Research Paper

Effects of angiotensin converting enzyme inhibition on adiponectin levels and lipid profile in the ovariectomized-aged rats

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ABSTRACT

Objective: To investigate the relationship between angiotensin converting enzyme (ACE) and adiponectin and lipid profile in the ovariectomized-aged rats. **Materials and Methods:** Wistar albino rats were first divided into two groups; control (C) and ovariectomized (OVX). Bilateral ovariectomy were carried out on rats (n = 30) except control group (n = 10). After 6 weeks from ovariectomy, ovariectomized rats were subdivided into three groups; one group received no treatment (OVX), two groups received low dose (OVX + Cap5; 5 mg/kg/day) and high dose (OVX + Cap20; 20 mg/kg/day) captopril (Cap). Body weights were monitored weekly. Adiponectin, triglyceride, cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and very low density lipoprotein cholesterol (VLDL-C) levels were measured at the end of the 6 weeks. **Results:** In the OVX group, body weights increased (P < 0.001). In the OVX + Cap20 group, body weights significantly decreased compared with the OVX group during weeks 5 and 6 (P < 0.05). While adiponectin levels increased in the OVX + Cap5 group (P = 0.014), triglyceride and cholesterol levels decreased in the OVX + Cap20 group (P = 0.016 and P < 0.001, respectively) compared to the OVX group. HDL-C and VLDL-C levels decreased only in OVX + Cap20 group (P < 0.005). **Conclusions:** ACE inhibitors may be decreasing the ovariectomy-induced weight gain by increasing adiponectin levels, and by affecting lipid profiles. The adipose tissue renin-angiotensin system (RAS) may be playing an important role in the development of adiposity.

Key words: Adiponectin, angiotensin converting enzyme, cholesterol, ovariectomy, triglyceride

INTRODUCTION

During menopause, with the dramatic fall in serum estrogen levels, together with relative hyperandrogenism which contribute to weight gain and changes in adipose tissue distribution, women tend to gain body fat.[1,2] It was shown that estrogen reduced adiposity, at least in part, in ovariectomized-mice by promoting the use of lipid as fuel. Estrogen was reported to cause this effect via enhancement of pathways that promote fat oxidation in muscle, by inhibiting fat storage (lipogenesis) in adipose tissue, liver and muscle, and increasing rates of adipocyte lipolysis.[1] The lack of estrogen causes obesity and metabolic syndrome after menopause. It is also one of the reasons for insulin resistance, dyslipidemia (elevated triglycerides, small dense low density lipoprotein cholesterol (LDL-C) particle, and reduced high density lipoprotein cholesterol (HDL-C)),

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elevated blood pressure, and increased hypercoagulability and proinflammatory state in blood.[3] It is known that estrogen prevents the progression of atherosclerosis by decreasing LDL-C, and increasing HDL-C plasma levels.[4]

Adiponectin, derived from adipose tissue, has roles in both carbohydrate and lipid metabolisms. Plasma adiponectin levels are affected by various factors and metabolic parameters. Adiponectin levels were reported to decrease in conditions such as obesity, type II diabetes, and atherosclerosis; and were shown to positively correlate with HDL-C levels.[5-7] Adiponectin levels are sex-dependent and were shown to be two- to three-fold higher in women than men.[8]

Angiotensin II type 1 (AT1) receptor which mediates most biological effects of angiotensin II (Ang II); such as vasoconstriction, aldosterone release, sodium and water retention, and cellular growth is one of the major components of the renin-angiotensin system (RAS). Estrogen induces down regulation of AT1 receptor expression, and shows protective effect against hypertension and atherosclerosis. Risks of cardiovascular disease and atherosclerosis increase in the menopause, because activation of AT1 receptor plays a key role in the regulation of blood pressure and fluid homeostasis.[9]

Several studies show that angiotensin converting enzyme (ACE) inhibitors change plasma adiponectin levels by affecting the hormonal function of adipose tissue.[10-12] Thus, ACE inhibitors which are frequently used for the treatment of hypertension and atherosclerosis in the postmenopause might lead to change of circulating adiponectin levels. Therefore, we investigated the effects of ACE inhibition on adiponectin levels and lipid profile in ovariectomized-aged rats.

MATERIALS AND METHODS

Six-month-old adult female Wistar albino rats (220 ± 25 g; $n = 40$) were obtained from the Experimental Animal Center, Adnan Menderes University, Aydin, Turkey. The protocol for the experiment was approved by the Animal Experimentation Ethics Committee of Medical School (2007/0056). Adult female Wistar rats were kept in conventional room with controlled light (12:12, dark:light), temperature (22 ± 1°C), relative humidity (40-50%), and ventilation (15 air changes per hour). Food and water were given *ad libitum*. They were allowed to adapt to their environment for one week prior to the experiments.

Rats were first divided into two groups; control (C) and ovariectomized (OVX). Rats in control group ($n = 10$) were not ovariectomized and did not receive any treatment. In the study group, ovariectomies were carried out intraperitoneally with ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg). The skin area was shaved from a hip to the lowest rib. Bilateral incisions were performed, and ovaries and the fat tissue around them were removed. Incision on the muscles and skin was sutured.[13]

After at least 6 months of recovery from the operation, ovariectomized rats were divided into three subgroups of ten animals each. First group (OVX) was given only water ($n = 10$). Second group of OVX rats received captopril (5 mg/kg/day) (OVX + Cap5; $n = 10$). Third group of OVX rats received captopril (20 mg/kg/day) (OVX + Cap20; $n = 10$). Captopril (C4042, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in water and given orally (p.o.) for 6 weeks. Body weights were monitored weekly.

Animals were decapitated under ketamine and xylazine anesthesia after 6 weeks. Blood was taken from the heart by midline laparotomy. Adiponectin, triglyceride, total cholesterol, HDL-C, LDL-C, and very low density lipoprotein cholesterol (VLDL-C) levels in blood were measured.

Laboratory assays

Blood samples were centrifuged at 4,000 rpm for 10 min at room temperature. Sera were removed and stored at -80°C for later studies.

Serum total cholesterol, HDL cholesterol, and triglyceride levels were determined with autoanalyzer (Architect C 8000, Abbott, Abbott Park, IL, USA). LDL-C and VLDL-C levels were calculated using "Friedewald" and "triglyceride/5" formulas, respectively. Rat adiponectin concentrations were determined with an ELISA kit (Linco Research Inc., St Charles, MO, USA) using a nonradioactive detection method.

Data analysis

Experimental values are expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by "Student-Newman-Keuls" multiple comparison test was used to compare the study groups. P values of less than 0.05 were considered statistically significant.

RESULTS

Weekly body weight changes

Changes in body weights were assessed within each group. Weights in OVX group increased throughout 6 weeks and changes were statistically significant in the later weeks compared to the first two weeks ($P < 0.001$). Weight gain was statistically significant in the OVX + Cap5 and OVX + Cap20 groups only in the 6th week when compared to week one ($P < 0.001$). Body weight changes in captopril-intake groups were not statistically significant from 3rd week onwards [Figure 1].

When changes in the body weights among groups were compared, the differences in the body weight between

the groups OVX and C were statistically significant from 3rd week on ($P < 0.05$), due to an increase in body weights in the OVX group. The increase was statistically significant in the OVX + Cap5 group compared to the control group in the 4th and 5th weeks ($P < 0.05$). In the OVX + Cap20 group, there was a statistically significant decrease in body weights compared to the OVX group ($P < 0.05$) [Figure 2].

Adiponectin

Plasma adiponectin levels increased in OVX group without any statistical significance. Adiponectin levels were higher in OVX + Cap5 group compared to the other three, being statistically significant between OVX + Cap5 group and controls. Adiponectin levels significantly decreased in the OVX + Cap20 group compared with the OVX + Cap5 group ($P = 0.014$) [Figure 3].

Lipid profiles

Triglyceride

Triglyceride levels were higher in OVX group than other groups, but not statistically significantly. They decreased in OVX + Cap5 and OVX + Cap20 groups, being statistically significant in the OVX + Cap20 group compared to the OVX group ($P = 0.016$) [Figure 4].

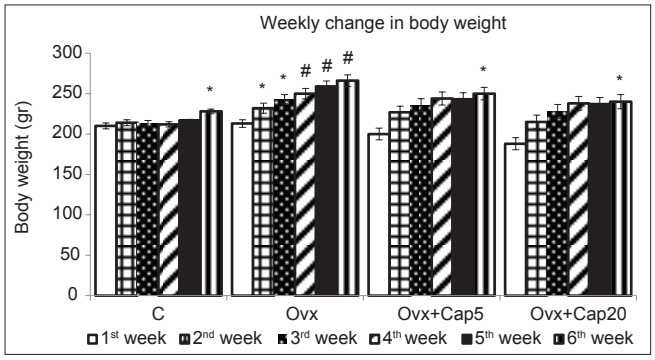

Figure 1: The comparison of the weekly changes in body weights within each group. Body weights increased throughout 6 weeks. Data were expressed as mean ± SEM. *$P < 0.001$ vs. 1st week, #$P < 0.001$ vs. 1st and 2nd week

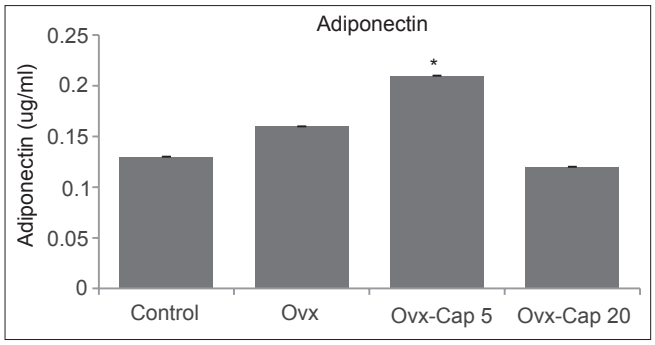

Figure 3: Changes in adiponectin levels among groups at the end of 6th week. Data were expressed as mean ± SEM. *$P = 0.014$ vs. C and OVX + Cap20

Cholesterol

Similar to the triglyceride levels, cholesterol levels were higher in the OVX group. In the OVX + Cap20 group, cholesterol levels were low with statistical significances between OVX + Cap20 group and all the others ($P < 0.001$) [Figure 5].

HDL-C, LDL-C, and VLDL-C

The HDL-C levels were higher in OVX group than the controls ($P = 0.001$). Low HDL-C levels in the OVX + Cap20 group were statistically significant compared to the OVX group ($P < 0.005$). The LDL-C did not show any significant changes among groups. The VLDL-C levels were lower in OVX + Cap20 group than the others. There were statistical significance between OVX + Cap20 group and groups of control, OVX and OVX + Cap5 ($P < 0.005$) [Figure 5].

DISCUSSION

One of the aims of the current work was to evaluate body weight changes after ovariectomy. We observed an increase in body weight after ovariectomy in association with lack of estrogen. We previously showed that weight gain due to increased adipose tissue in menopausal period might be

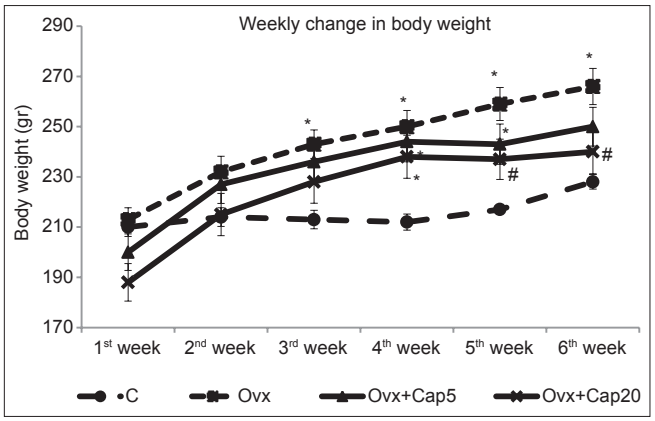

Figure 2: The comparison of weekly changes in body weights between groups. Data were expressed as mean ± SEM. *$P < 0.05$ vs. C, #$P < 0.05$ vs. OVX

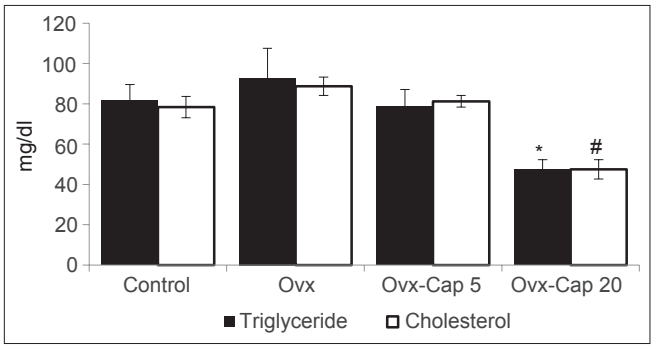

Figure 4: Changes in triglyceride and cholesterol levels. Data were expressed as mean ± SEM. *$P = 0.016$ vs. OVX, # $P < 0.001$ vs. C, OVX and OVX + Cap5

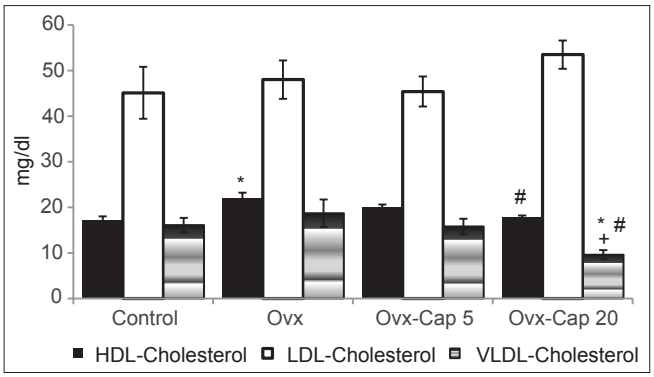

Figure 5: Changes in HDL-C, LDL-C, and VLDL-C levels. Data were expressed as mean ± SEM. *P = 0.001 vs. C, #P < 0.005 vs. OVX,+ P = 0.005 vs. OVX + Cap5

prevented by high-dose estrogen treatment (20 μg/kg).[14] Weight gain and obesity largely drives the increased prevalence of several disorders in postmenopausal women. These include increased central distribution of body fat, insulin resistance, dyslipidemia (elevated triglycerides, small dense LDL-C particle, and reduced HDL-C), elevated blood pressure, and increased hypercoagulability and proinflammatory state in blood.[3] Increased adiposity is associated with systemic loss of estrogen at menopause. Estrogen replacement alone or in combination with progesterone can prevent menopause-induced gains in adipose tissue mass. Reduction in adiposity is higher in intra-abdominal fat depots than subcutaneous, consistent with the observation in menopausal women that estrogen therapy primarily acts on intra-abdominal depots, thereby preventing central adiposity.[1]

Another aim of this study was to observe the effects of inhibition of ACE on body weight, lipid profile, and plasma adiponectin levels; and therefore captopril was used. Captopril treatment prevented body weight gain. While the classical RAS is known for its role in body fluid and cardiovascular homeostasis, there is an analogous RAS located in adipose tissue. Plasma angiotensinogen, plasma renin activity, plasma ACE activity, and adipose tissue angiotensinogen expression are positively correlated with adiposity in humans.[15] The adipose tissue RAS is thought to play a major role in the development of adiposity. Weisinger *et al*., reported that treatment with perindopril, an ACE inhibitor, from birth, reduced the body fat of rats maintained on a 7% fat diet.[16] In our study, body weight did not change in captopril-treated animals, and this may be related to its functions within adipose tissue. The RAS including Mas receptor is present in adipose tissue. It has been shown that captopril-induced weight loss may be related to activation of Mas receptor in the adipose tissue.[17] The decreased body weight is also associated with decreased body fat and plasma leptin and importantly, with increased adiponectin. Many studies have shown a relationship between plasma adiponectin levels and variety of diseases. First of all, it was demonstrated that plasma adiponectin levels were inversely correlated with body mass index. Reduction of body weight increases plasma adiponectin levels.[18]

Adipocytes secrete factors that play central roles in energy balance regulation, insulin sensitivity, immunological responses, and vascular diseases.[15,19] Adiponectin, released at high levels from differentiated adipocytes, is found at high levels in blood. Its expression is especially higher in subcutaneous adipose tissue. Plasma adiponectin levels decline before the onset of obesity in nonhuman primates, suggesting that hypoadiponectinemia contributes to the pathogenesis of this condition.[15] Plasma concentrations of adiponectin are significantly lower in obese subjects than in lean controls,[20] indicating a role for this adipocyte-secreted factor in the energy balance regulation. Adiponectin effects on energy homeostasis are mediated, at least in part, by increased free fatty acid oxidation in muscle. It was suggested that increasing free fatty acid oxidation pharmacologically in muscle might provide a new way to control body weight without interfering with food intake.[21] Kohlstedt *et al*., showed that ACE inhibitors, such as enalaprilat or ramiprilat, significantly increased soluble adiponectin production. In ACE-deficient 3T3 L1 cells, inhibitors of the enzyme did not affect the release of adiponectin. ACE inhibitors were shown to significantly increase the release of adiponectin from preadipocytes.[10] In the present study, we also observed that treatment with ACE inhibitor, captopril (5 mg/kg/day), significantly enhanced circulating adiponectin levels, whereas high-dose captopril did not have any effect.

The mechanism by which ACE inhibitors affect adiponectin levels is unknown, but it was indicated that ACE inhibitors modulated adipocyte gene expression via the induction of cellular retinol-binding protein 1 (CRBP1).[10] The effects of ACE inhibitor therapy are related to both changes in enzyme activity and ACE signaling cascade. Binding ACE inhibitors to the cytoplasmic site of the enzyme causes phosphorylation, activating specific signaling pathways affecting expression of several genes such as endothelial genes. Adipose tissue expresses all the components necessary for the renin-angiotensin system.[22] It has been reported that renin, angiotensinogen, aldosterone, and ACE levels were higher in obese women compared to slim women and low angiotensinogen gene expression in their adipose tissues, suggesting a correlation between RAS components and body weight. Weight reduction (≅5%) was shown to reduce angiotensinogen, renin, and aldosterone levels; and decrease ACE expression.[23]

Some of the ACE inhibitors such as perindopril and ramipril, exhibit a strong affinity for target tissues, while the others (enalapril and captopril) are characterized by relatively weak tissue affinity. Effects of ACE inhibitors on plasma adiponectin levels may show these differences. Although both

enalapril and perindopril increased plasma adiponectin level, perindopril was shown to have much stronger action than enalapril.[11] Here, we showed that adiponectin levels were higher in the low dose captopril group compared to the high dose one, indicating possible differences in pharmacokinetics and pharmacodynamics between various ACE inhibitors.

It has been generally accepted that HDL-C level decreased in women with menopause.[24] However, recent reports showed different results. Kim *et al*., showed that menopause might associate with the elevating of HDL-C. They also showed that cholesterol, triglyceride, LDL-C, and VLDL-C levels were higher in the postmenopausal women than the premenopausal and perimenopausal women.[25] In our study, HDL-C levels were significantly higher in ovariectomy group than the controls. Triglyceride and cholesterol levels were also increased in the OVX group, but LDL-C and VLDL-C levels did not change. ACE inhibitor, captopril changed the lipid profile. Increased triglyceride, cholesterol, HDL-C, and VLDL-C levels after ovariectomy were decreased by especially high dose of captopril. But, LDL-C level was not affected by captopril. These results showed that the adipose tissue RAS might play a major role in the development of adiposity.

It was well documented that adiponectin is associated with dyslipidemia. Plasma adiponectin levels correlate positively with HDL-cholesterol and negatively with triglyceride.[18,26] A study by Matsubara *et al*., on nondiabetic women reported that plasma adiponectin values correlated negatively with triglyceride, apolipoprotein B and E levels, and atherogenic index (total/HDL cholesterol); but positively with serum HDL-C and apoprotein A1 levels.[27] These results are compatible with our results. While low dose of captopril caused an increase in adiponectin levels, high-dose of the ACE inhibitor decreased triglyceride, cholesterol, HDL-C, and VLDL-C levels. These findings suggest that the hypoadiponectinemia observed in dyslipidemia may accelerate the atherosclerotic changes seen in the metabolic syndrome in postmenopausal period.

CONCLUSION

Ovariectomy-induced weight gain was decreased by ACE inhibitors. This effect may explain at least in part, increased adiponectin levels. Triglyceride, cholesterol, HDL-C, and VLDL-C levels were also decreased by ACE inhibitors. Our results demonstrate that the adipose tissue RAS may play a major role in the development of adiposity.

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