Reference Intervals for Plasma \( \text{L-Arginine} \) and the \( \text{L-Arginine:Asymmetric Dimethylarginine Ratio} \) in the Framingham Offspring Cohort\(^{1-3} \)

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Abstract

\( \text{L-Arginine} \), as a precursor of NO synthesis, has attracted much scientific attention in recent years. Experimental mouse models suggest that \( \text{L-Arginine} \) supplementation can retard, halt, or even reverse atherogenesis. In human studies, supplementation with \( \text{L-Arginine} \) improved endothelium-dependent vasodilation. However, \( \text{L-Arginine} \) levels are best interpreted in the context of levels of asymmetric dimethylarginine (ADMA), a competitive inhibitor of NO synthase. Thus, reference limits for circulating \( \text{L-Arginine} \) and the \( \text{L-Arginine:ADMA} \) ratio may help to determine the nutritional state of individuals at high cardiovascular risk in light of increased ADMA levels. We defined reference limits for plasma \( \text{L-Arginine} \) in 1141 people and for the \( \text{L-Arginine:ADMA} \) ratio in 1138 relatively healthy individuals from the Framingham Offspring Cohort. Plasma \( \text{L-Arginine} \) and ADMA concentrations were determined by using a stable isotope-based LC-MS/MS method. The reference limits (2.5th and 97.5th percentiles) for plasma \( \text{L-Arginine} \) were 41.0 \( \mu \text{mol/L} \) (95% CI = 39.5–42.5 \( \mu \text{mol/L} \)) and 114 \( \mu \text{mol/L} \) (95% CI = 112–115 \( \mu \text{mol/L} \)), whereas corresponding reference limits (2.5th and 97.5th percentiles) for the \( \text{L-Arginine:ADMA} \) ratio were 74.3 \( \mu \text{mol/L} \) (95% CI = 71.1–77.3 \( \mu \text{mol/L} \)) and 225 \( \mu \text{mol/L} \) (95% CI = 222–228 \( \mu \text{mol/L} \)). Plasma \( \text{L-Arginine} \) was positively associated with the estimated glomerular filtration rate (eGFR) and blood glucose levels, whereas the \( \text{L-Arginine:ADMA} \) ratio was positively associated with eGFR and diabetic blood pressure but inversely associated with homocysteine and (log)Creatine protein. We report reference levels for plasma \( \text{L-Arginine} \) and for the \( \text{L-Arginine:ADMA} \) ratio that may be helpful for evaluation of the effects of \( \text{L-Arginine} \) supplementation in participants with an impaired \( \text{L-Arginine:NO} \) pathway. J. Nutr. doi: 10.3945/jn.111.148197.

Introduction

\( \text{L-Arginine} \) (2-amino-5-guanidino-pentanoic acid) is a conditionally essential amino acid that is a natural constituent of dietary proteins, with the relative amount of \( \text{L-Arginine} \) ranging from 3 to 15% in various proteins. In addition to its role in protein anabolism, \( \text{L-Arginine} \) is involved in various metabolic pathways, such as the synthesis of creatine, \( \text{L-Ornithine} \), glutamate, and polyamines (1), and it is the substrate for synthesis of NO, one of the most potent endogenous vasodilators (2). NO is mainly released by the endothelium to regulate vascular tone and modulate the interaction of circulating blood cells with the vascular wall. The synthesis of NO by the endothelial NO synthase results in multiple vasoprotective effects that have been summarized as antiatherogenic (3).

Intracellular \( \text{L-Arginine} \) levels have been demonstrated to be considerably higher than those in the extracellular fluid or in plasma (3,4). However, plasma \( \text{L-Arginine} \) can be rapidly taken up by endothelial cells via the cellular \( \text{y+} \) transporter for cationic amino acids and can directly contribute to NO production (5), suggesting that plasma \( \text{L-Arginine} \) concentrations may influence endothelial NO production. Although a lack of \( \text{L-Arginine} \)-derived NO formation may result in endothelial dysfunction, a pathophysiologic finding that is common in patients with cardiovascular risk factors, reduced \( \text{L-Arginine} \) concentrations in plasma have rarely been described (6). Mouse and human...
studies that used $^{15}$N-labeled L-arginine as a precursor demonstrate that the major part of dietary L-arginine is metabolized in the liver and utilized in the hepatic urea cycle (7). Only a small portion of dietary L-arginine is converted to NO (8).

Additionally, besides its function as a substrate for NO synthase, L-arginine plays an important role in glucose metabolism. In adipocytes, L-arginine enhances glycogen synthesis in response to insulin, an effect that is independent of NO generation (9). Oral L-arginine supplementation during 6 mo in a referral sample has been reported to improve glucose tolerance and enhance insulin sensitivity (10).

One common cause of impaired L-arginine/NO metabolism is the presence of elevated levels of ADMA (11). ADMA displaces L-arginine from the substrate binding site of the NO synthase and thereby competitively inhibits this enzyme. ADMA is elevated in patients with renal failure and CVD and in advanced stages of diabetes mellitus (11–14).

In the present study, we determined reference limits for plasma L-arginine and the L-arginine:ADMA ratio, because these data may be of value for determining the nutritional state of participants at high cardiovascular risk due to their elevated ADMA levels. Such reference limits may also be useful for guiding nutraceutical interventions with L-arginine supplements. Accordingly, we measured plasma L-arginine and plasma ADMA concentrations in the Framingham Offspring Cohort and subsequently calculated the L-arginine:ADMA ratio. Plasma L-arginine and ADMA measurements were performed with a LC-MS/MS method that is characterized by high accuracy and precision (15,16). Plasma ADMA reference levels from this cohort have been reported elsewhere (17).

**Methods**

**Study sample.** The design and sampling strategy of the Framingham Offspring Study were previously described (18). In brief, in 1971 the Framingham Offspring Study began with the enrolment of 5124 participants who were the children of the original cohort or the spouses of these children. Of 3332 participants who attended the 6th examination cycle (1995 through 1998), we excluded participants with a serum creatinine level $>1.8 \times 10^{-4}$ mol/L, those with missing L-arginine, and attendees with missing covariate data. From this sample consisting of 3320 participants, smokers and individuals with CVD, obesity, hypertension, and diabetes were excluded in a hierarchical fashion (Supplemental Fig. 1). After the exclusions, 1165 participants comprised the reference sample for the present investigation. To create reference limits, outliers [defined as values $<Q1-1.5 \times$ IQR or $>Q3+1.5 \times$ IQR according to Solberg and Lahti (19)] were removed so that the final reference sample size comprised 1141 participants for analysis of plasma L-arginine and 1138 for analysis of the L-arginine:ADMA ratio, respectively. Hypertension was defined as increased BP (SBP $>140$ mm Hg or DBP $>90$ mm Hg) or use of antihypertensive medication. All participants provided written informed consent and the study protocol was approved by the Institutional Review Board of the Boston University Medical Center and by the Ethics Committee at the Hamburg Board of Physicians.

**Blood sample collection and analysis.** Laboratory assessment of several biomarkers was conducted on samples from fasting participants drawn at the 6th examination cycle; plasma/serum samples used for the present investigation were stored for $\sim8$ y at $-80^\circ$C without freeze-thaw cycles.

Phlebotomy was performed (typically between 0800 and 0900 h) on fasting participants. All participants were supine for $\sim5–10$ min. The blood was immediately centrifuged and plasma/serum was separated and stored at $-80^\circ$C until analysis. Serum high-sensitivity CRP was measured with an immunoprecipitation assay (20). Plasma homocysteine was analyzed by using HPLC with fluorometric detection (21). Serum blood lipids and creatinine were analyzed in the Framingham Heart Study laboratory with automated enzymatic assays (22).

Mass spectrometric determination of plasma L-arginine and ADMA was performed by using a fully validated, high throughput LC-MS/MS assay. The method details and stability of L-arginine and ADMA were previously described (15,16,23). In brief, plasma samples were analyzed using 96-well, 0.2-μm microfiltration plates precoated with 40 pmol of [UH]$^4$H$_4$-ADMA and 800 pmol of [UH]$^4$H$_4$-L-arginine (internal standards). After conversion to their butyl ester derivatives, analytes were analyzed on a Varian 1200L Triple Quadrupole MS (Varian) in the positive electrospray ionization mode. The sample run time was 1.6 min, with an intra- and inter-assay precision of 2.2 and 4.8% for L-arginine and 3.2% and 4.4% for ADMA, respectively.

**Statistical analysis.** The SAS statistical software (SAS Institute) was used for statistical analyses. A 2-sided $P$ value of $<0.05$ was considered significant. Data in the text are mean $\pm$ SD unless otherwise indicated. Associations between variables were assessed by Pearson correlation coefficients.

The calculation of the reference limits was done according to the recommendations of the International Federation of Clinical Chemistry as described elsewhere (24). Plasma L-arginine and ADMA levels were normally distributed in our sample and we analyzed these values as continuous dependent variables (without transformation). We constructed multivariable linear regression models with stepwise forward selection (significance criterion for entry of independent variables into the model, $P < 0.1$) using the following eligible covariates: age, sex, BMI, SBP, DBP total-HDL cholesterol ratio, TG, eGFR, homocysteine, alcohol consumption, glucose, and (log)CRP. To calculate the eGFR, we used the Modification of Diet in Renal Disease equation \[186.3 \times (\text{serum creatinine})^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ for women})\] (25).

**Results**

At baseline, the plasma L-arginine concentrations in the reference sample used for L-arginine analysis were 77.4 ± 18.2 μmol/L and the plasma ADMA concentrations were 0.53 ± 0.12 μmol/L (Table 1). The L-arginine:ADMA ratio in the reference sample was 150.3 ± 38. The reference limits (2.5th and 97.5th percentiles) for plasma L-arginine were 41.0 μmol/L (95% CI = 39.5–42.5 μmol/L) and 114 μmol/L (95% CI = 112–115 μmol/L) (Fig. 1A), whereas corresponding reference limits (2.5th and 97.5th percentiles) for the L-arginine:ADMA ratio were 74.3 μmol/L (95% CI = 71.1–77.3 μmol/L) and 225 μmol/L (95% CI = 222–228 μmol/L) (Fig. 1B).

The sex-specific 2.5th and 97.5th percentile reference limits for L-arginine were 42.0 μmol/L (95% CI = 39.5–44.2 μmol/L) and 113 μmol/L (95% CI = 111–116 μmol/L) in men and 40.8 μmol/L (95% CI = 38.8–42.6 μmol/L) and 113 μmol/L (95% CI = 111–115 μmol/L) in women. The sex-specific 2.5th and 97.5th percentile reference limits for the L-arginine:ADMA ratio were 72.0 (95% CI = 66.6–76.7 μmol/L) and 228 μmol/L (95% CI = 224–234 μmol/L) in men and 75.6 μmol/L (95% CI = 71.5–79.4 μmol/L) and 224 μmol/L (95% CI = 220–228 μmol/L) in women.

ADMA plasma concentrations were positively correlated with L-arginine (age- and sex-adjusted $r = 0.31; P < 0.0001$). A correlation between L-arginine and blood glucose was observed in the reference sample (unadjusted Pearson correlation $r = 0.09; P = 0.002$). Also, a correlation between L-arginine and eGFR

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11 Abbreviations used: ADMA, asymmetric dimethylarginine; BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; DDAH, dimethylarginine dimethylaminohydrolase; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.
The amino acid L-arginine has gained major scientific interest in recent years due to its multi-faceted roles in metabolism, among which the most prominent is its role as a precursor of NO synthesis. In this respect, not only L-arginine plasma concentrations per se but also its levels relative to its competitive antagonist, ADMA, are important; thus, the L-arginine:ADMA ratio has been inversely associated with homocysteine and logCRP (Table 3).}

### Discussion

The amino acid L-arginine has gained major scientific interest in recent years due to its multi-faceted roles in metabolism, among which the most prominent is its role as a precursor of NO synthesis. In this respect, not only L-arginine plasma concentrations per se but also its levels relative to its competitive antagonist, ADMA, are important; thus, the L-arginine:ADMA ratio has been inversely associated with homocysteine and logCRP (Table 3).

In most clinical conditions associated with endothelial dysfunction, circulating L-arginine concentrations have been observed to be within the usual normal range. Yet, it is intriguing that short-term dietary supplementation with oral L-arginine restores vascular function and improves clinical symptoms of diseases associated with vascular dysfunction [examples include angina pectoris (27), hypertension (28) erectile dysfunction (29,30), congestive heart failure (31), and pulmonary arterial hypertension (32)]. Many of these conditions are associated with elevated circulating ADMA levels and also a lower L-arginine ADMA ratio (28,33). Thus, an imbalance between L-arginine and ADMA can result in relative L-arginine deficiency. Our data therefore extend previous data on circulating L-arginine reference levels (34), because we also report reference intervals for the L-arginine:ADMA ratio in a healthy, community-based sample. Our data may help to better understand the roles of L-arginine and ADMA in the pathophysiology of various cardiovascular and metabolic conditions and also facilitate the interpretation of some of the inconsistent results of recent studies evaluating dietary supplementation with L-arginine.

Our reference sample was chosen to be free of CVD, hypertension, diabetes, obesity, and smoking, and participants with higher serum creatinine concentrations were also excluded. Therefore, we were unable to assess the influence of variables such as BP, serum lipids, BMI, and renal function on plasma L-arginine levels across their broad range of values.

We observed a mean decrease in the L-arginine plasma concentration of 0.05 μmol/L per 1 mL/min decline of eGFR. This may not necessarily point toward an association of L-arginine plasma concentration with glomerular filtration per se, because it is well known from experimental studies that the kidney releases more L-arginine into plasma than it extracts (35,36), suggesting that renal L-arginine synthesis outweighs L-arginine consumption. Thus, renal diseases leading to reductions

### Table 1: Clinical characteristics of the reference samples used for calculating the reference levels of L-arginine and L-arginine:ADMA ratio in the Framingham Offspring Cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference sample for L-arginine</th>
<th>Reference sample for L-arginine:ADMA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56 ± 9</td>
<td>56 ± 9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.1 ± 2.7</td>
<td>25.1 ± 2.7</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>118 ± 12</td>
<td>118 ± 12</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>72 ± 8</td>
<td>72 ± 8</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.2 ± 0.4</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.3 ± 0.9</td>
<td>3.3 ± 0.9</td>
</tr>
<tr>
<td>Total-HDL cholesterol</td>
<td>3.92 ± 1.29</td>
<td>3.93 ± 1.30</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.3 ± 0.7</td>
<td>1.3 ± 0.7</td>
</tr>
<tr>
<td>eGFR, mL/min</td>
<td>88.3 ± 21.4</td>
<td>88.6 ± 25.4</td>
</tr>
<tr>
<td>High sensitive CRP, mg/L</td>
<td>1.20 (0.59–2.79)</td>
<td>1.20 (0.60–2.80)</td>
</tr>
<tr>
<td>Total homocysteine, μmol/L</td>
<td>8.96 ± 3.05</td>
<td>8.98 ± 3.06</td>
</tr>
</tbody>
</table>

1 Values are means ± SD or median (Q1–Q3). All analytes were measured in serum. ADMA, asymmetric dimethylarginine; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.

### Table 2: Multivariable regression analysis of plasma L-arginine concentrations in 1141 healthy individuals from the Framingham Offspring Cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>β ± SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular filtration rate</td>
<td>0.05 ± 0.03</td>
<td>0.044</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.20 ± 0.06</td>
<td>0.002</td>
</tr>
</tbody>
</table>

1 Candidate variables that were considered for model entry were age, sex, BMI, SBP, DBP, the total-HDL cholesterol ratio, alcohol consumption, TG, glomerular filtration rate, homocysteine, fasting glucose, and CRP. CRP, C-reactive protein; DBP, diastolic blood pressure; SBP, systolic blood pressure.

2 All regression coefficients (β estimates) represent the estimated mean change in L-arginine (μmol/L) per 1-unit increase in the corresponding covariate.
arginine methylation, which might also contribute to this inverse relationship between homocysteine and ADMA pathways at the level of protein synthesis (40).

In conclusion, we report reference ranges for both plasma L-arginine and the L-arginine:ADMA ratio, which may reflect NO synthase substrate availability more closely than plasma L-arginine concentrations alone. These reference limits may facilitate the design of better clinical trials of L-arginine supplementation in those with relative deficiency of this amino acid as reflected by the L-arginine:ADMA ratio.

Literature Cited


