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Nephrotoxicity in rabbits after long-term nandrolone decanoate administration

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HIGHLIGHTS

- Nephrotoxic effects of nandrolone decanoate on young rabbits.
- Significant increase in serum urea and creatinine.
- Hypereamia, fibrosis and focal inflammation in kidney tissue of high-dosed rabbits.
- Increased telomerase activity in intramuscularly treated animals.
- Tissue TBARS and GSH levels were significantly altered.

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ABSTRACT

Among the various side effects of supra-physiological dose of anabolic androgenic steroids that are described, renal toxicity remains the least evaluated. The present study provides evidence that long-term administration of nandrolone decanoate could lead to alterations of renal function and structure in the experimental rabbit model. A pronounced increase in serum urea, creatinine, SGOT and SGPT is observed in the treated animals, with intramuscular administration being more detrimental. Histopathological evaluation of kidneys indicated hyperaemia, fibrosis and focal inflammation. Furthermore, the significantly increased telomerase activity found in the kidneys of the intramuscularly treated animals could possibly represent a counteracting survival mechanism. Oxidative stress markers that were influenced the most were TBARS, indicating lipid peroxidation, and GSH. An interesting finding in our study though, was that while intramuscular administration showed the highest biochemical derangement, oxidative stress markers provided mixed results between intramuscularly and subcutaneously treated rabbits. In conclusion, nephrotoxicity of nandrolone decanoate remains a multi-factorial, partly irreversible effect that involves augmented tissue oxidative status.

Abbreviations: AAS, anabolic androgenic steroids; SGOT, serum glutamil oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; ALP, alkaline phospatase; γGT, serum gamma-glutamyltransferase; TBARS, thiobarbituric acid reactive substances; TAC, total antioxidant capacity; GSH, glutathione.

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1. Introduction

Misuse of AASs is becoming a public health problem. AASs represent a group of steroidal hormones related to the male hormone, testosterone. Apart from increasing muscular development and strength, there is emerging evidence that a variety of pathological conditions may arise from their extensive and unsupervised abuse (Darke et al., 2014; Kanayama et al., 2008; Parssinen and Seppala, 2002a,b). Little is known about the potential effect of these drugs on renal function. Scientific knowledge on the subject usually is restricted to case reports or case series and a handful of animal studies. Since the potential effects of AASs on renal function have not been well characterized in humans, hypothesis is mostly driven by the fact that androgen receptors were identified in micro dissected murine glomeruli and cultured mesangial cells and by the knowledge that prognosis in men is worse for various types of chronic kidney disease (Herlitz et al., 2010).

Case reports linking AASs to renal damage include cases of acute kidney injury (Daher et al., 2009), acute renal failure as a complication of rhabdomyolysis (Hageloch et al., 1988), diffuse membrane proliferative glomerulonephritis (Revai et al., 2003), severe cholestasis with kidney failure (Nasr and Ahmad, 2009). Androgens are also known to induce oxidative stress and upregulate components of the renin–angiotensin system (Iliescu et al., 2007; McGuire et al., 2007). In a recent study, dose-related oxidative damage in the kidneys of nandrolone decanoate treated mice was also reported (Riezzo et al., 2014).

Among AASs, nandrolone decanoate possesses a dominant position. Nandrolone (19-nortestosterone, 17b-hydroxy-estr-4-en-3-one) was synthesized in the early 1950s and though it can be regarded as an "old" doping agent, it is still widely used to enhance muscular strength and performance in sports (Bricout and Wright, 2004; Hemmersbach and Grosse, 2010). At the same time, nandrolone therapeutic potential was evaluated, especially in the context of protein deficiency, for a variety of pathological conditions, as in aplastic anaemia, osteoporosis (Geusens, 1995), AIDS (Mulligan et al., 2005), cancer and protein deficiency of the elderly.

The aim of the present study was to investigate the possible detrimental effects of long-term nandrolone decanoate administrationon on renal function of rabbits by monitoring renal specific biochemical parameters, kidney histopathology and oxidative stress markers on serum and tissue level, along with telomerase kidney activity.

2. Methods and materials

2.1. Animals

Fourteen healthy New Zealand 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 groups. Group 1 and group 2 received intramuscularly 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 allegedly 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. The first sacrifice was performed after six months (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. 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. Biochemical markers

Blood samples were individually collected from the vena auricularis of each rabbit in the appropriate glass tubes in order to evaluate the concentration of the following biomarkers: urea, creatinine, SGOT, SGPT, ALP and γGT. Blood serum was separated by centrifugation at 4000 rpm for 15 min and then stored at −18 °C. All biomarkers were spectrometrically measured in Olympus AU2700.

2.3. Histopathological lesions

Kidney tissue block 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. Histopathological examinations were conducted blindly by histopathologists.

2.4. Telomerase activity

Telomerase activity in kidney tissue samples 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.

2.5. Oxidative stress biomarkers

Oxidative stress biomarkers (TBARS concentration, carbonyls, catalase activity, TAC) were measured as previously described (Germanakis et al., 2013; Tsitsimpikou et al., 2013; Zafiropoulos et al., 2014) in the animals' renal tissues. Previously published results on oxidative stress biomarkers in serum from the same animals (Vasilaki et al., 2016) are used for statistical analysis only. Due to sample failure only one tissue sample per HD group in the wash-out period was measured. Therefore the respective results are only discussed qualitatively and not presented.

Briefly, TBARS expressed in nmol/mg protein, were measured in renal tissue homogenate (diluted 1:2) 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 mmol diphenyl-1-picrylhydrazyl (DPPH)/mg protein reduced to DPPH:H. It was determined by the DPPH spectrophotometric assay using stable DPPH radical as reagent. Forty μL of renal tissue homogenate (diluted 1:10 with PBS) 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 (Aebi, 1984). Briefly, 40 μL of renal tissue homogenate (diluted 1:2) 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 renal tissue homogenate (diluted 1:2), as previously reported (Veskoukis et al., 2008). GSH was measured according to the methods of Reddy et al. (Reddy et al., 1981). In particular, 20 μL of renal tissue homogenate (diluted 1:2), treated with 5% TCA, was mixed with 660 μL of 67 mM sodium potassium phosphate (pH 8.0) and 330 μL of 1 mM 5,5′-dithiobis-2 nitrobenzoate (DTNB). The samples were incubated in the dark at room temperature for 45 min, and the absorbance was read at 412 nm.

2.6. Statistics

The statistical package SPSS 17.0 was used for statistical analysis. Normally distributed continuous variables are expressed as the means ± standard deviations (sd). The independent sample *t*-test was applied to compare means from two different groups ($n \geq 2$) (de Winter, 2013). One way Anova was also used in order to evaluate significant differences among treatment groups. Pearson and Spearman correlations between various parameters were also investigated. Differences between categorical variables were assessed by the Chi-square test. Statistical significant differences are considered for $p < 0.05$.

3. Results

3.1. Biomarkers indicative of renal function

Several biochemical markers were monitored. The results are summarized in Table 1. In general, intra-muscular administration seemed more detrimental to the biochemical status of the animals compared to subcutaneous one and most of the effects were reversible during the wash-out period. Urea and creatinine, indicative of renal function, showed a correlated increase ($r = 0.899$, $p = 0.001$) in all groups, with statistically significant differences only in the HD groups. More specifically, in the HDIM group creatinine increased up to 47%, $p = 0.024$ and values dropped during the wash-out period for about 20%. Urea, on the other hand, raised significantly both in the HDIM and the HDSC groups(56%, $p = 0.034$ and 21%, $p = 0.047$, respectively) and no significant changes were observed during the wash-out period.

3.2. Histopathological alterations of kidney tissue

Body weight and kidney weight of treated animals and controls are summarized in Table 2a. In Table 2b results of the histopathological evaluation of the renal tissue from each animal are summarized.

In the HDIM group hyperaemiain the glomeruli and the tubular interspace as well as focal stromal fibrosis and mild interstitial inflammation (Figs. 1 and 2) were observed. In the HDSC group, hyperaemia in the tubular interspace,vascular congestion and augmented vascular density (Figs. 3 and 4) were dominant. Also, mild focal interstitial inflammation was seen. In the LDIM groups only hyperaemiais observed along with mild chronic interstitial inflammation.

3.3. Telomerase activity in kidney tissue

Intramuscular administration lead to a significant increase in telomerase activity in kidney tissue only in the high dose group ($p = 0.020$) compared to controls. The dose-response effect was nearly significant ($p = 0.057$). In the HDSC group a non-significant relative decrease was observed (Fig. 5).

In the wash-out period,relative telomerase activity in high dose groups non-significantly dropped compared to the levels during the administration period (12% in the intramuscular group and 26% in the subcutaneously treated animals) and the decrease was more pronounced for the subcutaneous administration ($p = 0.04$).

3.4. Oxidative stress in blood and kidney tissue

The results of the oxidative stress parameters monitored in the animals' renal tissue during the administration period are summarized in Table 3. A high% coefficient of variation (CV) in all measurements is observed in the HD groups (Spanidis et al., 2016). In general, an advanced oxidative stress status is observed for all treated animals. During the wash-out period the levels of oxidative stress remained practically unchanged with a trend for increase in TBARs and carbonyl species in the high doses of the subcutaneous administration.

In animals treated with low doses of nandrolone, regardless of the administration mode, no significant changes in the oxidative stress are observed. High doses affected GSH and TBARS levels. More than 50% decrease ($p = 0.018$) in GSH was observed in the HDIM group, while in HDSC group the respective decrease was less pronounced (29%, $p = 0.046$). A two-times rise in the TBARS tissue levels ($p = 0.050$) in the HDIM was noticed, while the increase observed in the HDSC group reached even 252% in one animal. The individual variation was also high (% CV 69.6%). Similar results

Table 1
Levels of biomarkers during the administration and the wash-out period.

Biomarkers	Control Group	Administration period			Wash-out period	
		LDIM	HDIM	HDSC	HDIM	HDSC
Urea (ng/dl)	13.8 ± 6.38	20.0 ± 5.71	21.6 ± 9.12*	16.7 ± 7.34*	21.7 ± 5.21*	15.0 ± 7.16*
Creatinine (mg/dl)	0.372 ± 0.265	0.461 ± 0.270	0.546 ± 0.267*	0.359 ± 0.164	0.467 ± 0.081	0.352 ± 0.212
SGOT (U/L)	7.18 ± 4.34	15.4 ± 6.64**	11.3 ± 5.21	9.68 ± 7.21	8.30 ± 4.30	4.5 ± 3.5
SGPT (U/L)	7.30 ± 5.20	12.0 ± 4.91*	13.8 ± 7.21*	9.45 ± 3.26	10.7 ± 4.39	4.51 ± 0.752
γGT (U/L)	1.54 ± 1.12	2.09 ± 0.711	1.62 ± 0.612	2.51 ± 1.34	1.84 ± 0.433	1.00 ± 0.222
ALP (U/L)	13.8 ± 8.04	9.74 ± 3.6	8.83 ± 6.61	8.02 ± 4.71	7.06 ± 3.44	14.3 ± 9.04

* Statistically significant differences compared to control group, $p < 0.05$.
** Statistically significant differences compared to control group, $p < 0.01$.

Table 2a
Levels of body weight, kidney weight and kidney 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
Kidney weight(g)	12.6 ± 0.1	10.8 ± 2.0	11.1 ± 0.6*	10.8 ± 0.9*	12.2 ± 0.5	10.2 ± 0.1*
Kidney Weight/Body Weight ratio × 1000	3.1 ± 0.04	2.7 ± 0.55	2.7 ± 0.12*	2.7 ± 0.20	2.4 ± 0.25*	2.1 ± 0.23*

* Statistically significant differences compared to control group, $p < 0.05$

were found in the circulating levels of oxidative stress markers [previously published data (Vasilaki et al., 2016)].

The increase in TBARS kidney levels correlated with the observed decrease in kidney GSH ($r = -0.750$, $p = 0.020$), as well as with the ca 20% increase in kidney carbonyls ($r = 0.683$, $p = 0.042$). Interestingly enough, carbonyls levels in renal tissue positively correlated with circulating TBARS levels ($r = 0.667$, $p = 0.050$). Catalase levels in erythrocytes were found to be negatively correlated with tissue levels of TBARS ($r = -0.667$, $p = 0.050$) and positively correlated with tissue GSH ($r = 0.648$, $p = 0.043$), while a positive association with TBARS levels in plasma is also noticed ($r = -0.648$, $p = 0.043$, respectively). Kidney GSH changes were also associated with catalase in erythrocytes ($r = 0.648$, $p = 0.043$). A parallel increase of kidney TBARS and telomerase activity is noticed ($r = 0.695$, $p = 0.038$). Very strong negative correlations of telomerase activity in renal tissue with tissue GSH levels and TAC in plasma are observed ($r = -0.853$, $p = 0.002$; $r = -0.900$, $p < 0.001$, respectively), while the significant increases in creatinine and urea are strongly associated with tissue TAC levels, which appear non-significantly increased (ca 25%) in treated animals ($r = -0.851$, $p = 0.007$; $r = -0.878$, $p = 0.008$, respectively).

Table 2b
Histopathological evaluation of the renal tissue from all animals of the present study.

Rabbit	Findings
Control 1	*Normal*
Control 2	*Normal*
HDIM 1	↙ Hyperaemia and fibrosis of the tubular interspace
	↙ Hyperaemia of the glomeruli
	↙ Focal stromal fibrosis
	↙ Mild interstitial inflammation
HDIM 2	↙ Fibrosis of the tubular interspace
	↙ Hyperaemia of the glomeruli
	↙ Mild interstitial inflammation
HDIM 3 (wash-out)	↙ Hyperaemia in the glomeruli
	↙ Mild interstitial inflammation
HDIM 4 (wash-out)	↙ Fibrosis of the tubular interspace
	↙ Generalised hyperaemia
	↙ Mild interstitial inflammation
HDSC1	↙ Hyperaemia in the tubular interspace
	↙ Vascular congestion
	↙ Augmented vascular density
HDSC2	↙ Hyperaemia in the tubular interspace
	↙ Vascular congestion
	↙ Fibrosis
HDSC4 (wash-out)	↙ Hyperaemia in the tubular interspace
	↙ Augmented vascular density
LDIM 1	↙ Hyperaemia
	↙ Mild interstitial inflammation
LDIM 2	↙ Hyperaemia
	↙ Mild interstitial inflammation
LDIM 3	↙ Hyperaemia
	↙ Mild interstitial inflammation
LDIM 4	↙ Hyperaemia
	↙ Mild interstitial inflammation

One tissue sample of the HDSC group in the wash-out period could not be evaluated histopathologically due to sample failure.

4. Discussion

Among the various side effects of supra-physiological dose of AASs that were described, special attention was paid to the induced adverse effects on liver and the cardiovascular system. Few animal studies have been conducted in order to evaluate the impact of AASs on kidney function, while human studies still fail to clearly establish a direct cause–effect relationship between AASs abuse and renal injury (Ahmed Bin Bisher 2009; Hoseini et al., 2009; Riezzo et al., 2014; Zeier et al., 1998).

The present study, despite the limitation of the low number of animals especially in the wash-out period ($n = 2$), provides evidence that long-term administration of nandrolone decanoate could lead to alterations of renal function and structure in the experimental rabbit model. The rabbit model (average life time 4–8 years) is a useful tool for getting information about possible adverse effects in humans. The dosage scheme of the present study reflects the allegedly known administration scheme among steroids abusers, representing chronic exposure together with the period of withdrawal (Gronbladh et al., 2014; Sattler et al., 1999). Nandrolone decanoate acts really slowly and athletes that abuse nandrolone are advised to use the hormone for 10–12 weeks, although some may use it even up to 16 weeks. The recommended dose for a man of average 80 kg in order to enhance muscle growth is up to 500–800 mg per week. Such hormonal supplementation is recommended to be repeated regularly. Athletes allegedly use nandrolone both IM and orally. It has been reported that due to non-specialised personnel for administration, often IM administration ends up to be SC. This, due to the fact that nandrolone is lipophillic, results to much longer excretion time and much extended pharmacological effect. In addition, lately SC administration is propagandized in several bodybuilders' sites as an effective route of administration of testosterone. It should be noted that nandrolone is a prohibited substance for doping control, according to the World Anti-Doping Agency. The administration

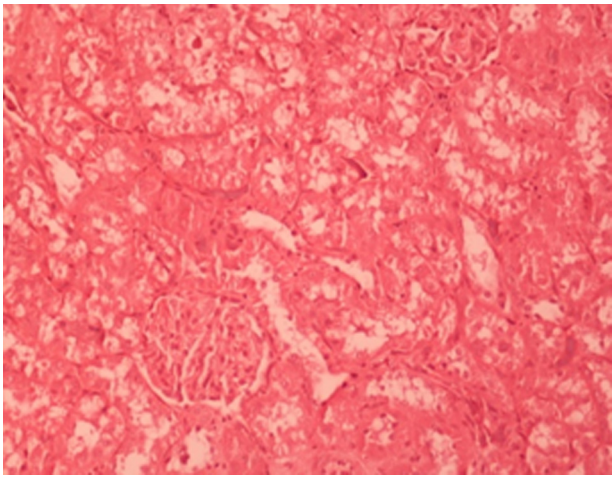

Fig. 1. Normal kidney tissue from control animals.

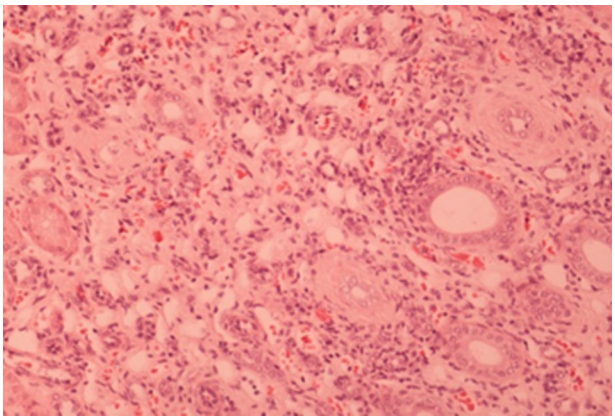

Fig. 2. Focal stromal fibrosis and mild interstitial inflammation in HDIM group.

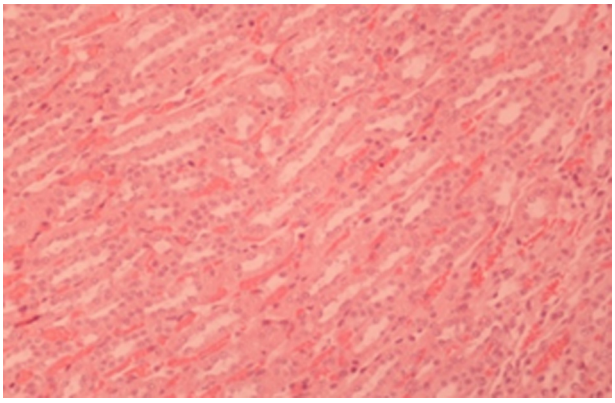

Fig. 3. Vascular congestion in HDSC group.

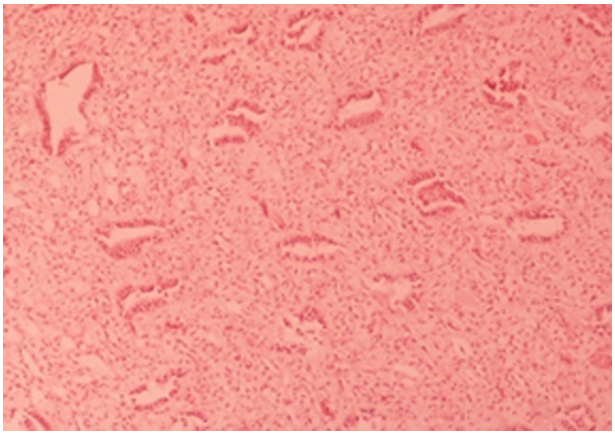

Fig. 4. Fibrosis and augmented vascular density in HDSC group.

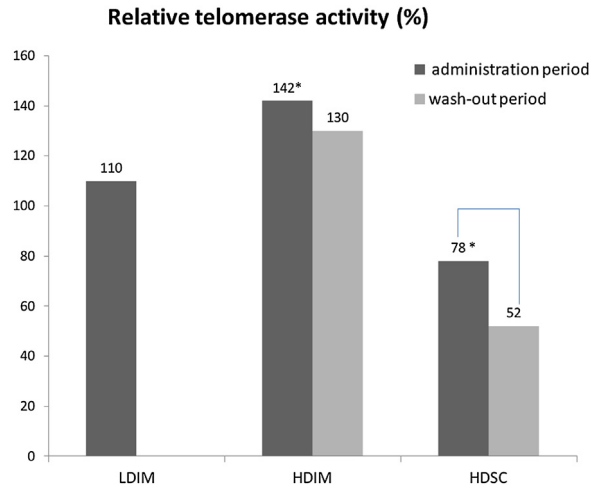

Fig. 5. Relative telomerase activity in kidney tissue in all treated animals. *Statistical significance $p \leq 0.05$.

scheme used in the rabbits of the present study is also in accordance with various animal studies (Ammar et al., 2004; Shokri et al., 2014).

The present study showed that the administration of nandrolone decanoate caused a pronounced increase in serum urea, creatinine, SGOT and SGPT in treated animals with the intramuscular mode of administration leading to more elevated biochemical levels, probably due to better absorbance (Simons et al., 2001). The hepatic adverse effects of AAS reported in the literature include slight alterations in the transaminase levels, moderate centrolobular inflammatory or degenerative lesions, and some cases of hepatocellular adenomas (Boada et al., 1999; Kiraly, 1988; Saborido et al., 1993). Usually transaminase levels and of ALP are increased in a dose-dependent manner (Vieira et al., 2008). The changes in creatinine are transient. These findings are in agreement with Tousson et al., who showed that intramuscular administration of boldenone, another well-known AAS, in rabbits led to significant changes of biochemical parameters of renal function (Tousson et al., 2016). Interestingly, the present study revealed that the elevated urea concentrations continued during the wash-out period in the intramuscularly high-dosed animals, indicating that this common route of administration contributes possibly to a more permanent renal derangement. In contrast, the values of creatinine returned to normal after the discontinuation of nandrolone decanoate. An interesting finding in our study though, was that while intramuscular administration showed the highest biochemical derangement, oxidative stress markers provided mixed results between intramuscularly and subcutaneously treated rabbits.

Histopathological evaluation of kidneys indicated hyperaemia, fibrosis and focal inflammation. Taken together with the said alterations of renal biochemical parameters, these findings suggest alterations in renal function and structure.

It is usually expected that reduced contractility of the heart can lead to renal failure in humans. In addition, even chronic dilated cardiomyopathy could lead to kidney malfunction. In our case, the% ejection fraction in the rabbits treated with nandrolone was not reduced and only the global myocardial performance indices were deteriorated in the high-dosed groups (Vasilaki et al., 2016). Nevertheless, on a tissue level, both the cardiac and the renal tissue suffered from fibrosis and inflammation.

Another interesting finding of this study was that animals' kidney weight was largely unchanged if not decreased, especially in the subcutaneous administration. In contrast, Hoseini et al. showed that in nandrolone decanoate treated animals the kidney's weight increased (Hoseini et al., 2009). In our case, it was the rabbits' total weight that seems to rise specifically during the wash out period, an alleged effect between athletes, too. More specifically, an increase of ca 25% of body weight in the wash-out period in all high-dosed groups is observed due to the long-lasting anabolic effect of nandrolone, which was made evident after the end of the treatment (Shahraki et al., 2015). Increased body mass due to nandrolone use requires an increase in

Table 3
Kidney tissue levels of oxidative stress parameters during the administration period.

Biomarkers	Control Group	LDIM	HDIM	HDSC
TAC (mmol DPPH/mg protein)	0.508 ± 0.119 (23.4)	0.502 ± 0.072 (14.3)	0.637 ± 0.406 (63.7)	0.571 ± 0.0015 (0.262)
TBARS (nmol/mg protein)	17.7 ± 4.06 (22.9)	27.0 ± 3.689 (13.7)	35.4 ± 7.18* (20.3)	41.8 ± 29.1 (69.6)
GSH (μmol/mg protein)	0.138 ± 0.004 (2.90)	0.116 ± 0.012 (10.3)	0.063 ± 0.020* (31.7)	0.098 ± 0.012* (12.2)
Catalase (Units/mg protein)	454 ± 239 (52.6)	451 ± 48.7 (10.8)	523 ± 343 (65.6)	543 ± 261 (48.1)
Carbonyls (nmol/mg protein)	139 ± 16.8 (12.1)	133 ± 23.3 (17.5)	161 ± 26.1 (16.2)	166 ± 74.8 (45.1)

Number in parenthesis represent% CV.
* Statistically significant differences compared to control group, $p < 0.05$.

glomerular filtration. Thus, the individual glomeruli adapt to hyperfiltration through hypertrophy. If these conditions persist, podocytes could eventually detach from the glomerular basal membrane, causing the development of a segmental scar (Herlitz et al., 2010; Riezzo et al., 2014), as this is probably the case in our study where hypertrophy was not present. Evidence for a possible chronic renal injury due to AASs, denoted by a glomerulus mass reduction that can progress to renal failure, was also provided by Alm-Eldeen et al., who studied the misuse of boldenone undecylenate, an anabolic steroid for veterinary use (Alm-Eldeen and Tousson, 2012).

Furthermore, the significantly increased telomerase activity found in the kidneys of the intramuscularly treated animals could possibly represent a counteracting survival mechanism. Our previous studies showed that telomerase activity increased significantly and in a dose-dependent manner in rabbits treated with nandolone decanoate (Vasilaki et al., 2016). 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 and oxidative damage (Ahmed et al., 2008).

The mechanism by which nephrotoxic activity is mediated is an intriguing question. It is well established that kidney is susceptible to free radical damage, after administration of drugs with toxic activity in animals (Divya et al., 2016). In the present study oxidative stress markers that were influenced the most were TBARS, indicating lipid peroxidation, and GSH. The above findings are in agreement with the results of Riezzo et al., who showed that long term administration of nandrolone promotes oxidative injury in strength-trained CD1 mice (Riezzo et al., 2014). Oxidative damage in kidneys of mice treated with nandrolone in the said study, was revealed by the increase of malondialdehyde levels (marker of lipid peroxidation) and by the reduction of antioxidant enzymes glutathione peroxidase and glutathione reductase activity resulting in the decreased ability of the kidney to scavenge toxic hydrogen peroxide and lipid peroxides. Frankenfeld et al., 2014 reported that the redox status in liver, heart and kidney of male Wistar rats was highly affected by AAS treatment, but the mechanisms of oxidative stress were found different between the three tissues. In the kidney, increase in protein carbonyl content and decrease of total reduced thiol residues and diminished catalase activity was observed, whereas in the liver, which was more prone to changes, NOX activity was increased, the antioxidant enzymes SOD and catalase were decreased (Frankenfeld et al., 2014). In addition, GSH has also been reduced in patients with diabetic nephropathy (Bhatti and Usman, 2015). GSH is usually the first endogenous antioxidant molecule which is sacrificed to counteract the production of free radicals (Lu and Holmgren, 2014).

Furthermore, high dose of nandrolone decanoate, particularly in HDIM group increased TBARS, supporting also an oxidative stress induction. This is consistent with previous results of our research group, in which high dose of nandrolone decanoate increased TBARS levels in plasma of rabbits (Vasilaki et al., 2016). The administration of the anabolic oral turinabol in high doses increased TBARS levels in rabbit plasma, too (Germanakis et al., 2013). Furthermore, the increase in TBARS kidney levels correlated significantly with the observed decrease in kidney GSH, suggesting that GSH was used to reduce lipid peroxidation. Protein carbonyl levels in kidney, indicative of protein oxidation, were not significantly affected by the administration of nandrolone decanoate at any dose, however they positively correlated with TBARS levels in kidney. Sometimes lipid peroxidation and protein oxidation are interrelated (Piccolomini et al., 2012). For example, tBHP has been shown to lead to the formation of tBO radicals that in turn lead to protein oxidation either directly by attacking the amino acyl side chains or indirectly by leading to lipid peroxidation (Hix et al., 2000).

Regarding TAC levels in kidney, no significant differences between the different groups were observed, however there was a negative correlation among them and the creatinine and urea levels. The latter suggested that the levels of TAC may play an important role in the kidney's function. Finally, catalase activity was not affected by administration of nandrolone decanoate at any dose, indicating that oxidative stress induction did not affect this antioxidant enzyme.

In conclusion, nandrolone decanoate administration to young rabbits led to significant deterioration of renal biochemical markers, coexisting with alterations of the kidney histology, possibly as a result of augmented oxidative stress. Nandrolone decanoate, being a testosterone derivative, has anabolic actions, even after the cessation of its administration, as documented by the continued weight gain of rabbits in the wash-out period. The renal effects of nandrolone administration are multifactorial in their origin, involving in certain stages of the pathophysiological pathway augmented oxidative damage.

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