Effect of the nandrolone decanoate on the efficiency of spermatogenesis of sedentary rats and rats subjected to physical training

Marcela de Paiva Foletto¹*, Cecilia Mareze-Costa¹, Fernanda Ferrari, Solange Franzói-de-Moraes¹ and Tânia Mara Segatelli²

¹Departamento de Ciências Morfofisiológicas, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil. ²Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270-910, Belo Horizonte, Minas Gerais, Brazil. *Author for correspondence. E-mail: mpfoletto@yahoo.com.br

ABSTRACT. Spermatogenesis is a complex, highly organized and coordinated process that results in the production of male gametes. This process is influenced by a number of drugs that enhance physical performance, which have been used mainly by young people, athletes or not, seeking to gain athletic performance or only a prominent social position. The objective of this study was to evaluate the effects of the anabolic steroid nandrolone decanoate on the efficiency of spermatogenesis in sedentary rats and rats subjected to physical training. Twenty-four male rats were divided into four experimental groups: sedentary control, sedentary treated, trained control and trained treated. Treated animals received intramuscular injection of nandrolone decanoate (0.5 mg kg⁻¹ body weight) during the last four weeks of physical training. The training program consisted of running on a programmable ergometer treadmill, tailored to train eight rats simultaneously, for nine weeks. We evaluated the morphological and morphoquantitative profiles of the male reproductive system. The results showed that the treatment causes a reduction in the efficiency of spermatogenesis; however, when associated with physical training, this compensates for the anabolic action, keeping the process of spermatogenesis within normality.

Key words: anabolic agents, germ cells, spermatogenesis, nandrolone decanoate, testis.

Introduction

Androgenic anabolic steroids (AAS) are a family of hormones that include testosterone, along with its many synthetic relatives, which show both anabolic – promote an increase of the skeletal muscle mass – and androgenic – masculinizing effects (KANAYAMA et al., 2008). The synthetic AAS analogues of testosterone were developed for the treatment of some diseases such as hypogonadism, refractory anemia and the conditions of malnutrition.
with loss of muscle mass (NOORAFSHAN et al., 2005). The anabolic effects of these drugs, however, called the attention of athletes and gym practitioners and body builders, and came to be improperly employed with the purpose of increasing skeletal muscle mass and improving performance in athletic competitions (NOORAFSHAN et al., 2005; SALAS-RAMIREZ et al., 2008).

Several side effects have been already verified in experimental animals treated with AAS and in humans making use of these drugs, such as cardiac problems, alterations of the adrenal gland (TAKAHASHI et al., 2004) and aggressive behaviors (SALAS-RAMIREZ et al., 2008). The increased risk of prostate cancer (POPE JUNIOR et al., 2000; SANTOS, 2003), problems related to absence of libido and sexual impotence (SANTOS, 2003) are also mentioned. High doses of AAS affect the estrous cycle and promote histological changes in the ovaries and uterus of female rats (BLASBERG et al., 1997; GEREZ et al., 2005), leading to a suppression of the reproductive capacity (MOBINI FAR et al., 2007). Normal males of several species of experimental animals showed decreased testicular size and weight after AAS treatment. Both parameters, when decreased, impair spermatogenesis, inhibit the release of luteinizing hormone and diminish the levels of endogenous testosterone (MOORE; PRICE, 1938; LUDWIG, 1950; DESJARDINS et al., 1973; BERNDTSON et al., 1974; BERNDTSON et al., 1979; MAUSS et al., 1975).

The use of anabolic hormones, despite being linked to many hazardous effects, has increased in recent years, becoming a problem no longer restricted to competitive sports, but reaching recreational sports and gym academies. Therefore, the purpose of this work was to evaluate the effects of nandrolone decanoate (Deca-durabolin®), one of the most commonly used AAS in gyms, on the spermatogenesis of sedentary rats and rats subjected to physical training.

Material and methods

Experimental animals

Male Wistar rats were kept in an animal house at a temperature of 23 ± 2°C, with light:dark cycle of 12/12h (lights on at 6:30 a.m.) and free access to water and rodent chow (Nuvital® Colombo, Paraná State). The experimental procedures were approved by the Committee of Ethics in the Use of Experimental Animals – CEAE (Protocol n°. 039/2008).

Experimental procedure

Twenty-four 45-days-old animals were allotted to four experimental groups: sedentary control (SCo), sedentary treated (STr), trained control (TCo) and trained treated (TTr), with six animals per group. The trained animals were subjected to physical exercise, which consisted of running (intensity of 60-65% of the VO₂ Max) in a programmable ergometer treadmill (Imbramed mod.KT3000) adapted for rat training. Before the beginning of the training, the animals had a week of adaptation, during which the speed of the treadmill increased progressively from 0.3 to 0.6 km h⁻¹ at stages lasting two minutes each. After this period, the speed and the duration increased gradually until 60 min. day⁻¹ at an average speed of 1.0 km h⁻¹ (NEGRÃO et al., 1992; DUFLOTH et al., 1997). The training was carried out four times a week, always at the same time (7:30 to 8:30 a.m.), during nine weeks. The treatment with nandrolone decanoate began on the fifth week and consisted of the intramuscular injection (gastrocnemius muscle) of Deca-durabolin® (0.5 mg kg⁻¹) diluted in vegetable oil, five times a week, during five weeks (PERES; LUCIANO, 1995; NOORAFSHAN et al., 2005). The control groups received intramuscular injections of the vehicle (vegetable oil). At the end of the training and treatment, the animals were anesthetized with sodium pentobarbital (Hypinol® 3%, 4 mg 100 g⁻¹ bw, i.p.) and subjected to abdominal-pelvic laparotomy to remove the seminal vesicles and testicles, which were weighted. Next, the animals were euthanized by anesthetic overload.

Histological processing of the testes

Testis samples were perfused-fixed with Karnovsky-fluid for 24 hours, at room temperature, and stored in 0.1 M phosphate buffer, pH 7.3 until histological processing. Samples were embedded in Historesin® (GMA, Leica Instruments), sectioned at 4 μm thickness, placed on glass slide, and stained with Schiff’s periodic acid, counter-stained with hematoxylin – PAS-H (SEGATELLI et al., 2004).

Cellular population at the stage VIII of the sec

All germ cell and Sertoli cell nucleoli present at stage VIII of the seminiferous epithelium cycle (SEC) were counted in 10 round or nearly-round seminiferous tubule cross-sections, chosen at random, for each animal. The following germ cells were counted: type-A spermatogonia, pachytene primary spermatocytes and round spermatids.

The cell numbers of the germ lineage were corrected for the nuclear diameter and the thickness of the histological section according to Abercrombie.
(1946), modified by Amann (1962). The mean nuclear diameter (DM) was obtained with the help of a 10X Olympus micrometer lens coupled to a 100X objective. The diameter of 10 nuclei of each cell type analyzed was measured. In the case of slightly ovoid nuclei (type-A spermatogonia), the value used was the mean of the largest and smallest diameters. The number of Sertoli cells was corrected for the nucleoli diameter and for the thickness of the histological sections; only the cells with clearly seen nucleoli were counted.

**Ratios between cell numbers**

With the purpose of evaluating the efficiency of spermatogenesis and the Sertoli cells, the ratios were calculated between the corrected numbers of the cells of the spermatogenic lineage and these numbers and the number of Sertoli cells. The following ratios were obtained:

- pachytene primary spermatocytes/ type-A spermatogonia, to estimate the coefficient of efficiency of spermatogonial mitosis;
- round spermatids/ type-A spermatogonia, to obtain the overall rate of spermatogenesis;
- round spermatids/ pachytene primary spermatocyte, to obtain the rate of the germ cell loss during meiosis (meiotic index);
- round spermatids/ Sertoli cell nucleoli, to estimate the Sertoli efficiency;
- total number of germ cells/ Sertoli cell nucleoli, to obtain the total support capacity of each Sertoli cell.

**Statistical Analysis**

The statistical analysis of the results was carried out using Student’s t-test for unpaired samples or ANOVA with Newman–Keuls multiple comparison test, when appropriate, setting the significance level at 95% (p < 0.05). The statistical tests were carried out using software Prism, v. 4.0 (GraphPad, USA).

**Results**

Table 1 shows the weight of the testes and seminal vesicles of the four experimental groups, corrected for 100 g body weight. It is noticed that the nandrolone decanoate treatment did not affect the weight of the testes, because there is no significant difference between the values found in groups SCo and STR, as well as in groups TCo and TTr. The seminal vesicles, however, were significantly heavier in the treated animals, both sedentary and subjected to physical training. Still concerning the results of table 1, but now focusing on the effects of physical training, a significant increase is verified in the testicular weight only when the trained groups (TCo and TTr) are compared with the sedentary treated group (STr), because when compared to the sedentary control group (SCo), the values are statistically similar. It is as well seen that the physical training did not cause a significant effect on the weight of the seminal vesicles. Finally, the association between physical training and anabolic hormone can be assessed comparing the group SCo with group TTr. This association caused a 10% increase in the testicular weight, an insignificant increase, while as for the seminal vesicles this weight increase was of 41.2% (p < 0.001), the treatment with the hormone being the major responsible.

**Table 1.** Effect of nandrolone decanoate on the weight of the testes and seminal vesicles (g 100 g⁻¹ bw) of sedentary and trained rats. SCo: sedentary control; STR: sedentary treated; TCo: trained control; TTr: trained treated.

<table>
<thead>
<tr>
<th>Weight</th>
<th>SCo</th>
<th>STR</th>
<th>TCo</th>
<th>TTr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes</td>
<td>101.26 ± 4.70</td>
<td>112.14 ± 10.47*</td>
<td>171.19 ± 7.28</td>
<td>159.72 ± 8.29</td>
</tr>
<tr>
<td>Seminal Vesicle</td>
<td>0.29 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.47 ± 0.03*</td>
<td>0.51 ± 0.03#</td>
</tr>
</tbody>
</table>
| Values express the mean±sem; n = 6 animals for each experimental group. * p < 0.05 relative to group STr; # p < 0.001 relative to groups SCo and TCo.

Table 2 shows the effects of the treatment with nandrolone decanoate and of physical training in the number of different cell types at the stage VIII of the seminiferous epithelium cycle (SEC). It is verified that the AAS did not significantly alter the numbers of the cell types evaluated; significant differences were not found either between the treated groups (STr and TTr) and the controls (SCo and TCo) as for the number of type-A spermatogonia and the number of Sertoli cells nucleoli. Nevertheless, considering the pachytene primary spermatocytes, it was observed that the training promoted a significant increase of 19.22% between the treated groups (STr and TTr). Comparing these same groups, significant differences could be found in the number of round spermatids and in the total number of germ cells, with increases of 21.28 and of 20.52%, respectively.

**Table 2.** Effect of the treatment with nandrolone decanoate in the number of different cell types – type-A spermatogonia (SPA), pachytene primary spermatocytes (SPI), round spermatids (SPids), total germ cells (Germ cell) and Sertoli cells (STL) of the stage VIII of the seminiferous epithelium cycle (SEC) of sedentary rats and rats subjected to physical training. SCo: sedentary control; STR: sedentary treated; TCo: trained control; TTr: trained treated.

<table>
<thead>
<tr>
<th>Cells</th>
<th>SCo</th>
<th>STR</th>
<th>TCo</th>
<th>TTr</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA</td>
<td>24.6 ± 1.52</td>
<td>28.39 ± 1.98</td>
<td>34.45 ± 2.69*</td>
<td>38.39 ± 2.45*</td>
</tr>
<tr>
<td>SP1*</td>
<td>34.45 ± 2.69*</td>
<td>38.39 ± 2.45*</td>
<td>42.93 ± 3.04</td>
<td>48.76 ± 3.68</td>
</tr>
<tr>
<td>Seminal Vesicle</td>
<td>0.29 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.47 ± 0.03*</td>
<td>0.51 ± 0.03#</td>
</tr>
<tr>
<td>Values express the mean±sem; n = 6 animals for each experimental group. * p &lt; 0.05 relative to group STr.</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
loss during the mitotic divisions in group STr, as can be observed by the coefficient of mitotic efficiency, reflecting the diminishment of the total number of cells produced at the end of spermatogenesis. However, when compared to the same parameter in the groups sedentary treated and trained treated, it is observed that training promotes a significant increase. There were no significant differences as for the meiotic index. Decreases in the Sertoli cells indices were demonstrated due to training, much as for the total capacity of support of the Sertoli cells.

Table 3. Effect of nandrolone decanoate on the ratios between corrected cell numbers at stage VIII of the seminiferous epithelium cycle (SEC) of sedentary rats and rats subjected to physical training. SCo: sedentary control; STr: sedentary treated; TCo: trained control; TTr: trained treated.

<table>
<thead>
<tr>
<th>SCo</th>
<th>STr</th>
<th>TCo</th>
<th>TTr</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP1h/SPA</td>
<td>19.28 ± 1.70</td>
<td>12.96 ± 1.27*</td>
<td>14.31 ± 1.69</td>
</tr>
<tr>
<td>SPhds/SPA</td>
<td>64.05 ± 4.83</td>
<td>41.50 ± 4.97*</td>
<td>51.02 ± 5.22</td>
</tr>
<tr>
<td>SPhds/STL</td>
<td>10.06 ± 0.79</td>
<td>0.38 ± 0.11</td>
<td>3.35 ± 0.09</td>
</tr>
<tr>
<td>Germ cell/STL</td>
<td>15.96 ± 1.11</td>
<td>14.62 ± 0.66</td>
<td>16.00 ± 0.73</td>
</tr>
</tbody>
</table>

Values express the mean ± SEM; n = 6 animals for each experimental group, * p < 0.05 relative to groups SCo and TTr; # p < 0.05 relative to group SCo.

**Discussion**

The mammalian testes are complex organs that have two morphofunctional compartments: (i) interstitial or intertubular, major source of androgens of the organism; (ii) tubular, where spermatogenesis takes place (RUSSELL et al., 1990). The seminiferous epithelium is composed of two types of cells: germ cells at different stages of maturation (spermatogonia, spermatocytes and spermatids); and Sertoli cells, which provide support to the germ cells, nutrition, phagocytosis, in addition to secreting a fluid that aids in the transport of the sperm (GARTNER; HIATT, 2008).

In the seminiferous tubules of sexually mature animals, the spermatogenic cells are not randomly arranged, but organized as a well-defined series of cellular associations or stages. The complete set of stages, organized in a progressive sequence of germ cell development in a given segment of the seminiferous tubule, is named seminiferous epithelium cycle (SEC) (CLERMONT, 1972; RUSSELL et al., 1990). In the study of the kinetics of spermatogenesis, the numbering of the cell types present at the several stages allows the follow-up, in quantitative terms, of the evolution of each type along the cycle. This analysis provides the estimation of the efficiency of spermatogenesis at any stage. The efficiency is translated by the ratios between the cells numbers obtained. Thus, by comparing the real ratio obtained by the direct count of nuclei with the theoretical ratio expected from the cell divisions of spermatogenesis, one can determine the efficiency of spermatogenesis at any point of the cycle. The real ratio is always lower than the theoretical (CASTRO et al., 2002).

Testicular size is an important parameter in the androgenic evaluation of mammals, reflects their normality and allows the inference of the sperm production rate (FRANÇA; RUSSELL, 1998). In general, there is no significant correlation between the size of the testis and body weight (FRANÇA, 1991). For each species, testicular size is determined in response to a variety of other factors, in addition to the primary influence dictated by body size. The number of Sertoli cells established during the tubular development represents the major factor responsible for the size of the testis and the sperm production (HESS et al., 1993). In this study, a reduction was verified in the testicular size in the sedentary animals treated with nandrolone decanoate. On the other hand, when the treatment was associated with physical training, there was a significant increase in the size of this organ. The seminal vesicle of the treated animals was heavier, either these animals were sedentary or trained. These results show that AAS treatment, even at low doses, causes changes that can be found macroscopically, and also indicate that the action of nandrolone decanoate on the reproductive system of rats suffers interference from physical activity.

In mammals, the apoptosis of germ cells is an integral part of spermatogenesis, occurring normally in several phases of development (BLANCO-RODRÍGUEZ; MARTÍNEZ-GARCÍA, 1996). The balance between proliferation and apoptosis plays a fundamental role in the regulation of the population of germ cells in the seminiferous epithelium, keeping the homeostasis of spermatogenesis and reflecting directly in the daily sperm production characteristic of each species (RUSSELL et al., 1990). Particularly during the spermatogonia phase, the homeostatic mechanism of regulation is considered density-dependent, limiting the amount of germ cells that enter meiosis to a number that can be sustained by the Sertoli cells (ROOIJ, 1998). Normally, apoptosis occurs during mitotic and meiotic divisions; in addition to limiting the population of germ cells, it eliminates cells with potential impairments or containing DNA mutations. Spontaneous degenerations of the germ cells result in losses of about 75% of the expected theoretical potential of mature sperm (RUSSELL; ROOIJ, 2000).

Considering that the rat has six generations of differentiated spermatogonia, the expected theoretical number would be 64 spermatocytes for...
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Each A1 spermatogonia. In this work, significant cell losses were observed in group STR, representing about 80% (Table 3). However, when the treatment was associated with physical training, these losses did not take place. During meiosis they were lower, considering that from each spermatocyte four spermatids are formed.

According to Boyadjiev et al. (2000), high doses of these drugs cause a marked diminishment of sperm in men, azoospermia being possible at the beginning of the treatment. The spermatogenic yield of rats is as well influenced by the administration of high doses of anabolic steroids, causing reduction in the amount and quality of the sperm produced (KARBALAY-DOUST et al., 2007). In this study, the spermatogenic yield was one of the parameters evaluated and the treatment with AAS caused reduction of the efficiency of spermatogenesis, but when the treatment was associated with physical training, there was normalization of spermatogenesis (Table 3), which could suggest that the treatment provided compensation, maintaining sperm production.

Conclusion

The results presented showed that the treatment with the anabolic steroid nandrolone decanoate significantly reduces the efficiency of spermatogenesis in sedentary rats, while physical training smoothed this negative interference.

References


Received on November 7, 2008. Accepted on May 26, 2009.

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