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Original Article

Turk J Med Sci
2012; 42 (2): 201-209
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1011-1237

Ovariectomy decreases biomechanical quality of skin via oxidative stress in rat

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Aim: To investigate the effect of ovariectomy on the skin using biomechanical, biochemical, and histological techniques in the ovariectomized rat model. Ovariectomy causes significant changes in the physical characteristics of the skin.

Materials and methods: Twenty female Wistar albino rats were divided into 2 groups, with each group consisting of 10 rats: the control group and the ovariectomized group. The ovariectomized group underwent bilateral ovariectomy via ventral incision and the control group underwent a sham operation. Skin biomechanics were measured 3 months following the ovariectomy with a tensile test using a biomaterial testing machine, and tensile strength, stress, strain, and toughness were calculated. Superoxide dismutase (SOD) and catalase (CAT) activities and the levels of malondialdehyde (MDA) in the skin of the rats were also measured using the biochemical methods. Thickness of the epidermis was determined by light microscopy.

Results: Strength, stress, strain, SOD activity, and thickness of epidermis values were significantly decreased and the MDA level was increased in the ovariectomized rats compared to the control group.

Conclusion: Our results showed that ovariectomy decreased the biomechanical quality of skin. This result may be related to reactive oxygen species generated by the induction of ovariectomy in rat skin damaging the connective tissue components of the dermis.

Key words: Menopause, ovariectomy, biomechanic, antioxidant, epidermis

Ovariektomi sıçanlarda derinin biyomekanik kalitesini oksidatif strese bağlı olarak azaltır

Amaç: Ovariektomi derinin fiziksel karakteristiklerinde değişikliklere neden olur. Bu çalışmada ovariektomili sıçan modeli kullanılarak ovariektominin deri üzerinde oluşturduğu biyomekanik, biyokimyasal ve yapısal değişikliklikliklerin incelenmesi amaçlanmıştır.

Yöntem ve gereç: 20 dişi Wistar albino sıçan her grupta 10 sıçan olacak şekilde kontrol ve ovariektomili olmak üzere iki gruba bölündü. Ovariektomili gruba ventral insizyon yoluyla çift taraflı ovariektomi ve kontrol gruba da sham operasyon yapıldı. Ovariektomiden üç ay sonra biyomateryal test cihazı kullanılarak germe testi ile deri biyomekaniği ölçüldü ve gerilme kuvveti, stres, strain ve dayanıklılık hesaplandı. Ayrıca deri örneklerinde biyokimyasal yöntemler kullanılarak süperoksit dismutaz (SOD) ve katalaz (CAT) aktivitesi ile malondialdehit (MDA) düzeyi ölçüldü. Epidermisin kalınlığı ışık mikroskobu kullanılarak saptandı.

Bulgular: Gerilme kuvveti, stres, strain, SOD aktivitesi ve epidermis kalınlığı ovariektomili grupta kontrol grubuna göre önemli miktarda azalırken, MDA düzeyi arttı.

Received: 08.11.2010 – Accepted: 02.03.2011

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Sonuç: Sonuçlarımız ovariektominin derinin biyomekanik kalitesini azalttığını gösterdi. Bu azalmanın sıçan derisinde ovariektominin reaktif oksijen türlerinin oluşumunu indükleyerek dermis bağ dokusunda hasara yol açması ile ilişkili olabileceğini düşündük.

Anahtar sözcükler: Menapoz, ovariektomi, biyomekanik, antioksidan, epidermis

Introduction

The skin is an organ functioning as a physical barrier to protect the body against hazards of the environment. Human skin in the postmenopausal period is affected by various factors such as senility, hormonal changes, sunlight, and smoking (1). The skin's complex mechanical properties include the elastic properties of solid materials and the viscous properties of fluids. As for the mechanical properties of the skin, tensile strength, breaking strength, strain, and toughness can be studied.

By causing hormonal changes, ovariectomy can affect the structural and mechanical changes in skin. It is possible to determine the physical characteristics of skin in ovariectomized animals and to draw conclusions by applying the obtained results to human beings. There are several studies available in the literature in which postmenopausal conditions were created through ovariectomy and the characteristics of the skin were studied (2-5); however, results of these studies are controversial. Özyazgan et al. reported a significant increase in the breaking strength, tensile strength, and Young's modulus of the skin (3). According to Brincat et al. (4) and Affinito et al. (5), ovariectomy decreased the collagen content of the skin. Because collagen is primarily responsible for the mechanical properties and strength of the skin, the strength of skin should decrease.

Reactive oxygen species (ROS) such as superoxide anion radicals, singlet oxygens, hydroxyl radicals, and perhydroxyl radicals play an important role in the pathogenesis of several diseases (6), including some skin diseases (7,8). ROS produced in excess may cause oxidative damage in biological molecules, cell membranes, and tissues. Malondialdehyde (MDA), the end product of lipid peroxidation, is a good marker of free radical-mediated damage and oxidative stress (9). Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) protect cells against oxidative stress (10).

Ovariectomized animals have been used in postmenopausal studies as a model, and an ovariectomized rat is the most frequently used animal model for postmenopausal osteoporosis in humans (11-13). The aim of the present study was to investigate the effect of ovariectomy on biomechanical, biochemical, and structural changes in skin.

Materials and methods

Animals

Twenty female Wistar rats, aged 5 months and with weights varying from 200 to 250 g, were divided into 2 groups. The first group (n = 10) was the control and the second (n = 10) was the ovariectomized group. The animals were acclimatized to laboratory conditions for 1 week prior to experimental manipulation and were exposed to a L:D photoperiod of 12:12 at a room temperature of 22 °C. They had free access to standard laboratory chow and water ad libitum. The rats in the ovariectomized group were anesthetized with ketamine hydrochloride (25 mg/kg i.p.) and xylazine (8 mg/kg, Rompun; Parke-Davis/Pfizer) before a bilateral ovariectomy was performed. With the rats of the control group, however, the abdomen was sectioned for a sham operation and closed without an ovariectomy.

Bone mineral density (BMD) measurement is widely used for detecting osteoporosis. For this reason, dual-energy X-ray absorptiometry (Norland XR-45, Norland Scientific Instruments, Fort Atkinson, WI, USA) was used with a scan speed of 1 mm/s and a resolution of 0.5 × 0.5 mm. Before taking the measurements, the instrument was calibrated with a Norland phantom. The BMD (in milligrams per square centimeter) was determined by the analysis of the femoral shaft.

The Institutional Animal Care and Use Committee at Mersin University's Medical Faculty approved the experiments described in this study, and care and experimentation of the animals were in accordance with National Institutes of Health principles of laboratory animal care. After 3 months, the rats were sacrificed with an overdose of ketamine hydrochloride and skin samples were excised. Tissue samples were used for biomechanical, biochemical, and histological evaluations.

Biomechanical test

The biomechanical properties of the skin were investigated using a tensile testing machine (MAY 03, BIOPAC, Santa Barbara, CA, USA) equipped with a 50-kg load cell. The tensile loading speed in all of the tests was 1 mm/min. Data were transferred to computers and translated to numerical signals by a 16-bit A/D converter for off-line analysis. The sampling rate was chosen as 1000 samples/s. Each specimen was subjected to a small initial preload (0.1 N) before actual testing. Load-displacement data were recorded using the BIOPAC MP100 Acquisition System Version 3.5.7 (BIOPAC). Strength represents the maximum tensile force applied until breaking occurred. Load-displacement recordings were normalized by a cross-sectional area, and this curve was converted to a stress-strain curve. Stress-strain curves for each specimen were generated and the ultimate stress, ultimate strain, and toughness were determined. The ultimate stress was calculated from the following equation (14):

$$\sigma = F/A,$$

where σ is the ultimate stress (MPa), F is the failure load (N), and A is the cortical area of the specimen (m^2).

The ultimate strain was calculated from the following equation:

$$\varepsilon = \Delta L/L_0.$$

Here, ε is the strain, ΔL is the change in the length (mm), and L_0 is the original length.

Elastic modulus was calculated from the following equation:

$$E = \sigma/\varepsilon.$$

Biochemical assay

After excision, fresh skin samples were homogenized with 50 mM phosphate buffer (pH 7.4). Homogenates were then centrifuged at 10,000 × *g* for 15 min at 4 °C. Supernatants were separated and kept at –20 °C until the enzyme activity and MDA measurements were performed.

Skin MDA level, as an index of lipid peroxidation, was determined by thiobarbituric acid reaction according to Yagi's method (15). This method relies on the measurement of the pink color produced by the interaction of thiobarbituric acid with MDA as a result of lipid peroxidation. The colored reaction of 1,1,3,3-tetraethoxypropane was used as the primary standard.

Tissue SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2^- generated by the xanthine/xanthine oxidase system as according to Sun et al. (16). One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate.

CAT activity of tissues was determined according to the method of Aebi et al. (17). The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. The values were expressed as IU per gram of wet tissue weight.

Tissue protein content was determined according to the method developed by Lowry et al. (18) using bovine serum albumin as standard.

Histological evaluation

Skin samples were placed in 10% formalin. Routine tissue processing for light microscopy was performed on all of the specimens. The skin samples were embedded in paraffin. Sections (5 μm) were cut by microtome and stained with Masson's trichrome. These sections were examined with an Olympus BX50 light microscope (Olympus Corporation, Tokyo, Japan). For all of the samples, 3 different areas were photographed with a digital camera (Nikon Coolpix 5000, Nikon, Tokyo, Japan). Ten areas were randomly selected and used for thickness of epidermis, dermis,

and hypodermis measurements. Measurements were performed using commercially available software (Item 5.0 Software, Olympus Soft Imaging Solutions GmbH, Münster, Germany).

Statistical analysis

Statistical analysis was performed using commercially available software (SPSS v. 10.0, SPSS Inc., Chicago, IL, USA). The data were expressed as a mean ± standard error. After documenting normal distribution (Kolmogorov-Smirnov), the statistical comparisons were performed using the Mann-Whitney U test. Values of $P < 0.05$ were considered statistically significant.

Results

The BMD values were 0.177 ± 0.012 in the control group and 0.112 ± 0.032 in the ovariectomized group. The BMD value in the ovariectomized group was significantly lower than that of the control group ($P < 0.05$).

The parameters investigated for biomechanical properties were strength, stress, strain, and toughness values. Their mean values and standard errors are represented in Figures 1, 2, 3, and 4, respectively. All of these parameters were observed to be significantly decreased ($P < 0.05$) in the ovariectomized group compared to the control group. The skin MDA level was increased almost 2 times in the ovariectomized group compared to the control group ($P < 0.05$) (Table 1). There were no significant differences for CAT activity between the ovariectomized and control groups ($P > 0.05$). Skin SOD activity decreased by 64.5% in the ovariectomized group compared to the control group (Table 1). The general appearance of the control and ovariectomized rat skins are shown in Figure 5. In the ovariectomized group, the epidermal thickness was significantly lower than that of the control group ($P < 0.05$) (Figure 6, Table 2). However, when dermal (Figure 7) and hypodermal thickness were compared (Figure 8), no significant difference was found between the control group and the ovariectomized group (Table 2).

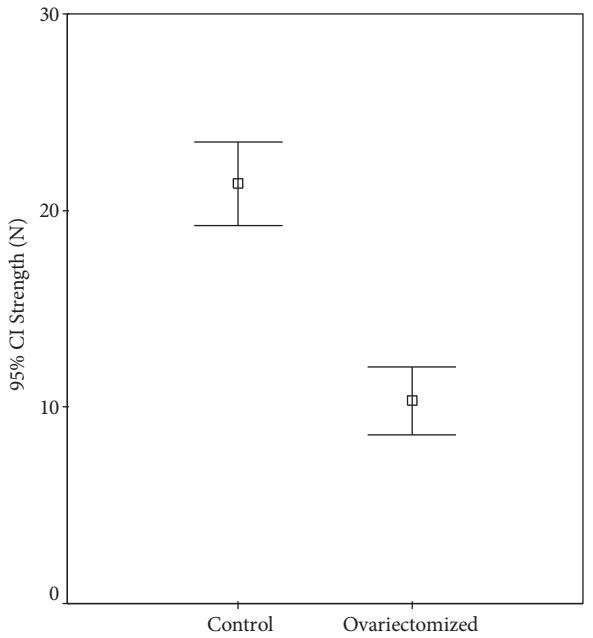

Figure 1. Mean skin strength values of groups. CI = confidence interval. Bars represent means ± standard errors. Strength was measured as 21.35 ± 2.12 and 10.31 ± 1.72 in the control and ovariectomized groups, respectively. There was a significant difference between the groups ($P = 0.04$) for strength value.

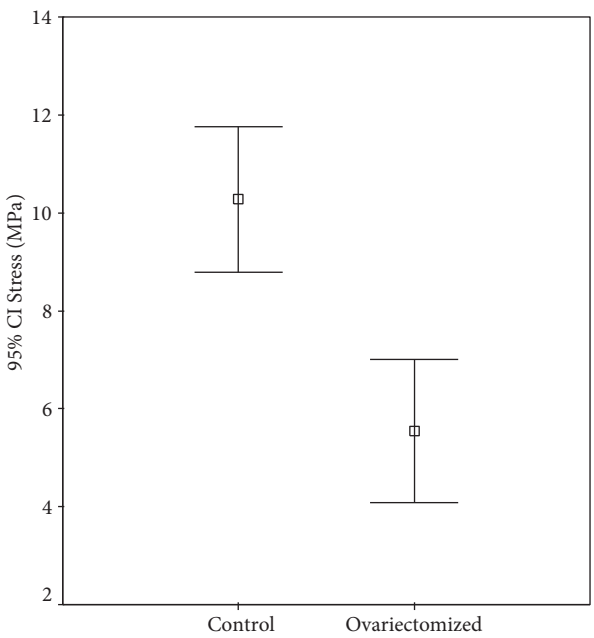

Figure 2. Mean skin stress values of the control and ovariectomized groups. CI = confidence interval. Bars represent mean ± standard errors. Stress was measured as 10.27 ± 1.48 and 5.54 ± 1.46 in the control and ovariectomized groups, respectively. There was a significant difference between the groups ($P = 0.039$) for stress value.

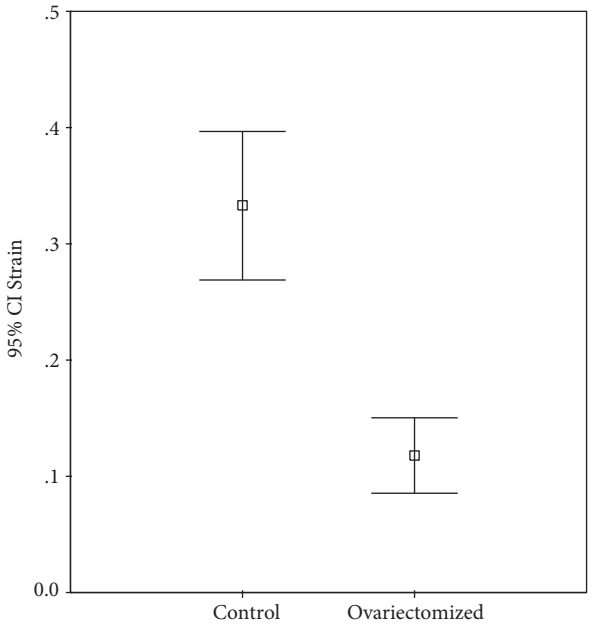

Figure 3. Mean skin strain values of control and ovariectomized groups. CI = confidence interval. Bars represent means ± standard errors. Strain was measured as 0.33 ± 0.06 and 0.11 ± 0.03 in the control and ovariectomized groups, respectively. There was a significant difference between the groups (P = 0.028) for strain value.

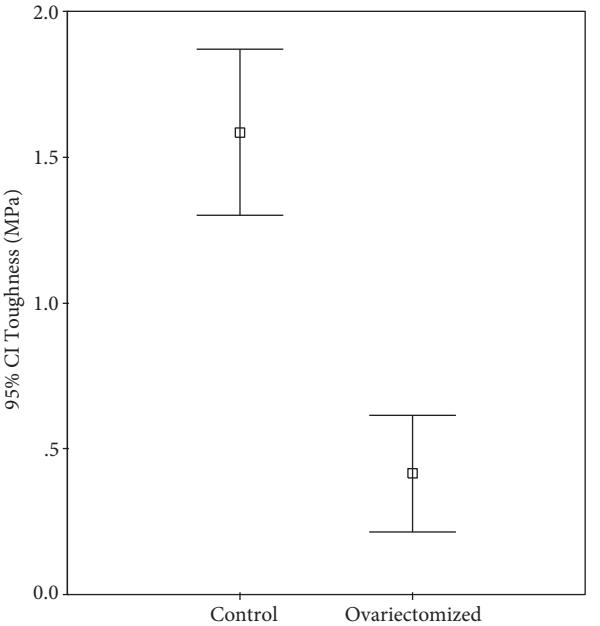

Figure 4. Mean skin toughness values of the control and ovariectomized groups. CI = confidence interval. Bars represent mean ± standard errors. Toughness was measured as 1.58 ± 0.28 and 0.44 ± 0.19 in the control and ovariectomized groups, respectively. There was a significant difference between the groups (P = 0.02) for toughness value.

Table 1. Mean MDA concentration and CAT and SOD activity in control and ovariectomized groups.

Variables	Control (n = 10)	Ovariectomized (n = 10)
MDA (nmol/mg protein)	70.48 ± 2.28	139.56 ± 5.84*
CAT (U/mg protein)	44.76 ± 3.19	40.88 ± 5.04
SOD (U/mg protein)	10.06 ± 1.4	5.23 ± 3.19*

*Significantly different from control (P < 0.05).

Discussion

In this study, we investigated the effect of ovariectomy on skin biomechanics, skin structure, and the skin antioxidant defense system. Compared with the control group, the ovariectomized group showed decreased formation in skin strength, stress, strain, toughness, and epidermal thickness; an increase in skin MDA; and a decrease in SOD levels. Thus, these results show that ovariectomy provokes significant structural, biomechanical, and biochemical changes at 3 months postsurgery.

The mechanical properties of skin are largely associated with collagen fibers (19,20). Affinito et al. reported decreases after menopause not only in the amounts of types I and III collagen independent of each other but also in the ratio of type III to type I (5). Brincat et al. found that skin collagen content decreased in postmenopausal women (4). Kafantari et al. investigated the effect of ovariectomy in rat skin and found that ovariectomy decreased skin collagen (2). The result of these structural changes is that the tensile strength and elasticity of the skin

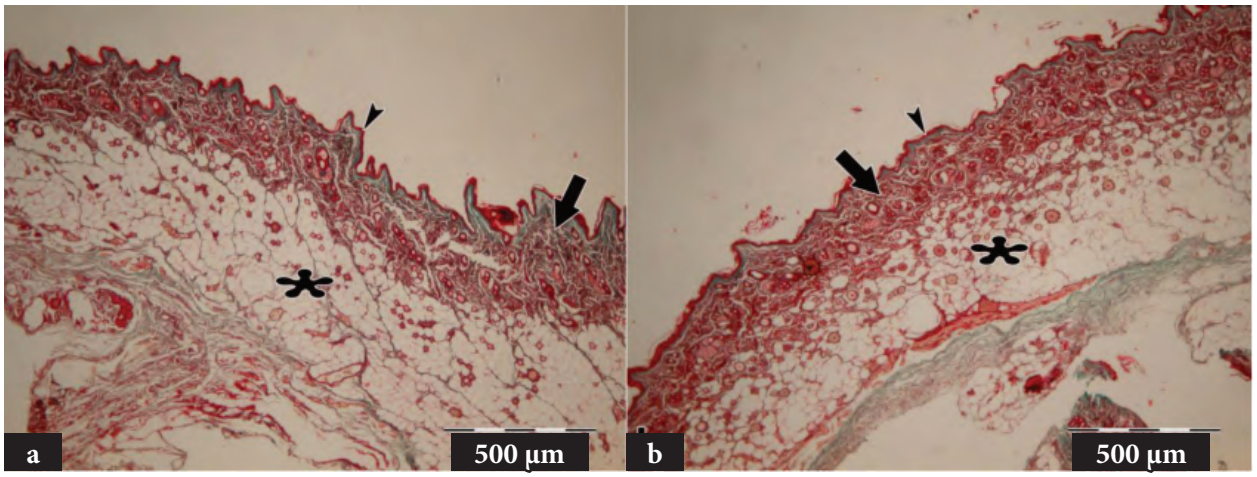

Figure 5. General appearance of skin: a) control group, b) ovariectomized group. Epidermis is shown with arrow head, dermis with arrow, hypodermis with asterisk. Rat skin samples were stained with Masson's trichrome and examined with an Olympus BX50 light microscope, 120×.

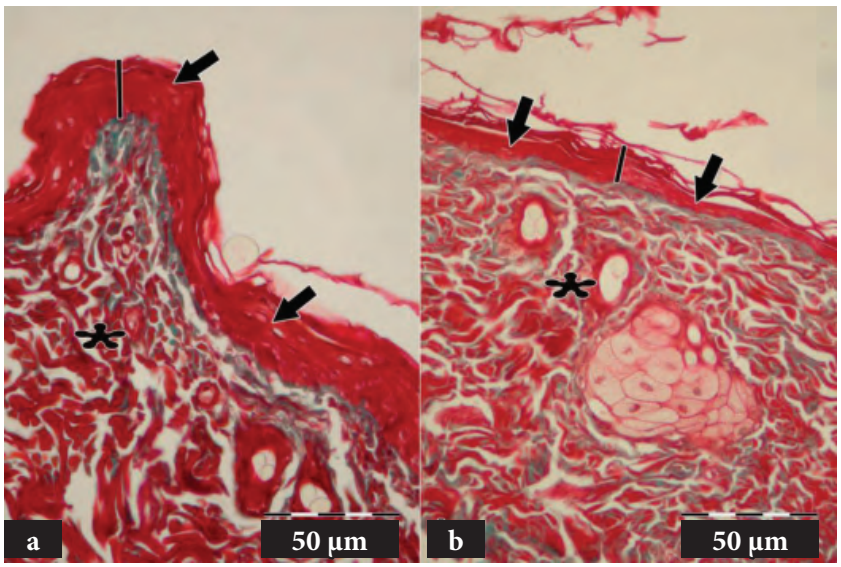

Figure 6. Epidermal thickness (black line) in a) control group and b) ovariectomized group. Epidermis is shown with arrow, dermis with asterisk. 1200×.

are attenuated (21). Our biomechanical findings in ovariectomized rats are consistent with those of these studies.

In the present study, our data show that the thickness of epidermis was decreased by 55.5% in the ovariectomized group when compared to the control group. Epidermis thickness is an important factor in the monitoring of skin health, aging, and photodamage (22). The thinning of the epidermis may affect the epidermal functions (barrier, lining, and protecting). Variations in epidermal thickness may influence penetration of ultraviolet (UV) radiation into the dermal layers. The amount of radiation passing through a specific area is inversely proportional to the square of the distance of that area from the energy source (23). This condition may affect the mechanical behavior of the dermis. The mechanical behavior of the dermis dominates the

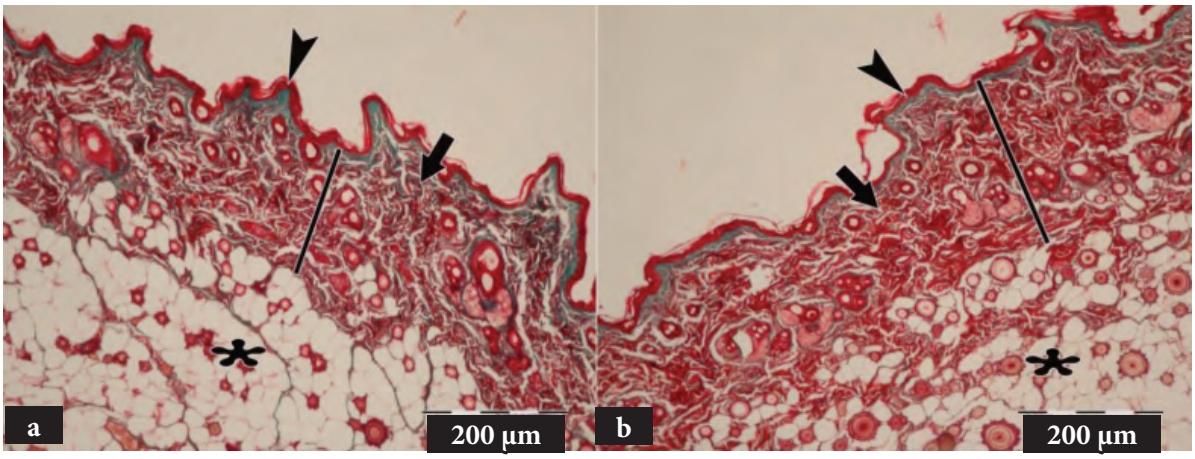

Figure 7. Dermal thickness (black line) in a) control group and b) ovariectomized group. Epidermis is shown with arrow head, dermis with arrow, hypodermis with asterisk. 300×.

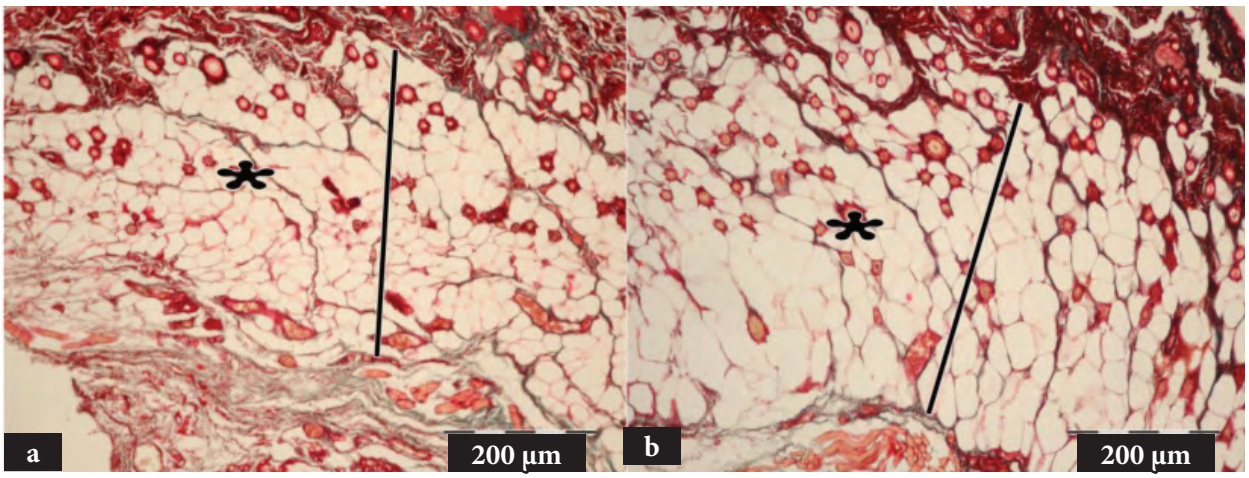

Figure 8. Hypodermal thickness (black line) in a) control group and b) ovariectomized group. Hypodermis is shown with asterisk. 300×.

Table 2. Mean epidermal, dermal, and hypodermal thickness in control and ovariectomized groups.

Variables	Control (n = 10)	Ovariectomized (n = 10)
Epidermal thickness (nm)	8.26 ± 1.23	4.59 ± 0.84*
Dermal thickness (µm)	0.81 ± 0.064	0.84 ± 0.046
Hypodermal thickness (µm)	1.11 ± 0.25	1.39 ± 0.41

*Significantly different from control ($P < 0.05$).

mechanical behavior of skin in normal conditions (24). The mechanical properties of skin are largely associated with the collagen fibers. Reduced mechanical properties may be related to the decrease of collagen synthesis or functional properties of collagen fibers (25-27). We found that the biomechanical parameters of the skin were reduced in the ovariectomized group. . Therefore, we think that these decreases may be related to skin collagen.

Our data showed that the level of MDA in skin was increased and SOD activity was decreased by ovariectomy. The increase in hydrogen peroxide might have induced the peroxidation of polyunsaturated fatty acids and led to the formation of MDA, one of the by-products of lipid peroxidation. Since MDA has high reactivity toward amino groups, it inhibits the synthesis of nucleic acids and proteins and also deactivates the enzymes (28). Thus, the decrease in skin antioxidant enzymes observed after ovariectomy may be due to heightened lipid peroxidation and the absence of estrogen. In the absence of estrogen, antioxidant enzyme activity decreases and hence oxidative tissue damage increases. In addition, the surface of the skin is vulnerable to ROS produced by physical processes, such as photochemical reactions with UV light and ionizing radiations. Elevation in lipid peroxidation may be dependent on a decrease of the epidermis thickness. Generally, attention has been focused on lipid peroxidation in various tissues induced by ovariectomy (29-31), but to our knowledge, there is no study investigating the effect of ovariectomy on lipid peroxidation in skin.

In conclusion, we suggest that ROS generated by induction of ovariectomy in rat skin damaged the connective tissue components of the dermis, which likely influences cell behavior via cell-matrix interactions. Therefore, the biomechanical properties of the skin were decreased.

Acknowledgment

The study was supported by the Mersin University Scientific Research Foundation.

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