Epidemiology

Brachial Artery Flow-Mediated Dilation and Asymmetrical Dimethylarginine in the Cardiovascular Risk in Young Finns Study

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Background—Elevated asymmetrical dimethylarginine (ADMA) is a novel risk factor for atherosclerosis that may impair endothelial function by interfering with endothelial nitric oxide synthesis. To gain insight into the effects of ADMA on systemic endothelial function, we examined the association between ADMA and brachial artery flow-mediated dilation (FMD) in a large population of young adults.

Methods and Results—Plasma ADMA and brachial FMD, as well as conventional cardiovascular risk factors, were measured in 2096 white adults aged 24 to 39 years. In univariate analysis, ADMA was inversely correlated with FMD (r = −0.07, P = 0.003). The inverse association between ADMA and FMD remained significant in a multivariable regression model adjusted for age, sex, conventional cardiovascular risk factors, estimated glomerular filtration rate, and brachial artery baseline diameter (β±SE = −1.56±0.62%, P = 0.01).

Conclusions—We conclude that elevated plasma ADMA concentrations are associated with decreased brachial FMD responses in healthy adults. These data provide evidence at the population level that ADMA levels are associated with endothelial function. (Circulation. 2007;116:1367-1373.)

Key Words: dimethylarginine ■ brachial artery ■ endothelium

In recent years, increased plasma levels of asymmetrical dimethylarginine (ADMA) have been shown to predict cardiovascular events. The adverse effects of ADMA on cardiovascular health are thought to be brought about by impaired endothelial function. Many antithrombogenic effects of endothelium, such as vasodilatation, inhibition of cellular adhesion, and modulation of vascular smooth muscle cells, are regulated by nitric oxide (NO) produced within the endothelium by the enzyme NO synthase, which uses the amino acid L-arginine as a substrate. ADMA is an endogenous analogue of L-arginine that may interfere with NO metabolism by competitively inhibiting the activity of NO synthase. Methylated arginine derivatives, including ADMA and symmetrical dimethylarginine (SDMA), are generated from arginine in reactions catalyzed by protein arginine N-methyltransferases. SDMA is an isomer of ADMA that is not directly capable of inhibiting NO synthase, but it may indirectly limit NO generation by reducing the intracellular availability of arginine.

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Editorial p 1338
Clinical Perspective p 1373

In addition to predicting future cardiovascular events, endogenous ADMA levels have been shown to be associated with markers of vascular endothelial dysfunction. In a recent report by Kielstein et al., it was also shown that systemic administration of exogenous ADMA influenced vascular tone in healthy subjects. Prior studies have been conducted mainly in small patient groups that consisted primarily of elderly subjects and subjects with chronic diseases. Endothelial function can be measured noninvasively from the brachial artery. In this method, increased blood flow is induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of >250 mm Hg for up to 5 minutes, followed by release. Mullen et al have shown that the brachial dilatation response for increased flow is mainly mediated by NO released from arterial endothelial cells, because the response can be blocked by simultaneous infusion of the arginine analogue N²-monomethyl-L-arginine.
The noninvasive brachial artery flow-mediated dilation (FMD) response correlates significantly with coronary and brachial endothelial function tested by invasive methods. To gain insight into the effects of endogenous methylarginines on endothelial function at the population level, we examined the relations of ADMA and SDMA to brachial FMD among 2096 healthy men and women aged 24 to 39 years as part of the Cardiovascular Risk in Young Finns Study.

Methods

Subjects

The Cardiovascular Risk in Young Finns Study is an ongoing 5-center follow-up study of atherosclerosis precursors of Finnish children and adolescents. The first cross-sectional survey was conducted in 1980, when 3596 participants aged 3, 6, 9, 12, 15, and 18 years were randomly chosen in each area from the national population register. In 2001, we reexamined 2283 of these individuals, then aged 24 to 39 years. In the present analysis, a total of 2096 subjects with data on ADMA and brachial FMD (both measured in 2001) were included. The study was approved by local ethics committees, and all subjects gave their written informed consent.

Clinical Characteristics and Risk Factors

Height and weight were measured, and body mass index was calculated. Blood pressure was measured with a random-zero sphygmomanometer; an average of 3 measurements were used in the analysis. Smoking habits were determined by use of a questionnaire. Venous blood samples were drawn after an overnight fast. All lipid determinations were performed on serum by standard methods. Plasma high-sensitivity C-reactive protein concentrations were analyzed by latex turbidometric immunoassay (Wako Chemicals GmbH, Neuss, Germany). Glucose concentrations (in 2001) were analyzed enzymatically (Olympus Diagnostica GmbH, Hamburg, Germany), and homocysteine concentrations (in 2001) were analyzed with a microparticle enzyme immunoassay kit (Inx assay, Abbott Laboratories, Tokyo, Japan).

Homeostasis model assessment (HOMA) index was calculated from the formula: HOMA = fasting glucose (in mmol/L) × fasting insulin (in μU/mL)/22.5. Serum insulin was measured with a microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot, Japan). Serum creatinine (in mmol/L) was measured with a microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot, Japan). Serum creatinine (in mg/dL) was determined by a single reader blinded to the individual subject’s details.

ADMA, SDMA, and Arginine

Arginine, ADMA, and SDMA concentrations were determined by isocratic high-performance liquid chromatography modified from the method described by Teerlink et al. Plasma proteins were precipitated from 200 μL of plasma with trichloroacetic acid, with 10 μmol/L monomethylarginine added as an internal standard and after centrifugation was applied on a solid-phase extraction cartridge (MCX, OASIS, Waters Corp, Milford, Mass.). After washing and elution, the eluate was dried with a stream of nitrogen and redissolved in dilute hydrochloric acid. The samples were derivatized with o-phthalaldehyde in an automatic sampler before chromatographic separation on a 5-μm Symmetry C18 (3.9×150 mm) column (Waters Corp). The mobile phase consisted of 50 mmol/L phosphate buffer, pH 6.5 acetonitrile:methanol (89.8:8.2), and the analytes were detected by fluorescence at 340/455 nm. The precision (coefficient of variation) for a plasma pool (n = 77) for arginine, ADMA, and SDMA within series was 7.5%, 5.7%, and 6.5% and between series, it was 12.9%, 10.6%, and 12.1%, respectively.

Brachial FMD

Brachial artery ultrasound studies were performed successfully for 2109 subjects, as reported previously. To assess brachial FMD, the left brachial artery diameter was measured both at rest and during reactive hyperemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 4.5 minutes, followed by release. Three measurements of arterial diameter were performed at end diastole at a fixed distance from an anatomic marker at rest and 40, 60, and 80 seconds after cuff release. The vessel diameter in scans after reactive hyperemia was expressed as the percentage relative to resting scan (100%). The greatest value between 40 and 80 seconds was used to derive the maximum FMD. The 3-month between-visit coefficient of variation was 3.2% for brachial artery diameter measurements and 26.0% for FMD measurements. All ultrasound scans were analyzed by a single reader blinded to the individual subject’s details.

Associations Between Methylarginines and FMD (FMD as Outcome Variable)

Correlation coefficients were calculated to assess univariate associations between methylarginines (ADMA, SDMA, and arginine/ADMA ratio) and FMD. Arginine/ADMA ratio was calculated because it has been suggested to be a more relevant marker for NO synthesis than absolute ADMA concentration. Next, multivariable models were constructed to study the effects of methylarginines on FMD, with other study variables taken into account as covariates. Possible risk factor × sex interactions were tested with linear regression models. Because no significant sex interactions were present when we analyzed the associations of ADMA, SDMA, and arginine/ADMA ratio with FMD, the analyses were performed with both sexes combined.

Associations Between Risk Factors and Methylarginines (Methylarginines as Outcome Variables)

To gain insight into the relations of methylarginines to risk factors and renal function at the population level, we constructed separate linear regression models to study the multivariable predictors of ADMA, SDMA, and arginine/ADMA ratio. In these models, the methylarginines were modeled as outcome variables, and the independent predictors included age, sex, cardiovascular risk factors, and renal function. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
mocysteine levels \((P=0.01)\) and the frequency of smoking \((<0.0001)\) increased.

**Associations of ADMA, SDMA, and Arginine/ADMA Ratio With Brachial FMD**

In univariate analysis, ADMA \((r=-0.07, P=0.003)\) and SDMA \((r=-0.11, P<0.0001)\) were inversely correlated with FMD, whereas the arginine/ADMA ratio did not correlate with FMD \((r=0.005, P=0.83)\). A decreasing linear trend also existed in FMD values across tertiles of ADMA and SDMA (Figure). The combination of the methylarginines (ADMA+SDMA) did not correlate with FMD better than each variable alone.

The inverse associations of ADMA \((P=0.01)\) and SDMA \((P=0.04)\) with FMD remained significant in multivariable models adjusted for cardiovascular risk factors, renal function, and brachial artery baseline diameter (Tables 2 and 3). When ADMA and SDMA were included in the same multivariable model, only ADMA \((P=0.01)\) remained significantly associated with decreased FMD.

**Population Predictors of ADMA, SDMA, and Arginine/ADMA Ratio**

Because information is lacking on the associations between cardiovascular risk factors (including renal function) and methylarginines in population-based samples, we also calculated multivariable linear regression models in which methylarginines were treated as outcome variables.

**Predictors of ADMA**

In multivariable analysis, male sex \((\beta \pm SE=-0.02 \pm 0.01, P=0.001)\), systolic blood pressure \((\beta \pm SE=-0.0006 \pm 0.0003, P=0.02)\), HDL cholesterol \((\beta \pm SE=-0.08 \pm 0.01, P<0.0001)\), triglycerides \((\beta \pm SE=-0.04 \pm 0.01, P<0.0001)\), insulin \((\beta \pm SE=-0.02 \pm 0.01, P=0.01)\), total cholesterol \((\beta \pm SE=0.009 \pm 0.004, P=0.04)\), smoking \((\beta \pm SE=-0.04 \pm 0.01, P<0.0001)\), and homocysteine \((\beta \pm SE=0.002 \pm 0.001, P=0.04)\) were statistically significantly associated with ADMA levels.

**Predictors of SDMA**

The factors with statistically significant associations with SDMA in the multivariable model were male sex \((\beta \pm SE=0.04 \pm 0.01, P<0.0001)\), age \((\beta \pm SE=-0.0014 \pm 0.0005, P=0.005)\), total cholesterol \((\beta \pm SE=0.008 \pm 0.003, P=0.006)\), body mass index \((\beta \pm SE=0.003 \pm 0.001, P=0.001)\), homocysteine \((\beta \pm SE=0.002 \pm 0.001, P<0.0001)\), GFR \((\beta \pm SE=-0.0010 \pm 0.0001, P<0.0001)\), HDL cholesterol \((\beta \pm SE=-0.04 \pm 0.01, P<0.0001)\), insulin \((\beta \pm SE=-0.02 \pm 0.01, P<0.0001)\), and triglycerides \((\beta \pm SE=-0.02 \pm 0.01, P<0.0001)\).

**Predictors of Arginine/ADMA Ratio**

In multivariable analysis, HDL cholesterol \((\beta \pm SE=46.2 \pm 5.0, P<0.0001)\), triglycerides \((\beta \pm SE=21.5 \pm 3.4, P<0.0001)\), insulin \((\beta \pm SE=9.6 \pm 2.9, P=0.001)\), and C-reactive protein \((\beta \pm SE=9.5 \pm 1.1, P<0.0001)\) were statistically significantly associated with arginine/ADMA ratio.
We observed that ADMA levels were inversely associated with brachial FMD in a sample of 2096 healthy young adults. Previously, an inverse relation between ADMA and FMD was reported in a small study of hypercholesterolemic patients,10 in patients with chronic kidney disease,13 and in subjects with type 2 diabetes mellitus.21 We have previously reported that plasma ADMA concentration is inversely related to dipyridamole-induced myocardial vasodilatory function in young men with borderline hypertension and familial hypercholesterolemia.11 However, in a recent study among 289 patients with coronary artery disease, no correlation existed between plasma ADMA concentration and coronary response to acetylcholine, adenosine, or nitroglycerin.22 In the present study, the associations between FMD and methylarginines were rather weak ($r = 0.1$). This may explain why the relation has not been detected in all studies. We have previously shown in this cohort an inverse correlation between FMD and systolic blood pressure and a direct correlation between FMD and HDL cholesterol that were of similar magnitude (both $r = 0.1$), thus suggesting a similar “effect” on FMD. The data of the present study extend previous knowledge and demonstrate for the first time in a large, population-based sample of young adults that plasma ADMA levels are inversely associated with brachial artery endothelial function in young, healthy subjects. This finding adds credit to the hypothesized physiological association between endogenous ADMA and endothelium-dependent vasodilation capacity.8

We also observed that SDMA levels were inversely associated with FMD. This may reflect the common pathway in the production of ADMA and SDMA. SDMA may also indirectly interfere with NO production, because it has been shown to reduce intracellular availability of arginine9 by interfering with L-arginine transport.23 Preliminary in vitro data also show that SDMA can (indirectly) inhibit NO production in endothelial cells.24 The inverse association between SDMA levels and FMD could also be a reflection of decreased renal function, because even mild impairment of renal function may be an independent cardiovascular risk factor.25 A recent meta-analysis by Kielstein et al26 showed that SDMA tightly correlates with the estimated GFR. In line with that study, we found that GFR levels were associated

![Graph A](image)

**Graph A**: Brachial FMD (mean ± SEM) according to tertiles of ADMA (A), SDMA (B), and arginine (C) in 2096 Finns aged 24 to 39 years. Probability values are from linear regression analysis.

### Discussion

We observed that ADMA levels were inversely associated with brachial FMD in a sample of 2096 healthy young adults. Previously, an inverse relation between ADMA and FMD was reported in a small study of hypercholesterolemic patients,10 in patients with chronic kidney disease,13 and in subjects with type 2 diabetes mellitus.21 We have previously reported that plasma ADMA concentration is inversely related to dipyridamole-induced myocardial vasodilatory function in young men with borderline hypertension and familial hypercholesterolemia.11 However, in a recent study among 289 patients with coronary artery disease, no correlation existed between plasma ADMA concentration and coronary response to acetylcholine, adenosine, or nitroglycerin.22 In the present study, the associations between FMD and methylarginines were rather weak ($r = 0.1$). This may explain why the relation has not been detected in all studies. We have previously shown in this cohort an inverse correlation between FMD and systolic blood pressure and a direct correlation between FMD and HDL cholesterol that were of similar magnitude (both $r = 0.1$), thus suggesting a similar “effect” on FMD. The data of the present study extend previous knowledge and demonstrate for the first time in a large, population-based sample of young adults that plasma ADMA levels are inversely associated with brachial artery endothelial function in young, healthy subjects. This finding adds credit to the hypothesized physiological association between endogenous ADMA and endothelium-dependent vasodilation capacity.8

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### Table 2: Multivariable Models of the Relationship of ADMA With Brachial FMD (%) Adjusted for Brachial Diameter and Cardiovascular Risk Factors in 2015 Subjects Aged 24 to 39 Years

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$</th>
<th>SE</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMA, $\mu$mol/L</td>
<td>-1.559</td>
<td>0.623</td>
<td>0.01</td>
</tr>
<tr>
<td>Male gender</td>
<td>1.493</td>
<td>0.308</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.021</td>
<td>0.020</td>
<td>0.30</td>
</tr>
<tr>
<td>Baseline brachial</td>
<td>-3.673</td>
<td>0.249</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>diameter, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.251</td>
<td>0.039</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure,</td>
<td>-0.021</td>
<td>0.008</td>
<td>0.009</td>
</tr>
<tr>
<td>mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L *</td>
<td>0.053</td>
<td>0.115</td>
<td>0.65</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.678</td>
<td>0.358</td>
<td>0.06</td>
</tr>
<tr>
<td>Triglycerides, mmol/L†</td>
<td>0.171</td>
<td>0.248</td>
<td>0.49</td>
</tr>
<tr>
<td>Insulin, IU/L†</td>
<td>-0.042</td>
<td>0.211</td>
<td>0.84</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>-0.192</td>
<td>0.114</td>
<td>0.09</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>-0.016</td>
<td>0.024</td>
<td>0.52</td>
</tr>
<tr>
<td>C-reactive protein, mg/dL†</td>
<td>-0.048</td>
<td>0.085</td>
<td>0.58</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>-0.002</td>
<td>0.005</td>
<td>0.69</td>
</tr>
<tr>
<td>Smoking, no/yes</td>
<td>0.109</td>
<td>0.220</td>
<td>0.62</td>
</tr>
<tr>
<td>Physical activity index</td>
<td>-0.008</td>
<td>0.005</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Results remained similar when total cholesterol was replaced with LDL cholesterol.
†Log-transformed values were used in analyses.
with SDMA in multivariable analyses; however, the association between FMD and SDMA remained significant after GFR was taken into account.

Several potential mechanisms could explain the inverse association between ADMA and FMD. First, it is thought that ADMA is a competitive inhibitor of NO synthases, thereby reducing NO formation. Vallance et al.27 initially demonstrated that ADMA is a competitive inhibitor of NO synthases, with genetically high ADMA levels having similar mean blood pressure values compared to control subjects. In line with those findings, experimental studies have shown that ADMA inhibits vascular NO production at concentrations within the physiological range.27,28 Second, ADMA, by competing with L-arginine, may also induce NO synthase uncoupling, leading to oxidative stress and increased inactivation of NO.29 Third, all methylarginines compete with cellular uptake of L-arginine and thereby limit NO generation by reducing intracellular arginine concentrations.30

We found that cigarette smoking was directly correlated with ADMA and that HDL cholesterol was inversely correlated with ADMA. These findings were in line with some previous reports.3,31 Zhang et al.32 recently demonstrated that ADMA is a competitive inhibitor of NO synthases and thereby reduces NO formation. Vallance et al.27 initially demonstrated that ADMA is a competitive inhibitor of NO synthases, with genetically high ADMA levels having similar mean blood pressure values compared to control subjects. In line with those findings, experimental studies have shown that ADMA inhibits vascular NO production at concentrations within the physiological range.27,28 Second, ADMA, by competing with L-arginine, may also induce NO synthase uncoupling, leading to oxidative stress and increased inactivation of NO.29 Third, all methylarginines compete with cellular uptake of L-arginine and thereby limit NO generation by reducing intracellular arginine concentrations.30

We found that cigarette smoking was directly correlated with ADMA and that HDL cholesterol was inversely correlated with ADMA. These findings were in line with some previous reports.3,31 Zhang et al.32 recently demonstrated that exposure to cigarette smoke increases ADMA concentrations in human endothelial cells in vitro, probably by inhibiting endothelial dimethylarginine dimethylaminohydroxide activity. Degradation of ADMA by dimethylarginine dimethylaminohydroxide plays a key role in the regulation of ADMA concentrations.33 Both isoforms of dimethylarginine dimethylaminohydroxide have been shown to be sensitive to oxidative stress,34 and ADMA concentrations tend to rise under conditions of oxidative stress.35 Thus, 1 plausible mechanism for the relation between smoking and increased ADMA may be increased oxidative stress associated with smoking.36

In the present study, insulin, triglycerides, and blood pressure were inversely associated with ADMA, which suggests enhanced insulin sensitivity in connection with higher ADMA concentrations. Previous studies have reported controversial results regarding the relations between ADMA and risk factors. Contrary to the present findings, some studies have found plasma ADMA levels to be directly correlated with triglycerides and insulin resistance37–40 and indirectly correlated with smoking.46 Most of these prior studies, however, have been conducted in case-control settings that included subjects with coronary heart disease or risk factors or in small study populations. A paucity of information exists on the associations between ADMA and risk factors at the population level of healthy adults. In line with the present findings, Päivä et al.41 observed significantly lower ADMA levels in insulin-resistant patients with type 2 diabetes mellitus than in healthy control subjects. Furthermore, they found that in patients with type 2 diabetes mellitus, low plasma ADMA levels were independently correlated with poor glycemic control and increased GFR.41 Eid et al.42 have recently shown that infusion of insulin during glucose clamping is accompanied by a significant reduction in circulating ADMA levels. Similarly, in experimental studies, the accumulation of ADMA in human endothelial cells was markedly inhibited by increasing doses of insulin. This decreased accumulation of ADMA was accompanied by a dose-related increase in the activity of the metabolic enzyme dimethylarginine dimethylaminohydroxylase.43 These observations are thus in line with the present finding of an inverse relation between insulin and ADMA.

We found an inverse association between ADMA and systolic blood pressure in the multivariable model. This was unexpected, because previous evidence had indicated higher blood pressure values occurred with increased ADMA levels. Miyazaki et al.44 reported a direct correlation between ADMA and mean arterial blood pressure in 116 subjects with a mean age of 52 years. In healthy men, an infusion of ADMA, which led to an increase in plasma ADMA from approximately 1 to 20 μmol/L, elevated mean arterial pressure modestly.45 Mice with genetically high ADMA levels have similar mean arterial pressure but higher systolic pressure than control mice.46 The apparent discrepancies regarding the relations between ADMA and blood pressure may reflect either complicated regulation mechanisms of ADMA concentrations or diverse effects of ADMA on biological processes in health and disease.

Study Limitations

We found relatively large long-term variation in FMD measurements,18 which is in agreement with several previous reports.47,48 On the other hand, the long-term reproducibility of brachial artery diameter measurements was excellent. In addition, recent experiments have shown that the reproducibility of FMD measurements is not essentially improved by use of automated measuring systems.49 This suggests that much of the long-term variation of FMD is due to physio-
logical fluctuation and not to measurement error. Considerable intra-assay (~6%) and interassay (~11%) variation also existed in ADMA and SDMA measurements; however, these values are in line with previous reports. 11 It may be argued that the observed association between ADMA, SDMA, and FMD would have been even stronger if the variations in these variables had been smaller. We did not perform any association studies regarding different determination methods of ADMA, such as a comparison with the ELISA method. This could be important, because high-performance liquid chromatography is a highly time-consuming method compared with an ELISA, which would be more suitable for routine use. However, the high-performance liquid chromatography method offers better sensitivity, selectivity, and, very importantly, simultaneous determination of ADMA, SDMA, and L-arginine. 50 Finally, because of the number of potential confounding factors, the unadjusted results in univariate analyses should be interpreted with caution.

Conclusions
We have observed in a large, population-based cohort of healthy young adults that ADMA is inversely associated with brachial FMD. This association remained significant after adjustment for conventional cardiovascular risk factors, thus providing evidence at the population level that the endogenous ADMA concentration is related to systemic endothelial function.

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Disclosures
Dr Raitakari has received research grants from the Academy of Finland. The other authors report no conflicts.

References
Increased plasma levels of asymmetrical dimethylarginine (ADMA) levels have been shown to predict cardiovascular events. The effects of ADMA on cardiovascular health are thought to occur via interference with endothelial nitric oxide synthesis. In support of this, high ADMA levels have been shown to be associated with markers of vascular endothelial dysfunction. However, prior studies have been conducted mainly in small patient groups in which the majority of subjects were elderly or had chronic diseases, and the role of ADMA in healthy subjects has not been assessed. In a large, population-based study of young adults (n = 2096, ages 24 to 39 years), we observed that ADMA levels were inversely related to endothelium-dependent brachial flow-mediated dilation. Because this association remained significant after adjustment for conventional cardiovascular risk factors and renal function, the present results provide evidence at the population level that ADMA levels are associated with endothelial function. These observations set the stage for future studies evaluating the role of ADMA as a clinically relevant biomarker of endothelial health.
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