Dietary Animal-derived L-Arginine Intakes and Risk of Chronic Kidney Disease
A 6-year Follow-up of Tehran Lipid and Glucose Study

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Introduction. There is inconsistent evidence regarding the potential role of L-arginine intake on kidney function. This study investigated the association of dietary L-arginine intake and the risk of chronic kidney disease (CKD) in adults.

Materials and Methods. We evaluated 1780 men and women participated in the Tehran Lipid and Glucose Study, followed for a median of 6.3 years. Dietary intakes of total L-arginine as well as animal- and plant-derived L-arginine were assessed using the validated semi-quantitative food frequency questionnaire, at baseline. Demographics, anthropometrics, and biochemical variables were evaluated at baseline and again after a 3-year and a 6-year follow-up. The incidence of CKD was assessed across tertiles of L-arginine and its categories using multivariable logistic regression models.

Results. The mean dietary intakes of total, plant-derived, and animal-derived L-arginine were 4.1 ± 1.5 g/d, 1.8 ± 0.9 g/d, and 2.1 ± 0.8 g/d, respectively. In the fully-adjusted logistic regression model, the highest compared to the lowest intakes of animal-derived L-arginine (2.57 g/d versus 1.05 g/d) increased the risk of CKD (relative risk, 1.54; 95% confidence interval, 1.06 to 2.14, \( P = .02 \) for trend). Animal-derived L-arginine was negatively associated with changes of estimated glomerular filtration rate and creatinine clearance rate during the follow-up. There was no significant association between total or plant-derived L-arginine intakes and the risk of CKD after 6.3 years of follow-up.

Conclusions. Our findings suggested an adverse effect of higher intakes of L-arginine from animal sources that could be a dietary risk factor for development of kidney disease.

INTRODUCTION

L-arginine, a main substrate for nitric oxide (NO) production, is involved in many physiological processes related to metabolic disorders and cardiovascular disorders. 1-5 L-arginine is synthesized endogenously in the kidney using L-citrulline. 6 A dual role of L-arginine on kidney has been reported in previous animal studies; it has been indicated that L-arginine could increase glomerular filtration rate (GFR) due to its potential to production of agmatine and stimulation of glucagon release. 7-11 Conversely, L-arginine may induce adverse effects on kidney function due to enhanced inducible NO synthase activity, overproduction of NO, generation of polyamine and proline, and consequently, promotion of proliferation and collagen formation. 12-14

Short-term beneficial properties of L-arginine administration have been reported in some
pathologic conditions including hypertension, hypertensive kidney disease, and cardiovascular disease (CVD)
however, the role of L-arginine in the pathogenesis and treatment of kidney disease is not completely understood. L-arginine could modify animal models of ischemic acute kidney failure and chronic kidney disease (CKD); however, L-arginine had no effect on kidney disease in humans with chronic glomerular diseases.

Long-term effect of L-arginine intake from usual amount of diet is unclear. In our previous studies, we showed that dietary intakes of L-arginine was related to NO metabolism, cardiometabolic risk factors, and the incidence of coronary heart disease (CHD). We also reported that increased levels of NO production and consumption of nitrate-rich foods, as exogenous pressures of NO, may be a risk factor for development of CKD. To the best of our knowledge, the association of L-arginine intakes from usual diet and the risk of kidney dysfunction is currently an important gap of knowledge. Therefore, we aimed to evaluate the association of total dietary L-arginine intake with the incidence of CKD in a national representative population. Considering some evidence regarding different physiological function of animal- and plant-based L-arginine in the body, we also conducted a separate analysis for both animal- and plant-derived L-arginine.

MATERIALS AND METHODS

Study Population

This study was conducted within the framework of the Tehran Lipid and Glucose Study (TLGS), an ongoing community-based prospective study being conducted to investigate and prevent noncommunicable diseases, in a representative sample in the district 13 of Tehran, the capital city of Iran. For the current analysis, 2823 men and women, aged 20 years and greater with complete data (demographics, anthropometrics, biochemical, and dietary data), participated in the 3rd TLGS examination (2006-2008), were recruited. After exclusion of 261 participants with under- or over-reported energy intakes (< 800 kcal/d or ≥ 4200 kcal/d) or specific diets (including dietary recommendations for hypertension, hyperlipidemia, or diabetes mellitus) and participants with prevalent CKD at baseline (n = 487), the remaining non-CKD participants were followed up to the 4th (2009-2011) and 5th (2012-2014) TLGS examinations.

Participants who had left the study before follow-up examinations without a diagnosis of CKD (n = 295) were also excluded and final analyses was conducted on 1780 adults (727 men and 1053 women; Figure).

Written informed consents were obtained from all participants, and the study protocol was approved by the ethics research council of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences.

Demographic, Anthropometric, and Clinical Measures

Trained interviewers collected demographic data using pretested questionnaires. Weight was measured to the nearest 100 g using digital scales, while the participants were minimally clothed, without shoes. Height was measured to the nearest 0.5 cm, in the standing position without shoes, using a tape meter. Body mass index was calculated as weight divided by square of the height (kg/m²). For blood pressure measurements, after a 15-minute rest in the sitting position, 2 measurements were taken, on the right arm, using a standardized mercury sphygmomanometer; the mean of the two measurements was considered as the participant’s blood pressure. Smoking status was obtained using face-to-face interviews; participants who smoked daily or occasionally were considered current smokers. Information on medication usage for treatment of diabetes mellitus, hypertension, and lipid disorders was collected.

Recruitment and follow-up flowchart of study.
Biochemical Measures

Fasting blood samples were taken after 12 to 14 hours, from all study participants at baseline and follow-up phases. Serum creatinine levels were assayed using a kinetic colorimetric Jaffe method. Fasting serum glucose was measured by the enzymatic colorimetric method using glucose oxidase. High-density lipoprotein cholesterol was measured after precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstic acid. Analyses were performed using Pars Azmoon kits (Pars Azmoon Inc, Tehran, Iran) and a Selectra 2 auto-analyzer (Vital Scientific, Spankeren, Netherlands). Both inter- and intra-assay coefficients of variation of all assays were < 5%. Serum NO metabolite concentration was measured by the Griess reaction which has been developed and validated in our laboratory. Inter- and intra-assay coefficients of variations of the assays were 5.2% and 4.4%, respectively.

Dietary Assessment

A validated 168-item food frequency questionnaire was used to assess typical food intake, and total L-arginine intake as well as L-arginine from animal and plant sources, at baseline. L-arginine content of food items (mg/100 g of foods), were multiplied by the amount of daily intake of food items; dietary total intake of the participants was estimated by summing up of the obtained values. To stratify dietary L-arginine sources, the amount of L-arginine intake was separately calculated by summing up of dietary L-arginine intake from plant sources (fruits, vegetables, nuts, legumes, and grains) and animal sources (meats, processed meats, dairy, and eggs).

Trained dietitians with at least 5 years of experience in the TLGS survey asked participants to designate their intake frequency for each food item consumed during the past year on a daily, weekly, or monthly basis. Portion sizes of consumed foods reported in household measures were then converted to grams. However, since Iranian Food Composition Table is incomplete, and has limited data on the nutrient content of raw foods and beverages, to analyze foods and beverages for their energy and nutrient content, we used the US Department of Agriculture Food Composition Table.

Definition of Terms

Chronic kidney disease was defined as an estimated GFR less than 60 mL/min/1.73 m². To calculate estimated GFR, the Chronic Kidney Disease-Epidemiology Collaboration creatinine equation was used as follows:

\[
GFR = 141 \times \min\left(\frac{\text{serum creatinine}}{\kappa}, 1\right) ^{\alpha} \times \max\left(\frac{\text{serum creatinine}}{\kappa}, 1\right) ^{-1.209} \times 0.993^{\text{age}} \times 1.018^{[\text{if female}]} \times 1.159^{[\text{if black}]}
\]

where \(\kappa\) is 0.7 and 0.9 for women and men, respectively; \(\alpha\) is -0.329 and -0.411 for men and women, respectively; \(\min\) indicates the minimum of serum creatinine divided by \(\kappa\) or 1, and \(\max\) indicates maximum of serum creatinine divided by \(\kappa\) or 1.

Creatinine clearance rate was estimated using the Cockcroft-Gault formula.

Diabetes mellitus was defined as a fasting serum glucose level of 126 mg/dL or greater, 2-hour serum glucose level of 200 mg/dL or greater, or use of antidiabetic medications. Cardiovascular disease was defined as any CHD or stroke. Coronary heart disease was defined as myocardial infarction, probable myocardial infarction, unstable angina pectoris, and angiographically proven CHD. Hypertension was considered as systolic blood pressure of 140 mm Hg or greater, diastolic blood pressure of 90 mm Hg or greater, or current use of antihypertensive medications. According to the World Health Organization classification, menopause was defined as the absence of spontaneous menstrual bleeding for more than 12 months for which no other pathologic or physiologic cause could be determined.

Statistical Analysis

Univariable analyses were performed on common risk factors of CKD or potential confounding variables, including age (< 56 versus ≥ 65 years), body mass index (< 25, 25 to 30, and ≥ 30 kg/m²), smoking, medications use, prevalent diabetes mellitus, history of CVD, hypertension, daily energy intake, dietary intake of fat, dietary intake of protein, and sodium-potassium ratio. Significant variables with \(P\) value less than .20 in the univariable analysis were selected for the final multivariable models.

We assessed dietary intakes of total L-arginine and its categories as both continuous and categorical variables in the models. In the categorical model, amount of L-arginine and its subgroups were
categorized into tertiles, given the 1st tertile as reference. In the continuous model, the risk of CKD was calculated for each 1 standard deviation increases in the intakes of L-arginine.

To assess the odds ratio (OR) and 95% confidence interval (CI) of CKD across tertiles of L-arginine intake, logistic regression models were used with adjustment for potential confounding variables. First, we obtained age- and sex-adjusted ORs, and then, we adjusted for body mass index, smoking, history of kidney disease, serum creatinine level, diabetes mellitus, hypertension, medications use, and history of CVD. Finally, we added energy intake, dietary intake of protein, total fat, and sodium-potassium ratio to the logistic regression model to examine whether the relationship was mediated by these confounding variables. In all multivariable models, the first tertile was considered as a reference. Since, the OR, which was estimated from the logistic regression models, may not be a valid estimation in binary outcome variables with a high prevalence (> 10%) in cohort studies, the adjusted ORs were corrected by the formula suggested by Zhang and Yu in this method, adjusted OR, obtained from logistic regression in a cohort study, is corrected by the following formula and represents the true relative risk (RR):

$$RR = OR / [(1-P_0) + (P_0 \times OR)]$$

where $P_0$ indicates the incidence of the outcome in the unexposed group.

To assess the overall trends of the ORs across quartile categories, the median of each tertile was used as a continuous variable in logistic regression models.

The association of L-arginine intakes and its categories with changes of creatinine clearance rate, estimated GFR, and blood pressure during the study follow-up was also assessed using linear regression analysis, with adjustment for all potential confounding factors.

All statistical analysis were conducted using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA), and $P$ values less than .05 were considered significant.

**RESULTS**

The mean age of the participants was $33.8 \pm 15.3$ years at baseline and 40.8% were men. The mean dietary intakes of total, plant-derived, and animal-derived L-arginine were $4.1 \pm 1.5$ g/d, $1.8 \pm 0.9$ g/d, and $2.3 \pm 0.8$ g/d, respectively. During the average of 6.3 years of follow-up, 318 participants (17.9%) experienced CKD.

Participants with CKD were more likely to be women, had a lower rate of smoking, and higher prevalence of kidney disease history at baseline ($P < .05$ for all); moreover, a higher rate of overweight (47.4% versus 44.0%) and obesity (32.4% versus 22.9%) was observed in CKD compared to non-CKD participants. Serum nitric oxide levels was significantly higher at baseline in CKD patients (34.1 μmol/L versus 30.6 μmol/L, in CKD compared to non-CKD, $P = .009$); serum nitric oxide-creatinine ratio was also higher in CKD compared to non-CKD participants at baseline (0.38 versus 0.34, $P = .004$). A lower rate of estimated GFR (68.9 ml/min/1.73 m² versus 80.7 ml/min/1.73 m², $P = .001$) as well as higher prevalence of CVD (14.5% versus 8.8%, $P = .002$) and hypertension (28.1% versus 10.0%, $P = .001$). There was no significant difference in total L-arginine intakes and its subclasses between the CKD and non-CKD participants (Table 1).

In Table 2, characteristics of the study population are compared across tertile categories of total L-arginine intakes. Serum nitric oxide levels ($33.3 \pm 26.6$ μmol/L versus $29.7 \pm 18.5$ μmol/L, in the last compared to the first tertile, $P = .01$) and nitric oxide-creatinine ratio ($0.37 \pm 0.29$ versus $0.32 \pm 0.21$, in the last compared to the first tertile, $P = .005$) were increased across increasing dietary intakes of L-arginine. There was no significant difference in estimated GFR and creatinine clearance rate across tertile categories of dietary intakes of L-arginine. Dietary intakes of total fat and sodium-potassium ratio decreased, whereas dietary intakes of protein and total fiber increased significantly across increasing L-arginine intakes.

Table 3 shows the association of L-arginine intakes and changes of blood pressures, estimated GFR and creatinine clearance during the study follow-up. Linear association of L-arginine intakes at baseline with changes of creatinine clearance rate, GFR, and blood pressure during the follow-up period showed a borderline negative association between plant-derived L-arginine and changes of both systolic and diastolic blood pressure ($β = -0.84; 95% CI, -2.17 to 0.01$ and $β = -0.78; 95% CI, -1.61 to 0.02$). Animal-derived L-arginine intakes were negatively related to 6 years’ changes of GFR ($β = -0.92; 95% CI, -2.34 to -0.05$) and creatinine clearance rate ($β = -0.59; 95% CI, -2.17 to 0.05$).
CI, -2.76 to -0.02). Total L-arginine intakes were not significantly related with changes of creatinine clearance rate, GFR, and blood pressure.

The risk of CKD across tertile categories of L-arginine intakes and its categories are shown in Table 4. There was no significant association
between intakes of total L-arginine and plant-derived L-arginine and the risk of CKD after 6.3 years of follow-up.

When animal-derived L-arginine intakes were considered as exposure in the fully-adjusted logistic regression model, participants in the highest compared to lowest tertile (2.57 g/d versus 1.05 g/d) had a significantly increased risk of CKD (RR, 1.54; 95% CI, 1.06 to 2.14). An increasing trend was also observed in the risk of CKD across tertiles of dietary intake of animal-derived L-arginine ($P = .02$).

Relative risk (95% CI) of CKD per 1 standard deviation increased intakes of total, animal-derived, and plant-derived L-arginine were 1.03 (0.67 to 1.57), 1.18 (0.89 to 1.57), and 0.81 (0.59 to 1.11), respectively, in the fully adjusted model.

**DISCUSSION**

Our results indicated that total and plant-derived L-arginine intakes had no significant effect on CKD incidence risk. In contrast, an increasing trend of CKD risk across increasing dietary intakes of animal-derived L-arginine, accompanied with a negative association with changes of creatinine clearance rate and estimated GFR during the follow-up, may reveal the fact that animal sources of L-arginine have adverse effects on kidney function. These findings raised the hypothesis that different sources of dietary L-arginine may have different effects on kidney function.

Similar findings were also observed in our previous study of dietary L-arginine and its sources and the risk of CHD; participants with higher intake of animal-derived L-arginine had a significantly increased risk of CHD events, whereas the risk of CHD had a decreasing trend across increasing plant-derived L-arginine intake ($P = .03$ for trend).18

It has been proposed that utilization of plant-derived L-arginine is better than animal-derived due to a higher ratio of lysine to L-arginine in

**Table 3. The Association of Dietary L-Arginine Intakes and Changes of Creatinine Clearance, Estimated Glomerular Filtration Rate, and Blood Pressures During the Study Follow-up**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total L-Arginine</th>
<th>Plant-derived L-Arginine</th>
<th>Animal-derived L-Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance rate</td>
<td>−0.75 (-1.60 to 0.08)</td>
<td>0.89 (-0.62 to 2.40)</td>
<td>−0.59 (-2.76 to -0.02)</td>
</tr>
<tr>
<td>Glomeration filtration rate</td>
<td>0.05 (-1.41 to 1.51)</td>
<td>0.82 (-0.25 to 1.89)</td>
<td>−0.92 (-2.34 to -0.05)</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>−0.33 (-1.40 to 0.73)</td>
<td>−0.84 (-2.17 to 0.01)</td>
<td>0.24 (-0.61 to 1.09)</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>−0.08 (-0.65 to 0.47)</td>
<td>−0.78 (-1.61 to 0.02)</td>
<td>0.51 (-1.07 to 2.11)</td>
</tr>
</tbody>
</table>

*Linear regression models were used with adjustment of age, sex, body mass index, smoking, serum creatinine, diabetes mellitus, hypertension, medications use, history of cardiovascular diseases, daily energy intake, and dietary intake of protein, total fat, and sodium-potassium ratio.

**Table 4. The Risk of Chronic Kidney Disease Across Tertile Categories of Dietary L-Arginine Intakes**

<table>
<thead>
<tr>
<th>Risk Factor and Model</th>
<th>Relative Risk (95% Confidence Interval) for Dietary L-Arginine Tertiles</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1 (n = 593)</td>
<td>Tertile 2 (n = 594)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total L-arginine</td>
<td>Model 1 Reference</td>
<td>1.17 (0.87 to 1.52)</td>
</tr>
<tr>
<td></td>
<td>Model 2 Reference</td>
<td>1.16 (0.81 to 1.62)</td>
</tr>
<tr>
<td></td>
<td>Model 3 Reference</td>
<td>1.22 (0.81 to 1.76)</td>
</tr>
<tr>
<td>Plant-derived L-arginine</td>
<td>Model 1 Reference</td>
<td>1.08 (0.80 to 1.44)</td>
</tr>
<tr>
<td></td>
<td>Model 2 Reference</td>
<td>1.30 (0.89 to 1.82)</td>
</tr>
<tr>
<td></td>
<td>Model 3 Reference</td>
<td>1.28 (0.88 to 1.81)</td>
</tr>
<tr>
<td>Animal-derived L-arginine</td>
<td>Model 1 Reference</td>
<td>1.12 (0.82 to 1.49)</td>
</tr>
<tr>
<td></td>
<td>Model 2 Reference</td>
<td>1.25 (0.89 to 1.71)</td>
</tr>
<tr>
<td></td>
<td>Model 3 Reference</td>
<td>1.35 (0.91 to 1.92)</td>
</tr>
</tbody>
</table>

*Logistic regression models were used. Model 1 was adjusted for sex and age; model 2, for additional adjustment for body mass index, smoking, serum creatinine, diabetes mellitus, hypertension, medications use, and history of cardiovascular diseases; and model 3, additionally for daily energy intake, dietary intake of protein, total fat, and sodium-potassium ratio.

A linear trend test was performed by considering each ordinal score variable as a continuous variable in the model.

The median values were 2.71 g/d, 3.83 g/d, and 5.42 g/d for dietary total L-arginine, 1.33 g/d, 1.97 g/d, and 2.92 g/d for dietary plant-based L-arginine, and 1.05 g/d, 1.66 g/d, and 2.57 g/d for dietary animal-based L-arginine, in the first, second, and third tertile category, respectively.
animal proteins; lysine can compete with arginine for intracellular transport, and higher ratio of lysine to L-arginine may indirectly affect arginine metabolism; accordingly, it seems that animal and plant sources of L-arginine induce different physiological consequence in the body.

Studies showed that L-arginine intake could modulate arginine bioavailability and promote nitric oxide synthesis. The physiological roles of nitric oxide in regulation of both glomerular blood pressure and GFR as well as kidney hemodynamics and growth have been identified; however, there are limited knowledge regarding the association of nitric oxide and kidney disease in human. In our study, we observed higher baseline serum nitric oxide level and creatinine-nitric oxide ratio, an indicator of the endogenous nitric oxide production, in CKD patients compared to the rest of the cohort. In our previous study, we found serum nitric oxide level to be an independent predictor of CKD. L-Arginine supplementation could enhance inducible nitric oxide synthase-dependent tissue fibrosis and injury by providing increased substrate. On the other hand, it has been reported that L-arginine administration had pro-inflammatory effects in models of kidney injury characterized by leukocyte influx.

The main strength of the current study was its population-based and prospective design. Another strength was the use of a validated food frequency questionnaire to assess dietary intakes of the participants that was completed by trained dietitians. Lack of data on serum levels of L-arginine may be considered as an important limitation of this study; however, an acceptable correlation has been reported between dietary L-arginine intakes and serum L-arginine, in previous studies. We also were unable to measure both endothelial nitric oxide synthase and inducible nitric oxide synthase activity to clarify sources of nitric oxide overproduction in CKD patients and compare the level of L-arginine-dependent nitric oxide production in CKD and non-CKD individuals.

Moreover, some inherent limitations of observational studies including selection bias, information bias in measuring exposure or outcome, and nondifferential misclassification should be considered in interpretation of the findings.

CONCLUSIONS
Our findings suggested an adverse effect of animal-based L-arginine on kidney function. Further clinical research on chronic outcomes of L-arginine supplementation from different dietary sources are required to confirm these findings.

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CONFLICT OF INTEREST
None Declared.

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