Relationship between serum DHEAS and oxidative stress levels of body mass index in healthy postmenopausal women

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Objectives: Menopause is a natural step in the process of aging. Postmenopausal women have decreased levels of antioxidants and increased oxidative stress, the latter of which plays an important role in atherogenesis. The aim of the present study was to evaluate the relationship of the body mass index (BMI) with serum catalase activity, malondialdehyde (MDA), and dehydroepiandrosterone sulfate (DHEAS) levels in healthy postmenopausal women and estimate whether the MDA/DHEAS ratio is a possible marker of oxidative stress for determining cardiovascular risk in these women.

Methods: We investigated serum catalase activity, MDA, and DHEAS levels, parity history, age, and BMI in 96 healthy postmenopausal women aged 50–82 years. The serum MDA levels and catalase activity were measured spectrophotometrically. The serum DHEAS levels were measured using an enzyme-linked immunosorbent assay. The ratio percentage of the serum DHEAS levels to serum MDA levels was designated as a biomarker for oxidative stress.

Results: The mean BMI of the patients was 31.72 ± 6.16 kg/m^2 (range = 20.5–47.94). The MDA/DHEAS ratio was significantly decreased in patients with a BMI over 30 compared to that of patients with a BMI between 25 and 30 ($P = 0.025$). Moreover, BMI was positively correlated with serum DHEAS levels ($r = 0.285$, $P < 0.01$) and negatively correlated with the MDA/DHEAS ratio ($r = -0.241$, $P < 0.05$) in postmenopausal women. Furthermore, BMI was observed to be a potential predictor of the MDA/DHEAS ratio based on covariance analysis ($P = 0.039$).

Conclusions: Our results indicate that healthy, obese, postmenopausal women have a decreased MDA/DHEAS ratio. Additionally, BMI was observed to be a potential predictor of the MDA/DHEAS ratio.

Keywords: Menopause, Number of pregnancies, Catalase, Oxidative stress, Body mass index, Dehydroepiandrosterone sulfate

Introduction

Dehydroepiandrosterone sulfate (DHEAS), which is secreted by the adrenal glands, comprises the largest quantity of the sex steroids in the human body.[1,2] The physiological significance of DHEAS is poorly understood. DHEAS levels decline markedly as a person ages, and the level of DHEAS at age 65 is less than one-fifth of the level measured at age 20.[3–5] The decline in DHEAS levels with age has led one to contemplate that DHEAS itself might play a role in the human lifespan. Low DHEAS levels in elderly men have been associated with increased mortality due to cardiovascular issues as well as other causes.[6–9] However, this association has not been clearly studied in either healthy women or women with a comorbidity.[6,8–12]

DHEAS has been shown to exert an antioxidant effect. Animal and *in vitro* tissue studies have shown that treatment with a DHEAS replacement lowers the oxidative stress levels.[13,14] Recent claims suggest that oxidative stress is associated with aging and that the degree of oxidative damage controls the rate of aging.[15]

Obesity is a chronic disease characterized by low-degree inflammation induced by oxidative stress and is a significant health problem in Western countries. Moreover, obesity is a critical risk factor for atherosclerotic cardiovascular disease.[16] Some studies have reported elevated levels of malondialdehyde (MDA),

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a marker of oxidative stress, in healthy obese subjects.[17,18] Although there are several reports of weight gain in menopausal women,[19,20] some studies have shown that weight gain due to menopause is associated with a decrease in estrogen levels and a reduced basal metabolism.[21,22]

In the present study, we evaluated the effect of the body mass index (BMI) on serum catalase activity and MDA and DHEAS levels in postmenopausal healthy women and estimated whether the MDA/DHEAS ratio could serve as a novel marker of oxidative stress for determining cardiovascular risk.

Materials and methods

Subjects

This study was performed between May and June 2014 at the Departments of Endocrinology and Metabolism and Internal Medicine of Yuzuncu Yil University, Faculty of Medicine. A total of 96 subjects older than 50 years of age were randomly selected from voluntary attendees during a routine checkup. Their current ages, age at menopause, and number of pregnancies and abortions were recorded.

Subjects who had diabetes mellitus, hyperlipidemia, chronic renal failure, congestive heart failure, postmenopausal bleeding, chronic liver disease, or rheumatologic disease were excluded from the study. Also smokers and patients who were treated with either hormone replacement therapy or steroid were excluded from the study. None of the patients were receiving regular antioxidant vitamin supplements, such as vitamins E and C.

All of the patients' basic physical parameters (e.g. weight and height) were measured, and the BMI values were calculated as follows: weight (kg)/height (m^2).

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 2000. The study protocol was accepted by the Ethical Committee of Yuzuncu Yil University (Van, Turkey), and informed consent was obtained from each subject.

Blood collection

Following a 12-hour fasting period, blood samples were collected at 8:00 and 11:00 a.m. after an overnight fasting period. The samples were collected into empty tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 1409*g* for 10 minutes. The resulting serum samples were stored at −20°C until they were used to measure catalase activity and the DHEAS and MDA levels.

Measurement of serum DHEAS levels

The serum DHEAS levels were measured using the microparticle chemiluminescence method on an ARCHITECT i2000SR device. The results are expressed as μg/dl.

Measurement of serum MDA levels

To determine the amount of lipid peroxidation in the serum, the MDA levels were analyzed spectrophotometrically using the modified thiobarbituric acid-reactive substance method as described by Yagi.[23] The results are expressed as nmol/ml.

Measurement of serum catalase activity

Catalase activity was measured using H_2O_2 as the substrate. The change in the H_2O_2 levels was followed at 240 nm. The enzyme activity is expressed as units per liter of serum (U/l) at 25°C.[24]

Statistical analysis

The study results were analyzed using the SPSS® for Windows computing program (version 16; SPSS, Inc., Chicago, IL, USA). Comparisons of three groups were performed with the analysis of variance test Variables potentially affecting the MDA/DHEAS ratio were analyzed using covariance analysis. The correlation between the variables was evaluated with the Pearson correlation. The results are expressed as the mean ± standard deviation (SD), and statistical significance was set at $P < 0.05$.

Results

A total of 96 postmenopausal women older than 50 years of age were included in the study. The mean age was 61 ± 8 years, and the mean BMI was 31.72 ± 6.16 kg/m^2. The average number of pregnancies and abortions were 8.67 ± 3.68 and 0.95 ± 1.39, respectively. The mean values of the demographic characteristics and laboratory tests are shown in Table 1.

The number of pregnancies in 28 of the subjects was less than 6, between 6 and 10 in 35 of the subjects, and greater than 10 in 33 of the subjects. Additionally, 41 of the 96 cases had a history of abortions. There was no statistically significant difference between the

Table 1 Description of the cases included in this study

	n	Minimum	Maximum	Mean ± SD
Age (year)	96	50	82	61.19 ± 8.11
Menopause age (year)	96	37	61	49.19 ± 4.98
Height (cm)	96	137	168	155.59 ± 5.33
Weight (kg)	96	43	109	76.59 ± 14.18
BMI (kg/m^2)	96	20.50	47.94	31.72 ± 6.16
Parity	96	0	18	8.67 ± 3.68
Abortus	96	0	7	0.95 ± 1.39
DHEAS (μg/dl)	96	2.10	236.3	84.38 ± 50.90
MDA (nmol/ml)	96	0.52	4.90	0.94 ± 0.70
Catalase (U/l)	96	0.025	8.49	0.64 ± 1.36

BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; MDA, malondialdehyde.

Table 2 Comparison of the BMI groups with regard to the demographic and laboratory data of the cases

	n	Mean ± SD	*P*
Age (year)			
BMI <25 kg/m^2	11	67.36* ± 7.00	
BMI 25–30 kg/m^2	32	60.44 ± 8.31	0.026
BMI >30 kg/m^2	53	60.36 ± 7.78	
Parity			
BMI <25 kg/m^2	11	8.55 ± 3.64	
BMI 25–30 kg/m^2	32	8.34 ± 3.42	0.803
BMI >30 kg/m^2	53	8.89 ± 3.88	
Abortus			
BMI <25 kg/m^2	11	0.82 ± 2.09	
BMI 25–30 kg/m^2	32	0.94 ± 1.16	0.953
BMI >30 kg/m^2	53	0.96 ± 1.36	
DHEAS (μg/dl)			
BMI <25 kg/m^2	11	67.35 ± 43.90	
BMI 25–30 kg/m^2	32	73.22 ± 53.54	0.086
BMI >30 kg/m^2	53	93.59 ± 45.30	
MDA (nmol/ml)			
BMI <25 kg/m^2	11	0.87 ± 0.21	
BMI 25–30 kg/m^2	32	1.06 ± 0.97	0.520
BMI >30 kg/m^2	53	0.89 ± 0.56	
Catalase (U/l)			
BMI <25 kg/m^2	11	0.86 ± 1.81	
BMI 25–30 kg/m^2	32	0.61 ± 1.47	0.849
BMI >30 kg/m^2	53	0.61 ± 1.21	
MDA/DHEAS			
BMI <25 kg/m^2	11	0.020 ± 0.021	
BMI 25–30 kg/m^2	32	0.031 ± 0.035	0.025
BMI >30 kg/m^2	53	0.014† ± 0.022	

*Comparison of BMI <25 kg/m^2 group to BMI between 25 and 30 and BMI >30 kg/m^2 group.
†Comparison of BMI >30 to BMI between 25 and 30.
BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; MDA, malondialdehyde.

number of pregnancies experienced and abortion history with regard to demographic characteristics and laboratory tests ($P > 0.05$).

The BMI was less than 25 kg/m^2 in 11 of the subjects, between 25 and 30 kg/m^2 in 32 of the subjects, and greater 30 kg/m^2 in 53 of the subjects. Comparative statistics of demographic characteristics and laboratory tests according to BMI groups are shown in Table 2. The mean age was significantly higher in women with BMI <25 kg/m^2 than in either women with a BMI between 25 and 30 kg/m^2 or women with a BMI >30 kg/m^2 groups ($P = 0.026$).

The MDA/DHEAS ratio was significantly lower in women with BMI >30 kg/m^2 than in women with a BMI between 25 and 30 kg/m^2 ($P = 0.025$). There were no statistically significant differences for the other parameters based on the BMI groups ($P > 0.05$) (Table 2).

The Pearson correlation analysis showed significant positive correlations between number of pregnancies and abortions ($r = 0.352$, $P < 0.01$). Catalase activity was positively correlated with the number of abortions ($r = 0.232$, $P < 0.05$), whereas the serum DHEAS levels were positively correlated with BMI ($r = 0.285$, $P < 0.01$). However, the MDA/DHEAS ratio was negatively correlated with the BMI ($r = -0.241$, $P < 0.05$) (Table 3).

Factors (e.g. age, number of pregnancies, number of abortions, and BMI) that might affect the MDA/DHEAS ratio were analyzed in a covariance analysis. BMI was found to be a potential predictor of the MDA/DHEAS ratio ($P = 0.039$) (Table 4).

Discussion

In the present study, we observed that the MDA/DHEAS ratio was significantly decreased in healthy, obese, postmenopausal women. To the best of our knowledge, this is the first study to investigate the MDA/DHEAS ratio in healthy, obese, postmenopausal women.

There are claims suggesting that oxidative stress is associated with aging and that the degree of oxidative damage controls the rate of aging.[15] Menopause normally manifests in women between ages 45 and 55 as

Table 3 Correlation analysis results of the cases

	Age	Menopause age	Menopause duration	BMI (kg/m^2)	Parity	Abortus	DHEAS (μg/dl)	MDA (U/l)	Catalase (nmol/ml)	MDA/DHEAS
Age	1									
Menopause age	0.172	1								
Menopause duration	0.828**	−0.409**	1							
BMI (kg/m^2)	−0.184	−0.172	−0.073	1						
Parity	0.184	0.159	0.080	0.071	1					
Abortus	−0.051	0.113	−0.112	0.046	0.352**	1				
DHEAS (μg/dl)	−0.171	−0.072	−0.118	0.285**	−0.132	0.010	1			
MDA (U/l)	−0.053	−0.063	−0.013	−0.103	−0.057	−0.122	−0.122	1		
Catalase (nmol/ml)	0.192	0.088	0.128	0.029	0.024	0.232*	0.003	−0.064	1	
MDA/DHEAS	0.006	0.0082	−0.041	−0.241*	0.085	−0.064	−0.589**	0.653**	−0.093	1

*$P < 0.05$.
**$P < 0.01$.
BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; MDA, malondialdehyde.

Table 4 Covariance analysis results for MDA/DHEAS

Covariate variables	MDA/DHEAS (*P* value)
Age	0.485
Parity	0.374
Abortus	0.704
BMI (kg/m^2)	0.039

BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; MDA, malondialdehyde.

a natural event in the process of aging. The estradiol level rapidly declines in women with the start of menopause. *In vitro* studies have shown that estrogens exert an antioxidant effect on membrane phospholipid peroxidation.[22,25] Compared with the decrease in estrogen levels during menopause, the decrease in androgen levels is slower and less pronounced.[26]

DHEAS comprises the largest quantity of the sex steroids in the human body.[2] DHEAS levels decline markedly as a person ages, and the level of DHEAS at age 65 is less than one-fifth of its measured level at age 20.[4,5] The results of *in vivo* and *in vitro* studies have shown that DHEAS limits lipid peroxidation.[27–29] DHEAS has also been shown to play a role in various physiological and pathological processes such as age-related cancer,[30] atherosclerosis,[30] obesity,[31] infections,[32] insulin sensitivity,[33] and aging.[34] The antioxidant characteristic of DHEAS can explain these effects.

In our study, we did not observe a statistically significant correlation between age and the DHEAS levels, probably due to the limited number of cases available to us as well as the intensity of the menopausal transition of some of our cases. Recently, it has been shown that during the menopausal transition, there is a modest increase in the production of DHEAS.[35]

MDA is one of the toxic end-products of non-enzymatic hydrolysis of oxidative lipid peroxides.[36] Recently, it has been shown that serum MDA levels increase with age in the 40–50 age group but remain constant after age 50.[37] In the present study, we found no correlation between age and MDA levels. All of our subjects were healthy women over age 50. Thus, the lack of an increase in the MDA levels after age 50 is consistent with data from the literature. Victorino *et al.*[38] observed no difference between premenopausal and postmenopausal women with regard to serum MDA levels. However, Sánchez-Rodríguez *et al.* [39] reported higher levels of MDA in postmenopausal women than in perimenopausal women. The effect of the aging process on serum MDA and dehydroepiandrosterone (DHEA) levels has also been assessed. Sreeramulu *et al.*[37] reported that serum MDA and DHEA levels in women are lower than those in men. They also observed a negative correlation between age and serum DHEA levels but a positive correlation between age and MDA levels.

In our study, we report lower levels of oxidative stress in our obese subjects. There was a negative correlation between the MDA/DHEAS ratio as an oxidative stress index and BMI in healthy postmenopausal women. However, there was no association between serum MDA levels and BMI.

When the BMI groups were compared, there was no significant difference between the groups with regard to the MDA and DHEAS levels, but the MDA/DHEAS ratio was significantly lower in women with a BMI over 30 than in women with a BMI between 25 and 30. The covariance analysis of the factors that can affect MDA/DHEAS ratio revealed that BMI was the only potential predictor.

In contrast to our study, Mittal and Kant[40] identified a positive correlation between MDA levels and body weight in healthy postmenopausal women. Though some studies have reported weight gain during menopause,[19,20] several studies have attributed weight gain during menopause to decreased estrogen levels and a lowered basal metabolism.[21,22] An increase in oxidative stress in conjunction with an increase in body weight can result in the development of metabolic syndrome, diabetes mellitus, hypertension, dyslipidemia, and atherosclerosis in obese subjects.[41] However, several authors have reported increased MDA levels (a marker of oxidative stress) in healthy obese subjects.[17,18]

Atherosclerosis is the major cause of morbidity and death in Western countries and involves complicated interactions between arterial cells, blood cells, and plasma lipoproteins.[42] Oxidative stress (i.e. an imbalance between the amount of reactive oxygen species and antioxidant defense mechanisms) plays an important role in atherogenesis.[43] Obesity is a chronic disease characterized by low-degree inflammation induced by oxidative stress. This oxidative stress plays a central role in the pathogenesis of obesity-associated disorders.[44] A Turkish study on the DHEAS levels in obese patients reported a positive correlation between the DHEAS levels and BMI in both women and men.[45] Likewise, Saruc *et al.* [46] reported that DHEA and DHEAS levels are positively correlated with the BMI and waist/hip ratio in Turkish postmenopausal women. Cao *et al.*[47] assessed the association between DHEAS and BMI in early and late postmenopausal women and observed higher levels of DHEAS in women with a BMI $\geq$24 kg/m^2 than in women with a normal body weight. Consistent with data published in the literature, we observed a positive correlation between the serum DHEAS levels and BMI.

Catalase is an antioxidant enzyme that contributes to the maintenance of oxidative balance by converting

hydrogen peroxide, a by-product of oxidative stress, into water and molecular oxygen.[48] Vaishali *et al*.[49] found that postmenopausal women had significantly higher levels of catalase than premenopausal women. Similarly, Arora *et al*. [50] reported higher levels of catalase in postmenopausal women than in premenopausal women. In our study, we observed no association between age and catalase levels because all of our cases were postmenopausal women. We also found no association between BMI and catalase levels. In contrast, Mittal and Kant[40] reported a positive correlation between catalase and body weight in healthy postmenopausal women.

In the present study, we measured the weight and height of each subject to calculate the BMI. We did not measure the waist/hip ratio because we were unaware of that type of obesity measurement for our subjects. Additionally, visceral fat is known to be associated with atherosclerosis, and perhaps our subjects have subcutaneous adipose tissue. Furthermore, the paradoxical results may be because none of the enrolled patients have a metabolic comorbidity such as hypertension, diabetes mellitus, or hypertriglyceridemia.

In conclusion, our study indicates that healthy, obese, postmenopausal women have a decreased MDA/DHEAS ratio. BMI was found to be a potential predictor of the MDA/DHEAS ratio, which can be used as a marker of oxidative stress in postmenopausal women.

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Disclaimer statements

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