Dietary Composition as a Determinant of Plasma Asymmetric Dimethylarginine in Subjects With Mild Hypercholesterolemia

Hannu Päivä, Terho Lehtimäki, Juha Laakso, Inkeri Ruokonen, Riitta Tervonen, Saara Metso, Matti Nikkilä, Erkki Wuolijoki, and Reijo Laaksonen

Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide synthase inhibitor that participates in the regulation of vasodilatory function and is also linked to hypertension, whereas its stereoisomere, symmetric dimethylarginine (SDMA), is biologically inactive. Dietary components influence vascular functions and a high-fat meal seems to increase postprandial plasma ADMA levels. However, it has not been published whether diet influences plasma ADMA levels. In this study, we investigated the impact of diet on plasma ADMA and SDMA levels. Thirty-four mildly hypercholesterolemic, otherwise healthy women (n = 14) and men (n = 20) with a mean age of 46.2 years (range, 35 to 62 years) participated in the study. The subjects were examined twice at intervals of 2 months. Seven-day food records were used to analyze diet and alcohol intake. ADMA was measured by using high-performance liquid chromatography (HPLC)–tandem mass spectrometry. In a multivariate analysis (R^2 = 0.20, P < .002), low amount of energy received from carbohydrates (r = −0.31, P = .009) and high plasma triglycerides (r = 0.30, P = .01) were predictors of high ADMA plasma levels. Alcohol drinkers had higher plasma ADMA concentrations than abstainers (0.50 ± 0.13 *v* 0.42 ± 0.11 μmol/L, P = .04). Plasma ADMA correlated with systolic (r = 0.60, P = .005) and diastolic blood pressure (r = 0.53, P = .02) in abstainers but not in alcohol drinkers. Plasma SDMA was not associated with any dietary components or with blood pressure. In conclusion, a high amount of dietary carbohydrates is strongly associated with low levels of plasma ADMA. Concentration of ADMA in plasma seems to be higher in alcohol drinkers than in abstainers.

DIETARY FACTORS play an important role in modulating endothelial function.[1] Clinical studies have demonstrated that even a single high-fat meal reduces endothelial function in healthy men[2] and that a high-fat meal in subjects with increased plasma levels of triglycerides is associated with acute impairment of endothelial function as compared with a carbohydrate-rich meal.[3]

Asymmetric dimethylarginine (ADMA) is an endogenous competitive nitric oxide synthase inhibitor, which can modulate NO production, whereas symmetric dimethylarginine (SDMA) is biologically inactive.[4] An association between increased plasma ADMA concentration and hypertension has been reported in several studies.[5-8] Moreover, myocardial vasodilator capacity has been found to have an association with the concentrations of plasma ADMA.[7,9] Recently, we have reported that high ADMA is a potent predictor of acute coronary events in nonsmoking middle-aged men.[10] These findings indicate that plasma ADMA represents a clinically significant marker for endothelial dysfunction.

Elevated plasma levels of ADMA have been observed in patients with hypercholesterolemia[9] and in type 2 diabetic patients after eating a high-fat meal. Elevation of plasma ADMA levels occur in association with increased plasma levels of triglycerides.[11] The current study was performed to examine further whether dietary factors are associated with plasma ADMA and SDMA concentrations.

MATERIALS AND METHODS

Selection of Subjects

Originally, 42 subjects participated in an intervention trial with rapeseed and olive oil. Of the original subjects, those receiving antihypertensive treatment (n = 8) were excluded. Thus, for the present study, a subgroup of 34 subjects aged 35 to 62 years (20 men and 14 women) were recruited. The inclusion criteria were age 30 to 70 years; normal liver, kidney, and thyroid function; serum total cholesterol greater than 5.0 mmol/L; and body mass index (BMI) 18 to 30 kg/m^2. Exclusion criteria were the use of lipid-lowering drugs, renal or liver dysfunction, diabetes, cancer, systemic corticosteroid medication, and vegetarianism.

Study Design

Basically, study subjects participated in an intervention study that had a cross-over design including a 4-week wash-out period between two 4-week treatment periods. The first baseline was before the first treatment period and the second baseline after the wash-out period. For the present study, we used only the data from the first and the second baseline. To control individual variation, eg, in blood pressure, we pooled results of these 2 baseline periods. This was possible because no carry-over effect was recorded for blood pressure, dietary compositions, or plasma concentrations of ADMA, SDMA, lipids, or lipoproteins between the periods. Thus, each subject has 2 sets of results for all variables.

Data on the risk factors of atherosclerosis, use of medication and

From the Department of Internal Medicine, Tampere University Hospital; Laboratory of Atherosclerosis Genetics, Department of Clinical Chemistry, Centre for Laboratory Medicine, University Hospital of Tampere; Medical School, University of Tampere, Tampere, Finland; Institute of Biomedicine, Pharmacology and the; Department of Clinical Pharmacology, University of Helsinki, Helsinki, Finland; FinnMedi Research, Ltd, Tampere; and Department of Internal Medicine, City Hospital of Tampere, Tampere, Finland.

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Address reprint requests to Hannu Päivä, MD, Department of Internal Medicine, University Hospital of Tampere, PO Box 2000, 33521 Tampere, Finland.

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Table 1. Baseline Characteristics of Patients (mean ± SD)

Variable	Subjects (N = 34)
Age (yr)	46.3 ± 7.5
Sex (M/F)	20/14
BMI (kg/m^2)	25.7 ± 3.9
Alcohol consuming (Y/N)	24/10
Antioxidative vitamins consuming (Y/N)	8/26
SBP (mm Hg)	132 ± 20
DBP (mm Hg)	83 ± 12
TC (mmol/L)	6.26 ± 0.89
LDL-cholesterol (mmol/L)	3.92 ± 0.84
HDL-cholesterol (mmol/L)	1.72 ± 0.44
Triglycerides (mmol/L)	1.36 ± 0.82
ADMA (μmol/L)	0.47 ± 0.13
SDMA (μmol/L)	0.34 ± 0.07

Abbreviations: M, male; F, female; Y, yes; N, no; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine.

hormones, and inclusion and exclusion criteria were collected in the case report forms. The study subject's blood pressure was determined with automatic blood pressure gauge and the mean of the 2 recordings was calculated after 10 minutes' rest in the supine position. Height and weight were recorded and BMI was calculated as weight/height square (kg/m^2).

Study Ethics

The study protocol was approved by the Ethics Committee of the Tampere University Hospital. Written informed consent was obtained from all participants.

Diet

Diet and alcohol intake of each subject were estimated from a 7-day food questionnaire (including 5 week and 2 weekend days) at the beginning of the first and the second baseline periods. The subjects recorded their food consumption after consulting a booklet containing photographs of food portions aimed to help them estimate portion energy content. A nutritionist advised the subjects on the practical management of the diets and checked their food records. The daily energy and nutrient intakes were calculated using a computerized, food table based Flamingo program (version 1.1, Helsinki, Finland). The program is based on the nutrient database of the FINELI, National Public Health Institute and national and international food composition.[12] Alcohol intake was recorded as a frequency of alcohol portions and as grams of 100% alcohol per day or as percent of total energy intake.

Blood Sampling

Blood samples were drawn into EDTA-tubes in ice after the study subjects had fasted 12 hours. Then plasma was separated by low-speed ultracentrifugation and stored at −80°C. Plasma total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, ADMA, and SDMA determinations were analyzed within 3 months.

Determinations of Plasma Lipids and Arginine Derivatives

The concentrations of plasma triglycerides, TC, and HDL-cholesterol were determined using a Cobas Integra 700 automatic analyzer with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). The LDL concentration was calculated using Friedewald's formula, since plasma triglyceride levels did not exceed 4.0 mmol/L.[13] The interassay coefficients of variation were 1.4% for TC, 1.0% for triglycerides, and 3.7% for the HDL-cholesterol assessments.

ADMA and SDMA determination was performed by employing high-performance liquid chromatography (HPLC)–tandem mass spectrometry. A 200-μL aliquot of plasma was diluted and applied on solid-phase silica column. After washing with methanol, arginine and its methylated derivatives were eluted into 4 mol/L NH_4OH in 50% acetonitrile and eluent was evaporated with N_2. Prior analysis dry residue were dissolved in HPLC mobile phase. Transitions used were *m/z* 203→70 for ADMA and SDMA, *m/z* 189→70 for monomethylarginine (LMMA), and *m/z* 175→60 for arginine. Analysis of every sample was performed in parallel with and without added SDMA. Area-normalized difference in SDMA peaks of samples run with and without added SDMA was used as an internal standard. The level of SDMA in each sample, in turn, was determined by the method of standard additions, using the normalized SDMA values. For ADMA total coefficient of variation (CV) was 10.3% and respective intra-and interday component CVs were 7.0% and 7.6%.[14]

Statistical Methods

The data were analyzed with the Statistica for Windows statistics program (StatSoft Inc, Tulsa, OK). The normality of each variable was studied by Kolmogorov-Smirnow test. Univariate correlation analysis was performed using Pearson's correlation test for normally distributed variables. Multivariate-regression analysis was used to find out the determinants of plasma ADMA levels. Data are presented as mean ± SD unless otherwise stated. A *P* value of less than .05 was considered statistically significant. The carry-over effect was tested by using an analysis of variance for repeated measures (RANOVA), utilizing crossover design in which the independent factor was treatment group, the dependent factors were changes in studied dietary factors, blood pressure, plasma ADMA, and SDMA, and the repeated measure factors were the first and the second periods of the crossover intervention.

RESULTS

Clinical Characteristics and Dietary Composition

The clinical characteristics of study subjects are given in Table 1. Study subjects consisted of 20 men and 14 women. They had normal to moderately elevated blood pressure and plasma TC. The mean daily intakes of major nutrients according to food records are given in Table 2. The diets used corresponded to the average Finnish diet.

Table 2. Mean Daily Intake of Nutrients According to Food Records

Nutrient	Subjects (N = 34)
Total energy (kJ)	8,505.3 ± 2,411.6
Fat (E%)	36 ± 6
Saturated fatty acids (E%)	14 ± 3
Monounsaturated fatty acids (E%)	12 ± 2
Polyunsaturated fatty acids (E%)	5 ± 1
Cholesterol (mg/d)	301.7 ± 131.5
Protein (E%)	17 ± 3
Carbohydrates (E%)	43 ± 6
Alcohol consumption (g/d)	10.4 ± 11.3
Alcohol (E%)	4 ± 4
Vitamin C (mg/d)	108.4 ± 58.7
α-tocopherol (mg/d)	10.6 ± 3.8

Abbreviation: E%, percent of energy.

Table 3. Predictors of Plasma ADMA, Based on a Stepwise Multivariate Regression Model

Independent Variable	Standardized Coefficient	*P* Value
Energy received from carbohydrates	−0.31	.009
Triglycerides	0.30	.01
Whole model	$R^2 = 0.20$	<.002

NOTE. Other explanatory variables in the model were total energy intake, amount of alcohol intake, and total cholesterol.

Arginine Derivatives and Lipids

The average plasma ADMA and SDMA concentrations in all subjects were 0.47 ± 0.13 and 0.34 ± 0.07 μmol/L, respectively. Neither TC nor LDL-cholesterol was associated with plasma ADMA concentration. However, a slight association ($r = 0.26$, $P = .04$) was observed between the concentrations of plasma ADMA and triglycerides. Plasma SDMA was not associated with plasma lipids.

Arginine Derivatives, Blood Pressure, and Major Dietary Components

In an univariate analysis, plasma ADMA concentrations were inversely correlated with carbohydrate-derived energy intake ($r = -0.35$, $P = .004$) and slightly with total energy intake ($r = 0.26$, $P = .04$) However, ADMA did not associate with energy derived from protein or total fat. Quantity of energy (E%) from polyunsaturated, monounsaturated or saturated fatty acids did not correlate with plasma ADMA. Moreover, by univariate analysis, neither dietary cholesterol, vitamin C, nor vitamin E was associated with plasma ADMA concentration. There were 10 abstainers among the study subjects and they had significantly lower plasma ADMA concentrations than did alcohol consumers (0.42 ± 0.11 *v* 0.50 ± 0.13 μmol/L, $P = .04$). Plasma SDMA did not associate with any dietary components.

Systolic and diastolic blood pressure were associated with total energy intake ($r = 0.33$, $P = .007$ and $r = 0.25$, $P = .04$), alcohol consumption ($r = 0.47$, $P < .001$ and $r = 0.43$, $P < .001$), and plasma triglycerides ($r = 0.24$, $P = .05$ and $r = 0.38$, $P = .002$).

Arginine Derivatives and Blood Pressure

In alcohol drinking subjects plasma ADMA did not associate with blood pressure, but in subjects avoiding alcohol, ADMA associated significantly with systolic ($r = 0.60$, $P = .005$) and with diastolic blood pressure ($r = 0.53$, $P = .02$). Plasma SDMA was not associated with blood pressure, not even in abstainers.

Multivariate Determinants of ADMA Plasma Levels

In forward stepwise multiple regression analysis ($R^2 = 0.20$, $P < .002$, Table 3) low amount of energy received from carbohydrates ($r = -0.31$, $P = .009$) and high plasma triglycerides ($r = 0.30$, $P = .01$) were the significant predictors of high plasma ADMA levels. The other explanatory variables in this model were total energy intake, TC, and amount of alcohol intake.

DISCUSSION

This study demonstrates that high amount of energy received from carbohydrates is significantly associated with low plasma ADMA levels. It also demonstrates that concentrations of plasma ADMA seem to be higher in alcohol drinkers than in abstainers.

We found, for the first time, that the high amount of energy received from carbohydrates is strongly associated with low plasma ADMA concentrations, suggesting that high-carbohydrate dietary intake may have beneficial effects by lowering ADMA levels. In fact, high intake of dietary carbohydrates has been observed to have an acute beneficial effect on brachial artery flow when compared with a high-fat meal, which also increased significantly plasma levels of triglycerides.[3] On the other hand, it has also been demonstrated that there were fewer new coronary lesions in patients who substituted fat calories with dietary carbohydrates than in those who substituted fat calories with monounsaturated or polyunsaturated fat.[10] Furthermore, in a stepwise multivariate-regression model, energy received from carbohydrates and plasma triglycerides were significant predictors of plasma ADMA. Based on this observation and on results received from a study by Fard et al,[11] it seems that there is an interplay between plasma ADMA, energy intake, and plasma triglyceride levels.

It has also been previously speculated that dietary antioxidant vitamins (vitamin C and E) may protect against endothelial dysfunction and atherosclerosis.[16] Therefore, we asked also whether plasma ADMA is associated with dietary antioxidant vitamins. We found no association between plasma ADMA levels and antioxidant vitamin intake, suggesting that antioxidant vitamins, at least in amounts received from normal diet, have no significant effect on plasma ADMA levels.

In previous studies, high plasma ADMA has been linked to hypertension.[5-8] It has also been shown that alcohol increases arterial blood pressure.[16,17] Intriguingly, we observed that plasma ADMA but not plasma SDMA concentrations were higher in alcohol drinkers than in abstainers. Furthermore, alcohol intake was linearly and significantly associated with systolic as well as with diastolic blood pressure. We hypothesized that increased plasma ADMA plays an important role in alcohol-induced hypertension. However, we did not find any association between blood pressure and plasma ADMA levels in alcohol drinking subjects. Instead, plasma ADMA was significantly associated with blood pressure only in abstainers. However, even in abstainers there was no association between blood pressure and SDMA, a biologically inactive stereoisomere of ADMA, suggesting that the association between ADMA and blood pressure in abstainers is significant. We can only speculate that in alcohol consumers there may be other more powerful mechanisms than ADMA, such as stimulation of the sympathetic nervous system, which influence on blood pressure and may even hide the effect of ADMA on blood pressure. Also, it is possible that the effect of ADMA inhibiting NO synthase will not enhance over the certain limit value. Despite a distinctive observation, we have to analyze this result

critically. First, the number of subjects avoiding alcohol was very small, although we pooled the data. Second, occasional blood pressure measurements are susceptible to misinterpretation due to stress and other circumstances. Although in previous studies blood pressure measurements were calculated using casual measurements, as we did in this study, we suggest that future studies determine blood pressure using ambulatory blood pressure measurements.

In conclusion, dietary carbohydrates were significantly associated with low ADMA. The possible relationship between blood pressure, alcohol, and ADMA must await future confirmation.

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