Dietary L-Arginine Intakes and the Risk of Metabolic Syndrome: A 6-Year Follow-Up in Tehran Lipid and Glucose Study

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ABSTRACT: This study was conducted to investigate whether regular dietary intake of L-arginine could affect the occurrence of metabolic syndrome (MetS). Eligible adult men and women (n=1,237), who participated in the Tehran Lipid and Glucose Study, were followed for a median of 6.3 years. Dietary intakes of L-arginine and serum nitrate and nitrite (NOx) concentration were assessed at baseline (2006~2008), and demographics, anthropometrics, and biochemical variables were evaluated at baseline and follow-up examinations. The occurrence of MetS was assessed in relation to total L-arginine, intakes of L-arginine from animal and plant sources, with adjustment of potential confounding variables. Participants who had higher intake of L-arginine also had higher serum NOx at baseline (35.0 vs. 30.5 μmol/L, $P<0.05$). After 6 years of follow-up, higher intakes of L-arginine from animal sources were accompanied with increased risk of MetS [odd ratios (OR)=1.49, 95% confidence interval (95% CI)=1.02~2.18]. Compared to the lowest, the highest intakes of L-arginine from plant sources were related to significantly reduced risk of MetS (OR=0.58, 95% CI=0.32~0.99). In conclusion, our findings suggest a potentially protective effect of plant derived L-arginine intakes against development of MetS and its phenotypes; moreover, higher intakes of L-arginine from animal sources could be a dietary risk factor for development of metabolic disorders.

Keywords: L-arginine, nitric oxide, metabolic syndrome

INTRODUCTION

L-Arginine, a conditionally essential amino acid, is the main substrate of nitric oxide (NO) synthase (NOS) family enzymes and is responsible for production of the endothelium-derived relaxing factor NO (1,2). Mean dietary intake of L-arginine is reported as 4~6 g/d (3,4). Amounts of L-arginine in different protein sources range from 3~15%, and soy protein, peanuts, walnuts, and fish are relatively rich in L-arginine whereas cereal proteins are poor sources (3~4% of total amino acids) (5). No recommended dietary allowance has yet been defined for L-arginine intake and differences in dietary patterns between populations may be responsible for differences in the mean intakes and plasma levels of L-arginine worldwide (5-8).

Several clinical studies indicated beneficial outcomes of L-arginine supplementation on diabetes, insulin resistance, hypertension, and vascular dysfunction (9-12). Data on the effects of L-arginine supplementation on im-provement of NO production are inconsistent (13-18).

The L-arginine-NO pathway is involved in many physiological processes and there is a growing body of evidence indicating that an impaired L-arginine-NO pathway may be an important determinant for development of cardiometabolic disorders, namely vascular dysfunction, cardiovascular disease, chronic kidney disease, endocrine disorders, insulin resistance, type 2 diabetes, and metabolic syndrome (MetS) (8,19-22). Due to the importance of NO in regulation of oxidative stress and inflammatory processes, an impaired L-arginine-NO pathway is considered a main risk for development of MetS (8). Dietary intakes of L-arginine in two cohort studies had no significant association with the incidence of hypertension, acute coronary heart disease (CHD), or cardiovascular mortality (23,24). We recently, in a 5-year follow-up of 2,284 adults, showed that higher intake of animal-derived L-arginine may be a risk factor for development of CHD events (25).

In this study, we aimed to evaluate the association of

dietary total L-arginine intakes and two different sources of L-arginine, both animal and plant sources, and the occurrence of MetS after a median 6.3 years of follow-up in a national representative population.

SUBJECTS AND METHODS

Study population

This study was conducted within the framework of the Tehran Lipid and Glucose Study (TLGS) (26). According to exclusion criteria, 1,237 adults (453 men, 784 women), aged 20~84 years, were included in the final analyses (Fig. 1). Written informed consents were obtained from all participants and the study protocol was approved (ethics committee number: 57ECRIES94/02/15) by the ethics research council of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences. The study protocol was conducted according to the principles of the Declaration of Helsinki.

Details of demographics, anthropometrics, and biochemical measures were reported elsewhere (26). Baseline measurements were conducted in 2006~2008; second and third examinations were carried out in 2009~2011, and 2012~2014, respectively.

Laboratory assays

Fasting blood samples were taken after 12~14 h from all study participants at baseline and again at the second and third follow-up examinations. Fasting blood glucose (FBG) was measured by the enzymatic colorimetric method using glucose oxidase. Enzymatic colorimetric analysis with glycerol phosphate oxidase was used to measure triglyceride (TG) levels. High-density lipoprotein cholesterol (HDL-C) 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). Inter- and intra-assay coefficients of variation of all assays were <5%.

Serum creatinine levels were assayed using the kinetic colorimetric Jaffe method. Serum nitrate and nitrite (NOx) concentration was measured by a rapid, simple spectrophotometric method (27-30).

Fasting serum insulin was measured in a subgroup of participants (n=1,141) at baseline and again in the second examination by the electrochemiluminescence immunoasaay, using Roche Diagnostics kits and the Roche/ Hitachi Cobas e-411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The intra- and inter-assay coeffi-
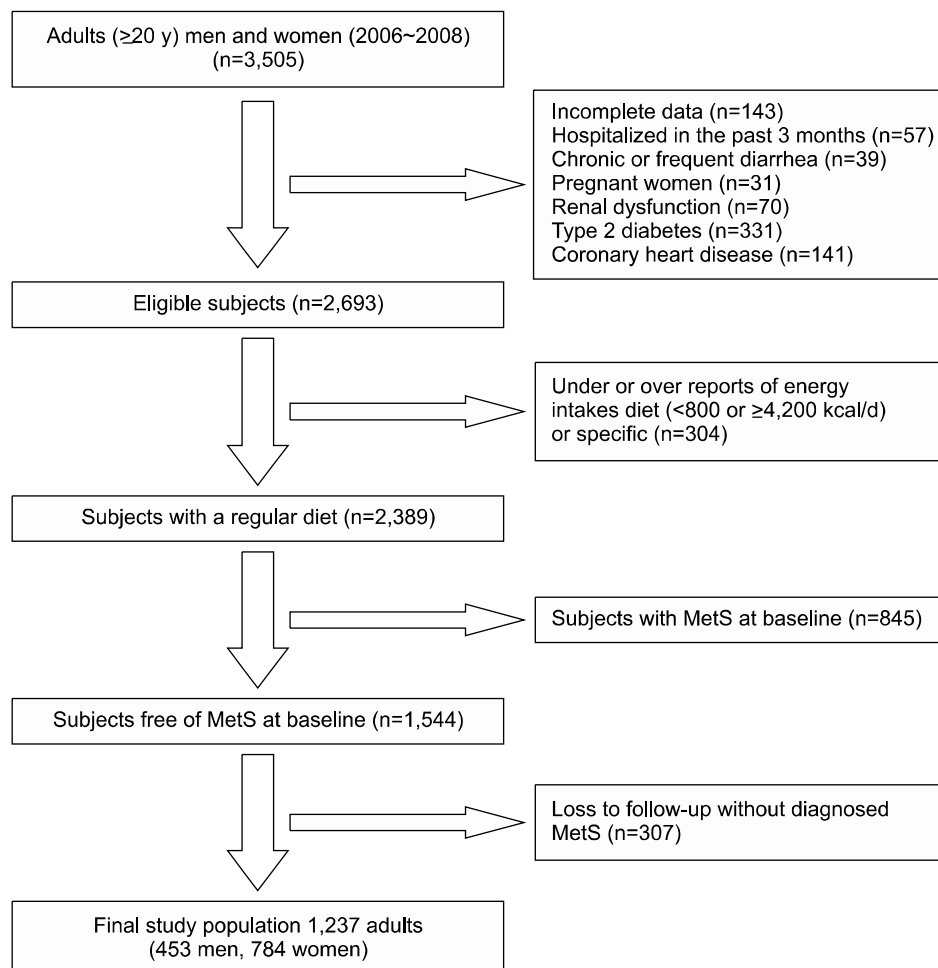

Fig. 1. Flowchart of the study population. MetS, metabolic syndrome.

cients of variation for insulin were 1.2 and 3.5 %, respectively. Homeostatic model assessment of insulin resistance (HOMA-IR) was also determined, as a simple and validated alternative tool for assessment of insulin resistance in epidemiological studies, by the following formula: fasting insulin (μU/mL)$\times$fasting glucose (mmol/L)/22.5 (31,32).

Dietary intakes

A validated (33) 168-item food frequency questionnaire (FFQ) was used to assess in typical food intakes, and to estimate total L-arginine intakes and L-arginine from animal and plant sources, over the previous year. 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 (34). We used the US Department of Agriculture Food Composition Table to analyze foods and beverages for their energy and nutrient content.

Definitions

Metabolic syndrome components were defined according to diagnostic criteria proposed by National Cholesterol Education Program—Adult Treatment Panel III (35), and the latest cutoff points of waist circumference for Iranian adults (36). Participants were considered to have metabolic syndrome at baseline if they have at least 3 of the metabolic abnormalities: 1) Hyperglycaemia as FBG $\geq$100 mg/dL (5.6 mmol/L) or drug treatment of impaired fasting glucose, 2) Hypertriglyceridemia as serum triglycerides $\geq$150 mg/dL (1.69 mmol/L) or drug treatment, 3) Low HDL-C as serum HDL-C <40 mg/dL (1.04 mmol/L) for men, and <50 mg/dL (1.29 mmol/L) for women or drug treatment, 4) Hypertension as blood pressure $\geq$130/85 mmHg or drug treatment for hypertension, and 5) Abdominal obesity as waist circumference $\geq$95 cm for both genders; names of MetS phenotypes, represented by any three or more combinations of the five MetS components, were defined in combination of following letters: W, elevated waist circumference; G, elevated blood glucose; T, elevated triglyceride levels; B, elevated blood pressure; H, low-HDL-C. Diabetes was defined as fasting serum glucose $\geq$126, 2 h serum glucose $\geq$200, or anti-diabetic medications (37). According to the World Health Organization classification, menopause was defined as the absence of spontaneous menstrual bleeding for over 12 months, for which no other pathologic or physiologic cause could be determined (38). Coronary heart disease was defined as definite myocardial infarction (MI) [with diagnostic electrocardiogram (ECG) and biomarkers], probable MI (positive ECG findings plus cardiac symptoms or signs plus missing bio-markers or positive ECG findings plus equivocal biomarkers), unstable angina (new cardiac symptoms or changing symptom patterns and positive ECG findings with normal biomarkers), angiographic proven CHD, and death from CHD (39).

Statistical analysis

Log-transformed variables with non-normal distribution (serum NOx and TG) were used in the analyses. Mean [$\pm$standard deviation (SD)] values and the proportions of baseline characteristics of the participants with and without the MetS, were compared, using the independent sample t test or chi-square test, respectively.

Dietary intake of L-arginine was adjusted for total energy intake, based on the residuals method (40). Dietary intakes of L-arginine were categorized into quartiles. The percentages of participants according to MetS phenotypes and L-arginine from animal and plant sources (quartile 4 vs. quartiles 1, 2, and 3) were analyzed using the chi-square test.

A univariate analyse was performed for each potential confounder including age (y), sex (male/female), MetS score (summing up of MetS risk factors including abdominal obesity, low-HDL-C, hyper-triglyceridemia, hypertension, and disglycemia), serum creatinine, menopause status (yes/no), using of medications (yes/no), smoking (yes/no), energy intakes (kcal/d), and dietary intakes of protein (g/d), carbohydrates (g/d), total fats (g/d), and fibre (g/d). Variables with P_E (P for entry) <0.2 in the univariate analyses were selected for the multivariable models; P_E determines which variables should be included in the final multivariable model.

To investigate the association of L-arginine intakes and changes of serum insulin and HOMA-IR, linear regression models were used with adjustment of the above mentioned confounding variables. Multivariable logistic regression models with adjustment for potential confounders were used to determine the incidence of MetS across quartiles of total L-arginine, and intakes of L-arginine from animal and plant sources.

All statistical analyses were conducted using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA), and P-values <0.05 were considered significant.

RESULTS

General characteristics of the participants

Mean age of participants (36.9% men) was 41.7$\pm$14.6 years. The cumulative incidence of MetS phenotype was 29.2% (34.6% in men, 26.1% in women) after a median follow-up of 6.3 years.

Baseline characteristics of the study population are presented in Table 1. Participants with diagnosed MetS

Table 1. Baseline characteristics of the participants (n=1,237)

	MetS⁻	MetS⁺	P
Age at baseline (y)	39.8±14.4	46.4±14.1	0.001
Men (%)	34.1	43.7	0.001
Smoking (%)	6.9	11.1	0.011
Body mass index (kg/m^2)	25.3±3.9	28.0±3.5	0.001
Waist circumference (cm)	83.8±10.9	93.1±9.6	0.001
Fasting blood glucose (mg/dL)	87.9±16.9	94.1±22.0	0.001
Serum triglycerides[1] (mg/dL)	97.5 (94.6~100)	136 (129~141)	0.001
HDL-C (mg/dL)	46.7±10.7	41.2±9.0	0.001
Systolic blood pressure (mm Hg)	108±14.4	115±15.2	0.001
Diastolic blood pressure (mm Hg)	69.2±9.05	73.6±8.8	0.001
Serum creatinine	1.03±0.16	1.05±0.15	0.044
Serum NOx[1] (μmol/L)	25.5 (24.5~26.3)	27.6 (26.0~29.1)	0.001
Total L-arginine (g/d)	4.1±1.5	4.0±1.5	0.55
L-arginine from animal sources (g/d)	1.7±0.94	1.9±1.0	0.12
L-arginine from plant sources (g/d)	2.3±1.8	2.1±1.8	0.18

MetS, metabolic syndrome; HDL-C, high-density lipoprotein cholesterol; NOx, nitrate and nitrite.
Data are mean±SD.
[1]Data are geometric mean (95% confidence interval).

were more likely to be older, and had higher body mass index, waist circumference, triglyceride levels, systolic blood pressure, diastolic blood pressure, serum creatinine, and lower HDL-C, at baseline. Dietary L-arginine intakes did not differ between subjects with and without MetS, but a higher serum NOx concentrations (27.6 vs. 25.5 μmol/L, P=0.001) was observed in subjects with MetS.

Dietary information
Mean dietary intakes of protein and L-arginine were 78.1 ±28.1 g/d and 4.08±1.46 g/d, respectively. Mean (SD) intake of L-arginine from animal and plant sources was 1.82±0.96 and 2.26±1.81 g/d, respectively. Lowest and highest categories of L-arginine intake, defined as the 10th and 90th percentile, were <2.42 and ≥6.11 g/d, respectively. Mean dietary intake of L-arginine to total protein ratio was 0.052±0.02, with 50.7±33.1% of total L-arginine intakes being from animal sources. At base-

line, serum NOx concentration was 30.5, 31.4, 32.6, and 35.0 μmol/L in the first, second, third, and fourth quartile categories of total L-arginine intake.

Table 2 shows dietary intakes of the participants across quartiles for total L-arginine intakes, indicating no significant difference in L-arginine intakes from animal sources across total L-arginine quartiles, whereas the relative amount of plant sources of L-arginine increased across increasing intakes of total L-arginine (4.37 vs. 0.64 g/d, P<0.01). Dietary intakes of energy, protein and fibre also increased across quartile categories of total L-arginine (P<0.01).

Metabolic syndrome and dietary L-arginine
The prevalence of MetS phenotypes across quartiles of L-arginine from plant sources are illustrated in Fig. 2A. Subjects in the highest compared to the lowest quartiles, had a significantly lower prevalence of WBH (9.7 vs. 14.3 %, P=0.002), WBT (7.8 vs. 12.5, P=0.002), BHT (9.2 vs.

Table 2. Dietary intakes of the participants across quartiles of total L-arginine intakes

	Q₁	Q₂	Q₃	Q₄
Total arginine (g/d)				
Range	<3.03	3.03~3.84	3.84~4.91	≥4.91
Median	2.55	3.45	4.34	5.72
Arginine from animal sources (g/d)	1.82±0.95	1.83±0.93	1.89±1.00	1.73±0.97
Arginine from vegetable sources (g/d)	0.64±1.02	1.60±0.96	2.46±1.05	4.37±1.59**
Energy intake (kcal/d)	1,533±362	2,050±384	2,508±463	3,118±565**
Carbohydrate (% energy)	57.3±7.7	57.2±7.1	57.8±6.8	56.9±7.4
Protein (% energy)	12.8±2.1	13.2±2.2	13.6±2.2	14.7±2.6**
Total fats (% energy)	32.0±7.8	31.9±7.5	31.1±6.5	30.9±6.6
Total fibre (g/d)	34.2±1.2	35.2±0.9	40.1±1.0	40.3±1.2**

Data are mean±SD.
**P<0.01 (analysis of variance or analysis of covariance with adjustment of total energy intake was used).
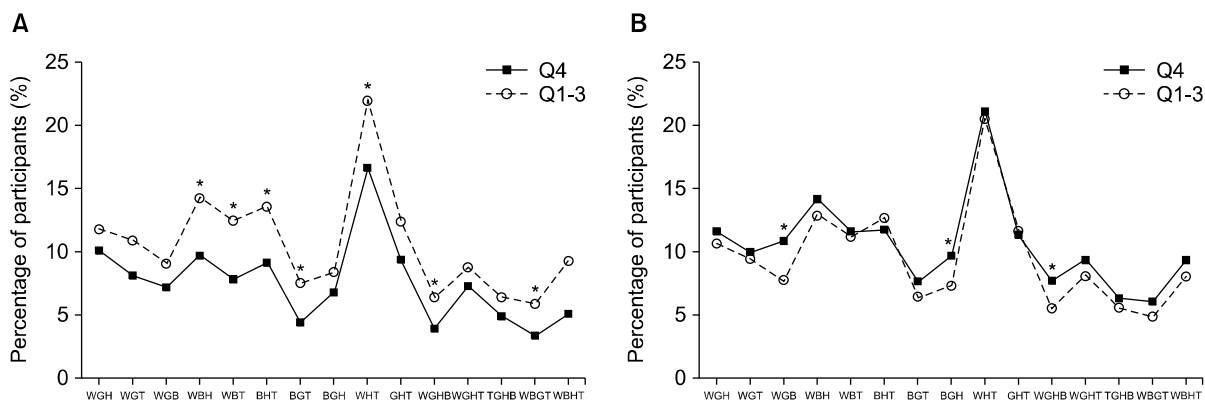
Fig. 2. The prevalence of metabolic syndrome (MetS) phenotypes across quartiles of L-arginine from plant sources (A) and animal sources (B). W, elevated waist circumference; G, elevated blood glucose; T, elevated triglyceride levels; B, elevated blood pressure; H, low-high-density lipoprotein cholesterol.

13.6, P=0.005), BGT (4.4 vs. 7.6, P=0.008), WHT (16.7 vs. 22.0, P=0.007), WGHB (3.9 vs. 6.4, P=0.031), and WBGT (3.4 vs. 5.9, P=0.02).

The prevalence of MetS phenotypes across quartiles of L-arginine from animal sources are illustrated in Fig. 2B. Subjects in the highest compared to the lowest quartiles, had a significant higher prevalence of WGB (10.9 vs. 7.8 %, P<0.05), BGH (9.7 vs. 7.4%, P<0.05), and WGHB (7.7 vs. 5.5%, P<0.05).

There was an inverse association for intakes of L-arginine from plant sources and changes of serum insulin [β=−2.59, 95% confidence interval (95% CI)=−5.32 and −0.12] and HOMA-IR (β=−2.82, 95% CI=−4.49, and −1.13); total intakes of L-arginine and L-arginine from animal sources had no significant association between changes of serum insulin and HOMA-IR (data are not shown).

The occurrence of MetS across quartiles of L-arginine intakes is presented in Table 3. After adjustment of all

potential confounding variables, a non-significant decreasing risk of MetS was observed in the highest, compared to the lowest, quartile of L-arginine intakes [odd ratios (OR)=0.83, 95% CI=0.38~1.31]. Higher intakes of L-arginine from animal sources were accompanied with increased risk of MetS (OR=1.49, 95% CI=1.02~2.18), whereas from plant sources, compared to the lowest intakes of L-arginine, the highest were related to a significantly reduced risk of MetS (OR=0.58, 95% CI=0.32~0.99).

DISCUSSION

Our findings showed a non-significant decreasing trend in the occurrence of MetS in subjects who had a regular L-arginine intakes ≥4.91 g/d. We also assessed the risk of MetS across animal and plant sources of L-arginine; our findings showed a protective effect of plant sources

Table 3. The occurrence of MetS across quartiles of total L-arginine, intakes of L-arginine from animal and plant sources (n=1,237)

	Q$_1$	Q$_2$	Q$_3$	Q$_4$
Total L-arginine (g/d)	<3.03	3.03~3.84	3.84~4.91	≥4.91
Model 1	1	1.1 (0.76~1.53)	0.99 (0.69~1.41)	0.96 (0.67~1.36)
Model 2	1	1.22 (0.84~1.76)	1.01 (0.69~1.47)	0.93 (0.64~1.36)
Model 3	1	1.21 (0.83~1.74)	0.98 (0.68~1.44)	0.83 (0.38~1.31)
L-arginine from animal sources (g/d)	<1.19	1.19~1.63	1.63~2.19	≥2.19
Model 1	1	0.88 (0.61~1.27)	1.09 (0.77~1.56)	1.28 (0.91~1.82)
Model 2	1	0.85 (0.59~1.24)	1.08 (0.75~1.55)	1.28 (0.89~1.83)
Model 3	1	0.88 (0.59~1.31)	1.22 (0.83~1.80)	1.49 (1.02~2.18)
L-arginine from plant sources (g/d)	<1.14	1.14~2.08	2.08~3.31	≥3.31
Model 1	1	1.15 (0.81~1.62)	0.96 (0.67~1.37)	0.82 (0.56~1.18)
Model 2	1	1.11 (0.78~1.58)	0.93 (0.65~1.33)	0.81 (0.57~1.17)
Model 3	1	1.12 (0.76~1.65)	0.85 (0.55~1.32)	0.58 (0.32~0.99)

Data are odds ratio (95% confidence interval).
Model 1, crude model; Model 2, adjusted for age and sex; Model 3, additional adjustment for baseline metabolic syndrome (MetS) score (summing up of MetS risk factors including abdominal obesity, low-high-density lipoprotein cholesterol, hyper-triglyceridemia, hypertension, and disglycemia), serum creatinine, smoking, use of medication, menopause status, energy intakes, fiber, fats, and protein intakes.
Median of total L-arginine intakes was 2.54, 3.46, 4.34, and 5.74, in the Q$_1$, Q$_2$, Q$_3$, and Q$_4$, respectively.

of L-arginine against the development of MetS whereas higher intakes of L-arginine from animal sources were accompanied with increased occurrence of MetS. Plant sources of L-arginine were also inversely related to changes of serum insulin and insulin resistance index during the follow-up. The prevalence of main MetS phenotypes was also lower in the highest compared to the lowest L-arginine intakes from plant sources.

The possible modulatory effects of L-arginine supplementation on NO-mediated pathways is currently considered as an effective strategy for prevention and treatment of MetS and its phenotypes including abdominal obesity, type 2 diabetes and dyslipidemia (41) whereas limited studies have examined the association of regular dietary intakes of L-arginine and the risk of cardio-metabolic disorders. L-arginine intake below the median range (3.8 g/d) was associated with higher levels of C reactive protein (CRP) and highest level of L-arginine intake (>7.5 g/d) were related to 30% less likely to have a CRP above 3.0 mg/L; moreover, a lower prevalence of elevated SBP and LDL-C was also observed in subjects who consumed >7.5 g/d L-arginine (4). Findings of a population-based cohort did not support the hypothesis that dietary arginine intake may lower the risk of coronary heart disease mortality (24). Similarly, in a 10-year follow-up of participants of Kuopio Ischemic Heart Disease Risk Factor Study, total L-arginine intake had no association with blood pressure or the risk of acute coronary events (23). In this study, the portion of animal derived L-arginine was higher than plant derived and there was an increasing trend in animal derived L-arginine across quartiles of total intakes of L-arginine (23).

It seems that animal and plant sources of L-arginine may induce different physiological effects in the body; it has been suggested that utilization of plant derived L-arginine is better than animal derived because higher ratio of lysine to L-arginine in animal proteins and competition of lysine with L-arginine for the same plasma membrane transport mechanism (42). It should also be noted that the different effects of animal- and plant-sources of L-arginine on metabolic disorders, observed in our study, may be due to different composition of animal and plant proteins or other nutrients such as fatty acids in animal-based foods or fibre and phytochemicals in plant-based foods. To statistically modify these dietary potential confounders, we therefore adjusted dietary intakes of protein, fibre and fats in logistic regression models.

The strengths of the current study were a population-based prospective setting, and use of a validated FFQ to assess regular dietary intake.

This study had several limitations. First, lack of data on serum levels of L-arginine was 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. Moreover, due to some inherent limitations of observational studies including selection bias, information bias in measuring exposure or outcome, and non-differential misclassification should be considered in interpretation of the findings. Inherent limitation of FFQ such as under or over estimations of dietary intakes was also another issue in our study. Dietary information was also assessed only at baseline examinations and possible changes in dietary patterns during the follow-up examinations have not been considered; however previous observations in our population indicated stability of major dietary patterns over the time.

In conclusion, our findings suggested a potential protective effect of plant derived L-arginine intakes against development of MetS and its phenotypes; moreover, higher intakes of L-arginine from animal sources could be a dietary risk factor for development of metabolic disorders. Considering the limited epidemiological studies available in relation to long-term effects of dietary L-arginine intakes on cardiometabolic outcomes including MetS, type 2 diabetes, hypertension and cardiovascular events, further cohort studies are required to clarify the possible association.

ACKNOWLEDGEMENTS

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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