Effect of protein and methionine intakes on plasma homocysteine concentrations: a 6-mo randomized controlled trial in overweight subjects[1–3]

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ABSTRACT

Background: A high plasma homocysteine concentration is an independent risk factor for cardiovascular disease. Homocysteine concentrations are thought to be raised by high protein and methionine intakes.

Objective: Our goal was to investigate the effects of high and low protein and methionine intakes on homocysteine in overweight subjects.

Design: Sixty-five overweight subjects were randomly assigned to a 6-mo intervention with a low-protein, low-methionine diet (LP: 12% of total energy, 1.4 g methionine/d; $n = 25$); a high-protein, high-methionine diet (HP: 22% of total energy, 2.7 g methionine/d; $n = 25$), both of which had similar fat contents (30% of total energy); or a control diet with an intermediate protein content ($n = 15$). All food was self-selected at a shop at the department. Protein intake was increased in the HP group mainly through lean meat and low-fat dairy products. Dietary compliance was evaluated by urinary nitrogen excretion.

Results: Homocysteine concentrations did not change significantly in the LP or control groups but were 25% lower in the HP group (NS). Homocysteine concentrations after the 3-mo intervention were inversely associated with vitamin B-12 intake and with weight change (by multivariate analysis performed for all subjects), but not with methionine or protein intake. Sixty-nine percent of the variation could be explained by baseline homocysteine ($P < 0.001$), 2% by vitamin B-12 ($P = 0.02$), and another 2% by weight change ($P = 0.06$). The plasma homocysteine concentration after 6 mo was associated only with baseline homocysteine ($P < 0.001$).

Conclusion: A high-protein, high-methionine diet does not raise homocysteine concentrations compared with a low-protein, low-methionine diet in overweight subjects.   *Am J Clin Nutr* 2002;76:1202–6.

KEY WORDS   Protein, methionine, homocysteine, diet, obesity, cardiovascular disease

INTRODUCTION

The prevalence of overweight and obesity is already high and is escalating in most parts of the world. Both overweight and obesity are accompanied by an increased risk of diabetes, cardiovascular disease, and premature death (1). Fat-reduced diets have been shown to induce spontaneous weight loss in overweight and obese subjects after a few weeks of intervention (2–4). One study showed that high-protein diets may induce weight loss in adults as a result of a higher satiating effect and a greater diet-induced energy expenditure than seen with carbohydrate diets (5). However, the results of methionine-loading tests suggest that protein, especially animal protein (which has a high content of methionine), may raise the plasma homocysteine concentration, an independent risk factor for cardiovascular disease (6). Dietary methionine is needed to synthesize plasma homocysteine (7–9).

Small increases in plasma homocysteine concentrations have been shown to be associated with increased risk of cardiovascular disease (10–12). Moreover, the results of a recent intervention trial showed that lowering homocysteine concentrations through dietary supplementation with a combination of folic acid, vitamin B-12, and pyridoxine decreased the rate of restenosis and the need for revascularization after coronary angioplasty (13).

Apart from a short-term intervention study (14), however, the associations between protein intake and plasma homocysteine concentrations have been investigated only in observational studies (6, 15, 16). The results of these studies are conflicting (6, 14–16). The effect of protein and methionine intakes on the plasma homocysteine concentration has not been investigated in a long-term intervention study. The present study investigated the effects of 6 mo of a high-protein, high-methionine diet and a low-protein, low-methionine diet on total plasma homocysteine in overweight and obese subjects.

SUBJECTS AND METHODS

Subjects

Sixty-five overweight and obese [25 < body mass index (BMI; in kg/m^2) < 35] men and women aged 18–56 y were included in

TABLE 1
Baseline characteristics of the study subjects[1]

	Low-protein group (n = 17 F, 6 M)	High-protein group (n = 18 F, 5 M)	Control group (n = 11 F, 3 M)
Sex (% female)	73	78	79
Age (y)	40 ± 2.2[2]	40 ± 2.0	37 ± 2.3
BMI (kg/m^2)	30.7 ± 0.4	30.0 ± 0.4	30.2 ± 0.7

[1] There were no significant differences between the groups by ANOVA.
[2] $\overline{x}$ ± SEM.

the study. Before inclusion, the subjects underwent a screening examination by a physician, which included a medical history, a routine physical examination, and blood tests. The tests were performed to ensure that no current or previous disorders, primarily concerning renal function, metabolic diseases, and cardiovascular disease, were discernable. All women were premenopausal. All the subjects had been weight stable, with no weight changes greater than ± 1 kg for ≥ 2 mo before enrollment. The subjects were randomly assigned to 2 dietary intervention groups (25 subjects in each group) or to a control group (15 subjects). To ensure group matching with respect to BMI, sex, age, and smoking habits, an independent third party who did not know the subjects or their identity reallocated 6 subjects.

A total of 60 subjects completed the trial, 23 in each intervention group and 14 in the control group. Two subjects dropped out of each intervention group because of a change of address or noncompliance. One subject was excluded from the control group because of surgery. The sex, age, and BMI of the remaining subjects are given in **Table 1**.

Approval was obtained from the Municipal Ethical Committee of Copenhagen and Frederiksberg, and the study was performed in accordance with the Helsinki II Declaration. Each subject signed an informed consent document before the start of the study.

Study design

Physical characteristics, habitual dietary intake, and total plasma homocysteine were measured at baseline. The study was conducted as a 6-mo, controlled dietary intervention study. The subjects were randomly assigned to either a low-protein, low-methionine diet (LP: 12% of total energy, 1.4 g methionine/d); a high-protein, high-methionine diet (HP: 22% of total energy, 2.7 g methionine/d), both of which were low in fat (30% of total energy); or to a control diet (habitual ad libitum diet). The macronutrient composition of the diet in the intervention groups was strictly controlled, but energy intake was unrestricted. All the food in the study period was collected from a shop at the department. The subjects were instructed not to change their physical activity pattern or smoking habits during the study.

Diets

The compositions of the diets are shown in **Table 2**. The subjects shopped twice a week, on average, with an interval of 3–4 d, and prepared the food at home. Food items could be chosen ad libitum within the framework of the project. Subjects were asked to obtain all their foods, including "empty" calories, and energy-containing beverages (excluding alcohol) at the shop. Any deviations were recorded analogous to recording of food waste, leftovers, food preparation method, and alcohol intake, weighed to the nearest 1 g. Uneaten foods and leftovers were taken into

TABLE 2
Dietary intakes at baseline and after 3 and 6 mo of the intervention[1]

	Low-protein group (n = 23)	High-protein group (n = 23)	Control group (n = 14)
Energy (MJ/d)			
Baseline	10.2 ± 0.5[a]	9.3 ± 0.5[b]	11.1 ± 0.6[a]
3 mo	12.0 ± 0.5[a]	9.6 ± 0.4[b]	10.5 ± 0.8[a]
6 mo	12.5 ± 0.6[a]	11.6 ± 0.5[a]	10.0 ± 0.6[b]
Carbohydrate (% of total energy)			
Baseline	42.8 ± 1.4	44.3 ± 1.0	46.1 ± 1.9
3 mo	57.8 ± 0.6[a]	45.4 ± 0.6[b]	46.3 ± 1.3[b]
6 mo	59.3 ± 0.7[a]	45.5 ± 0.5[b]	43.2 ± 1.7[b]
Fat (% of total energy)			
Baseline	36.2 ± 1.0[a]	31.5 ± 2.2[b]	35.1 ± 1.6[a]
3 mo	26.3 ± 0.4[c]	28.4 ± 0.4[b]	35.9 ± 1.2[a]
6 mo	25.5 ± 0.4[c]	28.1 ± 0.5[b]	37.8 ± 1.4[a]
Alcohol (% of total energy)			
Baseline	5.1 ± 0.9	4.9 ± 0.7	3.9 ± 1.0
3 mo	3.4 ± 0.6	3.1 ± 0.5	3.1 ± 0.8
6 mo	2.8 ± 0.4	3.4 ± 0.6	4.3 ± 0.9
Protein (% of total energy)			
Baseline	14.8 ± 0.5	15.6 ± 0.7	13.8 ± 0.6
3 mo	11.6 ± 0.2[c]	22.1 ± 0.3[a]	13.7 ± 0.8[b]
6 mo	11.6 ± 0.1[c]	22.1 ± 0.2[a]	13.6 ± 0.6[b]
Methionine (g/d)			
Baseline	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
3 mo	1.4 ± 0.1[b]	2.7 ± 0.1[a]	1.6 ± 0.1[b]
6 mo	1.5 ± 0.1[b]	3.0 ± 0.1[a]	1.5 ± 0.1[b]
Folate (μg/d)			
Baseline	281 ± 49	276 ± 15	278 ± 16
3 mo	540 ± 38[a]	441 ± 40[b]	256 ± 23[c]
6 mo	562 ± 39[a]	450 ± 22[b]	244 ± 20[c]
Vitamin B-12 (μg/d)			
Baseline	7.2 ± 1.0	5.3 ± 0.5	5.6 ± 0.7
3 mo	5.0 ± 0.5[b]	10.3 ± 1.3[a]	4.6 ± 0.5[b]
6 mo	6.0 ± 0.8[b]	9.8 ± 0.7[a]	4.3 ± 0.3[b]
Vitamin B-6 (mg/d)			
Baseline	1.5 ± 0.2	1.3 ± 0.1	1.4 ± 0.1
3 mo	2.6 ± 0.1[a]	2.4 ± 0.2[a]	1.2 ± 0.1[b]
6 mo	2.8 ± 0.2[a]	2.8 ± 0.1[a]	1.3 ± 0.1[b]

[1] $\overline{x}$ ± SEM. Means in the same row with different superscript letters are significantly different, $P < 0.05$. Time-by-treatment interactions were significant for all variables, $P < 0.01$.

account in the calculation of the energy content of the actual selection on each shopping visit. The control group was instructed to continue their habitual diet.

Shop diet

A 50-m^2 purpose-built shop was established at the department for the 2 intervention groups. The experimental shop facility was described in detail in an earlier publication (17). Opening hours were from 0730 to 1900 every weekday. Approximately 900 different food items were stocked, covering most common foods, and the selection was varied seasonally. Protein sources were primarily dairy and meat products; the latter consisted mainly of beef, pork, poultry, lamb, and fish. Individual preferences were met whenever sufficient information about the energy and macronutrient composition of the desired food item could be obtained. A computerized system was developed to register the selections made at each visit to the shop. All food items were bar-coded, and

the bar codes of the items selected were scanned by a dietitian to monitor the macronutrient distribution of the selection. When necessary, the dietitian assisted in altering the selection made to meet the required macronutrient distribution. The calculated energy content of the groceries selected was not made known to the subjects. The control group did not collect their food from the study shop, but continued to buy their food as normal.

Micronutrient and macronutrient intake, including registered alcohol consumption, was calculated by using the DANKOST 2000 and DANKOST 3000 software packages (National Food Agency of Denmark, Søborg, Denmark) and a database specific for the Danish diet (Levnedsmiddeltabeller; National Food Agency of Denmark). Calculations for the intervention groups were based on 7-d weighed-food records completed at baseline and on 14-d dietary records completed during the intervention. A 7-d dietary record was completed by the control group during the intervention. Intakes of vitamin and mineral supplements were included in all calculations.

Compliance with the diets was measured by 24-h urinary nitrogen excretion. Urine was collected at baseline and at 3 and 6 mo of the intervention.

Primary effect measures

The primary effect measure in the present study was change in total plasma homocysteine concentration. Results concerning dietary effects on body size, body composition, blood lipids, renal function, and compliance with the diet are reported elsewhere (17–19).

Measurement of plasma homocysteine

At baseline and after 3 and 6 mo of the intervention, venous blood samples were drawn into EDTA-containing tubes from an antecubital vein after the subjects had fasted overnight. Plasma was separated from blood cells by centrifugation at $3000 \times g$ at $20\,°C$ for 10 min within 1 h after sampling. Plasma was stored at $-80\,°C$ before analysis at the Department of Clinical Biochemistry, University Hospital of Aarhus. The total plasma homocysteine concentration was measured by using gas chromatography–mass spectrometry, with stable isotope dilution after reduction with dithiothreitol and solid-phase extraction (20). The interassay CV for the analysis was 5%, and the intraassay was 3%. The laboratory took part in an external proficiency testing protocol for this analyte.

Statistics

Unless otherwise stated, results are given as means ± SEMs. The significance level was set at $P < 0.05$. Data were normally distributed within the subgroups (HP, LP, and control). Test of normality for the pooled data for all 3 groups showed that the pooled data were not normally distributed. However, normal distribution of absolute homocysteine and intakes of vitamins were achieved by transformation to the inverse square root. Unless otherwise stated, all statistical tests were performed by using the computer software SAS (version 6.12; SAS institute Inc, Cary, NC).

Changes from baseline in protein intake as a percentage of total energy, methionine intake, B vitamin intake (all B vitamins including supplemental B vitamin intake), body weight, and total plasma homocysteine within groups were tested by using analysis of covariance with the baseline value as the covariate. Differences in protein intake as a percentage of total energy, methionine intake, B vitamins (all B vitamins including supplemental B vitamin intake), body weight, and total plasma homocysteine concentrations between the groups were tested by analysis of variance (ANOVA), with adjusted P values corresponding to multiple comparisons of least-squares means with the use of the Student-Neuman-Keuls test and Bonferroni correction. A two-factor repeated-measures ANOVA was conducted to assess the effects of a diet-by-time interaction. Linear regression analysis was performed to investigate the influence of possible confounders (change in body weight during the intervention, total plasma homocysteine concentration at baseline, intakes of B vitamins from foods and supplements, and intake of alcohol as a percentage of total energy) on the association between total plasma homocysteine and protein (as a percentage of total energy) and methionine intakes for all subjects together. This analysis was carried out in a stepwise manner, excluding nonsignificant factors.

RESULTS

Analyses of 24-h urinary nitrogen excretion showed the subjects' compliance with the intervention diets to be high. Twenty-four–hour urinary nitrogen increased in the HP group and decreased in the LP group ($P = 0.0001$), with no significant changes in the control group. The group differences remained throughout the intervention and were parallel to the changes in protein intake. These results are published in detail elsewhere (18). Weight loss in the HP group was larger than that in the LP group (9.4 compared with 5.9 kg; $P = 0.0003$).

No group differences in dietary intake of B vitamins or protein were observed at baseline, but significant time-by-treatment interactions were found for all intake variables reported in Table 2. After 3 and 6 mo, there were differences between all the groups in protein intake as a percentage of total energy: at 3 mo, LP versus HP and HP versus control, $P = 0.0003$, LP versus control, $P = 0.006$; at 6 mo, HP versus LP versus control, $P = 0.0003$. Methionine intake after 3 mo increased in the HP group (55%; $P = 0.0003$), tended to decrease in the LP group (-24%; $P = 0.06$), and remained unchanged in the control group. After 3 mo, the HP group had a higher intake of methionine than did the LP and control groups ($P = 0.0003$), whereas no significant differences were observed between the control and LP groups. Methionine intake in the HP group increased further from 3 to 6 mo (14%; $P = 0.03$). Group differences remained at 6 mo (HP versus LP and control, $P = 0.0003$).

Folate intake increased after 3 mo of the intervention in both the HP (60%; $P = 0.0003$) and LP (92%; $P = 0.0003$) groups and remained unchanged in the control group (Table 2). After 3 mo, the HP and LP groups both had higher intakes of folate than did the control group ($P = 0.006$ and $P = 0.0003$, respectively), whereas no significant differences were observed between the LP and HP groups. There were no changes in folate intake in any of the groups from 3 to 6 mo of the intervention. However, differences between all the groups were observed after 6 mo (HP and LP versus control, $P = 0.0003$; HP versus LP, $P = 0.02$).

Vitamin B-12 intake decreased in the LP group (-49%; $P = 0.0003$) and increased in the HP group (84%; $P = 0.0003$) after 3 mo of the intervention. After 3 mo, there were group differences between the LP and HP groups and between the HP and control groups ($P = 0.0003$ and $P = 0.0009$, respectively). There were no significant changes in vitamin B-12 intake in any of the groups from 3 to 6 mo. Group differences remained at 6 mo (LP versus HP and HP versus control, $P = 0.0003$).

TABLE 3
Mean total plasma homocysteine at baseline and after 3 and 6 mo of the intervention[1]

	Low-protein group (n = 23)	High-protein group (n = 23)	Control group (n = 14)
	μmol/L		
Baseline	10.9 ± 1.5	12.1 ± 2.3	10.8 ± 1.4
3 mo	10.6 ± 0.7	9.7 ± 0.7	9.8 ± 1.0
6 mo	10.4 ± 0.8	9.6 ± 0.7	10.3 ± 1.0

[1] $\bar{x}$ ± SEM. There were no significant differences between groups by ANOVA.

No significant differences in vitamin B-6 intake were seen between the HP and LP groups after 3 and 6 mo (Table 2), but the control group had a lower intake than did the 2 intervention groups ($P = 0.0002$). Vitamin B-6 intake increased from baseline to month 3 in the HP (77%; $P = 0.0002$) and LP (73%; $P = 0.0002$) groups. No significant changes were seen in any of the groups from 3 to 6 mo of the intervention.

A nonsignificant decrease in total plasma homocysteine ($\approx 25\%$) was observed in the HP group after 3 and 6 mo of the intervention (**Table 3**). No significant changes were observed in the LP or the control groups, and there were no significant differences between the groups. A two-factor repeated-measures ANOVA showed no diet-by-time interaction ($P = 0.233$).

We conducted a multivariate analysis on all the subjects in the study with homocysteine as the dependent variable and with weight change, folate, and intakes of riboflavin, vitamin B-6, and vitamin B-12 as independent variables. The homocysteine concentration after 3 mo of the intervention showed a significant positive association with baseline homocysteine and an inverse relation with vitamin B-12 and weight change. Sixty-nine percent of the variation could be explained by baseline homocysteine ($P < 0.001$), 2% by vitamin B-12 ($P = 0.02$), and another 2% by weight change ($P = 0.06$). The plasma homocysteine concentration after 6 mo was associated with baseline homocysteine only ($P < 0.001$).

DISCUSSION

The results of the present 6-mo intervention study clearly show that increasing dietary protein intake from 12% to 22% of total energy, with a corresponding increase in methionine intake, does not increase plasma homocysteine concentrations. Our finding is partially in agreement with the inverse association between dietary protein intake and plasma homocysteine concentration shown in a recent observational study by Stolzenberg-Solomon et al (6), although our study may have lacked the statistical power to detect the effect. The association remained after adjustment for folate and vitamin B-6 intake, and the association was also observed in the subset of subjects who had been taking a multivitamin supplement containing folate, vitamin B-12, and vitamin B-6 for 2–4 mo (16). Because these B vitamins are key players in homocysteine metabolism, we found it relevant to adjust the relation for such possible confounders, in particular for changes in body weight and intakes of vitamin B-12, vitamin B-6, and folate (7, 16, 21–23). We found that only vitamin B-12 and weight loss were inversely associated with homocysteine concentrations after the 3-mo intervention, whereas these predictors were not statistically significant after 6 mo. Our findings agree with those of a large

observational study by Shimakawa et al (15), who found that plasma homocysteine was inversely associated with intakes of folate, vitamin B-6, and vitamin B-12 but not with intakes of methionine and protein (15).

The sources of protein in the present study were primarily of animal origin (eg, dairy products, red meat, chicken, and fish) and consequently had a high methionine content. Previous short-term studies showed that methionine loading and protein loading induce hyperhomocysteinemia (14, 24, 25). Guttormsen et al (14) found that protein-rich breakfast and dinner meals produced acute diphasic changes in plasma methionine and homocysteine concentrations. However, it is difficult to compare the results of these studies with ours because the acute catabolic effects of protein and methionine intakes may be different from chronic effects after 6 mo of intake of a high-protein, high-methionine diet. The mechanisms behind the lack of association between protein intake and homocysteine concentration are not clear. An ex vivo animal study showed that a high methionine intake induces a more efficient homocysteine metabolism, as a result of an adaptive activation of the enzymes involved in the methionine cycle (26). This finding is supported by human studies assessing the effect of *S*-adenosyl-methionine loading, which showed that increases in plasma concentrations of 5-methyltetrahydrofolate are a marker of an increased rate of remethylation (27, 28). The results of our study support the hypothesis that an adaptation to a high methionine intake induces a more efficient homocysteine metabolism, thereby maintaining a normalized concentration of homocysteine in plasma. However, the mechanisms whereby protein intake may produce a low plasma homocysteine concentration should be investigated further, especially if further intervention studies confirm that lowering of plasma homocysteine reduces the development and progression of cardiovascular disease (13).

The major finding of the present study is that a diet rich in protein from lean meat and low-fat dairy products does not increase plasma homocysteine concentrations over 6 mo. That the high and low protein intakes were actually achieved in the 2 groups is convincingly shown by the changes in urinary nitrogen excretion during the 6-mo intervention. Thus, compliance with the dietary interventions was high. However, the specific effect of protein and methionine intakes was difficult to assess because this diet also provided a higher intake of vitamins that lower plasma homocysteine and that in theory could counterbalance a putative elevation of homocysteine caused by a high protein intake. ❧

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