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Asymmetric dimethylarginine association with antioxidants intake in healthy young adults: a role as an indicator of metabolic syndrome features

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Abstract

The purpose of this study was to evaluate the potential associations between serum asymmetric dimethylarginine (ADMA) and several anthropometric, biochemical, and lifestyle features in healthy young adults, emphasizing on the putative effects of the antioxidant intake on ADMA concentrations. Anthropometric and blood pressure measurements as well as lifestyle features and antioxidant intake were analyzed in 93 healthy young adults aged 18 to 34 years. Fasting blood samples were collected for the measurement of glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triacylglycerols, and ADMA concentrations, as well as erythrocyte glutathione peroxidase activity. Nail samples were collected for the analysis of selenium and zinc concentrations. Values of body mass index ($P = .004$), waist circumference ($P = .008$), waist-to-height ratio ($P = .046$), systolic blood pressure ($P < .001$), serum glucose ($P < .001$), and nail selenium ($P = .004$) and zinc ($P = .018$) were significantly different between subjects with serum ADMA higher and lower than the median (cutoff, 458 nmol/L). Furthermore, ADMA showed a positive association with several adiposity markers such as body weight ($P < .001$), body mass index ($P < .001$), waist circumference ($P = .006$), waist-to-height ratio ($P = .020$), body fat mass ($P = .001$), systolic blood pressure ($P = .001$), and serum glucose ($P < .001$), whereas erythrocyte glutathione peroxidase activity ($P = .021$) and nail selenium ($P = .040$) and zinc values ($P = .013$) were statistically significant negative predictors of ADMA concentrations. In conclusion, ADMA seems to be related with selenium and zinc status and several anthropometric and biochemical measurements linked to metabolic syndrome in apparently healthy young adults. These findings support a role for antioxidant/trace element intake in the modulation of ADMA, whose assessment may be a marker of metabolic syndrome manifestations.

1. Introduction

The role of inflammation and oxidative stress on several chronic diseases is receiving increasing attention because of their links with atherosclerosis, obesity, or type 2 diabetes mellitus [1,2]. Cause-effect relationships between inflammation, oxidative stress, and disease are not clear; but several studies associate metabolic syndrome features with higher concentrations of inflammatory biomarkers [3-5]. In this sense, beyond its energy storage function, adipose tissue is now recognized as a major endocrine organ synthesizing a variety of cytokines (adipokines) that are central in the control of energy balance and food intake [6]. Furthermore, it is now well established that at least some of these adipokines play a role in inflammation [7,8], endothelial dysfunction [9], and vascular repair [10].

In this context, asymmetric dimethylarginine (ADMA) is a naturally occurring amino acid that inhibits the activity of nitric oxide synthase (NOS) [11], whose increase in circulating levels is related with endothelial dysfunction, which is in turn associated with several manifestations of cardiovascular disease [12]. Because metabolic syndrome features are inflammation related [2] and ADMA concentrations are sensitive to inflammation [13], their study is a good target to better understand the associations between serum ADMA and several anthropometric, biochemical, and lifestyle features in healthy young adults, emphasizing on the putative effect of antioxidant intake on ADMA concentrations.

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2. Subjects and methods

2.1. Subjects

Ninety-three white healthy subjects were recruited to participate in the study (74 women and 19 men; age, 20.7 ± 2.7 years). Initial enrolment screening evaluations included a medical history, physical examination, and fasting blood profile to exclude subjects with evidence of any disease related to chronic inflammation, oxidative stress, hydric unbalance, nutrient absorption, or nutrient metabolism. Other exclusion criteria were drug or nutritional treatments up to 6 months before the participation in this study. In accordance with the Declaration of Helsinki (2000) and after a clear explanation of the study protocol, all subjects gave a written informed consent to participate in the study, which was previously approved by the Ethics Committee of the University of Navarra (ref 79/2005).

2.2. Anthropometric and body composition measurements

All anthropometric measurements were carried out with the subjects barefoot, wearing only their underwear, and after an overnight fast following standardized protocols [14]. All these determinations were carried out 3 times, but not consecutively. Body weight was measured to the nearest 0.1 kg and body fat to the nearest 0.1% by using a Tanita TBF 300 (Tanita, New York, NY). Body mass index (BMI) was calculated as body weight divided by squared height (in kilograms per square meter). Skinfold thicknesses (STs) were measured at the right side to the nearest 0.2 mm by means of a Holtain skinfold caliper (Holtain, Crymych, United Kingdom) at the triceps, the biceps, and the subscapular and suprailiac regions [15]. Waist and hip circumferences were measured with an inelastic tape to the nearest 1 mm. Blood pressure was measured by a mercury sphygmomanometer (Minimus II; Riester, Jungingen, Germany) to the nearest 2.5 mm Hg.

2.3. Analyses of biological samples

All serum blood samples were drawn after an overnight (12-hour) fast, centrifuged immediately for 15 minutes at 3500 rpm and 4°C, and stored at −80°C. Serum glucose, triacylglycerols, total cholesterol, and high-density lipoprotein cholesterol (HDL-c) were assessed by an automatized colorimetric assay (COBAS MIRA; Roche, Basel, Switzerland) with specific commercially available kits (ABX Pentra, Roche). The reported plasma low-density lipoprotein cholesterol (LDL-c) data were calculated by the Friedewald equation as described elsewhere [16]. Insulin concentrations were measured by using an enzyme-linked immunosorbent assay (ELISA) as described by the supplier (Mercodia, Uppsala, Sweden). For estimating insulin sensitivity [17], the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as glucose concentration × insulin concentration/22.5. Asymmetric dimethylarginine concentrations were evaluated by an ELISA kit (DLD Diagnostika, Hamburg, Germany) as described elsewhere [18]. Total serum complement factor 3 (C3) concentrations were determined using an automatized turbidimetric assay (COBAS MIRA). Plasma circulating concentrations of C-reactive protein (CRP) (Immundiagnostik, Bensheim, Germany) were evaluated by ELISA procedures. Glutathione peroxidase (GPx) activity was measured in erythrocytes by using a commercially available kit (Cayman Chemical, Ann Arbor, MI). Nail samples were collected at the time of interview and stored at room temperature in clean polypropylene bags labeled with subject identification numbers. Fingernails and toenails samples were treated with sub-boiling nitric acid in a high-pressure Teflon digestion vessel using a microwave digestion system (Ethos Plus; Millestone, Sorisole, Italy). A PerkinElmer (Norwalk, CT) Analyst 800 atomic absorption spectrometer, equipped with an air/acetylene flame and a graphite atomizer (transversely heated tube with end cap, Zeeman background corrector, and AS-800 Autosampler), was used for zinc and selenium measurements in sample digestions, respectively. A hollow cathode lamp and an electrodeless discharge lamp (PerkinElmer), operated at 15 and 280 mA with a spectral bandwidth of 0.7 and 2.0 nm, providing resonance lines of 213.9 and 196.0 nm were used for zinc and selenium, respectively. The concentration values of zinc and selenium obtained were adjusted for the sample weight and expressed as microgram or nanogram per gram of nail, respectively.

2.4. Dietary intake assessment

Habitual dietary intake was assessed with a semiquantitative 136-item food frequency questionnaire previously validated [19]. Each item in the questionnaire included a typical portion size. Daily food consumption was estimated by multiplying the portion size by the consumption frequency for each food item. Nutrient composition of the food items was derived from Spanish food composition tables [20,21].

2.5. Statistical analysis

The Shapiro-Wilk test was used to determine variable distribution. Accordingly, the parametric Student t test or nonparametric Mann-Whitney U test was performed to detect differences between subjects with ADMA concentrations higher and lower than the median value (cutoff, 458 nmol/L). The Spearman correlation coefficient was used to evaluate the statistical associations among variables. A multivariate linear regression model was fitted to further explain relationships between variables. Erythrocyte GPx activity and nail zinc concentrations were distributed into quintiles for the regression analysis to better understand their role in ADMA prediction. Results are presented as mean (standard deviation). Confidence intervals (95% CIs) were used to describe linear regression coefficient (B) values, and $P < .05$ was considered statistically significant. Statistical

analysis were performed by using SPSS version 13.0 (SPSS, Chicago, IL) for Windows XP (Microsoft, Redmond, WA).

3. Results

Anthropometric characteristics and biochemical determinations of subjects categorized according to serum ADMA concentrations are reported in Table 1. Sex distribution produced no differences between groups (data not shown). Interesting statistically significant differences ($P < .05$) between subjects with higher and lower concentrations of circulating ADMA (cutoff, 458 nmol/L) were found for body weight, BMI, visceral fat depots measurements such as waist circumference and waist-to-height ratio, and systolic blood pressure. Other adiposity measurements such as STs, body fat, and truncal vs total STs (percentage of truncal fat) followed similar trends, but did not change significantly when split by ADMA concentrations. Neither were significant changes found (Table 1) for circulating concentrations of insulin, HOMA-IR, total cholesterol, HDL-c, LDL-c, triacylglycerol, CRP, or C3. The only additional statistically significant differences in biochemical parameters were found for serum glucose, erythrocyte GPx activity, and nail selenium and zinc concentrations. Regarding the lifestyle features and the antioxidant intake analyzed (Table 2), only statistically significant changes in vitamin A and selenium intake were detected when split by ADMA levels. To further investigate the relationships between anthropometric and biochemical characteristics with serum ADMA concentrations as continuous variables, the following statistical correlations were identified: body weight ($r_s = 0.34$, $P = .001$), BMI ($r_s = 0.34$, $P = .001$), waist circumference ($r_s = 0.25$, $P = .015$), waist-to-height ratio ($r_s = 0.21$, $P = .043$), systolic blood pressure ($r_s = 0.43$, $P < .001$), serum glucose ($r_s = 0.48$, $P < .001$), erythrocyte GPx activity ($r_s = -0.25$, $P = .019$), nail selenium ($r_s = -0.28$, $P = .006$), and nail zinc ($r_s = -0.29$, $P = .005$). No associations were found between ADMA concentrations and smoking habits or physical activity. Linear regression analysis showed that ADMA concentration is a positive predictor of body weight, BMI, waist circumference, waist-to-height ratio, body fat, systolic blood pressure, and serum glucose after adjusting for sex,

Table 1
Anthropometric and biochemical data (mean ± SD) for young adults categorized by the median (cutoff, 458 nmol/L) of ADMA concentrations

	Serum ADMA <458 nmol/L (n = 46)	Serum ADMA >458 nmol/L (n = 47)	*P* value
Age (y)	20.8 ± 2.9	20.7 ± 2.5	.820
Body weight (kg)	57.0 ± 9.1	63.8 ± 12.1	.005
BMI (kg/m^2)	21.0 ± 2.2	22.5 ± 2.7	.004
Waist circumference (cm)	68.8 ± 6.6	73.0 ± 8.2	.008
Waist-to-height ratio[a]	0.42 ± 0.03	0.43 ± 0.04	.046
Tricipital ST (mm)[a]	16.1 ± 4.7	16.4 ± 4.2	.758
Sum of 4 STs (mm)	48.8 ± 16.2	48.6 ± 14.8	.936
Truncal fat (%)[a]	51.4 ± 6.0	49.3 ± 6.3	.100
Body fat (%)[a]	19.5 ± 6.2	21.5 ± 7.1	.157
Systolic blood pressure (mm Hg)	111.2 ± 11.6	120.7 ± 9.6	<.001
Diastolic blood pressure (mm Hg)	63.9 ± 8.5	64.8 ± 7.7	.640
Glucose (mmol/L)[a]	4.5 ± 0.4	4.8 ± 0.3	<.001
Insulin (μIU/mL)	8.3 ± 3.3	8.6 ± 3.5	.735
HOMA-IR[a]	1.7 ± 0.7	1.8 ± 0.8	.338
Total cholesterol (mmol/L)[a]	4.7 ± 0.9	4.5 ± 0.6	.217
HDL-c (mmol/L)	1.6 ± 0.4	1.6 ± 0.3	.866
LDL-c (mmol/L)[a]	2.7 ± 0.8	2.6 ± 0.5	.277
Triacylglycerol (mmol/L)	0.8 ± 0.3	0.8 ± 0.3	.923
GPx activity (nmol/[min mL])[a,b]	684.1 ± 23.1	615.0 ± 20.5	.028
Selenium (ng/g of nail)	476.9 ± 173.8	390.6 ± 86.6	.004
Zinc (μg/g of nail)	142.1 ± 119.2	123.9 ± 89.8	.018
CRP (mg/L)	1.1 ± 0.8	1.2 ± 0.9	.546
C3 (g/L)	1.1 ± 0.2	1.1 ± 0.2	.220
ADMA (nmol/L)	334.9 ± 71.9	651.3 ± 141.4	<.001

[a] Student *t* test for variables with normal distribution. The remaining variables were analyzed by Mann-Whitney *U* test.

[b] n = 44 for ADMA values less than 458 nmol/L; n = 45 for ADMA values greater than 458 nmol/L.

Table 2
Antioxidant/trace element intake and lifestyle features concerning young adults (mean ± SD) categorized by the median (cutoff, 458 nmol/L) of ADMA concentrations

	Serum ADMA <458 nmol/L (n = 46)	Serum ADMA >458 nmol/L (n = 47)	*P* value
Antioxidant daily intake			
Total energy intake (kcal/d)	2736.3 ± 961.4	2529.2 ± 915.9	.289
Fiber (g/d)	27.9 ± 14.1	23.2 ± 9.8	.189
Vitamin A (mg/d)	2171.6 ± 1734.9	1545.2 ± 1237.8	.013
Vitamin C (mg/d)	298.6 ± 139.6	259.7 ± 155.4	.108
Vitamin E (mg/d)	7.7 ± 3.8	7.6 ± 5.1	.329
Magnesium (mg/d)	444.3 ± 161.7	411.3 ± 133.7	.534
Selenium (μg/d)	100.7 ± 35.2	86.7 ± 26.8	.029
Zinc (mg/d)	19.6 ± 11.1	18.2 ± 10.5	.585
Lifestyle			
Vitamin supplementation users (%)[a]	37.0	29.8	.463
Smokers (%)[a]	23.9	17.0	.410
Smoking (cigarettes/d)	2.8 ± 4.6	2.5 ± 5.4	.293
Regular practice of sport (%)[a]	45.7	40.4	.611
Time spent practicing sport (h/wk)	1.5 ± 2.0	2.5 ± 6.7	.851
Family history of cardiovascular disease (%)[a]	39.1	29.8	.343

[a] χ^2 test for dichotomous variables.

Table 3
Multiple linear regression analysis with ADMA concentration (in micromoles per liter) as an independent variable (n = 87)

	ADMA coefficient (95% CI)	*P*	R^2
Model 1			
Body weight (kg)	17.330 (7.872 to 26.788)	<.001	0.402
BMI (kg/m^2)	4.616 (2.056 to 7.175)	.001	0.168
Waist circumference (cm)	7.056 (0.234 to 13.879)	.043	0.343
Waist-to-height ratio (cm/cm)	0.027 (-0.013 to 0.067)	.190	0.080
Body fat (%)	9.242 (3.109 to 15.374)	.004	0.295
Systolic blood pressure (mm Hg)	22.388 (11.324 to 33.452)	<.001	0.238
Serum glucose (mmol/L)	0.876 (0.486 to 1.265)	<.001	0.187
Model 2			
Body weight (kg)	19.039 (8.714 to 29.365)	<.001	0.404
BMI (kg/m^2)	5.368 (2.718 to 8.018)	<.001	0.235
Waist circumference (cm)	9.511 (2.793 to 16.230)	.006	0.446
Waist-to-height ratio (cm/cm)	0.043 (0.007 to 0.080)	.020	0.269
Body fat (%)	11.273 (4.960 to 17.586)	.001	0.352
Systolic blood pressure (mm Hg)	20.673 (8.729 to 32.617)	.001	0.221
Serum glucose (mmol/L)	0.879 (0.451 to 1.307)	<.001	0.167

Model 1: after adjusting for sex. Model 2: after adjusting for sex, age, smoking, physical activity, and family history of cardiovascular disease.

age, smoking, physical activity, and family history of cardiovascular disease (Table 3). Finally, a multiple linear regression analysis showed nail selenium ($P = .040$) as well as the fifth quintiles of zinc ($P = .045$) and erythrocyte GPx activity ($P = .021$) as significant negative predictors of serum ADMA concentrations after adjusting for sex, age, BMI, smoking, and physical activity (Table 4).

4. Discussion

Human adipocytes express the whole gene set that codes for the enzymatic system responsible for the biosynthesis and the degradation of ADMA, a methylarginine that is actually released by fat cells in culture [22]. However, other organs and tissues such as skeletal muscle are also important in ADMA production [23]. The concerned enzymes included protein arginine *N*-methyltransferases type I involved in ADMA synthesis [24,25], dimethylarginine dimethylaminohydrolases (DDAHs) responsible for ADMA degradation [26,27], as well as constitutive and inducible forms of NOS (ie, NOS1, NOS2A, and NOS3 genes), the main functional target of ADMA [22]. The reported associations between circulating concentrations of ADMA and body weight, BMI, and waist circumference confirm that ADMA is related with several adiposity markers. These findings are in accordance with previous studies concerning the interactions between ADMA and several adiposity measurements in adult subjects [28] in which ADMA concentrations were between 29% and 120% higher in obese than in lean subjects [28]. In other human studies, a strong relationship between BMI and plasma levels of ADMA in overweight subjects has been described [29]. Interestingly, weight reduction lowered ADMA in morbidly obese women [30].

Although in this trial we have not found any association between ADMA concentrations and the lipid profile of the healthy subjects' sample, in other studies, ADMA values also correlated with triglycerides [28,31]. Given the expression of ADMA and those enzymes involved in its metabolism in adipocytes [11] and that the inhibition of NO release by synthetic NOS inhibitors such as N^G-monomethyl-L-arginine in subcutaneous adipose tissue results in increased lipolysis in vivo [32], one could advance the hypothesis that ADMA would increase lipolysis, resulting in an augmented fatty acid flux eventually aggravating insulin resistance [11]. Thus, insulin tolerance is inversely related to plasma concentrations of ADMA in elderly subjects [33]. Indeed, in another study, ADMA also correlated with fasting insulin in healthy adults [28], supporting the reported association between glucose and serum ADMA. In the context of the metabolic syndrome, besides obesity and insulin resistance, hypertension and cardiovascular disease, both diseases related to ADMA [12], play an important role. Thus, ADMA tended to be associated with hypertension [31] in Turkish women, as occurs in our trial.

Because ADMA may be apparently associated with smoking status [31], risk factors of obesity [28-30], type 2 diabetes mellitus [33], cardiovascular diseases [9,11,12,34], and several complications associated with inflammation [13,35] and inflammatory biomarkers [13,35], ADMA might be a good candidate as a marker of metabolic syndrome

Table 4
Multiple linear regression analysis with ADMA concentration (in micromoles per liter) as a dependent variable[a]

	B coefficient (95% CI)	*P*
Model 1		
Selenium (μg/g of nail)	−0.271 (−0.528 to −0.013)	.040
Model 2		
Zinc (μg/g of nail)		
Q1 (31.2-93.6)	0 (ref)	
Q2 (95.8-107.3)	0.013 (−0.100 to 0.126)	.824
Q3 (108.2-119.9)	0.111 (−0.005 to 0.227)	.061
Q4 (120.1-134.9)	−0.051 (−0.164 to 0.062)	.375
Q5 (137.3-881.6)	−0.116 (−0.229 to −0.003)	.045
Model 3		
GPx activity (nmol/[min mL])		
Q1 (231.8-527.2)	0 (ref)	
Q2 (544.4-601.7)	−0.041 (−0.168 to 0.085)	.517
Q3 (608.1-662.2)	−0.064 (−0.194 to 0.066)	.328
Q4 (668.6-787.0)	−0.033 (−0.164 to 0.098)	.622
Q5 (789.5-964.6)	−0.146 (−0.269 to −0.022)	.021

Model 1: $R^2 = 0.162$, $P = .002$ for the model (n = 93). Model 2: $R^2 = 0.246$, $P < .001$ for the model (n = 93). Model 3: $R^2 = 0.167$, $P = .004$ for the model (n = 89).

[a] After adjusting for sex, age, BMI, smoking, and physical activity.

features. The fact that ADMA is an endogenous NOS inhibitor [11,22] supports a potential role related to oxidative stress. Moreover, ADMA is metabolized by DDAH [11,22], whose activity is positively regulated by probucol (a potent antioxidant) [36], taurine (a semiessential amino acid with antioxidant properties) [37], estradiol [38], fenofibrate, and pyrrolidine dithiocarbamate (an antagonist of nuclear factor–κB) [39] in vitro. Furthermore, studies have demonstrated the importance of redox interruption of DDAH activity, with a subsequent accumulation of cellular ADMA, which could be a starting point for further elevation of ADMA, an inhibition of NOS, and an increase in superoxide generation, thereby initiating a feed-forward reaction. In this sense, it has been described that a decrease in NO production is related with an increase in the expression of proinflammatory genes in the nucleus and the production of proinflammatory proteins such as chemokines and cytokines stimulated by nuclear factor–κB [40], whose signalling is inhibited by NO through cyclic adenosine monophosphate–dependent pathways [41]. Moreover, both oxidative status and low-grade inflammation indicators have been related with chronic disease development [2]. However, we could not directly correlate smoking status, physical activity, or inflammatory biomarkers with circulating ADMA.

Interestingly, we report here for the first time an association between circulating ADMA and nail selenium and zinc concentrations in young healthy people. Previously, it has been described that erythrocyte zinc, copper, and GPx levels as well as serum selenium values were lower, whereas ADMA levels were higher, in patients with chronic kidney disease than in controls [34]. Moreover, another study has linked nail selenium concentrations with C3, an inflammatory biomarker related to early metabolic syndrome features in healthy young adults [42]. Furthermore, serum selenium has been related to CRP, suggesting circulating CRP as a negative predictor of serum selenium [43]. On the other hand, vitamin and mineral supplementation is seemingly associated with lower concentrations of CRP in women, especially with selenium and vitamin E supplementation [44]. Moreover, supplementation with vitamin E significantly decreased ADMA levels after 8 weeks [45], but also after 24 months [46], suggesting a short- and long-term effect of antioxidant treatment in the regulation of ADMA concentrations. These assessments are in accordance with our findings, in which a high selenium and vitamin A intake was related to lower levels of serum ADMA. Interestingly, although we did not reach statistically significant results, the intake of other antioxidants such as vitamin E or zinc appeared to trend to lower values in subjects with ADMA concentrations higher than the median value.

Several diseases related with inflammatory or oxidative processes might change the relations between inflammatory markers and antioxidant status. According to findings described for other diseases, the selenium decrease in liver and plasma during an acute-phase response is associated with an increase of CRP synthesis in liver [47]. A decrease in nail selenium concentrations in subjects with inflammatory diseases compared with healthy subjects had been previously reported [48], highlighting the relation between inflammation and concentrations of antioxidants/trace elements.

These results should be examined with care because of the lack of suitable selenium and zinc food content tables, which are important to confirm that nail analysis in this sample is also a reliable marker of intake as has been suggested in previous studies [49,50]. Furthermore, we rely on self-reported information in the assessment of dietary intake. However, previous validation studies of the food frequency questionnaire [19] that we used have shown adequate quality of this information. Moreover, further research is needed with larger samples to confirm these translational data.

In conclusion, ADMA seems to be related with selenium and zinc status and several anthropometric and biochemical measurements linked to the metabolic syndrome in apparently healthy young subjects. These findings support a relevant role for antioxidant intake in the modulation of ADMA, whose assessment may be a marker of metabolic syndrome manifestations.

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