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Original Article

Comparative evaluation of the serum biochemical and lipid profile changes in spayed male and female rats

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Abstract

Background and aims: This study investigated the effects of gonadal removal in male and female rats on changes in serum biochemical parameters.

Methods: Twenty-eight adult male and female rats were divided into four groups of 7 animals for a period of 9 weeks. The first and second groups of intact male and female rats, as well as the third and fourth groups of male and female rats, were gonadectomized, respectively. At the end of the ninth week of the study, the rats were anesthetized with chloroform, and the amount of glucose, some lipid parameters in serum, and the activity of a number of serum enzymes were measured after taking blood from the heart.

Results: Alanine aminotransferase, and alkaline phosphatase (ALP) levels were higher in intact male rats than in intact female rats, respectively ($P=0.03$, $P=0.015$). The amounts of glucose and cholesterol of low-density lipoprotein (LDL) in intact female rats were higher than in the ovariectomized rats, respectively ($P=0.07$, $P=0.039$, $P=0.03$). The amount of lactate dehydrogenase (LDH) in spayed female rats demonstrated a significant increase compared to other groups ($P=0.001$). However, there was a significant decrease in calcium levels in gonadectomized rat groups in comparison to intact female rats ($P=0.02$). Finally, a significant increase was found in phosphorous levels in the intact male rats group compared to other groups ($P=0.002$).

Conclusion: Decreased sex hormones in gonadectomized rats compared with intact rats could lead to increased serum levels of LDH, ALP, and LDL cholesterol that may result in the development of metabolic and atherogenesis syndromes and acute liver failure.

Keywords: Orchidectomy, Ovariectomy, Gender, Serum, Lipid profile, Rat

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Introduction

The effects of ovariectomy and orchidectomy on insulin sensitivity, metabolic disorders, and molecular mechanisms have been studied by several researchers, although they have recorded various results in this regard (1).

According to the United Nations, by 2050, about 20% of the world's population will be over 60 years old. Statistics projected by the Center for Population Studies of Iran also indicate that by 2050, 26 million people (24%) of the country's population will be elderly, with a significant percentage of men (2). Menopause in women aged 45-54 years is characterized by a sudden drop in the serum levels of estrogen and progesterone but elevated follicle-stimulating hormone and luteinizing hormone levels (3,4), and an increased risk of coronary heart disease, diabetes, skeletal muscle wasting, and low bone density (5), as well as changes in body composition and fat parameters, which are associated with metabolic disorders (6). Sex hormones are the most important regulators of metabolic processes, including glucose metabolism, body weight, distribution of adipose tissue, fat absorption, and energy consumption patterns in men and women (5).

Recent studies have shown some associations between oxidative stress and decreased ovarian hormone activity (4,7,8). However, the decline in androgens (testosterone and dehydroepiandrosterone) in men, known as andropause, occurs steadily, gradually, and over decades with aging men (9). Approximately 95% of testosterone produced in men is produced by testicular interstitial cells, and the remaining 5% is produced by the adrenal cortex. Decreased production or decreased levels of androgens in elderly men, or in cases of testicular removal due to treatment of testicular cancer and advanced prostate tumors, can have multiple signs and symptoms; they include decreased bone density (10), decreased muscle mass, decreased libido, increased fat mass, insomnia and depression, increased triglycerides (TG) and blood pressure, the progression of Alzheimer's disease (2), and impaired carbohydrate and fat metabolism (11). Testosterone concentration is affected by a number of factors such as age, obesity, and binding protein concentration (albumin and sex hormone-binding globulin) (12). Removal of the testicles in male rats reduces food intake and weight loss but increases body fat percentage. Testosterone appears to have direct effects on glucose and fat metabolism

in rats (11). Due to the importance of andropause and menopause, the present study was performed to investigate and compare biochemical changes and parameters in male and female rats, which were orchidectomized (ORX) and ovariectomized (OVX), respectively, in order to understand the significant differences of sex hormones in serum biochemical parameters and appropriate evaluation in experimental treatments following gonadectomy rats and toxicological studies.

Materials and Methods

This experimental study was performed on 28 male and female Wistar rats with an average weight of 200-250 g for 10 weeks. The rats were purchased from the animal house of the Pasteur Institute Research Center in Tehran, Iran, and kept in polycarbonate cages at 20 ˚C, 60% humidity, and a 12-hour light-dark cycle at the Pathology Research Center of Shahrekord Islamic Azad University Veterinary Hospital. To adapt to the conditions of the new environment, all experiments were performed at least one week after the establishment of the animals. The rats had free access to standard water and food (13).

Operative technique for ovariectomy and orchidectomy of female and male rats

Male and female rats were anesthetized before surgery with a combination of ketamine and xylazine (Alfasan Holland) injection at doses of 50 and 5 mg/kg, respectively, intraperitoneally, and underwent surgery to remove female gonads as a menopausal model (14), and testicular removal as an andropause model (15) (Figures 1 and 2). The rats were then randomly divided into 4 groups of seven.

- Group 1: Female rats underwent a sham operation with similar procedures, except for the removal of ovaries, and were kept in standard conditions for 9 weeks.
- Group 2: Male rats underwent a sham operation with similar procedures, except for the removal of testes, and were kept in vitro for 9 weeks.
- Group 3: OVX rats were kept for 9 weeks after surgery and the removal of the ovaries.
- Group 4: Male ORX rats were kept for 9 weeks after surgery and testicle removal.

Determination of serum glucose and enzymes

At the end of the ninth week of the study, the rats were anesthetized with ether after one night of fasting while accessing water. The blood was drawn from the heart after the thoracic cavity was opened and then transferred into tubes without anticoagulant, and the serum was separated by a Hettich EBA 200 centrifuge (Germany) at 3000 rpm for 15 minutes. Serum glucose, aspartate

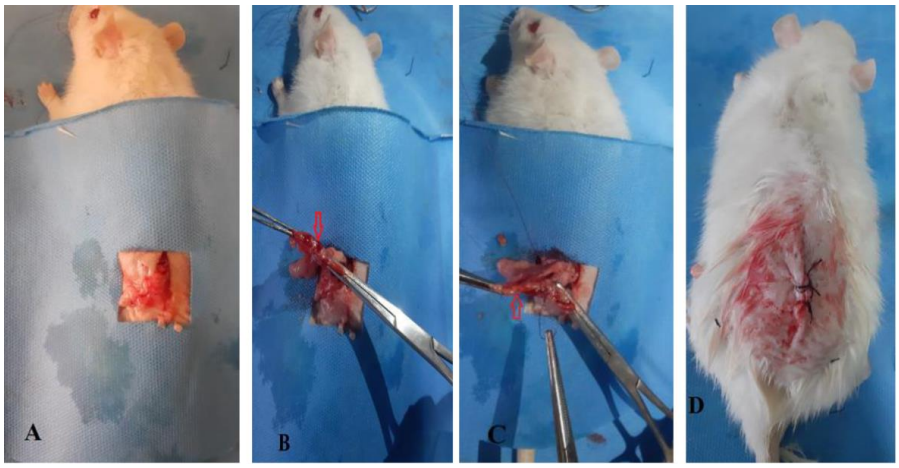

Figure 1. Stages of ovariectomy in rats (left to right). *Note*. The ovary is indicated by the red arrow

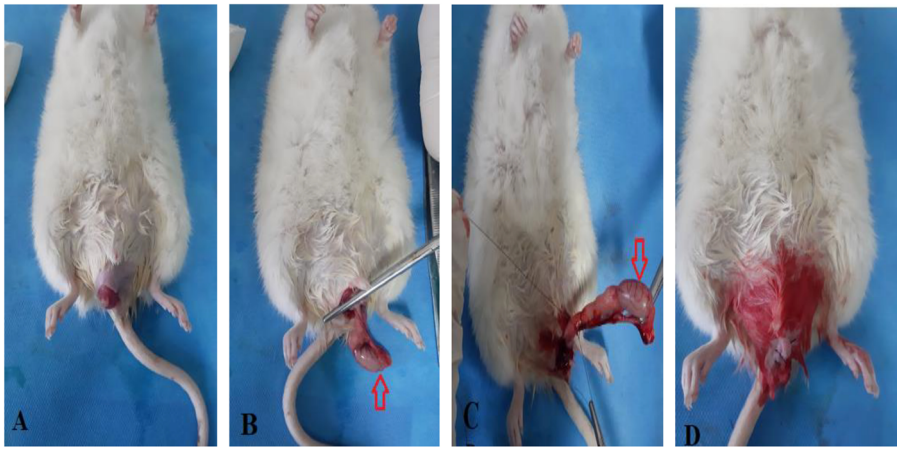

Figure 2. Stages of orchidectomy in rats (left to right). *Note*. The testes are indicated by the red arrow

aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine (Cr), cholesterol, TG, and low-density lipoprotein (LDL) were measured by a BT-3000 autoanalyzer device (Biotechnica Company, Italy) using Pars Azmon kits, Tehran (Iran) and according to the recommended instructions (13).

In addition, to measure high-density lipoprotein cholesterol (HDL), the deposition method was used so that, first, LDL and very low-density lipoprotein (VLDL) cholesterol were precipitated by a precipitating reagent, and then a clear supernatant containing HDL. The cholesterol in this clear layer was removed from the color intensity using a digital spectrophotometer and the amount of light absorption at 520 nm, and compared with the standard solution using an enzymatic method (13).

Statistical analysis

The collected data were calculated using SPSS (version 20) and presented as means and standard deviations. Considering the normal distribution of the data, they were analyzed by one-way analysis of variance, and in case where the difference was statistically significant ($P<0.1$), the data of each of the two groups were compared by the least significant difference test. Glucose, serum enzyme activity, and lipid profile serum parameters in the study groups were plotted using GraphPad Prism software, version 6.

Results

The serum glucose levels in intact female rats demonstrated a statistically significant increase compared to intact male rats, ORX, and female groups, respectively ($P=0.065$, $P=0.083$, and $P=0.07$, Figure 3). Serum cholesterol levels in the OVX group increased significantly compared to the intact female group ($P=0.03$). Further, the amount of cholesterol in the ORX rats group showed a significant increase in comparison to the intact female rats' group ($P=0.09$). The amount of TG in the healthy male rat group was higher than in the healthy female rat group ($P=0.05$, Figure 3). The amount of LDL in the intact male and OVX groups was significantly higher than in the group of intact female rats, respectively ($P=0.06$ and $P=0.02$). The amount of HDL in the group of intact female rats was significantly higher than that of OVX rats, intact male rats, and ORX rats, respectively ($P=0.08$, $P=0.09$, $P=0.04$, Figure 3). The amount of ALT in the group of intact female rats compared to intact male rats, ORX and OVX rat groups represented a significant decrease, respectively ($P=0.03$, $P=0.07$, $P=0.02$).

Furthermore, the amount of ALP in the ORX rats showed a significant increase compared to groups intact and OVX rats, respectively ($P=0.01$, $P=0.07$). The amount of serum LDH in the OVX rats was significantly higher than that of intact female, intact male, and ORX rats ($P=0.001$). Moreover, the amount of LDH in the ORX male rats group was higher than that of intact male rats ($P=0.06$, Figure 4).

Serum calcium concentration was higher in intact female rats than in the OVX and ORX rats, respectively ($P=0.01$, $P=0.02$). Additionally, serum calcium concentration was higher in the intact male rat group than in the OVX rat group ($P=0.07$, Figure 5). Serum phosphorus concentration in intact male rats was higher than that of the rats in other study groups ($P=0.02$). The amount of Cr in the intact female rat group was significantly higher than that in ORX male rats ($P=0.02$). In addition, the amount of Cr in intact female rats was significantly higher

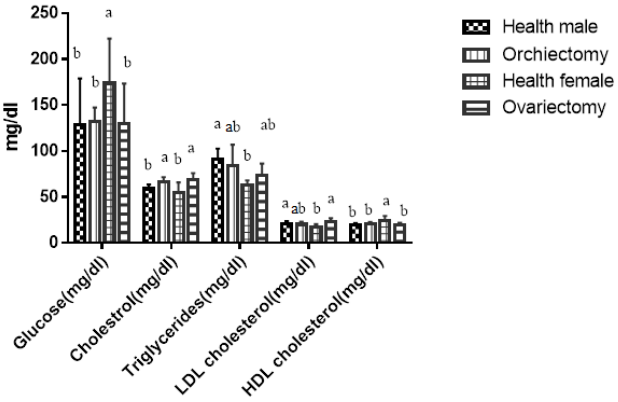

Figure 3. Biochemical changes in glucose and fat parameters (means ± standard deviations) in sterilized and healthy male and female rats. *Note*. The number of samples in each group (n = 7) and non-similar letters in each row indicate statistically significant differences ($P<0.1$)

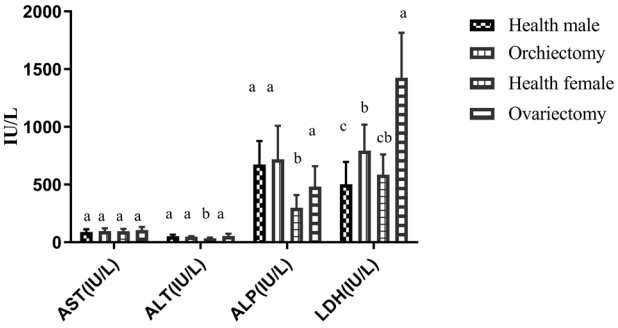

Figure 4. Biochemical changes in serum enzymes of aspartate aminotransferase, ALTALP, and LDH (Mean ± Standard Deviations) in Sterilized and Healthy Male and Female Rats. *Note*. ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase. The number of samples in each group (n = 7) and non-similar letters in each row represent statistically significant differences ($P<0.05$)

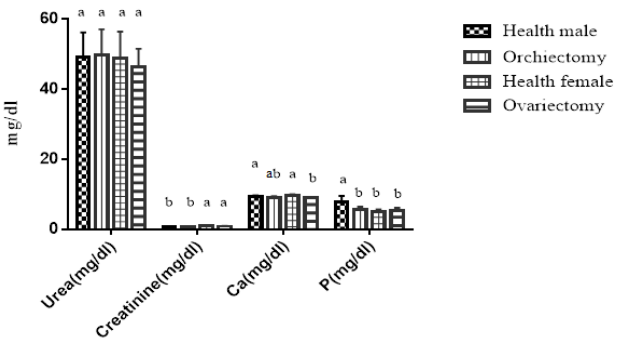

Figure 5. Biochemical changes in calcium, phosphorus, urea, and creatinine (means ± standard deviations) in sterilized and healthy male and female rats. *Note*. The number of samples in each group (n = 7), and non-similar letters in each row denote statistically significant differences ($P<0.05$)

than in intact male rats ($P=0.08$), and it was significantly higher in the OVX rats group than in the ORX male rats group ($P=0.07$, Figure 5).

Discussion

In this study, gonadectomized male and female rats were used as animal models to evaluate the changes in serum enzymes postmenopause due to easy handling, low maintenance costs, and an extremely fast physiological response compared to large animals and their similarity with women and men. Ovariectomy and orchiectomy animals are models for studying the effects of ovarian hormones and androgen deficiency (16,17). In the present study, a significant increase in cholesterol levels was observed in male and OVX rats compared to intact male and female rats. Studies have shown that it modulates testosterone and steroid hormones in animals that have access to a cholesterol-rich diet (18). In the present study, an increase in the concentration of cholesterol and LDL was found in the serum of OVX rats in comparison to intact female rats, confirming the positive role of estrogen in preventing high cholesterol than in the OVX animals (19). In the current study, there was an increase in the concentration of cholesterol and LDL in the serum of OVX rats compared to intact female rats, highlighting the positive role of estrogen and progesterone in preventing high cholesterol and regulating fat metabolism, and reducing fat breakdown in OVX animals (19). The results of the present study on lipid profiles demonstrated that in intact male rats, the levels of TG and LDL were higher than those in intact female rats, and the level of HDL was higher compared to other groups. According to previous results, the underlying mechanisms that cause this difference in serum lipid levels include sex gland hormones and sex chromosome XX (20). Studies reported that high concentrations of total cholesterol and LDL raise the risk of coronary heart disease, hypertension, and atherosclerosis (21). Gender differences play an important role in inducing hyperlipidemia and cardiovascular disease, and men are much more likely to develop cardiovascular disease than women of the same age, and postmenopausal women are just as likely to develop cardiovascular disease as men (22). The effects of estrogen on fat metabolisms, including decreased HDL catabolism by decreasing hepatic lipase activity and increased LDL catabolism, have been demonstrated by increasing the number of LDL receptors (23). Estrogen has antioxidant properties due to the presence of a phenolic group in the steroid structure, and it seems that the short-term deprivation of estrogen in female rats increases cellular oxidative damage in the tissue (12,24). Jones et al (2005) found that one of the causes of the atherosclerotic process and cardiovascular risk factors is a decrease in the serum testosterone level associated with aging, leading to increased cholesterol, dyslipidemia, and insulin resistance (25).

In the present study, the serum glucose levels of fasting intact female rats had a higher concentration compared to other groups, which is consistent with the results of He et al, which is probably due to energy demand, basal insulin secretion, and insulin sensitivity, along with the high ratio of insulin to blood glucose in males compared to females (26). In this study, serum ALT levels were higher in intact and ORX male rats in comparison to intact female rats. The amounts of transaminases in males were higher than in females because males had less subcutaneous fat storage, which is mainly spent on the production of sex hormones, and excess fat quickly enters the abdominal cavity, skeletal muscle, liver, and pancreas, increasing the amount of AST and ALT (27).

The results of a study by Yang et al revealed that the expression activity of the ALT type 2 isozyme gene in the muscles and liver of male rats was more than 4 times that of female rats, and the activity of the ALT liver of male rats was more than 30 percentages of this enzyme activity in female rats (28). In the current study, although the level of the enzymatic activity of ALT, or serum glutamic pyruvic transaminase, in intact male rats was extremely higher than that of intact female rats, increased serum ALT activity occurred in various conditions of liver damage, including viral infections, cirrhosis, fatty liver, and poisoning. In addition, increased serum ALT activity has been reported in cases such as muscle damage and celiac disease (28). The ALP enzyme is a membrane-bound glycoprotein that is found in various tissues such as the liver, bone, and in small amounts in the kidney, and is a biochemical indicator in the diagnosis of liver disorders (12). In the present study, the increase in ALP in intact male rats compared to healthy female rats could be due to the increased expression of androgen receptors in the growth of long bone and metaphyseal plates and the effect of androgens indirectly through aromatization to estradiol during puberty (29). Thus, increased turnover in bone is associated with increased levels of ALP (30).

LDH activity is present in all cells of the body and is found only in the cytoplasm of cells. Due to the widespread distribution of LDH in tissues, increased levels of this enzyme happen in a number of clinical conditions such as myocardial infarction, hemolysis, liver disorders, kidney, lung, and muscle. Increased LDH has also been observed in liver disease (31). In the present study, the increase in LDH activity in ORX male and female rat groups, compared to intact male and female rats, was associated with decreased activity of androgens and sex steroids. Tam et al concluded that a decrease in testosterone in castrated male rats induced oxidative stress and decreased antioxidant activity (32). Therefore, if the heart muscle, skeletal muscle, or other tissues are damaged, the LDH level increases significantly in the serum.

Creatinine and urea concentrations, as indicators of renal function, are associated with glomerular filtration and protein breakdown, respectively (33). The expression of the messenger RNA1 (mRNA) gene carrying organic kidney anion is higher in males than in females, which causes more creatinine excretion in males and is associated

with lower plasma creatinine (34). Other studies have also demonstrated that serum creatinine concentrations in female rats' increase after 16 hours of food deprivation (35).

In the current study, the serum calcium and phosphorus levels of ORX rats revealed a significant decrease compared to healthy male rats. The amount of hormones secreted by the testicles decreases following the removal of the testes. Testosterone aromatization products can increase the secretion of parathyroid hormone by affecting the parathyroid gland. This hormone also acts on the alpha enzyme hydroxylase in the kidney and produces the active metabolite of vitamin D (1,25 (OH) 2D). This substance acts in the intestine and increases the amount of calcium absorption. When the body's testosterone decreases, the amounts of active metabolites of vitamin D decrease, and calcium absorption is impaired, followed by a decrease in serum calcium (36). After the OVX of rats, an increase in calcium and phosphorus uptake from bone, and a decrease in their serum levels, due to a lack of absorption and renal excretion of phosphorus, the amount of phosphorus decreases in the serum (3). Serum phosphorus levels in intact male rats increased significantly compared to intact female rats, which contradicts the results of previous research (36). Chromosomal differences appear to naturally cause serum phosphorus levels to be higher in male rats than in female rats. The present study had a number of limitations such as the lack of specific kits for the determination of sex hormones and the non-availability of technology for the evaluation of the physiological activities of the heart, bone, and other organs.

Conclusion

The findings of this study confirmed that there were differences in the measurement of a number of biochemical parameters between male and female healthy and ORX rats, which is a good evaluation in interventional experimental and physiologic studies. Eventually, it should be noted that estrogen and testosterone hormones play an important role in regulating glucose, fat profiles, and a number of serum enzymes.

Authors' Contribution

Conceptualization: Abdolrasoul Namjou.
Data curation: Abdolrasoul Namjou.
Formal analysis: Abuzar Alikhani.
Investigation: Abdolrasoul Namjou.
Methodology: Esfandiar Heidarian.
Project administration: Esfandiar Heidarian.
Software: Abuzar Alikhani.
Writing–original draft: Abdolrasoul Namjou, Esfandiar Heidarian.
Writing–review & editing: Abdolrasoul Namjou.

Competing Interests

Although the second author of this article is the Editor-in-Chief of the journal, the entire process of evaluating and reviewing the article was the same as that of the other authors, and there is no conflict of interests.

Ethical Approval

All laboratory work methods used in this study were approved by the Ethics Committee of the Islamic Azad University, Shahrekord branch (Approval ID: IR.IAU.SHK.REC.2021.0097), and all ethical principles related to the tested animals were upheld during this study.

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