

Effects of single dose administered nandrolone decanoate on serum cytokine levels and some biochemical parameters in male and female rats.

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Abstract

The aim of this study was to determine the effects of single higher dose nandrolone decanoate (40 mg/kg) on serum IL-1 β , TNF- α , IL-10 and IL-6 levels and some biochemical parameters during 24 h in male and female rats. The blood samples were taken from 5 male and female rats to obtain their starting values. Then the blood samples were taken from 6 rats in sampling time at 1st, 2nd, 4th, 8th, 12th and 24th h following the nandrolone decanoate administration. There were significant increases determined in the serum IL-1 β levels, numerical increases in the TNF- α levels whereas decreased IL-6 levels were determined. IL-10 levels did not change during experimental period. While serum GGT and BUN levels increased towards the last h of the study, increased ALP levels were observed only in female rats. There were no significant changes determined in the other biochemical (ALT, AST, CK-MB and LDH) values. As a result, increased IL-1 β and TNF- α levels, proinflammatory cytokines, and some biochemical parameters may reflect that long term use of nandrolone decanoate causes organ damage and affects immune system function. However, effects of long term use of nandrolone decanoate on the immune system and organ functions should be detailedly evaluated.

Keywords: Nandrolone, Cytokines, Organ damage.

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Introduction

It is stated that use of doping has increased significantly in recent years, and it harms human health and at the same time, it also affects ethical values of sport negatively [1]. Doping refers to taking any impurity into the body illegally by athletes in order to gain an advantage over competitors in contests and competitions [2]. After the competitions, a science committee follows traces of various performance enhancers with blood and urine tests. This is very important in terms of protecting justice of the sport with athletes [3]. The fight against doping has become a political issue around the world for more than 60 years [4].

While AAS (Anabolic androgenic steroids) which are one of the synthetic derivatives of male sex hormones and testosterone [5-8] had been used for burns and traumas, radiation therapy and chronic weight loss diseases since the 1940s, they became even more important in treatments of AIDS related burnout syndrome and kidney diseases after 1985 [9].

It has been reported that anabolism takes part in enhancing protein synthesis to keep nitrogen in body mass [10], androgens take part in development of the male reproductive system [11].

Materials and Methods

Totally 35 male and 35 female rats were used. 5 male and 5 female rats were accepted as control time (0 hour), and rested. 30 male and 30 female rats were received at the dose of 40 mg/kg nandrolone decanoate (IP). After treatments, blood samples were collected at 1, 2, 4, 8, 12 and 24 hours from cardiac puncture under anesthesia. Serum ALP, ALT, AST, CK-MB, GGT, LDH and BUN levels were measured with auto-analyzer, while serum TNF- α , IL-1 β , IL-6 and IL-10 levels were determined with ELISA reader. Data were evaluated by ANOVA and Duncan test. $P < 0.05$ was accepted as statistically significant.

Results

Serum biochemical values of male and female rats are shown in Tables 1 and 2, respectively. Increased GGT and BUN levels were determined ($P < 0.05$) in male rats, whereas statistically significant fluctuations were determined ($P < 0.05$) in ALP, GGT, BUN and LDH levels in female rats.

Serum cytokine levels are presented in Tables 3 and 4, respectively. Increased IL-1 β levels were determined ($P < 0.05$) in male rats, whereas statistically significant fluctuations were

determined ($P < 0.05$) in IL-6 levels. Increased IL-1 β and decreased IL-6 levels were measured ($P < 0.05$) in female rats.

Table 1. Serum biochemical parameters of male rats after Nandrolon decanoate treatment (mean \pm SE).

Parameters	0 h	1 h	2 h	4 h	8 h	12 h	24 h
ALP, U/L	120.6 16.0	\pm 96.0 \pm 10.6	138.5 \pm 17.5	110.7 \pm 14.5	113.4 \pm 19.7	91.8 \pm 19.3	116.7 \pm 15.74
ALT, U/L	57.3 \pm 5.71	52.0 \pm 4.39	57.0 \pm 4.14	63.8 \pm 8.73	51.1 \pm 6.95	48.0 \pm 3.98	54.45 \pm 6.46
AST, U/L	95.1 \pm 16.5	100 \pm 14.5	120 \pm 19.7	154 \pm 26.1	104 \pm 13.30	94.0 \pm 11.9	112.3 \pm 14.4
CKMB, U/L	743 \pm 160	806 \pm 191	730 \pm 263	1421 \pm 474	674 \pm 217	779 \pm 251	952 \pm 234
GGT, U/L	1.06 0.16b	\pm 1.83 \pm 0.40ab	2.33 \pm 0.80ab	2.00 \pm 0.36ab	1.50 \pm 0.22ab	1.16 \pm 0.16b	2.84 \pm 0.66a
LDH, U/L	578 \pm 87.7	485 \pm 85.5	505 \pm 164	731 \pm 180	387 \pm 55.2	461 \pm 86.8	554 \pm 148
	48.0 1.54c	\pm 59.0 \pm 3.93abc	54.3 \pm 3.33abc	62.6 \pm 3.45ab	51.1 \pm 2.02bc	66.1 \pm 2.46a	64.4 \pm 4.6ab

a, b, c: Different letters along the same line are statistically significant (Duncan test, $p < 0.05$).

Table 2. Serum biochemical parameters of female rats after Nandrolon decanoate treatment (mean \pm SE).

Parameters	0 h	1 h	2 h	4 h	8 h	12 h	24 h
ALP, U/L	74.5 \pm 2.98 ^{ab}	68.6 \pm 4.75 ^b	69.3 \pm 4.87 ^{ab}	62.5 \pm 11.4 ^b	65.0 \pm 8.98 ^b	58.3 \pm 4.91 ^b	103 \pm 11.9 ^a
ALT, U/L	98.0 \pm 28.6	126 \pm 23.5	88.0 \pm 21.0	72.6 \pm 14.7	66.1 \pm 5.81	79.0 \pm 10.2	76.6 \pm 15.0
AST, U/L	152 \pm 24.4	205 \pm 44.2	159 \pm 28.3	122 \pm 30.2	117 \pm 15.5	154 \pm 18.6	165 \pm 30.1
CKMB, U/L	814 \pm 187	1223 \pm 575	843 \pm 132	538 \pm 49	778 \pm 228	707 \pm 198	1262 \pm 580
GGT, U/L	2.16 \pm 0.47 ^{ab}	1.50 \pm 0.22 ^{ab}	1.16 \pm 0.47 ^b	1.66 \pm 0.33 ^{ab}	1.24 \pm 0.22 ^b	1.33 \pm 0.21 ^b	3.66 \pm 0.98 ^a
LDH, U/L	891 \pm 101 ^a	774 \pm 154 ^{ab}	523 \pm 74 ^{ab}	287 \pm 43 ^b	458 \pm 142 ^{ab}	573 \pm 138 ^{ab}	783 \pm 182 ^{ab}
BUN mg/dl	59.8 \pm 12.7 ^{ab}	48.6 \pm 2.26 ^b	59.3 \pm 2.33 ^{ab}	55.0 \pm 3.84 ^{ab}	54.6 \pm 1.68 ^{ab}	78.6 \pm 4.33 ^a	53.8 \pm 1.88 ^b

a, b: Different letters along the same line are statistically significant (Duncan test, $p < 0.05$).

Table 3. Serum cytokine levels of male rats after Nandrolon decanoate treatment (mean \pm SE).

Parameters	0 h	1 h	2 h	4 h	8 h	12 h	24 h
TNF- α	4.27 \pm 0.76	4.54 \pm 1.20	4.74 \pm 1.34	4.40 \pm 0.96	5.41 \pm 1.74	6.08 \pm 1.86	5.76 \pm 1.44
IL-1 β	7.52 \pm 1.94 ^b	7.24 \pm 1.82 ^b	9.68 \pm 1.56 ^a	8.74 \pm 1.46 ^{ab}	10.12 \pm 2.94 ^a	9.85 \pm 1.44 ^a	9.72 \pm 1.52 ^a
IL-6	24.48 \pm 2.64 ^a	24.90 \pm 3.57 ^a	20.48 \pm 2.54 ^a	17.1 \pm 3.45 ^{ab}	22.34 \pm 5.60 ^a	14.84 \pm 3.86 ^b	18.42 \pm 2.78 ^{ab}
IL-10	6.84 \pm 1.08	6.76 \pm 1.14	7.80 \pm 1.06	8.08 \pm 2.14	7.96 \pm 3.08	8.48 \pm 2.17	8.12 \pm 2.64

a,b: Different letters along the same line are statistically significant (Duncan test, $p < 0.05$).

Table 4. Serum cytokine levels of female rats after Nandrolon decanoate treatment (mean \pm SE).

Parameters	0 h	1 h	2 h	4 h	8 h	12 h	24 h
TNF- α pg/mL	4.86 \pm 0.42	4.78 \pm 1.64	5.15 \pm 1.06	5.60 \pm 0.96	4.88 \pm 1.47	5.90 \pm 1.64	5.94 \pm 1.86
IL-1 β pg/mL	6.46 \pm 1.25 ^b	6.64 \pm 1.48 ^b	8.88 \pm 1.94 ^a	8.12 \pm 2.15 ^a	6.88 \pm 1.06 ^b	8.19 \pm 1.22 ^a	8.72 \pm 2.92 ^a
IL-6 pg/mL	20.12 \pm 3.85 ^a	19.46 \pm 2.98 ^a	18.42 \pm 2.08 ^a	16.54 \pm 1.13 ^a	17.55 \pm 3.72 ^a	16.57 \pm 2.92 ^{ab}	14.18 \pm 2.44 ^b
IL-10 pg/mL	6.78 \pm 1.20	6.66 \pm 2.16	7.84 \pm 2.26	6.98 \pm 2.14	7.89 \pm 2.85	7.92 \pm 3.02	8.06 \pm 2.54

a,b: Different letters along the same line are statistically significant (Duncan test, $p < 0.05$).

Discussion

Today, sudden cardiac death due to cardiovascular disease takes an important place in causes of death [12]. Because use of anti-androgenic drugs for treatment in medicine have increased rates of death due to cardiovascular function [13,14], this situation has revealed that there is a need for more in-depth investigation of the effects of androgenic hormones. It has been seen that application of testosterone above physiological doses decreases eNOS activity and increases oxidative stress [15]. As a matter of fact, in relation to the research done, it is reported that rate of death due to cardiovascular diseases has increased among ones misusing androgens and the reason of this might be due to endothelial dysfunction occurring [16].

Although it is reported that nandrolone decanoate increases $TNF\alpha$, IL-1 β and IL-4 production in cell cultures and suppresses IL-6 mRNA expression [17-19], in some studies, it has been determined that it has no effect on $TNF\alpha$ and IL-1 β mRNA expression and IL-2, IL-3 and IL-10 productions [18,20,21]. On the other hand, it has been reported that AAS inhibit activation of nuclear factor Kappa B (NF- κ B) [22] allowing expression of genes encoding proinflammatory cytokines [23].

The study done has shown that there is no statistical difference related to serum cytokine levels between male and female rats during first 24 h. After administration of ND, it has been determined in both genders that IL1- β levels increased after 2 h following administration ($p < 0.05$) and non-statistical numerical increase in $TNF-\alpha$ levels was observed. It has been seen that results obtained are compatible with findings of some researchers [17-19], they are not compatible with findings of Corrales et al. stating that there is a decrease in IL1- β and $TNF-\alpha$ levels in individuals receiving testosterone therapy. It has been interpreted that ND has much more androgenic effects than testosterone and this is the reason of using high dose of ND in the study and also 24-hour variation might vary. Also, Parrillo et al. and Dinarello have reported that IL1- β and $TNF-\alpha$ levels might be associated with multiple organ failure. In the light of this information, it has been thought that these increases observed in these two cytokines might be sign of results that may cause multiple organ failures.

In the study done, decreases in serum IL-6 levels have been observed in male and female rats during the h following ND administration and it has been observed that this decrease is compatible with some studies [17-19]. There has not been any change observed in IL-10 levels in male and female rats as compatible with findings of Thompson et al. and when results have been analyzed, it has been seen that although it doesn't make statistical sense, there is numerical increase as compatible with statements of Corrales et al.

In many studies in which effects of ASS on liver enzymes were researched, enzyme activities were examined and different results were obtained in terms of these activities [20-26]. In

some studies, any change has not been stated in AAS applications [27,28], but there has been some studies stating increases in AST and ALT activities [27,29-31].

In the study done, while hourly variation in any parameter has not been observed in male rats except GGT and BUN levels, this situation is also accompanied by ALP in females (Tables 4.1 and 4.2). However, when biochemical parameters are examined, there is numerical increase in especially AST and CK-MB levels in terms of h. Even if changes in biochemical parameters occurring in the first 24 h don't support increase in IL1-b and $TNF-\alpha$ levels completely, this has been thought as sign showing that ND applications will lead to various multiple organ failures particularly liver failure. As a matter of fact, although treatment was used in appropriate dose in a study in which ND was administered [32], with findings obtained at the end of 4 weeks, it was concluded that multiple organ failure might be encountered in rats.

Conclusion

As a result, it has been thought that these values are needed to be monitored longer than 24 h in order to understand changes in serum cytokine levels and biochemical parameters examined more clearly.

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