Variation in plasma cystathionine and its relation to changes in plasma concentrations of homocysteine and methionine in healthy subjects during a 24-h observation period1–3

Anne B Guttormsen, Einar Solheim, and Helga Refsum

ABSTRACT

Background: Plasma cystathionine measurement may be a useful complement to total homocysteine measurement in the assessment of B vitamin status. Information on the within-person variation in cystathionine is currently sparse.

Objective: The goal was to study the daily variation in plasma cystathionine concentrations in healthy subjects.

Design: Twelve subjects (aged 22–29 y) were followed for 24 h. During the observation period, the subjects received a breakfast (containing 15–18 g protein) at 0900 and a beef dinner (containing ~50 g protein) at 1500. Multiple blood samples for metabolite analyses were collected during the day, and a final sample was obtained the next morning. The results are expressed as medians and interquartile ranges.

Results: All subjects had normal fasting cystathionine concentrations [0.120 (0.100–0.160) μmol/L]. Cystathionine concentrations increased significantly after breakfast, reached a maximum after 4 h of 142.4% (100.0–170.3%) of the fasting concentration, and then declined to fasting concentrations before dinner. After dinner, plasma cystathionine started to increase within 0.5 h and reached a maximum after 6 h [281.3% (194.1–351.4%) of the concentration measured before dinner]. The changes in plasma methionine and total homocysteine concentrations during the day were less pronounced.

Conclusion: Food intake, even of foods with low protein content, causes an increase in plasma cystathionine concentrations that is more pronounced than the concomitant changes in total homocysteine and methionine. In studies including plasma cystathionine measurement, blood sampling in the fasting state should be considered. Am J Clin Nutr 2004;79:76–9.

KEY WORDS Cystathionine, homocysteine, diurnal variation, B vitamin status

INTRODUCTION

Cystathionine seems to be a useful marker of B vitamin status. According to Stabler et al (1), serum cystathionine is elevated during folate and cobalamin deficiencies. Furthermore, treatment with theophylline causes cystathionine concentrations to become elevated as a result of vitamin B-6 depletion (2).

Cystathionine is a thioether produced in a reaction in which homocysteine (Hcy) condenses with serine in a pyridoxal phosphate–dependent reaction catalyzed by cystathionine β-synthase (3). This reaction is the first step in the transsulfuration pathway. The second and final step, which cleaves cystathionine into cysteine and α-ketobutyrate, is catalyzed by another pyridoxal phosphate–dependent enzyme, cystathionine γ-lyase. The direction of Hcy into the transsulfuration pathway leads to the irreversible loss of methionine (3).

Two mechanisms regulate methionine-Hcy metabolism: 1) the tissue content of enzymes and their substrates and cofactors and 2) the intrinsic kinetic properties of the enzymes related to methionine and Hcy turnover (3). The enzymes may be categorized as methionine-conserving, low-Michaelis constant (K_m) enzymes or as methionine-catabolizing, high-K_m enzymes. Cystathionine β-synthase is a high-K_m enzyme, which removes excess methionine (3).

In 1988 Storch et al (4) showed that in the fed state, the flux of methionine increases through transmethylation, remethylation, and transsulfuration, and the incorporation of methionine into protein is increased. This had also been shown some years earlier in the elegant and comprehensive rat liver experiments and whole-body human studies conducted by Finkelstein, Mudd, and their colleagues in the 1970s and 1980s (5–8).

Despite the huge interest in Hcy both as a marker of B vitamin status and as a risk factor for cardiovascular disease and cognitive decline, relatively few studies have been published on cystathionine (1, 2, 9–15). In a previous study, we investigated the changes in plasma concentrations of total Hcy (tHcy) during a 24-h observation period (16); in the present article, we present the data for plasma cystathionine for the same study population.

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TABLE 1
Characteristics of the subjects

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<th>Methionine (μmol/L)</th>
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1 tHcy, total homocysteine.
2 Breakfast type: 1, bacon and egg; 2, bread with cheese.
3 A subject with folate deficiency who was excluded from the statistical analyses.

SUBJECTS AND METHODS

Subjects

Thirteen healthy volunteers, 10 women and 3 men aged 22–29 y, participated in the study. All participants except one had normal concentrations of folate, cobalamin, tHcy, and methionine. Compared with previously published data (9, 17), cystathionine concentrations in plasma were normal in all subjects. Subject no. 13 had mild folate deficiency with moderate hyperhomocysteinemia. This subject was excluded from the statistical analyses. All subjects provided written informed consent.

Protocol

The details of the study were published previously (9). Between 0900 and 1000, after an overnight fast, the participants consumed breakfast. Seven of the participants had a conventional Norwegian breakfast with bread, butter, and cheese, and 6 participants had a breakfast of bread, egg, and bacon. Estimated protein intake for both breakfasts was ≈15–18 g. Exactly 6 h after breakfast, a beef dinner with a protein content of ≈50 g was served (9).

Blood samples were obtained before and 0.5, 1, 2, 3, 4, and 6 h after breakfast. The 6-h sample was collected immediately before dinner, and, after dinner, blood was collected at 0.5, 1, 2, 3, 4, 6, and 8 h. Most of the participants had a meal before going to bed, and the next morning a new fasting blood sample was obtained.

Blood sampling and analytic methods

Blood was collected into cooled EDTA-treated evacuated tubes. Plasma for the determination of tHcy, methionine, and cystathionine was prepared by immediately centrifuging the blood at 2000 × g for 5 min at 0–2 °C. Plasma was stored at −20 °C until analyzed. Plasma concentrations of tHcy and methionine were measured by HPLC according to published methods (18, 19).

Cystathionine was analyzed by using a liquid chromatography–mass spectrometry–mass spectrometry method (Gutormsen et al, unpublished observations, 2003). Briefly, after the addition of dithioerythritol and deuterated standards (cystathionine and homocystine), the sample was acid precipitated and the supernatant fluid was injected onto a reversed-phase column. The sulfur amino acids were eluted by using an ethanol gradient in acetic acid and were then detected and quantified by using the transition from the precursor to the product ion for each of the compounds and their deuterated standards. The CV for the method was 5–10%, depending on the concentration of the analytes.

Statistical methods

In both the text and the figures, the results are expressed as medians and interquartile ranges. For comparison between groups, we used the Mann-Whitney U test. Changes in metabolites during the day were compared by using nonparametric tests (Friedman’s nonparametric analysis of variance followed by Wilcoxon’s signed-rank test). Correlation was performed by a Spearman rank correlation test. Variability during the day was calculated by measuring the difference between peak and nadir concentrations relative to the mean (maximum – minimum/average). The significance level was set at 0.05. For calculations and statistical analyses, SPSS version 10 for Macintosh was used (SPSS Inc, Chicago).

RESULTS

The characteristics of the participants and their basal concentrations of cystathionine, tHcy, methionine, folate, cobalamin, and creatinine are listed in Table 1. The median fasting cystathionine concentration was 0.120 (0.100–0.160) μmol/L, which is similar to that observed in other healthy adult populations (1, 9, 17). There was no significant difference in cystathionine response by type of breakfast consumed. Therefore, the data were pooled.
During the 24-h observation period, there was a marked increase in plasma methionine but before the increase in tHcy. Cystathionine started to increase in response to food occurred slightly later than the increase in methionine as does a beef dinner (≈1 g), it seems likely that the cystathionine derived from food causes the observed increase in all 3 compounds. This is supported by the fact that, in the present study, the average relative changes in cystathionine, methionine, and tHcy were much larger after a meal with a high protein content. Furthermore, the temporal pattern is consistent with methionine first being released from protein, followed by an increase in tHcy and cystathionine. We found, however, no correlation between the individual changes in the metabolites. This may reflect that the factors influencing the turnover of these 3 compounds differ and that the rates of metabolism may be tissue specific according to the content of the sulfur amino acid–related enzymes (3). In addition, other components in the meal, such as cysteine and folate, may change the rate of flow through the transsulfuration and remethylation pathways, respectively (3, 4).

During the 24-h observation period, there was a marked within-person variation relative to the mean for methionine (≈80%) and cystathionine (≈130%). In comparison, tHcy varied only ≈25% relative to the mean during the observation period (9). The changes in cystathionine were predominantly explained by food intake. The fluctuations did not correlate with

the observed changes in methionine or tHcy concentrations, nor were they correlated with body weight or serum concentrations of creatinine, folate, or cobalamin. We previously reported changes in cysteine and cysteine in this study population, but these sulfur amino acids responded to food intake with a decline in total plasma concentrations, probably as the result of changes in the plasma thiol redox status induced by homocysteine (16). Their changes in response to food intake did correlate with the observed changes in cystathionine.

Subject no. 13 had mild biochemical folate deficiency, without hematologic or clinical symptoms, that was associated with moderate hyperhomocysteinemia. In contrast with the concentrations reported in other subjects with folate deficiency (1), cystathionine concentrations in this subject were within the normal range. The diurnal fluctuations in cystathionine and tHcy were similar to the responses observed in the other subjects, whereas the methionine response was somewhat lower.

**DISCUSSION**

We investigated cystathionine concentrations during the day and in response to the intake of 2 meals with different protein contents. A low-protein meal, such as a typical Norwegian breakfast, was associated with relatively small but significant changes in methionine, tHcy, and cystathionine. After dinner, which comprised ≈3 times more protein than the breakfast did, plasma tHcy increased only 14.6% relative to predinner concentrations. In contrast, there was a striking increase in cystathionine concentrations of 181.3%. The postprandial time-concentration curves for cystathionine and methionine were similar, although the relative increase in methionine was less impressive.

Diurnal variations in plasma tHcy in response to food intake were reported previously (16, 20, 21). However, data on cystathionine are sparse. In a study by Frontiera et al (22), serum cystathionine was monitored for 48 h in 2 healthy subjects. During the observation period, both subjects showed marked variability in cystathionine, and the authors speculated that this variability was due to food intake or diurnal variations (22).

Hcy is derived from methionine (3), and it is well known that concentrations of both methionine and Hcy increase in response to pure methionine intake, as observed after a methionine-loading test (9, 23, 24). Although that test provides ≈5 times as much methionine as does a beef dinner (≈1 g), it seems likely that the methionine derived from food causes the observed increase in all 3 compounds. This is supported by the fact that, in the present study, the average relative changes in cystathionine, methionine, and tHcy were much larger after a meal with a high protein content. Furthermore, the temporal pattern is consistent with methionine first being released from protein, followed by an increase in tHcy and cystathionine. We found, however, no correlation between the individual changes in the metabolites. This may reflect that the factors influencing the turnover of these 3 compounds differ and that the rates of metabolism may be tissue specific according to the content of the sulfur amino acid–related enzymes (3). In addition, other components in the meal, such as cysteine and folate, may change the rate of flow through the transsulfuration and remethylation pathways, respectively (3, 4).

After dinner, plasma tHcy increased 1.15 times compared with predinner concentrations, whereas cystathionine increased 2.8 times at the most. In comparison, after methionine loading, tHcy increases ≈3 times (24) and cystathionine increases 10-fold.

**FIGURE 1.** Median (interquartile range) changes in plasma concentrations of methionine, total homocysteine, and cystathionine in 12 subjects during a 24-h observation period. Breakfast (B) and dinner (D) were served at 0900 and 1500, respectively. The fasting concentration measured the first morning was used as the reference and was set to 100%.
(9). Hence, these data suggest that plasma cystathionine increases much more than does plasma tHcy after intake of methionine. This is consistent with published data showing that cystathionine β-synthase is a high-K_m enzyme (3) and that superfluous methionine is directed into the transsulfuration pathway (4, 7).

Cystathionine in plasma was previously suggested as a marker in the assessment of folate and cobalamin deficiency (1, 2). Furthermore, plasma cystathionine has been suggested as a marker for vitamin B-6 deficiency. The physiologic basis of the latter finding is that cystathionine β-synthase has a higher affinity for the cofactor than does cystathionase, which then becomes a rate-limiting step for the removal of cystathionine.

Our data suggest that plasma cystathionine may be an earlier and more sensitive marker of changes in the flux through the transmethylation-transsulfuration pathway than is tHcy and that cystathionine may be a useful complement to tHcy measurement in human studies concerned with Hcy metabolism. Few studies have included plasma cystathionine measurement, and knowledge of the genetic, physiologic, and lifestyle determinants of cystathionine metabolism is limited. In patients with severe renal failure (25; AB Guttormsen, unpublished observations, 2002) or after methionine loading (2, 9, 22), the increase in cystathionine far exceeds the changes observed after a small meal. However, in mild renal impairment, vitamin deficiencies, homocystinuria, and various other clinical conditions or after use of drugs affecting Hcy metabolism (1, 2, 12, 22, 25, 26), cystathionine concentrations partly overlap those observed after food intake in our healthy subjects. Moreover, on the basis of findings in a healthy population (9), we observed that age- and sex-related differences are small and could be missed if food intake is not taken into account. Therefore, we suggest that in future studies including plasma cystathionine measurements, blood samples be collected from subjects in the fasting state; ie, ≥6 h after a light meal and ≥12 h after a heavy meal with a higher protein content. An alternative approach may be to adjust for time since the last meal.

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ABG was responsible for the planning and implementation of the project, collection of samples and data, analysis of the data, and preparation of a first draft of the article. ES was responsible for the analysis of the blood samples and revision of the article. HR was responsible for the planning and implementation of the project, collection of blood samples, analysis of the blood samples and the data, and critical revision of the article. None of authors had a financial or personal interest or advisory board affiliation in any company or organization sponsoring the research.

REFERENCES

5. Finkelstein JD, Kyle WE, Harris BJ. Methionine metabolism in mam-