Blood Pressure and Metabolic Changes During Dietary L-Arginine Supplementation in Humans

Alfonso Siani, Ermenegilda Pagano, Roberto Iacone, Licia Iacoviello, Francesco Scopacasa, and Pasquale Strazzullo

Dietary L-arginine supplementation has been proposed to reverse endothelial dysfunction in such diverse pathophysiologic conditions as hypercholesterolemia, coronary heart disease, and some forms of animal hypertension. In particular, chronic oral administration of L-arginine prevented the blood pressure rise induced by sodium chloride loading in salt-sensitive rats. To investigate the effects of L-arginine-rich diets on blood pressure and metabolic and coagulation parameters we performed a single-blind, controlled, crossover dietary intervention in six healthy volunteers. The subjects (aged 39 ± 4 years, body mass index [BMI] 26 ± 1 kg/m², mean ± SEM) received, in random sequence, three different isocaloric diets, each for a period of 1 week (Diet 1: control; Diet 2: L-arginine enriched by natural foods; Diet 3: identical to Diet 1 plus oral L-arginine supplement). Sodium intake was set at a constant level (about 180 mmol/day) throughout the three study periods. A blood pressure decrease was observed with both L-arginine-rich diets (Diet 2 v 1, SBP: −6.2 mm Hg [95% CI: −0.5 to −11.8], DBP: −5.0 mm Hg [−2.8 to −7.2]; Diet 3 v 1, SBP: −6.2 mm Hg [−1.8 to −10.5], DBP: −6.8 mm Hg [−3.0 to −10.6]). A slight increase in creatinine clearance (P = .07) and a fall in fasting blood glucose (P = .008) occurred after Diet 3 and, to a lesser extent, after Diet 2. Serum total cholesterol (P = .06) and triglyceride (P = .009) decreased and HDL cholesterol increased (P = .04) after Diet 2, but not after Diet 3.


KEY WORDS: L-arginine, diet, blood pressure, metabolism.

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Although a few studies have investigated the relationship between dietary protein intake and blood pressure, little interest has been paid thus far to the effect of single amino acids. Recently, the identification of nitric oxide (NO) as a product of the metabolism of L-arginine through the NO synthase pathway has opened a new avenue of research. L-arginine, a so-called “conditionally” essential amino acid largely present in several food items, plays a central role in a number of major metabolic pathways (3). Its average dietary intake is 5.4 grams/day, assuming a total daily protein intake of 100 grams/day (3). On clinical grounds, interest in the role of this aminoacid has been stimulated by the observation that L-arginine supplementation was able to attenuate the endothelial dysfunction associated with hypercholesterolemia (4) and coronary heart disease (5). Moreover, hypertension induced by sodium chloride loading in Dahl salt-sensitive rats was prevented by chronic dietary L-arginine supplementation. Yet in other studies the parenteral administration of large doses of L-arginine acutely lowered blood pressure in normotensive and in salt-sensitive hypertensive individuals. Against this background, the present study was designed to investigate the effects of increased L-arginine dietary intake—in the form of naturally arginine-rich foods or as a pharmacologic preparation—on blood pressure and metabolic and coagulation parameters in healthy humans.

MATERIALS AND METHODS

Six untreated healthy volunteers (aged 39 ± 4 years, body mass index [BMI] 26 ± 1 kg/m², mean ± SEM) gave their informed consent to the study and, after baseline evaluation, received, in random sequence, three different isocaloric diets each for a period of 1 week.

Dietary Intervention The diets were tailored for each patient by an expert dietitian to keep the caloric intake constant. Diet 1 (control) was a relatively low L-arginine diet (3.5–4.0 g/day). Diet 2 was an L-arginine–enriched diet (10 g/day) based on natural foods; dry legumes (lentils) and nuts (hazelnuts, walnuts, and peanuts) were chosen as major sources of L-arginine, due to their particularly high L-arginine content. Diet 3 was identical to Diet 1 (control diet), but was supplemented with 10 g/day of an oral L-arginine preparation (Bio-Arginina, Farmaceutici Damor s.p.a., Naples, Italy), given three times a day. The nutrient composition of the diets was calculated based upon updated tables of food composition. The calculated composition of the diets is reported in Table 1. The three diets were similar with regard to their macronutrient composition, with the exception of fiber intake, which was higher in Diet 2. The dietary content of L-arginine in Diet 2 was more than doubled as compared with the control diet. Sodium intake was set at a constant level throughout the three study periods (about 180 mmol/day). Meals were prepared in the research kitchen of the Institute of Food Sciences and Technology in Avellino. On weekdays, the participants had lunch on site and took their evening meals with them. On Friday, they received their weekend meals to be consumed off site. Two participants started the study with the control diet (Diet 1), two with the Diet 2, and two with the Diet 3.

Measurements Blood pressure and anthropometric, metabolic, and coagulation parameters were measured at baseline and at the end of each study period. Blood pressure was measured by a trained observer blind to the participant’s dietary regimen using a random-zero sphygmomanometer (Gelman Hawksley Ltd., Lancing, UK); after the participant had been sitting upright for at least 10 min, systolic and fifth-phase diastolic pressure were taken three times, 2 min apart. The average of the last two measurements was used in the analysis.

Serum cholesterol, triglycerides, and glucose were measured with automated methods (Cobas-Mira, Roche, Italy). High-density lipoprotein (HDL) cholesterol was measured by the precipitation method. Creatinine concentrations in blood and urine samples were measured by the picric acid colorimetric method. Urinary electrolytes were measured using an EA-2 Beckman Electrolyte Analyzer. Plasma insulin concentration was measured by radioimmunoassay (Insulina Lisophage, Technogenetics, Milano, Italy). Plasma fibrinolytic activity was measured on plasma euglobulin fraction by the fibrin plate method (Sigma Chemicals Co., St. Louis, MO) and was expressed as the euglobulin lysis area (ELA, mm²). PAI-1 antigen levels and t-PA antigen levels were determined by commercial double-antibody assay (American Diagnostica Inc., Greenwich, CT). Fibrinogen was determined by a.

### TABLE 1. CALCULATED COMPOSITIONS OF THE CONTROL DIET AND THE INTERVENTION DIET

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Diet 1 (Control)</th>
<th>Diet 2 (l-Arginine Rich)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total carbohydrate (% energy)</td>
<td>53</td>
<td>49</td>
</tr>
<tr>
<td>Protein (% energy)</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Total fat (% energy)</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Saturated (% energy)</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Polyunsaturated (% energy)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Fiber (g/day)</td>
<td>22</td>
<td>47</td>
</tr>
<tr>
<td>L-Arginine (mg/day)</td>
<td>4080</td>
<td>9650</td>
</tr>
</tbody>
</table>
one-stage clotting assay (Hemoliance, Cologno, Monzese, Italy). The concentration of fibrinogen was determined by comparison with a reference curve.

Statistical analysis Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS Italia, Bologna, Italy). Results are expressed as mean ± SEM or 95% confidence interval (CI; where appropriate). Differences between the arginine-rich diets and the control diet were analyzed for statistical significance by paired t test. The distributions of serum glucose and triglycerides, as well as of plasma insulin, were normalized by log transformation and log-transformed values were used in the analysis. Two-sided P values less than .05 were considered statistically significant unless otherwise indicated.

RESULTS

The main results are summarized in Table 2. All subjects but one had baseline blood pressure values within the normal range. A significant average blood pressure decrease was observed after both Diets 2 and 3, compared with the control diet. Figure 1 shows changes in individual systolic and diastolic blood pressure values; the increase in \( l \)-arginine intake, both from dietary sources and from pharmacologic supplements, resulted in a blood pressure fall in all subjects. The decrease in blood pressure was not significantly related to the initial blood pressure value.

After Diet 3, a significant reduction was observed in fasting blood glucose concentration; a similar trend was also apparent after Diet 2. A decrease in serum total cholesterol and triglycerides and an increase in HDL-cholesterol concentration were observed after Diet 2. Twenty-four–hour urinary sodium excretion was very close to the prefixed value (180 mmol/day) in all dietary periods, whereas a trend toward an increase in 24-h potassium excretion was observed after Diet 2. A trend toward an increase in creatinine clearance was also observed at the end of both Diets 2 and 3, the difference approaching statistical significance versus the control diet after Diet 3.

Body weight, plasma insulin levels, and coagulation factors (ELA, PAI-I, t-PA, fibrinogen) did not change throughout the study.

![FIGURE 1. Individual systolic and diastolic blood pressure changes after \( l \)-arginine supplemented diets (A: diet 2 vs diet 1; B: diet 3 vs diet 1). SBP: systolic blood pressure; DBP: diastolic blood pressure.](image-url)
DISCUSSION

To the best of our knowledge, there are no published data from controlled studies dealing with the blood pressure effects of l-arginine–rich diets in humans. The main finding of our work is that this dietary intervention was associated with a statistically and biologically significant blood pressure reduction, whether l-arginine was provided through natural foods or as a pharmacologic preparation.

A reduction in serum glucose and an apparent increase in renal glomerular filtration rate (as suggested by the higher creatinine clearance) were also observed. To our knowledge, this is also the first report of an effect of dietary l-arginine supplementation on glucose metabolism in humans. An effect of l-arginine on insulin release has been observed in acute experiments using the parenteral route; it is possible that a similar mechanism accounts for our finding during chronic oral administration, although no significant changes in fasting plasma insulin levels were observed in the present study. The increase in creatinine clearance is consistent with the recognized effects of an oral protein load or an infusion of amino acid mixtures on renal hemodynamics, although here again the available information stems essentially from acute experiments. As to the improvement in serum lipid profile after Diet 2, this was most likely due to the higher fiber content of that diet.

The explanation for the blood-pressure–lowering effect of l-arginine administration in our study is not apparent. Modulation of NO production may be critical for blood pressure control, particularly during dietary salt loading, and l-arginine feeding may actually correct a failure in NO production under these circumstances. A dysfunction in the l-arginine-nitric oxide pathway in the renal circulation has also been advocated by recent studies in patients with mild primary hypertension. Interestingly, in two of these studies the acute infusion of l-arginine reduced blood pressure also in normotensive control subjects, a finding consistent with our results. An unresolved issue, however, is that increased dietary l-arginine availability does not necessarily lead to enhanced NO production, as l-arginine supply is not normally rate limiting for NO synthesis. Alternative or additional mechanisms whereby l-arginine may affect blood pressure and renal function need to be investigated (direct effects of the amino acid, release of hormones, prostaglandins, etc.).

A few potential limitations of this study should be considered. First, the short-term duration of the intervention does not allow exclusion of the possibility that the blood-pressure–lowering effect of l-arginine may not be sustained over time. Second, we set out a high level of sodium intake for our experimental diets on the assumption that modulation of NO delivery is critical to blood pressure control during dietary salt loading. Thus we cannot exclude that the response may be limited to (or greater in) subjects eating a high-sodium diet, as we did not study subjects at different levels of sodium intake. Third, the possibility that dietary components other than l-arginine might at least partly explain the effect observed needs to be considered as well. The 24-h urinary potassium excretion of our participants increased after Diet 2, most likely due to the increase in legume and nut intake. In a previous long-term controlled trial we showed that increasing dietary potassium intake improved blood pressure control in hypertensive patients; however, in the present study, a blood pressure fall similar to the one observed with Diet 2 took place when l-arginine was given as pharmacologic supplementation to the control diet, thus ruling out the confounding influence of dietary components other than l-arginine. Finally, the dietary modifications adopted in our trial are not intended for use in clinical practice. Our dietary modifications were implemented to achieve the goal of consistently increasing l-arginine intake while avoiding, as much as possible, the influence of confounding factors on the experimental results. Interestingly, however, the magnitude of the blood pressure fall observed with both dietary and pharmacologic increase in l-arginine intake was similar to that reported by the DASH Collaborative Research Group and by McCarron et al, as a consequence of more complex dietary modifications aimed to provide, respectively, a dietary model for the prevention and treatment of high blood pressure and for the nutritional management of cardiovascular risk factors.

In conclusion, the present study indicates that an approximately twofold increase in dietary l-arginine intake had significant hemodynamic and metabolic effects in a group of healthy men. Further studies are warranted to confirm these findings over a long-term period and in a larger population; to evaluate the blood-pressure–lowering effect of oral l-arginine in hypertensive patients; and to elucidate the mechanism(s) involved.

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REFERENCES