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Cardiotoxicity in rabbits after long-term nandrolone decanoate administration

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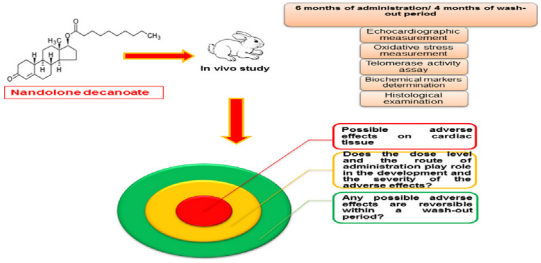
HIGHLIGHTS

- Cardiovascular effects of nandrolone decanoate on young rabbits.
- Focal fibrosis and inflammatory infiltrations of cardiac tissue in high dose groups.
- Preserved systolic performance, distorted MPI values, diastolic impairment.
- TBARS increased in high dose groups, troponin increased in wash-out period.
- Heart tissue relative telomerase activity increased dose-dependently.

GRAPHICAL ABSTRACT



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ABSTRACT

Abuse of anabolic androgenic steroids is linked to a variety of cardiovascular complications. The aim of our study was to investigate the possible cardiovascular effects of nandrolone decanoate on young rabbits using echocardiography, histology and monitoring of telomerase activity, oxidative stress and biochemical markers. Fourteen rabbits were divided into three administration groups and the control group. Doses of 4 mg/kg and 10 mg/kg of nandrolone decanoate, given intramuscularly and subcutaneously, two days per week for six months were applied. A 4-months wash-out period followed. Focal fibrosis and inflammatory infiltrations of cardiac tissue were observed in the high dose groups. Thiobarbituric acid-reactive species (TBARS) levels were significantly increased in the high dose groups, while catalase activity decreased. Myocardial Performance Index (MPI) is the main echocardiographic index primarily affected by nandrolone administration in rabbits. Despite the preserved systolic performance, histological lesions observed associated with distorted MPI values, point

Abbreviations: AAS, anabolic androgenic steroids; TBARS, thiobarbituric acid reactive species; TAC, total antioxidant capacity; LDH, lactate dehydrogenase; CpK, creatinine kinase; BNP, B-type natriuretic peptide; PW, pulsed wave Doppler; TDI, tissue Doppler imaging; MPI, myocardial performance index; LV, left ventricular.

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Telomerase activity
Biochemical markers

to diastolic impairment of the thickened myocardium due to nandrolone treatment. Oxidative stress accumulates and telomerase activity in cardiac tissue rises. Subcutaneous administration seems to be more deleterious to the cardiovascular system, as oxidative stress, telomerase activity and biochemical markers do not appear to return into normal values in the wash-out period.

1. Introduction

Anabolic androgenic steroids (AAS) are chemical derivatives of the endogenous hormone testosterone and exert two main physiological actions including the promotion of muscle growth and the development of the male reproductive system (Van Amsterdam et al., 2010; Thiblin and Petersson, 2005). Although AAS have valid medical applications, human and animal AAS are frequently misused in order to enhance performance, strength and even for improving the physical appearance and body image (Copeland et al., 2000; Darke et al., 2014; Hakansson et al., 2012; Kao et al., 2012; Larance et al., 2008; Petersson et al., 2010).

Abuse of AAS has become a public health issue, as there is mounting evidence suggesting that they affect the myocardial ventricular function through the androgen receptor pathway (Baggish et al., 2010; D'Andrea et al., 2007; Figueredo, 2011; Kasikcioglu et al., 2009; Krieg et al., 2007; Lane et al., 2006; Luijkx et al., 2013), as well as the cardiovascular system in general (Kanayama et al., 2010). Acute myocardial infarction (Fisher et al., 1996; Wysoczanski et al., 2008), cardiomyopathy (Ahlgrim and Guglin, 2009; Mark et al., 2005), severe arrhythmia (Lau et al., 2007; Sullivan et al., 1999) and even cases of sudden death (Fineschi et al., 2001, 2007; Di Paolo et al., 2007; Montisci et al., 2012; Petersson et al., 2006; Thiblin et al., 2000) have been described as the most dramatic cardiovascular manifestations caused by the excessive use of anabolic steroids. However, more clinical and mechanistic studies are needed to evaluate the prevalence of morbidity and mortality in users, as data up to now are scattered and circumstantial and based mainly on case reports.

Moreover, recent studies connect the administration of AAS with changes in oxidative stress, suggesting distinct and different patterns of oxidative stress systemic or local response per substance (Germanakis et al., 2013; Pey et al., 2003). Exercise training did not seem to affect the oxidative status of the individuals (Pey et al., 2003), whereas others report that treatment with stanozolol protected rat skeletal muscle mitochondria against oxidative damage of proteins and changes in membrane fatty acid composition induced by acute exercise (Saborido et al., 2011).

Oxidative stress is known to play a crucial role in the pathogenesis of heart failure. It induces damage or apoptosis of endothelial cells (Aoki et al., 2001; Matthews et al., 2006) and it has also been implicated in the development of atherosclerosis through a variety of mechanisms, especially those leading to endothelial dysfunction (Berliner et al., 1990; D'Agnillo et al., 2000). In addition, cultured vascular smooth muscle cells and endothelial cells exposed to oxidative stress, exhibit shortening of telomeres and accelerated cellular senescence (Matthews et al., 2006). Telomeres are indicators of oxidative stress (Saretzki, 2009). Telomeres and telomerase provide protection against threats to the genome that arise from aninherent difficulty in the asymmetric replication of DNA (Calado and Young, 2009). Recently, telomere and telomerase have been recognized as potential factors involved in the initiation and progression of cardiovascular disease (Samani and van der Harst, 2008; Fuster and Andres, 2006; Edo and Andres, 2005; Wong et al., 2008). There is accumulating evidence that connect telomere length with cardiovascular-related phenotypes, including atherosclerosis and heart failure (Wong et al., 2009). Moreover, alterations in telomerase activity have many clinical implications, such as aging, cancer, and diabetes mellitus (Blackburn, 2005).

Nandrolone (19-nortestosterone, 17b-hydroxy-estr-4-en-3-one) was synthesized in the early 1950s and although it can be regarded as an old doping agent, it is still used to enhance muscular strength and performance in sports and in horse racing. In fact, it is one of the most frequently detected doping agents worldwide (Bricout and Wright, 2004; Hemmersbach and Grosse, 2010; Sauer et al., 1998).

Nandrolone and its esters have been widely used as therapeutic agents mainly in protein deficiency diseases like aplastic anaemia (Gardner, 1985), osteoporosis (Geusens, 1995), AIDS (Mulligan et al., 2005; Storer et al., 2005), cancer (Puccio and Nathanson, 1997) and protein deficiency in the elderly.

The primary aim of our study was to investigate the possible adverse effects of nandrolone decanoate, one of the most commonly used pharmaceutical forms of nandrolone, on cardiac tissue of healthy rabbits. Secondary aim was to evaluate whether the dose level and the administration mode could play any further role and whether any observed adverse effects could be reversible within a wash-out period of 4 months. Thus, echocardiography was applied to the anabolic treated rabbits and histopathological examination of heart tissues was conducted. Furthermore, systemic oxidative stressmarkers and biomarkers related to normal cardiovascular function were measured. To our knowledge, this is the first study that examines all these parameters in order to evaluate the possible cardiotoxic action of nandrolone decanoate.

2. Methods and materials

2.1. Animals

Fourteen healthy New Zealand multicoloured male rabbits (3900–5500 g each, in the age of 10–15 months) were used for the purpose of this study. The animals were housed in individual metal cages and kept in a 12-h dark/light cycle, at a temperature between 20 and 23 °C, in the laboratory animal house facilities of the University Hospital of Heraklion, Crete. They were fed with commercial rabbit pellets ad libitum and provided with drinking (tap) water. The rabbits were acclimatized under laboratory conditions for 2 weeks, whereupon the treatment period begun.

The animals were divided into four treatment groups. Group 1 and group 2 received a high (HDIM) and a low dose (LDIM) of nandrolone decanoate (10 mg/kg and 4 mg/kg, respectively), two days per week for six months. Group 3 received subcutaneously a high dose (HDSC) of nandrolone decanoate (10 mg/kg) 2 days per week for 6 months. Group 4 served as the control group (C) and its animals were only treated with saline solution. The saline solution was administered intramuscularly. Originally, the appropriate amounts of anabolic were diluted in 2.0 ml of saline solution.

The experimental scheme of exposure was selected in order to simulate the claimed abuse of steroids by athletes and consisted of two periods: the administration period that lasted six months and the wash-out period, the duration of which was four months. Two animals of the high dosed groups were selected for monitoring in the wash-out period after ceasing administration. Two echocardiographic examinations were conducted, both of them the day before the sacrifice sessions. The first sacrifice was performed at

the end of the sixth month (end of the administration period) and the second at the end of tenth month (end of wash-out period). Serum was collected at baseline, every two months during the administration and wash-out period and at the day of the sacrifice sessions (Fig. 1). The animals were sacrificed by intravenous injection of 5 ml pentothal (Thiopental sodium solution, 25 mg/ml), according to the bioethical rules of the University of Crete. During the study period, the animals were weighed and their food consumption was recorded. All rabbits were regularly observed and their condition was closely monitored. No pathological clinical signs were observed at any point.

The present study was approved by the Veterinary Administration Office of Heraklion (Crete, Greece), the Animal Investigation Committee of the University of Crete (Heraklion, Crete, Greece) and conformed to the National and European Union directions for the care and treatment of laboratory animals. All efforts were made to minimize suffering.

2.2. Echocardiographic study protocol

The echocardiography protocol used has been previously described (Zafiropoulos et al., 2014). Briefly, following the subcutaneous administration of ketamine (17 mg/kg) and xylazine (7 mg/kg), the sedated rabbits, moved to the animal keeping lab special exam room, having their anterior chest and upper abdomen hair removed were placed in the supine position and studied by a high end echocardiographic system equipped with and 10 MHz phased array cardiac ultrasoundprobe. M-Mode, 2D imaging, Pulsed wave (PW) Doppler and tissue Doppler (TDI) recorded still frames and video loops were digitally stored allowing for offline analysis by a single observer with certified expertise in echocardiography. M-Mode and 2D-Mode measurements included documentation of radial left ventricular (LV) dimensions, systolic function (Fractional shortening (FS), Ejection Fraction (EF), Stroke Volume (SV), Cardiac Output (CO)) and myocardial mass estimation.

Availability of raw data allowed for anatomic M-Mode based estimation of longitudinal myocardial systolic function (Mitral and Tricuspid valve Annulus Peak Systolic Excursion (MAPSE and TAPSE) respectively,) as previously described (Germanakis et al., 2012). Pulsed wave (PW) Doppler and myocardial tissue Doppler imaging (TDI) were used to document diastolic flow and myocardial basal segment velocities and myocardial performance index (MPI) as a measure of global myocardial function. For each measured variable, the average value of three measurements corresponding to consecutive cardiac cycles was documented as a single value.

2.3. Histopathological lesions

Myocardial tissueblock samples, fixed in formalin, embedded in paraffin, and sectioned at 3 μm. Then, they were stained with eosin–hematoxylin and subsequently examined under light microscopy. In the samples that fibrosis was detected a histochemical Masson's trichrome stain was performed. Histopathological examinations were conducted blind by a histopathologist.

2.4. Oxidative stress biomarkers

Oxidative stress biomarkers (TBARS concentration, carbonyls, catalase activity and TAC) were measured as previously described (Germanakis et al., 2013; Tsitsimpikou et al., 2013; Veskoukis et al., 2008; Zafiropoulos et al., 2014). Briefly, TBARS expressed in μmol/l, were measured in blood plasma by mixing it with trichloacetic acid (TCA) Tris–HCl, Na_2SO_4 and thiobarbituric acid and incubated at 95 °C. TCA was added again, centrifuged and the absorbance was measured at 530 nm. TAC is expressed in mmoldiphenyl-1-picrylhydrazyl (DPPH)/L reduced to DPPH:H. It was determined by the DPPH spectrophotometric assay using stable DPPH radical as reagent. The plasma was mixed with PBS and DPPH, it was then incubated and centrifuged and the absorbance was measured at 520 nm. The determination of catalase activity was based on the method of Aebi (1984). Briefly, 4 μl of erythrocyte lysate (diluted 1:10) were added to 2991 μl of 67 mM sodium potassium phosphate (pH 7.4) and the samples were incubated at 37 °C for 10 min. Five microliters of 30% hydrogen peroxide (H_2O_2) were added to the samples and the change in absorbance was immediately read at 240 nm for 130 s. Calculation of catalase activity was based on the molar extinction coefficient of H_2O_2. Protein carbonyls, expressed in nmol/mg protein, were determined in plasma, as previously reported (Veskoukis et al., 2008).

2.5. Telomerase activity

The telomerase activity in cardiac tissue samples and peripheral blood monocytes (PBMNs) was measured using a commercial telomerase polymerase chain reaction—enzyme linked immune sorbent assay (PCR-ELISA) (Roche Diagnostics Corp., Indianapolis, IN, USA), based on the telomeric repeat amplification protocol, as previously described (Germanakis et al., 2013).

2.6. Biomarkers indicative of cardiovascularfunction

Blood samples were individually collected from the vena auricularis of each rabbit in the appropriate glass tubes in order to

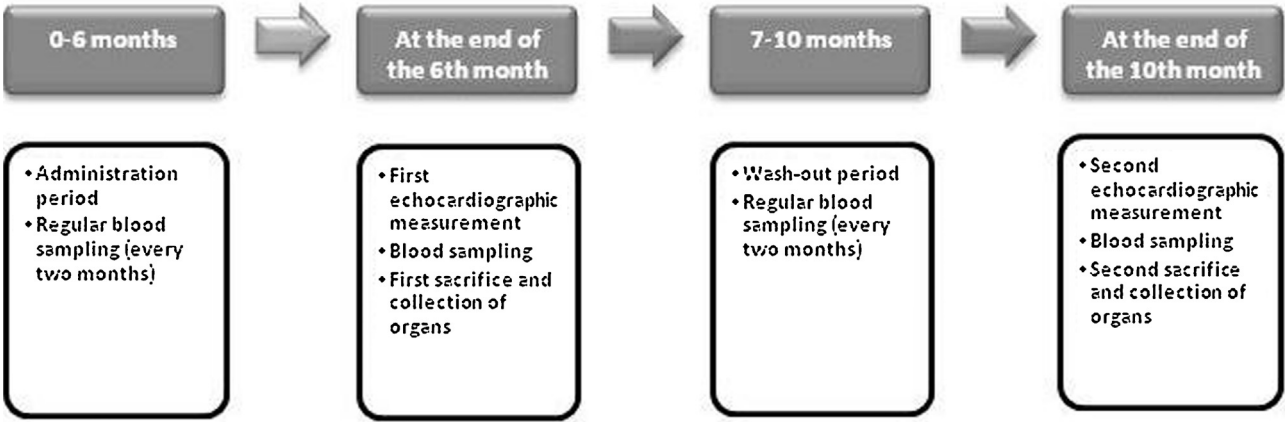

Fig. 1. Flow chart of the experimental procedure.

evaluate the concentration of the following biomarkers: LDH, TroponinI, CpK and BNP. Blood serum was separated by centrifugation at 4000 rpm for 15 min and then stored at −18 °C. LDH and CpK were spectrometrically measured in Olympus AU2700 while BNP and Troponin were measured in the ADVIA Centaur XP Immunoassay System (Siemens Healthcare Diagnostics).

2.7. Statistical analysis

All results are presented as mean values ± SD. Statistical analyses were performed with SPSS version 14 (SPSS Inc., Chicago, IL, USA). Significant differences between means for the same parameters were investigated with repeated measures ANOVA and paired *t*-test analyses. Independent *t*-tests were used to compare mean values between groups. Pearson and Spearman correlations and linear regression analysis was conducted to investigate associations between various variables. Differences between categorical variables were assessed by the chi-square test. A *p*-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Echocardiographic measurements

There were no significant changes both in the body weight and in the heart mass of all treated animals (Table 1). Anabolic treated animals in general demonstrated a trend for non-significant higher values of myocardial mass (myocardial mass mmode: treated animals 5.8 ± 1.3 g vs control group 5.0 ± 0.9 g, $p = 0.340$), which was associated with significant impairment of global myocardial performance indexes (MPI-PW: treated animals 0.73 ± 0.16 vs control group 0.52 ± 0.07, $p = 0.026$; MPI-TDI: treated animals 0.91 ± 0.09 vs control group 0.63 ± 0.02, $p = 0.001$). There was no correlation between heart weight/body weight ratio and MPI (both PW and TDI) ($p > 0.05$). Systolic performance showed no differences or a trend to ameliorate in anabolic treated animals (cardiac output: treated animals 0.42 ± 0.13 l/min vs control group 0.31 ± 0.71 l/min, $p = 0.152$). Animals treated with higher anabolic doses demonstrated more pronounced myocardial mass increase (myocardial mass-mmode: high-dose treated animals 6.0 ± 1.4 g vs low-dose treated animals 4.9 ± 0.31 g, $p = 0.343$) and more pronounced deterioration of global myocardial performance indexes (MPI-PW: high-dose treated animals 0.79 ± 0.11 vs low-dose treated animals 0.50 ± 0.47, $p = 0.001$; MPI-TDI: high-dose treated animals 0.90 ± 0.09 vs low-dose treated animals 0.89 ± 0.13, $p = 0.000$).

3.2. Histopathological alterations of the cardiac muscle tissue

Focal fibrosis and a mild chronic inflammation of cardiac tissue were observed at high doses (HDIM and HDSC group respectively) in contrast to the LDIM group, where only a mild focal fibrosis was observed. In animals treated subcutaneously, edema was also observed (Figs. 2–6). The extent of fibrosis was statistically correlated with the heart weight/body weight ratio ($p = 0.045$).

3.3. Systemic oxidative stress biomarkers

Compared to the control group, TBARS levels were significantly increased ($p < 0.05$) in HDIM and HDSC groups. For carbonyls and TAC, no statistically significant difference was observed in any of the administration groups. Catalase levels were non-significantly decreased in HDSC and HDIM group ($p = 0.238$ and $p = 0.237$, respectively). In LDIM group, the levels of all oxidative stress biomarkers remained practically unchanged (Fig. 7). Comparing the two different administration modes, there were no differences in the biomarkers monitored. A significant dose response in the intramuscular administration mode was observed in the TBARS levels ($p = 0.01$). In the wash-out period, TBARS levels and catalase in the HDIM group returned to normal levels ($p > 0.05$). For the HDSC group, a significant increase in the TBARS levels was observed ($p = 0.01$) and catalase returned to the normal values ($p > 0.05$).

3.4. Telomerase activity

During the administration period, heart tissue relative telomerase activity in all administration groups increased significantly and in a dose-dependent manner compared to controls (LDIM 230% vs HDIM 552%, $p = 0.004$; and HDSC 212%) (Fig. 8). Intramuscular administration seemed to further increase inflammation, as depicted by telomerase activity in PBMNs (HDIM 652% vs HDSC312%, $p = 0.003$; LDIM 330% vs HDSC 312%, $p = 0.20$). In the wash-out period, telomerase activity in HDIM and HDSC groupdid not return to normal values ($p < 0.05$).

3.5. Biomarkers indicative of cardiovascular function

CpK was universally but non-significantly increased, while LDH showed a mild rise more pronounced in the intramuscular administration group (26%). Significantly higher troponinI levels were observed in the HDSC group ($p = 0.024$), which continued to

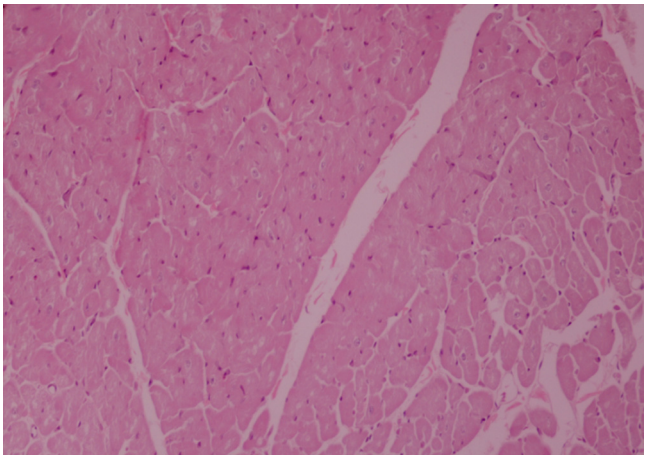

Fig. 2. Normal cardiac muscle tissue from control animals (Hematoxylin Eosin).

Table 1
Levels of body weight, heart weight and heart weight/body weight ratio.

	Control group	Administration period			Wash-out period	
		LDIM	HDIM	HDSC	HDIM	HDSC
Body weight (g)	4025 ± 35	4050 ± 70	4050 ± 70	3950 ± 71	5100 ± 566	4800 ± 120
Heart weight (g)	8.40 ± 0.14	7.90 ± 0.10	8.75 ± 0.14	8.70 ± 1.27	11.4 ± 1.6	10.1 ± 0.5
Heart weight/body weight ratio ×1000	2.00 ± 0.05	1.95 ± 0.05	2.15 ± 0.05	2.20 ± 0.03	2.5 ± 0.7	2.20 ± 0.10

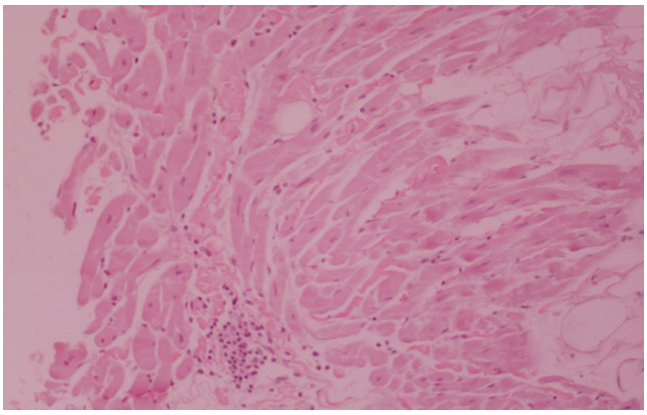

Fig. 3. Inflammation of cardiac muscle tissue in HDSC group (Hematoxylin Eosin).

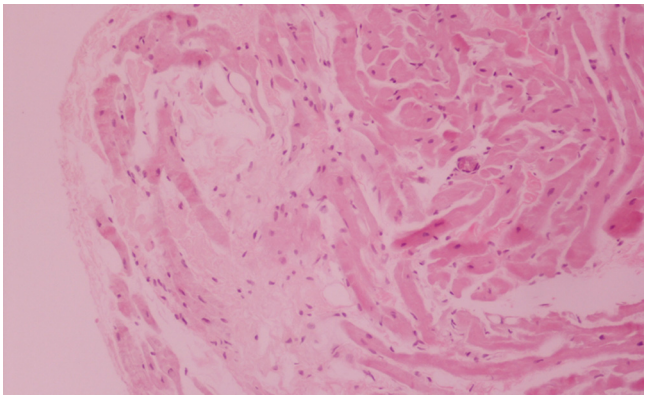

Fig. 4. Fibrosis of cardiac muscle tissue in HDIM group (Hematoxylin Eosin).

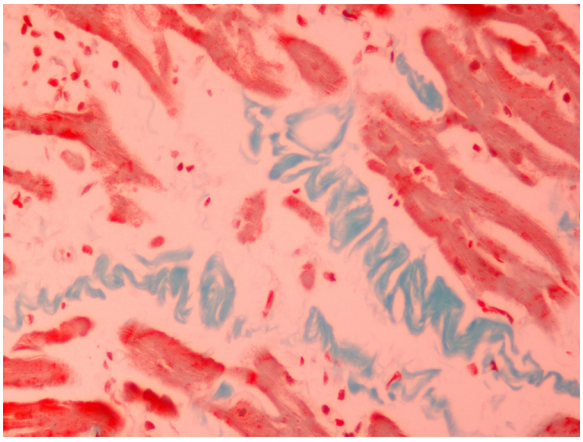

Fig. 5. Inflammatory infiltrations, edema and fibrosis of cardiac muscle tissue in HD group (Masson Trichrome).

rise during the wash-out period and correlated with an abrupt increase in BNP levels (7.3 ± 3.6 pg/mg) at that time (Table 2).

4. Discussion

Anabolic steroids have become a popular drug among athletes and are known to have a multitude of pathological effects when administered in supraphysiologic doses. Long term illicit use of supraphysiologic doses of AAS may cause adverse cardiovascular effects, but these remain poorly understood (Kanayama et al., 2008; Parssinen and Seppala, 2002; Plehn et al., 1993).

Previous studies reported that long-term illicit use of supra-pharmacological doses of AAS was associated with reduced LV diastolic functions (impaired relaxation and reduced compliance of LV), increased LVmass, LV/atrial hypertrophy, subclinical systolic impairment, increased myocardial stiffness and myocardial fibrosis, and altered cardiac autonomic system regulation (Basaria, 2010; D'Andrea et al., 2007; Deligiannis and Kouidi, 2006; Thompson et al., 1992; Varró and Baczkó, 2010). Moreover, the diastolic dysfunction was found to be correlated with the dosage and the duration of use (Basaria, 2010; Kouidi et al., 2008).

A previous study (D'Andrea et al., 2007) investigating left ventricular dysfunction, after chronic misuse of AAS in athletes showed that power athletes had a subclinical impairment of both systolic and diastolic myocardial functions, being the dysfunction associated with mean dosage and duration of AAS use (Martinez-Quintana et al., 2013). In contrast, short-term administration of AAS for periods up to 16 weeks did not lead to detectable echocardiographic alterations of heart morphology and systolic and diastolic function in experienced strength athletes (Hartgens et al., 2003).

Animal model studies have been conducted to evaluate the impact of AAS supraphysiological doses on the cardiovascular system and on myocardial injury and to understand the pathogenesis of ventricular remodeling and dysfunction, of ventricular arrhythmias and of sudden cardiac death associated with AAS-abuse (Turillazzi et al., 2011). The rabbit model (average life time 4–8 years) is a useful tool for exporting information about possible adverse effects in humans, especially in the heart (Milani-Nejad and Janssen, 2014). The dose scheme of the present study was selected in order to simulate the allegedly known administration scheme among steroids abusers representing chronic exposure together with the period of withdrawal (Sattler et al., 1999; Grönbladh et al., 2014). Nandrolone decanoate acts really slowly and athletes are advised to use the hormone for 10–12 weeks, even though some may use it up to 16 weeks. The recommended dose for a man of average 80 kg in order to enhance muscle built, is up to 500–800 mg per week. Such hormonal supplementation is recommended to be repeated regularly. The administration scheme used in rabbits of the present study is also in accordance with various other animal studies (Shokri et al., 2014; Ammar et al., 2004).

The present study tried to assess the long-term effects on heart's function of similar dosage of nandrolone decanoate as athletes' claimed abuse. This is one of the first attempts to evaluate cardiac function by echocardiography in anabolic treated animals. In order to assess systolic, diastolic, and global myocardial performances of rabbits that were administered nandrolone decanoate, several echocardiographic techniques and indices were used, some of them being conventional (M-, PW Doppler) (Fontes-Sousa et al., 2006; Plehn et al., 1993), while others being cutting edge including TDI, Myocardial Performance Index (MPI) (Moura et al., 2009; Stypmann et al., 2007) and MAPSE assessments by anatomic M-Mode, rarely applied in animal models (Germanakis et al., 2012).

Anabolic administration seems to be correlated with increased myocardial mass due to eccentric left ventricular hypertrophy (increase both in myocardial wall thickness as well as increase in left ventricular cavity enddiastolic diameter), in agreement with the anabolic nature of nandrolone. These changes are associated with a possible better systolic performance of the heart or at least not of deterioration of systolic performance. Of concern, however, is the observed impairment of global myocardial function indices, in all anabolic treated animals, which was also statistical significant. This global myocardial function impairment is suggestive of changes in the histology and relaxation properties of the

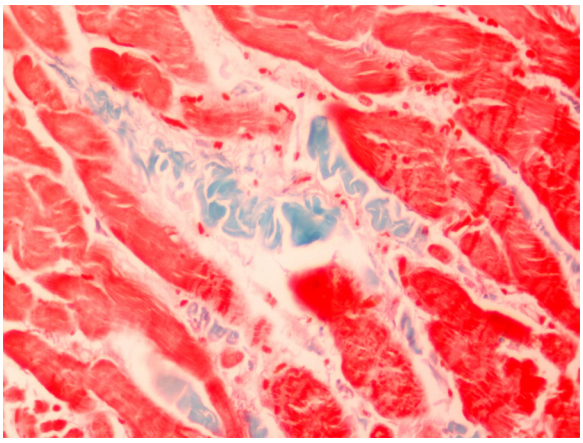

Fig. 6. Fibrosis of cardiac muscle tissue in HD group (Masson Trichrome).

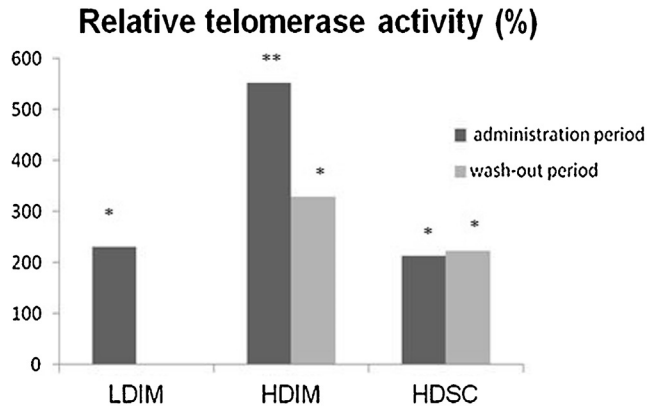

Fig. 8. Relative telomerase activity in heart tissue in all treated groups. Symbols mark the statistically significant levels as follows: (*) and (**) indicate $p < 0.05$ and $p < 0.01$, respectively, as compared to control group.

thickened myocardium following anabolic administration, especially at higher doses. Indeed, focal fibrosis and inflammatory filtrations were observed in heart tissue of high dosed rabbits. Therefore the "improved" myocardial mass and "improved" systolic myocardial performance are achieved at a cost of changes of diastolic function/relaxation properties of the thickened myocardium. The long term significance of alternations of mechanical properties of the thickened myocardium could not be assessed in the present study. Since young rabbits have been used, before having reached their maximum myocardial mass, our findings might represent a mild myocardial cardiotoxicity combined with a probable inhibition of normal myocardial heart growth. Inhibition of normal myocardial growth following administration of cardiotoxic agents, has previously been well described in anthracycline cardiotoxicity, which results in thin walled ventricles with diastolic and systolic dysfunction (Germanakis et al., 2006).

Results from this study are consistent with previous findings showing that treatment with nandrolone decanoate developed cardiac hypertrophy in rats and was associated with myocyte hypertrophy and augmented heart collagen deposition, alone or in combination with physical training (Do Carmo et al., 2011; Franquniet al., 2013; Tanno et al., 2011). Moreover, from a previous study, an important localized cardiotoxic effect was presented after short term administration to young rabbits of low dose anabolic steroid. Methanabol administration compared to turinabol was associated with a trend for worse myocardial function indexes and greater negative impact on myocardial mass growth (Germanakis et al., 2013).

The heart is the most frequently affected organ by administration of exogenous steroids (Riezzo et al., 2011). It is well established that the heart is susceptible to free radical damage, due to its

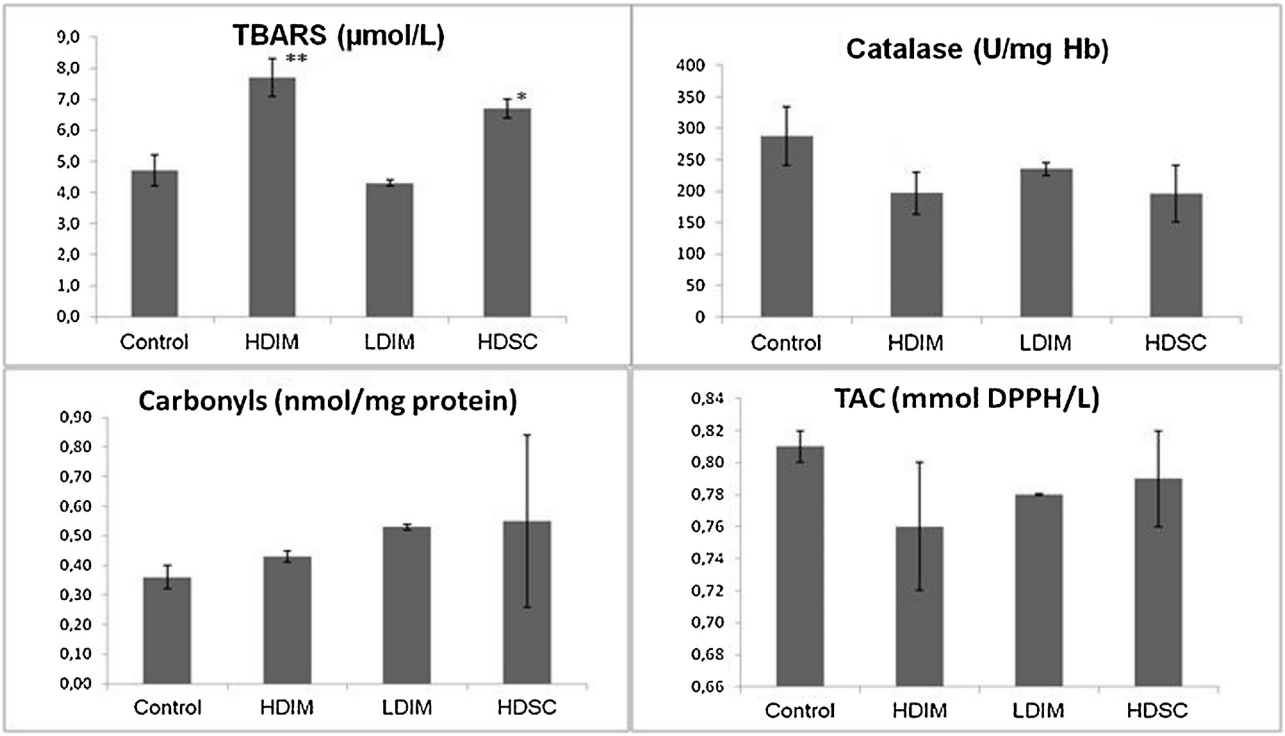

Fig. 7. Oxidative stress biomarkers at the end of the administration period. Symbols mark the statistically significant levels as follows: (*) and (**) indicate $p < 0.05$ and $p < 0.01$, respectively, as compared to control group.

Table 2
Levels of biomarkers during the administration and the wash-out period.

Biomarkers	Control group	Administration period			Wash-out period	
		LDIM	HDIM	HDSC	HDIM	HDSC
CpK (U/L)	332 ± 253	645 ± 442	1149 ± 733	461 ± 314	553 ± 353	351 ± 333
LDH (U/L)	117 ± 133	253 ± 121	183 ± 142	126 ± 81	125 ± 60	52 ± 36
Troponin I (U/L)	0.000	0.0015 ± 0.0015	0.003 ± 0.002	0.007 ± 0.0015*	0.3 ± 0.08[a]	1.2 ± 1.07[a]

* Indicates $p < 0.05$ as compared to control group.

intrinsic elevated oxidative metabolic activity and its fragile antioxidant resistance, in comparison to other parts of the body.

Regardless the route of administration of nandrolone decanoate, increased lipid peroxidation in plasma, as evidenced by the elevated TBARS levels, was observed at high doses. Moreover, in the same group not significantly decreased activity of catalase, the most important antioxidant enzyme, was noticed.

Protein carbonyls and TAC levels remained unchanged in high as well as in low dose of nadrolone decanoate. Our findings suggest an oxidative stress induction in such an extent level that could not be outbalanced by antioxidant mechanisms.

A recent study investigating the effects of supraphysiological administration of nandrolone decanoate on rat heart redox metabolism in sedentary and exercised animals, demonstrates that high doses of AAS hampered the cardioprotection provided by exercise by blocking its positive effects on antioxidant enzymes activities. Further research is required to determine the exact mechanisms by which nadrolone decanoate mediates these effects on heart physiology and redox metabolism (Chaves et al., 2013). Similarly, another study on the effects of testosterone on rat heart physiology and redox homeostasis under non-ischemic conditions indicated that testosterone promoted lipid peroxidation in sedentary and exercised animals in a dose-dependent manner. Also, in sedentary animals, high testosterone doses significantly reduced heart GPx and GR activities but not catalase, whereas in exercised rats the activities of all these enzymes were strongly reduced by this steroid (Sadowska-Krepa et al., 2011). Together, these data reveal that the cardiotoxic effects associated to AAS abuse are mediated by reduced heart antioxidant capacity.

Telomerase is a principal target for regulatory mechanisms, while simultaneously being highly susceptible to oxidative stress, since it plays a crucial role in the maintenance of steady state telomere length (Rentoukas et al., 2012). The significantly increased telomerase activity found in the heart of the anabolic treated animals, which corresponds to an extension of the life span of the cells, could possibly represent a counteracting survival mechanism (Lopez-Diazguerrero et al., 2012; Serra et al., 2003). Such a protective function has already been shown for telomerase, which is excluded from the nucleus under oxidative stress and is localized in the mitochondria in order to protect them from stress (Ahmed et al., 2008).

BNP is a cardiac hormone and a well-established biomarker, extensively used for the diagnosis and prognosis of patients with heart failure. The higher the plasma levels of BNP the more severe the condition of heart failure is. The progression of heart failure is associated with a progressive loss of cardiomyocytes that can be detected clinically by increased serum levels of troponins. CpK is expressed by various tissues and cell types. Elevated levels of CpK in the blood are associated with damaged tissue, clinically referring to myocardial or skeletal muscle damage. In our study elevated levels of CpK were observed in all treatment groups compared to controls during the administration period. The rise of CpK levels did not coincide with significant troponin elevation. This suggests a predominant skeletal muscle origin of CpK elevation, probably due to a concomitant process of skeletal muscle myocyte death, regeneration and hypertrophy during anabolic administration.The high levels of troponin and BNP in HDSC group that are persistent during the wash-out period and the moderate elevated levels in HDIM group at the same period can be attributed to more pronounced evidence of heart failure with time. Troponin and BNP elevations are prominent markers of heart failure establishment.

Anabolic steroids use is a growing problem affecting both amateur and professional athletes. Current knowledge of adverse myocardial implications due to anabolic steroids use comes from echocardiographic studies in athletes that report anabolic use usually for several years and most times using a mixture of anabolic steroids. The current study is the first echocardiographic study in animals treated with nandrolone at a prespecified dose and administration scheme that allows us to verify the extent and the mechanisms of anabolic steroid cardiotoxicity. Troponin rise in the wash-out period is an alarming finding for a prolonged or delayed deteriorated action of anabolic steroids to the heart, while differentiation in cardiotoxicity via the administration route is a finding that demands further study. As the generation of athletes that heavily abused anabolic steroids in the 1970s and 1980s presents nowadays its first cardiological implications, providing an insight of the adverse cardiac results of anabolic steroids and the mechanism of toxicity is the first step in the application of specific therapeutic protocols and general measures of myocardial function salvation. In a recent cutting edge review, the pathophysiological role of oxidative stress in systolic and diastolic heart failure was highlighted along with the potential of angiotensin converting enzyme inhibitors and exercise to counteract oxidative stress. In the same direction novel peptides are also tested (Münzel et al., 2015). Early recognition of myocardial cardiotoxicity due to nandrolone use could allow the prompt implementation of suitable and specific therapy.

In conclusion, the long term administration of nandrolone decanoate leads to the impairment of global myocardial function indices, affecting mainly the diastolic function. Oxidative stress, as depicted by TBARS and catalase activity is now generating. The histopathological findings in heart tissue point to local tissue damage, while the increased telomerase activity in heart observed could represent a drive towards myocardial salvation. Moreover, the obtained data depict that high dose intramuscular administration affects the cardiovascular system more than the low dose respectively and also, subcutaneous administration seems to lead to more consistent effects than intramuscular one.

Conflict of interest

The authors declare that there are no conflicts of interest.

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