Acute Elevations of Plasma Asymmetric Dimethylarginine and Impaired Endothelial Function in Response to a High-Fat Meal in Patients With Type 2 Diabetes


Abstract—Asymmetric dimethylarginine (ADMA), a compound detectable in human plasma, is an endogenous inhibitor of NO synthase. Endothelial dysfunction is an early event in atherogenesis, and large-vessel atherosclerosis is a major cause of morbidity and mortality in patients with type 2 diabetes mellitus. Fifty patients with type 2 diabetes mellitus were studied at baseline and 5 hours after ingestion of a high-fat meal. Plasma ADMA measured by using high-performance liquid chromatography increased from 1.04±0.99 to 2.51±2.27 μmol/L (P=0.0005). Brachial arterial vasodilation after reactive hyperemia, a NO-dependent function, measured by high-resolution ultrasound, decreased from 6.9±3.9% at baseline to 1.3±4.5% (P<0.0001). These changes occurred in association with increased plasma levels of triglycerides and very low density lipoprotein triglycerides, with reduced low density lipoprotein cholesterol and high density lipoprotein cholesterol, and with no changes in total cholesterol. The increase in plasma ADMA in response to a high-fat meal was significantly and inversely related to the decrease in percent vasodilation. In 10 of the subjects studied with a similar protocol on another day, no significant changes in the brachial artery flow responses or in plasma ADMA were observed 5 hours after ingestion of a nonfat isocaloric meal. The data suggest that ADMA may contribute to abnormal blood flow responses and to atherogenesis in type 2 diabetics. (Arterioscler Thromb Vasc Biol. 2000;20:2039-2044.)

Key Words: asymmetric dimethylarginine ■ nitric oxide ■ vasodilation ■ triglyceridemia ■ diabetes mellitus

Patients with type 2 diabetes mellitus have increased risk of atherosclerosis and its complications and frequently manifest postprandial hypertriglyceridemia, which has been shown to be an independent predictor of coronary artery disease.1,2 Endothelial cell dysfunction in large arteries is an important early event in the pathogenesis of atherosclerosis.3 Abnormal endothelial cell–mediated vasodilation has been demonstrated in patients with established atherosclerosis and in healthy subjects with risk factors for atherosclerosis4-5 as well as in patients with type 2 diabetes mellitus.6-8 In patients with clinically evident atherosclerosis or risk factors for atherosclerosis, reduced endothelial cell–dependent vasodilatory responses of large arteries have been found and attributed to diminished synthesis of NO and/or to increased inactivation of NO in the vessel wall.9,10 Endothelial cell–mediated brachial artery flow responses were reported to decline after ingestion of a high-fat meal in normal subjects11,12 and in patients with type 2 diabetes mellitus.13

In 1992, Vallance and colleagues14,15 demonstrated that Nω,Nω-dimethyl-L-arginine (asymmetric dimethylarginine [ADMA]) is detectable in plasma and is an endogenous competitive inhibitor of NO synthase (NOS). Nε,Nε-dimethyl-L-arginine (symmetric dimethylarginine [SDMA]) is also found in human plasma but does not inhibit NO synthesis.14 ADMA is synthesized in many tissues, including vascular endothelial cells, and is thought to be derived from the hydrolysis of methylated proteins.16 ADMA is excreted by the kidneys, and elevated plasma levels have been found in patients with uremia.17 Elevated plasma levels of ADMA have been reported in animals and patients with hypercholesterolemia and with atherosclerosis.17,18 The purpose of the present study was to investigate the changes in plasma ADMA and endothelial cell–mediated brachial artery vasodilation that occur in subjects with type 2 diabetes mellitus after the ingestion of a high-fat meal.

Methods

Thirty-four men and 16 women with type 2 diabetes mellitus (aged 42 to 75 years, mean 62±9 years) were admitted to the General Clinical Research Center of the Columbia-Presbyterian Campus of New York Presbyterian Hospital. The study protocol was approved by the Columbia-Presbyterian Institutional Review Board, and each subject gave written informed consent.
A careful clinical history was taken, and a physical examination was performed. Cardiovascular risk factors (eg, arterial hypertension, cigarette smoking, family history, and history of cardiovascular disease) were noted, and laboratory tests, including complete blood count, plasma electrolyte, glucose, hemoglobin A1C, urinary protein, and liver function tests, were performed. Individuals with nephrotic-range proteinuria, uncontrolled thyroid disease, or hematologic, hepatic, or renal disease were excluded from the study, as were patients with a history of ketoacidosis or severe hypertriglyceridemia (>400 mg/dL) and patients taking corticosteroids, angiotensin-converting enzyme inhibitors, antioxidants, or estrogen replacement. Statin medications were withheld for 2 weeks before the study.

Study Design
After an overnight fast, noninvasive assessment of brachial arterial vasoreactivity in response to reactive hyperemia was performed after the collection of blood for determination of fasting levels of plasma total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides (TGs), VLDL TGs, and ADMA. The subjects were asked to ingest a liquid meal of 75% fat, 15% carbohydrate, and 10% protein. Plasma glucose, total cholesterol, LDL cholesterol, HDL cholesterol, TG, and VLDL TG levels were measured at baseline and 3, 5, 7, and 10 hours thereafter. Plasma ADMA, SDMA, and L-arginine levels were measured at baseline and 5 hours after ingestion of the high-fat meal, at which time brachial artery vasodilation in response to reactive hyperemia was again measured.

Ten volunteers were randomly selected to return on a separate day for additional studies. They were fed an isocaloric liquid meal that contained a carbohydrate content equivalent to the high-fat meal but that did not contain fat. Plasma lipids, dimethylarginines, L-arginine, and brachial artery vasodilation in response to reactive hyperemia were measured at baseline and 5 hours later.

To exclude the effects of the high-fat meal on endothelium-independent vasodilation, 5 volunteers also returned on another day. Baseline flow-mediated vasodilation of the brachial artery was measured. Fifteen minutes after acquisition of the postocclusion image, the baseline image was regained, after which nitroglycerin was added to plasma was 78.9 μmol/L (n = 10).

Preparation of High-Fat and Nonfat Meals
The high-fat meal was composed of heavy cream, ice cream, safflower oil, a powdered whey protein, syrup, and Lactaid (McNeil Consumer Products). It provided 1265 calories with 105 g fat (75% of total calories), 52 g saturated fat, 48 g carbohydrate (15% of total calories), 32 g protein (10% of total calories), and 300 mg cholesterol. Vitamin A (100 000 IU/m2 body surface area) was also added.

The isocaloric nonfat meal contained whey protein, skim milk (250 g), evaporated skim milk (63 g), syrup (40 g), and granulated sugar (2.4 g).

Determination of Plasma Lipids
Venous blood from the forearm not used for the study of reactive hyperemia was collected into sterile polypropylene tubes containing EDTA and immediately centrifuged at 4°C and 2500g for 20 minutes.

Total plasma cholesterol and TG levels in plasma and VLDL were determined by standardized enzymatic procedures with use of a Hitachi 704 automatic spectrophotometer. HDL cholesterol levels were measured after precipitation of plasma apoB-containing lipoproteins with phosphotungstic acid.19 LDL cholesterol levels were calculated only for subjects with TG levels <400 mg/dL by the Friedewald formula.20

Assessment of Brachial Artery Vasodilation in Response to Reactive Hyperemia
Brachial artery vascular reactivity was measured by using an HP 2500 high-resolution ultrasound machine equipped with a 7.5-MHz linear-array transducer (Hewlett Packard), according to a protocol described in detail.21 Image analysis was performed on a personal computer that was equipped with image analysis software. The images recorded on videotape were analyzed by an investigator blinded to all clinical information. Arterial diameter was measured from the intimal-luminal interface on the anterior wall to the intimal-luminal interface on the posterior wall 1 minute after cuff deflation.21 Paired measurements along a 10-mm length of the artery were performed, and the mean diameter was calculated by averaging these pairs and reporting them in millimeters with the use of calibration factors. An average of 3 separate measurements before and after hyperemia was calculated. Intraobserver and interobserver variability was 1.3% and 2.7%, respectively (n = 10).

Determination of Dimethylarginine and L-Arginine Levels
Plasma concentrations of ADMA, SDMA, and L-arginine were measured by high-performance liquid chromatography (HPLC) by a modification of a method previously described.14 Briefly, plasma samples were loaded onto solid-phase extraction cartridges (CBA Bond Elut, Varian). Eluates were dried over low-flow nitrogen in a 60°C water bath and resuspended in double-distilled water for HPLC. HPLC was performed on a computer-controlled Hewlett Packard 1090 HPLC system equipped with an automatic injector and a variable wavelength UV detector. Separation of the amino acids was achieved on an ODS C18 column (Fisher Scientific) by a mobile phase containing 25 mmol/L phosphoric acid buffer (pH 5.0), 10 mmol/L hexane sulfonic acid, and 1% (vol/vol) acetonitrile. Amino acids were detected by UV absorbance at 205 nm, and the absorption units were converted to moles per liter on the basis of UV measurements of known concentrations of ADMA, SDMA, and L-arginine standards. The identity of the endogenous compounds was confirmed by adding known concentrations of the synthetic compounds to the plasma samples and detecting a proportional increase in absorbance peak at the same elution time. Recovery of standards added to plasma was 78.9 ± 18.3%. Intrassay variability was 1.2% with an ADMA detection limit of 0.1 μmol/L (n = 10).

Statistical Analysis
Values are reported as mean ± 1 SD. Because ADMA levels were not normally distributed, the significance of changes from baseline to 5 hours was assessed by the Wilcoxon paired sample test. A paired t test was used to evaluate the changes in percent brachial artery dilation. Changes in lipids and glucose from baseline to 3, 5, 7, and 10 hours after ingestion of the high-fat meal were analyzed by repeated-measures ANOVA with post hoc analysis of changes from baseline assessed by Dunnett test. Nonparametric Spearman rank correlation was used to assess the significance of the association of ADMA with lipids and brachial arterial dilation.

Results
Subject characteristics are given in the Table. There were 34 males and 16 females, of whom 9 (6 men and 3 women) were smokers. There were 20 whites, 1 African American, 26 Hispanics, and 2 Asians.

Changes in Plasma Lipids After Ingestion of High-Fat Meal
The baseline plasma TG level was 186 ± 111 mg/dL. Baseline VLDL TG level was 117 ± 90 mg/dL. Mean plasma TG levels and VLDL TG levels peaked at 5 hours (485 ± 270 and 354 ± 228 mg/dL, respectively; P < 0.01 versus baseline) and declined thereafter (Table, Figure 1).

After ingestion of the high-fat meal, no significant changes in plasma total cholesterol were observed at 3, 5, 7, or 10 hours. HDL cholesterol decreased from 32 ± 10 to 27 ± 8 mg/dL at 5 and 7 hours (P < 0.05 versus baseline). LDL cholesterol, calculated for subjects with TG levels <400 mg/dL (n = 48 at baseline, n = 31 at 3 hours, and n = 23 at 5 hours)
Effects of High-Fat Meal on Endothelial Function
Brachial arterial diameter was 0.41 ± 0.08 cm at baseline and 0.43 ± 0.08 cm 5 hours thereafter \( (P < 0.01) \). Percent brachial arterial vasodilation in response to reactive hyperemia before the high-fat meal averaged 6.9 ± 3.9%. It was significantly reduced to 1.3 ± 4.5% when measured 5 hours after the meal \( (P < 0.0001) \), Figure 2A).

Effects of High-Fat Meal on Plasma Dimethylarginine and \( \text{L-Arginine Levels} \)
HPLC analysis showed the presence of compounds that eluted with the same retention times as those of synthetic ADMA, SDMA, and \( \text{L-Arginine standards} \) (Figure 3), similar to those published by others.\(^{15,16} \) The ADMA absorption peaks increased significantly 5 hours after the high-fat meal, whereas the peaks other than ADMA showed only minimal changes (Figure 3).

The mean plasma baseline ADMA concentration was 1.04 ± 0.99 \( \mu \text{mol/L} \). At 5 hours after the high-fat meal, ADMA increased significantly \( (P < 0.0005) \) to 2.51 ± 2.27 \( \mu \text{mol/L} \) (Figure 2B).

Baseline mean \( \text{L-arginine concentration} \) was 49.80 ± 16.09 \( \mu \text{mol/L} \) and did not change significantly at 5 hours (48.35 ± 15.76 \( \mu \text{mol/L} \)). Baseline mean SDMA concentration was 0.73 ± 0.34 \( \mu \text{mol/L} \) and also did not change significantly at 5 hours (0.81 ± 0.33 \( \mu \text{mol/L} \)). The \( \text{L-arginine/ADMA ratio} \) was lower at 5 hours compared with that at baseline (46.17 ± 43.30 versus 72.31 ± 41.87, respectively; \( P < 0.005 \)).

Correlations
The change in plasma ADMA concentration did not correlate significantly with the change in plasma glucose, TG, VLDL TG, total cholesterol, LDL cholesterol, or HDL cholesterol at baseline. There was a statistically significant inverse correlation between the plasma ADMA level and the percent brachial arterial dilation \( (r = -0.32, P = 0.02) \) and between the ADMA level at 5 hours after the fatty meal and the percent vasodilation at 5 hours \( (r = -0.49, P = 0.0003) \); Figure 4A). In addition, there was a statistically significant inverse relationship between the change in percent arterial dilation at 5 hours and the fatty meal–induced change in ADMA at 5 hours \( (r = -0.37, P = 0.007) \); Figure 4C). There was no significant correlation between the change in plasma TG or in VLDL TG and the change in arterial reactivity.

Effects of Nonfat Meal on Plasma TG, ADMA, \( \text{L-arginine, and Endothelial Function} \)
Ten of the 50 subjects received an isocaloric nonfat meal as a control on a separate day. Although TG levels increased from 185 ± 135 to 241 ± 144 \( \mu \text{mol/L} \) 5 hours after ingestion of the nonfat meal, there were no significant changes in percent arterial dilation \((5.4 ± 5.1\% \text{ versus } 6.6 ± 4.0\% \text{ at baseline and after 5 hours, respectively}) \) or in ADMA concentration \((0.90 ± 0.82 \text{ versus } 1.09 ± 1.65 \mu \text{mol/L at baseline and after 5 hours, respectively}) \). In these 10 subjects, the response to the nonfat meal was significantly different from the response to
Effects of High-Fat Meal on Endothelium-Independent Vasodilation

Flow-mediated and endothelium-independent brachial arterial dilation before and 5 hours after the high-fat meal were assessed in 5 of the subjects on a separate day. Preprandial flow-mediated vasodilation averaged 5.4 ± 2.3% and was significantly impaired at 5 hours after the high-fat meal (−0.3 ± 3.6%, \( P < 0.001 \)). Vasodilation in response to nitroglycerin was not significantly affected (11.4 ± 1.9% versus 11.2 ± 2.3% at baseline versus 5 hours after the high-fat meal, respectively.)

Discussion

Basal endothelial cell–dependent vasodilator responses in patients with non–insulin-dependent type 2 diabetes have been found to be depressed in most published reports.6–8 The basal brachial arterial vasodilator responses to reactive hyperemia observed in the present study were also moderately but significantly reduced below the responses observed in normal subjects (not age-matched) studied in this laboratory. In response to a high-fat meal, however, the brachial artery vasodilator response declined significantly at 5 hours in the type 2 diabetic subjects. This depression of the brachial vasodilator response was directionally opposite the increased flow responses reported to occur between morning and noon in studies of diurnal variation of endothelial cell–mediated flow responses,22 and it was accompanied by a significant increase in the mean plasma level of ADMA. Neither the vasodilator response nor the plasma ADMA changed significantly between hours 0 and 5 in control studies of 10 type 2 diabetics fed an isocaloric nonfat meal.

The inverse correlations between brachial artery vasodilation and plasma ADMA suggest that inhibition of NOS by ADMA may have contributed to the abnormal brachial arterial vasodilator responses after ingestion of the high-fat meal. Studies of mesenteric and cerebral vascular responses in rats and rabbits indicate that ADMA concentrations of 1 to 300 \( \mu \text{mol/L} \) impair endothelial cell–mediated vasodilation of vascular rings in a dose-dependent fashion.23,24 Vallance et
HPLC peaks, was highly accurate, and was sensitive to extraction approached 80%; the method produced sharp
indicated that concentrations of ADMA from 1 to 10
m
and NOS-3.14,23,24
significantly inhibited the enzyme activity of NOS-1, NOS-2,
vascular risk factors.18 In response to the high-fat meal, the
reported by other groups in normal subjects without cardio-
low-fat meal. Plasma ADMA was not measured in those
reduction in the mean percent brachial arterial vasodilation in response to reactive hyperemia after the ingestion
of a high-fat meal observed in the type 2 diabetics is also consistent with results obtained by Vogel and colleagues11,12
consistent with our data, Vogel and colleagues found that the brachial artery vasodilator response declined in normal subjects after ingestion of a high-fat meal but that it did not decline after ingestion of an isocaloric low-fat meal. Plasma ADMA was not measured in those studies.

In the present study, plasma ADMA was measured by a modification of the method of Vallance et al.14 The mean extraction approached 80%; the method produced sharp HPLC peaks, was highly accurate, and was sensitive to ADMA concentrations of 0.1 μmol/L; and the variability of repeated measurements was low (1.3%). The control plasma ADMA concentration obtained in the type 2 diabetic subjects averaged 1.04±0.99 μmol/L, an amount similar to that reported by other groups in normal subjects without cardiovascular risk factors.18 In response to the high-fat meal, the mean plasma ADMA concentration increased significantly within 5 hours, a transient response that has not been reported previously. Plasma SDMA and plasma l-arginine did not change significantly. The levels of ADMA observed after the high-fat meal in the type 2 diabetic patients (2.51 ± 2.27 μmol/L) are similar to those that have been reported in animals and in patients with hypercholesterolemia and with atherosclerosis who had impaired NO-mediated arterial vasodilation.17,18,25 In a study of patients with peripheral artery atherosclerosis, Böger et al25 found that increased ADMA levels of this magnitude were associated with reductions in urinary nitrate and cGMP excretion rates, findings suggestive of inhibition of NO synthesis in the patients.

The stimulus and the source of the rise in ADMA concentrations in the present study have not been defined and are the subjects of current investigation. Elevated ADMA due to reduced renal excretion is unlikely because the subjects had normal levels of serum creatinine.15 The observations that ADMA and brachial flow responses did not change significantly in the type 2 diabetic subjects fed the nonfat meal suggests that fat ingestion was involved. After the high-fat meal, plasma TG and VLDL TG levels rose significantly, but there were no significant correlations between the rise in plasma TG and VLDL TG and either the increase in ADMA or the reduction in brachial arterial dilation in response to reactive hyperemia. In the studies of Vogel and colleagues involving normal subjects, there was also no correlation between the change in plasma TG levels and the vasodilatory responses. In a study of normal subjects without risk factors for coronary artery disease, Lundman et al26 reported that the intravenous infusion of intralipid, a TG emulsion, was associated with a 4-fold increase in serum TG levels and a decline in endothelial cell–mediated brachial artery vasodilation.

Increased biosynthesis and diminished degradation of ADMA by endothelial cells are other possibilities.16,27–29 Work by Ito et al30 indicates that dimethylarginine dimethylaminohydrolase (DDAH)-1 may be subject to biological regulation. They reported that incubation of transformed umbilical endothelial cells with oxidized LDL and with tumor necrosis factor-α resulted in increased accumulation of ADMA in the culture media along with diminished DDAH enzyme activity but no change in DDAH-1 expression. Very recent studies by Leiper et al30 have indicated that DDAH-2 is expressed in vascular and other tissues in which NOS-3 expression is high, whereas DDAH-1 is expressed in tissues in which NOS-1 expression is high. Thus, it is possible that the increased plasma ADMA observed in the type 2 diabetics after the high-fat meal resulted from a reduction in the expression or enzyme activity of DDAH-2.

In addition to vasodilation, NO has actions that are antiatherogenic. NO inhibits the proliferation and migration of vascular smooth muscle cells, inhibits monocyte/macrophage adhesion to and transmigration across the endothelium, and inhibits platelet adhesion and aggregation. When NOS enzyme activity is inhibited, these antiatherogenic effects of NO may be reduced. Experiments in cholesterol-fed rabbits and in LDL receptor–knockout mice indicate that inhibition of NOS increases the extent of aortic atherosclerosis, whereas feeding of l-arginine reduces it.31–35 It seems reasonable to speculate that during the hours after eating a high-fat meal (when ADMA levels are increased and NOS is presumably inhibited), the type 2 diabetic patients are in a proatherogenic state, which may contribute to the development of atherosclerotic vascular complications. Consistent with this idea is a recent study by Miyazaki et al30 of 116 subjects with no symptoms of coronary or peripheral artery disease. They found that plasma ADMA levels were positively correlated with several risk factors for atherosclerosis and were also independently correlated with the intima-media thickness of the carotid artery.

In summary, the present study demonstrated that in patients with type 2 diabetes, plasma levels of ADMA (the endogenous inhibitor of NOS) are acutely elevated after the ingestion of a high-fat meal. This occurred in association with a reduction in the vasodilator response of the brachial artery to reactive hyperemia, an endothelium-dependent response. The

\begin{figure}
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  \includegraphics[width=\textwidth]{figure5}
  \caption{Changes in plasma TG, plasma ADMA, and percent dilation 5 hours after a nonfat and high-fat meal.}
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data suggest that changes in plasma ADMA levels in response to a high-fat load may contribute to abnormal blood flow responses and, possibly, to atherogenesis in patients with type 2 diabetes.

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References
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