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Original Article

# The Effect of Nandrolone Decanoate on the Body, Testis and Epididymis Weight and Semen Parameters in Adult Male Rats

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**Abstract**

**Background:** Anabolic-androgenic steroids are used at high doses by athletes for improving athletic ability, physical appearance and muscle mass. Therefore, the abuse of these steroids has been significantly increased. Many undesirable side effects of these steroids on the male reproductive function have been reported, however, little is known about their effects on sexual behavior and tissues of the reproductive system. The aim of this study is to identify the effects of anabolic-androgenic steroids on the body, testis and epididymis weight, as well as semen parameters.

**Method:** Five groups of Sprague-Dawley adult male rats (n=72) were used. Two experimental groups were medicated with intramuscular injection of 3 and 10 mg/kg body wt/wk of nandrolone decanoate and two vehicle groups with same doses of sweet almond and olive oils, respectively, for 14 weeks. The control group received no injection. One week after the last injection, rats were sacrificed and the weights of the body, testis and epididymis and also semen parameters were assessed.

**Results:** The weights of testis and epididymis, as well as, sperm count and motility rate were significantly decreased in experimental groups than in the vehicle and control groups. Morphologically abnormal sperms were observed.

**Conclusion:** We found that anabolic-androgenic steroids affect fertility parameters and cause testis atrophy.
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**Keywords** ● Nandrolone decanoate ● body weight ● testis ● epididymis ● sperm count ● rat

## Introduction

Sport represents a significant part of our lifestyle.[1] Despite of its positive features, one of the incredible aspects of sport is to "win at all times and costs," so that some persons may not obey the generally-accepted rules of not using banned substances, mainly for doping.[2] From ancient times, athletes have used plants or natural and synthetic drugs to increase their performance[3].

Currently, the most commonly-abused drugs for doping are anabolic-androgenic steroids (AASs).[3] AASs are synthetic derivatives of testosterone and are important pharmacologically for treatment of growth deficiency, some blood disorders and

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osteoprosis. They have also been reported to increase muscle mass, strength and libido.[4,5] Many of the abusers believe that the side effects of AASs are neither serious nor permanent. Nonetheless, some researchers reported that AASs have negative health consequences including endocrine, hepatic, cardiovascular and behavioral disturbances.[3,6]

The effects of AAS compounds on male reproductive tissue are equivocal; some investigators reported that spermatogenesis still continued after administration of AASs;[7,8] others showed that AASs decrease density, motility and normal morphology of sperm in men.[9] Ludwig found that spermatogenesis in rat was continued normally following treatment with AASs.[10] On the contrary, some investigations have shown depletion of Leydig cells and arrest of advanced steps of spermatogenesis.[11] Few studies identified that AASs cause atrophy of testis and reproductive accessory organs and also decrease sperm production.[5] We have previously shown that reduction of testis volume and length of seminiferous tubules still remained 14 weeks after the last injection of a high dose (10 mg/k/wk) of nandrolon decanoate.[12] Torres-Calleja, et al, reported that sperm count and the number of sperms with normal morphology were significantly reduced in AASs abuser bodybuilders.[13]

Sperm count and sperm quality have a wide range of effects on male fertility. Abuse of AASs is a frequent cause of male infertility.[14] The objective of this study was to assess the sperm count and quality and also body, testis and epididymis weight of male rats.

## Materials and Methods

*Animal treatment*

Seventy-two adult Sprague-Dawley male rats weighing 180–210 g were randomized into two experimental (n=38), two vehicle (n=24) and one control (n=10) groups. Experimental groups were medicated with intramuscular (IM) injections of 3 and 10 mg/kg body wt/wk, nandrolone decanoate (ND). Vehicle groups received the same doses of sweet almond and olive oils and control group received no injection for 14 weeks.[4,5] The volume of injection was 0.1 mL/200 g of body weight. The administered doses were selected based on previous studies in which the effect of ND treatment on muscle mass and physical performance of rats have been studied.[5,15-18] We also performed a pilot study to confirm the adequacy and bioavailability of these doses of ND. The animals were housed at 22–25 ºC with 12-h light/dark cycle. The weight of rats was measured weekly until one week after the last injection when the rats were sacrificed and their testes and epididymis were removed and weighed. The organs to body weight ratio were then calculated.

*Sperm parameters assessment*

To collect semen and assess semen parameters we used the method described by Seed, et al.[19]

The diffusion method was used for sperm collection.[15,19] A small piece (10 mm) of the vas deferens was placed in a petri dish containing five mL Hank's balanced salt solution (HBSS) and the sperms were allowed to diffuse into the buffer. After five min, the vas deferens was removed and the suspension was gently shaken to homogenize and spread the sperms.

Sperm motility was classified as: a) fast progressive (FP) when sperms moved rapidly in linear directions; b) slow progressive (SP) when sperms moved slowly in linear directions; c) non-progressive (NP) when sperms moved in circular directions; and d) immotile (IM) when sperms had neither linear nor circular movements.[19]

To count the sperms, we used a hemocytometer as was recommended by Seed, et al.[19] Four replicate counts were used for each subject.

An aliquot of sperm suspension was placed on a slide and air dried and then stained with Eosin. To assess morphology, the sperms were classified into two main categories: a) normal and b) abnormal, which included sperms with amorphous head, bicephal, fused body, bicudate and normally-shaped head with separated flagellum.[19]

*Statistical analyses*

One-way analysis of variance (ANOVA) followed by Duncan and LSD tests were used to compare the mean weight of body, testis and epididymis as well as sperm count among the studied groups. Mann Whitney U test was used to compare the sperm motility and morphology among the studied groups. A p value $<0.05$ was considered statistically significant.

## Results

*Effects of ND on the body weight*

The body weight was progressively increased in all rats during the treatment period but no significant difference was observed among the studied groups (fig 1). The mean±SEM weight in low-dose ND-treated rats (TLD) (234.70±6.97 g) and high-dose ND-treated rats (THD) (234.66±5.46 g) were lower than those of the control rats (CO) (241.72±7.02 g), low-dose vehicle-treated (VLD) (242.04±6.94 g) and high-dose vehicle-treated (VHD) (258.31±23.66 g) groups.

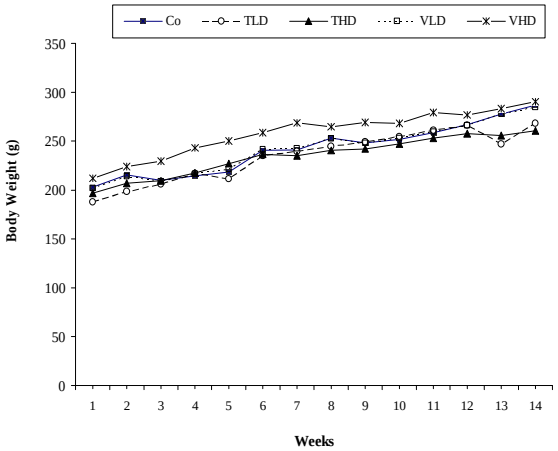


**Fig 1:** Mean body weight of rats in all groups. There are no significant differences among groups.

*Effects of ND on the weight of testis and epididymis*

The weight of testis and epididymis was significantly reduced in experimental groups compared with those of the control and vehicle groups ($p<0.01$ for testis weight, $p<0.05$ for epididymis weight of THD group and $p<0.001$ for epididymis of TLD group). Compared to the THD group, the weight of testis and epididymis was lower in TLD group (table 1 and figs 2 and 3). Table 1 also shows testis and epididymis weight/sacrificed body weight ratio.

*Effects of ND on the sperm parameters*

Sperm motility was totally decreased in experimental groups. It was significantly different from those of the control and vehicle groups ($p<0.001$). Percentage of the FP motility was significantly different among the experimental, control and vehicle groups and also between THD and TLD groups ($p<0.01$; table 2, fig. 4).

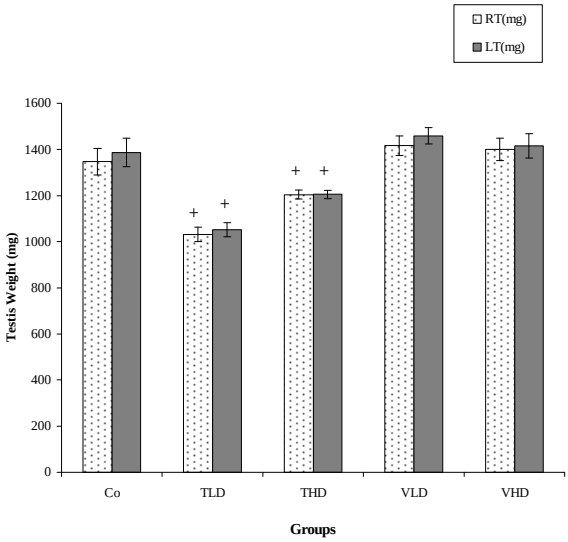


**Fig 2:** Mean±SEM of right (RT) and left (LT) testes (mg) in all rats, + shows significant differences ($p<0.01$) between low-dose (TLD) and high-dose treatment (THD) and other groups. CO: control group; VLD: low-dose vehicle; VHD: high-dose vehicle.

**Table 1:** Mean±SEM of testis and epididymis weight (mg) in all groups. Significant differences are seen between low-dose (TLD) and high-dose treatment (THD) and other rats.

| Groups | n | RT(mg) | RT/SBW | LT(mg) | LT/SBW | RE(mg) | RE/SBW | LE(mg) | LE/SBW |
|---|---|---|---|---|---|---|---|---|---|
| Control | 10 | 1348.00 ±57.90 | 0.47 ±0.19 | 1387.00 ±61.77 | 0.48 ±.20 | 554.00 ±18.98 | 0.19 ±0.87 | 583.00 ±18.95 | 0.2 ±0.04 |
| TLD | 19 | 1031.57$^{+}$ ±32.25 | 0.39$^{*}$ 0.17 | 1052.10$^{+}$ ±30.4 | 0.39$^{*}$ ±.16 | 281.05$^{*}$ ±19.62 | 0.11$^{*}$ ±0.92 | 337.38$^{*}$ ±24.36 | 0.12$^{*}$ ±0.08 |
| THD | 19 | 1204.21$^{+}$ ±19.53 | 0.36$^{\times}$ ±0.18 | 1204.73$^{+}$ ±18.40 | 0.46$^{\times}$ ±.10 | 444.21$^{\times}$ ±13.34 | 0.17 ±0.63 | 471.05$^{\times}$ ±16.32 | 0.18 ±0.07 |
| VLD | 12 | 1416.66 ±42.84 | 0.51$^{\times}$ ±.15 | 1459.16 ±35.66 | 0.53$^{\times}$ ±.11 | 556.66 ±24.38 | 0.19 ±0.76 | 550.00 ±22.08 | 0.2 ±0.07 |
| VHD | 12 | 1400.00 ±48.35 | 0.48 ±.15 | 1415.00 ±52.77 | 0.49 ±.16 | 532.50 ±25.34 | 0.17 ±0.89 | 535.00 ±22.61 | 0.18 ±0.07 |

VLD: low-dose vehicle, VHD: high-dose vehicle, RT: right testis, LT: left testis, RE: right epididymis, LE: left epididymis, SBW: sacrificed body weight. *$p<0.001$, $^{+}$ $p<0.01$, $^{\times}$ $p<0.05$

**Table 2:** Percentage of sperm motility and sperm count as well as sperm morphology in all groups. Significant differences are seen between low-dose (TLD) and high-dose treatment (THD) and other rats.

| Groups | n | Sperm motility (%) | | | | IM % | Count (million) | NM % | AM % |
|---|---|---|---|---|---|---|---|---|---|
| | | FP | SP | NP | Total | | | | |
| Control | 10 | 5.84 ±1.05 | 17.74 ±1.61 | 54.51 ±1.21 | 78.09 ±1.30 | 21.9 ±1.11 | 8.89 ±0.32 | 96.84 ±0.64 | 3.21 ±0.62 |
| TLD | 19 | 2.02$^{+}$ ±0.43 | 4.41$^{+}$ ±1.06 | 33.09$^{+}$ ±3.71 | 39.54$^{*}$ ±4.83 | 60.45$^{*}$ ±4.83 | 5.83$^{*}$ ±0.69 | 83.67$^{*}$ ±3.99 | 16.39$^{*}$ ±3.98 |
| THD | 19 | 1.88$^{+}$ ±0.73 | 4.18$^{+}$ ±1.08 | 29.64$^{+}$ ±3.58 | 36.39$^{*}$ ±4.38 | 63.61$^{*}$ ±5.13 | 4.11$^{*}$ ±0.62 | 88.87$^{*}$ ±1.74 | 11.12$^{*}$ ±1.74 |
| VLD | 12 | 5.49 ±0.98 | 13.29 ±1.52 | 49.24 ±5.65 | 68.02 ±6.76 | 31.93 ±6.77 | 8.98 ±0.78 | 95.84 ±0.59 | 4.15 ±0.59 |
| VHD | 12 | 4.9 ±0.72 | 10.59 ±1.83 | 53.95 ±5.54 | 69.44 ±6.54 | 30.55 ±6.54 | 9.23 ±0.96 | 95.93 ±0.52 | 4.06 ±0.52 |

VLD: low-dose vehicle, VHD: high-dose vehicle, FP: fast progressive, SP: slow progressive, NP: non-progressive, IM: immotile sperm, NM: normal morphology AM: abnormal morphology. $^{*}$ $P< 0.001$, $^{+}$ $p< 0.01$

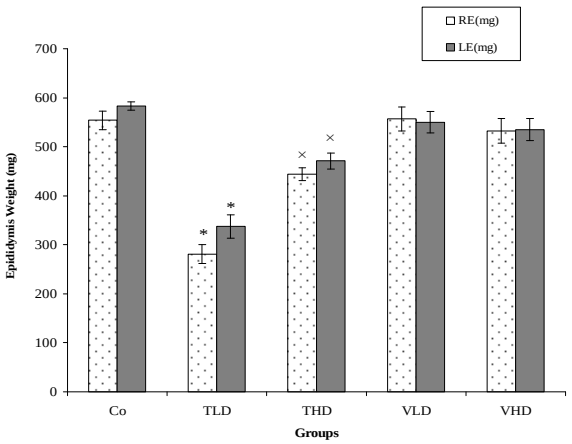


**Fig 3:** Mean±SEM weight of right (RE) and left (LE) epididymis (mg) in all rats. × and * show significant differences (p<0.05 and p<0.001, respectively) between low-dose (TLD) and high-dose treatment (THD) and other groups. CO: control group; VLD: low-dose vehicle; VHD: high-dose vehicle.

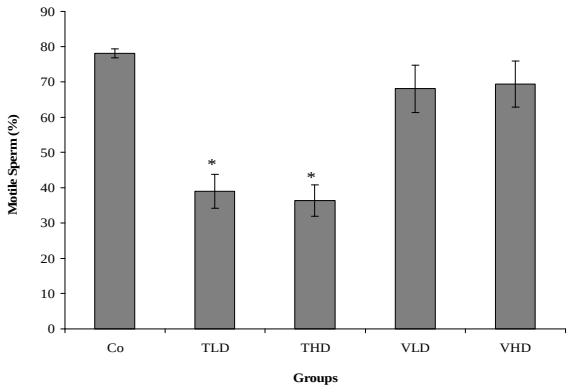


**Fig 4:** Asterisks show significant differences (p<0.001) between total sperm motility (%) of high-dose (THD) and low-dose treatment (TLD) rats and other groups. CO: control group; VLD: low-dose vehicle; VHD: high-dose vehicle.

Table 2 shows that the sperm count was lower in THD and TLD groups compared to other groups. Significant differences were observed among experimental, control and vehicle rats (p<0.001, fig. 5).

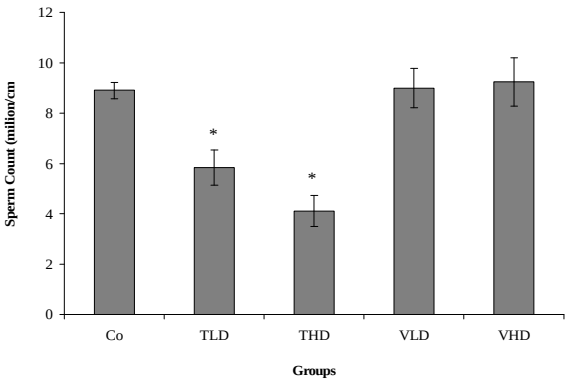


**Fig 5:** Sperm count in all rats. Asterisks show significant differences (p<0.001) between sperm count of high-dose (THD) and low-dose treatment (TLD) rats and other groups. CO: control group; VLD: low-dose vehicle; VHD: high-dose vehicle.

A higher prevalence of morphologically abnormal sperms was observed in both THD and TLD groups; it was significantly different from those of the control and vehicle groups (p<0.001, table 2, fig. 6).

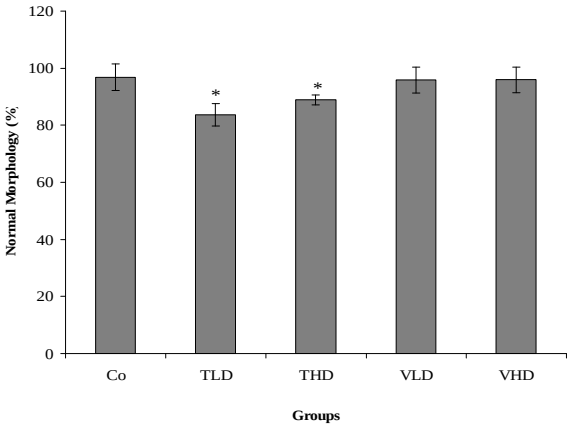


**Fig 6:** Sperm morphology. Asterisks show significant differences (p<0.001) between morphologically normal sperms of high-dose (THD) and low-dose treatment (TLD) rats and other groups. CO: control group; VLD: low-dose vehicle; VHD: high-dose vehicle.

## Discussion

Although, the body weight was progressively increased in all rats during the treatment period, the rate of body weight increment was lower in the treatment groups. Many researchers found that AASs have no effects on body weight of animals and human subjects.[4,5,18,20-24] There is an acceptable consequence that supraphysiological doses of AASs can inhibit body growth and weight gain.[25] Ann Sophie reported that the reduced body weight, observed in ND-treated rats, was probably the result of lowered food intake, but the physiologic mechanism was not investigated.[22] Based on Jonas' findings, the inhibitory effect of ND on body weight gain may be the result of alteration in the melanocortin system.[18]

On the other hand, there are numerous studies indicating increase in body weight in animals and human.[26-32] Bouhlel clearly showed that treatment with AASs can prevent atrophy and functional changes of rat skeletal muscles.[33] Some authors however, believe that the gain in the body weight is apparently due to the muscular exercise rather than an anabolic effect.[27,31] Others reported that increase in body weight may be attributed to the accumulation of fluids and sodium in body.[29,30] Few investigations showed that the body weight gain is indirectly a result of decreasing fatigue and increasing anxiety which may increase the exercise tolerance by skeletal muscles.[6,32] Presence of slower weight gain in our study was in agreement with Scott's findings,[34] (fig 1).

In this study, the weight of testis and epididymis of experimental rats were significantly lower than that in the control and vehicle groups, which is in agreement with other reports.[4,5,35] Also, McIntyre reported that the weight of testis was significantly lower in the pubertal male mice receiving the high dose (7.5 mg/kg/day) of AASs (17 α-MeT).[24] Schurmeyer showed that in human the mean±SEM volume of testicles decreased from 39±3 to 21±3 mL after treatment with 19-nortestosterone hexoxyphenlpropionate.[27]

It is clearly known that, gonadotropin releasing hormones from pituitary gland (FSH and LH) have growth promoting effects on testis development and that administration of exogenous androgens suppresses the serum LH and FSH level in human and rats.[6,13,35] The exogenous testosterone has negative feedback effects on hypothalamic-pituitary-gonadal axis.[10] Under normal condition, LH is regulated by GnRh which is released by hypothalamus. LH interacts with receptors on the Leydig cells to produce testosterone which is then transported to the testis and accessory reproductive organs for regulation of growth and maintenance of these tissues. Following administration of exogenous AASs, the high level of androgen causes a decrease in LH release from the pituitary gland, which in turn results in suppression of endogenous testosterone.[4,10,36] Consequently, for decreased level of testosterone, testicular atrophy occurrs.[10] Table 1 shows the mean±SEM weight of testis and epididymis of TLD rats which was lower than that of THD rats. It seems that the biphasic responses of reproductive tissue are responsible for this observation. Therefore, high dose of AASs has a direct protective effect on reproductive organs and prevents more atrophy of the testis.[37]

The fertility parameters (sperm count, motility and normal morphology) in experimental rats were significantly lower than those of other groups (table 2, figs 4-6). Torres also found that the sperm count and the percentage of sperms with normal morphology were significantly lower in 15 body-builders abused AASs; azoospermia was reported in three of them.[13] Sever oligospermia and azoospermia were also reported in those who were given supraphysiological doses of AASs.[27,38]

The percent of sperm with FP motility, which plays an important role in conception, was significantly less in THD (1.88 ±0.73) and TLD (2.02 ±0.43) rats. It is well-known that the sperm parameters play important role in male fertility.

It is clearly described that exogenous AASs affect hypothalamo-pituitary-gonadal axis and reduce serum LH and FSH level.[10] As a result, supporting role of sertoli cells on spermatogenesis is decreased, and under the influence of FSH, sertoli cells secret androgen binding protein (ABP) which has an important role in transport of testosterone to seminiferous tubule, which affects spermatogenesis.[36] Boyadjiev recommended that the only possible way for recovery of spermatogenesis is removal of exogenous testosterone.[39] Schurmeyer also reported that suppression of spermatogenesis is apparently caused by suppression of pituitary gonadotropine secretion.[27]

In conclusion, administration of low and high doses of AAS compounds has no effects on body weight gain, but decreases weight of testis and epididymis and sperm parameters in rats. Excess AASs cannot mimic the roles of endogenous gonadotropins. Therefore, administration of AASs affects the functions of gonadotropin and as a result, the weight of testis and accessory reproductive tissues as well as fertility parameters. All these findings indicate that there is a high degree of reproductive risk associated with use of AASs.

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