Plasma Concentrations of Homocysteine and Other Aminothiol Compounds Are Related to Food Intake in Healthy Human Subjects[1,2]

ANNE B. GUTTORMSEN,[3,4] JØRN SCHNEEDE, TORUNN FISKERSTRAND, PER M. UELAND AND HELGA M. REFSUM

Department of Clinical Biology, Division of Pharmacology, University of Bergen, N-5021 Bergen, Norway

ABSTRACT We investigated total, free and protein-bound plasma homocysteine, cysteine and cysteinylglycine in 13 subjects aged 24–29 y after a breakfast at 0900 h containing 15–18 g of protein and a dinner at 1500 h containing ~50 g of protein. Twelve subjects had normal fasting homocysteine (mean ± SD, 7.6 ± 1.1 μmol/L) and methionine concentrations (22.7 ± 3.5 μmol/L) and were included in the statistical analyses. Breakfast caused a small but significant increase in plasma methionine (22.2 ± 20.6%) and a brief, nonsignificant increase followed by a significant decline in free homocysteine. However, changes in total and bound homocysteine were small. After dinner, there was a marked increase in plasma methionine by 16.7 ± 8.9 μmol/L (87.9 ± 49%), which was associated with a rapid and marked increase in free homocysteine (33.7 ± 19.6%, 4 h after dinner) and a moderate and slow increase in total (13.5 ± 7.5%, 8 h) and protein-bound (12.6 ± 9.4%, 8 h) homocysteine. After both meals, cysteine and cysteinylglycine concentrations seemed related to changes in homocysteine, because there were parallel fluctuations in the free:bound ratios of all three thiols. Dietary changes in plasma homocysteine will probably not affect the evaluation of vitamin deficiency states associated with moderate to severe hyperhomocysteinemia but may be of concern in the risk assessment of cardiovascular disease in patients with mild hyperhomocysteinemia. Synchronous fluctuations in the free:bound ratio of the plasma aminothiol compounds indicate that biological effects of homocysteine may be difficult to separate from effects due to associated changes in other aminothiol compounds. J. Nutr. 124: 1934–1941, 1994.

INDEXING KEY WORDS:

- *homocysteine* • *aminothiol compounds*
- *food* • *humans*

Plasma homocysteine is a risk factor for cardiovascular disease (Kang et al. 1992, Ueland et al. 1992). Moreover, it has been shown to be a valuable measure to assess the function of cobalamin and folate (Ueland et al. 1993). For these reasons, homocysteine determination in serum or plasma may become a useful routine clinical assay. Recommended procedures for sample collection, preparation and storage are therefore warranted (Ueland et al. 1993).

In several clinical studies on plasma or serum homocysteine, fasting blood samples have been collected, but samples from non-fasting subjects have also been used, especially in prospective studies based on stored samples (Arnesen, E., Refsum, H., Bønaa, K. H., Ueland, P. M., Førde, O. H. and Nordrehaug, J. E., unpublished results; Stampfer et al. 1992). Firm recommendations regarding the necessity of fasting samples cannot be made because data on the effect of food intake on plasma homocysteine are sparse.

We have previously observed that in cancer patients, urinary homocysteine excretion shows diurnal changes, being low at night and higher during the day (Refsum et al. 1986). Similar diurnal homocysteine fluctuations have not been looked for in plasma but it has been reported that plasma samples from fasting and non-fasting subjects have similar homocysteine concentrations (Malinow et al. 1989). In a recent study, it was shown that plasma homocysteine even declines after a breakfast with moderate protein content (Ubbink et al. 1992). This is a somewhat unexpected finding because protein contains methionine, which is the precursor of homocysteine (**Fig. 1**) (Finkelstein 1990).

[1]Supported by the Norwegian Research Council and the Norwegian Council on Cardiovascular Diseases.

[2]The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[3]Anne B. Guttormsen is a Fellow of the Norwegian Council on Cardiovascular Diseases.

[4]To whom correspondence should be addressed.

0022-3166/94 $3.00
Manuscript received 15 November 1993. Initial review completed 6 January 1994. Revision accepted 6 April 1994.

The absence of an increase in homocysteine after food intake (Malinow et al. 1989, Ubbink et al. 1992) may possibly be related to methionine content of the meal, or that the intake of nutrients may stimulate homocysteine metabolism (Ubbink et al. 1992). An alternative explanation is that the enhanced homocysteine formation is buffered by the concurrent production of cysteine, glutathione and cysteinylglycine, which interact with plasma homocysteine through complex processes (Mansoor et al. 1992 and 1993), including competition for protein-binding sites (Wiley et al. 1988 and 1989) and thiol-disulfide exchange reactions (Munday 1989) (Fig. 1).

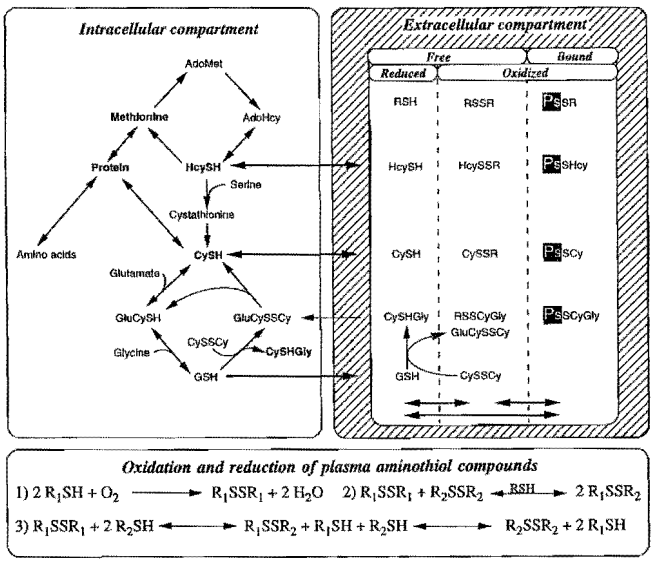

FIGURE 1 Interrelation among different forms of homocysteine, cysteine and cysteinylglycine. *Upper left panel*: The formation of aminothiol compounds from protein and methionine in the intracellular compartment. *Upper right panel*: The extracellular compartment with the plasma compartment (*inner square*). A minor fraction of the plasma aminothiol compounds is reduced, i.e., they exist as thiols. The remainder has undergone oxidation with formation of disulfide bonds with another free circulating thiol (free (mixed) disulfide) or with thiol groups in protein (protein-mixed disulfide). Cysteinylglycine in plasma is derived from glutathionine and cystine in plasma. *Lower panel*: Oxidation and reduction reactions of the aminothiol compounds include *1*) autoxidation, *2*) disulfide-disulfide exchange reactions in the presence of catalytic amounts of a thiol, and *3*) thiol-disulfide exchange reactions.

Abbreviations used: AdoHcy, adenosylhomocysteine; AdoMet, adenosylmethionine; CySH, reduced cysteine; CySSCy, cystine; CySSR, free (mixed) disulfide of cysteine; CySHGly, reduced cysteinylglycine; GSH, reduced glutathione; HcySH and HcySSR, reduced and free (mixed) disulfide of homocysteine; P, protein; RSH, reduced undefined plasma thiol; RSSCyGly, (mixed) disulfide of cysteinylglycine; RSSR, (mixed) disulfide of undefined plasma thiols; PSSCy, PSSCyGly, PSSHcy and PSSR, protein-bound forms of cysteine, cysteinylglycine, homocysteine and undefined thiol, respectively.

The aim of the present study was to investigate whether food intake affects plasma concentration of homocysteine and other aminothiol compounds. Plasma homocysteine and the two major circulating aminothiol compounds, cysteine and cysteinylglycine, were measured in healthy subjects who received two meals over a 14-h period. The first meal (at 0900 h) had a moderate protein content, which is characteristic of breakfast, lunch and evening meals in Norway. The dinner, served at 1500 h, provided ~70% of average daily protein intake. Multiple blood samples were obtained to follow both the early response reported for plasma sulfur amino acids derived from digested protein (Ashley et al. 1982, Ljungqvist 1978) and possible late effects related to metabolic formation, release into and elimination of homocysteine and related aminothiol compounds from plasma.

SUBJECTS AND METHODS

Subjects. Thirteen healthy volunteers, 10 women and three men aged 24–29 y (**Table 1**), participated in the study. Subjects 1–12 had concentrations of folate, cobalamin, total homocysteine, total cysteine, methionine and creatinine in plasma within the normal range. Subject 13 had a mild folate deficiency with moderate hyperhomocysteinemia and was excluded from the statistical analyses. The characteristics of the participants and the fasting concentrations of homocysteine, cysteine, methionine, folate, cobalamin and creatinine are listed in Table 1.

All subjects gave their written informed consent for participation in the study.

Protocol. Between 0900 and 1000 h, after overnight fasting, seven persons received a breakfast consisting of two slices of bread with butter and cheese, coffee or tea, and a glass of orange juice, and six persons had one egg and two strips of bacon, a slice of bread (without butter and cheese), orange juice, and coffee or tea. Estimated amounts for energy and protein intake (Blaker and Rimestad 1991) for the first group were 1860 kJ and 18.6 g protein (12 g was derived from animal or milk protein). The second group received 1200 kJ and 15 g of protein (11.5 g of animal origin). The protein content in the breakfast was about 20–25% of the average daily intake of protein by Norwegians (Drevon and Sander 1992). Because the animal-derived protein contents were quite similar, the amount of methionine probably did not substantially differ in the two types of breakfast (Orr and Watt 1968).

Dinner was served exactly 6 h after breakfast, i.e., between 1500 and 1600 h. All participants received the same meal, which consisted of a small steak (150–200 g), two to four potatoes, and a mixed salad. They had only water to drink. For dessert, they had a

TABLE 1

Characteristics of subjects

Subject	Age	Sex[1]	Weight	Height	Breakfast[2]	Hcy[3]	Cysteine	Methionine	Folate	Cobalamin	Creatinine
	y		*kg*	*cm*		*μmol/L*	*μmol/L*	*μmol/L*	*nmol/L*	*pmol/L*	*μmol/L*
1	25	M	100	192	1	10.1	252.2	23.3	7.0	362	108
2	23	F	54	168	1	6.5	245.7	19.7	15.4	274	90
3	25	F	50	165	1	6.5	261.5	27.8	18.3	663	85
4	23	F	67		1	7.2	237.5	26.8	16.8	505	101
5	23	F	60	166	1	6.9	247.8	24.7	11.6	393	93
6	27	F	54	170	1	7.5	226.0	21.9	8.9	615	87
7	23	F	57	173	2	7.4	254.7	16.6	11.3	401	95
8	22	M	64	174	2	8.3	294.5	23.2	12.4	322	92
9	24	F	70	175	2	8.0	268.8	18.8	13.6	338	93
10	22	F	54	166	2	6.9	254.0	26.1	11.3	437	79
11	24	F	55	163	2	6.9	262.3	24.1	11.8	294	86
12	29	F	62	172	2	9.4	273.0	19.1	19.5	364	103
Mean	24		62	171		7.6	256.5	22.7	13.2	414	93
SD	2		13	8		1.1	17.7	3.5	3.7	123	8
13[4]	22	M	67	181	1	32.0	298.2	27.8	4.8	286	115

[1]M = male, F = female.
[2]Breakfast type 1: bacon and egg. Breakfast type 2: bread with cheese. See text for details.
[3]Hcy = homocysteine.
[4]Subject with folate deficiency. Excluded from the statistical analyses.

cream cake and black coffee. Estimated energy intake was ~2750 kJ. Protein content was ~51 g, of which 43 g was of animal or milk origin. This meal contained ~70% of the daily protein intake for Norwegians (Drevon and Sander 1992).

To obtain blood samples, a canula (1.7 mm) was inserted into a cubital vein. Immediately prior to breakfast, blood was collected for determination of plasma folate, cobalamin and creatinine and fasting concentrations of the plasma aminothiol compounds and methionine. Blood samples were collected 0.5, 1, 2, 3, 4 and 6 h after breakfast. The sample collected at 6 h was immediately before dinner. Blood samples were collected at 0.5, 1, 2, 3, 4, 6 and 8 h after dinner. The subjects then went home, and most subjects had a meal before going to bed. The next morning a blood sample was obtained from each subject in the fasting state.

Sample collection and processing. Blood was collected into a cooled evacuated tube with EDTA that was immediately placed on ice. Plasma used for determination of total aminothiol levels was prepared by centrifuging the blood at 2000 × *g* for 5 min at 0–2°C.

Acid-precipitated plasma was used for the determination of free concentrations of the aminothiol compounds. Blood cells were immediately removed by centrifugation for ~0.5 min at 10,000 × *g*. Within 2 min after collection of the blood, plasma (1 mL) was mixed with 100 μL of 500 g/L sulfosalicylic acid. The acid-treated plasma was then allowed to stand for 5–15 min in ice before removal of the acid precipitate by centrifugation for ~1 min at 10,000 × *g* (Fiskerstrand et al. 1993).

Plasma and acid-precipitated plasma were stored at −20°C until analysis.

Analytical methods. The method for determination of total and free plasma homocysteine, cysteine and cysteinylglycine was described previously (Fiskerstrand et al. 1993, Refsum et al. 1989). Briefly, a programmable sample processor was used for automated sample derivatization and sample injection. Oxidized species of aminothiol compounds in plasma or the acid supernatant were reduced by adding $NaBH_4$. The sample was then diluted with an ethylmorpholine buffer and the aminothiol compounds derivatized with monobromobimane. The reaction was stopped by adding glacial acetic acid, and the sample was injected into a 150 × 4.6 mm ODS Hypersil column, equilibrated with an ammonium nitrate-ammonium formate buffer, pH 3.65 (total concentrations) or pH 3.5 (free concentrations). The three thiols were eluted from the column, using a linear acetonitrile gradient, and the concentrations were measured by fluorescence detection. The temperature used was 50°C, and the flow rate was 2 mL/min. The retention times for cysteine, cysteinylglycine and homocysteine were approximately 8, 9 and 10.5 min, respectively. The between-day CV of this method is 2–4% for both free and total aminothiol compounds (Fiskerstrand et al. 1993).

Methionine was determined in deproteinized plasma with an assay based on derivatization with *o*-phthaldialdehyde and fluorescence detection (Krishnamurti et al. 1984).

Plasma cobalamin was determined with a microparticle enzyme intrinsic factor assay run on an IMx system from Abbott (Abbott Park, IL), and plasma folate was assayed using the Quantaphase folate radioassay produced by BioRad (Hercules, CA).

Statistical methods. Results are expressed as means ± SD. In the figures, means with a 95% confidence interval (CI) are shown. Comparison between the two different breakfasts was performed using the Mann Whitney *U* test (Johnson and Wichern 1982). In addition to confidence intervals, changes in plasma aminothiol compounds during the day were compared using nonparametric tests (Friedman nonparametric ANOVA and the Wilcoxon signed rank test) (Johnson and Wichern 1982). Correlation between variables was tested by using a Spearman's Rank correlation coefficient (Johnson and Wichern 1982). Correlation in fluctuations in free:bound ratios during the day was tested by categorizing a change from one time point to the next either as a reduction (including unchanged) or as an increase in free:bound ratio and then performing a Spearman's Rank correlation coefficient. Significance level was set at 0.05.

RESULTS

Plasma methionine. Plasma methionine started to increase within 1 h after breakfast, reached a maximum (22.2 ± 20.6% above fasting level; $P < 0.05$) after 2 h, and returned to the fasting level after 4 h (**Fig. 2**). There was no significant difference in methionine response between subjects fed the two types of breakfast (data not shown).

After dinner, plasma methionine response was much higher, and plasma methionine started to increase within 30 min and reached a plateau of ~90% above pre-dinner level ($P < 0.05$) after 4–6 h. Plasma methionine then decreased slowly toward fasting concentrations (Fig. 2).

Maximum increase in plasma methionine (16.7 ± 8.9 μmol/L) after dinner was 3.3 times higher than after breakfast (5.0 ± 4.6 μmol/L), and in line with the ratio (3.6:1) of animal-derived protein content in the two meals. This confirms that there is a relation between methionine content in the food and plasma methionine response. Still, there were marked interindividual differences in maximal methionine increase both after breakfast (range 0–15.1 μmol/L above fasting concentration) and after dinner (range 8.2–64.8 μmol/L above pre-dinner concentration), which were not related to body weight, serum folate, serum cobalamin or the maximal homocysteine response (data not shown). This agrees with previous studies that the amount of protein digested does not correlate well with postprandial plasma amino acid concentration (Longenecker and Hause 1959, Sarwar 1987, Yearick and Nadeau 1967).

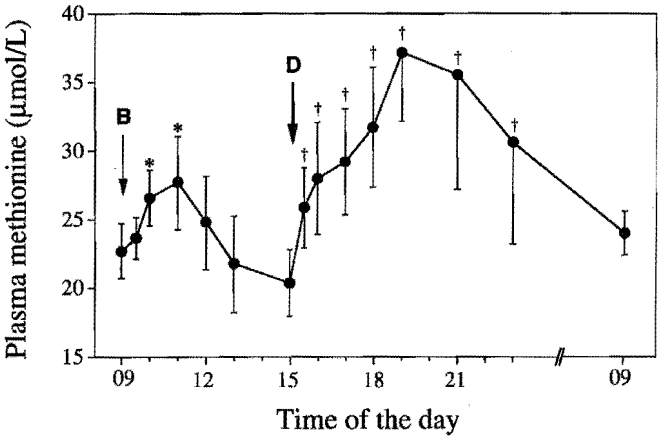

FIGURE 2 Plasma methionine concentrations in 12 subjects with normal total plasma homocysteine after receiving breakfast (B) and dinner (D). Breakfast contained 15–18.6 g of protein; dinner contained ~51 g of protein. Values shown are means with a 95% confidence interval. *Significantly different from fasting level ($P < 0.05$). †Significantly different from level immediately prior to dinner ($P < 0.05$).

Plasma homocysteine, cysteine and cysteinylglycine. There was no significant difference in the plasma aminothiol response between subjects fed the two types of breakfasts (data not shown), and they are therefore treated as one group.

Total, free and protein-bound homocysteine showed small variations for 6 h after breakfast (**Fig. 3**). After a transient increase in free homocysteine, there was a small decrease in total and free homocysteine, which reached significance for the free faction after 4 h. Protein-bound homocysteine tended to change in the opposite direction of free homocysteine (a decrease followed by increase) in this time interval, creating a biphasic curve for the free:bound ratio (**Fig. 4**). The levels of total, free and protein-bound cysteine and cysteinylglycine showed profiles similar to those of the homocysteine species (Fig. 3).

After dinner, total homocysteine started to rise slowly, and maximum increase (13.5 ± 7.5%, 1.0 ± 0.6 μmol/L, $P < 0.05$) was measured after 8 h. Among the subjects, maximum increase varied from 3 to 21% and was observed 3–8 h after dinner. Bound homocysteine followed a similar response pattern, with maximal increase (12.6 ± 9.4%, 0.7 ± 0.5 μmol/L, $P < 0.05$) after 8 h. Free homocysteine increased rapidly and more extensively. Already 30 min after dinner, it was significantly higher (14 ± 11%, $P < 0.05$) than before dinner, and after 4 h, the maximum increase was 33.7 ± 19.6% (0.6 ± 0.3 μmol/L, $P < 0.05$) above the pre-dinner concentration.

The changes in plasma cysteine and cysteinylglycine concentrations after dinner were similar, but they differed from that of homocysteine. Except for a brief but significant (at 30 min post-meal) increase in total cysteine, total concentrations of these thiol

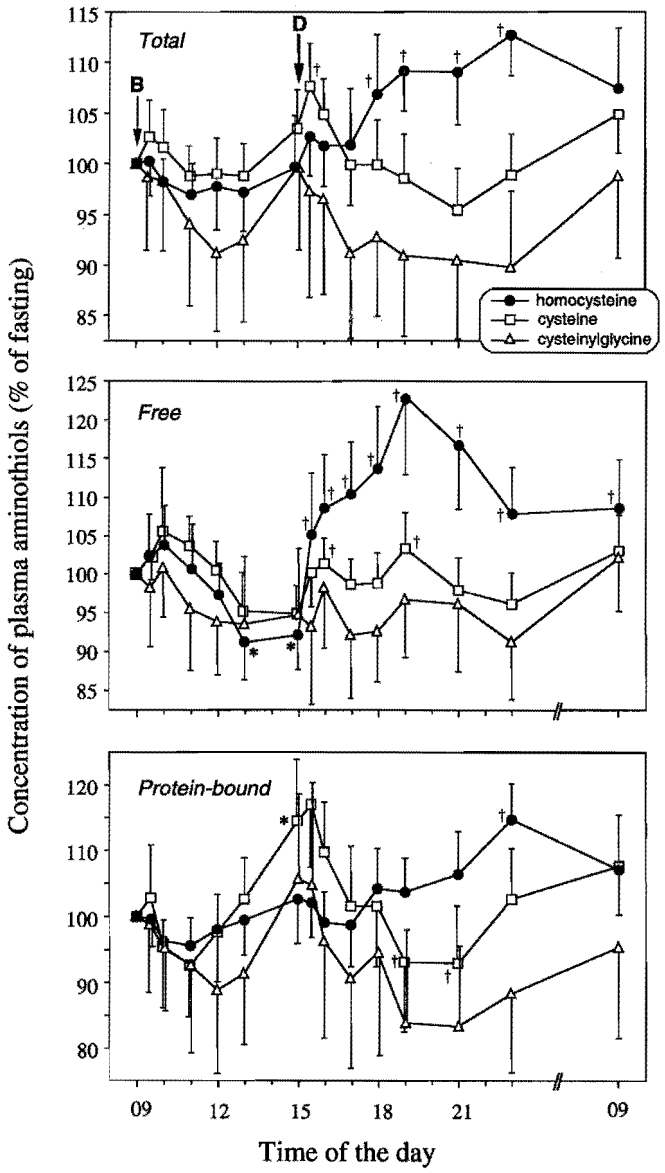

FIGURE 3 Percentage change in total, free and bound plasma homocysteine (Hcy), cysteine and cysteinylglycine in 12 subjects with normal total plasma Hcy after receiving breakfast (B) and dinner (D). The mean (± SD) total, free and bound fasting concentrations were 7.6 ± 1.1, 2.1 ± 0.2 and 5.6 ± 1.0 μmol/L for Hcy, 213.7 ± 14.7, 112.1 ± 15.2 and 101.7 ± 21.9 μmol/L for cysteine and 25.8 ± 4.7, 14.2 ± 1.7 and 11.6 ± 4.1 μmol/L for cysteinylglycine. Values shown are means with a 95% confidence interval. *Significantly different from fasting level ($P < 0.05$). †Significantly different from level immediately prior to dinner ($P < 0.05$).

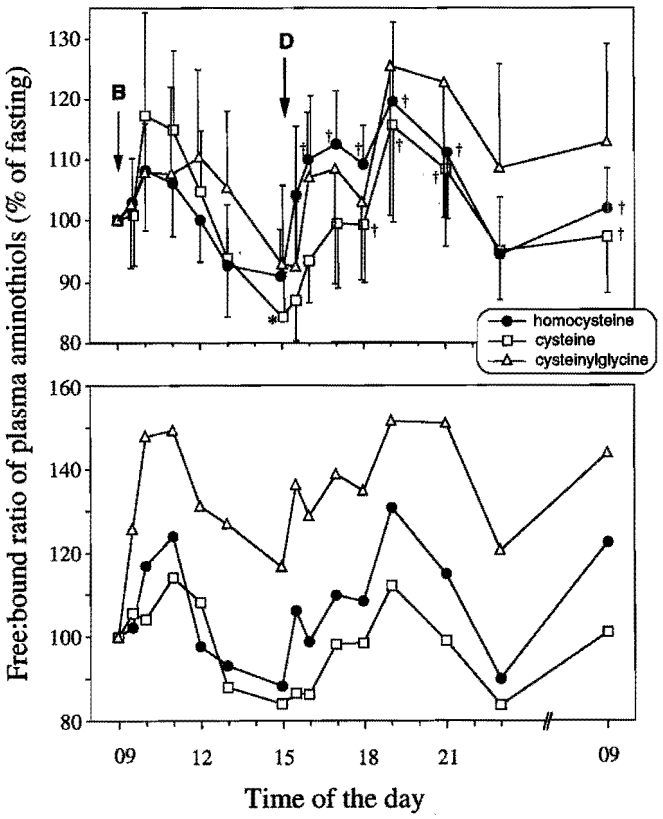

FIGURE 4 Percentage change in free:bound ratio of plasma homocysteine (Hcy), cysteine and cysteinylglycine after breakfast (B) and dinner (D). *Upper panel*: Subjects (n = 12) with normal total plasma Hcy. The mean (± SD) free:bound ratio before breakfast was 0.38 ± 0.05 for Hcy, 1.17 ± 0.38 for cysteine and 1.38 ± 0.55 for cysteinylglycine. Values shown are means with a 95% confidence interval. *Significantly different from fasting level ($P < 0.05$). †Significantly different from level immediately prior to dinner ($P < 0.05$). *Lower panel*: Subject 13 with elevated total plasma Hcy (fasting level 32.0 μmol/L) due to a mild folate deficiency. The free:bound ratios before breakfast was 0.34, 1.08 and 1.18 for Hcy, cysteine and cysteinylglycine, respectively.

compounds declined (by 5–12%) in the time interval 2–8 h after dinner. There was a more pronounced reduction in bound concentrations (almost 20%). This corresponded to a mean reduction of bound cysteine and cysteinylglycine of 20 and 2.7 μmol/L, respectively. Notably, these changes in cysteine and cysteinylglycine caused fluctuations in the free:bound ratios, which were similar and significantly correlated ($P < 0.05$) for homocysteine and cysteine after breakfast and for all three thiol compounds after dinner ($P < 0.05$) (Fig. 4, *upper panel*).

The next morning, plasma homocysteine and the other aminothiol compounds had not returned to the levels measured before dinner. In particular, the concentration of free plasma aminothiol compounds was still markedly elevated (Fig. 3). Compared with values for the previous morning, both free and total plasma homocysteine were significantly higher ($P < 0.05$).

During the 24-h follow-up of these subjects who received two ordinary meals, the maximum change in total homocysteine in the individuals was between 15 and 39% of the morning fasting level (mean 24 ± 8%). The same values for free homocysteine were 23 and 60% (mean 38 ± 14%). The ratio between free and bound plasma homocysteine showed the greatest change during the 24 h, with maximum variation

between 23 and 81% (mean 49 ± 16%) (data not shown).

Plasma aminothiol compounds in a folate-deficient subject. Subject 13 had low serum folate and hyperhomocysteinemia (Table 1) but no clinical symptoms. Total, free and protein-bound forms (data not shown) and the free:bound ratios (Fig. 4, *lower panel*) of homocysteine, cysteine and cysteinylglycine in plasma from this subject showed the same profile as the average response of the 12 subjects with normal plasma homocysteine. Also in this subject, the fluctuations in the free:bound ratios of the three aminothiol compounds were significantly correlated ($P < 0.05$). The maximum change during the day was 9.4 μmol/L (29% of morning fasting level) for total homocysteine and 2.8 μmol/L (35% of morning fasting level) for free homocysteine.

DISCUSSION

The fluctuations in plasma homocysteine during the day may be related to at least two different processes. First, intake of food is associated with increased consumption of the amino acids, and second, these diet-induced changes may lead to altered distribution between free and bound forms of homocysteine and other aminothiol compounds in plasma.

Plasma homocysteine and food intake. The essential amino acid methionine, which is especially rich in protein of animal origin (Orr and Watt 1968), is the only source of homocysteine in humans (Finkelstein 1990). Homocysteine is formed when methionine is utilized in the numerous adenosylmethionine-dependent transmethylation reactions (Fig. 1) and represents a branch-point in methionine metabolism (Finkelstein 1990). During periods of adequate intake of methionine, a greater fraction of homocysteine becomes irreversibly degraded via the transsulfuration pathway. Conversely, during fasting, most of the homocysteine formed is remethylated to methionine (Fig. 1) (Storch et al. 1988).

We have shown that homocysteine export increases from a variety of cell types with increasing extracellular methionine concentrations in vitro. Except for liver cells, the most marked increase occurs between 15 and 150 μmol/L of methionine (Christensen et al. 1991). Liver cells export more homocysteine than any other cell type investigated, also at low methionine concentrations (Christensen et al. 1991). These in vitro data indicate that a meal leading to moderately enhanced plasma methionine may lead to increased plasma homocysteine concentration.

In the present study, we found that breakfast led to a small increase in plasma methionine (~5 μmol/L) (Fig. 2), which was associated with a brief elevation of free homocysteine followed by a decline until intake of dinner. Total homocysteine and protein-bound homocysteine were essentially stable. Notably, two different types of breakfasts with approximately the same amount of methionine (Orr and Watt 1968) were associated with the same response in both plasma methionine and the other plasma aminothiol compounds. Thus, the small amount of methionine furnished by an ordinary breakfast is probably not sufficient to induce a markedly enhanced cellular homocysteine export. These results confirm previous studies that have shown that there is no difference between samples from fasting and non-fasting subjects (Malinow et al. 1989) or that a meal may even be associated with a decline in plasma homocysteine (Ubbink et al. 1992).

In contrast to results following breakfast, there was a marked and persistent increase in plasma methionine following dinner (Fig. 2). The increase in plasma methionine was associated with a concurrent increase in free homocysteine. Total and protein-bound homocysteine also increased, but the elevation was delayed and less pronounced (Fig. 3). The homocysteine response showed similarities with the elevation of homocysteine species provoked by a methionine-loading test. After intake of pure methionine (100 mg methionine/kg body wt), maximum plasma methionine is obtained within 1–2 h, free plasma homocysteine peaks after 2–4 h, and total homocysteine and bound homocysteine reach a maximum 2–4 h later and remains elevated for 12–24 h (Mansoor et al. 1992, Refsum et al. 1989, Sardharwalla et al. 1974, Ueland et al. 1992). The homocysteine response following dinner is probably due to a supply of methionine from proteins, and the slow kinetics relative to that of a methionine loading is probably related to the time required for protein breakdown and methionine absorption. These results demonstrate that a protein-rich meal may affect plasma homocysteine for at least 8 h.

In the fasting blood samples obtained the following morning, both total and free plasma homocysteine were significantly higher ($P < 0.05$) than on the previous morning. This day-to-day variation was higher than, but not significantly different ($P > 0.05$) from, the day-to-day variation in total and free plasma homocysteine in nine healthy subjects (Guttormsen, A. B. and Refsum, H. M., unpublished results). Most of the participants in the present study had eaten a small meal between 2400 and 0200 h, which may explain the elevated plasma homocysteine concentration the second morning. However, it may also be a persistent effect of the protein-rich dinner. This is supported by our recent finding that homocysteine is slowly eliminated from plasma with a half-life of about 3–4 h at both high (Guttormsen et al. 1993) and normal (Guttormsen, A. B., Christensen, B., Mella, O., Lønning, P. Ø., Ueland, P. M. and Refsum, H. M.,

unpublished results] plasma homocysteine concentrations. Such kinetics predicts elevated plasma homocysteine for 12–20 h following intake of a protein-rich meal. Thus, the decline in plasma homocysteine after breakfast observed by Ubbink et al. [1992] and by us [Fig. 3] may represent elimination of plasma homocysteine derived from a precedent protein-rich meal.

Cysteine and cysteinylglycine. We observed that elevation of plasma homocysteine was associated with a marked decrease in protein-bound cysteine and cysteinylglycine and moderate decrease in the total levels, whereas free concentrations of these aminothiol compounds were relatively stable [Fig. 3]. The resulting profiles for free:bound ratios of homocysteine, cysteine and cysteinylglycine showed a remarkable synchrony [Fig. 4].

The changes in cysteine and cysteinylglycine mimic the alterations in these aminothiol compounds observed during the marked hyperhomocysteinemia following methionine [Mansoor et al. 1992] or homocysteine loading [Mansoor et al. 1993]. Such a response may be related to competition for protein-binding sites, i.e., that bound cysteine and cysteinylglycine are displaced by homocysteine. This is in accordance with the presence in plasma of binding sites with apparent higher affinity for homocysteine than for cysteine [Smolin and Benevenga 1984, Wiley et al. 1988]. However, the reduction of protein-bound cysteinylglycine and cysteine occurred 4 h before and was 35 times higher than the maximum increase in bound homocysteine [Fig. 3], which suggests that displacement reactions cannot fully explain the observed changes. The synchronous fluctuation in free:bound ratios for the three thiols points to a probable explanation, i.e., that change in one of the thiol compounds, possibly homocysteine, results in a cascade of thiol-disulfide exchange reactions [Fig. 1], leading to altered ratios between free and bound plasma aminothiol compounds.

Implications. Our data show that a protein-rich meal leads to elevated plasma homocysteine concentration. The duration [>8 h] of the homocysteine response after a protein-rich meal points to dietary protein content as an important determinant of plasma homocysteine level. Total homocysteine seemed to undergo less variations than free homocysteine, and this supports the view [Ueland et al. 1993] that measurement of total homocysteine is preferable in the clinical setting.

The daily variation in total homocysteine observed in our subjects was ~25%. This may be even greater in other subjects such as those heterozygous for cystathionine β-synthase deficiency, who respond with marked elevation of plasma homocysteine after a peroral methionine load [Boers et al. 1985, Clarke et al. 1991]. Variations in plasma homocysteine related to food intake will probably not affect the diagnostic value of plasma homocysteine concentration in folate or cobalamin deficiencies [Ueland et al. 1993] or in homocystinuria [Mudd et al. 1989]. In contrast, moderate elevation in plasma homocysteine is associated with increased risk of cardiovascular disease [Ueland et al. 1992], and it has been shown that for every 4 μmol/L increase in homocysteine, the risk of developing a cardiac infarction increases 40% [Arnesen, E., Bønaa, K., Førde, O. H., Nordrehaug, J. E., Refsum, H. and Ueland, P. M. unpublished results]. In an adult male population in Norway, a variation of 25% in plasma homocysteine [~2.5 μmol/L] represents 25% difference in risk. Thus, in risk assessment for cardiovascular disease, attention should be paid to sampling time in relation to food intake.

This study confirms previous results [Mansoor et al. 1992 and 1993, Wiley et al. 1989] that there is a dynamic relation between plasma homocysteine and other aminothiol compounds. In addition, we showed that even small fluctuations in total concentrations of plasma aminothiol compounds lead to marked changes in free and bound concentrations of all three aminothiol compounds. These findings indicate that the biological effects of increased plasma homocysteine in vivo may be difficult to separate from associated changes in other plasma aminothiol compounds. Interestingly, most in vitro effects of homocysteine related to atherogenesis are not restricted to homocysteine but can also be produced by other thiols, including cysteine [Dudman et al. 1991, Fryer et al. 1993, Harpel et al. 1992, Jia and Furchgott 1993, Stamler et al. 1988, Ueland et al. 1992].

ACKNOWLEDGMENTS

The technical assistance of Elfrid Blomdal and Gry Kvalheim is highly appreciated.

LITERATURE CITED

Ashley, D. V., Barclay, D. V., Chauffard, F. A., Moennoz, D. & Leathwood, P. D. [1982] Plasma amino acid responses in humans to evening meals of differing nutritional composition. Am. J. Clin. Nutr. 36: 143–153.

Blaker, B. & Rimestad, A. H. [1991] Statens Ernæringsråds Matvaretabell, 6th ed. Landsforeningen for Kosthold og Helse, Oslo, Norway.

Boers, G.H.J., Smals, A.G.H., Trijbels, F.J.M., Fowler, B., Bakkeren, J.A.J.M., Schoonderwaldt, H. C., Kleijer, W. J. & Kloppenborg, P.W.C. [1985] Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N. Engl. J. Med. 313: 709–715.

Christensen, B., Refsum, H., Vintermyr, O. & Ueland, P. M. [1991] Homocysteine export from cells cultured in the presence of physiological or superfluous levels of methionine: methionine loading of non-transformed, transformed, proliferating and quiescent cells in culture. J. Cell. Physiol. 146: 52–62.

Clarke, R., Daly, L., Robinson, K., Naughten, E., Cahalane, S., Fowler, B. & Graham, I. [1991] Hyperhomocysteinemia: an independent risk factor for vascular disease. N. Engl. J. Med. 324: 1149–1155.

Drevon, C. A. & Sander, J. (1992) Enteral ernæring. In: Norsk Legemiddelhåndbok 1992–93 for Helsepersonell (Vennerød, A. M., ed.), pp. 725–742. Norsk Legemiddelhåndbok Interessentskap, Oslo, Norway.

Dudman, N.P.B., Hicks, C., Wang, J. & Wilcken, D.E.L. (1991) Human arterial endothelial cell detachment in vitro: its promotion by homocysteine and cysteine. Atherosclerosis 91: 77–83.

Finkelstein, J. D. (1990) Methionine metabolism in mammals. J. Nutr. Biochem. 1: 228–237.

Fiskerstrand, T., Refsum, H., Kvalheim, G. & Ueland, P. M. (1993) Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin. Chem. 39: 263–271.

Fryer, R. H., Wilson, B. D., Gubler, D. B., Fitzgerald, L. A. & Rodgers, G. M. (1993) Homocysteine, a risk factor for premature vascular disease and thrombosis, induces tissue factor activity in endothelial cells. Arterioscler. Thromb. 13: 1327–1333.

Guttormsen, A. B., Mansoor, M. A., Fiskerstrand, T., Ueland, P. M. & Refsum, H. (1993) Kinetics of plasma homocysteine in healthy subjects after peroral homocysteine loading. Clin. Chem. 39: 1390–1397.

Harpel, P. C., Chang, V. T. & Borth, W. (1992) Homocysteine and other sulfhydryl compounds enhances the binding of lipoprotein(a) to fibrin: a potential biochemical link between thrombosis, atherogenesis, and sulfhydryl compound metabolism. Proc. Natl. Acad. Sci. U.S.A. 89: 10193–10197.

Jia, L. & Furchgott, R. F. (1993) Inhibition of sulfhydryl compounds of vascular relaxation induced by nitric oxide and endothelium derived relaxing factor. J. Pharmacol. Exp. Ther. 267: 371–378.

Johnson, R. A. & Wichern, D. W. (1982) Applied Multivariant Statistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.

Kang, S.-S., Wong, P.W.K. & Malinow, M. R. (1992) Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Annu. Rev. Nutr. 12: 279–298.

Krishnamurti, C. R., Heindze, A. M. & Galzy, G. (1984) Application of reversed-phase high-performance liquid chromatography using pre-column derivatization with *o*-phthaldialdehyde for the quantitative analysis of amino acids in adult and fetal sheep plasma, animal feeds and tissues. J. Chromatogr. 315: 321–331.

Ljungqvist, B. G. (1978) Plasma amino acid response to single test meals in humans. Res. Exp. Med. 174: 1–12.

Longenecker, J. B. & Hause, N. L. (1959) Relationship between plasma amino acids and composition of the ingested protein. Arch. Biochem. Biophys. 84: 46–59.

Malinow, M. R., Kang, S. S., Taylor, L. M., Wong, P.W.K., Inahara, T., Mukerjee, D., Sexton, G. & Upson, B. (1989) Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. Circ. Res. 79: 1180–1188.

Mansoor, M. A., Guttormsen, A. B., Fiskerstrand, T., Refsum, H., Ueland, P. M. & Svardal, A. M. (1993) Redox status and protein-binding of plasma aminothiols during the transient hyperhomocysteinemia that follows homocysteine administration. Clin. Chem. 39: 980–985.

Mansoor, M. A., Svardal, A. M., Schneede, J. & Ueland, P. M. (1992) Dynamic relation between reduced, oxidized and protein-bound homocysteine and other thiol components in plasma during methionine loading in healthy men. Clin. Chem. 38: 1316–1321.

Mudd, S. H., Levy, H. L. & Skovby, F. (1989) Disorders of transsulfuration. In: The Metabolic Basis of Inherited Disease (Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D., eds.), pp. 693–734. McGraw-Hill, New York, NY.

Munday, R. (1989) Toxicity of thiols and disulfides: involvement of free-radical species. Free Radical Biol. & Med. 7: 659–673.

Orr, M. L. & Watt, B. K. (1968) Amino Acid Content of Foods. Home Economics Report #4. U.S. Department of Agriculture, Washington, DC.

Refsum, H., Helland, S. & Ueland, P. M. (1989) Fasting plasma homocysteine as a sensitive parameter to antifolate effect: a study on psoriasis patients receiving low-dose methotrexate treatment. Clin. Pharmacol. & Ther. 46: 510–520.

Refsum, H., Ueland, P. M. & Kvinnsland, S. (1986) Acute and long-term effects of high-dose methotrexate treatment on homocysteine in plasma and urine. Cancer Res. 46: 5385–5391.

Refsum, H., Ueland, P. M. & Svardal, A. M. (1989) Fully automated fluorescence assay for determining total homocysteine in plasma. Clin. Chem. 35: 1921–1927.

Sardharwalla, I. B., Fowler, B., Robins, A. J. & Komrower, G. M. (1974) Detection of heterozygotes for homocystinuria: study of sulphur-containing amino acids in plasma and urine after L-methionine loading. Arch. Dis. Child. 49: 553–559.

Sarwar, G. (1987) Digestibility of proteins and bioavailability of amino acids in foods. Effects on protein quality assessment. World Rev. Nutr. Diet. 54: 26–70.

Smolin, L. A. & Benevenga, N. J. (1984) The use of cyst(e)ine in the removal of protein-bound homocysteine. Am. J. Clin. Nutr. 39: 730–737.

Stamler, J., Cunningham, M. & Loscalzo, J. (1988) Reduced thiols and the effect of intravenous nitroglycerin on platelet aggregation. Am. J. Cardiol. 62: 377–380.

Stampfer, M. J., Malinow, M. R., Willett, W. C., Newcomer, L. M., Upson, B., Ullmann, D., Tishler, P. V. & Hennekens, C. H. (1992) A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in United States physicians. J. Am. Med. Assoc. 268: 877–881.

Storch, K. J., Wagner, D. A., Burke, J. F. & Young, V. R. (1988) Quantitative study in vivo of methionine cycle in humans using [methyl-$^{2}H_3$] and [1-^{13}C]methionine. Am. J. Physiol. 255: 322–331.

Ubbink, J. B., Vermaak, W.J.H., Vandermerwe, A. & Becker, P. J. (1992) The effect of blood sample aging and food consumption on plasma total homocysteine levels. Clin. Chim. Acta 207: 119–128.

Ueland, P. M., Refsum, H. & Brattström, L. (1992) Plasma homocysteine and cardiovascular disease. In: Atherosclerotic Cardiovascular Disease, Hemostasis, and Endothelial Function (Francis, R. B., Jr., ed.), pp. 183–236. Marcel Dekker, New York, NY.

Ueland, P. M., Refsum, H., Stabler, S. P., Malinow, M. R., Andersson, A. & Allen, R. H. (1993) Total homocysteine in plasma or serum: methods and clinical applications. Clin. Chem. 39: 1764–1779.

Wiley, V. C., Dudman, N.P.B. & Wilcken, D.E.L. (1988) Interrelations between plasma free and protein-bound homocysteine and cysteine in homocystinuria. Metabolism 37: 191–195.

Wiley, V. C., Dudman, N.P.B. & Wilcken, D.E.L. (1989) Free and protein-bound homocysteine and cysteine in cystathionine beta-synthase deficiency: interrelations during short-term and long-term changes in plasma concentrations. Metab. Clin. Exp. 38: 734–739.

Yearick, E. S. & Nadeau, R. G. (1967) Serum amino acid response to isocaloric test meals. Am. J. Clin. Nutr. 20: 338–344.