Arginine intake, blood pressure, and the incidence of acute coronary events in men: the Kuopio Ischaemic Heart Disease Risk Factor Study1, 2

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ABSTRACT

Background: Evidence suggests that dietary supplementation of L-arginine, the precursor of nitric oxide, may protect arteries against atherosclerosis.

Objective: We tested the hypothesis that dietary arginine intake is associated with a decreased risk of acute coronary events in Finnish men aged 42–60 y.

Design: We investigated this association in a prospective cohort study of men who were free of prior coronary artery disease and who were examined in 1984–1989 in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD). The dietary arginine intake of 1981 men was assessed by a 4-d food intake record during the baseline phase of the KIHD.

Results: Men in the highest quintile of dietary arginine intake (≥5691 mg/d) did not have a significantly lower risk of acute coronary events than did men in the 4 lower quintiles (relative risk after adjustment for potential coronary risk factors: 1.28; 95% CI: 0.85, 1.94). The covariates were age; examination years; body mass index; systolic blood pressure; serum total, HDL, and LDL cholesterol; serum triacylglycerols; urinary excretion of nicotine metabolites; maximal oxygen uptake in an exercise test; and alcohol intake. Splitting arginine intake into deciles or analyzing plant- and animal-derived arginine separately did not show any association between dietary arginine intake and the risk of acute coronary events. Arginine intake was also not consistently associated with blood pressure.

Conclusion: Dietary arginine intake is not associated with the risk of acute coronary events in middle-aged men in eastern Finland.


KEY WORDS Acute coronary events, arginine intake, nitric oxide, myocardial infarction, population studies, prospective studies, L-arginine, Kuopio Ischaemic Heart Disease Risk Factor Study, Finnish men

INTRODUCTION

Endothelial dysfunction is a well-known feature of atherosclerotic arteries (1, 2). It is related to a decreased biological activity of nitric oxide (NO), which is produced by the endothelium from the amino acid precursor L-arginine (3, 4). NO has been shown to be involved in vasorelaxation and inhibition of platelet aggregation (3, 5), leukocyte adhesion (5), and smooth muscle cell proliferation (5) in the vessel wall. Both animal and human studies suggest that acute infusion or chronic oral administration of L-arginine improves peripheral endothelium-dependent vasodilation, inhibits platelet aggregation, and may reduce atherosclerosis (6–10). Theoretically, dietary arginine could be involved in coronary artery disease (CAD) mediated by the effects of NO. The human body apparently utilizes plant-derived arginine better than it does animal-derived arginine (11, 12). This is thought to occur because animal proteins have a higher ratio of lysine to arginine than do vegetable proteins (13) and because lysine competes with arginine for the same plasma membrane transport mechanism.

We found only one previous study concerning dietary arginine intake and CAD risk (14). In the present study we tested the hypothesis that total and plant-derived dietary arginine intake is associated with a decreased risk of acute coronary events in middle-aged Finnish men free of prior CAD.

SUBJECTS AND METHODS

Study population

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) is an ongoing population-based study of risk factors for CAD, atherosclerosis, and related outcomes (15). The study protocol was approved by the Research Ethics Committee of the University of Kuopio. All study subjects gave their written, informed consent. The study population is a random sample of men living in the city of Kuopio or neighboring rural communities, stratified and balanced in 4 strata by age at the baseline examination: 42, 48, 54, or 60 y. The baseline examinations were carried out between 1984 and 1989. Of 3235 eligible men, 2682 (82.9%) participated. Men with prevalent CAD at baseline (n = 677) were
excluded from the present analyses. Prevalent CAD was defined as having a history of myocardial infarction or angina pectoris, having angina pectoris on effort, or taking nitroglycerin tablets ≥1 time/wk (16). Of the remaining 2005 men, food intake data were available for 1981 men.

Ascertainment of follow-up events

Acute coronary events that occurred between 1984 and 1992 were registered as part of the multinational MONICA (MONItoring of Trends and Determinants in CArdiovascular Disease) project (17). Data on coronary events between 1993 and 1997 were obtained by record linkage from the national computerized hospital discharge registry. Diagnostic classification identified with that of the MONICA project was used. The cohort was followed on average for 10 y, which corresponded to ≈27 000 person-years. In the present study, 199 men had their first acute coronary event by the end of 1997. According to the diagnostic classification of the events, there were 104 definite and 61 possible acute myocardial infarctions and 34 typical prolonged chest pain episodes.

Measurement of nutrient intake

The consumption of foods was assessed at the time of blood sampling during the baseline phase of the KIHD study. Subjects were instructed on how to use household measures to record their food intake during 4 d. A nutritionist gave the instructions and checked the completed food intake records. The intake of nutrients, including amino acids, was estimated with the use of NUTRICA software (version 2.5; National Public Health Institute, Turku, Finland). Data on the amount of amino acids in Finnish food items was obtained from the Fineli food composition data bank from the National Public Health Institute of Finland. We analyzed the arginine content of the 308 foodstuffs that contain the most arginine and that are the most commonly consumed by Finnish men. On the basis of these values, the arginine content of mixed dishes was calculated. The major arginine sources were calculated on the basis of randomly selected food intake records from 10 men who had an acute coronary event and from 50 who did not. All nutrients were energy adjusted by using the residual method (18). The residuals were standardized to the mean nutrient intake of a study subject consuming 10 MJ/d, the approximate average total energy intake in this study population.

We also validated food intake records with the use of serum arginine measurements in another study of a subset of 45 middle-aged Finnish men from the Antioxidant Supplementation in Atherosclerosis Prevention study (19). In this study the mean (±SD) serum concentration of arginine was 130 ± 27 μmol/L, and the mean (±SD) intake of total energy-adjusted arginine (measured by a 4-d food intake record) was 3750 ± 700 mg/d. We found a significant correlation between dietary and serum arginine (r = 0.31, P = 0.039) measured at baseline.

Amino acid analysis

Arginine concentrations were analyzed after deproteinizing plasma samples with 5% (by vol) sulfosalicylic acid. One part 5% sulfosalicylic acid containing norleucine as an internal standard was added to 2 parts plasma, and the plasma was mixed thoroughly and kept for 1 h at 4°C. The samples were centrifuged at 12 000 × g for 15 min at 4°C, and the supernatant fluid was used for analysis. Arginine concentrations were analyzed with the use of a Biochrom 20 amino acid analyzer (Pharmacia LKB Biochrom Ltd, Cambridge, United Kingdom), which is based on continuous flow ion exchange chromatography with ninhydrin detection of separate amino acids and lithium as the exchanging ion.

Assessment of covariates

Assessment of demographic variables, medical history, use of medications, and blood pressure was carried out as described previously (16). The collection of blood specimens (16) and the measurement of serum lipids (20), lipoproteins (21), maximal oxygen uptake in an exercise test (22), 24-h urinary excretion of nicotine metabolites (23), and sodium excretion (24) were described previously.

Statistical analysis

The means of the baseline characteristics of the men who had an acute coronary event and of those who did not were compared with the use of analysis of variance. One-way analysis of variance was used to test the significance of trends across quintiles of total arginine intake. Subjects were classified into quintiles according to their mean total, plant-derived, and animal-derived arginine intake. The Cox proportional hazards model was used to analyze the relations between total, plant-derived, and animal-derived arginine intake and the risk of acute coronary events. The CIs were estimated by assuming asymptotic normality of the estimates. Correlations were computed as Pearson’s coefficients. All tests of significance were two-tailed. Statistical computations were performed with SPSS for WINDOWS (version 10.0; SPSS Inc, Chicago).

RESULTS

The dietary sources of arginine are shown in Table 1. We calculated the percentages of food groups contributing to arginine intake, and the major sources for the total arginine intake were meat and meat products (38%), cereal (24%), milk and milk products excluding cheese (23%), cheese (6%), and potatoes (4%).

During the average of 10 y of follow-up, 199 men experienced an acute coronary event. The distributions of the major known CAD risk factors for the men who had an acute coronary event

<table>
<thead>
<tr>
<th>Source</th>
<th>Arginine intake</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Animal derived</td>
<td>Plant derived</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Meat and meat products</td>
<td>38</td>
<td>57</td>
<td>72</td>
</tr>
<tr>
<td>Cereal</td>
<td>24</td>
<td>72</td>
<td>28</td>
</tr>
<tr>
<td>Milk and milk products</td>
<td>23</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>Cheese</td>
<td>6</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>Potatoes</td>
<td>4</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>Nuts and pulses</td>
<td>2</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>Vegetables and roots</td>
<td>2</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>Fruits and berries</td>
<td>1</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
and for those who did not are shown in Table 2. Risk factors for which there were significant differences between the men who had an acute coronary event and those who did not were age; serum total, HDL, and LDL cholesterol; serum triacylglycerols; urinary excretion of nicotine metabolites (an indicator of the amount of smoking); maximal oxygen uptake in an exercise test; body mass index; and systolic blood pressure.

The mean total arginine intake of the men who had an acute coronary event was 5061 mg/d, ranging from 1646 to 8424 mg/d. Of the mean total arginine intake, 29% was derived from plants and 71% was of animal origin. The mean total arginine intake of the men who did not experience an acute coronary event was 4983 mg/d, ranging from 1646 to 8424 mg/d. Of the mean total arginine intake, 29% was derived from plants and 71% was of animal origin. The mean total arginine intake of the men who had an acute coronary event was 5061 mg/d, ranging from 1646 to 8424 mg/d. Of the mean total arginine intake, 29% was derived from plants and 71% was of animal origin.

We divided the participants into quintiles according to the dietary intake of total, plant-derived, and animal-derived arginine. The characteristics of the study population by quintile of total dietary arginine intake are shown in Table 3. In the Cox proportional hazards model with adjustment for age and examination years, there were no associations between the dietary intake of total, plant-derived, or animal-derived arginine and the risk of coronary events (Table 4). The men in the highest quintile of total arginine intake (≥5691 mg/d) did not have a significantly lower risk of acute coronary events than did the men in the lowest quintile (<4230 mg/d) (relative risk: 1.33; 95% CI: 0.88, 1.99). Splitting arginine intakes into deciles or adjustment for any coronary risk factor (body mass index; systolic blood pressure; serum total, HDL, and LDL cholesterol; serum triacylglycerols; urinary excretion of nicotine metabolites; or maximal oxygen uptake in an exercise test) did not show an association between dietary arginine intake and the risk of acute coronary events (relative risk: 1.28; 95% CI: 0.85, 1.94). After additional adjustment for age, examination years, dietary intake of alcohol, saturated fatty acids, fiber, and vitamins C and E, the relative risk was still the same (ie, 1.28; 95% CI: 0.84, 1.94). We also checked the results by using only the data from the men with definite infarction, and the results did not change.

Pearson’s coefficient for correlation between plant-derived and animal-derived arginine was 0.24 (P < 0.001). In a linear regression model (adjusted for age; examination years; body mass index; serum total, LDL, and HDL concentration; serum triacylglycerols; urinary excretion of nicotine metabolites; and maximal oxygen uptake in an exercise test), the regression coefficients between systolic blood pressure and the intake of total, animal-derived, and plant-derived arginine were 0.01 (P = 0.674), < 0.01 (P = 0.931), and 0.02 (P = 0.420), respectively. The regression coefficients between diastolic blood pressure and the intake of total, animal-derived, and plant-derived arginine were −0.01 (P = 0.746), 0.01 (P = 0.831), and −0.03 (P = 0.195), respectively.

**DISCUSSION**

Our main finding was that arginine intake from a normal diet, as assessed by a 4-d food intake record, was not associated with the risk of acute coronary events in middle-aged Finnish men who were free of CAD. Arginine intake was also not consistently associated with blood pressure.
### TABLE 3
Characteristics of the study population by quintile of total dietary arginine intake

<table>
<thead>
<tr>
<th>Variable</th>
<th>Quintile of total dietary arginine intake $^1$</th>
<th>P for trend $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 ($\leq 4230$) (n = 396)</td>
<td>2 (4231–4700) (n = 396)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.6 ± 4.5</td>
<td>24.9 ± 6.6</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>3.9 ± 0.9</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>144 ± 30</td>
<td>144 ± 30</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>17.7 ± 4.0</td>
<td>17.5 ± 3.7</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>132 ± 27</td>
<td>134 ± 27</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>182 ± 26</td>
<td>183 ± 26</td>
</tr>
<tr>
<td>Total body mass index (kg/m²)</td>
<td>27 ± 8.1</td>
<td>27 ± 8.1</td>
</tr>
<tr>
<td>Dietary factor</td>
<td>1.0 0.63 (0.41, 1.05)</td>
<td>1.0 0.63 (0.41, 1.05)</td>
</tr>
<tr>
<td>Arginine (mg/dL) $^3$</td>
<td>8.4 ± 2.6</td>
<td>8.7 ± 2.3</td>
</tr>
<tr>
<td>Saturated fatty acids (% of total energy)</td>
<td>18.8 ± 4.5</td>
<td>18.3 ± 4.5</td>
</tr>
</tbody>
</table>

$^1$ Intake in mg/d is given in parentheses.
$^2$ $^{77}$ ± SD.
$^3$ One-way ANOVA.
$^4$ Adjusted for energy intake by using the residual method. The residuals were standardized to the mean nutrient intake of a study subject consuming 10 MJ/d, the approximate average total energy intake in this study population.

The mean arginine intake in our cohort was 4990 mg/d. The average intake of arginine from the US diet has been estimated to be similar (ie, 5397 mg/d; 11). In the above-mentioned Dutch study, the mean baseline arginine intake was 4350 mg/d (14). In our study, 29% and 71% of the mean arginine intake of 4990 mg/d was of plant and animal origin, respectively. The human body

### TABLE 4
Relative risk (95% CI) of acute coronary event by quintile of dietary arginine intake

<table>
<thead>
<tr>
<th>Quintile of dietary arginine intake</th>
<th>Adjustment for age and examination years</th>
<th>P for trend $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 ($\leq 4230$) (n = 396)</td>
<td>2 (4231–4700) (n = 396)</td>
</tr>
<tr>
<td></td>
<td>1.0 $^1$ 0.65 (0.41, 1.05)</td>
<td>1.0 1.03 (0.67, 1.58)</td>
</tr>
<tr>
<td></td>
<td>1.0 $^1$ 1.00 (0.63, 1.37)</td>
<td>1.0 1.00 (0.63, 1.37)</td>
</tr>
<tr>
<td></td>
<td>1.0 $^1$ 1.07 (0.68, 1.69)</td>
<td>1.0 1.07 (0.68, 1.69)</td>
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<tr>
<td></td>
<td>1.0 $^1$ 0.43 (0.19, 0.91)</td>
<td>1.0 0.43 (0.19, 0.91)</td>
</tr>
<tr>
<td></td>
<td>1.0 $^1$ 0.93 (0.59, 1.47)</td>
<td>1.0 0.93 (0.59, 1.47)</td>
</tr>
<tr>
<td></td>
<td>1.0 $^1$ 1.12 (0.71, 1.78)</td>
<td>1.0 1.12 (0.71, 1.78)</td>
</tr>
<tr>
<td></td>
<td>1.0 $^1$ 1.03 (0.67, 1.58)</td>
<td>1.0 1.03 (0.67, 1.58)</td>
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<tr>
<td></td>
<td>1.0 $^1$ 1.04 (0.65, 1.65)</td>
<td>1.0 1.04 (0.65, 1.65)</td>
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<tr>
<td></td>
<td>1.0 $^1$ 1.12 (0.67, 1.86)</td>
<td>1.0 1.12 (0.67, 1.86)</td>
</tr>
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</table>

$^1$ Comparison of the highest quintile with the lowest (Cox proportional hazards model).
$^2$ Over all 5 quintiles in Cox model.
$^3$ Total arginine.
$^4$ Plant-derived arginine.
$^5$ Animal-derived arginine.
$^6$ Adjusted for age, examination years, and coronary risk factors (BMI; systolic blood pressure; serum total, HDL, and LDL cholesterol; serum triacylglycerols; urinary excretion of nicotine metabolites; and maximal oxygen uptake in an exercise test).
$^7$ Adjusted for age, examination years, and potential confounders (alcohol, saturated fatty acids, energy-adjusted fiber, and vitamins C and E).
apparently utilizes plant-derived arginine better than it does animal-derived arginine because foods containing vegetable proteins have a higher ratio of arginine to lysine than do foods containing animal proteins (13) and because lysine competes with arginine for the same plasma transport mechanism (11, 12). Our results, however, do not support this theory, because neither the plant-derived nor the animal-derived arginine had any association with the risk of acute coronary events.

In our study dietary arginine intake was assessed with a 4-d food intake record. The number of days needed for a reliable estimation of protein intake is 5–7 (25) and is probably even longer for a reliable estimation of the intake of a single amino acid. It is important to realize that a method to measure dietary intake takes an extended period of time, because the diet of most subjects is complex and varies over time. Nevertheless, a sufficient sample size should reveal any important relation between a dietary factor and disease (26). Although 4 d may be too short a time to estimate the exact long-term arginine intake for each individual, we found a significant correlation between the total arginine intake assessed by a 4-d food intake record at the KIHD baseline and at the 11-y follow-up (r = 0.40, P < 0.001). We also found a significant correlation between dietary and serum arginine. Because 50–70% of dietary arginine is metabolized by the intestinal mucosa and does not enter the circulation (27), this correlation indicates good accuracy of the dietary arginine assessment.

The use of supplements in this study population is very low, and no commercial arginine supplements were available in Finland in the 1980s. Thus, our findings could not have been confounded by a possible use of dietary arginine supplements.

The endothelium is an important modulator of coronary vascular tone through the release of endothelium-derived relaxing factors and vasoconstrictors. Early coronary atherosclerosis is related to coronary endothelial dysfunction, which is characterized by an attenuated or absent endothelium-dependent vasodilatation (28). There is evidence that dietary supplementation of arginine improves endothelial function, probably by a number of mechanisms (10). L-Arginine is the substrate for nitric-oxide synthase (EC 1.14.13.39), the enzyme that catalyzes the vascular endothelial cells to produce NO (3). L-Arginine mainly has its effects through NO, but nitric-oxide synthase could also have other effects. Asymmetrical dimethylarginine, an endogenous inhibitor of nitric-oxide synthase, has been found to accumulate in the serum of cholesterol-fed rabbits and to reduce intracellular concentrations of L-arginine. Dietary supplementation with L-arginine overcomes the effect of asymmetrical dimethylarginine (10, 29). L-Arginine may also improve endothelial function indirectly through other vasoactive factors. For example, L-arginine has been shown to decrease the production of a vasoconstrictor, endothelin-1 (10). Understanding of the mechanisms remains incomplete.

There are also opposite findings concerning the effects of dietary arginine. Dietary L-arginine supplementation for 14 wk did not permanently increase plasma arginine concentrations in hypercholesterolemic rabbits (30). The investigators suggested that a homeostatic mechanism restores plasma arginine concentrations to control values. Supplementation of 21 g L-arginine/d for 3 d had no significant effect on arterial reactivity in healthy subjects with normal endothelium-dependent dilatation (31).

The NO system is important for vascular tone, which is essential for the regulation of blood pressure (5). Endothelium-dependent relaxation is impaired in patients with essential hypertension (32). Arginine treatment has been shown to prevent the increase in blood pressure in animals prone to hypertension (33), and arginine infusion rapidly reduces systolic and diastolic pressures in healthy humans and patients with essential hypertension (34). Yet, despite the potent action of NO in blood pressure regulation, reports estimating its possible role in hypertension have been highly conflicting (35). In our study there was no consistent, significant association between arginine intake and blood pressure. A possible reason for this negative finding is that the arginine intake of our study participants was too low and too homogeneous, as in the recent Dutch study (14). The dose of oral L-arginine in supplement trials has been much higher, usually 21 g/d. In addition, normal endothelium, which already secretes NO in the basal state, may not produce significantly more NO even if extra substrate is available (31). However, in more severe hypercholesterolemia, in which NO may be catabolized at an increased rate, supplementation of excess L-arginine may improve endothelium-dependent dilatation (31).

In conclusion, the present study indicates that dietary intake of arginine is not associated with the risk of acute coronary events or with blood pressure in middle-aged Finnish men free of CAD. Compared with the intakes of dietary L-arginine in supplementation studies, the intakes from normal food items in the present study may have been too low to improve endothelial function. This may be the main explanation for the negative results of the present study.

We thank our staff of almost 35 people for helping with data collection and Jaakko Tuomilehto and Kalevi Pyörälä for providing access to the FINMONICA registry data.

REFERENCES