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Effect of Supplemental Methionine on Plasma Homocysteine Concentrations in Healthy Men: A Preliminary Study

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Abstract: Hyperhomocysteinaemia is an established risk factor for vascular disease. The only source of homocysteine in humans is the amino acid methionine found in dietary protein. In an 8-week study, fasting plasma homocysteine concentrations were examined in a group of healthy male subjects (n = 6) under usual dietary conditions (weeks 1–4) and in response to weekly graded (25, 50 and 75mg/kg/d) supplementary methionine (weeks 5, 6, 7). Nutrient intakes, including methionine, were calculated from 4×3 day food records. Under usual dietary conditions (mean methionine intake; 0.95 ± 0.51 mg/d) weekly mean plasma homocysteine concentrations for the group were not significantly different (ANOVA) from each other ranging from 6.82 ± 1.77 to 9.42 ± 2.73 μmol/l. Doubling (supplementing with 25 mg/kg/d; + 2.04 g/d) or quadrupling (50 mg/kg/d; + 4.08 g/d) methionine intakes did not result in a significant increase in plasma homocysteine (8.56 ± 3.68 μmol/l and 13.37 ± 5.09 μmol/l respectively). A significant increase, however, was achieved when diets were supplemented with methionine at the highest level of 75 mg/kg/d (+ 6.14 g/d) resulting in a mean plasma homocysteine concentration of 18.05 ± 11.8 μmol/l. Mean plasma homocysteine concentration returned to baseline (8.76 ± 3.42 μmol/l), 10 days post-supplementation. The results of this study indicate that an increased dietary methionine will only cause elevated fasting homocysteine concentrations if ingested at intakes equivalent to five times usual intake. Because it is very unlikely that such levels could be achieved through dietary means alone we conclude that plasma homocysteine is unlikely to be affected by longer-term changes in food methionine intake.

Key words: Human, supplementary methionine, chronic, intervention, homocysteine

Abbreviations used: SAM, s-adenosylmethionine; MTHFR, 5,10-methylenetetrahydrofolate reductase; CβS, cystathionine-β-synthase; SF, serum folate; RCF, red-cell folate; BMI, Body Mass Index.

Introduction

A moderately increased plasma homocysteine concentration (hyperhomocysteinemia) is a strong independent risk factor for vascular disease in the general population [1–3]. A deficiency of one or more of the B-vitamins (folate, B_{12} or B_6), which serve as co-factors in the principal enzymatic pathways of homocysteine metabolism is the most common cause of elevated homocysteine [4]. Intervention with folic acid alone or in combination with vitamins B_{12} and B_6, is well established as being a very effective means of lowering homocysteine concentration [5, 6]. The possibility that plasma homocysteine is influenced by nutrients other than the B-vitamins is not well understood.

The only dietary source of plasma homocysteine is the amino acid, methionine, which is rich in protein of animal origin. Methionine is activated by ATP to yield the methyl donor S-adenosylmethionine (SAM) under normal dietary conditions [7]. SAM influences the metabolic co-ordination of remethylation and transsulfuration pathways by allosterically inhibiting the enzyme 5,10 methylenetetrahydrofolate reductase (MTHFR; required for the production of the methyl donor 5-methyltetrahydrofolate) and by activating cystathionine-β-synthase (CßS; a co-enzyme required for the condensation of homocysteine to serine in the transsulfuration pathway) [7]. When SAM levels are low, remethylation is enhanced and cystathionine formation is reduced. In contrast, elevated levels of SAM promote transsulfuration while inhibiting remethylation [7].

The acute response of plasma homocysteine to methionine is routinely assessed in the methionine load test. The aim of the methionine-load test is to stress the homocysteine metabolising pathways in order to investigate the response of plasma homocysteine 4 to 6 hours after a large dose of methionine (usually 100 mg/kg body weight) has been administered orally [8, 9]. Results provide information on disturbances in the transsulfuration pathway and the acute response of plasma homocysteine to increased methionine [10].

The ingestion of a methionine-rich meal is also known to result in a transient rise in homocysteine concentration, which peaks after 8 hours [11]. Little information, however, is documented on the intermediate to long-term effect of an increased methionine intake on homocysteine concentration. Several authors have proposed that variations in homocysteine concentration could be related to methionine intake [12, 13] although to our knowledge, this has never been confirmed in a human intervention study. Therefore, the effect of dietary methionine on homocysteine remains unknown. The aim of this study was to investigate the response of plasma homocysteine to longer-term chronic intervention with supplementary methionine at doses of relevance to usual dietary intake and above.

Subjects and methods

Subject recruitment

Healthy male volunteers, aged 20 to 30 years (n = 6), were recruited from the student population at the University of Ulster, Coleraine. Prior to commencing the study, all subjects were asked to complete a brief medical questionnaire and give their written informed consent. Subjects were excluded if they were found to be suffering from vascular, hepatic, renal disease or haematological disorders. Ethical approval for the study was granted by the Research Ethical Committee at the University of Ulster. Prior to recruitment and throughout the study period, none of the subjects was reported to be taking any form of medication or B-vitamin supplement.

Study design

Weeks 1 to 4 of the eight-week study represented the baseline period; during this time subjects were instructed to continue their habitual diet and weekly fasting blood samples were taken. On the three days immediately prior to each sample, food diaries were completed by subjects. Blood samples were taken on different days each week to account for any possible day-of-the-week effects on food and nutrient intakes. Supplementation began at week 5; subjects ate their usual breakfast and approximately two hours later (under supervision) received L-methionine (Scientific Hospitals Supply) orally in an orange drink (200 mls) at a dose of 25 mg/kg/d, which represented approximately double their usual intake. This was continued daily for one week. At week 6, the daily dose of L-methionine was increased to 50 mg/kg/d and continued for a further week. A final dose of 75 mg/kg/d was administered during week 7. At the end of weeks 5, 6 and 7 fasting blood samples were collected. A final fasting blood sample was taken 10 days post-completion of the intervention phase.

Biochemical measurements

Fasting venous blood samples (20 ml) were collected. Whole blood was collected into evacuated tubes containing EDTA for plasma homocysteine, whole blood folate and full blood count analysis, and into non-heparinised integrated serum separator tubes for serum folate (SF) and serum vitamin B_{12} determination. Samples collected for homocysteine analysis were placed immediately on ice until centrifuged (within 1 hour). Following centrifugation at 2000 g for 15 minutes, separated plasma and serum samples were stored at –20°C until analysis. Lysates of red cell folate (RCF) were prepared by dilution (1 in 10) of whole blood with freshly prepared ascorbic acid (1%). Aliquots were mixed thoroughly on a roller mixer, incu-

bated at 37°C for 30 minutes, and stored at –20°C. Full blood count analysis was carried out by automated coulter counter at Causeway laboratories, Coleraine on the day of sample collection.

Analysis for total plasma homocysteine was carried out by isocratic HPLC [14] using ammonium-7-flurobenzone-2-oxa-1,3-diazole-4-sulphonate (SBD-F) [15]. The microbiological assay was used for the determination of SF and RCF [16] and serum B_{12} [17].

Dietary assessment

Dietary intakes were assessed by the three-day food diary method. Prior to the collection of each of the four baseline blood samples, subjects were provided with three-day food diaries and instructed to record all food and beverages consumed using a variety of household measurements. Portion sizes were subsequently quantified using published food portion sizes [18]. Methionine and B-vitamin intakes were calculated using the nutrient analysis programme Comp-Eat (Lifeline; Nutritional Services ltd., England).

Statistical methods

Results are presented as mean and standard deviation. Baseline data were compared and responses to intervention were assessed by one-way analysis of variance (ANOVA), with post-hoc analysis (LSD). Correlation coefficients were calculated using Pearson Product-Moment Correlation statistics. The Data Desk statistics programme for Macintosh, version 4.1 (Data Descriptions) was used for all statistical analyses.

Results

Baseline data

The mean age of subjects was 23.6 years and body mass index (BMI) ranged from 21.4 to 26.2 kg/m^2. Dietary and haematological data for the baseline period (weeks 1 to 4) are presented in Table I. Over the 4-week baseline period, dietary methionine intake, which was highly correlated with total dietary protein ($r = 0.773$, $p \leq 0.001$), did not alter significantly with methionine intakes (mean ± SD; g/d) for the group ranging from 1.22 ± 0.42 in week 1 to 0.74 ± 0.75 in week 4.

The plasma homocysteine concentration (mean ± SD) for week 1 was 9.42 ± 2.73 µmol/l, a value which was not significantly higher than the mean values reported for weeks 2, 3 and 4 (Table I). No significant correlation was found between plasma homocysteine and SF, RCF or serum B_{12}. Plasma homocysteine values over the baseline period were significantly correlated with dietary methionine intake ($r = 0.443$, $p = 0.03$) but not with total dietary protein or any other dietary variable examined. All subjects had normal B-vitamin status as determined by dietary and biochemical data (Table I).

Response to intervention

Mean supplementary methionine doses during the intervention period were 2.04, 4.08 and 6.14 g/d in weeks 5, 6 and 7 respectively, corresponding to the supplementary levels of 25, 50 and 75 mg/kg/d. These doses, together with usual dietary methionine, yielded total mean methionine intakes (g/d) of 2.99, 5.01 and 7.07 (Fig. 1). The effect of methionine supplementation on plasma homocysteine concentration is summarised in Figure 1. Responses to intervention were assessed (ANOVA) using a baseline value of 7.46 µmol/l derived from the mean of the values obtained in weeks 1, 2, 3 and 4 (Fig. 1). In response to the lowest supplementary dose of methionine (given daily for a week), no significant increase in plasma homocysteine (mean ± SD) was observed (week 5) with a value of 8.56 ± 3.68 µmol/l reported. Plasma homocysteine appeared to rise (5.91 µmol/l increase from baseline; not significant) when the dose of supplementary methionine was increased to 50 mg/kg/d for a further week. Intervention with the highest dose (75 mg/kg/d) for the

Table I: Dietary data and Laboratory indices of subjects (n=6) for the baseline period (weeks 1 to 4)

	Week 1	Week 2	Week 3	Week 4	*P* value
Dietary data					
Methionine (g/d)	1.23 ± 0.42	0.89 ± 0.59	0.99 ± 0.43	0.75 ± 0.43	0.4564
Folate (µg/d)	438 ± 196	319 ± 177	362 ± 180	348 ± 131	0.7478
Vitamin B_{12} (µg/d)	6.63 ± 1.4[a]	4.46 ± 1.2[b]	4.51 ± 1.31[b]	4.28 ± 1.31[b]	0.0170
Vitamin B_6 (mg/d)	4.15 ± 1.09	2.5 ± 1.34	2.96 ± 1.38	2.99 ± 0.95	0.1410
Protein (g/d)	102 ± 20	88 ± 20	89 ± 23	85 ± 17	0.4693
Laboratory indices					
Plasma Homocysteine (µmol/l)	9.42 ± 2.73	6.83 ± 1.30	6.82 ± 1.77	7.05 ± 1.74	0.0900
Serum folate (µg/l)	15.5 ± 5.44	14.02 ± 3.33	13.18 ± 2.58	14.50 ± 4.03	0.7768
Serum B_{12} (ng/l)	542 ± 222	555 ± 253	541 ± 207	534 ± 254	0.9989

Values are expressed as mean ± (SD;
[ab] Values across rows with different superscripts are significantly different (ANOVA, $P < 0.05$; LSD).

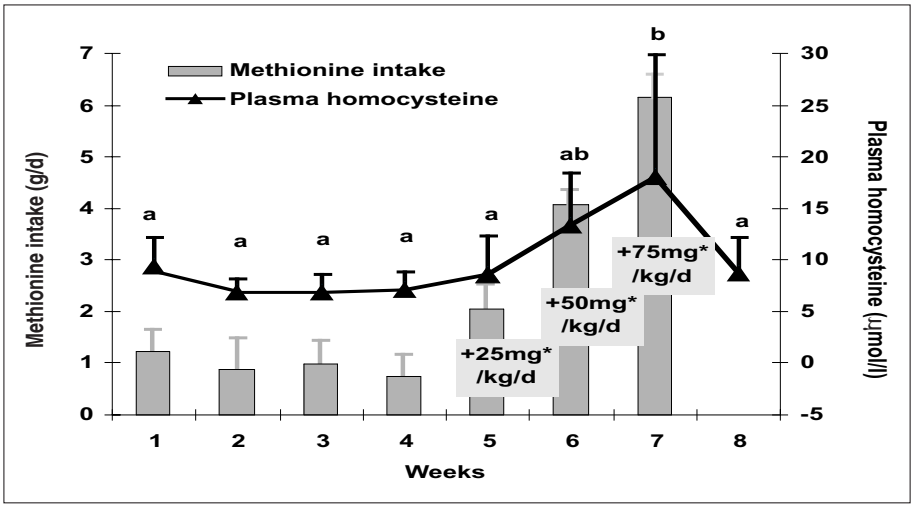

Figure 1: Plasma homocysteine (µmol/l) over time under normal dietary conditions (weeks 1 to 4), in response to methionine supplementation at increasing doses of 25, 50 and 75 mg/kg/d (weeks 4 to 7) and post-supplementation (week 8) in healthy male subjects (n = 6). Values are expressed as mean ± sd.
* mg L-methionine (Scientific Hospitals Supply)
[ab] Values with different superscripts are significantly different (ANOVA) ($p < 0.05$; LSD).

final week, however, resulted in a significant increase (10.59 µmol/l increase from baseline) compared with either the baseline or week 5 value but the increase from week 6 (4.68 µmol/l) was not significant. By 10 days post-supplementation, plasma homocysteine concentration had returned to a value (8.76 ± 3.42 µmol/l) not significantly different from baseline (Fig. 1). As expected over the 7-week study period SF concentration (11.8 ± 4.88 to 14.3 ± 4.90 µg/l), and serum vitamin B_{12} concentration (507 ± 140 to 543 ± 228 ng/l) remained unchanged.

Discussion

In the present study, we evaluated the potential role of methionine intake as a determinant of plasma homocysteine concentration, by investigating the response of plasma homocysteine to normal dietary methionine fluctuations and to supplementary methionine at increasing doses, in a group of healthy males with no evidence of elevated plasma homocysteine concentration.

Under habitual dietary conditions with reported methionine intakes ranging from 0.22 to 2.14 g/d, a wide variation in individual methionine intake within the group was observed over the 4-week baseline period. The mean intake for the group (0.95 ± 0.37 g/d) compared well those reported by others [13]. Over the four week baseline period, no significant difference, in plasma homocysteine was noted, suggesting that homocysteine concentration is unaffected by fluctuations in dietary methionine within the normal dietary range. Surprisingly, no significant correlations were observed between homocysteine and dietary or laboratory indices of the B-vitamins (folate and B_{12}) during this period. This lack of association, however, was most likely owing to the small number (n = 6) included in the investigation. Nonetheless, despite the small numbers and contrary to the findings of others [19, 20], a significant correlation was observed between plasma homocysteine and dietary methionine (although not dietary protein) over the baseline period of measurement.

Plasma homocysteine showed no response to chronic supplementation with either the lowest dose of supplementary methionine (25 mg/kg/d; resulting in a total mean intake of 2.99 g/d, more than double usual methionine intake) or a dose of 50 mg/kg/d (which yielded a total methionine intake of 5.01 g/d). This is in agreement with Andersson and colleagues who observed no change in homocysteine concentration in subjects fed supplementary methionine at doses ranging from 1.6 to 2.44 g/d [21]. In the current study there was a significant increase in response to the highest dose of 75 mg/kg/d methionine, which resulted in a total methionine intake of 7.07 g/d during the final week of intervention. This latter dose represented an increase of 500% compared to usual diet and, thus, is unlikely ever to be achieved through dietary means alone. On an individual basis, the response to the lowest supplementary dose of methionine was similar in all subjects. Marked differences in individual response to the higher doses of 50 and 75 mg/kg/d, however, were observed; homocysteine concentrations in some subjects displayed little or no change over the period of intervention while an increase in homocysteine from baseline of 24.0 µmol/l in response to the highest dose of methionine was achieved by one subject. Individual subject differences in terms of ability to maintain plasma homocysteine concentrations in the face of high exogenous methionine may be partly explained by differences in genetic disposition (not measured in this study), namely the MTHFR genotype. Individuals who are homozygous for a common

variant of the MTHFR gene have low enzyme activity and, therefore, have decreased ability to generate 5-MTHFR, the substrate for the remethylation pathway of homocysteine metabolism [22]. Such individuals may therefore have a poorer ability to maintain similar plasma homocysteine concentrations when faced with equivalent methionine intakes to those without the variant. Despite subject difference in response, homocysteine concentration in all subjects had returned to a level not significantly different from baseline by 10 days post-supplementation.

Results of this study show that under normal dietary conditions and in response to increases of up to approximately 200% of usual methionine intake, methionine metabolism appears to be tightly regulated. Therefore while it is possible to achieve significant increases in plasma homocysteine by chronic ingestion of supplementary methionine in some (not all) subjects, the quantity required to bring about such increases is unlikely ever to be achieved by dietary means alone.

In conclusion, results obtained from both the baseline and supplementary methionine intervention periods of this study provide strong evidence to suggest that methionine intake is not a major determinant of plasma homocysteine concentration in healthy male subjects.

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