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ASYMMETRIC DIMETHYLARGININE: RELATIONSHIP WITH CIRCULATING BIOMARKERS OF INFLAMMATION AND CARDIOVASCULAR DISEASE RISK IN UNCOMPLICATED OBESE WOMEN

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In recent years, the link between obesity, inflammation and atherosclerosis has attracted increasing interest. Recently, besides the classical inflammatory markers, the competitive nitric oxide synthase antagonist asymmetric dimethylarginine (ADMA) has been shown to be involved in the pathogenesis of atherosclerosis and cardiovascular diseases. Since obese people present a condition of chronic low-grade inflammation and endothelial dysfunction, in the present study we quantified ADMA levels in uncomplicated obese women (with no clinical, cardiac or metabolic complications) and normal-weight control subjects. We investigated the relationship of ADMA with some anthropometric measurements, abdominal visceral and subcutaneous adipose tissue accumulation, and biochemical and pro-inflammatory factors of the subjects [interleukin-6 (IL-6), soluble IL-6 receptor (sIL-6R), IL6-R/IL-6 ratio, tumor necrosis factor alpha (TNFα), homocysteine (Hcy) and plasminogen activator inhibitor-1 (PAI-1)]. ADMA and all the other pro-inflammatory parameters resulted higher in obese patients than in healthy subjects. ADMA significantly correlated with Hcy, PAI-1, TNFα and with sIL-6R/IL-6 ratio but not with other anthropometric and biochemical parameters. In a stepwise regression analysis ADMA correlated most closely with Hcy and TNFα. In conclusion, in our obese uncomplicated patients TNFα and Hcy emerged as strong predictors of ADMA which might be a potential mediator of the effects of different risk factors affecting the cardiovascular system.

It is now well established that obesity is one of the most important independent risk factors for the development of atherosclerosis and cardiovascular diseases (CD) (1). Chronic immune-mediated inflammation, which involves adipokines, chemokines, cytokines and their receptors, and vascular endothelial cell dysfunction, characterized by a reduced bioavailability of the signaling molecule nitric oxide (NO), are both critically involved in the onset and progression of atherosclerosis (1-2).

Key words: asymmetric dimethylarginine, cardiovascular risk, obesity, inflammation

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Obesity, and in particular visceral obesity, is an important predictor of CD (3). At present, a great deal of evidence demonstrates that obesity appears to be associated with a low-grade state of inflammation, probably as a consequence of the secretion of pro-inflammatory cytokines by adipocytes (4). Adipose tissue, beyond its energy-storage function, is now recognized as an important regulator of the development and progression of chronic low-grade inflammation (5-6) and vascular endothelial dysfunction (7) present in obese subjects. In fact, adipose tissue produces and releases different cytokines and hormone-like proteins, such as interleukin-6 (IL-6) (8) and tumor necrosis factor-a (TNF-α) (9), that may be relevant to the process of atherogenesis (10-11). In particular, high levels of TNF- α and IL-6 have been associated with elevated body fat (12) and with visceral fat deposition directly measured by computed tomography (CT) (11, 13).

In recent years, the link between obesity, inflammation and CD has attracted increasing interest and, recently, besides the classical inflammatory markers, the competitive NO synthase antagonist asymmetric dimethylarginine (ADMA) has been shown to be involved in the pathogenesis of CD (14). Moreover, adipose tissue has also been shown to produce ADMA and to express the full enzymatic machinery responsible for ADMA metabolism (15).

Previous studies (16-17) which evaluated the role of ADMA in the link between obesity and CD have been generally carried out on groups of obese subjects already affected by other complications. In this study we aim to quantify ADMA level in obese uncomplicated women (with no clinical, cardiac or metabolic complications) and in normal-weight controls and to investigate the relationship of ADMA with some anthropometric measurements, abdominal visceral (VAT) and subcutaneous (SAT) adipose tissue accumulation, biochemical, pro-inflammatory and pro-atherosclerotic factors of the subjects.

MATERIALS AND METHODS

Subjects

Twenty-four obese women with no clinical, cardiac or metabolic complications and 12 normal-weight age-matched female controls were selected at the IRCCS Policlinico San Donato, Milan, Italy. The subjects gave informed consent to the examination protocol, conducted in accordance with the Declaration of Helsinki, as revised in 2000. All patients were premenopausal, normotensive (systolic -SBP- and diastolic blood pressure -DBP- < 130 and < 80 mmHg, respectively), normoglycemic (fasting glucose < 110 mg/dL), normolipidemic (total cholesterol < 220 mg/dL, HDL-cholesterol > 50 mg/dL, triglycerides < 150 mg/dL), not dyspnoeic and drug-free to exclude potential confounding factors. All subjects underwent oral glucose tolerance test (75 g) and none had 2-h post-load glucose > 140 mg/dL. Blood samples were collected after an overnight fasting and plasma was stored at -20°C for the assays. Fasting glucose, insulin, total cholesterol, HDL-cholesterol and triglycerides were assayed as previously reported (18). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: HOMA-IR = fasting insulin [μU/mL] x fasting glucose [mmol/L].

Anthropometric measurement and VAT-SAT areas assessment

All the subjects were measured for height, weight, waist circumference and hip circumference; BMI and waist/hip ratio (WHR) were calculated. Adipose tissue accumulation in the obese group was assessed by computed tomography at the L4-L5 level. VAT and SAT areas were measured using a single scan at the L4 level and computed with attenuation values between 2150 and 250 Hounsfield units. The VAT/SAT area ratio was calculated.

Biochemical assays

Plasma ADMA, IL-6, sIL-6R, TNFα and PAI-1 concentrations were evaluated using Enzyme-Linked ImmunoSorbent Assay kits (DLD DIAGNOSTIKA GmbH, Hamburg, Germany, for ADMA, and R&D Systems, Minneapolis, MN, USA for all the others). Hcy levels were measured by chemiluminometric assay (Immulite, LosAngeles, CA, USA). The lowest limits of sensitivity were 0.05 mol/L for ADMA, 0.7 pg/mL for IL-6, 6.5 pg/mL for sIL-6R, 0.06 pg/mL for TNFα, 3 ng/mL for PAI-1 and 0.3 μmol/L for Hcy.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). The normality of data distribution was assessed by the Kolmogorov-Smirnoff test. Comparison between groups was performed using Student's two-tailed *t* test. Pearson's simple correlation model was used to compare different variables and stepwise regression analysis was performed to evaluate the independent relationships. All statistical analyses were performed using STATISTIX 7.0 (Analytical Software, Tallahassee, FL). A p value < 0.05 was considered significant.

RESULTS

Some clinical and anthropometric characteristics of obese and healthy subjects are shown in Table I. In the obese group the means of anthropometric measurements, fasting glucose, fasting insulin, HOMA-IR and triglycerides values were significantly higher, whereas HDL level resulted lower when compared to healthy subjects. However, all biochemical data of the obese group were within the normal ranges. Obese patients had higher ADMA ($p<0.001$), IL-6 ($p<0.005$), sIL-6R ($p<0.0001$), sIL-6R/IL-6 ratio ($p<0.01$), TNFα ($p<0.005$), PAI-1 ($p<0.0001$) and Hcy ($p<0.0001$) levels than healthy subjects (Fig. 1). In Pearson's correlation analysis, ADMA significantly correlated with Hcy ($p<0.002$), PAI-1 ($p<0.02$), TNFα ($p<0.02$) and with sIL-6R/IL-6 ratio ($p<0.05$), but not with IL-6 or sIL-6R alone (Table II), whereas there was no correlation between ADMA level and body mass index (BMI), waist, waist-to-hip ratio, VAT area, SAT area, VAT/SAT ratio, fasting glucose, fasting insulin, HOMA-IR, triglycerides, total and HDL-cholesterol, SBP and DBP (Table II).

To investigate which parameters (independent variable) could account for the association with ADMA (dependent variable), a stepwise regression analysis was carried out. The independent variables included in the model were those significantly correlated with ADMA in univariate analysis: ADMA correlated most closely with Hcy (t value 3.63, $p<0.002$) and TNFα (t value 2.69, $p<0.02$); the other indices did not enter the model.

DISCUSSION

In the present study we observed that in young pre-menopausal, normotensive, uncomplicated obese patients, ADMA plasma concentration was higher compared to age- and sex-matched normal weight subjects and that Hcy and TNFα were independent predictors of ADMA level. According to previous studies, we observed that obese subjects have higher levels of IL-6, sIL-6R, sIL-6R/IL-6 ratio, TNFα,

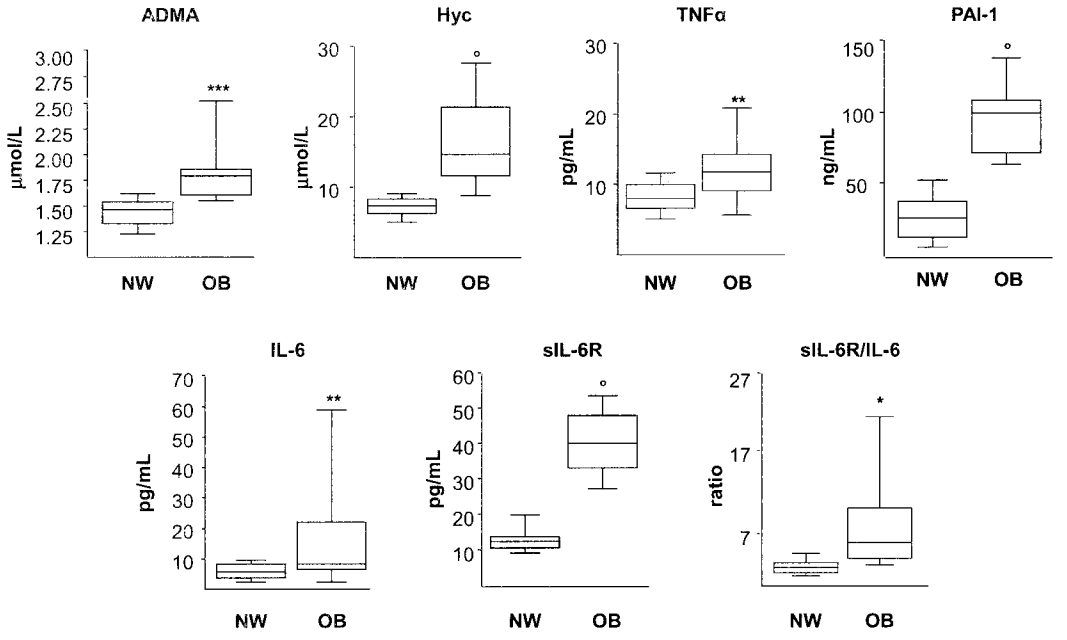

Fig. 1. *Asymmetric dimethylarginine (ADMA), interleukin-6 (IL-6), soluble interleukin-6 receptor (sIL-6R), soluble interleukin-6 receptor/ interleukin-6 ratio (sIL-6R/ IL-6 ratio), tumor necrosis factor* alpha (TNFα), plasminogen activator inhibitor-1 *(PAI-1) and homocysteine (Hcy) levels in normal weight (NW) and obese patients (OB). The smallest observation (sample minimum), lower quartile (25^{th}), median (50^{th}), upper quartile (75^{th}), and largest observation (sample maximum) are shown. *$p<0.01$, ** $p<0.005$, ***$p<0.001$ and °$p<0.0001$ vs NW.*

Table I. *Characteristic of normal-weight and obese subjects.*

	Normal-weight (n = 12)	Obese (n = 24)	*p* value
Age	36.8±8.2	31.9±8.3	0.1
Weight (kg)	58.0±7.5	108.2±12.2	<0.0001
Height (cm)	162.2±8.6	157.4±4.7	0.1
BMI (Kg/m^2)	22.0±1.8	43.7±5.0	<0.0001
Waist circumference (cm)	75.2±4.8	116.1±12.7	<0.0002
Waist-to-hip ratio	0.8±0.04	0.9±0.1	<0.03
Fasting glucose (mg/dL)	75.4±8.0	88.8±7.1	<0.0001
Fasting insulin (μU/mL)	7.4±2.1	12.1±6.0	<0.005
HOMA-IR	1.5±0.6	2.6±1.3	<0.0005
Total Cholesterol (mg/dL)	163.3±17.0	174.1±25.1	0.13
HDL cholesterol (mg/dL)	64.0±5.8	56.4±4.7	<0.001
Triglycerides (mg/dL)	82.1±28.5	111.4±35.0	<0.05
Blood pressure (mmHg)			
Diastolic	78.8±4.8	75.8±6.7	0.14
Systolic	117.9±6.2	119.6±8.0	0.5
VAT (cm^2)		152.76±63.31	
SAT (cm^2)		526.53±98.41	
VAT-to-SAT ratio		0.29±0.16	

Data are expressed as mean ± SD. BMI: body mass index; HDL: high density lipoprotein; HOMA-IR: homeostatis model assessment of insulin resistance; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue.

PAI-1 and also Hcy, which have all been correlated to increased risk of CD (5, 18-20). In our study we did not find any relationship between ADMA and any of the anthropometric and biochemical parameters evaluated. Previous studies which investigated the relationship of ADMA with obesity, observed, in particular, a correlation of ADMA with BMI, glucose and insulin levels (16, 21-22). Although our study did not confirm the existing evidence, the lack of correlations may be explained considering the limited sample size and the characteristics of our population. The development of obesity-related CD, in fact, may be considered the final step of a chronic degenerative process that grows gradually and requires the onset of a chronic inflammatory status which in turn promotes an insulin-resistant condition. Considering that our study was performed on young, pre-menopausal, normotensive obese subjects without any sign of hyperglycemia and insulin resistance, it is clear that our patients should be considered in the early stage of the disease. Thus, the lack of hyperglycemia and insulin resistance in our subjects seems to be important to explain our results.

Relative to lipid metabolism, the association of ADMA with dislypidemia is not clear and our data are in agreement with those studies which failed to confirm a correlation with ADMA (16-17).

In diseases different from obesity, a significant correlation between ADMA and pro-inflammatory cytokines, such as TNFα and IL-6, has been observed and the reduced activity of dimethylarginine dimethylaminoidrolase (DDAH), the enzyme responsible for ADMA degradation, has been indicated as the main mechanism by which these cytokines contribute to increase ADMA concentration (23-25). In particular, in an *in vitro* study carried out on human umbilical vein endothelial cells, TNF-α was shown to increase the accumulation of ADMA and to decrease that of DDAH (25). Moreover, in patients with acute liver failure, elevated plasma concentration of ADMA was shown to correlate with the concentrations of TNF-α, possibly through the regulation of ADMA metabolism (24). Lastly,

Table II. *Pearson's coefficients between ADMA and relevant risk variables in obese patients.*

	ADMA	
	r	p
BMI	0.09	0.67
Waist circumference	0.15	0.49
Waist -to-hip ratio	0.09	0.69
VAT	0.12	0.57
SAT	-0.15	0.48
VAT/SAT	0.19	0.36
Glucose	0.28	0.18
Insulin	-0.08	0.72
HOMA-IR	-0.04	0.84
Triglycerides	0.38	0.14
Total Cholesterol	0.17	0.43
HDL	0.19	0.37
SBP	0.02	0.93
DBP	0.38	0.07
IL-6	-0.31	0.14
sIL-6R	0.17	0.43
sIL-6R/IL-6	0.44	**<0.05**
TNFα	0.50	**<0.02**
Hcy	0.62	**<0.002**
PAI-1	0.50	**<0.02**

ADMA: asymmetric dimethylarginine; BMI: body mass index; HOMA-IR: homeostatis model assessment of insulin resistance; HDL: high density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure; IL-6: interleukin-6; SAT: subcutaneous adipose tissue; sIL-6R: soluble interleukin-6 receptor; TNFa: tumor necrosis factor a; Hcy: homocysteine: PAI-1: activator inhibitor-1; VAT: visceral adipose.

the observation that also in our uncomplicated obese patients, a positive correlation between increased ADMA and TNF-α levels, which further resulted as independent predictor of ADMA level, exists, supported the hypothesis of an association between a cytokine-driven inflammatory response and ADMA level.

Interestingly, we also observed a positive association with sIL-6R/IL-6 ratio but not with IL-6 and sIL-6R alone. A key feature in the regulation of IL-6 responses has been the identification of a soluble interleukin 6 receptor (sIL-6-R), which forms a ligand-receptor complex, capable of either stimulating several cellular responses, including proliferation and differentiation, and critically involved in the transition between acute and sustained inflammation state and in the maintenance of chronic inflammatory diseases (26-27). Thus, the complex, and not IL-6 alone, may be regarded as the active cytokine, and our study disclosed a novel biological effect of this complex, which appeared to be distinct from sIL-6R and IL-6 alone, in a condition of chronic inflammation. However, in multiple testing the correlation was not significant.

Increased PAI-1 levels have been shown to promote the progression of CD. PAI-1 levels were significantly increased in our obese subjects and are consistent with previous studies (28). ADMA and PAI-1 are both specific circulating markers associated with impaired endothelial function reflecting altered vascular tone and fibrinolysis, respectively. Our regression analysis revealed a significant positive correlation of ADMA with PAI-1. On the other hand, this correlation resulted not significant in multiple testing. Thus, it remains speculative whether PAI-1 may be associated with ADMA in obese subjects.

Hcy was proposed as an important risk factor for atherosclerosis and CD (29). Some of the mechanisms by which Hcy induces atherosclerosis were shown to be mediated by ADMA-reduced NO production. Moreover, the relationship between ADMA and Hcy has also been supported by *in vitro* studies which suggested that Hcy may affect ADMA by reducing DDHA activities. Furthermore, the association between ADMA and Hcy has been described in patients with atherosclerosis or cardiovascular events or complicated obesity (30-31). In our study we observed this correlation also in uncomplicated obese patients who did not display symptoms of CD. Although the association between Hcy metabolism and ADMA has been extensively investigated, to our knowledge no previous reports have described this relationship in obese patients without evident CD. Moreover, since Hcy emerged as a strong and independent predictor of ADMA, it is possible that Hcy may indirectly promote endothelial dysfunction and progression of atherosclerosis via ADMA.

Future studies will be designed to better correlate in such a population ADMA, Hcy and inflammation with specific markers of atherosclerosis such as plaque formation, aortic intima-media thickness, calcification index and elongation.

In conclusion, in the present study we demonstrated that in uncomplicated obese patients with no apparent CD, the increased inflammatory status is linked to ADMA level. Thus ADMA may be considered a potential biomarker reflecting the effects of different risk factors which affect the cardiovascular system.

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