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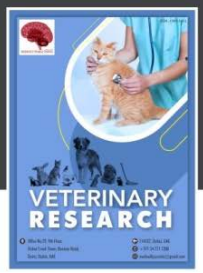

The Effect of Nandrolone and Testosterone on Cholesterol in Rabbits During Puberty

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Abstract: Puberty is a period in which dramatic changes occur in human life. This study was conducted to determine the effects of nandrolone and testosteron on some blood parameters in rabbits. In this study 60 days old, healthy, weighing nearly each other, 30 New Zealand rabbits were used. Animals were separated in 2 main groups as control (7♂; 8♀) and experimental (7♂; 8♀). The 10 mg kg^{-1} nandrolone and 10 mg kg^{-1} testosterone was injected subcutaneously to experimental group 1 day of per week. The application was continued for 90 days. In the plasma samples taken on the 45 and 90th days of the study, total cholesterol, HDL cholesterol and LDL cholesterol concentrations were determined. LDL cholesterol levels increased both in female and male experimental groups in each sampling time (45 and 90th days) and total cholesterol levels increased only 90th days ($p<0.05$). On 45th day, HDL cholesterol level was tending to decrease in female experimental group but this decrease was significantly ($p<0.05$) in male experimental group. Reduction in the level of HDL was also significantly in both experimental groups on 90th day ($p<0.05$). Consequently, the findings received from the study which resulted in the side effects of nandrolon+testosterone application regarding the parameters, seems important for that it reveals the effects of AAS in puberty.

INTRODUCTION

Throughout the sports history many young athletes have used "Anabolic Androgenic Steroid" (AAS) drugs intensively as doping and this emerges as a major issue. Research studies have indicated that effects of the anabolic androgenic steroids abuse on many systems differ with respect to the dose and type of the steroid used by athletes (Samieinasab *et al*., 2015).

AAS drugs are synthetic ester or alkylated derivatives of the male hormone testosterone (Marshall-Grandisnik *et al*., 2009). More than 100 different AAS drugs have been developed: oxymethelone, oxandrolone, methandrostenolone, stanozolol as most commonly used ones for oral administration and nandrolone decanoat, nandrolone phenpropionate, testosteron cypionate, testosterone propionate and boldenone undecylenate as most commonly used ones for parenteral administration (Vardar *et al*., 2002; Evans,

2004). While, these drugs possess both anabolic and androgenic effects their anabolic and androgenic efficacy ratios are different (Evans, 2004). Nandrolone is one of the most widely used AAS (Maravelias *et al*., 2005). AAS drugs are currently used to enhance exercise tolerance and sporting performance (doping effect) or to improve physical appearance for professional and cosmetic purposes in addition to their medical indications. Most of these drugs are marketed as unprescribed nutritional supplements in sports markets (Vardar *et al*., 2002; Sahin *et al*., 2006; Min and Lee, 2018).

Research studies on anabolic-androgenic steroids have shown that the use of AAS is similarly increasing among adult athletes and non-athletes (Min and Lee, 2018). However, these drugs also bring some physical and psychiatric adverse effects while they improve muscle growth and physical performance (Parssinen and Seppala, 2002).

In addition, adverse effects including hypertension, atherosclerosis, blood clotting, jaundice, hepatic neoplasms, carcinoma, tendon damage and behavioral disorders have also been reported (Maravelias *et al*., 2005). It has also been stated that AAS lead short-term side effects such as liver function disorder, atherogenic blood-fat profile, gonadal dysfunction as well as dermatological changes (Min and Lee, 2018). Drugs such as testosterone, nandrolone and stanozolol which are commonly used by athletes, possess several hepatic, cardiovascular, laryngeal, endocrin, dermatological, hematological, immunological, urinary and psychological adverse effects on reproductive system in men and women (Maravelias *et al*., 2005).

Despite, the positive effects of AAS in improving physical performance, many studies have reported that the use of high dose AAS raises the risk of cardiovascular diseases as it increases levels of total cholesterol, low density lipoprotein, blood pressure, thrombosis, myocardial infarct and heart failure and decreases levels of high density lipoprotein (Chaves *et al*., 2006).

In this study it was aimed to determine total cholesterol, HDL cholesterol and LDL cholesterol concentrations in “nandrolone and testosteron” treated rabbits during puberty.

MATERIALS AND METHODS

Experimental design: In the study, a total of 30 New Zealand breed rabbits, 14 male and 16 female were obtained from Selcuk University Experimental Medicine Research and Application Center were used. Animals were divided into two groups as control (7♂; 8 () and trial (7♂; 8 () after health checks. The rabbits were kept in standard cages during the trial. The research project was approved by the ethics committee of the Faculty of Veterinary Medicine of Selcuk University (2009/78).

Table 1: Composition of the feed

Feed materials	Percentage
Barley	20.00
Egypt	48.80
Wheat bran	10.00
Soy	10.50
Fish meal	2.00
Meat bone meal	4.00
Salt	0.90
D. fosfat	0.60
Limestone	2.80
Methionine	0.20
Vitamin and mineral mix *	0.20

*Ca: 1.5%; P; 0.8%, Na: 0.35%; Mn: 8 mg kg^{-1}; Zn: 50 mg kg^{-1}; A vit: 8000 IU kg^{-1}; E Vit: 10 mg kg^{-1}; K vit: 1 mg kg^{-1}; D vit: 800 IU kg^{-1}; B2 vit: 3 mg kg^{-1}; B12 vit: 5 mcg kg^{-1}

The animals were fed with ad libitum with standard rabbit feed which is presented in Table 1 and clean water was kept in front of them.

The rabbits in the control group did not receive any treatment. Each animal in the experimental group was given 10 mg kg^{-1} nandrolone (Nandrolone deconate, Deca Durabolin-Organon, 100 mg mL^{-1}) and 10 mg kg^{-1} testosterone (Testosterone propionate-Sustanon 250 mg mL^{-1}) subcutaneously for 90 days.

Blood analysis: On the 45 and 90th day of the study, blood samples taken from the ear veins of anticoagulant (sodium citrate) tubes were centrifuged. The resulting plasmids were stored at -80°C until the analysis. In plasma samples, total cholesterol, HDL and LDL levels were determined in the autoanalyzer (ILAB 300 Plus, Italy) using commercial kit (IL Test™).

Statistical analysis: It the end of the research, the arithmetic mean and standard errors of the parameters of all groups and the importance of differences between the groups were determined by using Duncan, in-group differences using the Indipendent-test and SPSS 10.0 Package Program (SPSS 16.0).

RESULTS AND DISCUSSION

Results obtained from all groups were given in Table 2. Plasma cholesterol, HDL and LDL levels emerged in this study were found to be within or close to the reference limits suggested for rabbits (Ammar *et al*., 2004; Aydin, 2009; Donmez and Keskin, 2008). Plasma cholesterol level decreased in all groups on the 90th day comparing to the values on the 45th day, however, this reduction was not statistically significant.

Plasma cholesterol levels increased in male and female experimental groups at both sampling times in comparison to their control groups and the increase was significant (except for the 45th day in females) ($p<0.05$). Plasma cholesterol levels found at both sampling times

Table 2: Plasma cholesterol, HDL and LDL levels determined in male and female rabbits in control and experimental groups (X±SX)

		Cholesterol (mg dL^{-1})		HDL (mg dL^{-1})		LDL (mg dL^{-1})	
Groups	n	45. gun	90. gun	45. gun	90. gun	45. gun	90. gun
Female control	8	57.62 ± 1.91^{B}	56.62 ± 1.48^{C}	25.12 ± 1.48^{AB}	26.12 ± 0.74^{A}	28.87 ± 0.83^{Cb}	31.37 ± 1.48^{Ca}
Female trial	8	62.87 ± 1.43^{AB}	61.50 ± 1.63^{AB}	22.37 ± 0.80^{ABa}	17.25 ± 1.42^{Bb}	33.87 ± 0.95^{ABb}	36.87 ± 0.71^{Ba}
Male control	7	59.25 ± 1.97^{B}	57.12 ± 1.32^{BC}	26.87 ± 2.81^{A}	25.50 ± 2.09^{A}	31.00 ± 1.71^{BC}	31.50 ± 2.00^{C}
Male trial	7	65.25 ± 1.91^{A}	63.75 ± 1.67^{A}	19.37 ± 1.93^{Ba}	13.00 ± 0.92^{Cb}	36.25 ± 1.67^{Ab}	44.62 ± 2.48^{Aa}

[a,b]The difference between the average values indicated by the different letters of the same parameter on the same line is important ($p<0.05$); [A-C]The difference between the mean values in the same column between different groups is significant ($p<0.05$)

were higher in the male control group than those of the female control group and again in the male experimental group than those of the female experimental group without suggesting any significance.

The findings suggested that there were no differences in plasma HDL levels between the control groups of both genders depending on sampling times. On the other hand in both experimental groups, plasma HDL levels were observed to be decreasing on the 90th day ($p<0.05$). Plasma HDL levels were lower in both experimental groups at both sampling time compared to their control groups and the decrease was significant ($p<0.05$) (except for the 45th day sampling in females). In addition, the plasma HDL values recorded in both male and female control groups were at similar levels at the time of sampling but the values obtained in male experimental groups were lower than female experimental groups especially at the time of sampling on the 90th day by revealing significance ($p<0.05$).

According to the results, plasma LDL levels were higher in all research groups on day 90 than the levels of day 45 and there was a significant difference between the female experimental and control groups ($p<0.05$). Plasma LDL levels also increased significantly at both sampling periods and in both experimental groups compared to the control groups ($p<0.05$). The findings suggested to significance between male and female control groups in terms of plasma LDL levels obtained on days 45 and 90. Still at both sampling times, plasma LDL levels of male experimental group were higher than the female experimental group and the difference was significant at the sampling on the 90th day ($p<0.05$).

The first issue to be emphasized in the study was decreasing plasma HDL and increasing plasma LDL levels in testosterone+nandrolone applied male and female experimental groups compared to the control groups levels. This finding is similar to the previous assertions which suggest that AASs increase hepatic lipase activity and in that way may cause an increase in blood LDL levels and a decrease in HDL levels. It is further similar to the argument that AAS medications are effective on lipoprotein metabolism, lowering LDL levels in blood and lowering HDL levels (Ammar *et al*., 2004; Kayaalp, 2005; Ozdemir and Gulturk 2008; Perret *et al*., 2002).

Similar to the results found in this study, many other studies suggested that especially testosterone and HDL are negatively correlated (Gul, 2008; Kaushik *et al*., 2009; Urhausen *et al*., 2003). It is argued that long-term testosterone therapy reduces HDL levels in hypogonadal men (Kamischke *et al*., 2002). Additionally, it is thought that in men with pre-andropause period, testosterone negatively affects the lipid profile, resulting in an increase in total cholesterol and LDL levels and a decrease in HDL levels (Gul, 2008). In a study investigating the relationship between sex hormones with lipid anomalies (Kaushik *et al*., 2009) it was observed that there was a correlation between testosterone and high LDL and low HDL levels in men.

However, in a study to determine the risk profile of long-term use and misuse of AAS drugs (Urhausen *et al*., 2003), no significant change was observed in total cholesterol and LDL cholesterol levels while HDL cholesterol levels were significantly lower.

In recent clinical studies, it is stated that AAS drugs are safer in pharmacological and sup pharmacological doses when they are used for short period of time. It has been suggested that administration of AAS drugs up to 20 weeks does not show any toxicity except for a few laboratory abnormalities (decreased serum HDL value, increased hemoglobin and increased liver enzymes) (Bhasin *et al*., 2001; Ozdemir and Gulturk, 2008). In fact, Hartgens *et al*. (2004) reported that only HDL was low at the end of the 6.5 week AAS drug application and other parameters were not affected. In a study conducted by Urhausen *et al*. (2003) on older AAS drug users, HDL cholesterol levels were reported to be normal again within a year after discontinuation of AAS. In another study in which bodybuilding and weightlifting athletes were used as subjects in order to reveal the effects of long-term abuses of AAS drugs on blood cells, fats, liver functions and hormones (Urhausen *et al*., 2003). While, low cholesterol and LDL cholesterol levels were found to be similar in both groups.

The second point to be emphasized in the study was that the plasma HDL level was high in the female experimental group and the cholesterol and LDL levels were low in the female experimental group at both sampling times. This difference in the experimental groups may be because of the fact that the change of

nandrolone to estrogen is more than testosterone. Indeed, by suppressing the hepatic lipase activity of estrogens it is reported that HDL level increases and LDL level decreases (Ammar *et al*., 2004; Estrogens, 2011).

CONCLUSION

It was concluded that the findings of the study showed that AAS drugs had negative effects on some lipid parameters such as plasma HDL and LDL cholesterol in male and female rabbits and thus it was found to be beneficial in terms of revealing the risks to cardiovascular system in athletes who are AAS users. The ethical issue, care and handling of animals followed the internationally accepted procedures according to, the Institute for Laboratory Animal Research's Guide for the Care and Use of Laboratory Animals.

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