Meal Amino Acids with Varied Levels of Arginine do Not Affect Postprandial Vascular Endothelial Function in Healthy Young Men¹,²

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Abstract

Postprandial endothelial dysfunction is a key event mediating the effects of diet on early atherogenesis. The potential effects of protein intake have been overlooked in the past, although amino acids are precursors for homocysteine and nitric oxide (NO). Our objective was to study the effect of amino acids on postprandial vascular function, in relation to the utilization of meal arginine for NO production. In a crossover design, 9 men ingested 50 g of a complete amino acid mixture, trace-labeled with ¹³C-glycine and ¹⁵N₂-arginine, without (meal A) or with (meal B) 3 g extra arginine. The postprandial utilization of meal arginine for NO production was determined from urinary ¹⁵NO₃. We monitored endothelial function of the brachial artery, the stiffness of the common carotid artery, aortic pulse wave velocity and soluble markers related to endothelial function for 8 h. Meal A did not significantly increase plasma homocysteine and did not alter endothelial function markers. The amount of NO synthesized from meal arginine doubled after meal B (107.1 ± 16.5% increase vs. meal A, P < 0.01) but was very low (271 ± 84 ppm vs. 332 ± 73 ppm, P < 0.05, respectively). After meal B, flow-mediated and nitroglycerine-induced dilation decreased but common carotid artery compliance, pulse wave velocity, plasma soluble intercellular adhesion molecule-1, and von Willebrand factor, and urinary cGMP did not differ when compared with meal A. Together, the data indicate that, in healthy men, meal amino acids do not adversely affect endothelial function, and meal arginine only slightly enters the NO pathway. Unexpectedly, arginine in physiological amounts may acutely lessen smooth muscle cell reactivity to a high dynamic NO release. J. Nutr. 137: 1383–1389, 2007.

Introduction

Vascular endothelial dysfunction, an early initiating factor in atherogenesis, can be transiently elicited during the postprandial period. Postprandial endothelial dysfunction has been repeatedly observed after a high-fat meal in healthy subjects and after an oral glucose load (1–6). Endothelial dysfunction is associated with the elevation of other emerging cardiovascular risk factors related to hemostasis, oxidative stress, and inflammation and is closely related to postprandial metabolic excursion. Postprandial elevations of triglycerides and glucose are related to endothelial dysfunction probably in a cumulative manner (2). Therefore, growing evidence indicates that postprandial homeodynamic perturbations are everyday challenges to long-term metabolic homeostasis and endothelial health; therefore, many consider atherogenesis a postprandial phenomenon (2,4,7,8), as originally proposed by Zilversmit (9). This paradigm is important when studying the effect of diets and nutrients on cardiovascular risk (10).

However, to our knowledge, until recently (11), no studies have addressed the postprandial effect of amino acids on endothelial function, although many mechanisms exist whereby dietary proteins and amino acids could affect postprandial endothelial function. Whereas “physiological” doses of oral methionine have induced transient hyperhomocysteinemia with a reduction in flow-mediated dilation in healthy subjects (12), arginine supplementation has been observed to favorably affect vascular function (13–15), most probably because arginine is the precursor of NO, the central molecule in the regulatory system of endothelial cells (16,17). However, it is of note that the favorable effects of oral arginine on vascular function have generally been documented in subjects at risk or in disease states, with few acute studies in healthy humans at physiological doses (18,19). Indeed, although there is still no clear explanation for the beneficial effect, or absence of an effect, of oral arginine on vascular function (13), the main standing paradigm is that oral arginine per se can be used as a substrate for NO production (20). Little is known about the dynamics of arginine metabolism (21), but the literature indicates that arginine intake contributes...
largely to total arginine flux (22,23) and that circulating arginine is the major precursor pool for NO synthesis (24). Therefore, it remains likely that oral or meal arginine does enter the NO production pathway during the postprandial period, but, to our knowledge, this hypothesis has never been tested directly.

Accordingly, our aim in this study was to investigate the acute postprandial effect of a mixture of dietary amino acids (in the same relative proportion as in milk protein) on endothelial vascular function, in relation to the utilization of meal arginine for NO production, in healthy humans. To ascertain the effect of arginine, we studied 2 levels of arginine in the meal, using a crossover design.

Materials and Methods

Study subjects. Nine healthy young men participated in the study, which used a randomized crossover design. The subjects were nonsmokers and in good health, as determined by a thorough medical examination. In particular, they were normotensive and did not exhibit abnormal cardiovascular function, and none were taking regular medication (Table 1). The protocol was approved by the Institutional Review Board for the Saint-Germain-en-Laye Hospital. All subjects gave full written consent to participate in the study after the experimental protocol was explained to them in detail.

Meal protocol. Each meal consisted of 50 g amino acids (food grade, kindly provided by Ajinomoto), further supplemented (meal B) or not (meal A) with 3 g L-arginine, suspended in 500 mL regular bottled spring water (Cristalline). The standard 50 g amino acid mixture (Table 2) was based on a milk protein composition as reported by the USDA database (25), with aspartic acid:asparagine and glutamic acid:glutamine ratios, as reported elsewhere for casein (26). The meals also contained 70 mg [1-15N]-L-glutamine and 100 mg (meal A) or 170 mg [15N2-(guanido)]-arginine·HCl (meal B).

Randomized crossover blind trial design. Each subject was examined on 2 occasions separated by a period of at least 5 wk. Their diet was not controlled prior to the experimental sessions. Subjects did not know the nature of the meal given to them. On each study day, overnight-fasted volunteers arrived at the laboratory in the morning. After baseline measurements and blood samples were taken, subjects consumed either meal A or meal B within a 5-min period and were monitored subsequently for 8 h. The volunteers also ingested 100 mL water (Cristalline) immediately after the meal and again every hour, starting 2.5 h after ingestion, to facilitate urine collection. After their arrival, subjects rested for 30 min in a supine position before the baseline vascular examinations. A catheter was then inserted into a superficial hand vein for blood sampling; baseline blood and breath were sampled and urine collected before volunteers consumed the test meal. After meal ingestion, we sampled the breath every 30 min, blood every hour, collected urine every 2 h, and performed all vascular examinations every 2 h.

Laboratory analyses. Plasma glucose and urea and urinary creatinine were measured using automated assays on a Hitachi 917 analyzer (Roche Diagnostics). We measured plasma insulin using a radioimmunoassay assay method (INSIK-5 Diasorin) and urinary nitrate concentrations according to a fluorimetric method (27). We measured plasma total homocysteine (Diazyme Laboratory), asymmetric dimethyl-arginine (ADMA6; Cardiovascular Medical Science Laboratory) and soluble intercellular adhesion molecule-1 (sICAM-1; R&D systems) and urinary cGMP (Assay Designs), using commercial ELISA, and plasma von Willebrand factor (vWF) by immunoturbidimetry (STA-LIATEST vWF) on a STA automaton (Diagnostica Stago). We determined amino acids by ion-exchange chromatography with postcolumn ninhydrine detection (Amino-System 2500; Bio-Tek), as previously described (28). All samples were batch-processed at the end of the study. Arginine percentage of oral dose recovery per hour, as previously described (28). Arginine and inosine areas under the curve (AUC) were calculated using the trapezoidal rule.

Nitric oxide production from meal arginine. We determined the 15N enrichment in urinary nitrate by adapting previously described methods (29,30) (Paul D. Brooks, UC Berkeley; Sylvie Recous, INSRA; and Steven R Silva, U.S. Geological Survey; personal communications). Urinary nitrate was first extracted on a highly selective ion-exchange resin (IMAC-HP-555, Rohm&Haas) and eluted with NaCl (25%) before conversion into ammonia, using the microdiffusion method. Briefly, the 10 mL eluate was transferred to a glass bottle, and combined with 800 mg KCl, 50 µL Tween20, 400 mg Devarda’s alloy, and 200 mg MgO. The solution was then sealed with a suspended 6 mm filter paper disk saturated with 10 µL KH2SO4 (2.5 mol/L), and incubated for 7 d at 65°C. The disks were then combusted in an elemental analyzer, coupled with an IRMS (Micromass). Background contamination was determined with serial blanks and enrichments, corrected using the nitrogen levels in samples

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographics of study subjects</th>
</tr>
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<tbody>
<tr>
<td>Subjects</td>
<td>Age (y)</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>26.2 ± 8.6</td>
</tr>
</tbody>
</table>

1 600 mL water and tracers were added to meals.

| TABLE 2 | Amino acid composition of experimental meals
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Amino acid, g</td>
<td>Meal A</td>
</tr>
<tr>
<td>l-Arginine</td>
<td>1.727</td>
</tr>
<tr>
<td>l-Tryptophan</td>
<td>0.688</td>
</tr>
<tr>
<td>l-Threonine</td>
<td>2.148</td>
</tr>
<tr>
<td>l-Isoleucine</td>
<td>2.873</td>
</tr>
<tr>
<td>l-Leucine</td>
<td>4.658</td>
</tr>
<tr>
<td>l-Lysine</td>
<td>3.773</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>1.190</td>
</tr>
<tr>
<td>l-Cystine</td>
<td>0.435</td>
</tr>
<tr>
<td>l-Phenylalanine</td>
<td>2.293</td>
</tr>
<tr>
<td>l-Tyrosine</td>
<td>2.293</td>
</tr>
<tr>
<td>l-Valine</td>
<td>3.193</td>
</tr>
<tr>
<td>l-Histidine</td>
<td>1.292</td>
</tr>
<tr>
<td>l-Alanine</td>
<td>1.640</td>
</tr>
<tr>
<td>l-Aspartic acid</td>
<td>2.024</td>
</tr>
<tr>
<td>l-Asparagine</td>
<td>1.590</td>
</tr>
<tr>
<td>l-Glutamic acid</td>
<td>4.985</td>
</tr>
<tr>
<td>l-Glutamine</td>
<td>4.985</td>
</tr>
<tr>
<td>l-Glycine</td>
<td>1.001</td>
</tr>
<tr>
<td>l-Proline</td>
<td>4.615</td>
</tr>
<tr>
<td>l-Serine</td>
<td>2.583</td>
</tr>
<tr>
<td>Total</td>
<td>50.0</td>
</tr>
</tbody>
</table>

6 Abbreviations used: ADMA, asymmetric dimethylarginine; AUC, area under the curve; FMD, flow-mediated dilation; GTN, nitroglycerine; NO, nitric oxide; sICAM-1, soluble intercellular adhesion molecule-1; vWF, von Willebrand factor.
and blanks as determined by the elemental analyzer. The linearity of the method ($R^2 = 0.995$) was checked using urine spiked with $^{15}$NO$_3$-N. For each 2-h urine collection, we used a standard isotopic dilution equation to calculate the amount of nitrate originating from the guanidium of meal arginine, i.e., the amount of NO produced from meal arginine: $Q \times E/Emeal$, where $Q$ is the amount of nitrate in the collection, $E$ the $^{15}$NO$_3$ enrichment (in percentage of atom excess), and $Emeal$ the $^{15}$N enrichment on the guanidium moiety of meal arginine.

Vascular measurements. The same person performed all measurements and image analyses throughout the study (L.S.) and was unaware of the nature of the meal. The mechanical properties of the common carotid artery were determined as previously described by our group (31). Briefly, the right common carotid artery was examined 2 cm proximal to the carotid bifurcation using real-time, B-mode ultrasound imager (Acuson XP 128) with a 7-MHz vascular probe. Systolic and diastolic diameters, as determined after the offline analysis of ECG-gated echographic images, and systolic and diastolic blood pressures (recorded automatically by an oscillometric recorder, Dynamap model 8100, Critikon) were used to calculate cross-sectional compliance (31). Flow-mediated and nitroglycerine-mediated dilations of the brachial artery were determined using a high-resolution vascular ultrasound system, as previously described (31). Aortic pulse wave velocity was determined using aplanatonyometry at the site of the common carotid artery and femoral artery and calculated as the delay between the femoral pulse wave and the carotid pulse wave, divided by the distance between the 2 sites.

Statistical analysis. Results are reported as means ± SEM. We analyzed data according to a mixed model for repeated measures, with the nature of the meal and the time after ingestion as independent, fixed (repeated) factors and the subject as a random factor (SAS Institute). Furthermore, the sequence of the treatment (i.e., which of the meals was tested first), as nested into the subject, and the number of the sequence (i.e., first or second visit) were also included into the model. Comparisons between meals at specific time points were made using ad hoc orthogonal contrasts under the mixed models, when main effects or interactions were significant or tended to be significant ($0.05 < P < 0.01$). Significance was determined at $P < 0.05$.

Results

Plasma arginine concentrations increased during the postprandial period after both meals (Fig. 1A) but more so after meal B than after meal A ($P < 0.001$). Plasma arginine was higher for the first 4 h ($P < 0.01, 62 ± 7%$ higher) after meal B than meal A (4 h-AUC after meal B was $2.6 ± 0.3$-fold that after meal A). We observed a transient and moderate increase in plasma insulin during the first 2 h, with no significant difference between meals (Fig. 1B; AUC $151.6 ± 19.8$ and $163.9 ± 29.2$ pmol/l·h after A and B, respectively). The excretion of $^{13}$CO$_2$ in the breath (Fig. 1C), originating from the postprandial catabolism of the meal labeled glycine, had similar kinetics and total final recovery of the tracer after both meals (meal A: $38.9 ± 3.8%$ and meal B: $39.9 ± 6.7%$). Labeled glycine was used to trace the kinetics of appearance of the bulk of amino acid (28) 1 to test for a potential difference in gastric emptying between meals and 2 to determine whether this nonspecific amino acid would be catabolized at a different rate as a function of the nature of the meal. The results show that the amino acids were available and catabolized at the same rates, irrespective of the nature of the meal.

Although plasma methionine concentrations increased markedly after both meals (3-fold of baseline 1–2 h after ingestion, data not shown), plasma homocysteine ($7.55 ± 0.22$ μmol/L) and ADMA ($0.147 ± 0.004$ μmol/L) concentrations did not increase during the postprandial period and were unaffected by the nature of the meal (Table 3).

The postprandial excretion of nitrate and creatinine did not differ between meal, and over 8 h of recovery, the total amount excreted was: $413.9 ± 31.9$ and $426.5 ± 62.7$ μmol nitrate, and $5.67 ± 0.28$ and $5.80 ± 0.36$ mmol creatinine, after meals A and B, respectively, data not shown. The amount of nitrate originating from meal arginine (Fig. 2A) doubled with meal B compared with meal A ($107.1 ± 16.5%$ increase). Relative to the amount of ingested arginine ($1.73$ g in meal A vs. $4.73$ g in meal B), the utilization of meal arginine for NO production (Fig. 2B) was slightly but significantly lower after meal B than meal A, with a $23.0 ± 6.1%$ decrease in the relative entry of meal arginine into the NO synthesis pathway following meal B.

The urinary excretion of cGMP (the second messenger of NO) varied over time and increased significantly more 2 and 4 h after meals than 6 and 8 h after, but did not differ as a function of the meal (Table 3). Plasma sICAM-1 ($254.9 ± 7.1$ μg/L) and vWb ($81.8 ± 3.3%$) concentrations remained steady during the postprandial period (Table 3).

Blood pressure did not vary as a function of either treatment or time after the meal. The postprandial arterial pressure was $80.8 ± 1.8$ mm Hg after meal A and $80.8 ± 1.6$ mm Hg after the meal.
meal B. Flow-mediated dilation (FMD), nitroglycerine (GTN), and aortic pulse wave velocity did not differ significantly before consuming meal A than before consuming meal B (data not shown). After meal A, FMD (Fig. 3A) did not differ as a function of time. However, FMD tended to differ depending on the meal (meal × time effect, P = 0.075), and FMD was markedly and significantly lower 4 h after ingesting meal B than after meal A (Fig. 3A). GTN-mediated dilation (Fig. 3B) decreased significantly more with time after meal B than after meal A. Aortic pulse wave velocity (Fig. 3C) differed significantly with time and tended to be lower 4 h after ingestion than at baseline (P = 0.074) but was unaffected by the nature of the meal. Compliance of the common carotid artery (Table 3) was not affected by time or by the nature of the meal.

**Discussion**

The major findings of this study are: 1) meal amino acids do not adversely affect vascular endothelial function during the postprandial phase; 2) meal arginine is used for NO synthesis depending on the amount in the meal, but the relative utilization of meal arginine is low; 3) accordingly, meal arginine does not affect basal endothelial function, but 4) it may lessen the sensitivity of smooth muscle cells to a very high and dynamic NO release (such as that which occurs during FMD or GTN experimental testing). All effects of the amino acid mixtures in the present work refer only to the acute response during the postprandial phase.

Our data show that the ingestion of 50 g of an amino acid mixture did not induce endothelial dysfunction, as evidenced by all indicators of endothelial function. First, all vascular endpoints (flow-mediated dilation of the brachial artery, common carotid artery compliance, and aortic pulse wave velocity) did not decrease for 8 h after ingestion, clearly showing no alteration in vascular function. We studied all of these variables because they are NO-dependent variables that complementarily describe vascular function in different types of arteries. During acute studies, aortic pulse wave velocity and arterial compliance were shown to be relevant indicators, as is the FMD, of a transient alteration to endothelial function (32–34). Second, the excretion of cGMP was higher in the first urinary collections (0–2 h and 2–4 h) than in later collections (4–6 h and 6–8 h). These later urinary collections represented postsorptive urine cGMP excretion that should have been very similar to the basal urinary excretion of cGMP before meals, which we did not measure. Therefore, the cGMP excretion pattern clearly indicated that there was indeed a postprandial increase in the basal activity of guanylate cyclase during the first hour after both meals, which suggests an increase in NO bioavailability and downstream signaling. Third, the soluble markers of endothelial function remained steady during the postprandial period. Conversely, it has been widely documented that in healthy humans, a high-fat and a high-sucrose load decreases flow-mediated dilation (1,2,5,35) and increases plasma ADMA, an endogenous inhibitor of NO synthase (40,41). However, because the amino acid intake in this experimental meal (50 g) was close to the (daily) recommended dietary allowance and ~50% of the mean daily protein intake in Western countries (42), our experimental setting is relevant to a normal (physiological) amino acid challenge. The amino acid composition, which was based on milk protein, should also be considered as

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**TABLE 3** Plasma homocysteine (Hcy), ADMA, sICAM-1, and vWb, urinary excretion of cGMP, and aortic pulse wave velocity of healthy men during the postprandial period after ingesting a standard 50 g mixture of amino acids without (meal A) or with (meal B) 3 g extra arginine

<table>
<thead>
<tr>
<th>Meal</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy, μmol/L</td>
<td>7.4 ± 0.7</td>
<td>9.3 ± 1.4</td>
<td>7.6 ± 0.5</td>
<td>8.3 ± 0.7</td>
<td>7.5 ± 0.5</td>
<td>7.2 ± 0.5</td>
<td>7.2 ± 0.6</td>
<td>7.2 ± 0.3</td>
<td>6.8 ± 0.5</td>
<td>7.2 ± 0.7</td>
</tr>
<tr>
<td>ADMA, nmol/24 h</td>
<td>136 ± 12</td>
<td>133 ± 10</td>
<td>145 ± 17</td>
<td>150 ± 13</td>
<td>157 ± 11</td>
<td>152 ± 10</td>
<td>138 ± 11</td>
<td>151 ± 9</td>
<td>146 ± 11</td>
<td>158 ± 10</td>
</tr>
<tr>
<td>Urinary cGMP³</td>
<td>nd²</td>
<td>70.8 ± 9.3</td>
<td>87.0 ± 15.5</td>
<td>49.1 ± 7.7</td>
<td>51.5 ± 8.9</td>
<td>nd</td>
<td>84.0 ± 15.7</td>
<td>75.7 ± 13.0</td>
<td>52.8 ± 6.5</td>
<td>40.4 ± 11.5</td>
</tr>
<tr>
<td>sICAM-1, μg/L</td>
<td>271.9 ± 30.7</td>
<td>254.0 ± 24.5</td>
<td>243.8 ± 21.7</td>
<td>247.8 ± 19.0</td>
<td>248.7 ± 24.2</td>
<td>263.0 ± 19.1</td>
<td>257.1 ± 23.2</td>
<td>260.6 ± 22.2</td>
<td>246.2 ± 23.8</td>
<td>252.6 ± 19.7</td>
</tr>
<tr>
<td>vWb, %</td>
<td>86.0 ± 8.0</td>
<td>nd</td>
<td>83.9 ± 12.4</td>
<td>nd</td>
<td>80.8 ± 5.4</td>
<td>80.0 ± 7.6</td>
<td>nd</td>
<td>81.2 ± 9.0</td>
<td>nd</td>
<td>78.6 ± 6.6</td>
</tr>
<tr>
<td>CCA compliance,⁴</td>
<td>0.117 ± 0.017</td>
<td>0.141 ± 0.029</td>
<td>0.132 ± 0.014</td>
<td>0.130 ± 0.017</td>
<td>0.106 ± 0.012</td>
<td>0.109 ± 0.010</td>
<td>0.097 ± 0.011</td>
<td>0.121 ± 0.017</td>
<td>0.124 ± 0.017</td>
<td>0.105 ± 0.015</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEM, n = 9.
² nd, not determined.
³ Significant effect of time, P < 0.05 (repeated measures ANOVA).
⁴ CCA, common carotid artery.

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**FIGURE 2** Postprandial amounts of nitrate in healthy men originating from the ingestion of meal arginine, as traced by meal [¹⁵N₂]-guanidino-arginine, expressed either as absolute amounts, with the 2 h-collection details (A), or as relative to the amount of arginine in the meal, with mean (bold line) and individual data (B), after ingestion of a standard 50 g mixture of amino acids without (meal A) or with 3 g extra arginine (meal B). Data are means ± SEM, n = 9. Asterisks indicate different from meal A, *P < 0.05, **P < 0.01.
standard, although it was low in arginine, as are other important protein sources for human nutrition, such as wheat. It should also be noted that because no other nutrients were included in the meal except amino acids, the results cannot be extrapolated directly to complex meals.

Oral arginine is usually considered to be a potent stimulator of insulin secretion, although physiological amounts have only marginal effects on insulin secretion in humans (43). Our study showed that insulin secretion was not modified by the addition of 3 g arginine to the amino acid meal. Furthermore, oxidation of the indicator amino acid was completely unaffected, indicating no change in overall amino acid catabolism. It has been reported that an increase in the arginine flux does not affect total NO production as assessed at the steady state (44). However, the massive level (~36 g) of arginine used during that study (as in many other studies) also markedly increased plasma insulin and had a large impact on nitrogen and urea kinetics (44), so that the result was difficult to interpret in terms of normal postprandial metabolism. Indeed, it is clear that high, nonphysiological levels of arginine supplementation can result in nonspecific effects. Moreover, because of the profound impact of an increase in insulin secretion on vascular function, it is not possible to understand the specific impact of any manipulation, such as high arginine supplementation, that also affects plasma insulin concentrations (45). We recently reported that, in contrast with studies involving infusions of high levels of arginine, a chronic oral administration of arginine that resulted in ~70% higher fasting plasma arginine concentrations did not increase plasma insulin and had moderate effects on homeodynamics than those elicited by an arginine infusion (46). Similarly, during our study, the results could be ascribed specifically to a physiological modulation of arginine availability, virtually free of nonspecific and nonphysiological effects on NO production or vascular function.

Unexpectedly, the addition of arginine to the amino acid mixture resulted in a significant transient decrease in flow-mediated dilation. Although between-subject variations were considerable, there was a marked decrease in FMD \( P < 0.05 \) 4 h after ingestion of the arginine-supplemented meal compared with the standard meal, but the overall effect was only a trend \( P = 0.075 \). Furthermore, the decrease was also delayed when compared with the difference in the plasma arginine increases between meals, although the marked compartmentalization of arginine metabolism might offer an explanation for the delay between arginine exposure and its physiological impact. Furthermore, and more importantly, the nonendothelial mediated function, as tested with an exogenous NO donor, was also largely impaired during the postprandial phase by the addition of arginine. GNT-mediated dilation is a useful control when interpreting effects on FMD. When GNT-mediated dilation is determined and remains steady across treatments, changes to flow-mediated dilation can be interpreted as changes to the ability of the endothelium to transduce a physiological stimulus into a NO signal. Because the decrease in GNT-mediated dilation in our study indicated a postprandial impairment in smooth muscle cell reactivity to NO stimulation, FMD could not be interpreted as endothelial dysfunction. Therefore, taken together, the unexpected FMD results are clearly difficult to interpret, and further studies are needed to understand our observations. Indeed, other markers of vascular endothelial function, which are sensitive markers of endothelial function (as stated above), indicate no alteration to the basal (unstimulated as opposed to stimulated, as tested in the FMD paradigm) endothelial function. Lastly, NO downstream signaling, determined by postprandial cGMP excretion, did not differ between meals, although it is unclear whether this marker was sensitive enough to detect slight variations in guanylate-cyclase activation by NO. Overall, these results indicate that arginine supplementation did not affect basal (unstimulated) endothelial function. On the other hand, the higher arginine availability after meal B clearly resulted in a paradoxical reduction of NO signaling in smooth muscle cells under stimulation by nitroglycerine. Because we detected no changes in vascular function (aortic pulse wave velocity, common carotid artery compliance), we can conclude that the reduction in smooth muscle sensitivity to NO may be limited to the situations of intense, short-term increases in NO availability to the smooth muscle cells that occur during postischemic dilation or the administration of nitroglycerine.

We note that it is unlikely that the absence of effect regarding vascular and/or endothelial function markers was the result of low statistical power. Although the number of subjects per se was quite small \( n = 9 \), the power of the tests was markedly enhanced by the dual repetition on factors (crossover and after meals) that, besides absorbing interindividual effects, enabled
showed, in 2 healthy humans, that the asymptotic recovery of consistent with the pioneering pilot work by Leaf et al. who from 0.14 to 1.2%, (47)]. This low utilization of oral arginine is the plasma arginine flux into NO in the steady state [ranging under a "saturation" kinetics, as often inferred from studies the higher dose, the marked increase in NO produced from meal relative utilization for NO synthesis was only slightly lower with NO production during the postprandial period. Because the relative utilization for NO synthesis was only slightly lower with the higher dose, the marked increase in NO produced from meal arginine revealed that the process was certainly far from being under a “saturation” kinetics, as often inferred from studies using only indirect markers of total NO production. However, the utilization of meal arginine for NO production was low after both meals compared with estimates concerning conversion of the plasma arginine flux into NO in the steady state [ranging from 0.14 to 1.2%, (47)]. This low utilization of oral arginine is consistent with the pioneering pilot work by Leaf et al. who showed, in 2 healthy humans, that the asymptotic recovery of $^{15}$N as urinary nitrate after a single oral 6-g dose of totally labeled $^{15}$N$_2$-arginine was only $\sim$700 ppm (48).

The compartmentment of arginine metabolism is complex. In particular, NO synthase are expressed in structural microdomains (the caveolae) that are considered to define a kinetically and structurally separate pool of arginine for NO synthesis, with most of the arginine directed toward NO synthesis presumably being channeled from specific pathways (49). In this background, our data offer the first in vivo evidence that, in healthy humans, exogenous arginine only marginally enters the NO production pathway. This metabolic feature may thus account for the lack of a favorable effect of meal arginine on NO-related physiological functions during our study. Lastly, because high levels of ADMA (which may be elicited at an early stage in many pathophysiological situations) are proposed to be the rationale for the benefits of chronic oral arginine supplementation in many diseases (50), the present set of results could also be related to the low and steady plasma ADMA levels found in our healthy young subjects.

It would be interesting to test this translational approach in the many early pathophysiological processes where NO production is thought to be jeopardized, so as to inspect more closely the potential impact of dietary arginine on NO synthesis and thereby the maintenance of vascular health.

**Acknowledgments**

We would like to thank Sophie Daré for amino acid chromatography and Dr. Donald J. Herman for useful information about $^{15}$NO$_3$ enrichment determinations. We thank Ajinomoto Europe for kindly providing the amino acids and Rohm&Hass for their gift of the special ion-exchange resin.

**Literature Cited**


