Arginine Intake and Risk of Coronary Heart Disease Mortality in Elderly Men

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Abstract—From experimental studies, the hypothesis is derived that the amino acid arginine, the precursor of NO, could restore the impaired endothelial function and increased platelet activation observed in atherosclerosis. We investigated whether dietary intake of arginine is associated with reduced coronary heart disease risk in elderly persons. The study population consisted of 806 men aged 64 to 84 years at baseline who participated in the Zutphen Elderly Study, a population-based cohort followed up for 10 years. Information about habitual food consumption was collected by use of the cross-check dietary history method. Ninety (11.2%) of the 806 men died from coronary heart disease. Mean±SD baseline arginine intake was 4.35±1.07 g/d. Meat was the main source of arginine intake (37.1%), followed by bread (13.1%) and milk and milk products (12.1%). Arginine intake was not associated with coronary heart disease mortality. After adjustment for age, the relative risk (RR) for the medium tertile of arginine intake was 0.72 (95% CI 0.44 to 1.18), and the RR for the highest tertile was 0.71 (95% CI 0.43 to 1.19, *P* for trend=0.19) compared with the lowest tertile of arginine intake. After additional adjustment for history of coronary heart disease and diabetes mellitus, energy intake, body mass index, smoking habit, physical activity, and other relevant dietary and biological risk factors, the RR was 1.86 (95% CI 1.06 to 3.27) for the medium intake and 1.56 (95% CI 0.83 to 2.93) for the highest intake (*P* for trend=0.17). These results do not support the hypothesis that dietary arginine intake lowers the risk of coronary heart disease mortality. (*Arterioscler Thromb Vasc Biol*. 2000;20:2134-2139.)

Key Words: arginine ■ diet ■ coronary heart disease ■ elderly ■ epidemiology

The discovery of NO as a signaling molecule in the cardiovascular system was rewarded with the 1998 Nobel Prize in Medicine. The semiessential amino acid L-arginine is the precursor of NO. It has been demonstrated that administration of L-arginine can improve endothelium-dependent vascular relaxation through the release of NO.[1] Chronic dietary supplementation of L-arginine in hypercholesterolemic animals has been associated with reduced atherosclerosis and antiatherogenic effects, such as reduction in surface area and intimal thickness of atheromatous lesions, decrease of platelet aggregation, and attenuation of cell proliferation and of vascular monocyte accumulation.[2–8]

In humans, endothelial dysfunction could be restored after dietary supplementation[9–12] and intravenous administration of L-arginine[13–15] in patients with hypercholesterolemia,[9,13,14] heart failure,[12] and nonobstructive or advanced coronary artery disease.[10,11,15] Reduction in platelet aggregation by L-arginine has also been observed.[16–18]

Consequently, arginine was recently presented as a possible new pharmacological therapy for the atherogenic process leading to coronary heart disease.[19] Antiatherogenic effects of arginine are especially demonstrated in human subjects with more or less advanced atherosclerosis. Coronary atherosclerosis is increasingly present in the elderly. Possibly, arginine is associated with reduced coronary heart disease risk in elderly persons. Therefore, we investigated the hypothesis that dietary arginine intake is inversely associated with coronary heart disease mortality among participants in the Zutphen Elderly Study.

Methods

Study Population

The study population consisted of men who participated in the Zutphen Elderly Study, an extension of the Zutphen Study, the Dutch contribution to the Seven Countries Study.[20] In 1960, the Zutphen Study started with a cohort of 878 men from Zutphen (the Netherlands) born between 1900 and 1919. In 1985, 367 of the 555 participants who were still alive were reexamined. In addition, 711 other men from the town of Zutphen in the same age category were asked to participate. A total of 939 men (response rate 74%) were examined in 1985, and complete information on diet and risk factors was available for 806 men. In 1990, 781 of these 939 men were still alive, and 560 took part in a follow-up examination. Dietary intake in 1990, together with complete information on diet and risk factors at baseline (1985) was available for 508 men.

Data Collection

Dietary and medical examinations were completed between March and June 1985 and after 5 years of follow-up, between March and

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June 1990. Information about the habitual food consumption was collected by use of the cross-check dietary history method, adapted to the Dutch situation.[21] Participants, together with the person who prepared the meals, were interviewed about their usual food consumption pattern during weekdays and weekends for the 2 to 4 weeks preceding the interview. Quantities of food bought per week were used to calculate and verify the food consumption of a participant on an average weekday.

Nutrient intake data, including ethanol, were based on the Dutch food table.[22] Because of lack of information on the amino acid composition of Dutch foods, arginine contents of chemical analysis were used, preferably from the United Kingdom[23] and subsequently from the United States.[24] The arginine contents were converted by use of the protein contents from the Dutch food table.[22]

During medical examinations, nonfasting venous blood samples were taken. Serum total cholesterol and HDL cholesterol were determined enzymatically.[25,26] In 1995, serum total homocysteine was measured in serum stored at $-20°C$, as described previously.[27] Weight and height were measured while participants were wearing underwear only, and body mass index was calculated (kilograms per meter squared). At the end of the physical examinations, blood pressure was measured twice with participants in the supine position. Information on smoking status (nonsmoker, exsmoker, or current smoker), medication use, and history of hypertension and diabetes mellitus was obtained by a standardized questionnaire. Patients with insulin-dependent and non–insulin-dependent diabetes mellitus were considered in the present study. Minutes of physical activity (mainly walking, cycling, hobbies, sports, gardening, and work) per week were calculated by use of a self-administered questionnaire originally designed for retired men.[28] Information regarding a history of coronary heart disease was obtained by using the Dutch translation of the Rose questionnaire.[29]

Follow-Up

Information involving the vital status of all participants was obtained from the municipal registries until January 1995. Three participants were lost to follow-up. Information about the cause of death was obtained from the Dutch Central Bureau of Statistics for deaths occurring between baseline assessment and June 1990, after verification with hospital discharge data and information from the deceased's general practitioners, and from the hospital discharge data and/or participants' general practitioners for deaths occurring thereafter, and if such information was not available, from the Dutch Central Bureau of Statistics. Causes of death were coded according to the ninth revision of the International Classification of Diseases (ICD). Coronary heart disease refers to ICD codes 410 to 414. Because the underlying cause of death in the elderly is often difficult to determine, the primary (n=84) and the secondary (n=6) causes of death were both considered in the analysis.

Statistical Methods

Differences in baseline characteristics of the participants between tertiles of arginine intake were evaluated by ANOVA for normally distributed variables, by Kruskal-Wallis test for skewed variables, and by χ^2 test for categorical variables. Pearson correlation coefficients (r_p) were calculated between the intake of arginine and other dietary factors.

Energy-adjusted nutrient intake (ie, arginine) was computed as residuals from the regression model, with energy intake as the independent variable and nutrient intake as the dependent variable.[30] Before computing the residuals of nutrient intake, the logarithmic transformation of each nutrient variable, including arginine, was calculated to improve the normality. Cox proportional hazards (survival) analysis was used to calculate crude and adjusted relative risks (RRs), 95% CIs, and probability values for linear trend. The lowest tertile of arginine intake was taken as a reference group. Several multivariate analyses were performed to take into account potential confounding by major risk factors such as age, history of coronary heart disease, smoking status, body mass index, and other factors associated with arginine intake and potentially associated with coronary heart disease mortality. To evaluate confounding, a value of $P<0.2$ was considered. Energy-adjusted analysis included the energy-adjusted tertiles of arginine intake plus a term for energy

intake. Interactions between arginine intake and relevant factors, such as age, history of coronary heart disease, and smoking status, were explored to determine whether the association between arginine intake and coronary heart disease mortality differed among subgroups of the population. Proportional hazards analyses, including arginine intake and all the covariables as time-dependent covariates, were also performed. The baseline measurement (1985) for the first 5 years of follow-up and the measurement for the second 5 years of follow-up (1990) were used. When the measurement for 1990 was not available, only the baseline measurement was used for the total follow-up period.

Results

Baseline

Mean±SD baseline arginine intake of the 806 participants was $4.35±1.07$ g/d. Meat provided the largest contribution to the total arginine intake (37.1%), followed by bread (13.1%) and milk and milk products (12.1%). A relatively large amount of arginine was also provided by peanuts (2.5%); this was due to the high percentage of arginine in the protein of peanuts (13.0%).

We examined the distribution of risk factors within categories of arginine intake. Arginine intake was inversely associated with age, history of coronary heart disease, and serum homocysteine levels and positively associated with physical activity, alcohol use, and history of diabetes mellitus (Table 1). However, the distributions of other risk factors did not differ appreciably across the arginine categories. Concerning dietary factors, arginine intake was positively associated with the intake of (saturated and unsaturated) fat, carbohydrates, protein, fiber, cholesterol, and total energy (Table 2).

Daily intake of arginine was strongly correlated with the intake of energy ($r_p=0.65$, $P=0.001$). After adjustment for energy, the associations between arginine intake and dietary factors were weakened (Table 3). Energy-adjusted arginine intake remained significantly positively associated with protein, dietary cholesterol, and fiber. In addition, energy-adjusted arginine intake was positively associated with history of diabetes mellitus and body mass index and inversely associated with age and serum homocysteine levels (data not shown).

Follow-Up

During 10 years of follow-up, 374 participants died, of which 90 (11.2%) died from coronary heart disease. In the crude analysis, arginine intake was inversely associated with the risk of coronary heart disease, with an RR of 0.63 (95% CI 0.38 to 1.04) for the highest intake (P for trend=0.07, Table 4). In different multivariate models, with potential confounding by factors associated with arginine intake and potentially associated with coronary heart disease mortality taken into account, no inverse association between arginine intake and coronary heart disease mortality was observed. Especially because of the adjustment for energy intake, the RRs adjusted for age, history of coronary heart disease, history of diabetes mellitus, and energy intake were higher, 1.55 (95% CI 0.93 to 2.58) for the medium tertile and 1.19 (95% CI 0.68 to 2.07) for the highest tertile of arginine intake. After adjustment for body mass index, cigarette smoking, physical activity, alcohol consumption, and energy-adjusted intake of saturated fatty acids, polyunsaturated fatty acids, cholesterol, and fiber,

TABLE 1.   The Zutphen Elderly Study: Baseline (1985) Characteristics According to Tertiles of Arginine Intake in 806 Men

Characteristic	Tertiles of Arginine Intake, g/d			P*
	0–3.85 (Low) (n=268)	3.86–4.65 (Medium) (n=269)	>4.65 (High) (n=269)	
Age, y	72.3±5.4	71.7±5.3	70.0±4.8	0.0001
Body mass index, kg/m^2	25.6±3.2	25.4±2.6	25.5±3.5	0.57
Smoking, %				
Current smoker	29.9	29.0	31.2	0.85
Past smoker	51.9	52.8	49.8	0.78
Alcohol users, %	66.4	77.3	75.5	0.01
Physical activity, min/wk	490±484	642±562	660±559	0.0001
Systolic blood pressure, mm Hg	151±22	152±21	149±20	0.25
Serum total cholesterol, mmol/L	6.12±1.18	6.01±1.08	6.21±1.07	0.12
Serum HDL cholesterol, mmol/L	1.11±0.33	1.13±0.29	1.13±0.26	0.16
Serum homocysteine, μmol/L	17.8±10.5	15.2±6.8	13.9±6.0	0.0001
Medication use, %				
Hypertension	11.9	14.5	9.3	0.18
Cholesterol lowering	2.6	1.1	0.8	0.17
History of coronary heart disease, %	23.9	16.4	16.0	0.03
History of diabetes mellitus, %	3.0	6.7	7.8	0.02
History of hypertension, %	22.8	21.2	17.1	0.24

Values are mean±SD.

*ANOVA for normal distributed variables, Kruskal-Wallis test for skewed variables, and χ^2 test for dichotomous variables.

the RRs for the medium and high tertiles compared with the lowest were 1.68 (95% CI 0.97 to 2.90) and 1.48 (95% CI 0.80 to 2.72), respectively. Tests for linear trend were not statistically significant. The inclusion of serum total and HDL cholesterol, blood pressure, and serum homocysteine as potential confounders in the analysis increased the RRs further (Table 4).

It may be questioned whether energy intake is a confounder in this population, because the association between energy intake and coronary heart disease mortality was not statistically significant in the adjusted analysis. Therefore, we repeated the multivariate analysis (the full model, with systolic blood pressure, cholesterol, and homocysteine) without adjustment for energy intake. The RR was 0.88 (95% CI

TABLE 2.   The Zutphen Elderly Study: Baseline (1985) Mean Daily Nutrient Intake According to Tertiles of Arginine Intake in 806 Men

Nutrient	Tertiles of Arginine Intake, g/d			P*
	0–3.85 (Low) (n=268)	3.86–4.65 (Medium) (n=269)	>4.65 (High) (n=269)	
Arginine, g	3.31±0.42	4.22±0.23	5.50±0.90	...
Energy, MJ	8.0±1.7	9.4±1.6	11.0±2.0	0.0001
Total fat, g	83.2±24.3	101.6±27.8	121.3±33.6	0.0001
Saturated fatty acids, g	36.3±11.6	43.7±12.7	51.1±15.2	0.0001
Monounsaturated fatty acids, g	31.1±10.5	37.9±12.2	46.0±14.8	0.0001
Polyunsaturated fatty acids, g	12.6±6.2	16.2±7.7	19.5±8.8	0.0001
Carbohydrates, g	206±60	228±56	265±65	0.0001
Total protein, g	62.9±8.7	78.8±6.6	98.5±14.5	0.0001
Vegetable protein, g	20.2±5.1	23.9±5.4	29.6±8.2	0.0001
Animal protein, g	42.9±8.4	55.1±8.5	69.1±14.4	0.0001
Alcohol, g	12.1±16.1	14.7±18.6	12.8±16.2	0.07
Fiber, g	21.2±5.6	25.1±6.4	29.9±7.9	0.0001
Cholesterol, mg	275±95	340±102	399±123	0.0001

Values are mean±SD.

*ANOVA for normal distributed variables, Kruskal-Wallis test for skewed variables, and χ^2 test for dichotomous variables.

TABLE 3.   The Zutphen Elderly Study: Baseline (1985) Mean Daily Nutrient Intake According to Tertiles of Energy-Adjusted Arginine Intake in 806 Men

| Nutrient | Tertiles of Energy-Adjusted Arginine Intake, g/d | | | |
	0–3.91 (Low) (n=268)	3.92–4.53 (Medium) (n=269)	>4.53 (High) (n=269)	P*
Energy, MJ	9.5±2.3	9.5±2.1	9.4±2.1	0.67
Total fat, g	103.4±34.5	102.2±31.6	100.6±32.2	0.63
Saturated fatty acids, g	44.8±15.8	44.2±14.1	42.1±13.6	0.07
Monounsaturated fatty acids, g	38.4±14.8	38.5±12.9	38.1±14.3	0.94
Polyunsaturated fatty acids, g	15.8±8.3	15.6±7.4	17.0±8.6	0.11
Carbohydrates, g	243±67	236±65	220±61	0.0001
Total protein, g	68.5±8.7	79.5±13.1	92.2±17.4	0.0001
Vegetable protein, g	23.0±6.4	24.5±6.6	26.3±8.8	0.0001
Animal protein, g	45.7±12.1	55.3±10.7	66.2±14.9	0.0001
Alcohol, g	14.8±18.2	12.5±17.8	12.3±14.9	0.47
Fiber, g	23.3±6.9	25.6±6.4	27.3±8.3	0.0001
Cholesterol, mg	316±122	338±107	360±122	0.0001

Values are mean±SD.

*ANOVA for normal distributed variables, Kruskal-Wallis test for skewed variables, and χ^2 test for dichotomous variables.

0.49 to 1.56) for the medium intake and 0.99 (95% CI 0.50 to 1.98) for the highest intake (*P* for trend=0.98).

We further examined whether the association between arginine intake and coronary heart disease mortality was modified by other risk factors, with use of an interaction term in the multivariate analysis. Only for age was a significant interaction observed. The association was significant and more strongly positive in men below the median age of 71 years and inverse (but not significant) in men aged >71 years.

Finally, to reduce the likelihood that our results were biased by changes in dietary intake and lifestyle, analyses were also performed with the use of time-dependent covariates. This resulted in significantly increased risks; in the full multivariate model, the RR was 2.19 (95% CI 1.22 to 3.95)

for medium intake and 2.44 (95% CI 1.30 to 4.59) for the highest intake (*P* for trend=0.01).

Discussion

This is the first observational study investigating the association between dietary arginine intake and coronary heart disease mortality. We observed no beneficial effect of dietary arginine intake on the risk of coronary heart disease in elderly men.

Our results regarding arginine intake and coronary heart disease mortality are not consistent with the antiatherogenic effects of arginine experimentally demonstrated in human subjects with more or less advanced atherosclerosis.[9–18] In healthy subjects, some experimental studies observed no effect on endothelial function after infusion or supplementa-

TABLE 4.   RRs and 95% CIs for Mortality From Coronary Heart Disease in 806 Men During 10-Year Follow-Up According to Tertiles of Arginine Intake

| Coronary Heart Disease Mortality | Tertiles of Arginine Intake* | | | |
	Low (n=268)	Medium (n=269)	High (n=269)	P for Trend
No. of deaths	36	28	26	
Mortality rate, per 1000 person-years	18.8	13.6	12.0	
RRs				
Crude	1.0	0.72	0.63	0.07
95% CI		0.44–1.19	0.38–1.04	
Age-adjusted	1.0	0.72	0.71	0.19
95% CI		0.44–1.18	0.43–1.19	
Multivariate†	1.0	1.87	1.58	0.16
95% CI		1.06–3.29	0.84–2.96	

*Cutoff points were 3.85 and 4.66 g/d for the tertiles unadjusted for energy intake and 3.91 and 4.53 g/d for tertiles adjusted for energy intake (residual from regression analysis with energy intake).

†Factors include age, history of coronary heart disease, history of diabetes mellitus, energy intake, body mass index, smoking, alcohol, physical activity, intake of saturated fatty acids, polyunsaturated fatty acids, cholesterol, fiber (all as residuals from the regression model with energy intake), systolic blood pressure, total and HDL cholesterol, and homocysteine.

tion of arginine.[31,32] Plasma arginine levels in these subjects were also unaffected after supplementation.[32] This finding suggests that at least under normal conditions, sufficient endogenous arginine can be synthesized to maintain the rate of NO synthesis.[33] However, some findings in healthy young subjects showed that infusion of arginine could increase vasodilatation and blood viscosity and reduce platelet aggregation.[34] Studies using a tracer for arginine also have shown that dietary arginine can affect the endogenous arginine metabolism and plasma arginine levels in humans.[33,35] These findings and those from experiments in humans with a high risk profile for coronary heart disease suggest a relative lack of endogenous arginine for the synthesis of NO, which could be restored by arginine administration. Uncertainty remains regarding the exact conditions in which insufficient endogenous arginine is present.

An important limitation of the present study was that it included only men aged 64 to 84 years at baseline. The beneficial effects of arginine on vasodilatation might be higher in younger populations because stiff arteries are not as prevalent in the young. However, in the elderly, atherosclerosis is increasingly present, just as in the subjects of experiments in which the antiatherogenic effects of oral L-arginine supplementation were demonstrated. Subgroup analysis indicated that in the oldest men, arginine was inversely associated with coronary heart disease mortality, albeit nonsignificantly. On the basis of these data, however, generalizability with respect to other age groups and women is uncertain.

In experiments in which the antiatherogenic effects of oral L-arginine supplementation were demonstrated, subjects were supplemented with 3 to 21 g L-arginine per day during a short[10,17] or long[9,11,12] period. The dose used in those experiments was generally much higher than the average arginine intake in the present study. Therefore, it cannot be excluded that the effects of arginine on atherosclerosis are especially present when large amounts of arginine are supplemented and that the range of dietary arginine intake in our cohort was not wide enough to observe an association.

A true inverse association between arginine intake and coronary heart disease mortality could also be missed if the measurement of the arginine content of the diet is imprecise. Habitual food composition was measured by use of the cross-check dietary history method, which is acknowledged as a valid method in an epidemiological setting.[21] Random misclassification of dietary exposure due to error in the quantification of arginine in food tables cannot be excluded. Plasma arginine levels were not measured; however, these might reflect short-term rather than long-term dietary intake because of endogenous synthesis and conversion of arginine.[35] The estimate of daily average arginine intake of 4.4 g of the Zutphen elderly population was comparable to the estimated arginine intake of 4.2 g/d after 5 years of follow-up ($r_p=0.50$, $P=0.001$) and to the estimated average arginine intake of the American diet of 5.4 g/d, which is based on the arginine contents of foods available in 1970.[36] In addition, by use of the baseline measurement of arginine and the measurement after 5 years of follow-up, arginine intake was even more strongly positively associated with coronary heart disease mortality compared with the measurement using arginine intake at baseline only. Thus, it is unlikely that misclassification of dietary exposure explains our findings.

Because higher arginine intake was associated with a healthier lifestyle and diet, including a high energy intake, in our study population, we used several multivariate models to reduce residual confounding. A higher risk of coronary heart disease mortality with arginine intake was observed with the multivariate model. This apparent positive association is probably due to chance, because there is no biological explanation for this finding. Furthermore, the effect of L-arginine on coronary heart disease mortality could be confounded by other dietary factors that were not included in our analysis. Because arginine is derived from different foods, other dietary factors occurring in the same foods could have affected the results for arginine. For instance, in addition to arginine, nuts also contain other potentially protective constituents.[37] Furthermore, arginine intake is highly associated with the intake of other amino acids, such as methionine ($r_p=0.89$, $P=0.001$). However, our results are adjusted for serum homocysteine, the intermediate in the potential association between methionine and coronary heart disease. Also, other dietary factors influencing the arginine-NO pathway, such as L-glutamine[38,39] or vitamin C,[40] could have interfered with the role of arginine. Vitamin C could inhibit the inactivation of NO by oxygen-derived free radicals. However, adjustment for intake of vegetables, fruit, or vitamin C in the analysis did not appreciably change the results (data not shown).

In conclusion, we observed no association between dietary arginine intake and coronary heart disease mortality in a cohort of elderly men. However, this result cannot be generalized because of the elderly population studied and unmeasured dietary factors (potentially) associated with arginine. Additional observational studies are needed, preferably in other age groups in populations with a relatively high arginine intake or on more specific parameters, such as endothelial function. Furthermore, other lines of research, including studies involving biomarkers for arginine intake, should be carried out to confirm or reject the beneficial impact of arginine on coronary heart disease suggested by human and animal experiments.

Acknowledgment

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