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High dietary methionine intake increases the risk of acute coronary events in middle-aged men

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Abstract *Background and aim:* Homocysteine, a methionine metabolite, is suggested to be a risk factor for cardiovascular diseases (CVD). To date, the effects of dietary intake of methionine, the key amino acid in homocysteine metabolism, on CVD have not been studied. Our aim was to examine the effects of dietary methionine intake on the risk of acute coronary events.
Methods and results: We examined the effects of dietary methionine intake, assessed with 4-d food record, on acute coronary events in a prospective cohort study consisting of 1981 coronary disease free men from eastern Finland, aged 42–60 years at baseline in 1984–89, in the Kuopio Ischaemic Heart Disease Risk Factor (KIHD) Study. During an average follow-up time of 14.0 years, 292 subjects experienced an acute coronary event. In a Cox proportional hazards model adjusting for age, examination years, BMI, urinary nicotine metabolites and protein intake (excluding methionine) the relative risks of acute coronary event in the three highest quarters of dietary methionine intake were 1.31 (95% CI: 0.92, 1.86), 1.31 (95% CI: 0.88, 1.96) and 2.08 (95% CI: 1.31, 3.29) as compared with the lowest quarter. Further adjustments did not change the results. However, opposite association was observed with total protein intake, which tended to decrease the risk.

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Conclusions: The main finding of this study is that long-term, moderately high dietary methionine intake may increase the risk of acute coronary events in middle-aged Finnish men free of prior CHD. More prospective research is needed to confirm the role of dietary methionine in the development of CVD, and whether its effects are independent of homocysteine.

Introduction

Methionine is a sulphur-containing amino acid naturally present in the diet. It is a precursor of S-adenosylmethionine (SAM) which, following the removal of the methyl group, forms homocysteine (Hcy) (Fig. 1). Hcy can be either remethylated back to methionine by a methyl group from N-5-methyltetrahydrofolate or betaine or irreversibly metabolized to cystathionine by transsulfuration with serine [1]. There is normally a strict balance between Hcy formation and elimination [2]. However, defects in Hcy metabolism can lead to rise in blood total Hcy (tHcy). This may be due to genetic defects in Hcy metabolism or nutritional deficiencies in one or more vitamins that participate in Hcy metabolism [1]. In addition, if dietary intake of methionine is high, as in the methionine loading test, the transsulfuration capacity is exceeded and Hcy is excreted from cells and blood concentration of tHcy increases [1,2]. Elevated blood concentrations of tHcy were found to be an independent risk factor for cardiovascular diseases (CVD) in most but not all studies [3,4].

There is very limited data from prospective cohort studies about the effects of normal dietary intakes of amino acids, which participate in Hcy metabolism, on the risk of CVD. Thus, the purpose of this study was to test the hypothesis that methionine intake from normal diet is associated with the risk of acute coronary events in middle-aged Finnish men free of prior coronary heart

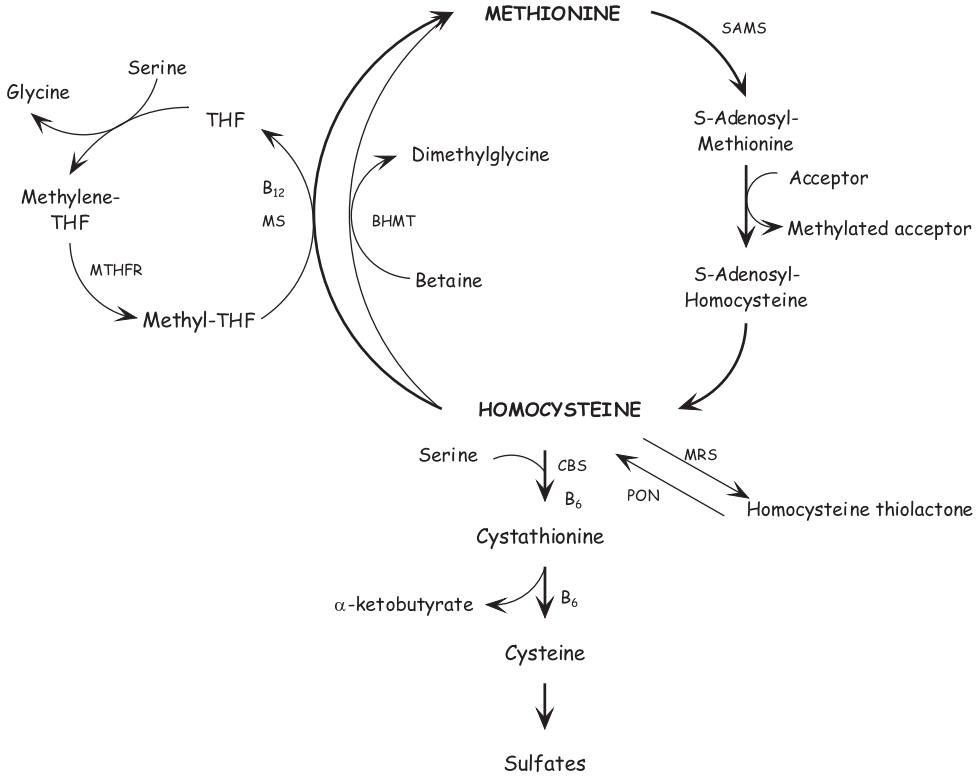

Figure 1 Homocysteine metabolism. BHMT = betaine-homocysteine methyltransferase, CBS = cystathionine β-synthase, CL = cystathionine γ-lyase, PON = paraoxonase, MRS = methionyl-tRNA synthase, MS = methionine synthase, MTHFR = methylenetetrahydrofolate reductase, SAMS = S-adenosylmethionine synthetase, THF = tetrahydrofolate.

disease (CHD) at baseline. Since methionine is a part of total protein intake, we also wanted to test whether total protein intake is associated with the risk.

Methods

Study population

The KIHD study is a population-based study of risk factors for CHD, atherosclerosis and related outcomes [5]. 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 neighbouring rural communities, stratified and balanced in four strata: 42, 48, 54 or 60 years at the baseline examination. The baseline examinations were carried out between 1984 and 1989. Of 3235 eligible men, 2682 (82.9%) participated. Men with prevalent CHD at baseline ($n=677$) were excluded from the main analyses. Of the remaining 2005 men, food record data were available for 1981 men.

Assessment of nutrient intakes

The consumption of foods was assessed at the time of blood sampling at the KIHD study baseline and at the one-year follow-up with an instructed 4-day food recording by household measures. The instructions were given and the completed food records were checked by a nutritionist. The intakes of nutrients were estimated using the NUTRICA® version 2.5 software. The content data of amino acids in Finnish food items was obtained from the Fineli® databank at the National Public Health Institute (Fineli-Food composition databank of Finland, http://www.ktl.fi/fineli) [6]. Intakes of 350 foodstuffs rich in methionine and recipes containing these foodstuffs were assessed. The intakes of nutrients used as covariates in the Cox models were energy adjusted by the residual method [7,8]. The major methionine sources were calculated based on randomly selected food records from 10 cases and from 50 others. We replicated the baseline food record data by 4-day food records ($n=50$) used in the 1-year follow-up visit of the KIHD study.

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 [9]. Data on coronary events between 1993 and 2002 were obtained by record linkage from the national computerized hospitalization registry. Identical diagnostic classification with that of the FINMONICA project was used. The mean follow-up time of the cohort was 14.0 years. The cases of the present study were the 292 men who had their first acute coronary event by the end of 2002. According to the diagnostic classification of the events there were 156 definite and 79 probable acute myocardial infarctions (AMI) and 57 typical prolonged coronary chest pain episodes leading to hospitalization.

Homocysteine measurements

Plasma tHcy measurements were available for 174 acute coronary event cases and 160 others. Cases and others were matched for age, examination year and residence. EDTA blood samples were obtained from subjects between 8.00 and 10.00 AM after an overnight fast. Subjects were instructed to abstain from alcohol for at least one week. The subjects were also instructed to avoid strenuous exercise during the previous 24 hours. Plasma was separated within 60 min and stored at -20 °C until analysis. The plasma tHcy concentrations were analyzed in 1998 at the Department of Clinical Pharmacology, Rigshospitalet, Copenhagen, Denmark by gas-chromatography mass spectrometry using isotope dilution method [10,11].

Assessment of covariates

Assessment of demographic variables, medical history, medications, and blood pressure was carried out as described previously [12]. The collection of blood specimens [12], and the measurement of serum lipids and lipoproteins [13], maximal oxygen uptake [14] and 24-hour urinary excretion of nicotine metabolites [15] have been previously described. Body mass index (BMI) was computed as the ratio of weight in kilograms to the square of height in meters.

Statistical analysis

The means of the baseline characteristics between men who experienced an event (the ''cases'') and the others were compared with ANOVA. Correlations between plasma tHcy and risk factors studied were estimated with Pearson correlation coefficients (r). Subjects were classified into quarters according to the daily mean methionine intake. The Cox proportional hazards models were used to analyze the relationship between dietary intake of

methionine and protein and the risk of acute coronary events. Risk factor adjusted hazard rate ratios (RR) were estimated as the antilogarithms of coefficients from multivariable models. The covariates used in the initial model were age, examination years, serum LDL and HDL cholesterol and triglyceride concentrations, BMI, diabetes, urinary nicotine metabolites, family history of ischemic heart disease, maximal oxygen uptake, systolic blood pressure, annual income, alcohol usage, serum ferritin, serum creatinine, plasma ascorbic acid, and dietary intakes of vitamin B_6, B_{12}, C and E, beta-carotene, fiber, folate, saturated fatty acids and protein (excluding methionine; only for the analyses with methionine intake). The exclusion of a covariate from this initial model was based on a change in RR for methionine or protein intake quarters of less than 10%. Age and examination years were forced in the models. When this criterion was used, BMI, urine nicotine metabolites and protein intake remained in the final models in addition to the age and examination years in the analyses with methionine intake. For the analyses with dietary protein intake, only age and examination years remained in the final models. An eigenanalysis of the predictor correlation matrix using the full model was carried out to detect multicollinearities among the predictor variables. All tests of significance were two-tailed. SPSS for Windows version 11.5 statistical software (SPSS Inc., Chicago, IL, USA) was used.

Results

The major dietary sources of methionine were meat and meat products (31.8%), milk and milk products (31.7%), cereal (17.7%) and fish (9.8%). The men in the highest quarter of dietary methionine intake compared with the lowest quarter had a higher BMI and annual income, higher prevalence of diabetes and ischemic heart disease history in family, higher serum ferritin concentration and lower plasma tHcy concentrations (Table 1). The intakes of nutrients and foods in the quarters of energy adjusted methionine intake are also shown in Table 1. When crude (not energy adjusted) values were used, the men in the highest methionine intake quarter consumed significantly more energy, protein, saturated fat, folate, vitamin B_6, vitamin B_{12}, vitamin E, vitamin C, fiber and β-carotene than men in the lowest quarter ($P<0.001$ for each nutrient, except for β-carotene $P=0.002$). In addition, the consumption of meat, fish, milk products, eggs, vegetables (excluding potatoes), fruits and berries and coffee was significantly higher in the highest quarter of not energy adjusted methionine intake ($P<0.001$ for each food, except for fruits and berries $P=0.002$).

Crude intake of dietary methionine correlated with intakes of dietary protein ($r=0.92$), vitamin B_6 ($r=0.68$), folate ($r=0.58$), saturated fatty acids ($r=0.53$), vitamin E ($r=0.50$), fiber ($r=0.42$), vitamin B_{12} ($r=0.27$), vitamin C ($r=0.18$) and beta-carotene ($r=0.06$). The correlation between methionine and total energy intake was 0.71.

The median of the daily methionine intake for the cases was 2.01 g per day and for the others 1.97 g per day. For the cases the methionine intake represented 2.30% and for the others 2.22% of the daily total protein intake (P for difference <0.001). After adjustment for age, examination years, BMI, urine nicotine metabolites and protein intake (excluding methionine) the RRs of acute coronary events in the three highest quarters of energy adjusted methionine intake were 1.31 (95% CI, 0.92 to 1.86), 1.31 (95% CI, 0.88 to 1.96) and 2.08 (95% CI, 1.31 to 3.29) as compared with the lowest quarter. Further adjustments for cardiovascular risk factors did not change the result. Fig. 2 presents survival curves for the quarters of energy adjusted dietary methionine intake, adjusted for age, examination years, BMI, urea nicotine metabolites and protein intake (excluding methionine).

We also assessed the effect of total protein intake on the risk of acute coronary events. The mean ($\pm$SD) intake of protein was 90.7 ± 25.1 g/day (15.5 ± 2.6% of energy). The age and examination year adjusted RRs of acute coronary event in quarters of energy adjusted protein intake were 1.0, 0.89 (95% CI, 0.66 to 1.22), 0.86 (95% CI, 0.63 to 1.18) and 0.78 (95% CI, 0.56 to 1.09). If energy adjusted methionine intake was added in the models, the RRs for quarters of energy adjusted protein intake (excluding methionine) were 1.0, 0.76 (95% CI, 0.55 to 1.05), 0.63 (95% CI, 0.44 to 0.91) and 0.43 (95% CI, 0.27 to 0.70). Further adjustments did not change the result.

We had plasma tHcy measurements available for 174 cases and 160 others. The mean ($\pm$SD) plasma tHcy concentration in the cases was 11.2 ± 2.9 μmol/L and in the others 11.3 ± 3.4 μmol/L, P for difference 0.867. The correlation between plasma tHcy and dietary methionine intake was −0.088 and between dietary protein intake −0.121.

Discussion

The main finding of this study is that long-term, moderately high dietary methionine intake may increase the risk of acute coronary events in

Table 1 Comparison of baseline characteristics according to the quarter of energy adjusted dietary methionine intake[a]

	Quarter of dietary methionine intake, g/d				P [b]
	<1.7 (n=496)	1.7–2.0 (n=495)	2.0–2.2 (n=495)	>2.2 (n=495)	
Age, y	52.6±5.0	52.4±5.3	52.4±5.4	52.4±5.4	0.898
Serum total cholesterol, mmol/L	5.84±1.10	5.91±1.02	5.86±1.03	5.84±1.02	0.618
Serum LDL cholesterol, mmol/L	3.97±0.98	4.04±0.97	4.01±0.99	4.00±0.98	0.724
Serum HDL cholesterol, mmol/L	1.32±0.30	1.29±0.28	1.29±0.30	1.32±0.29	0.350
Serum triglycerides, mmol/L	1.21±0.72	1.27±0.74	1.27±0.80	1.25±0.66	0.504
Systolic blood pressure, mm Hg	133±15	134±16	135±17	135±17	0.169
Serum ferritin, μg/L[c]	155.4±165.0	167.7±143.5	159.6±130.7	183.4±146.3	0.005
Serum creatinine, μmol/L[d]	88.7±12.5	91.0±16.3	89.4±13.5	88.8±13.1	0.915
Plasma ascorbic acid, mg/L[e]	8.3±4.3	8.3±4.1	8.4±4.1	8.7±3.9	0.069
BMI, kg/m^2	26.1±3.1	26.5±3.4	26.7±3.5	27.6±3.7	<0.001
Urea nicotine metabolites, mg/d	5.6±8.1	5.7±8.3	5.7±7.6	5.5±7.9	0.968
Diabetes, %	2.2	3.6	3.2	7.5	<0.001
Family history of ischemic heart disease, %	43.2	43.8	45.9	51.3	0.044
Maximal oxygen uptake, L/min	2.55±0.56	2.55±0.58	2.52±0.55	2.58±0.59	0.451
Annual income, thousand euros[f]	13.1±8.3	14.4±9.4	14.3±10.1	14.9±9.4	<0.001
Plasma total homocysteine, μmol/L[g]	11.9±2.7	11.2±3.2	11.2±3.6	10.9±3.0	0.029
Use of antioxidant vitamin supplements, %	13.5	12.3	12.5	12.9	0.779
Nutrient intakes					
Methionine, mg/d	1610±430	1800±380	2060±390	2590±540	
Protein, E%	12.9±1.4	14.9 ±1.4	16.1±1.7	18.0±2.5	<0.001
Saturated fat, E%	18.4±4.4	18.0±4.1	17.8±4.1	17.3±3.9	<0.001
Folate, μg/d	251±75	247±70	257±73	281±86	<0.001
Vitamin B_{12}, μg/d	7.1±8.4	9.4±8.2	10.0±8.2	11.8±11.3	<0.001
Vitamin $B_{6,}$ mg/d	1.8±0.5	1.8±0.5	1.9±0.5	2.2±0.6	<0.001
Vitamin E, mg/d	8.9±3.0	8.7±2.9	9.0±2.9	9.8±3.2	<0.001
Vitamin C, mg/d	75.2±55.2	69.1±44.0	74.2±51.9	78.7±56.0	0.333
Fiber, g/d	26.2±9.1	24.4±8.3	24.5±8.0	26.7±9.3	0.424
β-carotene, mg/d	2.5±3.3	2.3±2.1	2.3±2.3	2.7±2.6	0.425
Food intakes					
Meat, g/d	140±73	148±72	160±74	206±103	<0.001
Fish, g/d	25±31	37±37	49±48	72±75	<0.001
Milk products, g/d	512±332	547±328	664±348	776±424	<0.001
Eggs, g/d	30±23	31±22	35±27	38±32	<0.001
Vegetables, g/d	120±95	118±73	125±79	144±96	<0.001
Cereals, g/d	270±100	250±90	251±87	261±96	0.173
Fruits and berries, g/d	170±147	163±131	164±154	168±144	0.861
Coffee, mL/d	572±293	556±274	560±315	569±307	0.902
Alcohol, g/wk of pure alcohol	64.2±95.3	70.4±116.3	70.1±123.6	86.2±120.1	0.019

[a] Values are means±SD, unless otherwise indicated.
[b] The differences between the highest and lowest quarter were compared with ANOVA.
[c] Data available for 1979 men.
[d] Data available for 1820 men.
[e] Data available for 1936 men.
[f] Data available for 1953 men.
[g] From 174 acute coronary event cases and 160 others.

middle-aged Finnish men free of prior CHD. High total protein intake may be associated with lower risk, however. These associations remained after extensive adjustment for other CHD risk factors. The data concerning dietary methionine intake and CHD from other prospective cohort studies is very limited. Rimm et al. studied 658 incident cases of nonfatal myocardial infarction and 281 cases of fatal CHD in a prospective Nurses' Health Study cohort of 80,082 women [16]. In this study population methionine intake was not associated with the risk of CHD.

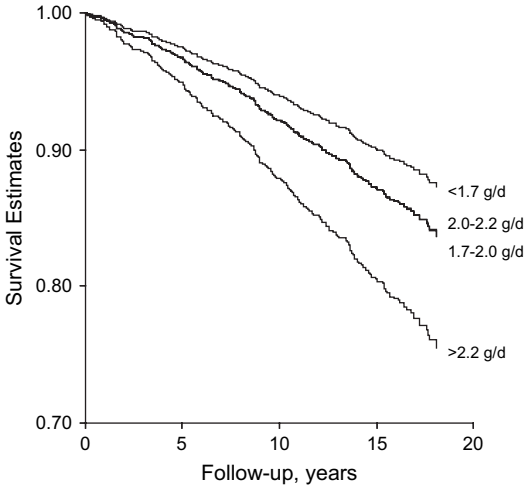

Figure 2 Survival curves for quarters of energy adjusted dietary methionine intake, adjusted for age, examination years, BMI, urea nicotine metabolites and protein intake (excluding methionine) in the Cox proportional hazards model in 1981 middle-aged men from eastern Finland.

Since Hcy is formed by methionine, increased dietary methionine intake could lead to an increase in blood tHcy, thus increasing the risk for CHD. Elevation of blood tHcy occurs in the oral methionine loading test, in which a large dose of methionine (0.1 g/kg body weight of L-methionine) is ingested to diagnose hyperhomocysteinemia [2]. Thus, it could be speculated that a long-term moderately high dietary methionine intake could lead to a modest but chronic elevation in blood tHcy concentrations. In our study, however, plasma tHcy was inversely associated with methionine intake. In addition, in a recent randomized controlled trial, a six-month high-protein, high-methionine diet did not raise plasma tHcy concentrations compared with a low-protein, low-methionine diet [17]. Similar results have been observed in other studies [18—22]. It is not clear what causes the lack of association between methionine or protein intake and blood tHcy. One hypothesis is that the enzymes in the methionine cycle (Fig. 1) adapt to the high methionine intake and thus maintain a normal concentration of Hcy in circulation [17].

There are several prospective studies concerning the association between plasma/serum tHcy and the risk of CHD [3,4]. Although case-control studies suggest that tHcy is a strong and graded risk factor for CHD, it is still uncertain whether it is a risk factor itself or just a marker of some other causal risk factor [23]. Since in our recent study tHcy was not found to be associated with the risk of acute coronary events [24], it seems that tHcy is not a risk factor in this study population. Unfortunately, the number of tHcy measurements available for the present study was low and the subjects were not randomly selected (a nested case-control design), which makes it difficult to draw any conclusions about relationships between methionine intake and tHcy. Lack of relationship between methionine intake and plasma tHcy could also be explained by the higher intakes of vitamins B_6 and B_{12} in the highest methionine intake quarter. Deficiencies in these vitamins have been associated with higher tHcy levels [1].

Although the possible atherogenic effect of dietary methionine intake in humans is still to be determined, there is considerable evidence from animal experiments. Feeding animals a methionine-rich diet has induced atherosclerosis independently of high plasma tHcy [25,26]. In one study ApoE-deficient mice were fed with experimental diets designed to achieve three different conditions: (a) high methionine intake with normal blood tHcy, (b) high methionine intake with B vitamin deficiency and hyperhomocysteinemia, and (c) normal methionine intake with B vitamin deficiency and hyperhomocysteinemia [25]. Hyperhomocysteinemia alone did not contribute to the atherosclerotic process, but mice fed a methionine-rich diet had significant atheromatous pathology even with normal plasma tHcy levels. In another study pigs were fed a methionine-rich diet, which induced hyperhomocysteinemia and atherosclerosis [26]. However, although folate supplementation normalized plasma tHcy levels, it did not prevent the methionine-induced arterial lesions. The mechanisms by which methionine could contribute to the progression of atherosclerosis are not fully understood. However, it has been suggested that the atherogenic effects are mediated by stimulation of free radical and lipid peroxidation processes [27].

Nevertheless, as the main dietary sources of methionine are animal products, we cannot fully exclude the possibility that high methionine intake could also be only a marker of an unhealthy diet that contains low amounts of fruits and vegetables and moderate to high amounts of animal products accompanied with saturated fat. However, since the covariates used in the statistical models included saturated fat intake and the most important known protective and risk increasing dietary factors for CHD, it is unlikely that the methionine intake would be only a marker of unhealthy diet. Also, the higher consumption of vegetables, fruits and berries and higher intakes of folate, vitamin E, vitamin C, fiber and beta-carotene in the highest methionine intake quarter speak against the

marker theory. The higher plasma ascorbic acid concentration in the highest vs. lowest methionine intake quarter reflects most likely higher fruit, berry and vegetable intake, since use of vitamin supplements is very low in this study population. Furthermore, the use of vitamin supplements did not differ between the quarters of methionine intake (Table 1).

The inverse association between energy adjusted methionine intake and saturated fat, as seen in Table 1, indicates that although the absolute saturated fat intake increases with increasing methionine intake, its relative proportion of total energy intake decreases. That may indicate that the subjects who consumed more methionine also consumed more energy, but chose protein (and thus methionine) sources with low saturated fat content. Interestingly, total protein intake was not associated with increased risk of acute coronary events, but rather tended to modestly decrease the risk, consistent with previous findings in women [28]. The risk was further decreased when the intake of methionine was taken into account in the models, which supports our findings of the risk increasing effects of high methionine intake. This could also explain why the increased risk associated with high methionine intake became more apparent only after total protein intake (excluding methionine) had been taken into account in the statistical models. In other words, the risk reduction associated with total protein intake masks the risk associated with its minor component, methionine. There is limited epidemiological data available about dietary protein intake and risk of CVD in addition to the findings by Hu and colleagues [28]. Very low levels of animal protein intake have been associated with increased risk of hemorrhagic stroke in women [29], and low total intake of protein with hemorrhagic stroke in Japan [30]. Furthermore, a significant inverse association between dietary protein and blood pressure in both sexes has been found in a meta-analysis of cross-sectional studies [31]. In addition, recent studies have found that short-term high protein diets decrease triglyceride and LDL cholesterol and increase HDL cholesterol concentrations or have no significant effect on blood lipid measures, although the concurrent weight loss may explain at least part of the effect [32]. However, it seems that high protein diets may be beneficial for cardiovascular health, although longer-term studies are needed for more definite evidence.

In this study dietary methionine and protein intakes were assessed with a single four-day food record at the study baseline. The number of days needed for a reliable estimation of protein intake is 5—7 days [33] and for single amino acids probably even longer. It is important that a method measuring dietary intake concerns an extended period of time, since the diet of most subjects is complex and varies over time. However, a sufficient sample size should reveal any important relationship between a dietary factor and disease [34]. Although four days may be too short a time to estimate the exact long-term methionine intake for each individual, we found a significant correlation between methionine intake assessed by a four-day food record at the KIHD Study baseline and at one-year follow-up ($r=0.36$, $P=0.023$).

In conclusion, the results of this study support the idea that high dietary methionine intake may increase the risk of acute coronary events, although high total protein intake was associated with decreased risk. In addition, it may be that methionine contributes to the atherosclerotic processes through other mechanisms than by increasing plasma tHcy concentrations, since in this study population tHcy has not been found to increase the risk of CHD. Additional prospective epidemiological studies in different populations concerning the effects of dietary components on homocysteine metabolism and subsequently on CVD are needed.

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