Oxidative stress and myocardial dysfunction in young rabbits after short term anabolic steroids administration

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A B S T R A C T

The present study focuses on the short term effects of repeated low level administration of turinabol and methanabol on cardiac function in young rabbits (4 months-old). The experimental scheme consisted of two oral administration periods, lasting 1 month each, interrupted by 1-month wash-out period. Serial echocardiographic evaluation at the end of all three experimental periods was performed in all animals. Oxidative stress markers have also been monitored at the end of each administration period. Treated animals originally showed significantly increased myocardial mass and systolic cardiac output, which normalized at the end of the wash out period. Re-administration led to increased cardiac output, at the cost of a progressive myocardial mass reduction. A dose-dependent trend towards impaired longitudinal systolic, diastolic and global myocardial function was also observed. The adverse effects were more pronounced in the methanabol group. For both anabolic steroids studied, the low dose had no significant effects on oxidative stress markers monitored, while the high dose created a hostile oxidative environment. In conclusion, anabolic administration has been found to create a possible deleterious long term effect on the growth of the immature heart and should be strongly discouraged especially in young human subjects.

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1. Introduction

Anabolic androgenic steroids (AASs) are synthetic derivatives of the male hormone testosterone and their use has been associated with the attempts of athletes to improve their performance. Despite the fact that AAS belong to the WADA’s prohibited list of substances, they constitute the leading group in the statistics of doping cases and in adverse analytical findings. Over the last decades, the scientific community was aware of their possible deleterious cardiac effects, since the case reports that link, directly or not, the abuse of AAS with cardiovascular effects are continuously increasing. The abuse of AAS is associated with a variety of different cardiovascular side effects including left ventricular hypertrophy (Mark et al., 2005; McKillop et al., 1986), hypertension, fibrosis, arrhythmias, altered serum lipoprotein profile (Bonetti et al., 2008; Hartgens et al., 2004), acute myocardial infarction (Fisher et al., 1996; Wysoczanski et al., 2008) and even, sudden death (Di Paolo et al., 2007; Fineschi et al., 2007; Thiblin et al., 2000).

Methanabol (methandienone) was originally developed by John Ziegler and released in the United States in the early 1960s by the Swiss chemical company Ciba. It was used as an aid to muscle growth by bodybuilders until it was banned by the FDA under the Controlled Substances Act. However, methanabol continues to be used illegally to this day (Mosler et al., 2012).

Turinabol, the chlor-substituted compound of methandienone, is another synthetic oral anabolic androgenic steroid with reported abuse by athletes in East Germany in the late 1980s. This steroid was first synthesized in 1960 (Ho et al., 2007).

Oxidative stress, independent of its origin, is considered the major cause of endothelial dysfunction (von Zglinicki, 2002). Administration of anabolic steroids either alone or in conjunction with training, induced changes in oxidative stress biomarker levels and antioxidant defence systems both in rat liver and skeletal muscle and in human heart (Figueredo, 2011; Pey et al., 2003; Saborido...
et al., 2011). Cultured vascular smooth muscle cells and endothelial cells exposed to oxidative stress, exhibit increased shortening of telomeres and accelerated cellular senescence (Matthews et al., 2006). In addition, telomerase activity decreases in response to oxidative stress, which is thought to be a direct consequence of oxidative stress, rather than the result of premature senescence (Kurz et al., 2004).

The aim of our study was to evaluate the possible adverse effects of turinabol and methanabol, on young rabbit’s heart tissue both regarding the functional status and in a molecular level during short term, low dose administration. Thus, echocardiography was applied to the anabolic treated rabbits and histopathological examination of heart tissues was conducted. Oxidative stress (through the determination of total antioxidant capacity – TAC, thiobarbituric acid reactive species – TBARS, catalase and reduced glutathione – GSH) induced in rabbits by the short term administration to methanabol and turinabol was also monitored.

2. Materials and methods

2.1. Animals

Six healthy young New Zealand white female rabbits (weighing between 3400 and 4200 g each, age 4 months) were used for the aims 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. As confirmed by daily inspection, animals were consuming all water made available to them (250 ml of tap water per day). The rabbits were acclimatized under laboratory conditions for 2 weeks, whereupon the treatment period began.

Anabolic treated animals (n = 4) were administered Turinabol (TUR) and Methanabol (METH) both at a low (0.6 mg/kg/d) and at a high (1 mg/kg/d) dose, respectively. Control animals (n = 2) received only tap water. Originally, the appropriate amounts of anabolics were diluted in 500 ml tap water.

The experimental scheme of exposure was selected in that way, in order to simulate the allegedly reported athlete’s abuse of anabolic steroids regime. It consisted of two oral administration periods, lasting 1 month each, interrupted by 1-month wash-out period (total duration 3 months). All animals underwent three serial complete echocardiographic evaluations under sedation following the first anabolic administration, at the end of the wash-out period and at the end of the re-administration period. During the study period, the animals were regularly observed, their condition was closely monitored, they were weighed every month and their food consumption was recorded. No pathological clinical signs were observed at any point. At the middle of the second administration period, the high-dose methanabol treated animals started to show signs of anxiety and aggressiveness. At the end of both administration periods, serum samples were collected.

2.2. Ethics

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee of Ethics of the Medical School of the University of Crete.

2.3. Oxidative stress

TBARS concentration, reduced glutathione (GSH) concentration, catalase activity and total antioxidant capacity (TAC) were measured as previously described (Vesoukis et al., 2008). Briefly, total antioxidant activity (TAC) expressed in mmol dityrosine-1-pipecolyhydrayzide (DPPH)/L, reduced to DPPH.H, 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 read at 520 nm. Thiobarbituric acid-reactive species (TBARS), expressed in μmol/L, were measured in blood plasma by mixing it with TCA, Tris–HCl, Na2SO4 and thiobarbituric acid and incubated at 95 °C. TCA was added again, centrifuged and the absorbance was read at 530 nm. Reduced glutathione (GSH) and catalase, expressed in μmol/gHb and U/mgHb, respectively, were determined in red blood cell lysate. Each assay was performed in triplicate (Vesoukis et al., 2008).

2.4. Echocardiographic study protocol

Following subcutaneous administration of ketamine (16 mg/kg) and xylazine (6 mg/kg), the sedated rabbits, 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 ultrasound probe. 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 Anulus Peak Systolic Excursion (MAPSE and TAPSE) respectively), as previously described (Germánakis et al., 2012).

Pulsed wave (PW) doppler and myocardial Tissue Doppler Imaging (TDI) was 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.

2.5. Statistical analysis

Appropriate non-parametric tests for related samples (Friedmann, Wilcoxon sign tests) were used to evaluate for significant differences between paired or all available measurements. Similarly, intergroup comparison based on anabolic agent (turinabol vs. methanabol) or level of exposure (low vs. higher) was performed by using non-parametric tests for unrelated samples (Mann Whitney U-test). By using the same analysis, anabolic treated animal values were also compared against control values, in each time point, and the distribution of oxidative stress values between cases and controls, as well as between treatment subgroups, was compared. A P-value < 0.05 was considered as the level of statistic significance.

3. Results

3.1. Serial echocardiographic measurements and comparison to control values

Anabolic treated animals demonstrated significantly (p = 0.021) higher left ventricular mass (0.69 vs 0.48 g/kg) and EF (64% vs 55%) values compared to controls after the first anabolic administration, and increased CO values during both administrations (0.064 and 0.072 vs 0.049 L/min). Additionally, a trend for impaired longitudinal systolic, diastolic and global myocardial function indexes was observed.

Following the first anabolic administration, anabolic treated animals showed significantly increased indexed to body weight myocardial mass (0.69 g/kg) and cardiac ejection fraction and output (EF = 64%, CO = 0.23 L/kg/min), which normalized at the end of the wash out period (0.58 g/kg, 56%, 0.2 L/kg/min, respectively). Re-administration was associated with a new increase of cardiac output (EF = 65%, CO = 0.29 L/kg/min), at the cost though of a...
progressive myocardial mass reduction (0.54 g/kg) (Figs. 1 and 2). Observed differences between serial measurements were statistically significant (p values = 0.038–0.05). Furthermore, anabolic administration was associated with a trend in a dose-dependent manner towards impaired longitudinal systolic, diastolic and global myocardial function, as expressed by decreased MAPSE (Fig. 3), reduced E/A mitral valve inflow ratio and increased MPI values (Fig. 4). All the above parameters recovered during the wash-out period.

Methanabol administration compared to turinabol was associated with a trend for worse myocardial function indexes and greater negative impact on myocardial mass growth.

3.2. Systemic oxidative stress parameters were increased upon turinabol and methanabol administration

Levels of systemic oxidative stress parameters monitored in the present study are presented in Fig. 5. Low doses of both anabolic steroids studied, did not significantly affect any of the oxidative stress markers monitored. The high dose of turinabol increased catalase activity by 11% and TBARS by 46%, while no effect was observed on GSH and TAC levels. High dose of methanabol exhibited a significant trend for reduction of TAC levels by 46%, while GSH increased by 198%.

4. Discussion

The term anabolic androgenic steroids refers to a group of compounds that are structurally related to testosterone and exert two main physiological effects including muscle growth and masculinization (van Amsterdam et al., 2010). Until recently, research activity was mainly focused on nandrolone and stanozolol, due to their wide-spread use. Myocardial dysfunction in rats was observed in the study by Tanno et al. who found that nandrolone induced cardiac hypertrophy, reduced the cardiac index in treated animals compared to controls and reduced the ratio of maximum early to atrial transmitral flow (Tanno et al., 2011).

The cardiac effects of long-term anabolic steroid use remain inadequately characterized. Short term anabolic use, up to 16 weeks, in strength athletes was not associated with any morphologic or functional impact on the heart (Hartgens et al., 2003). However, in a recent studies using sensitive modern echocardiographic indexes, such as strain and TDI, weight lifters and power athletes having admitted use of anabolic steroids in the past, appeared to have more severe cardiac dysfunction than previously reported and sufficient to increase the risk of heart failure as anabolic steroids users presented lower LV ejection fraction, longitudinal strain, and radial strain and decreased diastolic function compared to non users (Baggish et al., 2010; D’Andrea et al., 2007).

Aim of the present study was to assess the short term effects on heart function of a repeated transient low level administration of turinabol and methanabol, simulating thus the situation of athletes’ doping practices. Furthermore, young animals have been used, before having reached sexual maturity age, in order to evaluate any further adverse effects of anabolics on the normal growing of the heart.

Any early “improvement” in myocardial mass and function, observed after initial evaluation of the animals, following the first administration period, was then associated with a gradual decrease in myocardial mass of the growing animals, as well as a constant trend for deterioration of most indexes of systolic, diastolic and global myocardial function. 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).

Although anabolic steroids are considered as promoting left ventricular hypertrophy, the findings of the present study are...
consistent with a diminished myocardial mass growth induced by the repeated, even short-lasting anabolic administration in young subjects, having not having reached their heart growth potential.

Methanabol, widely used by athletes, causes overt heart dysfunction with excessive loss of myocardial contractility. This is the first time to the authors’ knowledge that methanabol effects on the heart is assessed by echocardiography in animals. The maximum methanabol dose used in the present study (i.e. 1 mg/kg/day), is still lower than 1.5 mg/kg reported as high dose administration in previous studies in animals (Rämö et al., 1987; Takala et al., 1991) and humans (D’Andrea et al., 2007), ensuring that the results observed are not due to any poisoning effect following an over-dose.

Reduced myocardial performance under various loading conditions invasively assessed has been described in animals receiving anabolics (Rämö et al., 1987). Similarly in the present study, by using modern echocardiographic indexes, a trend for progressive deterioration in longitudinal systolic (diminishing mitral annulus peak systolic excursion – MAPSE) and global (increasing myocardial performance index – MPI) myocardial performance in the methanabol high-dose group was detected, as well as a trend for worse global myocardial performance at higher compared to lower doses for both anabolics tested. In general, more mild effects were observed in turinabol-treated animals.

Recent findings on physiological testosterone replacement therapy demonstrate that in women with chronic heart failure (CHF) testosterone supplementation improves functional capacity and insulin resistance (Jellamo et al., 2010). Our findings of impaired myocardial mass gain and a trend to impaired global myocardial function in young subjects treated with anabolic steroids, point out the necessity of conducting age-specific studies before introducing anabolic steroids replacement as an alternative treatment in CHF. Furthermore, it becomes evident that any effect is substance-specific and cannot be generalized within the chemical group of anabolic steroids.

The mechanism by which cardiotoxic activity is mediated is an intriguing question. It is well established that the heart is susceptible to free radical damage, due to its intrinsic elevated oxidative metabolic activity and its fragile antioxidant resistance, in comparison to other parts of the body. Our findings indicate that administration of turinabol and methanabol induced oxidative stress. However, the results from the oxidative stress markers suggest distinct and different patterns of oxidative stress systemic response for turinabol and methanabol, in the rabbit model. More specifically, the high-methanabol dose decreased TAC levels indicating an oxidative stress induction. At the same time, the increase of GSH levels observed could correspond to a probable rescue mechanism to counteract oxidative stress induction. On the other hand, high dose of turinabol increased TBARS, a lipid peroxidation marker, suggesting an oxidative stress induction. In contrast to methanabol administration, an antioxidant response to oxidative stress was manifested with an increase in catalase activity. Although, a decrease in GSH or catalase activity is expected under oxidative stress, this is not always the case. In several studies (Douarre et al., 2012; Tchantchou et al., 2004), there is an increase either in GSH or other antioxidant factors under oxidative stress. Moreover, we have also observed increase in GSH levels in rat plasma after induction of oxidative stress by two insecticides, diazinon and propoxur (Tsitsimpikou et al., 2012). The explanation for this apparent contradiction is that sometimes under oxidative stress conditions the organism increases antioxidant molecules as a compensatory mechanism against free radicals.

The main findings of our study should be viewed though within the study limitations, namely the number of animals allocated per...
dosage group. However, the consistent pattern of observed differences, the concordance of echocardiographic findings with oxidative stress marker documentation, along with the presence of some significant (although limited) findings, argue for a potential hazardous effect of anabolics when applied in the growing heart.

Summarizing, based on echocardiographic findings, an important localized cardiotoxic effect of low dose anabolic steroids, especially methanabol, has been identified, after short-term administration to young rabbits. Furthermore oxidative stress, as depicted by GSH and TAC levels in serum, is starting to accumulate.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References


