The effect of menopause status on lipid profile and oxidative stress in women

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Abstract
Aim: The aim of the study was to compare the lipid profile and oxidant/antioxidant status between pre-menopausal and post-menopausal women and to evaluate the correlations between these variables.

Material and Methods: This study included 50 healthy women, of which 21 are in pre-menopausal and 29 are post-menopausal status. We analyzed lipid profile in serum samples and oxidative stress in RBC samples. Oxidant status was assessed by measurement of malondialdehyde, whereas antioxidant defence was evaluated by analysis of catalase and glutathione.

Results: Compared to pre-menopausal women, triglycerides, total cholesterol and low-density lipoprotein-cholesterol levels were higher and high-density lipoprotein-cholesterol levels were lower in post-menopausal women. However, difference was statistically significant only for triglycerides levels. The levels of oxidant/antioxidant system members did not differ between the groups.

Conclusion: Postmenopausal status is associated with dyslipidemia, however the alterations in at oxidative status level might be reduced with the use of hormone replacement therapy, or might vary inter-individually.

Keywords: Menopause; oxidative stress; antioxidant; lipid profile.

INTRODUCTION
Menopause is a physiological condition, defined as the cessation of the menstrual period and ovarian function because of altered hormone levels. Follicle stimulating hormone (FSH) levels increase and estradiol and inhibin levels decrease during the course of the menopause. Physical appearance of most women also changes in terms of greater adiposity and body mass after transition to menopause.

Most studies show that deteriorated lipid balance in favor of total cholesterol (TC) triglycerides (TG), and low density lipoprotein-cholesterol (LDL-C) levels is the reason for increased rate of cardiovascular diseases (CVD) in post-menopausal women together with decreased high density lipoprotein-cholesterol (HDL-C) levels (1). The lowered estrogen levels following the menopause decreases the energy expenditure of the organism, and together with imbalances in lipid profile and glucose/insulin metabolism, the risk of CVD and atherosclerosis increases (2). Inflammatory response and oxidative stress (OS) have been shown to play important roles in the development of CVD (3). OS is the imbalance between oxidant and antioxidant molecules, causing elevated production of reactive oxygen species, which contributes in development of a wide variety of diseases including CVD.

Malondialdehyde (MDA) is a lipid peroxidation product and has been implicated as a marker of oxidative status of the organism. Catalase (CAT) and glutathione (GSH) are cellular free radical scavengers, which convert oxidant molecules into less reactive ones, or inactivate them. Various studies have shown decreased estrogen levels results in an imbalance between oxidant/antioxidant systems after the menopause (4,5).

Hence, the aim of the study was to compare the lipid
profile and oxidant/antioxidant status between pre- and post-menopausal women and to evaluate the correlations between these variables.

**MATERIAL and METHODS**

**Study subjects**
This study included 50 healthy women, of which 21 are in pre-menopausal and 29 are post-menopausal status. The pre-menopause group consisted of women ranging from 30 to 40 years of age, whereas the post-menopause group consisted of women ranging from 48 to 61 years of age.

Eligibility criteria were regular menstrual cycle for pre-menopause group, and amenorrhea ≥12 months for post-menopause group.

The ethical committee of Istanbul University Cerrahpaşa Medical Faculty approved the study. All participating patients provided informed consent, and the study was performed according to the Helsinki Declaration.

**Biochemical measurements**
Blood samples were drawn in the morning following at least 8 hours of fasting. The samples were centrifuged and transported to the Central Clinical Chemistry Laboratory of our institute. The laboratory tests (TC, TG, LDL-C, HDL-C) were performed as recommended by the manufacturer using a COBAS 6000 automated analyzer (Roche Diagnostics, Germany).

Since we lack the information regarding waist circumference, blood pressure and fasting glucose levels for each subjects, the cases with a TG level ≥150 mg/dL along with HDL-C levels <50 mg/dL were considered as having metabolic syndrome (MetS) since the presence of these two symptoms for MetS diagnosis is adequate (6).

**Preparation of the hemolysate and plasma**
Blood collected in EDTA containing tubes and centrifuged at 3000 rpm for 15 minutes in order to separate the plasma and red blood cells (RBC). The RBCs were washed thrice with 5-fold volume of ice-cold phosphate buffer saline at pH 7.4 and centrifuged. Then, the hemolysate was stored at -80°C until analysis (7).

**MDA assay**
The levels of MDA were determined using the thiobarbituric acid (TBA) method (8). Peroxidation was measured as the production of MDA, which, in combination with TBA, forms a pink chromogen compound. Then, absorbance at 532 nm was measured with spectrophotometry. The concentration of MDA in the sample was calculated using a plot table obtained using absorbance’s of standard solutions.

**CAT assay**
CAT activity was determined according to the method of Aebi (9). CAT catalyses the breaking of H₂O₂ into the water and molecular oxygen. The activity of CAT is measured as the conversion of H₂O₂ into H₂O and O₂ in 1 minute under standard conditions.

**GSH assay**
GSH levels were determined according to the method of Beutler et al (10). Briefly, 0.1 ml of blood sample was treated with 1.5 mL of precipitation solution (1.67 g MPA+0.2 g EDTA+30 g NaCl dissolved in 100 ml water) was added and incubated for 5 minutes. After the addition of phosphate buffer and 5 '5' dinitrobenzoic acid (DTNB), the absorbance was measured at 412 nm. All spectrophotometric measurements were done with a Shimadzu ultraviolet (UV)-1208 spectrophotometer.

**Statistical analyses**
Data were expressed as the mean ± SD (standard deviation) and they were compared using Student's t-test between the pre- and post-menopause groups. Spearman's correlation coefficient analysis was performed for the calculations of correlations between the analytes. A p value of <0.05 was considered statistically significant.

**RESULTS**
Table 1 shows the baseline values of the variables. As for lipid variables, TC, TG, and LDL-C levels were lower and HDL-C levels were higher in the pre-menopause group. However, only TG levels reached statistical significance (TC: 183.3±37 vs. 213±47.5 mg/dL, p=0.07; TG: 110.64±3 vs. 172.7±90 mg/dL, p<0.05; LDL-C: 109.8±29.9 vs. 133.1±38.3 mg/dL, p=0.08; HDL-C: 51±15.3 vs. 45.2±9.7 mg/dL, p=0.28).

Results showed that levels of the MDA, a lipid peroxidation product and antioxidant molecules CAT and GSH did not differ between pre- and postmenopausal women.

Before menopause, a significant positive correlation was found between TC and LDL-C concentrations, and between TC and HDL-C concentrations whereas a negative correlation between TG and HDL-C concentrations (r=0.96, p<0.01; r=0.58, p<0.01; r=-0.66, p<0.01, respectively). In group of postmenopausal women, a positive correlation was found between TC and LDL-C concentrations (r=0.92, p<0.01) (Table 2).

According to the criteria established by National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), TG levels >150 mg/dL and HDL-C levels < 40 mg/dL for men and 50 mg/dL for women are among the diagnostic factors for the MetS together with blood pressure, fasting glucose level and waist circumference (11). Using the cut-off for TG and HDL-C according to the NCEP-ATP III criteria, we observed that the prevalence of metabolic syndrome was present in the 14.2% (3/21) of the pre-menopausal and 34.4% (10/29) of the post-menopausal women. However, statistical analysis of these ratios did not show a significant difference between the groups.
Table 1. Results of serum lipid profile and RBC MDA, CAT and GSH measurements of the pre- and post-menopause groups (M: mean, SD: standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>Pre-menopause group (n=21) (M±SD)</th>
<th>Post-menopause group (n=29) (M±SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>183±37</td>
<td>213±47.5</td>
<td>0.07</td>
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<tr>
<td>Triglyceride (mg/dl)</td>
<td>110.6±43.0</td>
<td>172.7±90</td>
<td>&lt;0.05*</td>
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<td>LDL-C (mg/dl)</td>
<td>109.8±29.9</td>
<td>133.1±38.3</td>
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<tr>
<td>HDL-C (mg/dl)</td>
<td>51±15.3</td>
<td>45.2±9.7</td>
<td>0.28</td>
</tr>
<tr>
<td>RBC MDA (nmol MDA/g Hb)</td>
<td>22.7±4.1</td>
<td>21.7±2.3</td>
<td>0.53</td>
</tr>
<tr>
<td>RBC CAT (U/g Hb)</td>
<td>119.6±130.5</td>
<td>122.5±155.5</td>
<td>0.96</td>
</tr>
<tr>
<td>RBC GSH (mg%)</td>
<td>20.8±9.1</td>
<td>20±14.1</td>
<td>0.86</td>
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</tbody>
</table>

Table 2. Correlation coefficients (r) of the analytes in the pre- and post-menopause groups. Statistically significant values are defined in bold characters

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>RBC MDA (nmol MDA/g Hb)</th>
<th>RBC CAT (U/g Hb)</th>
<th>RBC GSH (mg%)</th>
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<tr>
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<tr>
<td>LDL-C</td>
<td><strong>0.96</strong></td>
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<tr>
<td>HDL-C</td>
<td><strong>0.58</strong></td>
<td><strong>-0.66</strong></td>
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<td>0.39</td>
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<tr>
<td>RBC MDA</td>
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<td>0.24</td>
<td>-0.004</td>
<td>1.00</td>
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<tr>
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<td>-0.25</td>
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<tr>
<td>Total Cholesterol</td>
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<tr>
<td>Triglyceride</td>
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<tr>
<td>LDL-C</td>
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<td>-0.16</td>
<td>0.48</td>
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<tr>
<td>RBC MDA</td>
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<td>-0.62</td>
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<td>RBC GSH</td>
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<td>0.31</td>
<td>0.42</td>
<td>-0.44</td>
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</table>

*Significant at the <0.01 level
DISCUSSION

A worsened lipid profile and balance between oxidant and antioxidant system members are usually observed and expected following the menopause (12). In the present study, we detected increased levels of TG and a significant positive correlation between TC and LDL-C levels in post-menopausal subjects. Additionally, the ratio of metabolic syndrome was 20.2% higher in the post-menopause group (not statistically significant).

It has been shown that aging has a decreasing effect on HDL-C and increases TC, LDL-C, and TG levels (13). Since post-menopausal women are at least a few decades older than the pre-menopause group, the alterations in the lipid profile might be a result of the aging process. According to previous reports, lipid profile is worsened also in aging men because of decreasing testosterone and increasing free androgens index levels (14). Thus, the higher lipid levels in post-menopausal women, which reached statistical significance in terms of TG levels might be a result of aging process as well as the menopausal status. While women of similar ages were compared regardless of the menopause status, higher body adiposity, metabolic syndrome ratio and poorer lipid profile were correlated with the chronological aging (13). However, replacing decreased estrogen levels with hormone replacement therapy (HRT) in post-menopausal women was shown to improve lipid profile (15). Furthermore, lipid profile has been worsened in women who underwent surgical menopause, suggesting that menopause status has an exacerbating effect on lipid metabolism regardless of the age (16). The levels of some phosphatidylethanolamines and ceramides, the lipid-derived products were increased in post-menopausal women, suggesting these alterations were also present in the lipidomic level (17). Although we could not find a statistically significant difference between pre- and post-menopausal women in terms of TC and HDL-C levels, the levels of non-HDL-C were higher in the latter group, suggesting that, alone or together, menopause and aging are risk factors for CVD and MetS. Supporting this, we found a higher ratio of metabolic syndrome in our post-menopause subjects.

However, while we were evaluating the MetS status, we only used TG and HDL-C levels, and other components such as waist circumference, blood pressure and fasting blood glucose were not taken into account. Thus, recruitment of these variables in the classification of subjects for MetS status might yield differing ratios between two groups.

Lipid profile and MetS status are affected also from socio-demographic factors such as parity, educational level, lifestyle, physical activity level and marital status. Yet, we did not collect data regarding these variables, thus it is not possible to make a conclusion regarding the effects of these factors on lipid profile of the study groups.

In our study, the positive correlation between TC and HDL-C in pre-menopausal women is suggestive of a balance, working to regulate the lipid profile, which is worsened following the menopause. Advocating this hypothesis, we could not be able to find this correlation in the post-menopausal women. Estrogen, the female hormone, levels of which decreases following the menopause was shown to have antioxidant effects and reduce OS (18). In a cross-sectional study, OS in post-menopausal women have been shown to be related with higher depression score, psychological disturbances and low quality of life (19).

It is fair to expect increased oxidant capacity in post-menopausal women. However, we did not find a difference in the levels of both oxidant and antioxidant system members when we compared our study groups. Because of deteriorated lipid status, lipid peroxidation marker, MDA was expected to increase after the menopause. Furthermore, there are studies supporting this hypothesis (20).

HRT, using a combination of estrogen and progestin increased blood Mn-Superoxide Dismutase (MnSOD) and CuZnSOD activity in postmenopausal women, leading to a mitigated plasma antioxidant profile (21). Moreover, in an animal study of rats, estrogen therapy reduced OS in ventrolateral medulla, decreasing sympathetic outflow, thus blood pressure (22).

Similar levels of oxidant/antioxidant molecules in our study groups might be a result of the sample type we chose. Since we measured the levels of these analytes in RBCs of the women, serum or plasma measurements might result in significant differences. In a study, decreased expression of Glutathione peroxidase (GPx1) and increased expression of SOD1 genes have been shown in post-menopausal subjects, suggesting that OS might be induced by menopause (23). Thus, analyzing other determinants of redox status such as SOD, GPx activity and genetic polymorphisms of the genes that encode these proteins might give a better understanding of the OS status of post-menopausal women. We did not study the levels of GPx since GSH is the product of the Gpx metabolism, hence the levels of GSH might be reflecting the Glutathione cycle in the organism. On the other hand, the evaluation of SOD levels in our study group might yield interesting results, however the group is not familiar with the laboratory process regarding the measurement of this variable.

We note that this study has the limitation of a small sample size and lacks the knowledge of demographic and socio-demographic factors. Additionally, we did not collect information on which type of HRT combinations were prescribed for our post-menopause subjects.

CONCLUSION

In conclusion, the present study underlies the exacerbation of lipid profile and increased ratio of MetS among women following the menopause. Reflection of oxidative status at systemic level in post-menopausal women might be a subjective phenomenon, and needs further research with large epidemiological data sets.
Competing interests: The authors declare that they have no competing interest.

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Ethical approval: This work has been approved by the Institutional Review Board.

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