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RESEARCH ARTICLE

Effects of Testosterone Undecanoate Treatment on Serum Biochemical Parameters in New Zealand Rabbits

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Yeni Zelanda Tavşanlarında Testosteron Undecanoate Uygulamasının Serum Biyokimyasal Parametrelere Etkileri

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Öz

Amaç: Çeşitli hastalıkları tedavi etmek için kullanılan anabolik androjenik steroidler (AAS) aynı zamanda sporcuların sportif performansın iyileştirilmesi amacıyla kullandıkları doping maddeleridir. Bu çalışmanın amacı, uzun süreli Testosteron Undecanoate (TU) uygulanan Yeni Zelanda tavşanlarında bazı serum biyokimyasal parametrelere etkilerini değerlendirmektir.

Gereç ve Yöntem: Sunulan çalışmada 5-6 haftalık 21 adet erkek Yeni Zelanda tavşanı, Kontrol, Hint yağı ve Hint yağında çözündürülen TU grubu olmak üzere 3 gruba ayrıldı. Hint yağı ve Hint yağında çözündürülmüş TU (10 mg/kg) haftada 5 gün olmak üzere 6 hafta boyunca 0.2 ml olarak ilgili gruplara kas içi olarak uygulandı.

Bulgular: Toplam 22 biyokimyasal parametrenin değerlendirildiği çalışmanın sonuçları, aspartat aminotransferaz, amilaz, trigliserit, kolesterol, total protein, kreatinin, ürik asit, fosfat, potasyum, sodyum, kalsiyum ve glukoz düzeylerinin TU grubunda kontrol ve hint yağı gruplarına göre istatistiksel olarak anlamlı derecede yüksek olduğunu ve yüksek yoğunluklu lipoprotein düzeylerinin diğer gruplarda kontrol grubuna göre anlamlı derecede düşük olduğunu göstermiştir.

Öneri: Sonuç olarak Yeni Zelanda tavşanlarında TU uygulamasının serum biyokimyasını olumsuz yönde etkileyebileceği ve farklı testosteron analogları ile multidisipliner çalışmaların yapılması gerektiği ifade edilebilir.

Anahtar kelimeler: Serum, Tavşan, Testosteron undekanoat

Abstract

Aim: Anabolic androgenic steroids (AAS), used to treat various diseases, are also doping substances for athletes to improve their sportive performance. The aim of this study is to evaluate some serum biochemical parameters in New Zealand rabbits treated with long-term Testosterone Undecanoate (TU).

Materials and Methods: In the present study, 21 male New Zealand rabbits aged 5-6 weeks were divided into 3 groups; control, castor oil group, and TU group (in which TU was diluted in castor oil). In the Castor oil and TU diluted in castor oil (10 mg/kg) were injected intramuscularly in 0.1 ml for 6 weeks, 5 days a week in the related groups.

Results: : The results of the study, in which a total of 22 biochemical parameters were evaluated, showed that aspartate aminotransferase, amylase, triglyceride, total cholesterol, total protein, creatinine, uric acid, phosphate, potassium, sodium, calcium, and glucose levels were statistically significantly higher in the TU group than in the control and castor oil groups, and high-density lipoprotein levels were significantly lower in the other groups compared to the control group.

Conclusion: In conclusion, it can be stated that TU administration may adversely affect serum biochemistry in New Zealand rabbits and multidisciplinary studies with different testosterone analogues should be performed.

Keywords: Serum, Rabbit, Testosterone undecanoate

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Introduction

Testosterone a steroid hormone responsible for the development of reproductive function in males was isolated and characterised in 1935, and various derivatives were synthesised (David et al 1935, de Souza and Hallak 2011, Walker and Cooke 2023). Testosterone and its derivatives, known as anabolic steroids originate from cholesterol (de Souza and Hallak 2011, Craig et al 2023). Nandrolone, decanote, testosterone, stanozolol, methandienone, fluoxymesterone, oxymetholone, methyltestosterone and methenolone are the most commonly used androgens (Fink 2018, Sessa 2018, Albano 2021). Anabolic androgenic steroids (AAS) are essentially synthetic derivatives of testosterone. Its examples include Testosterone enanthate (TE), Testosterone cypionate, and Testosterone Undecanoate (TU) (de Souza and Hallak 2011). Testosterone is esterified in order to prolong its half-life and dissolved using oils such as castor oil and tea seed oil for sustained release into the bloodstream. Different testosterone esters differ in terms of absorption kinetics. TU, among the testosterone esters, has an 11-carbon side chain, while TE has a 7-carbon side chain. Absorption time increases with longer side chains that are not esterified. Additionally, the viscosity of the oil used as a solvent and volume and site of injection may affect the absorption kinetics (Bond 2022, Figueiredo et al 2022). TU is a semi-synthetic androgen with a molecular weight of 456.7 that forms by esterification of natural testosterone with undecanoic acid at the 17-β position (Edelstein and Basaria 2010). Although the acid length used for esterification affects the duration of the anabolic effect, derivatives containing the 17β- group can be administered parenterally (Báthori et al 2008). The results of the pharmacokinetic analysis indicate that TU has a longer half-life when used in castor oil than that when used in tea seed oil (Behre et al 1999) ous side effects appeared in patients (Nieschlag et al 1999). Furthermore, TU is a preparation used in the treatment of male hypogonadism (Meriggiola et al 2005, Gu et al 2009, Zhang et al 2016). Depending on the AAS used, general side effects include testicular atrophy (Király et al 1987), hypertension (Khalid et al 2002) gastrointestinal disorders, acne, liver disorders, hypogonadotropic, hypogonadism, infertility, gynecomastia, head and stomach pain, premature hair loss, insulin resistance, salt and water retention, early myocardial infarction, atherosclerosis, thromboembolism, hypertension and changes in blood cholesterol (Rashid et al 2007, Badawy 2018, Liu 2019, Çınaroğlu 2023). It has also been reported that the severity of side effects may vary depending on factors such as dose, duration of use, individual response, sensitivity, and gender (Yavari 2009).

Although they are used to treat different diseases, AAS are molecules that are used to improve sportive performance, especially in athletes, but their use is prohibited due to various fatal side effects. Rabbit, rat, mouse are used as experimental animals for ASS applications. It is among the information that the effects of the ASSs used are affected by many factors such as dose, duration and gender.

The aim of this bresearch was to determine of the potential harmful effects of TU on enzyme activities and some biochemical values in New Zealand rabbits.

Material and Methods

The study was carried out on 21 male New Zealand rabbits aged 5–6 weeks, procured from a private Experimental Animal Production Centre (Abdeham) licenced by the Ministry of Agriculture and Forestry. The rabbits, which were fed ad libitum during the study, were kept in equal numbers in standard cages and divided into 3 groups: control (n:7), castor oil (n:7), and TU (n:7). While the control group was not subjected to any treatment, the castor oil group was injected with 0.1 ml of castor oil- carrier substance- (intramuscularly 5 days a week for 6 weeks), and the TU group was injected with TU diluted in castor oil (Nebido® 250 mg/ml inj, Bayer Türk Kimya San. Ltd. Şti, İstanbul, Türkiye) at a dose of 10 mg/kg (intramuscularly 5 days a week for 6 weeks) (Moeloek et al 2008).

At the end of the experiment, all the rabbits were deeply sedated with xylazine (5 mg/kg IM, Rompun® inject.) + ketamine (40 mg/kg IM, Ketasol® inject.) and euthanized with IM injection of a high dose of anaesthetic.

Blood samples collected from the (intracardiac) animals under anaesthesia were centrifuged at 3000 rpm for 15 min, and sera were extracted. Commercial kits and an autoanalyser (Architectc8000) were used to analyse the serum samples for GGT (Gamma-glutamyl Transferase), AST (Aspartate Aminotransferase), ALT (Alanine Transaminase), ALP (Alkaline phosphatase), HDL (High-density lipoprotein), glucose, triglycerides, total cholesterol, lipase, total protein, albumin, phosphate, urea, creatinine, uric acid, magnesium, iron, calcium, chlorine, potassium, sodium, and amylase (Architectc8000).

Statistical Analysis

The data was analysed using SPSS 22 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical software. ANOVA and Tukey, as post hoc test were run to analyse the data. Descriptive statistics (mean, standard deviation) were provided for categorical and continuous variables. A value of $p<0.05$ was considered as statistically significant.

Results

Table 1 shows the results of biochemical parameters obtained from serum samples for each group.

Table 1. Changes in some serum biochemical parameters in healthy (control), castor oil (10 mg/kg, IM, for 6 weeks, 5 days a week) and TU (10 mg/kg, IM, for 6 weeks, 5 days a week) groups on New Zealand rabbits (mean±SE).

Parameters	Unit	Control (n=7)	Castor Oil (n=7)	TU (n=7)
ALT	U/L	16.57±1.46^{ab}	11.29±2.19^{b}	20.29±2.53^{a}
AST	U/L	7.86±0.67^{b}	9.43±1.62^{b}	25.86±6.60^{a}
GGT	U/L	4.24±0.35^{a}	4.20±0.22^{a}	4.81±0.33^{a}
ALP	U/L	76.29±8.43^{a}	57.00±14.33^{a}	78.29±5.82^{a}
Amylase	U/L	103.57±11.22^{b}	86.57±18.94^{b}	164.14±18.36^{a}
Lipase	U/L	290.57±13.66^{ab}	232.43±33.17^{b}	358.86±37.85^{a}
Triglyceride	mg/dl	33.71±3.31^{b}	57.00±21.37^{b}	187.14±40.57^{a}
Total Cholesterol	mg/dL	17.43±1.49^{b}	14.86±3.33^{b}	32.14±4.08^{a}
HDL	mg/dl	15.44±1.48^{a}	10.66±0.68^{b}	11.24±0.70^{b}
Total protein	g/dL	2.00±0.08^{b}	1.53±0.26^{b}	3.03±0.35^{a}
Albumin	g/dL	1.46±0.06^{ab}	1.09±0.20^{b}	1.96±0.17^{a}
Creatinine	mg/dl	0.50±0.02^{b}	0.41±0.02^{b}	0.65±0.06^{a}
Urea	mg/dL	26.86±0.91^{a}	22.43±1.02^{a}	27.14±1.83^{a}
Uric acid	mg/dL	0.88±0.01^{b}	0.89±0.03^{b}	1.06±0.03^{a}
Phosphate	mg/dL	3.07±0.13^{b}	2.73±0.29^{b}	4.44±0.41^{a}
Magnesium	mg/dl	1.24±0.06^{ab}	1.04±0.11^{b}	1.67±0.18^{a}
Potassium	mmol/L	2.83±0.07^{b}	2.84±0.11^{b}	3.44±0.11^{a}
Sodium	mmol/L	97.29±0.57^{b}	99.86±0.67^{b}	108.14±3.79^{a}
Chlorine	mmol/L	70.00±0.76^{ab}	66.29±2.29^{a}	76.57±2.53^{a}
Iron	ug/dL	104.00±5.00^{ab}	73.43±10.04^{b}	126.71±11.67^{a}
Calcium	mg/dL	6.83±0.19^{b}	5.93±0.63^{b}	8.93±0.74^{a}
Glucose	mg/dL	111.29±3.83^{b}	103.57±8.17^{b}	150.71±14.86^{a}

$^{a, b}$ Different letters in the same line are statistically significance ($p<0.05$).

AST, amylase activities, triglyceride, total cholesterol, total protein, creatinine, uric acid, phosphate, potassium, sodium, calcium, and glucose levels were significantly higher in the TU group compared to the control and castor oil groups and HDL levels were significantly lower in the other groups compared to the control group. Althought there was no statistical difference in the TU group compared to the control group, but statistical insignificant decreased were detected in ALT, ALP, GGT, lipase enzyme activities, albumin, magnesium, iron, urea, and chlorine levels.

Discussion

The most well-known and widely used doping substances used by athletes are AASs. The major cause for athletes to use these substances, which are legally prohibited, is not only success and financial gains but also the notion that these substances are also used by their competitors. The use of these substances, which violate sports ethics, also leads to serious health problems as they cause serious irreversible harm to athletes.

Testosterone is known to regulate many physiological

processes, including muscle protein metabolism, sexual and cognitive functions, erythropoiesis, and bone metabolism. Testosterone increases bone and skeletal muscle mass by increasing the uptake of amino acids and elevating the serum level of the insulin-like growth factor IGF I. Second messengers, such as calcium, are mediating for these effects. Calcium is required for muscle contraction, activation of different energy pathways, and cellular proliferation and maturation (Samaha et al 2008).

It has been reported that testosterone levels are positively correlated with changes in haemoglobin levels, while they are negatively correlated plasma HDL and fat mass (Samaha et al 2008). This study showed a statistically significant rise in the protein level in the TU group compared to the control group. AAS used in animals (Soma et al 2007) and humans (Schänzer et al 1996) are testosterone-related synthetic substances used to improve strength and endurance by boosting muscle protein production (Kicman 2008).

It has been reported that 17-α alkyl derivative AAS (methyltestosterone, fluoxymesterone, oxymetholone, oxandrolone, stanozolol, etc.) may have hepatotoxic effects depending on the dose and duration and may elevate some enzyme activities such as ALT, AST, ALP, and GGT in liver (Pärssinen and Seppälä 2002, Sekuła et al 2020). As a result of intramuscular injection of high (10 mg/kg) and low (4 mg/kg) doses of nandrolone decanoate two days a week for six months in New Zealand rabbits, it was reported that ALT activities, urea and creatinine levels were elevated in the group treated with a high dose compared to the control group; AST activities did not differ in the group treated with a high dose compared to the control group; and there was no difference between the groups in GGT and ALP activities (Tsitsimpikou et al 2016). Besides, a study conducted on New Zealand rabbits reported that the changes in ALP, ALT, AST activities, creatinine, and urea levels were within the reference range after long-term administration (Tasgin et al 2011). Another study conducted on New Zealand rabbits showed that intramuscular injection of 5 mg/kg BOL undecylenate elevated ALT, AST activities, creatinine, and urea levels, and these findings indicated that improper use of the growth promoter BOL undecylenate may lead to genital, renal, or hepatic diseases by contributing to permanent impairment of testicular, renal, and hepatic functions (Tousson et al 2013). Consistent with some of the foregoing reports Pärssinen and Seppälä (2002), Tousson et al (2013) statistically significant elevations in serum AST activities and numerically significant elevations in ALT activities were observed in the groups treated with TU in the study, but no significant change was found in GGT and ALP activities. Although it is considered that the elevation in AST activities might be due to skeletal muscle damage, it was concluded that it would be more convenient to evaluate creatine kinase (CK-MB) activities together in future studies in order to further support this finding. Furthermore, besides the studies reporting the effect of AAS on liver damage, there are also studies reporting the effect of AAS on liver damage within the reference range (Tasgin et al 2011). It is considered that the outcomes of the studies may be due to differences in the drug, dose, duration of treatment, and animal breed, and it would be useful for future studies to evaluate all these considerations together with histopathologic examinations for liver damage.

It has been reported that AASs affect lipoprotein metabolism and elevate serum low-density lipoprotein (LDL) in the blood or affect these levels insignificantly and lower HDL levels; thus, they may pose a risk of atherosclerosis (Eckardstein and Wu 2003, Kaushik et al 2010). Changes in liver transaminase activities or HDL and LDL profile may suggest anabolic steroid use in patients (Rashid et al 2007). It was reported that the experimental groups treated with 25 mg/kg nandrolone decanoate and 50 mg/kg testosterone enanthate in New Zealand rabbits had significantly lower HDL levels compared to the control group, and the group treated with nandrolone decanoate had statistically higher LDL levels, while there was no significant change in serum total cholesterol and triglyceride levels (Ammar et al 2004). The finding that serum HDL levels were lower in the experimental groups compared to the control groups in the present study was found to be compatible with the related reports, but a fall in serum HDL levels in the castor oil group suggested that it may also be due to a solvent-induced drop. Furthermore, statistically significant elevations in triglyceride and total cholesterol levels were noticed. Although this suggests the possibility of hypercholesterolemia and atherosclerosis risk, it was concluded that it would be better to evaluate LDL levels in future studies.

Although it was reported that there was no difference between groups in total protein, albumin, globulin, urea, and creatinine levels after IM injection of 5 mg/kg of boldenone undecylenate in New Zealand rabbits Alm-Eldeen and Tousson (2012), this study showed an elevation in total protein and creatinine levels in the TU group, while no change was noticed in urea and albumin levels. It is clear that the differences in the results depend on the ASS used and the duration of use. Furthermore, it was believed that it would be useful to perform biochemical parameters and histopathological examinations of the organs together in future studies.

Girard et al (2005) reported that the administration of castor oil caused diarrhoea in rats, which may result in water and electrolyte (sodium, potassium, and chloride) changes. Another study Chen et al (1999) reported that castor oil altered water and electrolyte secretion, and lowered serum potassium and sodium levels but did not

induce hypokalaemia or hyponatremia. The present study showed that there was no statistical difference in parameters such as ALT, AST, ALP activities, total protein, albumin, chlorine, sodium, potassium, and urea in the castor oil group compared to the control group.

Many steroid hormones can increase sodium reabsorption in the distal tubules of the kidney (Guyton and Hall 2001). It is also reported that ASS may improve the reabsorption of substances such as calcium, potassium, chlorine, and phosphate (Kayaalp 2005). Parallel to the existing knowledge, phosphate, potassium, calcium, and sodium levels were found to be significantly high in the TU group in the study. The statistically significant elevation of calcium levels in the TU group in the present study suggests that ASS might cause hypercalcemia due to reduced urinary excretion of calcium. It is useful that future studies should be conducted together with urine analysis.

Guo et al (2020) reported that testosterone treatment of iron-deficient mice improved bone marrow erythropoiesis and furthermore raised the rate of early erythroid ancestors and late erythroblasts. They stated that anabolic steroids may induce erythropoiesis by stimulating erythropoietic stimulating factor (Gabr et al 2009). Hussein et al (1999) also reported that serum trace elements, including copper, iron, zinc, and manganese concentrations, showed a significant fall after castration in New Zealand rabbits. It was thought that the statistically insignificant elevation in iron level in the TU group compared to the control group in the study could be accounted for by erythropoietic mechanisms (Guo et al 2020, Gabr et al 2009, Hussein et al 1999), and the mechanism could be more clearly understood with the different doses and administration times. Androgens are also reported to be beneficial in anemia (García-Arnés 2022).

The most commonly used parameters to assess renal functions are serum Na, K, Cl, HCO_3, creatinine, uric acid, and urea nitrogen (BUN) (Gowda et al 2010). The study showed that creatinine, uric acid, sodium, and potassium levels were significantly higher in the TU group compared to the control group, while urea and chlorine levels showed an elevation with no statistical difference. These results suggest that the use of AASs may damage the kidneys.

The administration of exogenous synthetic testosterone may inhibit follicle-stimulating hormone (FSH) and luteinising hormone (LH) production by inhibiting gonadotropin-releasing hormone (GnRH) with negative feedback in the hypothalamus-pituitary axis. Inhibition of LH, required for testosterone production in the testes, consequently lowers intra-testicular testosterone levels and overall testosterone production (Crosnoe et al 2013). It has been reported that testosterone is one of the most important regulators of the biological activity of insulin in the liver, adipose tissue, and skeletal muscle of men, and low testosterone levels are correlated with insulin resistance (Bianchi and Locatelli 2018). Therefore, it is thought that the elevated blood glucose levels observed in the study can be explained by the above mechanisms, and it is also possible that AASs, which can damage many organs, also cause pancreatic damage, and it would be appropriate to plan new studies combined with histopathological studies as well as insulin hormone levels in this regard.

Amylase and lipase are important digestive enzymes secreted by the pancreas. Amylase is also secreted by the salivary glands. Although their activities are elevated in many disease processes, they are mostly used in the diagnosis of pancreatitis (Sher 1982). The lipase among these appears to be more sensitive and specific for pancreatic disease and is elevated over a longer period of time; therefore, it is recommended to use lipase for the diagnosis of pancreatitis (Sepulveda 2019). Samaha et al (2008) reported in a case study that a male patient who took amino acid and anabolic supplements had hypercalcaemia, elevated liver enzymes, and elevated amylase, lipase, and creatine protein kinase levels. The study showed a statistically significant increase in amylase, calcium with AST activities but a statistically insignificant increase in lipase and ALT activities in the TU group compared to the control group. Furthermore, hypercalcemia was observed in the animals, suggesting that acute pancreatitis and acute renal failure may develop if the administration time of ASS is prolonged.

Conclusion

In conclusion, it may be stated that AASs may cause hepatotoxicity and changes lipid and electrolyte metabolism. It is considered that the planning of multidisciplinary studies about ASS administration at different doses and durations will be useful in better understanding the effects of doping substances.

Conflict of Interest

The authors declare no conflict of interest.

References

Albano GD, Amico F, Cocimano G, Liberto A, et al; 2021. Adverse Effects of Anabolic-Androgenic Steroids: A Literature Review. Healthcare (Basel). 9(1), 97. https://doi.org/10.3390/healthcare9010097

Alm-Eldeen A, Tousson E, 2012. Deterioration of glomerular endothelial surface layer and the alteration in the renal function after a growth promoter boldenone injection in rabbits. Hum Exp Toxicol, 31(5), 465-472. https://doi.org/10.1177/0960327111420745

Ammar EM, Said SA, Hassan MS, 2004. Enhanced vasoconstriction and reduced vasorelaxation induced by testosterone and nandrolone in hypercholesterolemic rabbits. Pharmacol Res, 50(3), 253-259. https://doi.org/10.1016/j.phrs.2004.03.010

Badawy AA, 2018. Modulation of tryptophan and serotonin metabolism as a biochemical basis of the behavioral effects of use and withdrawal of androgenic-anabolic steroids and other image-and performance-enhancing agents. J Tryptophan Res, 11, 1178646917753422. https://doi.org/10.1177/1178646917753422

Báthori M, Tóth N, Hunyadi A, Márki A, et al; 2008. Phytoecdysteroids and anabolic-androgenic steroids--structure and effects on humans. Curr Med Chem. 15(1), 75-91. https://doi.org/10.2174/092986708783330674

Behre H, Abshagen K, Oettel M, Hübler D, et al; 1999. Intramuscular injection of testosterone undecanoate for the treatment of male hypogonadism: phase I studies. Eur J Endocrinol, 140(5), 414–419. https://doi.org/10.1530/eje.0.1400414

Bianchi VE, Locatelli V, 2018. Testosterone a key factor in gender related metabolic syndrome. Obes Rev, 19(4), 557-575. https://doi.org/10.1111/obr.12633

Bond P, Smit DL, de Ronde W, 2022. Anabolic-androgenic steroids: How do they work and what are the risks? Front Endocrinol (Lausanne), 13, 1059473. https://doi.org/10.3389/fendo.2022.1059473

Chen C-C, Ng W-W, Chang F-Y, Lee SD, 1999. Magnesium citrate–bisacodyl regimen proves better than castor oil for colonoscopic preparation. J Gastroenterol Hepatol, 14(12), 1219–1222. https://doi.org/10.1046/j.1440-1746.1999.02032.x

Craig M, Yarrarapu SNS, Dimri M, 2023. Biochemistry, Cholesterol. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing

Crosnoe LE, Grober E, Ohl D, Kim ED, 2013. Exogenous testosterone: a preventable cause of male infertility. Transl Androl Urol, 2(2), 106-113. https://doi.org/10.3978/j.issn.2223-4683.2013.06.01

Çınaroğlu M, 2023. Exercise, anabolic steroid dependence, muscle dysmorphia, performance enhancement drug abuse. Nişantaşı Üniv Sosyal Bil Der, 11(2), 443-454. https://doi.org/10.52122/nisantasisbd.1348564

David K, Dingemanse E, Freud J, Laqueur E, 1935. Uber krystallinisches mannliches Hormon aus Hoden (Testosteron) wirksamer als aus harn oder aus Cholesterin bereitetes Androsteron. Hoppe Seylers Z Physiol Chem, 233, 281-283. https://doi.org/10.1515/bchm2.1935.233.5-6.281

de Souza GL, Hallak J, 2011. Anabolic steroids and male infertility: a comprehensive review. BJU int, 108(11), 1860-1865. https://doi.org/10.1111/j.1464-410X.2011.10131.x

Eckardstein AV, Wu FCW, 2003. Testosterone and atherosclerosis. Growth Horm IGF Res. Suppl A, 72-84. https://doi.org/10.1016/s1096-6374(03)00059-5

Edelstein D, Basaria S, 2010. Testosterone undecanoate in the treatment of male hypogonadism. Expert Opin Pharmacother, 11(12), 2095-2106. https://doi.org/10.1517/14656566.2010.505920

Figueiredo MG, Gagliano-Jucá T, Basaria S, 2022. Testosterone therapy with subcutaneous injections: a safe, practical, and reasonable option. J Clin Endocrinol Metab, 107(3), 614-626. https://doi.org/10.1210/clinem/dgab772

Fink J, Schoenfeld BJ, Hackney AC, Matsumoto M, 2018. Anabolic-androgenic steroids: procurement and administration practices of doping athletes. Phys Sportsmed , 17(1), 10–14. https://doi.org/10.1080/00913847.2018.1526626

Gabr FI, Hassan TA, Abo El-Maaty AM, Aotifa AM, 2009. Effects of growth promoter boldenone undecylenate on weaned male lambs. Nature and Science, 7(3), 61-69.

García-Arnés JA, García-Casares N, 2022. Doping and sports endocrinology: anabolic-androgenic steroids. Rev Clin Esp (Barc), 222(10), 612-620. https://doi.org/10.1016/j.rceng.2022.09.003

Girard P, Pansart Y, Coppe MC, Gillardin JM, 2005. Saccharomyces boulardii inhibits water and electrolytes changes induced by castor oil in the rat colon. Dig Dis Sci, 50(11), 2183-2190. https://doi.org/10.1007/s10620-005-3029-3

Gowda S, Desai PB, Kulkarni SS, Hull VV, et al; 2010. Markers of renal function tests. N Am J Med Sci, 2(4), 170-173.

Gu Y, Liang X, Wu W, Liu M, et al; 2009. Multicenter contraceptive efficacy trial of injectable testosterone undecanoate in Chinese men. J Clin Endocrinol Metab, 94(6), 1910–1915. https://doi.org/10.1210/jc.2002-020447

Guo W, Abou Ghayda R, Schmidt PJ, Fleming MD, et al; 2020. The role of iron in mediating testosterone's effects on erythropoiesis in mice. Fed Am Soc Exp Biol, 34(9), 11672-11684. https://doi.org/10.1096/fj.202000920RR

Guyton AC, Hall JE, 2001. Textbook of Medical Physiology. Ninth Edition, 9nd ed., 1011-1013. WB Saunders Company.

Hussein SA, Azab ME, Abdel-Maksoud H, 1999. Metabolic changes concerning the effect of castration on some blood constituents in male rabbits (Abstract). DTW, 106(3), 113-118.

Kayaalp SO, 2005. Rational Therapy in Terms of Medical Pharmacology. 11th Edition, Feryal Press, Ankara.

Khalid M, Ilhami N, Giudicelli Y, Dausse JP, 2002. Testosterone dependence of salt-induced hypertension in Sabra rats and role of renal α2-adrenoceptor subtypes. J Pharmacol Exp Ther, 300(1), 43-49. https://doi.org/10.1124/jpet.300.1.43

Kaushik M, Sontineni SP, Hunter C, 2010. Cardiyovaskuler disease and androgens. Int J Cardiol. 142(1), 8-14. https://doi.org/10.1016/j.ijcard.2009.10.033

Kicman AT, 2008. Pharmacology of anabolic steroids. Br J Pharmacol, 154(3), 502-521. https://doi.org/10.1038/bjp.2008.165

Király CL, Collan Y, Alén M, 1987. Effect of testosterone and anabolic steroids on the size of sebaceous glands in power athletes, Am J Dermatopathol, 9(6), 515– 519. https://doi.

org/10.1097/00000372-198712000-00008

Liu JD, Wu YQ, 2019. Anabolic-androgenic steroids and cardiovascular risk. Chin Med J (Engl). 132(18), 2229-2236. https://doi.org/10.1097/CM9.0000000000000407

Meriggiola MC, Costantino A, Saad F, D'Emidio L, et al; 2005. Norethisterone enanthate plus testosterone undecanoate for male contraception: effects of various injection intervals on spermatogenesis, reproductive hormones, testis, and prostate. J Clin Endocrinol Metab. 90(4), 2005-2014. https://doi.org/10.1210/jc.2004-1852

Moeloek N, Asmarinah A, Siregar NC, Ilyas S, 2008. Testosterone undecanoate and depo medroxyprogesterone acetate induced azoospermia through increased expression of spermatogenic cell caspase 3. Med J Indones, 17(3), 149-156. https://doi.org/10.13181/mji.v17i3.313

Nieschlag E, Büchter D, Von Eckardstein S, Abshagen K, et al; 1999. Repeated intramuscular injections of testosterone undecanoate for substitution therapy in hypogonadal men. Clin Endocrinol (Oxf), 51(6), 757-763. https://doi.org/10.1046/j.1365-2265.1999.00881.x

Pärssinen M, Seppälä T, 2002. Steroid use and long-term health risks in former athletes. Sports Med, 32(2), 83–94. https://doi.org/10.2165/00007256-200232020-00001

Rashid H, Ormerod S, Day E, 2007. Anabolic androgenic steroids: what the psychiatrist needs to know. Adv Psychiatr Treat, 13(3), 203-211. https://doi.org/10.1192/apt.bp.105.000935

Samaha AA, Nasser-Eddine W, Shatila E, Haddad JJ, et al; 2008. Multi-organ damage induced by anabolic steroid supplements: a case report and literature review. J Med Case Rep, 2(1), 340. https://doi.org/10.1186/1752-1947-2-340

Schänzer W, Delahaut P, Geyer H, Machnik M, et al; 1996. Long-term detection and identification of metandienone and stanozolol abuse in athletes by gas chromatography-high-resolution mass spectrometry. J Chromatogr B Biomed Appl, 687(1), 93-108. https://doi.org/10.1016/s0378-4347(96)00187-9

Sessa F, Salerno M, Di Mizio G, Bertozzi G, et al; 2018. Anabolic Androgenic Steroids: Searching New Molecular Biomarkers. Front Pharmacol, 9, 1321. https://doi.org/10.3389/fphar.2018.01321

Sekuła M, Świerczyńska B, Smoluchowskı K, Undziakiewicz A, et al; 2020. Hepatotoxicity of anabolic androgenic steroids in sport. J Educ Health Sport. 10(9), 349-356. https://doi.org/10.12775/JEHS.2020.10.09.040

Sepulveda JL, 2019. Accurate Results in the Clinical Laboratory || Challenges in routine clinical chemistry analysis. 2nd ed., 141–163. https://doi.org/10.1016/B978-0-12-813776-5.00010-8

Sher PP, 1982. Drug interferences with clinical laboratory tests. Drugs, 24, 24-63. https://doi.org/10.2165/00003495-198224010-00002

Soma L, Ubo CE, Guan F, McDonnell S, et al; 2007. Pharmacokinetics of boldenone and stanozolol and the results of quantification of anabolic and androgenic steroids in race horses and nonrace horses. J Vet Pharmacol Ther, 30(2), 101–108. https://doi.org/10.1111/j.1365-2885.2007.00824.x

Tasgin E, Lok S, Demir N, 2011. Combined usage of testosterone and nandrolone may cause heart damage. Afr J Biotechnol, 10(19), 3766-3768.

Tousson E, El-Moghazy M, Massoud A, El-Atrash A, 2013. Physiological and biochemical changes after boldenone injection in adult rabbits. Toxicol Ind Health, 32(1), 177–182. https://doi.org/10.1177/0748233713501365.

Tsitsimpikou C, Vasilaki F, Tsarouhas K, Fragkiadaki P, 2016. Nephrotoxicity in rabbits after long-term nandrolone decanoate administration. Toxicol Lett, 259, 21–27. https://doi.org/10.1016/j.toxlet.2016.06.1122

Yavari A, 2009. Abuse Of Anabolıc Androgenıc Steroıds. J Stress Physiol Biochem, 5(3), 22-32.

Zhang XW, Zhang C, Zhang W, Yang D, 2016. Suppression of spermatogenesis by testosterone undecanoate-loaded injectable in situ-forming implants in adult male rats. Asian J Androl, 18(5), 791–797. https://doi.org/10.4103/1008-682X.160886

Walker WH, Cooke PS, 2023. Functions of Steroid Hormones in the Male Reproductive Tract as Revealed by Mouse Models. Int J Mol Sci, 24(3), 2748. https://doi.org/10.3390/ijms24032748

Author Contributions

Motivation/Concept: BSA, MOD, SH; Design: NK, MOD; Control/Supervision: BSA, SH; Data Collection and/or Processing: BSA, AC, MOD, SH; Analysis and/or Interpretation: BSA, AC, MOD, SH; Literature Review: BSA, AC, MOD, NK; Writing the Article: MOD, NK; Critical Review: BSA, AC, MOD, SH

Ethical Approval

This study ethical approval (Approval no: 2022/51) was obtained from Selcuk University Veterinary Faculty Experimental Animal Production and Research Center Ethics Committee (SÜVDAMEK).