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Original research paper

Chronic nandrolone decanoate administration effects on antioxidant parameters in Wistar rats

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Abstract

Chronic nandrolone decanoate exposure is associated with a modified redox status in the favor of oxidants. Paraoxonase and myeloperoxidase activities are considered sensitive biomarkers of oxidative stress and inflammation, being also predictors for coronary artery disease. The aim of our study was to investigate the impact of chronic nandrolone administration on paraoxonase and myeloperoxidase activities in Wistar rats. 16 Wistar rats were divided into two groups (n=8 for each of them): nandrolone decanoate (A) treated group and control group (C). After 12 weeks of nandrolone decanoate administration we analyzed several plasma oxidative stress parameters: TROLOX Equivalent Antioxidant Activity, paraoxonase and myeloperoxidase activities, total thiols, and advanced oxidation protein products. Our results showed a significant decreased total antioxidant activity in nandrolone treated group compared with controls, while paraoxonase activity and total thiols levels were significantly increased. Myeloperoxidase activity and advanced oxidation protein products were not significantly different between the two studied groups. In conclusion decreased plasma total antioxidant activity suggested increased oxidative stress induced by chronic high doses of nandrolone. Despite oxidative stress presence high doses of nandrolone induced also increased plasma paraoxonase activity.

Keywords Anabolic androgenic steroids; nandrolone decanoate; oxidative stress; TROLOX Equivalent Antioxidant Activity; paraoxonase and myeloperoxidase activities; total thiols and advanced oxidation protein products.

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Introduction

Nandrolone decanoate (DECA) belongs to class II androgenic anabolic steroids (AAS), having a similar chemical structure to testosterone, with one extra CH_3 group. Like all other AAS it has pleiotropic actions on the human body with various effects depending on dose, time, and physiological state. It has been used in different medical conditions including anemia associated with renal failure or neoplasia, HIV cachexia, and osteoporosis (SAHA, 2009, SHEASHAA, 2005, FLICKER, 1997, AL-SHAREFI, 2019). Apart from its therapeutic use, DECA is also a widespread abused drug in athletes and non-athletes population, especially men (POPE and KANA-YAMA, 2012). Total used doses are much higher than the pharmacological ones, and have been associated with important side effects, especially on the cardiovascular system, liver, or kidney (FRANKENFELD, 2014). Our previous results also showed the deleterious impact of DECA supraphysiological administration on haemostasis, lipid profile, and blood pressure in rats (ROSCA, 2013, ROŞCA, 2013, ROSCA, 2019, ROŞCA, 2016).

Decreased HDL cholesterol has been reported as one of the consequences of chronic DECA exposure (GREVIK, 2011, SAMIEINASAB, 2015, ROSCA, 2019). Apart from cholesterol modulation, there are also other properties of HDL particles considered very important in terms of cardio-vascular protection (RE. et al, 2014). For instance, HDL particles can be modified in inflammatory conditions, through inflammation-related proteins binding, such as amyloid A and complement C3 (GUNTHER, 2013). HDL also forms the ternary complexe of paraoxonase-HDL-myeloperoxidase, which can modulate the levels of actual oxidants and antioxidants (HUANG, 2013) Plasma para-oxonases are antioxidant enzymes synthesized by the liver and associated mainly with HDL particles (FURLONG, 2016). Described initially as esterases or lactonases, para-oxonases gained increased attention after their proved antioxidant action on LDL (MACKNESS, 1991). Opposite to paraoxonase, the myeloperoxidase enzyme activity is associated with increased oxidative stress and inflammation (KLEBANOFF, 2005). Both myeloperoxidase and paraoxo-nase are bound to HDL particles, this further suggesting they are capable to interact and modulate the oxidants levels in specific conditions (HUANG, 2013).

Disruption of antioxidant/oxidant balance represents one potential mechanism of high doses DECA-induced toxicity (TSITSIMPIKOU, 2016, VASILAKI, 2016). However, the impact of supraphysiological DECA admi-nistration on paraoxonase/myeloperoxidase systems has been poorly studied. To our knowledge, there is only one previous study assessing the influence of DECA on paraoxonase activity, which has been conducted in hemodialyzed patients, an inflammatory condition per se (GHORBANIHAGHJO, 2005). The aim of the present study was therefore to investigate the effects of chronic, high doses DECA administration on paraoxonase/myeloperoxidase activity in Wistar rats.

Material and Methods

Study design

Sixteen adults male Wistar rats (14 weeks age) were included in the study. The rats were housed in individual cages, floored with wood shavings, in a room with constant temperature (23°C), with free access to rat chow and water and 12 h light–dark cycle (lights on at 07:00 h). They were randomly assigned to two groups of study: androgen group (A) and control group (C), (n=8 for each group). A group was treated with a high dose of DECA (Norma Hellas Pharmaceutical Industry, Menandrou 54, 10431 Athens, Greece), 10 mg/kg body weight, a single injection once a week in the gluteus medium muscle, for 12 weeks. DECA dose was comparable to that frequently abused by athletes: ± 600 mg/week, or approximately 8 mg/kg/week. C group received only weekly intramuscular vehicle injection. At the end of the experimental study (after 12 weeks) blood samples obtained by cardiac puncture under ether induced and maintained anaesthesia, were collected into lithium heparin containing tubes. Plasma was separated by centrifugation and stored at -70°C until assayed. The rat's weight was determined at the beginning and at the end of the study.

All animal procedures were approved by local ethics committee for animal research and were carried out in accordance with the guiding principles for biomedical research involving animals as stated by the European Communities Council Directive 86/609/EEC.

The following oxidative stress parameters have been assessed on plasma samples: total antioxidant status, total thiols levels, paraoxonase activity toward paraoxon (NaCl stimulated and basal activity), myeloperoxidase activity, and the level of advanced oxidation protein products.

Trolox equivalent antioxidant capacity

Plasma total antioxidant activity was determined based on the 6-hydroxy-2,5,7,8-tetramethylchroman-2 carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay developed by Miller et al (MILLER and RICE-EVANS, 1996) with minor modifications (RE R, 1999). The TEAC assay measures the relative abilities of antioxidants to scavenge the 2, 2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTSx+) in comparison with the antioxidant potency of standard amounts of Trolox, the water soluble vitamin E analogue. The ABTS radical was generated from the interaction between ABTS and potassium persulphate. Solution containing ABTSx+ was added to the serum samples and the absorbance was read after 1 min at 734 nm against a reagent blank (prepared with 5 mM

phosphate buffer treated as the sample). The percentage inhibition of the absorbance, which is directly proportional to the antioxidant activity of the sample, was calculated. The assay was calibrated against a calibration curve with Trolox as standard, and the results were expressed as mmol/L Trolox.

PON activity against paraoxon

To measure paraoxonase activity, serum was incubated in Tris-HCl buffer (100 mmol/l; pH 8.0) containing 5.5 mmol/l paraoxon (O,O-diethyl O-p- nitrophenyl phosphate; Sigma-Aldrich Chemie, Steinheim, Germany) and 2 mmol/l CaCl2 either with 1 mol/l NaCl (salt-stimulated activity: PON_s) or without NaCl (basal activity: PON_b). The rate of product generation, p-nitrophenol, was monitored at 412 nm. Enzyme activity was calculated from its molar extinction coefficient 18,290 M^{-1} cm^{-1}. One unit of PON is defined as 1 nmol of p-nitrophenol per milliliter formed per minute under the above-described assay conditions. Results were expressed as units per g protein (AVIRAM, 1998).

Total plasma thiols level

For the estimation of total -SH groups content aliquots of plasma were mixed with phosphate buffer (pH = 8) and 10% sodium dodecyl sulphate. Then Ellman reagent was added and samples were incubated at 370°C for one hour. The absorbance was read at 412 nm against a reagent blank. Results were calculated using a calibration curve with GSH as standard and were expressed as µM/mg protein (HIMMELFARB, 2008).

Advanced oxidation protein products

The advanced oxidation protein products (AOPPs) in plasma samples were measured by spectrophotometry on a Tecan SunRise microplate reader calibrated with chloramine-T (Sigma) (DHIMAN, 2009). Briefly, in 96-well plates, serum samples (1:10 dilution in phosphate-buffered saline [PBS]; 200 µl/well) were mixed in triplicate with 10 µl of 1.16 M potassium iodide and 20 µl of 100% acetic acid. The change in absorbance was immediately read at 340 nm. A standard curve was prepared using chloramine-T (linear range, 0 to 100 µmol), and the AOPP concentration was expressed as µmol chloramine-T equivalents per g protein.

Myeloperoxidase assay

The samples were assayed for myeloperoxidase MPO activity by the dianisidine-peroxyde method (DHIMAN, 2009), measuring the change in OD at 460 nm using kinetic readings over 3 min (200 µl sample with 800 µl reaction buffer containing 50 mM potassium phosphate buffer, 0,53 mM of o-dianisidine dihydrochloride, and 0,15 mM H_2O_2). After incubation for 5 min at room temperature, the reaction was stopped with 30% sodium azide, and the change in absorbance was measured at 460 nm ($\varepsilon = 11{,}300\ M^{-1}\cdot cm^{-1}$). Results were expressed as units of MPO/mg protein, whereby 1 unit of MPO was defined as the amount of enzyme degrading 1 nmol H_2O_2 per min at 25°C. Sample protein concentrations were determined by Bradford assay (BRADFORD, 1976).

Statistical analysis

Data are expressed as mean ± SEM. For all the data collected two tailed student *t* test with was performed. GraphPad InStat software (GraphPad Software Inc. La Jolla, United States) was used for statistical analysis. A p-value < 0.05 was considered to be statistically significant.

Results

Using two tailed student *t* test we have obtained a significant difference between groups regarding plasma TEAC, PON_b, PON_s and SH groups.

There were found no statistically significant differences between A and C groups regarding the weight of the rats at the beginning (327.50±11.76 g vs. 321.25±11.08 g,) and at the end of the study (381.25±9.9 g vs. 391.25±10.22 g,).

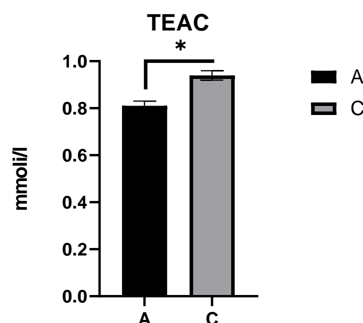

Figure 1. Data were expressed as mean ±SEM (n=8). Student *t* test was used for statistical analysis of data. *significantly different from control group $p < 0.001$.
A = nandrolone, C = control group;

Following student *t* test analysis, TEAC (Fig. 1) was significantly reduced in nandrolone treated group compared with controls (0.81±0.02 mM/l vs. 0.94±0.02 mM/l, $p < 0.001$).

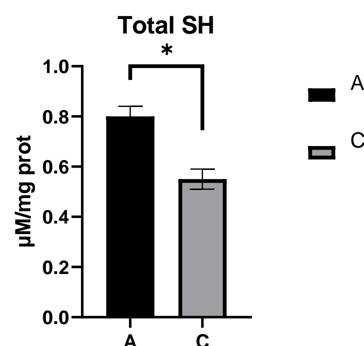

Figure 2. Data were expressed as mean ±SEM (n=8). Student *t* test was used for statistical analysis of data. *significantly different from control group $p < 0.001$.
A = nandrolone, C = control group;

Total thiols groups (Fig. 2) were significantly increased in nandrolone treated group compared with control group (0.8±0.04 vs. 0.55±0.04 μM/mg prot, $p < 0.001$).

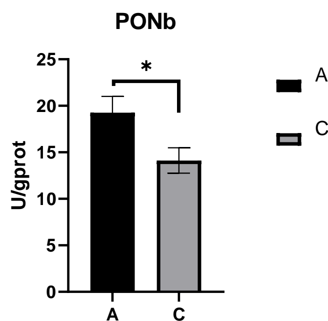

Figure 3. Paraoxonase basal activity. Data were expressed as mean ±SEM (n=8). Student *t* test was used for statistical analysis of data. *significantly different from control group $p < 0.05$. A = nandrolone, C = control group;

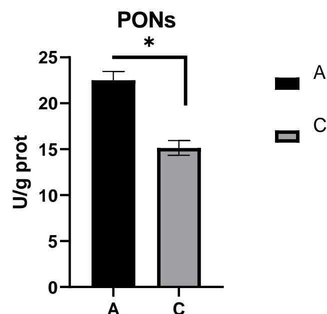

Figure 4. Paraoxonase stimulated activity. Data were expressed as mean ±SEM (n=8). Student *t* test was used for statistical analysis of data. *significantly different from control group $p < 0.001$. A = nandrolone, C = control group;

Paraoxonase activities, both basal and salt stimulated, in nandrolone treated group were significantly increased compared with control group (Fig. 3 and Fig. 4). Student *t* test identified an increase of PON_b and PON_s in nandrolone treated group compared with controls (19,25±1,76 vs. 14,12±1,37 U/g prot, $p < 0.05$ and 22,51±0,96 vs. 15,14±0,8 U/g prot, $p < 0.001$ respectively).

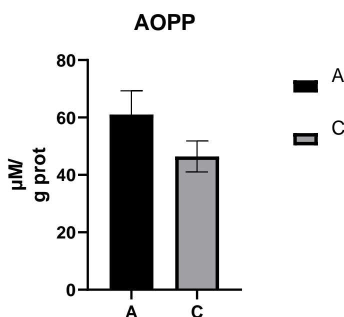

Figure 5. Advanced oxidation protein products. Data were expressed as mean ±SEM (n=8). A = nandrolone, C = Control group;

Advanced oxidation protein products AOPP (Fig. 5) and myeloperoxidase activity (Fig. 6) are not significantly different between groups (AOPP: 61,05±8,25 vs. 46,43±5,43 μM/g prot, and MPO: A=74.40±9.95 U/mL and C=82.51±13.54 U/mL).

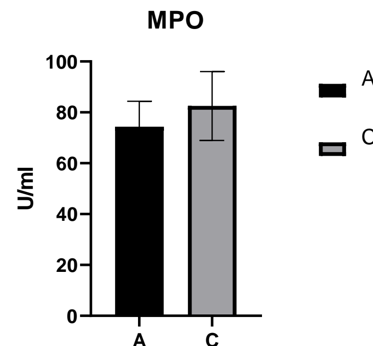

Figure 6. Myeloperoxidase activity. Data were expressed as mean ±SEM (n=8). A = nandrolone, C = control group;

Discussion

Total antioxidant activity is significantly decreased in the DECA group compared with controls. The reduced TEAC observed in our study can be considered an indicator of the oxidative stress presence, following DECA administration in chronic high doses. Our result supports previous data in the literature showing an increased oxidative stress induced by androgen excess (LIU, 2010, LU, 2010).

We also found an increased SH level in DECA treated group, when compared with controls. Generally, oxidative stress conditions are supposed to consume the thiol groups (JONES, 2008). However, there are also several other studies noting increased total thiol levels in conditions associated with high oxidative stress. Ji et al. reported an increased SH levels after exercise-induced oxidative stress in healthy subjects (JI, 1999). Plasma SH were also found to be increased in chronic renal failure patients undergoing haemodialysis, a condition associated with enhanced oxidative stress (JACKSON, 1995, MIRCESCU, 2005, CAPUSA, 2012).

DECA treatment induced an increase of PON activity, basal and also salt stimulated, when compared with controls. Paraoxonase (PON) is an enzyme synthesised primarily in the liver and secreted in plasma associated with HDL3 particles (GOSWAMI et al, 2009). PON is acting on various substrates, having esterase or lactonase activity, being able to hydrolyse organophosphoric insecticides, endogenous lactones (lactones of arachidonic acid metabolites, thiohomocysteine lactones), as well as exogenous lactones (certain antibacterial prodrugs) (GOSWAMI, 2009). Paraoxonase is also protecting HDL and LDL against oxidation (BAILEY, 2012). Paraoxonase activity (PON) is influenced by genetic polymorphism, nutritional factors and pharmacological agents (COSTA, 2005). As far as we know, there is only one study that has

investigated DECA influence on paraoxonase activity (GHORBANIHAGHJO, 2005). This study reported a decreased PON level after clinical accepted doses of DECA treatment in haemodialyzed patients. The differences between their conclusion and ours probably result from the completely different experimental study design. Their study was performed in humans, with the presence of an initial inflammatory condition (haemodialysis), while our study was conducted in healthy rats. Moreover, Ghorbanihaghjo' study assessed only enzymatic activity toward paraoxon, and not MPO activity.

Increased PON activity along with increased SH levels, despite the presence of oxidative stress conditions, are interesting findings emerging from our study. Analysed inflammation markers – MPO activity and AOPP levels – were not modified by DECA treatment. This is somehow surprising, as DECA administration has been previously associated with increased markers of inflammation in different organs (RIEZZO, 2014, AHMED, 2015). Unchanged MPO activity and AOPP levels in our study do not exclude the presence of organ inflammation, as it is a known fact that most plasma MPO is associated with PON and HDL, and we found a significantly increased PON activity in DECA group. Several other studies reported a negative correlation between MPO and PON activities (ZSÍROS, 2016, VARIJI, 2019, RAZAVI, 2013). Unchanged AOPP levels found in our study are practically in consensus with the unmodified MPO activity.

Additional studies are required in order to better understand the causes and implications of increased PON activity induced by chronic high doses of AAS exposure.

Conclusion

Decreased plasma total antioxidant activity suggest increased oxidative stress presence induced by chronic high doses of nandrolone.Despite oxidative stress presence high doses of nandrolone are also inducing increased plasma paraoxonase activity.

Author Contributions

A.E.R and *L.I* equally contributed to this work as first authors. All authors have contributed to all of the following: the conception and design of the study, acquisition of data, analysis, data interpretation, drafting, and revision of the article. *I.S* has coordinated the overall work.

Conflict of Interest

The authors have no conflict of interest to declare.

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