Obesity is associated with decreased total antioxidant capacity in apparently healthy postmenopausal women

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ABSTRACT

Objectives: Postmenopausal period is associated with the decline in antioxidant levels due to gradual loss of estrogen, increased body weight and central adiposity. The present study aimed to evaluate association of adiposity and regional fat distribution with total antioxidant capacity in postmenopausal women.

Methods: This cross-sectional study included 90 apparently healthy postmenopausal women. We measured anthropometric indices including body mass index (BMI), waist circumference (WC) and waist-hip ratio (W/H ratio), Total fat mass (TFM), total lean mass (TLM), percentage fat mass (%FM), visceral fat diameter (VFD) and subcutaneous fat diameter (SFD) were measured using ultrasound. Serum total antioxidant capacity (TAC) was measured by quantitative colorimetric determination using Total antioxidant Capacity -QuantiCromAntioxidant Assay Kit (BioAssay systems, USA; DTAC-100).

Results: Out of 90 postmenopausal women, 35.9% were overweight and 25.0% obese, while 60.9% had central obesity. Postmenopausal obese women had significantly lower median TAC level [308.3 (283.0-375.1)] mM Trolox equivalents compared to overweight [383.38 (356.5-389.4) mM Trolox equivalents; p<0.001] and normal weight women [376.3 (318.0-388.7) mM Trolox equivalents; p<0.005]. Serum logTAC level was inversely associated with BMI, TFM, TLM and WC in postmenopausal women. However, when stratified by central obesity, inverse associations between serum logTAC level and BMI (r=-0.503; p<0.001), TFM (r=-0.383, p=0.004) and WC (r=-0.408; p=0.002) were observed only in postmenopausal women with central obesity.

Conclusion: Our results provide evidence that obesity and central obesity during postmenopausal period are associated with decreased total antioxidant capacity and depleted antioxidant defenses possibly due to elevated oxidative stress. Larger prospective studies are needed to evaluate whether obese postmenopausal women might benefit from antioxidants supplementation for the prevention of obesity related diseases.

Keywords: total antioxidant capacity, oxidative stress, obesity, overweight

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INTRODUCTION

Oxidative stress is a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. Perturbations in the normal redox state leads to increased production of free radicals that can damage cell proteins, lipids, and DNA. At the same time the decrease in antioxidant defense leads to impaired ability of the cell to detoxify the damage caused by free radicals. Total Antioxidant Capacity considers the cumulative action of all the antioxidants present in plasma and body fluids [1]. As the body ages, antioxidant levels decline, leaving the human body susceptible to a variety of age-related pathologies, such as non-alcoholic liver cirrhosis and atherosclerotic heart disease. This decline combined with a gradual loss of estrogen in postmenopausal women is highly associated with the various sequelae of menopause such as heart disease, vasomotor disturbances, and osteoporosis. The marked reduction in estrogen has been shown to increase levels of oxidative stress in the body [2]. Previous studies have shown that obesity and central fat accumulation are associated with chronic inflammatory state and increased oxidative stress (OS) [3,4]. Postmenopausal women tend to gain body fat due to the decline in estrogens synthesis and reduced ener-
energy expenditure leading to increased oxidative stress. Besides the decrease in circulating estrogens, during menopause transition, the adrenal glands continue to secrete androgen precursors which are aromatized to estrogens in adipose tissue. The relative increase in the androgen/estrogen ratio is likely to be important for the fat distribution shift [5]. Postmenopausal women manifest this shift in abdominal fat distribution (centripetal type of obesity) [6]. The present study aimed to evaluate association of adiposity and regional fat distribution with total antioxidant capacity in postmenopausal women.

**Materials and Methods**

**Subjects**

This cross-sectional study included 90 apparently healthy postmenopausal women who were originally selected from an osteoporosis screening study which was conducted from 2010 to 2013 and included in total 210 postmenopausal women. Women were initially referred to the University Clinical Centre Sarajevo (UCCS) by their primary health care practitioners and from the Centre for healthy aging for osteoporosis screening. For the purpose of this study, we included only postmenopausal women with preserved bone mass determined by their total hip and/or total lumbar bone mineral density measured by densitometry (n=107). Further selection of the participants was performed upon interview on menstrual cycles and the date of the last menstrual cycle. Postmenopausal status was initially defined as absence of menstruation for at least 12 months and confirmed by elevated serum FSH levels as provided by our laboratory reference values for postmenopausal women. Participants were excluded if they were receiving hormone replacement therapy or had received it within the last year before the start of the study or had cancer, cardiovascular disease, diabetes mellitus and other endocrinal disorders, bronchial asthma, acute or chronic inflammatory diseases, autoimmune diseases and rheumatic diseases (n=17). The final study sample consisted of 90 postmenopausal women. All participants signed informed written consent after the explanation of the study procedure. All procedures in this study were conducted in accordance with the guidelines of The Declaration of Helsinki.

**Anthropometric measurements**

The women height and weight were measured with women dressed in indoor clothes and with no shoes. Height was measured to the nearest 0.5 centimeters using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg on a seca digital scale. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m²). Waist circumference (WC) was measured halfway between the lower rib and the iliac crest, and hip circumference was measured at the level of the greater trochanter. BMI values in the range 19-25 kg/m² were considered normal weight, while BMI ≥25 kg/m² and ≥30 kg/m² were the cut-off levels for overweight and obese subjects, respectively. Central obesity was defined according to NCEP ATP III guidelines with a waist circumference of 88 cm or greater for women [7]. We also measured mid-thigh circumference in the middle third of thigh in women free of clothes and the value was later used in the model for total body fat mass calculation.

**Assessment of body fat and regional fat distribution by ultrasonography**

Ultrasonography was used to measure the maximum subcutaneous fat thickness (SFT), visceral fat thickness (VFT), total body fat (TBF) and percentage of body fat (%BF). The same examiner performed all ultrasonographic measurements at the Clinics for Radiology UCCS. SFT was defined as the distance between the external face of the rectoabdominal muscle and the internal layer of the skin. VFT was defined as the distance between the anterior wall of the aorta and the internal layer of the rectoabdominal muscle perpendicular to the aorta. Both SFT and VFT were measured using a 3.5 MHz probe located 1 cm above umbilicus in the midline of the abdomen. Application of the transducer on the body surface was done without undue pressure that would alter the body layer contour and thickness. All measurements were performed at the end of a quiet inspiration. Each distance was measured at 3 positions, and each measurement was performed three times. Total body fat and percentage of fat mass was determined indirectly measuring the skinfolds at specific body sites by ultrasound using the model validated by Pineau et al. [8]. The model estimating the %BF with ultrasound in women was found to have high accuracy and is strongly correlated with the values obtained by DXA (r=0.96). The model includes, besides standard anthropometric measurements such as body weight and height, midnight and waist circumference, a subcutaneous fat tissue diameter at two sites: 1) subcutaneous fat tissue above posterior iliac crest near lumbar spine located in a horizontal plane with an approximately 45° vertebral axis at the umbilical level and 2) subcutaneous fat tissue diameter in the middle of tight anteriorly. Abdominal and midthigh subcutaneous fat was measured using a 5.0 MHz linear array probe. The formula for calculating total body fat and body fat percentage using the validated model [8]:

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\text{Total body fat (kg)} \ (\text{TBF}) = 0.44 \ \left[\text{body weight (kg)}\right] - 0.143 \ \left[\text{body height (cm)}\right] + 0.425 \ \left[\text{lumbar skinfold thickness (mm)}\right] - 0.017 \ \left[\text{midthigh skinfold thickness (mm)}\right] + 0.144 \ \left[\text{waist circumference (cm)}\right] - 0.254
\]


[midnight circumference (cm)] + 3.84.

Body fat percentage (%BF) = total body fat / body weight x 100.

**Assessment of serum Total Antioxidant Capacity**

Analysis of TAC was performed at Department of Physiology, Faculty of Medicine Sarajevo. From each subject 5 mL of blood sample were taken from the antecubital vein, in fasting state. Blood samples were centrifuged at 4°C for 10 min at 2500 rpm to obtain serum. The serum samples were stored at -80°C until analysis. The samples were thawed at room temperature only once at the time of assay. Serum total antioxidant capacity was measured by quantitative colorimetric assay, using Total antioxidant Capacity - QuantiCromAntioxidant Assay Kit (BioAssay systems, USA; DTAC-100). By this method Cu²⁺ is reduced by antioxidant to Cu⁺. The resulting Cu⁺ specifically forms a colored complex with a dye reagent. The color intensity at 570 nm is proportional to TAC in the sample. Serum total antioxidant capacity is expressed in mM Trolox equivalents. Referral TAC values using this kit is within the linear range of 1.5-1000 mM Trolox equivalents.

**Statistical analysis**

All statistical calculations were performed with the SPSS 16 software (version 16.0, SPSS Inc, Chicago, Illinois, USA). The distribution of variables was tested by Kolmogorov-Smirnov or Shapiro-Wilk test. Values with normal distribution were expressed as mean±standard deviation, while those without normal distribution were shown as median and interquartile range. For continuous variables, a comparison between two groups was made by using Student’s t-test or the Mann-Whitney U-test. Since TAC, visceral fat diameter and subcutaneous fat diameter did not follow normal distribution; the values were transformed into the logarithmic scale and subjected to Pearson correlation analysis. P-values less than 0.05 were considered statistically significant.

**RESULTS**

Demographic and anthropometric characteristics of postmenopausal women are shown in Table 1. Mean BMI and waist circumference were in the overweight and central obesity range (BMI 26.7±4.3 kg/m²; WC 90.8±11.6 cm). In our study, there were 35.9% overweight and 25.0% obese women, while 60.9% of postmenopausal women had central obesity (Table 1).

Median serum TAC level in total study sample of postmenopausal women was 373.2 (311.3-386.7) mM Trolox equivalents. Postmenopausal obese women had significantly lower TAC level [308.3 (283.0-375.1) mM Trolox equivalents] compared to overweight [383.38 (356.5-389.4 mmol/L) mM Trolox equiv-
Serum logTAC level was significantly inversely associated with BMI, TFM, TLM and WC in postmenopausal women (Table 2). When we stratified women across different BMI ranges, significant negative association was observed between logTAC and waist circumference only in obese postmenopausal women ($r=-0.511$; $p<0.05$) (Table 2).

In postmenopausal women with central obesity serum logTAC level was inversely associated with BMI ($r=-0.503$; $p<0.001$) (Figure 2A), TFM ($r=-0.383$, $p=0.004$) (Figure 2B) and WC ($r=-0.408$; $p=0.002$) (Figure 2C).

**Discussion**

In our study, we found that postmenopausal obese women had significantly lower levels of TAC compared to overweight and normal weight women. However, there was no significant difference in serum TAC level between overweight and normal weight women. Our
study suggests that obese postmenopausal women have lower TAC levels indicating a compromised systemic antioxidant defense possibly due to the increased oxidative stress. Oxidative stress (OS) is associated with increased production of oxidizing species, particularly reactive oxygen species, and/or a decrease in the effectiveness of antioxidant defenses [9]. Depleted antioxidant capacity due to increased oxidative stress during postmenopausal period might be attributable to increased adiposity and decreased estrogen synthesis.

Obesity is recognized as an important health problem reaching pandemic proportions and represents main risk factor for the development of diabetes, hypertension, cardiovascular disease and malignancies [10-13]. In our study, over 60% of postmenopausal women were overweight and obese, and also over 60% had central obesity characterized by accumulation of fat in the abdomen. Recent study found that central obesity in normal weight people places them at greater risk of death than does overall overweight or obesity in people without the excess of abdominal fat [14].

Several mechanisms are involved in generating OS in obesity. Obesity may induce systemic OS and, in turn, OS is associated with an irregular production of adipokines, which contributes to the development of the numerous diseases [4,15].

Our results are also consistent with the results from Amirkhizi et al. [16] who found that plasma TAC levels were significantly lower in obese women compared to healthy women group. Also, they did not find significant difference between overweight and normal weight women in plasma TAC levels. In addition, women with central body fat distribution had higher malondialdehyde (MDA) and lower TAC levels compared to women with normal body fat distribution. In our study, serum TAC levels decreased with increasing body mass, fat mass and waist circumference in postmenopausal women. The results suggest that increased adiposity leads to increased oxidative stress which in turn lowers TAC levels countering increased radical production. Additionally, we found strong inverse association between fat mass and waist circumference only in women with central adiposity but not in women without central obesity. Our results suggest that there could be a certain cut-off at which accumulation of central fat increases reactive oxygen species (ROS) production to the point when the depletion of antioxidant stores becomes evident. Recent studies have also shown that antioxidant defense markers are lower according to the amount of body fat and central obesity [17,18].

Amirkhizi et al. [16] found significant negative correlation between plasma TAC levels and weight, BMI, waist circumference and waist-to-hip ratio among all subjects. These data suggest that obesity and, in particular abdominal adiposity leads to oxidative stress, which in turn may contribute to obesity related diseases. Chrysohoou et al. [17] found an inverse relationship between body fat, central adiposity, and antioxidant capacity. They showed that women with central fat exhibited 7% lower TAC concentrations and obese or overweight women had 10% lower TAC concentrations. Possible reasons for the findings might be attributable to the fact that visceral fat mass is more metabolically active compared to subcutaneous fat mass. Adipose tissue secretes various adipokines acting in autocrine, paracrine and endocrine manner. Excessive storage of adipose tissue, specifically in the abdomen, leads to disturbances in adipokines secretion. This promotes endothelial dysfunction and chronic low grade pro-inflammatory state leading to numerous diseases. In physiological and, even more, in pathological conditions, adipokines induce the production of ROS, thus generating oxidative stress [15]. However, our study results do not support the hypothesis that visceral fat mass alone contributes to decrease in total antioxidant capacity, but rather the total fat mass and body mass index as measures of overall adiposity. In that context, our results indicate that rather increased inflammatory response in obese postmenopausal women leads to increased oxidative stress. It has been shown that excessive adipose tissue is a source of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6 [19]. Upon activation, many immune cells generate free radicals and, in the same way, the synthesis of ROS promotes an inflammatory status [4].

Additionally, it has been shown that the decrease in estrogen synthesis could be associated with an increase in oxidative stress in postmenopausal women. Previous studies have shown that estrogens exert antioxidant property, particularly 17α-estradiol and some estradiol derivatives by preventing intracellular peroxide accumulation [20]. Uppoor et al. [20] showed increase in oxidative stress in postmenopausal women compared to normal menstruating women. They also showed that oxidative stress markers were significantly higher in obese postmenopausal women when compared to normal weight postmenopausal women. Authors suggested that increasing BMI can play an important role in maximizing the oxidative stress along with increasing age and depleting estrogen level. According to authors, level of oxidative stress among premenopausal women can be used as an early indicator. To prevent future cardiovascular complications, these women need to be recommended to make significant life style changes and diet control.

Obese persons may have an antioxidant deficit as a result of poor antioxidant intake or activities of the major antioxidant enzymes may be inadequate [21]. In recent
years, antioxidants have been used extensively to overcome the effects of excess reactive oxygen species in several pathological conditions. Some of the common antioxidants, vitamins E and C, coenzyme Q, α-lipoic acid, lycopenes, and polyphenols might have beneficiary effects in obese postmenopausal women.

**CONCLUSIONS**

Our results provide evidence that obesity and in particular central obesity during postmenopausal period is associated with decreased total antioxidant capacity and depleted antioxidant defenses possibly due to elevated oxidative stress. Larger prospective studies are needed to evaluate whether obese postmenopausal women might benefit from antioxidants supplementation for the prevention of obesity related diseases.

**DECLARATION OF INTEREST**

The authors report no conflicts of interest.

**REFERENCES**


