training induces pathological cardiac hypertrophy. Nandrolone per se increased collagen in the left ventricle, decreased the cardiac output index, and was capable of damaging the beneficial effects of resistance training on ventricular β-MHC mRNA expression. The association of resistance training with nandrolone induced the re-expression of Atrial Natriuretic Peptide and skeletal α-actin, which was parallel to diastolic and systolic function impairment. Thus, these results may contribute to understanding the underlying mechanisms involved in the pathological cardiac hypertrophy promoted by AAS abuse, and represent a potential tool for understanding the sudden death of strength trained athletes who are AAS users.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors thank Margery Galbraith for editing the English in the manuscript and Gustavo Narvaes Guimarães for technical assistance with the collagen analysis. This work was supported by grants from the State of São Paulo research support foundation—FAPESP 05/60284-6, FAEPEX/UNICAMP and CNPq. A.P.T., V.J.N. and F.C.L.G. were recipients of FAPESP scholarships and T.S.C. was the recipient of a CNPq scholarship.

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