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The Effects of Vitamin E and Hormone Replacement Therapy on Serum Leptin Levels of Female Rats After Ovariectomy*

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To evaluate the effects of hormone replacement therapy and vitamin E on weight and serum leptin levels in female rats before and after surgical menopause.

A total of 38 Wistar Albino female rats with an average weight 200 g were divided into five groups randomly; sham group, ovariectomy + saline group, ovariectomy + 17ß–estradiol group, ovariectomy + 17ß–estradiol + progesteron group, ovariectomy + vitamin E group. At the end of the study, blood was taken by decapitation and serum estradiol, progesteron and leptin levels were measured. Kolmogorov Smirnov Z and One Way Anova tests were used in statistical analyses. $p < 0.05$ was considered statistically significant.

Weight gain in rats after ovariectomy after third month was not significantly higher than the control. However, leptin levels were found to be significantlyu decreased in the ovariectomised group compared to control group. Serum leptin levels were increased significantly in the ovariectomy + estradiol and ovariectomy + estradiol + progesteron groups but decreased in the vitamin E group. No weight change was observed in estradiol, estradiol + progesteron or vitamin E groups.

The decrease in leptin levels after ovariectomy in rats can be prevented by estrogen and/or progesteron treatment. Vitamin E may not prevent the decrease in serum leptin levels due to menopause.

Key Words: *Ovariectomy, estradiol, progesterone, leptin, vitamin E.*

Dişi Ratlarda Ovariektomi Sonrası Vitamin E ve Hormon Replasman Tedavisinin Serum Leptin Düzeylerine Etkisi

Dişi ratlarda cerrahi menopoz öncesi ve sonrası hormon replasman tedavisi ve vitamin E kullanımının, ağırlıklarına ve serum leptin düzeylerine etkilerinin incelenmesi.

Ortalama ağırlıkları 200 g olan 38 adet Wistar Albino cinsi dişi rat, randomize şekilde beş gruba ayrıldı; sham grubu, ovariektomi + Serum Fizyolojik grubu, ovariektomi + 17ß–estradiol grubu, ovariektomi + 17ß–estradiol + progesteron grubu, ovariektomi + vitamin E grubu. Deney sonunda dekapitasyonla kanları alınan ratların serumlarında estradiol, progesteron ve leptin düzeyleri ölçüldü. İstatistiksel analizlerde Kolmogorov Smirnov Z test ve One Way Anova testleri kullanıldı. $p < 0.05$ istatistiksel olarak anlamlı kabul edildi.

Ovariektomi sonrası ratların ağırlıklarında üç ayın sonunda kontrol grubuna göre anlamlı artış olmadı. Leptin düzeyleri ovariektomili grupta sham grubuna göre düşük bulundu. Estradiol, estradiol + progesteron verilen ratlarda serum leptin düzeylerinde belirgin artış olurken, vitamin E grubunda düşüş gözlendi. Östrojen, östrojen + progesteron, vitamin E alan ratların ağırlıklarında anlamlı bir değişim olmadı.

Ovariektomi sonrası leptin düzeylerindeki azalma, östrojen ve/veya progesteron tedavisi ile önlenebilir. Vitamin E, menopozla ortaya çıkan leptin düzeylerindeki azalmayı önleyemeyebilir.

Anahtar Kelimeler**:** *Ovariektomi, estradiol, progesteron, leptin, vitamin E.*

Introduction

As estrogen levels in women decrease with menopause, testosterone level increases. This situation causes central obesity and the change of fat distribution in body (1). The obesity seen at ovariectomized rats also supports these findings. In addition, the increase in hypertrophy of adipocyte and epidermal growth factor levels in these animals explain the reason for obesity. It has been shown that there is an increase in number of estrogen receptors in adipose tissue and this can be reversed by estrogen replacement therapy (2-4).

The mechanism between changes in body fat distribution and body weight as a result of estrogen loss occurred in menopause could not be explained completely. In many studies it has been emphasized that there is relation between the

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synthesis and release of estradiol and leptin. It has been shown that estrogen given to post menopausal women cause more leptin synthesis from omental fat tissue and increase leptin serum levels (5-7).

Leptin is adipostatic and antiobesity hormone synthesized from fat tissue (8). It is coded by obesity gene and a hormone in polypeptide structure with 16 kilodalton weight, composed from 167 amino acids. The obesity gene in human is localized in 7q31 and its DNA consists of 1500 basic couples. Leptin being a product of Ob gene makes duty as weakening hormone, controlling food intake (9-11).

Vitamin E (alfa-tocopherol acetate) is a significant antioxidant for biological systems. It has significant roles in the protection of cell membrane integrity. It is an important vitamin melting in water and fatty acids are needed for its absolute absorbtion (12, 13). Tocopherol's highest antioxidant activity is in its α-tocopherol form. Alfa-tocopherol prevents the formation of free radicals, hindering lipid peroxidation (14). Epidemiological studies has shown that in general population and postmenopausal women, the risk of having heart disease is lower for the persons consuming a diet rich in vitamin E (15).

The relation between vitamin E and estrogen could not be revealed completely. The decrease in serum vitamin E levels has been determined with respect to women using oral contraceptive including estrogen (16). In addition, vitamin E can be beneficial to postmenopausal women (17).

In our study, we have examined the effect of treatment of hormone replacement and use of vitamin E on weights, serum, leptin, estradiol and progesterone levels of these rats. In this way we aimed to examine the effects of estrogen and/or progesterone and also alternatively vitamin E treatments used in menopause on serum leptin levels and compare these with control groups. Such a treatment is recommended as an alternative to these agents and may have preventive roles for some negative results (such as cardiovascular disease) of menopause.

Materials and Methods

A total of 38 female Wistar Albino rats having average weight of 200 g (180-220) were used in the study. Rats were preserved in the laboratory of test animals of our faculty. Rats were hold in plastic cages in four and five groups in the laboratory with air conditioning system. Ambient kept in room temperature. Rats hold in 12 hours light and 12 hours darkness. Rats were fed with ready pellets in special steel pots and their waters were given by ball feeding bottle made from stainless steel. The cages of rats were cleaned daily. Rats were divided into 5 groups randomly:

Group 1 (n = 7): In this group, sham (only abdomen opened and closed, ovariectomy was not made) operation was applied to rats with 206 gr. average weight under general anesthesia (giving Rompun/Ketamine; ¾, 0.4 ml/rat im). Rats received no medical treatment. At the end of the study, decapacitation was made and their serums were taken in glass tubes.

Ovariectomy

Ovariectomy was performed in 31 rats other than rats in sham group. After application of general anesthesia (giving Rompun/Ketamine; ¾, 0,4 ml/rat im), the hairs on lower abdomen region of rats were shaved, abdomen was opened through a midline incision. Tubes were found and connected bilaterally and ovaries were taken out. After completing operation, the abdomen of rats were closed and waited for their healing. Rats in these groups were divided into four subgroups following the healing period.

Group 2 (n = 9): In this group, ovariectomy was performed in the way that was mentioned previously to rats with an average weight of 246 gr. After 3 months of healing period, 0,2 ml/rat/day SF was applied under skin during one month. Decapacitation was made at the end of one month and blood was taken to normal glass tubes.

Group 3 (n = 7): In this group, ovariectomy with the same technique was performed to rats having an average weight of 260 gr. and after 3 months healing period, 17β Estradiol in the dose of 30 mg/kg/day was administered under skin for a period of one month. Decapacitation was made at the end of one month and blood sample was taken in normal glass tubes from each rat.

Group 4 (n = 7): In this group, rats had an average weight of 262 gr. After 3 months of a healing period following ovariectomies, 30 mg/kg/day 17β Estradiol + 2 mg/rat/day progesterone was given under skin for one month. Blood sample was obtained by decapitation at the end of one month.

Group 5 (n = 8): Rats in this group with an average weight of 240 gr underwent ovariectomy and after 3 months of healing period, 12,5 mg/day intraperitoneal vitamin E was administered for one month. Decapacitation was made at the end of one month and blood was taken in normal glass tubes.

Rats were weighed before operation, before giving the test agents and before predecapacitation. In order to prevent the formation of stress, decapitation was performed within 5-10 seconds. While consequtive decapitation operations were made, guillotine was washed by tap water, cleaned and dried before each operation. The bloods of animals taken by decapitation were placed in normal glass tubes, and kept at +4 °C in 10 minutes. It was centrifuged for 10 minutes. Serums separated were preserved at –20 °C in deep freeze to measure leptin, estradiol and progesterone levels.

The measurement of leptin, estradiol and progesteron levels in blood serum samples taken from rats was made by radioimmune assay system.

In statistical examination, at first it has been assessed that whether data shows normal in-group distribution by Sample Kolmogorov Smirnov Z test from nonparametric tests. When it was shown that all groups have normal distribution, the comparison among groups were made by One Way ANOVA test. Data was analyzed by post-hoc Tukey test. Significance level was accepted $p < 0.05$. The analysis of data was made by SPSS 15 Windows packaged software.

Results

Weight measurement of 38 rats was made at the begining of our study and average weight was found to be 200 g (180-220). Weight measurements before and after drugs administrations were shown in Table 1.

Table 1. Average weights and their standard deviations of rats before and after medicine.

	Before medicine (g)	After medicine (g)
1. group (Sham)	206,42 ± 18,8	206,42 ± 18,8
2. group (Ovx + SF)	262,22 ± 9,3	257,77 ± 15,8
3. group (Ovx + E2)	246,42 ± 24,1	245,00 ± 19,5
4. group (Ovx+ E2 + Prog)	260,71 ± 43,4	240,00 ± 25,1
5. group (Ovx + vit E)	240,62 ± 25,5	237,50 ± 29,1

The average weights of 7 rats which sham operation was made was 206 g before the medicine to other groups, it was measured 206 g at the end of test. A statistical difference was not determined.

Daily SF, steadily estradiol + progesteron and vitamin E injections were made to 31 rats which ovariectomy was applied during 1 month. At the end of 1 month, weight measurements were made again. While the average weight of SF group was 262 g, it was measured 258 g before decapitation. A statistical difference was not determined.

The average weight of 7 rats which received estradiol before treatment was measured as 246 g, whereas their weights were measured 245 g after the treatment. A statistical difference was not determined.

Although the average weights of the group which estradiol + progesteron were given decreased from 260 g to 240 g, the difference was not found statistically significant.

The average weight before giving medicine to 8 rats which vitamin E was administered was 240 g, whereas their weights after medicine were measured as 237 g. No statistically significant difference was determined.

Serum leptin levels were shown in table 2. There was a statistically significant difference between sham group and other four groups with respect to serum leptin levels ($p<0.05$). On the contrary, no statistically significant difference was present between the group which estradiol was given and the group which estradiol and progesteron were administered ($p=0.898$). It has been determined that serum leptin level decreased in SF group and vitamin E group and increased in the group which estradiol was given and the group which estradiol and progesterone were given compared to control group.

Table 2. Average values of serum leptin, estradiol, and progesteron levels for all groups and their standard deviations.

	Leptin level (ng/ml)	Estradiol level (pg/ml)	Progesteron level (ng/ml)
Sham	3,00 ± 0,05	65,00 ± 2,6	0,47 ± 0,06
Ovx+SF	1,87 ± 0,07	33,55± 2,9	0,13 ± 0,02
Ovx+E2	3,65 ± 0,05	130,00 ± 4,5	0,15 ± 0,01
Ovx+E2+Prog.	3,68 ± 0,06	134,42 ± 3,6	0,52 ± 0,03
Ovx+vit.E	1,99 ± 0,03	36,75 ± 2,49	0,14 ± 0,01

After ovariectomy, average estradiol levels in the group which estradiol was given and in the estradiol plus progesterone were found to be significantly higher than the control group. However, when the group which estradiol was given and the group which estradiol and progesteron were given compared between each other, statistically significant difference was not determined ($p=0.156$). Estradiol levels were significantly decreased in the groups which vitamin E and SF were given compared to control group after ovariectomy. In the comparison of both groups, a significant difference was not seen in either in terms of the decrease in estradiol levels. ($p=0.357$). All data was summarized in table 2.

Progesteron levels and their standard deviations were shown in Table 2. Statistically significant difference was determined only for the group which estradiol and progesteron were given compared to control group. Statistically significant decrease was determined for other groups. When these three groups were compared, statistically significant difference was not found between each other.

Discussion

The synthesis and release of leptin increase proportional to increasing body fat mass. It has been shown that leptin blood level is two fold more in obese persons compared to non-obese. At the same time, it has also been shown that overnutrition increase leptin synthesis and release through excess release of insulin and cortisone (18, 19). It was stated that the most significant hormonal factor affecting leptin release and synthesis may be insulin. It was determined that long term hyperinsulinemia stimulate leptin secretion in abdominal adipocytes and cause increase in serum leptin levels (19).

In ovariectomised rats and women, estrogen replacement therapy increases ob gene mRNA expression and thereby raises leptin production in retroperitoneal and subcuteneus adipose tissue (20). This study is significant because it shows the effect of

estrogen on production of leptin in women who is in menopause and having changes occurred in fat distribution.

It is not known definitely that which mechanism or mechanisms increase leptin levels. However, it is thought that estrogen shows its effect with the effects of direct leptin synthesis and release from binding proteins and adiposits in serum (21). Estrogen has direct effects for leptin synthesis and release. As shown by Murakami *et al.* (22) it is known that estrogen cause the increase in adiposits and ob mRNA. On the other hand, in an *in vitro* study carried out by Casabiella *et al*. (23) it was shown that estrogen cause release of leptin from omental fat tissues, which is contrary to the same effect in omental tissues of men. In addition, Montaque *et al.* (24) has shown that subcutaneous fat tissue synthesize more mRNA than intraabdominal fat tissue and that's why women in the period before menopause have two fold more leptin level compared to men. However, all these data partially explain the relation between leptin and estrogen.

We have observed in our study that serum estradiol levels have a linear relation with serum leptin levels and 17ß Estradiol given from outside the body cause an increase in leptin levels. In our study, in order to evaluate the effect of progesteron on leptin levels, we have applied progesterone in addition to estradiol. Despite of an increase in leptin levels was found, no statistical difference was determined between both groups was not available. This effect of progesteron can be formed due to its use together with estradiol. Thus, new studies are needed to observe the further effects of progesteron.

Messinis *et al.* (6, 25) have shown that leptin levels increases in ovariectomised women and in women with normal menstrual cycle when estrogen and progesteron are given together. However, this effect was not observed when estradiol given alone. In addition Hardie *et al.* (26) have found a significant relationship between leptin and progesteron levels in the study which they have carried out on women having normal menstruation or pregnancy. It has been suggested based on all these factors that estradiol prepares adipocytes to stimulate leptin production through follicular phase of menstrual cycle. Therefore it has been thought that the main factor providing production of leptin is not estradiol but can be progesterone (25). In addition, why leptin follows higher values in luteal phase compared to follicular phase can be explained by this hypothesis. However there is not enough study made on this subject at molecular and histopathological level. We have observed in our study that only estradiol increase serum leptin levels.

In addition, we have examined whether estradiol and progesteron and vitamin E has any effects on changes in the body weight. We determined no significant increase in the body weights of rats after ovariectomy. Although externally administered estrogen and/or progesteron increased leptin levels in the rats, no change in the weights of ovariectomized rats were detected. Probably, in this study, duration of application of agents were not sufficient to observe a significant effects on body weights. Therefore, comprehensive and long term studies are needed to further evaluate this reason.

In this study, we examined the effect of vitamin E melting in fat and its role for a significant antioxidant on body weight and serum leptin, estradiol and progesteron levels. Although it is known that vitamin E, melting in fat, has preventive effect on coronary heart disease (15), it is still not known completely that whether a relation among vitamin E and estrogen and obesity exists. Nirwana *et al.* (27) evaluated the relation between vitamin E and serum leptin levels and shown that vitamin E deficiency cause decrease in body weight in normal and ovariectomized rats. However the relation of serum leptin levels and vitamin E deficiency and its treatment have not been examined. We have observed in our study that the vitamin E treatment in ovariectomized rats did not cause any change in their weights but caused decrease in serum leptin, estradiol and progesteron levels. This effect can be dose and time dependent.

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