Serum Asymmetric Dimethylarginine and Nitric Oxide Levels in Obese Postmenopausal Women

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Background: It has been reported that estrogen deficiency after menopause might cause a decrement in nitric oxide (NO) bioavailability by increasing the level of asymmetric dimethylarginine (ADMA), a major endogenous nitric oxide synthase inhibitor, thus leading to abnormalities in endothelial function. Because NO plays an important role on feeding behavior, ADMA may be involved in the pathogenesis of obesity, too. This cross-sectional study aimed to evaluate the relations of ADMA and NO with the obesity-linked peptides, such as ghrelin, leptin, and adiponectin in postmenopausal women free of hormone replacement therapy. **Methods:** Adiponectin, ghrelin, leptin, ADMA, and NO_x (total nitrite/nitrate) were measured in 22 obese (BMI: 30–47 kg/m²) and 19 normal weight (BMI: 21.5–26 kg/m²) postmenopausal women.

Anthropometric measurements (height, weight, BMI, waist, and hip circumferences) were recorded. Statistics were made by the Mann–Whitney *U*-test. **Results:** Ghrelin and adiponectin levels were significantly lower ($P < 0.001$), whereas ADMA and leptin levels were higher in obese women than in normal weight controls ($P < 0.01$ and 0.001 , respectively). BMI was correlated negatively with adiponectin and ghrelin and positively with ADMA and leptin levels. No correlation existed between ADMA and NO. **Conclusion:** Estrogen deficiency alone may not cause an increase in ADMA levels unless the women are prone to disturbances in energy homeostasis. In spite of the high ADMA levels, the unaltered NO levels in plasma may be owing to ongoing inflammatory conditions. *J. Clin. Lab. Anal.* 25:174–178, 2011. © 2011 Wiley-Liss, Inc.

Key words: asymmetric dimethylarginine; nitric oxide; obesity; ghrelin; adiponectin

INTRODUCTION

Obesity-related studies have presented a complex interaction among a variety of peptides, such as ghrelin, leptin, and adiponectin, all of which have important roles on feeding behavior. Ghrelin is a peptide hormone that modulates energy balance and weight regulation, and its dysregulation is an important factor in the onset of obesity (1). A significant decrease in plasma ghrelin concentrations was observed in obese subjects compared with those in lean subjects (2,3). Leptin is produced predominantly by the adipocytes and acts opposite to ghrelin on energy homeostasis (4). Another adipose tissue-released peptide is adiponectin that seems to regulate energy homeostasis and functions in combination with leptin (5). Besides its antiatherogenic properties, adiponectin improves insulin sensitivity and inhibits vascular inflammation (6). Although adiponectin is produced by the adipocytes, its level in serum was reported to be low in obese subjects (6,7).

It has been shown that both adiponectin and leptin modulate the activity of nitric oxide synthase (NOS), an enzyme responsible for the generation of nitric oxide (NO) (8–10). Asymmetric dimethylarginine (ADMA), a major endogenous NOS inhibitor, may alter NO bioavailability under physiological and pathological conditions (11,12). Reports related to plasma ADMA concentrations in obese individuals are contradicting (13–15). Insulin resistance was shown to associate with elevated plasma ADMA levels (16), and this elevation was accompanied with obesity (17). On the other hand,

Grant sponsor: Istanbul University; Grant numbers: UDP-1771/23112007; BYP-881/06012006.

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Received 30 July 2010; Accepted 28 January 2011

DOI 10.1002/jcla.20452

Published online in Wiley Online Library (wileyonlinelibrary.com).

ADMA levels were found increased by the onset of menopause, which could be significantly reduced by the estrogen replacement therapy (18,19). Estrogen deficiency after menopause might accelerate abnormalities in endothelial function by increasing the level of ADMA (20). Inhibition of NO bioavailability through ADMA might alter the feeding behavior, because administration of competitive NOS inhibitors had decreased food intake and body weight in animal experiments (21–23).

In this study, we sought to characterize the relations of ADMA and NO with obesity at postmenopausal stage. To better understand their possible role in obesity-linked disorders, we also evaluated the circulating levels of peptides functioning in energy homeostasis, such as adiponectin, ghrelin, and leptin.

SUBJECTS AND METHODS

The study population ($n = 41$) comprised 22 obese postmenopausal women, with a median age of 51 years (range 39–75 years, BMI: 30–47 kg/m²), and 19 healthy volunteers with a median age of 56 years (range 45–84 years, BMI: 21.5–26 kg/m²). Obese subjects were recruited from the Endocrinology Department of the Taksim Training and Educational Hospital, Istanbul. Obesity was defined as having a BMI ≥ 30 kg/m². All subjects had normal physical examinations and normal electrocardiograms, and underwent a series of laboratory tests (e.g., blood urea nitrogen, creatinine, hepatic enzymes, protein, lipid profiles, and urine analysis) for any evidence of hepatic or renal disease, malignancies, any secondary cause of obesity, and also for any personal history of mental diseases. The subjects were not under any medication and were free of a systemic disease or infection in the month before entry to the study. Subjects from similar socioeconomical status were chosen. Alcoholics and heavy smokers were excluded. All subjects reported that their body weight had been stable for at least 3 months before the study. The procedures were in accordance with the revised form of the Helsinki Declaration 2004 and all participants signed an informed consent form.

Anthropometric Measurements

Height and weight were measured with the subjects standing in light clothes without shoes. BMI was calculated as body weight divided by height squared (kg/m²). Waist-to-hip ratio was calculated as the ratio of waist and hip circumferences.

Analytical Methods

Venous blood samples were taken at 08:00 hr following a 12 hr starvation period, immediately centrifuged and

stored at -80°C until analytical measurements were performed. Glucose, total cholesterol, LDL and HDL cholesterol, and triglyceride were determined using automated routine methods by Roche/Hitachi Modular P autoanalyzer (Roche Diagnostis, Mannheim, Germany). FT₃, FT₄, and TSH were measured by the chemiluminescent microparticle immunoassay (DPC, Los Angeles, CA).

Adiponectin was determined by the enzyme-linked immunosorbent assay (Linco Research, Missouri; www.lincoresearch.com). The limit of sensitivity of this assay was 0.78 ng/ml, the intraassay coefficient of variation (CV) $< 15\%$.

Total ghrelin levels (acetylated plus nonacetylated) were determined in duplicate using the commercial enzyme immunoassay kit (Phoenix Pharmaceuticals Inc; www.PhoenixPeptide.com). The detection limit was 0.08 ng/ml, the interassay CV was $< 14\%$, and intraassay CV $< 5\%$.

Serum leptin levels were determined using the commercial solid phase *enzyme amplified sensitivity immunoassay* performed on microtiter plate (BioSource Europe S.A, Belgium; www.biosource.com.au). The minimum detectable concentration was 0.1 ng/ml.

ADMA levels were measured in serum samples by a commercially available ELISA kit (DLD Diagnostika, Hamburg, Germany; www.dld-diagnostika.de). The reference ranges were between 0.4–0.75 $\mu\text{mol/l}$. The intraassay and interassay CVs were < 8 and $< 10\%$, respectively.

Serum levels of NO_x (total nitrite/nitrate) were analyzed using total NO assay kit (R&D System Europe, Abingdon, UK; www.RnDSystems.com). The minimum detectable dose ranged from 0.09 to 0.78 $\mu\text{mol/l}$, the interassay CV was $< 4.6\%$, and intraassay CV $< 2.5\%$.

Statistical Analyses

All statistical evaluations were performed using the Statistical Package for the Social Sciences (Version 12.0 for Windows; SPSS, Inc., Chicago, IL). Data were expressed as means $\pm$ SD. The significance of differences among groups was assessed using the Mann–Whitney *U*-test. The correlations between anthropometric and biochemical measurements were estimated by the Spearman correlation test. Linear regression analysis was performed by the Analyse-it for Microsoft Excel Version 2.20 program.

RESULTS

Anthropometric characteristics and data from routine laboratory analyses of the study subjects are shown in Table 1. The BMI, weight, waist, hip circumferences, glucose, total cholesterol, and LDL cholesterol levels were higher in obese group than those in controls.

TABLE 1. Anthropometric Characteristics and Laboratory Data of the Study Subjects

	Normal weight (<i>n</i> = 19)	Obese (<i>n</i> = 22)
Age (years)	58.6 ± 10.3	53.0 ± 8.5
Height (cm)	156 ± 9.0	155 ± 6.5
Weight (kg)	60.5 ± 7.5	80.7 ± 10.5**
BMI (kg/m ²)	25.2 ± 1.7	34.1 ± 4.0**
Waist (cm)	83.0 ± 7.3	96.6 ± 8.9**
Hip (cm)	105 ± 6.0	118 ± 9.1**
WHR	0.78 ± 0.04	0.81 ± 0.02
Total cholesterol (mmol/l)	4.87 ± 0.41	5.44 ± 0.90*
LDL cholesterol (mmol/l)	2.74 ± 0.48	3.23 ± 0.79*
HDL cholesterol (mmol/l)	1.52 ± 0.36	1.47 ± 0.40
Triglyceride (mmol/l)	1.37 ± 0.45	1.51 ± 0.82
Glucose (mmol/l)	4.71 ± 0.48	5.36 ± 0.61
FT ₃ (pmol/l)	4.79 ± 0.92	5.42 ± 1.06
FT ₄ (pmol/l)	16.5 ± 2.58	16.7 ± 1.80
TSH (mIU/l)	2.6 ± 1.41	2.9 ± 1.41

Values are means ± SD. WHR, waist-hip ratio.

P* < 0.05; *P* < 0.001.

TABLE 2. Adiponectin, Ghrelin, Leptin, ADMA, and NO_x (Nitrite/Nitrate) Levels in the Study Subjects

	Normal weight (<i>n</i> = 19)	Obese (<i>n</i> = 22)
Ghrelin (ng/ml)	0.61 ± 0.34	0.22 ± 0.13**
Leptin (ng/ml)	7.61 ± 3.34	19.1 ± 11.2**
Adiponectin (μg/ml)	23.9 ± 10.3	13.0 ± 4.5**
ADMA (μmol/l)	0.82 ± 0.33	1.34 ± 0.58*
NO _x (μmol/l)	104 ± 47.5	134 ± 56.2

Values are means ± SD.

P* < 0.01; *P* < 0.001.

TABLE 3. Correlation Coefficients Between the Biochemical Variables and the BMI (by the Spearman Correlation Test, *n* = 41)

	Glucose	Leptin	Adiponectin	Ghrelin	ADMA
BMI	0.537**	0.753**	-0.497**	-0.497**	0.470**
Glucose	—	0.453*	-0.441*	-0.206	0.321
Leptin	—	—	-0.310	-0.470**	0.423*
Adiponectin	—	—	—	0.421*	-0.206
Ghrelin	—	—	—	—	-0.186

P* < 0.05; *P* < 0.01.

Serum ghrelin and adiponectin levels were found significantly low in obese postmenopausal women when compared with the age-matched controls (*P* < 0.001; Table 2). Serum ADMA and leptin levels were significantly higher in obese group (*P* < 0.01 and *P* < 0.001, respectively). No difference in NO levels was observed between obese and normal weight subjects (Table 2).

The correlation coefficients calculated by the Spearman test are shown in Table 3. The concentrations of

Fig. 1. The correlation between ADMA and leptin in study subjects (*r* = 0.423, *P* = 0.011). Lines representing the 95% confidence interval and the 95% prediction interval of the regression line are demonstrated. **Fig. 2.** The correlation between ADMA and BMI in study subjects (*r* = 0.470, *P* = 0.003). Lines representing the 95% confidence interval and the 95% prediction interval of the regression line are demonstrated.

adiponectin and ghrelin were found correlated negatively with the BMI and leptin, whereas leptin was correlated positively with the BMI. ADMA levels were positively correlated with those of leptin (Fig. 1) and with the BMI (Fig. 2), but no correlation existed between ADMA and NO.

DISCUSSION

This study reveals that serum ADMA and leptin levels are increased in postmenopausal obese women while ghrelin and adiponectin levels are lowered in comparison to their age-matched, normal weight counterparts.

Several reports have indicated obesity-linked changes in plasma leptin and ghrelin levels (24,25). Both ghrelin and leptin are known to participate in the regulation of cardiovascular system and sympathetic nerve activity by interacting with NO (26,27). The decrement in NO production through inhibition of central NOS activity was reported to be responsible for the anorectic effects of leptin (28). In our study, however, no difference was observed in serum total nitrite/nitrate levels of obese and nonobese individuals, despite high levels of circulating leptin in the former group. Accordingly, no correlation existed between plasma NO and leptin concentrations. An inhibition on central NOS activity had been observed following the intracerebroventricular injection of leptin (28). Therefore, it may be concluded that leptin–NOS interaction is tissue specific.

Plasma ADMA concentrations are increased in many pathological conditions, such as atherosclerosis, diabetes, and hyperhomocysteinemia, in parallel to the NO deficiency (28). On the other hand, estrogens can alter the catabolism and release of ADMA, and thus reduce the plasma concentrations (18). In our study, plasma ADMA levels in obese postmenopausal subjects were found significantly higher than those obtained from women with normal BMIs. This finding led us to suggest that estrogen deficiency alone may not cause an increase in ADMA levels unless the women are prone to disturbances in energy homeostasis. Our results with regard to the high plasma ADMA levels in obese subjects are in good agreement with the previous studies (13,14). Recently, Andersson et al. reported that overweight postmenopausal women exhibited significantly lower adipose tissue blood flow (ATBF) compared with normal weight postmenopausal women, and a negative correlation existed between fasting ATBF and ADMA (29). Their observation also indicates the link between ADMA and metabolic–vascular complications after menopause.

The lack of any correlation between ADMA and NO levels suggested that both the level of L-arginine, which reverse the effects of ADMA (30), and ADMA/L-arginine ratio are more important for NOS function and NO bioavailability than ADMA alone, as reported previously (31). Additionally, ADMA concentration inside the cells should be more relevant for NOS function than that in the circulation. An approximately 60% increase in plasma ADMA levels observed in our study may not be sufficient to induce a significant NOS

blockade in the cells. Furthermore, obesity is associated with infiltration of macrophages into adipose tissue, and macrophages are important sources of NO. In spite of the high ADMA levels, unaltered NO levels in plasma may be owing to ongoing inflammatory conditions.

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