v. 3, n. 73, 2023

International Journal of Health Science

CHRONIC USE OF STEROIDS AND THEIR RENAL EFFECTS

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Abstract: Anabolic androgenic steroids (AAS) are synthetic components derived from the hormone testosterone. Despite its importance in medicine, its therapeutic use is irrelevant when compared to its illegal abuse, both among professional and amateur athletes, often an exclusive use for aesthetic purposes. The present study sought to evaluate the relationship between types of steroid (Nandrolone Decanoate, Testosterone Cypionate and Mix of the two steroids), in different doses (5, 10 and 15 mg/kg), in animals undergoing a physical exercise protocol resistance (Vertical Ladder) on biochemical parameters (Urea and Creatinine) and renal lipid peroxidation of male Wistar rats. There were changes in analyzed biochemical parameters, in addition to an increase in renal lipid peroxidation levels. It is concluded that there is an apparent relationship between increasing the steroid dose and the greater propensity for the appearance of side effects, both in biochemical parameters and in the parameter related to oxidative stress, corroborating the hypothesis widely discussed in the scientific literature about the relationship between dose and the duration of steroid use with the adverse effects presented.
Keywords: Nandrolone Decanoate, Testosterone Cypionate, steroid, Serum analysis.

INTRODUCTION

Anabolic androgenic steroids (AAS) are synthetic compounds derived from the hormone testosterone, presenting both anabolic and androgenic effects, since both occur via the same receptors (Pope; Khaisa; Bhasin, 2017; Joksmovic et al., 2017).

EAAs are used in medicine due to their anabolic effects and also because they inhibit protein catabolism, thus being useful in the treatment of debilitated states, such as major burns and acquired human immunodeficiency syndrome (Lusseti et al., 2015). However, the predominance of the use of such substances is via illegal abuse, mainly among young adults who aim to increase performance in sports or physical improvements, exclusively for aesthetic purposes (Niedfeldet et al., 2018).

Brazil is one of the largest steroid-consuming countries (Abrahin et al., 2017). But the use of steroids grows annually around the world, being considered a global public health problem (Pope, Khaisa, Bhasin, 2017; Mahamid, Ismalit, 2020; Aidarwee shi, Alhajjaj, 2020).

The side effects related to the abuse of these substances are the most diverse, ranging from hepatic (Solimini et al., 2017), renal (Luchi et al., 2015; Lewczuck et al., 2019), to behavioral and neurotoxic effects (Bueno et al., 2017; Ribeiro et al., 2019). Among the side effects described in the scientific literature, cardiac side effects are the most commonly related to cases of death, with left ventricular hypertrophy being described in both humans and animals (Shahsavari et al., 2014; Poscidônio et al., 2019; 6). Many of the commonly described effects are related to cellular oxidative stress, an increase in parameters related to oxidative stress have already been observed in several organs and tissues (Bond et al., 2016; Bueno et al., 2017).

Despite the wide variety of side effects already described in rats, mice and humans, they seem to be related to some factors, of which two are worth highlighting: the duration of steroid use and the doses used (Lusseti et al., 2015).

Studies demonstrate changes in biochemical parameters in animals and humans subjected to the use of steroids, in various experimental models, with changes related to Total Cholesterol, HDL Cholesterol and the liver enzymes Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) being common (Severo et al, 2012; Gaverick et al., 2016).

MATERIAL AND METHODS

We used 77 male Wistar rats, aged 8 weeks and weighing between 250 and 250 grams, from the Central Animal Facility of ``Universidade Federal de Alfenas`` (UNIFAL-MG). The rats were divided into 11 groups, namely: Control (C), Vehicle (V - peanut oil), Nandrolone Decanoate (DN) at doses 5, 10 and 15 mg/kg, Testosterone Cypionate (CT) at doses 5, 10 and 15 mg/kg and Mix of the two steroids, also in doses 5, 10 and 15 mg/kg. The animals received two weekly doses, subcutaneously, and were subjected to three sessions of resistance physical exercise per week. The model chosen for resistance physical exercise was the Vertical Ladder model, described in the work of Cassilhas and collaborators (2012).

After eight weeks of treatment, totaling 16 applications of steroid or peanut oil and 24 physical training sessions, the animals were decapitated and the blood and organs collected for analysis. The blood was collected in a tube without anticoagulant, centrifuged to obtain the serum, which was frozen for biochemical analysis and total testosterone measurement, while the liver and kidneys were stored in phosphate buffer (PBS, pH 7.2) for analysis of lipid peroxidation.

The serum obtained from centrifugation and stored at -20° C was used to measure Urea and Creatinine, determined using an automatic analyzer (LabMax Plenno®, labtest Diagnostica, Brazil) and commercial kits (Labtest®). Controls and standards were evaluated before each determination, and the values obtained for the different biochemical parameters were performed in triplicate.

Lipid peroxidation was determined by measuring the peroxidation products that react with thiobarbituric acid (TBA) through the thiobarbituric acid reactive species (TBARS) test (Silva et al., 2016). Aliquots of 150 µL of the homogenates were mixed with 1.22M phosphoric acid (750 µL), deionized water (1350 µL) and TBA (0.67%, 750 µL) and incubated in water for one hour at 95 °C.

After incubation, a mixture of 1000 µL of the sample with 1800 µL of methanol and 200 µL of 1M NaOH is placed in an ice bath at 4 °C and added to a cuvette. The concentration of TBARS is estimated from a standard curve of MDA (malonic dialdehyde). MDA/TBARS are quantified using a Varian Cay Eclipse spectrofluorimetric detector (λexcitation = 532 nm; λemission = 563 nm). The result is expressed in µmol MDA/mol protein. Total protein concentration is determined by the Bradford method.

RESULTS

Table 1 presents the results obtained with the analysis of biochemical parameters, in mean and standard deviation format:

Treatment with DN showed changes in biochemical parameters in the three doses studied. The dose of 15 mg/kg of DN increased the serum activity of the Creatinine enzyme, when compared to the Vehicle. It is worth mentioning that all the elevated parameters compared to the Vehicle were also higher than those presented by the 5 mg/kg DN group. Treatment with CT also showed changes in the parameters studied, Creatinine concentration (0.74 ± 0.11), when compared to the vehicle group.

Treatment with the Mix of the two steroids also changed some biochemical parameters, such as at a dose of 10 mg/kg, with an increase in Creatinine (0.81 ± 0.07). Parameters elevated at a dose of 10 mg/kg were greater Creatinine was even higher than that presented by the group that received the lowest dose of Mix, compared to that presented by the group that received the highest dose. The dose of 15 mg/kg also increased serum creatinine (0.64 ± 0.05)

Figure 1 shows the results of renal lipid

	Treatment	Control	Vehicle	DN		CT			MIX			
	Parameter/ Dose	-	-	5	10	15	5	10	15	5	10	15
Renal Profile	Urea	40.18 ± 7.99	43.14 ± 8.45	47.14 ± 11.54	57.43 ± 11.47	52 ± 3.21	35.57 ± 8.01	44.87 ± 7.99	42.71 ± 7.09	42.73 ± 9.46	51.43 ± 8.77	41.71 ± 6.68
	Creatinine	0.46 ± 0.05	0.48 ± 0.05	0.37 ± 0.09	0.54 ± 0.09	0.63 ± 0.06*#	0.42 ± 0.09	0.74 ± 0.11*#	0.5 ± 0.07°	0.47 ± 0.06	0.81 ± 0.07*#	0.64 ± 0.05*#°

Table 1 – Biochemical analysis of the renal profile

Where DN = Nandrolone Decanoate, CT = Testosterone Cypionate and M = mix. * significant difference when compared to the Vehicle group ($p<0.05$); # significant difference when compared to the 5mg/kg group within the same steroid ($p<0.05$) and ° difference between the 10 and 15 mg/kg groups within the same steroid ($p<0.05$).

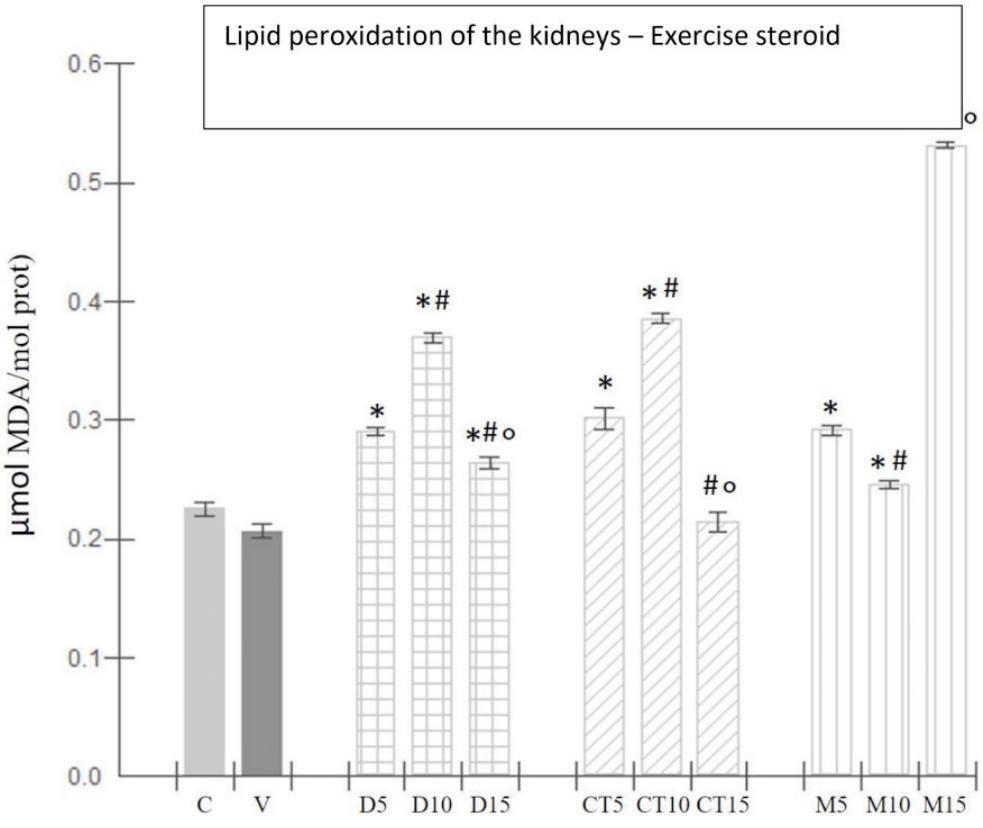

Figure 1 – Renal lipid peroxidation (MDA)

peroxidation.

It can be seen in figure 2 that the renal effects, when compared to MDA levels, were more significant in the three steroids, at all doses studied. Interestingly, the result does not reflect changes in the renal serum parameters studied, Urea and Creatinine. Urea did not change in any of the groups studied, however, creatinine increased in the groups DN 15 mg/kg, CT 10 mg/kg and in the two highest doses of Mix, 10 and 15 mg/kg.

DISCUSSION

Steroid abuse is generally associated with an increase in the activity of liver enzymes in serum and plasma (Hartgens, Kuipers, 2004). The AST and ALT enzymes are present in large quantities in hepatocytes, and an increase in their plasma levels reflects hepatocellular damage or at least an increase in the permeability of the hepatocellular membrane (Niedfeldet et al., 2018). Increases in the levels of these enzymes caused by steroid abuse are in the range of 2 to 3 times higher than the dosages of these enzymes in non-users (Hartgens, Kuipers, 2004).

However, only the increase in liver enzyme levels in plasma demonstrates a very simplistic relationship between the use of steroids and possible hepatic toxic effects, since the increase in the AST enzyme also occurs in bodybuilders and animals subjected to physical activities even without the use of steroids, related exclusively to muscle damage caused by exercise. This is a very important fact, as lack of knowledge can lead to a false association between EAA use and liver damage. It is believed that an increase in plasma AST and ALT levels, without an increase in GGT (Gamma glutamyl transpeptidase) levels, cannot in itself be related to hepatotoxicity caused by anabolic steroid abuse (Hartgens, Kuipers, 2004).

According to Bond and colleagues (2016), steroids are believed to cause hepatotoxicity through increased oxidative stress in hepatocytes, a conclusion they reached by observing the following points: (1) increased oxidative stress in liver cells; (2) Activation of androgen receptors can increase reactive oxygen species (ROS); (3) Constantly activated androgen receptors generate an increase in mitochondrial oxidation; (4) Antioxidants can protect the liver from the side effects of steroids ("liver protector"); (5) Metabolic resistance and androgenic potency appear positively correlated with reduced hepatotoxicity.

It is believed that hepatocyte hyperplasia (Karbalay-Doust, Noorafshan, 2009), perhaps, is one of the main factors for the increase in liver enzymes AST and ALT in the blood of anabolic steroid users (Urhausen, Torsten, Wilfried, 2003; Vieira, 2008; Venâncio et al., 2010).

The studies by Venâncio et al. (2010) and Vieira et al. (2008) found an increase in the serum activity of both liver enzymes (AST, ALT and Alkaline Phosphatase), and Vieira et al. (2008) also found a dose-dependent effect for these parameters, concluding that the increase in the three parameters studied for liver function are the result of abusive use of the steroid Nandrolone Decanoate.

Some studies, however, such as that by Frankenfield et al. (2014) and Almeida and Lima (2019) do not find changes in the serum activity of the ALT enzyme. While Samieinasab and colleagues (2015) found a reduction in AST activity and an increase in ALT activity.

Tasgin et al. (2017) observed the effect of a single high dose of Nandrolone Decanoate (40 mg/kg) on biochemical parameters of male and female Wistar rats. In male animals, they observed that after 1, 3, 4, 8, 12 and 24 hours, there was no significant increase in the serum activities of the AST and ALT enzymes,

however, when they analyzed Alkaline Phosphatase, they observed that its activity decreases in the first hours after application and rises afterwards. They concluded, in their study, that the harmful effects commonly related to the use of anabolic steroids, including biochemical changes, are derived from the prolonged use of these substances.

In a study with 32 body-builders, Urhansen, Tosten and Wilfrie (2003) concluded that the adverse effects of steroids on the hepatic system and other body systems are reversible after at least one year of ceasing use. In this study, biochemical and hormonal parameters were compared between 15 former users (without using steroids for at least a year) and 17 users. Among the users, 16 of the 17 had AST and ALT enzymatic activities up to ten times above the reference values. In these users, ALT activity was greater than AST, while the activity of the alkaline phosphatase enzyme showed no difference between the groups. Although several analyzed parameters returned to reference values after more than a year of stopping steroid use, 4 of the former users had ALT values above the reference value, which could be indicative of chronic liver damage.

In general, considering the large number of illicit EAA users, the number of reports of hepatotoxicity is considerably low, with the majority of them being related to the use of steroids administered orally (Niedfeldet et al., 2018) and not to injectable use. of these. Observing the results of the current study, it is noted that in only two groups, DN 15 mg/kg and Mix 10 mg/kg, there was an increase in serum AST and ALT activity, in the other groups, only in AST. Since the ALT enzyme is more specific for the liver than the AST enzyme, it is believed that the source of elevation is not the liver, which can also be seen in graph 2, referring to hepatic lipid peroxidation. As the time between the last session of resistance physical exercise and blood collection is considered sufficient for muscular AST not to interfere with the results, the main effect may be cardiac, another common source of AST.

The decrease in the HDL cholesterol fraction after the use of steroids has been reported for a few decades (Vieira et al., 2008; Bonetti et al., 2008). It is believed that this effect may be derived from the increased activity of hepatic triglyceride lipase (HTGL) (Shahid et al., 2001; Hartgens, Kuipers, 2004). Despite the no change in HDL values when compared to the vehicle groups in the current study, the excessive increase in Total Cholesterol and the Non-HDL cholesterol fraction are alarming.

Contrary to the studies presented and the current results, the study by Vieira et al. (2008) observed a decrease in the serum dosage of Total Cholesterol and fractions, in addition to a decrease in triglyceride levels, which they claim is the result of a dysfunction in the hepatocytes and consequent inability to synthesize lipoproteins, or a simple effect of the decrease in feed consumption, which was also not observed in most groups in the current study.

The study by Urhause et al. (2003) compared the lipid profile of users and former users of steroids and bodybuilders. They observed that HDL levels were reduced in users compared to former users, however, total cholesterol and LDL fraction levels were the same. Granados et al. (2013) observed a lower level of HDL, a higher level of LDL and an increase in the Total Cholesterol/HDL ratio in men aged between 18-35 years, using the oral prohormone androstenedione. Hartgens et al. (2004) observed a decrease in some subtypes of HDL and an increase in LDL, however, they did not find significant differences in serum levels of triglycerides and total cholesterol.

In a study, Naqhvi and Flaherty (2016) list the most common changes in the lipid profile of steroid users (Increase in LDL and

decrease in HDL) as independent factors, directly related to the greater probability of developing heart problems, including atherosclerosis. Jarallah et al. (2018) report a decrease in the HDL fraction and an increase in Total Cholesterol and LDL fraction in users of anabolic steroids, in addition to linking their use with heart problems, such as left ventricular hypertrophy (Lusseti et al., 2015). Fett et al. (2018) found an increase in the LDL fraction and a decrease in the HDL fraction in steroid users, in addition to an increase in the Total Cholesterol/HDL and LDL/HDL ratio, according to him, both capable of increasing cardiac risks. Nishizawa et al. (2002) showed that Nandrolone Decanoate reduces a plasma protein called adiponectin, which has anti-atherogenic and antidiabetic effects, and that its reduction may be directly related to the effects on the cardiovascular system of EAA users.

Niedfeldet (2018) reports that the use of steroids, when done quickly, tends to change the lipid profile of users little, and that the tendency after discontinuing use is to revert the changes to normal. However, it reports that prolonged use can reduce HDL levels and increase LDL levels and, regarding total cholesterol, it reports that there are cases where it is increased and cases without changes, and that oral steroids increase total cholesterol more frequently than injectable steroids, probably due to their greater hepatotoxicity.

A study by Urhasen and collaborators (2003) reveals that one year after stopping steroid use, HDL values tend to return to normal, a fact that did not occur in two of the former users, who continued to have serum HDL levels lower than the reference values. Studies have assessed that the side effects of anabolic steroids on hepatic, renal and lipid biochemical parameters tend to be reversed between 5 months and one year after ceasing steroid use.

Skogatierna and collaborators (2013) observed in their study that supraphysiological doses of testosterone enanthate can induce endothelial dysfunction, directly related to cardiovascular problems. They also observed that the treatment inhibited the expression of the gene that produces endothelial nitric oxide (NO), an important vasodilator in our body. A result that, together with the changes observed in the lipid profile in the current study, demonstrates the danger of steroid abuse for the cardiovascular system.

Pozzi and collaborators (2012) observed genetic damage to kidney cells in male Wistar rats subjected to subcutaneous applications of Nandrolone Decanoate, mainly at a dose of 15 mg/kg. In the current study, a dose of 15 mg/kg of DN increased levels of renal lipid peroxidation, but only creatinine showed an increase in this group, urea showed no difference when compared to the vehicle group.

Frankenfield and collaborators (2014) studied the effect of DN on parameters related to oxidative stress in the liver, kidneys and heart of Wistar rats. DN treatment increased NADPH oxidase activity in the heart and liver. Catalase enzyme activity decreased in the liver and kidneys, while superoxide dismutase (SOD) was decreased in the liver. Thiol residues decreased in the liver and kidneys, while carbonyl residues decreased in renal analysis. Taken together, the changes observed in the study allow us to conclude that treatment with DN induces a dysregulation in the cellular redox balance in the organs studied in the rats, thus generating a state of oxidative stress, consistent with current theories about the damage caused by anabolic steroids.

CONCLUSIONS

It is concluded from the data obtained that the use of anabolic steroids can induce changes in biochemical parameters and also in parameters related to oxidative stress. It is important to consider the relationship between the type of steroid used, the dose used and the time of use, factors that are inseparable from side effects. The most serious renal effects, such as renal infarction, usually appear later, users who develop more serious pathologies, including some athletes between 25-35 years old, have started to die more frequently in recent years, usually with problems related to the cardiovascular system, such as heart attacks, aneurysms and strokes. Recently, there has been a movement of bodybuilder athletes on social media asking non-athletes not to use steroids, due to the growing number of health problems generated, whether physical or psychological.

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