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ORIGINAL PAPER

Unchanged asymmetric dimethylarginine levels in non-diabetic, premenopausal obese women who have common risk factors for cardiovascular disease

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Abstract This study was performed to test whether plasma asymmetric dimethylarginine (ADMA) concentrations are related to obesity and obesity complications including decrement in insulin sensitivity and adiponectin levels, dyslipidemia and low-grade inflammation. Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) concentrations were analyzed by HPLC in 17 overweight (BMI ≥ 25 kg/m^2) and 40 obese (BMI ≥ 30 kg/m^2) premenopausal women. Age-matched healthy women were studied as controls. Obesity did not give rise to a significant change in circulating ADMA levels but reduced in SDMA levels. As compared with control subjects (0.441 ± 0.102 μM), ADMA values in overweight and obese subjects were found to be as 0.412 ± 0.102 and 0.436 ± 0.093, respectively. No Pearson's association of ADMA with relevant risk variables for cardiovascular disease, including blood pressure, insulin sensitivity, inflammatory markers, lipid and adiponectin levels. However, in linear regression analysis, BMI, diastolic blood pressure, glucose, insulin, and IL-8 emerged as significant predictors of ADMA. In spite of obese women have elevated hs-CRP, triglyceride levels and decreased insulin sensitivity, adiponectin and HDL-cholesterol levels, all of which is closely linked risk factors for cardiovascular disease, circulating ADMA levels remained unchanged in obese individuals as compared with controls.

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Introduction

Nitric oxide (NO) is synthesized from the amino acid L-arginine by NO synthases and regulates vascular tone, thrombocyte activation, neurotransmission, and host defence. Deficiency of NO increases vascular resistance and promotes atherogenesis.

Impaired vasodilation has been suggested to be caused by inhibition of NO generation by the amino acid, asymmetric dimethylarginine (ADMA), a recently described important endogenous competitive inhibitor of nitric oxide synthetase (NOS) [1]. ADMA has also been shown to increase oxidative stress by uncoupling of electron transport between NOS and L-arginine, and hence decrease both the production and availability of endothelium-derived NO [2]. ADMA is consistently produced in the course of normal protein turnover in many tissues, including vascular endothelial cells, and is derived from hydrolysis of methylated proteins. It appears that both synthesis and degradation of ADMA and other methylarginine, symmetric dimethylarginine (SDMA) are highly actively regulated, and dysregulation of either of these pathways may result in increased levels of free methylarginines [3, 4].

Although SDMA is produced in equivalent amounts, has no effect on NO synthesis [5]. ADMA is mainly metabolized by dimethylarginine dimethylaminohydrolase (DDAH) in to citrulline and dimethylamine [6] and additionally eliminated by the kidney [7]. However, SDMA is eliminated only via renal excretion [7]. Therefore SDMA is

more closely related to the glomerular filtration rate compared to ADMA [8].

Increased ADMA concentrations have been described in hypercholesterolemia [9], hypertension [10], arterial occlusive disease [11], chronic renal failure [1], and type 2 diabetes [12].

However, ADMA concentrations in obese patients who are at risk for diabetes mellitus and cardiovascular disease are not well established.

We have therefore quantified ADMA and SDMA plasma levels in non-diabetic overweight and obese-premenopausal women and controls and investigated the relationships of these parameters to insulin sensitivity, dyslipidemia, low-grade inflammation and hypoadiponectinemia observed in obese patients.

Material and methods

Patients

We studied 17 overweight women, with a median age of 33 years (range 19–46 yrs) and 40 obese women, with a median age of 37 (range 21–49 yrs), and 29 healthy control with a median age of 29 (range 19–45 yrs). Overweight was defined on the basis of a body mass index (BMI) of ≥25 kg/m^2 and obese was defined on those of ≥30 kg/m^2. All women were spontaneously menstrually active and non-diabetic. In obese group, the prevalence of hypertension (systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg) and that of hyperlipidemia (triglyceride levels of ≥200 mg/dl) were 10%. None of the subjects had any known cardiovascular disease, familial hyperlipidemia, chronic renal failure, and thyroid disorders. No subjects were taking any medications.

Each subject gave informed consent to participate in the study.

Analytical methods

Blood samples were taken and immediately centrifuged and stored at –80°C. Glucose, total cholesterol, LDL- and HDL-cholesterols, triglyceride, creatinine, and uric acid were determined by an automated analyzer. Insulin was measured by means of an enzyme-amplified chemiluminescence assay.

Insulin resistance was evaluated by ''homeostasis model assessment (HOMA)'' formula. This formula was calculated by fasting plasma insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5. To analyze high sensitivity C-reactive protein (hs-CRP), we used highly sensitive immunoassay (Cobas Integra 400 chemistry analyzer: Roch Diagnostics). Blood concentrations of tumor necrosis factor-α (TNF-α) (Bio Source International, CA, USA), interleukine-8 (IL-8) (Beckman Coulter Comp, Marseille Cedex, France), and adiponectin (B-Bridge International, CA, USA) were determined by enzyme-linked immunosorbent assays. Nitrate was analyzed using Total Nitric Oxide Assay kit (R&D System Europe, Abingdon, UK).

Plasma concentrations of ADMA, SDMA, and L-arginine were measured by high-performance liquid chromatography (HPLC) and precolumn derivatization with *o*-phthaldialdehyde (OPA) are previously described in detail [13].

Statistical analysis

Data are presented as means ± SD. With continuous variables, group mean values were compared using the unpaired Student's *t*-test, as long as the variables were normally distributed. If the data distribution did not follow the normally assumption, Mann–Whitney *U*-test was utilized. Pearson's correlation coefficients (*r*) were computed to explore the correlation between two variables. If variables were not normally distributed, it was log-transformed prior to correlations. Further, linear regression analyses were performed to evaluate the independent relationships.

Results

Table 1 presents the subject characteristics.

ADMA concentrations of overweight and obese patients were not found to be different from those of controls.

However, SDMA values were significantly lower in obese patients. No difference in L-arginine levels, L-arginine/ADMA ratio and nitrate levels was detected between groups. Plasma concentration of ADMA was not found to be different in smokers (0.449 ± 0.092) as compared with non-smokers (0.430 ± 0.091).

In Pearson's correlation analysis, there were no correlations between ADMA levels and relevant risk variables (Table 2). No correlations between L-arginine/ADMA ratio, as an index of the bioavailability of plasma L-arginine, and relevant risk factors were detected in our study. Creatinine was found to be correlated with SDMA levels, not ADMA levels (Fig. 1).

A multiple linear regression analysis was carried out with ADMA as dependent variable and BMI, blood pressure, L-arginine, glucose, insulin, adiponectin, and inflammatory markers as independent variables by using stepwise regression model (Table 3). According to this, BMI, diastolic blood pressure, glucose and insulin and IL-8 levels

Table 1 Characteristics of controls, overweight, and obese subjects. Data are mean ± SD

	Control	Overweight	Obese
Age (years)	30.34 ± 7.13 (n:29)	32.00 ± 8.14 (n:17)	35.82 ± 6.81 (n:40)
BMI (kg/m^2)	21.49 ± 2.19 (n:29)	26.87 ± 1.7 (n:17)***	37.08 ± 5.37 (n:40)***^{c}
SBP (mmHg)	105.4 ± 10.4 (n:21)	105.6 ± 15.6 (n:15)	116.3 ± 21.1 (n:40)
DBP (mmHg)	69.5 ± 7.4 (n:21)	73.5 ± 8.4 (n:15)	78.7 ± 11.2 (n:40)**
Glucose (mg/dl)	79.17 ± 7.13 (n:29)	81.23 ± 8.43 (n:17)	88.15 ± 9.16 (n:40)***^{a}
Insulin (μU/ml)	5.85 ± 2.42 (n:28)	7.34 ± 3.10 (n:16)*	13.55 ± 5.96 (n:39)***^{c}
HOMA	1.21 ± 0.60 (n:27)	1.51 ± 0.67 (n:15)	2.92 ± 1.24 (n:39)***^{c}
Adiponectin (μg/ml)	13.04 ± 3.89 (n:29)	10.07 ± 3.63 (n:17)*	7.47 ± 2.94 (n:40)***^{b}
Triglyceride (mg/dl)	64.6 ± 22.3 (n:28)	75.5 ± 28.3 (n:17)	117.5 ± 64.6 (n:40)***^{b}
Total cholesterol (mg/dl)	177.0 ± 41.7 (n:28)	175.2 ± 33.5 (n:17)	182.5 ± 33.7 (n:40)
LDL-cholesterol (mg/dl)	99.4 ± 33.2 (n:28)	100.9 ± 24.6 (n:17)	110.4 ± 26.7 (n:40)
HDL-cholesterol (mg/dl)	57.9 ± 14.3 (n:28)	51.9 ± 7.9 (n:17)	44.5 ± 9.2 (n:40)***^{b}
hs-CRP (mg/l)	1.11 ± 1.25 (n:26)	2.16 ± 1.99 (n:14)	4.13 ± 3.24 (n:31)***^{a}
TNF-α (pg/ml)	12.37 ± 36.10 (n:29) (median: 4.16)	21.26 ± 28.60 (n:15) (median: 6.16)	11.70 ± 18.38 (n:30) (median: 5.43)
IL-8 (pg/ml)	32.06 ± 34.31 (n:24) (median: 17.25)	18.69 ± 14.54 (n:14) (median: 19.39)	44.79 ± 75.92 (n: 34) (median: 27.88)
Creatinine (mg/dl)	0.685 ± 0.090 (n:27)	0.723 ± 0.083 (n:17)	0.695 ± 0.098 (n:40)
Uric acid (mg/dl)	3.35 ± 0.49 (n:18)	3.82 ± 0.98 (n:16)	4.47 ± 0.78 (n:39)***^{a}
ADMA (μmol/l)	0.441 ± 0.089 (n:25)	0.412 ± 0.102 (n:16)	0.436 ± 0.093 (n:37)
SDMA (μmol/l)	0.313 ± 0.064 (n:25)	0.311 ± 0.064 (n:16)	0.270 ± 0.064 (n:36)*
ADMA/creatinine (μmol/mg)	0.065 ± 0.018 (n:22)	0.057 ± 0.015 (n:16)	0.064 ± 0.016 (n:37)
SDMA/creatinine (μmol/mg)	0.046 ± 0.011 (n:23)	0.042 ± 0.009 (n:16)	0.038 ± 0.009 (n:36)**
L-arginine (μmol/l)	78.70 ± 17.84 (n:25)	79.30 ± 18.67 (n:16)	75.02 ± 18.47 (n:37)
L-arginine/ADMA	181.2 ± 50.8 (n:25)	201.6 ± 59.0 (n:16)	177.0 ± 47.2 (n:37)
Nitrate (μmol/l)	39.43 ± 23.01 (n:28)	31.19 ± 14.29 (n:15)	38.97 ± 19.00 (n:32)

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared with control group

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ as compared with overweight group

Table 2 Pearson correlations between ADMA, L-arginine/ADMA, and relevant risk variables in all groups. * log transformed

	ADMA		L-arginine/ADMA	
	r	*p*	*r*	*p*
BMI	0.077	0.50	–0.130	0.25
Glucose	–0.028	0.80	0.098	0.38
Insulin	–0.051	0.65	0.036	0.75
HOMA	–0.087	0.45	0.063	0.59
Triglyceride	–0.016	0.88	0.038	0.73
HDL	0.053	0.63	–0.036	0.75
SBP	0.110	0.35	–0.230	0.05
DBP	0.160	0.18	–0.180	0.12
Hs-CRP*	0.091	0.45	–0.034	0.78
Adiponectin	0.009	0.93	0.016	0.88
Creatinine	–0.120	0.27	0.086	0.44

emerged as significant predictors of plasma ADMA concentrations.

A linear regression analysis was also performed with BMI as independent variable and blood pressures, lipid profile, inflammatory markers, HOMA index, and adiponectin as independent variables. The percentage of variance of BMI by these parameters was 76.5% (*p*: 0.000). BMI emerged as a significant predictor of insulin sensitivity and hs-CRP (Table 4).

Discussion

The endogenous nitric oxide synthase inhibitor asymmetric dimethyl arginine (ADMA) is elevated in patients with increased risk for arteriosclerosis. Obesity is a risk factor for cardiovascular disease. The well-known cardiovascular

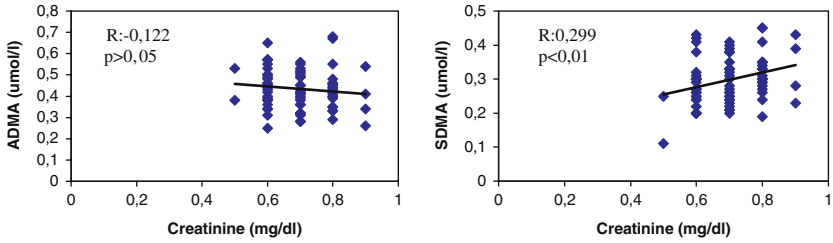

Fig. 1 Pearson correlations of creatinine with ADMA and SDMA. Pearson's correlation coefficient (r) and p-values are indicated

Table 3 Linear regression analysis of variables associated with ADMA in all groups. * log transformed

	Estimate	p
BMI	2.532	**0.020**
SBP	–1.510	0.147
DBP	3.808	**0.001**
TNF-α *	–0.855	0.403
IL-8*	4.469	**0.000**
Glucose	–2.912	**0.009**
Insulin	–2.870	**0.009**
Adiponectin	2.023	0.057
L-arginine	1.744	0.096
Uric acid	–1.347	0.193

Table 4 Linear regression analysis of variables associated with BMI in all groups. * log transformed

	Estimate	p
HOMA index	4.162	**0.000**
Hs-CRP*	3.358	**0.002**
TNF-α*	1.492	0.145
Triglyceride	0.314	0.755
HDL-cholesterol	–1.213	0.233
Adiponectin	0.725	0.473
SBP	2.732	**0.010**
DBP	–1.182	0.245

risk factor such as type-2 diabetes, dyslipidemia, and low-grade inflammation have been defined in obese subjects [14–16]. ADMA is also a potential candidate enhanced vascular complications in obese individuals.

In the present study, low-grade inflammatory response, increased triglyceride levels, decreased insulin sensitivity, HDL-cholesterol, and adiponectin levels, all of which is closely linked risk factor for cardiovascular disease, were detected in obese patients (Table 1). However, ADMA plasma levels remained unchanged in overweight and obese patients as compared with controls. But symmetric dimethyl-L-arginine (SDMA), its biologically inactive stereoisomer, showed a significant decrement in obese patients. As SDMA is eliminated only via renal excretion, low levels of circulating SDMA may indicate its high renal clearance in obese subjects. Meanwhile, creatinine levels, a strong predictor for the renal outcome, was found to be correlated with SDMA but not ADMA in our study (Fig. 1), as confirmed previously report indicating a strong relation of SDMA to renal excretion [17]. In addition, a decrement in SDMA/creatinine ratio in obese patients may show that SDMA is eliminated more efficiently than creatinine in this group. On the other hand, ADMA, SDMA, and L-arginine share a common pathway for entry in to the cell competitively [18]. A decline in plasma concentration of SDMA may, therefore indirectly modify the transport of ADMA and L-arginine into the cells even if the plasma levels of last two remained unchanged in obese individuals. For this reason, nitrate levels, a stable metabolite of NO was also measured in this study. We could, however, not demonstrate any significant change in nitrate concentrations between groups.

In previous studies investigating the relationship of ADMA with obesity, ADMA concentrations of obese patients have been reported to be significantly higher than non-obese healthy subjects [19, 20]. Although our study could not confirm the existing evidence, the unchanged ADMA levels as compared with controls may be partly explained with the different impact of several parameters changing with obesity on ADMA production.

In this study, we tried to select factors independently related to ADMA by using stepwise regression model (Table 3). We found independently positive correlations of ADMA with diastolic blood pressure, IL-8 and BMI and negative correlations of ADMA with glucose and insulin concentrations. The percentage of variance of ADMA levels predicted by these measured parameters was 70.1% (p: 0.002). According to this finding, we may say that high BMI and high levels of glucose and insulin in obese patients may have been differently affected circulating ADMA levels.

Insulin, at normal physiologic concentration, was reported to increase skeletal muscle blood flow in healthy people [21]. It is reasonable that insulin exhibits this effect as a vasodilatator by modulating ADMA levels, consequently NO production. As a matter of fact, it was recently reported that in human cultured endothelial cells, ADMA accumulation decreases with increasing insulin doses via

dose-related increase in the activity of the metabolic enzyme DDAH [22], confirming the independent inverse association between insulin and ADMA levels in our study.

In spite of ADMA was observed an independent predictor of diastolic blood pressure (DBP), it does not seem possible that ADMA was responsible for the high DBP in obese patients. In the previous studies, plasma ADMA concentrations were also reported not to correlate with blood pressure in type 2 diabetes [23] and morbidly obese women [19]. Although plasma ADMA was also positively and independently associated with BMI in regression analysis, plasma ADMA concentration in our obese patients was not found to be high as compared with controls.

The plasma levels of ADMA in the control subjects in present study were 0.441 ± 0.089 μmol/l, which were much lower than those reported before [24, 25], but were similar to those reported by Miyazaki et al. [26], Volkanen et al. [27] and Teerlink et al. [28]. These variable results may be likely explained by methodologic and/or ethnic differences.

It was previously reported that chronic subclinic inflammation is closely related to obesity and reduction of BMI results in a decrease of inflammatory markers [29]. Hs-CRP levels, a strong inflammatory marker for cardiovascular risk [30] was found to enhance in our obese population. We also detected a profound independent association between BMI and hs-CRP (Table 4). However, a relationship between hs-CRP and ADMA was not observed. Nevertheless, ADMA emerged a significant predictor of IL-8 concentration in our study, consistent with previous study reporting that exogenous ADMA stimulates secretion of IL-8 in endothelial cells [31]. IL-8 is a cytokine, which promotes pivotal steps in initiation of atherosclerosis [32]. Increased levels of ADMA could influence vascular function by modulating IL-8 production. But this speculation must be further investigated.

In conclusion, our data supports that risk factors such as elevated hs-CRP, triglyceride levels and decreased insulin sensitivity, adiponectin, and HDL-cholesterol levels seem to play a more important role for the cardiovascular outcome of obese patients than ADMA levels.

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