Electrocardiographic Profile and Muscle Glycogen Content of Rats Treated with Nandrolone

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Abstract

Background: **We considered both the indiscriminate use of steroids by top athletes and by physically active individuals.**

Objective: **To evaluate the effects of nandrolone decanoate on the electrocardiographic profile, glycogen content and total-protein profile of skeletal and cardiac muscles, as well as the plasma albumin concentrations.**

Methods: **The drug was administered subcutaneously, at a concentration of 5 mg/kg, twice a week for three weeks, to animals in the treated group. Once a week, the rats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and they underwent an electrocardiogram (ECG). After the trial period, samples of the cardiac muscle (left ventricle - LV), soleus muscle (S), white gastrocnemius muscle (WG), red gastrocnemius muscle (RG), pectoral muscle (P), intercostal muscle (IC) and diaphragm muscle (D) were promptly collected and analyzed. An analysis of variance (ANOVA) and then a Tukey test ($p>0.05$) were carried out to assess the data (mean ± sem).**

Results: **There were changes in the following parameters of rats in the treated group: QRS interval, QTc interval and heart rate, characterized by an increase in these parameters, with the peak being reached in the period between the pre-treatment week and the first week. There was an increase of 127% in glycogen reserves in the LV. In relation to the total-protein amount, the significant difference was found in S, RG and D. As for the hematocrit and biochemical profile, it was possible to notice an increase in the percentage of erythrocytes.**

Conclusion: **The study shows that major cardiac changes are triggered at an early stage, which indicates a hierarchy in the sequence of changes that compromise the homeostasis of the body. (Arq Bras Cardiol 2010;95(6):720-725)**

Keywords: **Decanoates/adverse effects; nandrolone/adverse effects; steroids/contraindications; electrocardiogram pattern; rats.**

Introduction

High-performance athletes try to maximize the results at any cost and, to do that, they often associate physical training with the use of substances that are illegal in the sporting world, such as androgenic anabolic steroids (AAS). Such substances are natural or synthetic compounds, made up of testosterone and its derivatives, and they are divided into two groups, namely: esterified derivatives and alkaline derivatives. The first ones are represented by testosterone propionate, testosterone enanthate and testosterone cypionate. They are administered preferably via intramuscular route and remain active for days and weeks. The second group, however, is administered orally[1].

Recent studies aimed at evaluating the pharmacological behavior of these agents indicate a prescription targeted at catabolic processes manifested in specific situations and diseases, such as chronic infections, extensive surgery, testosterone deficiency, malnutrition, aplastic anemia, male impotence (caused by testicular failure), delayed male puberty, eunuchism (castration), menopause, in people with AIDS (decreased degradation of muscle and maintenance of muscle mass), treatment of hereditary angioedema, hypogonadism and decreased dehydroepiandrosterone and DHEAS, which typically affects the elderly, treatment of patients for weight gain, after serious trauma or continuous infection, besides enabling, in animals, the regeneration of tissues such as blood, cornea, among others[2,3].

In the 70s, an increasing number of people became interested in the topic and studies were carried out with the purpose of maintaining and reestablishing the muscular strength of young or elderly people[4]. After that, a study carried out by Ryan[5] demonstrated that during World War Two, AAS were widely used to restore the positive nitrogen balance in malnourished victims that had been subjected to forced fasting.

Developed by laboratory Organon and introduced in the market in 1962, nandrolone decanoate or Deca-Durabolin™, whose active ingredient is nandrolone, is one of the AAS most

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widely used in the world[6]. It is available commercially as an injectable anabolic preparation and its action lasts for up to three weeks, after intramuscular administration in humans[7]. Compared to testosterone, nandrolone shows more anabolic action and less androgenic activity[8].

All steroids that are considered anabolic are compounds that derive from testosterone. When these anabolic steroids work on androgen receptors, they modulate, in an inseparable way, both the anabolic effects and the androgenic effects. These substances vary in relation to the androgenic anabolic activity and there is a hierarchy with respect to their ratios. Thus, methandrostenolone is 2-5 times more potent than testosterone, and the ratios of oxymetholone, oxandrolone, nandrolone and stanozolol are 9, 10, 10, 30 times higher, respectively. On the other hand, no drug currently available is able to trigger only anabolic effects[9].

AAS have attracted the attention of health researchers because athletes have been using this drug without prescription and at high doses, with the purpose of increasing muscle mass, for aesthetic purposes or to improve physical performance[10]. This use has several side effects, such as atrophy of testicular tissue, prostate tumors[11], changes in lipid metabolism[12] emotional changes[13] and the development of psychotic symptoms[14].

In addition, there are studies that associate the abuse of AAS with cardiovascular changes, such as a predisposition to hypercoagulability, increased platelet aggregation, decreased fibrinolysis[15], increase in interventricular septum thickness, but with preservation of normal systolic and diastolic functions[16], ventricular thrombosis, systemic embolism[17], dilated cardiomyopathy, acute myocardial infarction caused by blockage of the left anterior descending artery and sudden death caused by left ventricular hypertrophy[18].

For some time, energy metabolism, mainly in relation to the muscle glycogen content, has formed the basis for the research of many scientists. All of such scientists agree that the endurance time for a given exercise is related to the amount of muscle glycogen available.

Thus, high contents refer to improvement in performance, and low reserves are directly related to exhaustion[19]. As the exercise progresses, the muscle glycogen reserves decrease progressively, and part of the energy spent in the effort starts to be provided by triglycerides, by glucose and free fatty acids (FFA) circulating in plasma[20,21].

This study was based on the assessment of the effects of nandrolone at a dose of 5 mg/kg/week, which is equivalent to the dose mentioned in the literature. This dose is regarded as excessive and it is usually administered to athletes at the beginning of sports practice[6]. In the proposal presented herein, the evaluation was directed at the electrocardiographic profile, glycogen content total-protein content of skeletal muscles and heart muscles, as well as plasma albumin concentrations.

Material and methods

Three-month-old Wistar rats, purchased from company ANILAB™, were used. The animals were housed in collective cages with no more than four animals per cage. The cages were kept in a temperature-controlled room (23 ± 2°C) with 12/12h light/dark cycle and received water and feed ad libitum. All procedures adopted in this experiment were in line with the standards of COBEA (Brazilian College of Animal Experimentation) and the Guidelines of the Department of Comparative Medicine at the University of Toronto[22]. The animals were randomly divided into two experimental groups, which were named "Control" (n = 10) and "Treated with Nandrolone Decanoate" (n = 13) (Deca-Durabolin™; 5 mg/kg) with n=23. The rats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and their cardiac electrical activity (ECG) was evaluated in the week before the treatment and in the three subsequent weeks, with the device ECG 98 - HEART WARE™. Subcutaneous injections of nandrolone decanoate were administered to the treated group twice a week for three weeks, between 10 a.m. and 10:30 a.m., and injections of PBS (phosphate buffer solution) were administered to the control group on the same days and at the same time. After the trial period, samples of the cardiac muscle (left ventricle - LV), soleus muscle (S), white gastrocnemius muscle (WG), red gastrocnemius muscle (RG), pectoral muscle (P), intercostal muscle (IC) and diaphragm muscle (D) were promptly collected and the glycogen content was evaluated, as proposed by Siu et al[23], with the values being expressed in mg/100 mg of wet weight. The muscle samples were also used to evaluate the amount of total proteins (g/dl), and blood samples were used for evaluating the glycemia and concentration of free fatty acids[24]. An analysis of variance (ANOVA) and a Tukey test ($p>0.05$) were carried out to evaluate the data (mean ± sem).

Results

Initially, we direct the evaluation to the behavior of the following electrocardiographic parameters: QRS duration, QT interval, QTc and resting heart rate (HRest).

When we evaluated the electrical behavior of cardiac muscles of rats treated with nandrolone, we saw that there was an increase in the QRS interval between the week before the treatment and the second week of treatment, as shown in Table 1. With respect to the QT interval, there was homogeneousness in the behavior of such interval, as shown in the same table. On the other hand, the QTc interval reached the acrophase in the first week of treatment, getting to values that were 19% higher, and then where was a reduction of 10% and 17% respectively. In the analysis of the heart rate, it was possible to note a significant increase of approximately 10% in the period between the pre-treatment week and the first week of treatment ($p > 0.05$). After that, there was a small decrease of 2%, which continued in last week (Table 1).

Then, we evaluated the muscle glycogen reserves, and it was possible to see that the control group had a pattern of glycogen reserves that was similar to that mentioned in recent publications of the literature[25-27]. With respect to the group treated with nandrolone, as one may see in Figure 1, with the treatment, there changes only in the glycogen reserves of the ventricular chamber, with a reserve that was 127% greater than the control group's reserve. As for the other muscles, there was no statistical difference. In the evaluation of total-protein content, there was a significant increase in

the soleus muscle, red gastrocnemius muscle and diaphragm muscle. However, in the white gastrocnemius muscle, pectoral muscle and intercostal muscle, no significant difference was found (Figure 2).

Finally, we assessed the plasma biochemical profile and hematocrit profile. With respect to changes in the metabolism, there was no difference in blood glucose levels and in the plasma concentration of free fatty acids. Similarly, there was difference neither in body weight nor in the following structures: heart, prostate or epididymal fat. However, in the hemacrocrit evaluation, it was possible to see that the percentage of erythrocytes in the treated group was 18% higher than in the control group. However, it is important to highlight that the other parameters evaluated were not statistically significant (Table 2).

Discussion

The classic therapeutic indication of AAS is intended to address androgenic deficiencies or pathological conditions that may cause deficiency in the protein metabolism[10]. It is known that, in and out of sports, nandrolone is widely used. This steroid is administered at high doses with the purpose of making aesthetic changes and improving performance. However, together with this practice, the literature shows an increasing rate of mortality among users of this substance[28]. The electrocardiographic evaluation revealed changes in the electrical pattern of the cardiac muscle represented by an increase in the time to propagate the signal in the ventricular chamber, as the QRS became longer. This fact was accompanied by a similar event that was observed in the QTc interval, which was also high and indicated a delay in the processes that are inherent in the re-polarization of ventricular chambers.

A point to consider is that the presence of estrogen receptors has been demonstrated in a variety of tissues, including in the cardiovascular system[29]. In particular, in relation to muscle tissue, the expressed receptors are of the ERa type. This expression has already been characterized in mice, rats, cattle and humans, and its action is linked to the regulation of cellular energy metabolism that can act both through the nongenomic, cytosolic pathway and through the genomic pathway[30]. As these receptors act on metabolism, the fact that the action of the nandrolone is multifactorial and represented by the capacity to modify the expression of androgen receptors, the affinity and activity of post-receptor pathways. Moreover, there is a direct relationship between changes in adrenergic sensitivity of the sinoatrial node and the presence of steroids. This may indicate

Table 1 - Electrocardiographic parameters and heart rate of rats in the group treated with nandrolone (Deca-Durabolin™; 5 mg/kg). The values correspond to the mean ± sem, n=23

Weeks	QRS (ms)	QT (ms)	QTc (ms)	HR (bpm)
Pre-treatment	92.57 ± 5.42	210.14 ± 8.55	341.09 ± 15.06	186.46 ± 8.53
Week 1	94.46 ± 7.62	210.3 ± 8.47	408.1 ± 1.9*	203.69 ± 4.43*
Week 2	128.36 ± 9.53*	200.3 ± 6.65	367.07 ± 1.2*	199.61 ± 4.08
Week 3	106.18 ± 8.25	196.92 ± 5.4	338.75 ± 1.79	199.83 ± 8.72

** Significant (p> 0.05), compared to the pre-treatment week.*

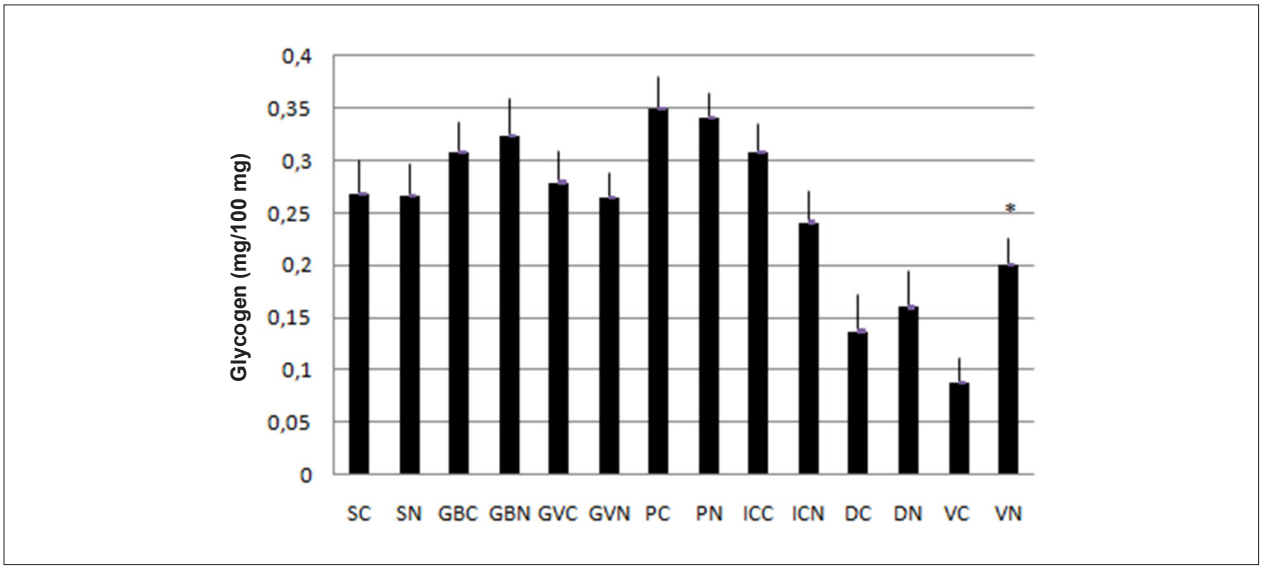

Figure 1 - *Glycogen content (mg/100 mg) of soleus muscle (S), white gastrocnemius muscle (WG), red gastrocnemius muscle (RG), pectoral muscle (P), intercostal muscle (IC), diaphragm muscle (D) and left ventricle (V) of rats in the control group (C) and in the group treated with nandrolone (N, Deca-Durabolin™; 5 mg/kg). The values correspond to the mean ± sem, n=23. * p<0.05, compared to control.*

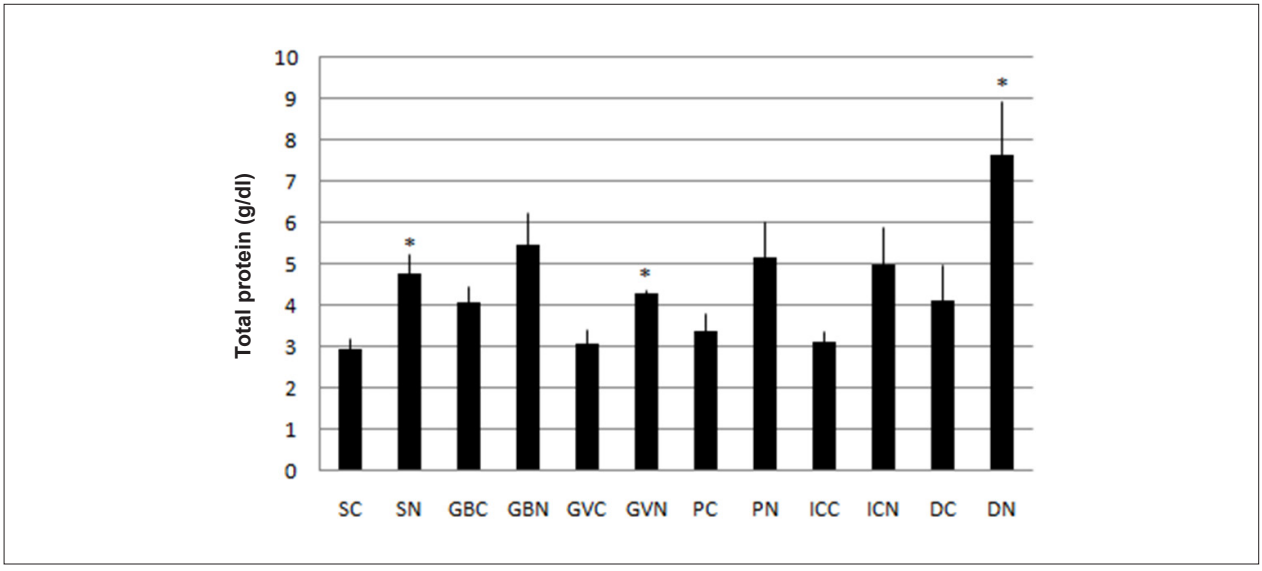

*Figure 2 - Content of total proteins (g/dl) in the soleus muscle (S), white gastrocnemius muscle (WG), red gastrocnemius muscle (RG), pectoral muscle (P), intercostal muscle (IC) and diaphragm muscle (D) of rats in the control group (C) and the group treated with nandrolone (N, Deca-Durabolin™; 5 mg/kg). The values correspond to the mean ± sem, n=23. * p<0.05, compared to control.*

Table 2 - Body weight, biochemical and hematological parameters of rats in the control group and the group treated with nandrolone (Deca-Durabolin™; 5 mg/kg). The values correspond to mean ± sem, n=23

	Control	Nandrolone
FFA (mmol/l)	0.41±0.1	0.47±0.07
Blood glucose (mg/dl)	139.42±11	139.42±9.1
Epididymal fat (g)	6.32±0.49	7.11±0.8
Prostate (g)	0.64±0.06	0.57±0.04
Albumin (g/dl)	1.69 ± 0.07	1.93 ± 0.05
Body weight (g)	404.46 ± 9.3	433.68 ± 6.3
Heart weight (g)	1.382 ± 0.4 g	1.430 ± 0.4
Hematocrit (%)	43.9 ± 1.6	51 ± 2.4*

** Significant (p> 0.05) compared to controls.*

that in the presence of a supraphysiological dose of nandrolone, there may be changes in the sensitivity of the heart, which can be expressed by the increase in the population of adrenergic β receptors, thereby causing super-sensitiveness in the cardiac pacemaker, as suggested by Norton et al[31]. It is important to note that heart rate also rose, indicating a change in the sensitivity/activity interface of the sinoatrial node.

If we consider that nandrolone may increase metabolic patterns, another option here is directed at a cardiotropic action with a biochemical base, because the nandrolone can stimulate the activation of the glucose-6-phosphate, 6-phosphogluconate dehydrogenase and isocitrate dehydrogenase enzymes that are present in the cardiac muscle[32]. At first, this change in the biochemical pattern of cardiac fibers causes an increase in the generation of NADPH and consequently, a change in the time to convey the electrical signal, thereby overloading the cardiac function and increasing the systole time of the ventricular chamber.

When we evaluated the glycogen content of the cardiac muscles, we observed that in the presence of nandrolone, there was an increase in reserves. This increase in glycogen content may reflect the action of the steroid, which is capable of changing the tissue responsiveness to other hormones, such as the IGF, which is the insulin-like growth factor. This shows some potent action on the glycogenic pathway, thereby favoring the formation of this reserve of metabolizable substrates. Thus, we suggest that, besides the increase in the functional requirement and metabolic activity of the cardiac muscle, changes occur in the functional relationships that can be characterized by an increase in the uptake and metabolism of substrates, with consequent increase in energy use, as suggested Falkenberg et al[15]. In other words, nandrolone acts on the cardiac muscle and stimulates a rise in heart rate and metabolic rate, but in contrast, it may increase energy reserves, causing the heart to have a greater intake of metabolizable substrates and implementing a metabolic status that meets the largest demand. In this sense, a sign that suggests a rise in energy needs may be the rise in hematocrit, indicating that it is part of the responsiveness that is enabled in the early moments.

One needs to consider that the anabolic action, which is expressed by an increase in weight of some organs and which is inherent in nandrolone, has not been verified and it is possibly due to the experimental observation period, since it deals with actions related to nuclear codes expressed in the long term. This fact suggests that the effects that are inherent in the action of steroids may have a time relationship.

Conclusion

The study shows that major cardiac changes are triggered at an early stage, which indicates a hierarchy in the sequence of changes that compromise the homeostasis of the body. Therefore, we recommend carrying out further studies to clarify

the relationship between nandrolone and the genesis of these changes, which indicate overloading of cardiac muscles, which is something that can cause arrhythmia and sudden death.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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