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ORIGINAL ARTICLE **OPEN ACCESS**

The Effects of Nandrolone and Testosterone Application on Calcium and Parathormone Levels in Rabbits

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ABSTRACT
The study aims to research the effects of nandrolone and testosterone, which are AAS and being used as doping agents as well, on plasma calcium, calcitonin and parathormone levels in rabbits. Total 60-days-old 30 New Zealand race rabbits, 14 male, 16 female, were used as material in the study. Within the group of rabbits, separated into two groups as control (7 males, 8 females) and experimental (7 males, 8 females); 10 mg/kg nandrolone (nandrolone deconate) and 10 mg/kg testosterone (testosterone propionate) were injected to each animal generating the experimental group as subcutaneous for a day in a week for 90 days. From blood samples taken during 45th and 90th days from the animals forming both of the groups,plasma calcium, calcitonin and parathormone levels were determined. In the study, nandrolone (nandrolone deconate) and testosterone (testosterone propionate) has been ascertained to have a significant effect (P<0.05) on plasma calcium and PTH levels for both rabbit genders and to be able to change parameters regarding bone metabolism.
Keywords: Nandrolone, Testosterone, Calcium, Parathormone

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INTRODUCTION

Anabolic-androgenic steroids (AAS) are the similar synthetic derivatives of testosterone [1,2], and they are commonly used today in order to increase tolerance against competitive and power requiring performance and exercise apart from the medical indications [3] or to enhance physical appearance with cosmetic purposes [1,4]. Testosterone is an endogenous hormone having anabolic and androgenic steroidal activity [5]. Its androgenic effects are to increase skeletal muscle mass, to ensure the development of bones with genitals and secondary sex characteristics; its anabolic effects can be summarized as: to cause calcium, phosphate, sodium, potassium, chlorine, and water retention, to increase the mass of back and muscle tissue by stimulating protein synthesis, and to increase mineral content of the bones by blocking osteoclast activity [6,7].

Calcium is the most abundant inorganic substance in the human body. Total body calcium is about 1000-1200 gr. More 90% of this is present in bones and teeth; approximately 10% is present in extracellular fluids, soft tissues, and different membrane structures. Most of the calcium in the bones is stored in the form of hydroxyapatite. 50% of plasma calcium is present in the ionized form, 40% is bound to protein, and 10% is in the form of complexes formed with anions such as phosphate, citrate, and bicarbonate [5,8,9,10].

Calcitonin (CT) is a polypeptide hormone secreted significantly by thyroid C cells (clear cell). Calcitonin or similarly effective hormone is produced in the body aside from thyroid gland; calcitonin is reported to be present in cerebrospinal fluid, pituitary gland, thymus, liver, lung, colon, and bladder. The basic metabolic effect of the hormone is keep the increased calcium and phosphate level in the plasma within the normal range by reducing their levels [11,12,13].

It is the physiological antagonist of parathyroid hormone (PTH) [14,15,16]. Parathyroid hormone (PTH) is a single chain peptide hormone secreted by the parathyroid glands located on the back of the thyroid gland. Its main task is to keep the reduced amount of ionized calcium in the blood plasma within the physiological range by increasing its level [13,17,18].

Aim of this study is to investigate the effects of nandrolone (nandrolone deconate) which is an anabolic-androgenic the steroid (AAS) and widely used as a doping agent and the effects of testosterone (testosterone propionate) on plasma calcium, CT and PTH levels of the rabbits.

MATERIALS AND METHODS

Experimental design and laboratory animals

30 New Zealand rabbits composed of 60 days old 14 males and 16 females were used in the study. The animals were obtained from Selcuk University Experimental Medicine Research and Application Center. Rabbits classified as control (7 males, 8 females) and experimental (7 males, 8 females) were kept in standard cages throughout the experiment, and they were fed ad libitum with commercial rabbit feed. 10 mg/kg Nandrolone deconate (100 mg/ml) and 10 mg/kg Testosterone propionate (250 mg/ml) were injected subcutaneously to each animal in the experimental group once a week (at the same day of the week) during 90 days.

Blood analyzes

Blood was collected into anticoagulant (sodium citrate) tubes from the ears of the animals of both groups on day 45 and 90. Plasma obtained after centrifugation of collected blood samples was stored at -80 °C freezer until the analysis. Calcium levels in the blood plasma samples were detected spectrophotometrically (Siemens Dimension device) with "Siemens Bayer" commercial kit, and calcitonin and parathyroid hormone levels were detected with radioimmunoassay method by using "Siemens Immulite 2000" commercial kit (Siemens Immulite 2000).

Statistical analysis

Variance analysis (ANOVA) was used in the control of significance of differences among groups relating to the parameters obtained in this study results, and Duncan test was utilized. Paired t-test was used in the detection of differences within the group.

RESULTS AND DISCUSSION

Obtained results belonging to plasma calcium, CT and PTH levels determined in all groups are given in table 1 and 2.

Table 1. Plasma calcium, CT and PTH levels detected on day 45 in female and male rabbits of control and experimental groups (X ± SEM).

PARAMETERS	Calcium (mg/dl)	CT (pg/ml)	PTH (pg/ml)
(Group = n)	(45th day)	(45th day)	(45th day)
Female control = 8	13.95 ± 0.20 B	41.78 ± 0.62 B	11.96 ± 0.25 AB
Female experimental = 8	14.80 ± 0.22 AB b	55.19 ± 3.13 A	8.23 ± 1,11 B a
Male control = 7	14.78 ± 0.43 AB	47.26 ± 0.39 AB	13.78 ± 0.64 A
Male experimental = 7	15.28 ± 0.53 A b	50.28 ± 3.05 AB	8.60 ± 0,31 B a

A,B: Difference between intergroup mean values indicated by different letters in the same column is significant ($P<0.05$) , a,b: Difference between mean values indicated by different letters in the same row and belonging to the same parameter is significant ($P<0.05$).

Table 2. Plasma calcium, CT and PTH levels detected on day 90 in female and male rabbits of control and experimental groups (X ± SEM).

PARAMETERS	Calcium (mg/dl)	CT (pg/ml)	PTH (pg/ml)
(Group = n)	(90th day)	(90th day)	(90th day)
Female control = 8	14.44 ± 0.54 B	44.73 ± 0.89 B	11.95 ± 0.35 A
Female experimental = 8	16.41 ± 0.51 A a	57.79 ± 4.61 A	5.37 ± 0.73 B b
Male control = 7	14.71 ± 0.64 B	48.52 ± 1.03 AB	12.60 ± 0.47 A
Male experimental = 7	16.94 ± 0.65 A a	52.20 ± 5.60 AB	5.94 ±0.27 B b

A,B: Difference between intergroup mean values indicated by different letters in the same column is significant ($P<0.05$) , a,b: Difference between mean values indicated by different letters in the same row and belonging to the same parameter is significant ($P<0.05$).

Primary target tissues for the anabolic effects of AAS are skeletal muscles and bones, it was reported that there is a positive relationship between muscle mass and bone mass [19,20] It is stated that they cause an increase in muscle mass and tension, and an increase in muscle strength and growth by creating a positive nitrogen balance especially during puberty [2,11]. It is also recorded that it can provide muscle development in adult males in the case of regular physical exercise [7]; it shortens the healing process by increasing the protein synthesis after muscle injury [21].

It is reported that anabolic steroids increase length, thickness, and durability of bone by providing deposition of calcium in bones, and they provide narrower and longer pelvis structure in males with the fusion (epiphyseal closure) of the articular cartilage in puberty [22,23,24,25]. AAS can lead to an increase in body weight in addition to anabolic effects since they increase sodium, potassium, calcium, chlorine, and phosphate retention and water reabsorption [5]. It is also recorded that AAS increase calcium absorption from the distal tubules by increasing renal sensitivity against PTH [18].

Besides all these literature reports, it is also recorded that AAS is having psychological, physiological, morphological, morphometric and pathological changes and some adverse effects on experimental animals adversely affects many systems in athletes (Cardiovascular, hepatic, dermatological, endocrine and reproductive) when used in an uncontrolled way or unconsciously [1,3,26,27,28]**.** Use of AAS at high dose and for a long time, particularly in prepubertal age, causes stop in growth as a result of the early closure of the epiphyseal plate in long bones and abnormal growth and development disorders resulting in the premature sexual development [29,30,31].

Other important factors that influence bone metabolism in addition to anabolic steroids are calcium, PTH, CT, and D-hormone. These factors arrange calcium levels by changing serum calcium levels, bone resorption and formation, intestinal calcium absorption and excretion from the kidney [14,32].

While plasma calcium levels determined in control groups belonging to both genders and both sampling times in the study were close to reference values for the rabbits reported by some researchers [33,34,35], they were slightly higher than the values determined in some studies [36,37,38]. Differences in the reports belonging to plasma calcium levels can be caused by the factors such as age, gender, race, diet of the animals and differences in methods.

It was observed that plasma calcium levels of all groups determined on day 90 increased compared to day 45, and this increase was significant in the experimental group ($P<0.05$). Plasma calcium levels of both experimental groups were higher than both control groups on both day 45 and 90, and the differences on day 90 were found to be statistically significant ($P<0.05$).

Despite the reports in which AAS was found to provide calcium deposition in bones, to increase bone length, thickness and density [22,23,24,25], to repress cytokines reported to be effective on bone destruction [32,39,40,41,42], to stimulate osteoblastic cells via androgenic receptors [18], and to bloke the function of osteoclasts [30], plasma calcium levels of experimental groups in the study were found to be significant. The reason of a significant increase in plasma calcium levels in AAS applied experimental groups can be attributed to increasing effect on renal sensitivity and therefore calcium reabsorption by providing an increase in steroids possibly kidney PTH receptors [7,18,43]. In addition, increasing intestinal calcium absorption by enhancing D-hormone synthesis of androgens may also have contributed to this as a minor factor [5,14,15].

Although this increase detected in plasma calcium levels in experimental groups of the study was similar to the results obtained in the study in which the effects of nandrolone in the treatment of osteoporosis and bone mineralization in New Zealand rabbits by Aithal et al. [38], it does not support the reports in which increase in serum calcium level was not significant in nandrolone applied group of the study conducted on rats by Saranteas et al. [44], and long-term application of testosterone to the rats by Lok et al. [45] caused significant decrease in plasma calcium levels.

It is reported that blood concentration increased on 4th and 6th month in wistar rats applied with testosterone propionate and methenolone enanthate for humeral osteotomy treatment [46].

In a study of male lambs, it was recorded that there were no differences in calcium values between control and nandrolone groups on 4th, 6th and 10th weeks, calcium values were higher in nandrolone group compared to control group on the 12th week [47].

Increase in serum calcium level was reported to be increased as a result of testosterone propionate application in castrated heifers [48].

Need et al. reported that there was an increase in mineral density of the forearm and a significant increase in plasma calcium level due to increase in calcium reabsorption in renal tubules after the nandrolone application in osteoporotic, postmenopausal women [49,50]. Again, Chesnut et al. [51] reported 32% decrease in urinary calcium excretion as a result of the anabolic steroid (stanozolol) application during 8-32 months in 23 women with postmenopausal osteoporosis. In a study conducted with joint patients, it is reported that the bone mineral density and radiocalcium absorption in the intestine are increased as a result of 50 mg nandrolone decanoate once in 3 weeks during 2 years in 10 women [52].

Within the concept of the above literature, it was concluded that the effects of AAS on blood calcium level can vary depending on living species, applied steroid type, especially application period of steroid and dosage.

In the studies conducted on different cell types of human and rats, it is noted that norandrostenedione with testosterone and nandrolone also increase intracellular calcium level [53,54,55,56].
Although there was no gender-related statistically significant difference between male and female control and male and female experimental groups for both sampling times in terms of plasma calcium level in the study, plasma calcium level detected in male groups was slightly higher than females generally at the same sampling times.
It is reported that the mechanism controlling CT secretion was not fully understood [10], the physiological importance was contradictive [5] and its duration of action was short; however increase in plasma calcium levels up to approximately 10% increased the CT secretion 3-6 folds in less than one hour. It is recorded that this effect of CT provides the quick storage of calcium in bones of especially young living beings [8]. It was observed that plasma CT levels determined in all groups on day 45 were slightly higher compared to day 45 although it was not different.
It is reported that plasma CT levels in males are significantly higher compared to females, a decrease in estrogen level as a result of menopause or ovariectomy in females causes a decrease in CT level [5,57]. However, in a study of Heath III and Sizemore [58], they found plasma level as 49 pg/ml in males and 31 pg/ml in females, while Lambert et al. [59] found this parameter level as 48 pg/ml in males and 31 pg/ml in females.
It is reported that CT levels fluctuate between 5-100 pg/ml in humans [5]. Serum CT level of racehorses is recorded as 20.0 pg/ml [60], and plasma CT level of laying hens is recorded between 550-3240 pg/ml [61].
While plasma Ct levels in the study was found to be high in experimental groups of the same gender compared to male and female control group in both sampling times (day 45 and day 90), the difference between female control group and the female experimental group was significant for both sampling times ($P<0.05$). Although CT level of the male control group was higher than female control group without statistical difference in both sampling times, CT level of the female experimental group was slightly higher than the male experimental group.
Obtained results related with plasma Ct levels in the study failed for adequately discussion since we have not seen the original studies about this subject on rabbit or other laboratory animals in the literature.
In the study, it was determined that PTH level decreased on day 90 compared to day 45, and this decrease was significant in both experimental groups ($P<0.05$). The reason of decrease in plasma PTH levels in experimental groups was attributed to activation of phospholipase-C and inhibition of adenylate cyclase with more binding of calcium to parathyroid gland membrane receptors depending on significant increase in plasma ionized calcium levels on day 90, therefore decrease in intracellular cAMP amount and hormone synthesis, also inhibition of PTH secretion with negative feedback mechanism between D-hormone and parathyroid gland.
In the study, it was determined that plasma PTH levels decreased in both sampling times (day 45 and day 90) and in both experimental groups compared to control groups, and this decrease (except day 45 sampling in females) was significant ($P<0.05$). Plasma PTH levels determined at both sampling times were higher in the male control group compared to female control group, and higher in male experimental group than the female experimental group without significance.
Aithal et al. [39] reported that plasma PTH level in new Zealand rabbits was 20.443 pg/ml on day 1, 24.869 pg/ml on day 15, 16.759 pg/ml on day 30, 17.755 pg/ml on day 60 in control group, it was 19.984 pg/ml on day 1, 19.086 pg/ml on day 15, 18.531 pg/ml on day 30, 16.133 pg/ml on day 60 in nandrolone applied group, and stated that parathyroid hormone level was lower in nandrolone applied experimental group compared to control group on day 15 and 60, as a result nandrolone increased bone mineralization in rabbits. These statements of Aithal et al. [39] show similarity with the findings of the study.
In another study in which physiological effects of AAS drug use on some hormonal parameters and kidney function in male guinea pigs were investigated [62], it was reported that PTH level was determined as 2.95 pg/ml in control group, 2.87 pm/mg in 15 mg/kg steroid injected group for 6 weeks, and there was no significant difference between the groups. Difference between the results obtained in the study by Bisher [62] and the results of our study may be caused by the differences such as material, trial period, sampling time and diet.
Although there was no gender-based statistical differences between male and female control groups, and male and female experimental groups for both sampling times in terms of PTH level in the study, plasma PTH level determined in male groups was slightly higher than the females generally at the same sampling times.
As a result, it was concluded that there was significant importance of nandrolone (nandrolone deconat), and testosterone (testosterone propionate) both of which are AAS on plasma calcium and PTH levels for both genders of the rabbits ($P<0.05$); however, there is a need for larger scale studies including the

measurements of bone forming parameters such as serum alkaline phosphatase, osteocalcin, type 1 collagen propeptide levels besides the measurement of bone mineral density, and the measurements of bone resorption parameters such as serum acid phosphatase, type 1 collagen N and C-telopeptide crosslinks, urinary hydroxyproline, pyridinoline, deoxypyridinoline collagen N and C-telopeptide crosslink levels.

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