Kinetics of total plasma homocysteine in subjects with hyperhomocysteinemia due to folate or cobalamin deficiency[1–3]

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ABSTRACT Hyperhomocysteinemia in cobalamin and folate deficiency reflects an imbalance between influx and elimination of homocysteine (Hcy) in plasma. We investigated the kinetics of total Hcy (tHcy) in plasma after peroral Hcy administration in 19 volunteers with hyperhomocysteinemia ($\bar{x}$ ± SD: 67.1 ± 39.5 μmol/L; range: 23.5–142.8 μmol/L) before and after supplementation with cobalamin and/or folate. Vitamin therapy decreased plasma tHcy to 21.8 ± 14.1 μmol/L (range: 9.6–57.9 μmol/L) but caused only a marginal decline in the area under the curve (AUC) by 8% and plasma half-life by 21%. Using the equations for steady-state kinetics, these data indicate that mean plasma tHcy clearance is normal and that massive export of Hcy from tissues into plasma is the major cause of hyperhomocysteinemia in cobalamin or folate deficiency. However, the spread in AUC and plasma half-life values was large in hyperhomocysteinemic subjects, suggesting marked individual variability in tHcy clearance. Plasma methionine after Hcy loading did not increase before (0.9 ± 6.8 μmol/L) but increased normally (12.8 ± 4.6 μmol/L) after vitamin therapy, and the methionine response discriminated between vitamin-deficient and vitamin-replete subjects. In cobalamin- or folate-deficient subjects, only 6.5 ± 3.0% of the Hcy dose was excreted unchanged in the urine, demonstrating that urinary Hcy excretion does not explain normal tHcy plasma clearance in subjects with impaired Hcy remethylation. Our data suggest that hyperhomocysteinemia in folate and cobalamin deficiency is related to increased influx of Hcy to plasma, and that the methionine synthase function is not an important determinant of elimination of Hcy from plasma. The large interindividual difference in Hcy clearance may be explained by variable adaptation to impaired methionine synthase function through increased Hcy flux through alternate metabolic pathways. *Am J Clin Nutr* 1996; 63:194–202.

KEY WORDS Homocysteine, kinetics, cobalamin deficiency, folate deficiency

INTRODUCTION

The total concentration of homocysteine [tHcy; the sum of free (fHcy; non-protein-bound Hcy) and protein-bound Hcy] in plasma recently gained considerable interest as a useful marker of impaired function of cobalamin and folate. Moreover, an elevated plasma tHcy concentration has been identified as an independent risk factor for atherosclerotic disease (1). These data have encouraged the search for determinants of plasma tHcy concentration.

Hcy (Hcy indicates that the oxidation status of the thiol group of Hcy is not specified and includes both the thiol and disulfide forms of Hcy) in plasma derives from cellular Hcy, which is a product of adenosylmethionine-dependent transmethylation reactions (2). Intracellular Hcy is either converted to cystathionine through the action of cystathionine β-synthase (EC 4.2.1.22.) or remethylated to methionine. Formation of methionine is catalyzed by two enzymes. 5-Methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase, EC 2.1.1.13.) is widely distributed and requires methyltetrahydrofolate as a methyl donor and cobalamin as a cofactor. Betaine-homocysteine methyltransferase (EC 2.1.1.5.) is probably confined to the liver and kidney and uses betaine as a methyl donor (2). The Hcy metabolizing enzymes (3), their substrates (methyltetrahydrofolate and betaine), and their cofactors (vitamin B-6 and vitamin B-12) are recognized as important determinants of the plasma Hcy concentration (3–6).

Conceivably, the high plasma Hcy concentration in cobalamin and folate deficiency is due to an imbalance between the rate of entry into and removal from plasma. Both these processes may be disturbed. In vitro studies with whole blood, fresh blood cells, and permanent cell lines (7–9) have shown that all cell types investigated export Hcy. Notably, cells with disturbed remethylation of Hcy to methionine increase their Hcy export to the extracellular compartment (10–13). These data suggest that enhanced Hcy export from cells can contribute to the hyperhomocysteinemia in cobalamin and folate deficiency.

The fate of plasma Hcy is unknown. In healthy subjects, renal excretion is small relative to the amount of Hcy formed (14–16) and a minor fraction of administered Hcy is recovered in the urine (17). This indicates that plasma Hcy is taken up by cells and metabolized. Notably, it has been shown in rats that

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Hcy can function as the sole source of sulfur amino acids as long as the concentrations of cofactor and substrates of the Hcy metabolizing enzymes are adequate (18). Most benign cell types in culture are able to utilize Hcy as a methionine source (19), but cells with disturbed methionine synthase function are often unable to grow under such conditions (20, 13).

Recently, we performed a study on healthy young subjects receiving peroral Hcy, which increased plasma tHcy to 57.4 ± 9.9 μmol/L above the fasting concentration (17). The elimination half-life of tHcy from plasma was 3.7 ± 0.8 h and obeyed first-order kinetics in the time interval 2–6 h after administration of Hcy. Only ≈2% of the Hcy dose was excreted unchanged in the urine. There was a substantial dose-dependent increase in plasma methionine (17), suggesting that remethylation of administered Hcy to methionine could be an important elimination pathway of plasma Hcy.

The hyperhomocysteinemia in folate- or cobalamin-deficient patients is explained by inhibition of methionine synthase (1). Data from the experimental studies cited above showed enhanced Hcy export from cells with impaired remethylation (10–13) but also the ability of cells and animals to utilize Hcy as a methionine precursor, suggesting reuptake of extracellular Hcy (18, 19). The present work was undertaken to distinguish between enhanced Hcy release into the extracellular compartment or decreased clearance of plasma tHcy as the cause of hyperhomocysteinemia in cobalamin or folate deficiency, and thereby identify the possible role of folate- and cobalamin-dependent Hcy remethylation in the elimination of Hcy from plasma. The aim of the study was to determine plasma tHcy kinetics after Hcy loading in 19 subjects with hyperhomocysteinemia and low or borderline concentrations of the vitamins cobalamin and folate, before and after vitamin supplementation.

SUBJECTS AND METHODS

Subjects

Nineteen volunteers, 13 men and 6 women, aged 18–73 y ($\bar{x}$ ± SD: 49 ± 16 y) were enrolled in the study. Ten subjects were recruited from a population-based study (n = 18 000) of plasma tHcy in the county of Hordaland in Norway (21) and nine subjects were diagnosed in the routine laboratory of the Haukeland University Hospital, Bergen, Norway. All subjects had low or borderline serum concentrations of folate and/or cobalamin and plasma tHcy concentrations ranging from 23.5 to 142.8 μmol/L.

The participants were categorized into three groups:

1) Folate-deficient subjects (n = 6) had normal serum methylmalonic acid (MMA) concentrations (< 0.37 μmol/L) but low serum folate concentrations (≤ 3.7 nmol/L), and responded to folate substitution with a reduction in plasma tHcy.
2) Cobalamin-deficient subjects (n = 6) had a normal serum folate concentration (> 3.7 nmol/L), a low serum cobalamin concentration (< 150 pmol/L), and an elevated serum MMA concentration (> 0.37 μmol/L), and responded to cobalamin substitution with a reduction in both plasma tHcy and serum MMA.
3) The miscellaneous group (n = 7) included those who had a combined deficiency (subjects 17 and 18) or those who did not fulfill all of the above criteria. One subject had an elevated MMA concentration, but normal concentrations of both cobalamin and folate, and responded to folate supplementation (subject 13). Three subjects had normal serum concentrations of cobalamin, folate, and MMA, and responded to cobalamin (subject 15) and/or folate (subjects 14–16) supplementation. Subject 19 had a low serum folate concentration but normal concentrations of MMA and cobalamin, and responded to cobalamin injections with a reduction in tHcy concentration, but had no response after 10 d of folic acid therapy.

Subject characteristics before vitamin supplementation are given in **Table 1**. The participants had provided their written, informed consent and the protocol was approved by the regional ethical committee of western Norway.

Study design

Each subject received two peroral Hcy loads, one before and one 2–6 wk after the start of therapy with cobalamin, folic acid, or both (*see* below). Before administration of Hcy, a fasting blood sample was collected for determination of fasting plasma and serum concentrations of Hcy, MMA, methionine, creatinine, folate, and cobalamin. Hemoglobin concentration and the mean corpuscular volume of red cells were also determined (Table 1).

The Hcy solution, corresponding to 65 μmol Hcy/kg body wt, was prepared immediately before administration by dissolving L-Hcy thiolactone in 5 mL 5 mol NaOH/L to cleave the thiolactone ring (22). After 5 min, the pH was adjusted to between 4 and 5 by adding 5 mol HCl/L. A mixture of water and apple cider (to mask the unpleasant taste of Hcy) was added to a total volume of 200 mL. This solution was swallowed by the subject in < 2 min. For ≥ 2 h after administration, subjects refrained from eating but were allowed to drink water and apple cider freely. During the next 24 h, 11 blood samples were routinely collected, ie, 0.3, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after Hcy administration.

During the first and second loads, urine was collected in fractions for 24 h from 13 and 11 participants, respectively. Before the second load of Hcy, the subjects received intramuscular injections with cobalamin (3–5 mg in 2–3 wk) and/or peroral folic acid (5 mg/d for 10 d). Cobalamin-deficient subjects received only cobalamin injections. Subjects in the folate-deficient and miscellaneous groups who had serum cobalamin concentrations > 200 pmol/L were given folic acid, whereas those who had a serum cobalamin concentration < 200 pmol/L first received cobalamin injections. If the plasma tHcy concentration remained elevated, therapy was continued with peroral folic acid (5 mg/d for 10 d).

Laboratory procedures

During the first 8–12 h after administration of Hcy, blood samples were collected through a 1.7-mm cannula inserted into a cubital vein. Later, blood samples were obtained by venous puncture. Whole blood was drawn into evacuated tubes containing EDTA as anticoagulant and immediately transferred to 1.5-mL vials that were centrifuged at 13 000 × g for 0.5 min. This procedure allows separation of the plasma fraction from the blood cells within 3 min. One milliliter of plasma was immediately deproteinized by adding 100 μL of 2 mol sul-

TABLE 1
Characteristics of subjects before vitamin supplementation[1]

Category	Age	Plasma Hcy			Urinary Hcy	Plasma methionine	Serum MMA	Serum cobalamin	Serum folate	MCV	Hb	Serum creatinine
		Total	Free	Free:total								
	y	*μmol/L*			*μmol/h*	*μmol/L*	*μmol/L*	*pmol/L*	*nmol/L*	*fL*	*g/L*	*μmol/L*
Folate deficiency												
1, F	37	48.9	13.2	0.27		23.6	0.35	440	1.8	90	166	90
2, M	41	31.4	8.4	0.27	0.8	23.5	0.17	311	3.7	96	155	86
3, F	43	79.0	27.0	0.34	4.7	19.7	0.13	208	2.2	89	136	75
4, M	42	75.6	24.0	0.32	5.4	16.8	0.06	141	2.4	108	152	78
5, F	41	34.4	10.2	0.30	0.9	22.4	0.08	173	3.4	89	128	86
6, F	35	23.5	6.0	0.26	0.8	22.8	0.07	125	2.1	76	125	76
$\bar{x}$	40	48.8	14.8	0.29	2.5	21.5	0.14	233	2.6	91	144	82
SD	3	23.6	8.7	0.03	2.3	2.7	0.11	121	0.8	11	16	6
Cobalamin deficiency												
7, M	48	120.0	49.1	0.41	—	11.6	46.86	72	21.7	129	120	91
8, M	57	131.8	59.9	0.45	—	20.3	39.25	83	13.2	117	127	98
9, M	53	38.9	13.7	0.35	—	30.3	2.44	97	12.9	112	138	96
10, M	69	94.0	33.1	0.35	—	20.7	17.44	69	9.8	128	111	90
11, M	66	142.8	53.9	0.38	19.1	20.0	78.10	42	28.0	114	144	103
12, M	73	137.1	50.6	0.37	10.1	32.9	22.20	73	20.4	129	102	94
$\bar{x}$	61	110.8	43.4	0.39	14.6	22.6	34.38	73	17.7	122	124	95
SD	10	39.2	17.1	0.04	6.4	7.8	26.63	18	6.9	8	16	5
Miscellaneous												
13, M	21	37.0	9.5	0.26		25.6	0.45	279	4.5	88	149	110
14, M	41	48.5	15.0	0.31	2.4	24.0	0.15	356	5.2	93	144	95
15, F	62	38.8	8.4	0.22	1.0	20.5	0.22	156	3.9	95	121	100
16, F	44	38.2	9.8	0.26	0.8	19.6	0.13	193	8.4	93	124	66
17, M	67	66.5	22.1	0.33	3.5	28.5	0.44	128	2.9	97	148	116
18, M	67	33.6	9.0	0.27	0.9	20.1	0.46	135	2.2	110	139	84
19, M	18	54.7	15.0	0.27	3.4	17.1	0.15	150	3.0	87	163	96
$\bar{x}$	46	45.3	12.7	0.27	2.0	22.2	0.29	200	4.3	95	141	95
SD	21	11.9	5.0	0.04	1.3	4.0	0.16	86	2.1	8	15	17
All subjects combined												
$\bar{x}$	49	67.1	23.1	0.31	4.1	22.1	11.02	170	8.0	102	136	91
SD	16	39.5	17.7	0.06	5.2	4.9	21.52	107	7.8	16	17	12

[1] Established normal values: total plasma Hcy, 5–15 μmol/L; serum MMA < 0.37 μmol/L; serum cobalamin, 150–840 pmol/L; serum folate > 3.7 nmol/L; MCV, 80–102 fL; Hb, 132–166 g/L (men) and 116–160 g/L (women); serum creatinine, 60–125 μmol/L (men) and 55–110 μmol/L (women). Hcy, homocysteine and its oxidized species; MMA, methylmalonic acid; MCV, mean corpuscular volume; Hb, hemoglobin; F, female; M, male.

fosalicylic acid/L, and the acid supernate was collected after centrifugation. Plasma and the acid supernate were stored at −20 °C until analyzed for total and free plasma Hcy and methionine concentrations. Blood samples for preparation of serum were also collected.

The concentration of Hcy in untreated plasma (tHcy), in acid-precipitated plasma (fHcy), and in urine was determined by using a modified version of an automated procedure developed for the determination of plasma tHcy (23). The between-day CV is ≈3–5%.

Serum MMA was determined by using a newly developed method based on capillary electrophoresis with laser-induced fluorescence detection (24). Briefly, serum was precipitated with a mixture of acetonitrile and methanol containing an internal standard, ethylmalonic acid. To the supernate, 1-pyrenyldiazomethane was added, which forms a highly fluorescent 1-pyrenylmethyl monoester with MMA and other dicarboxylic acids. After overnight incubation at room temperature, the sample was subjected to capillary electrophoresis. The CV varies between 3% and 12%, depending on the MMA concentration.

Plasma methionine was determined with an assay based on derivatization with *o*-phthaldialdehyde and fluorescence detection (25). Serum cobalamin was determined with a microparticle enzyme intrinsic factor assay run on an IMx system from Abbott (Abbott Park, IL), and serum folate was assayed by using the Quantaphase folate radioassay produced by Bio Rad (Hercules, CA).

Calculation of kinetic indexes

The elimination of tHcy after peroral loading obeys first-order kinetics between 2 and 6 h, and is consistent with a one-compartment, open pharmacokinetic model. The half-life was calculated in the interval 2–6 h by using these equations (26):

$$C = C_0 e^{-kt} \quad (1)$$

$$\text{Half-life} = \ln 2/k \quad (2)$$

where C is the plasma concentration at time t, C_0 is the extrapolated plasma concentration at $t = 0$, and k is the rate

constant of the elimination. $AUC_{0-24\ h}$ is the area under the plasma concentration time curve from 0 to 24 h after the administration of Hcy, measured by the trapezoidal rule (26). To calculate the variables, we used KALEIDAGRAPH software (version 2.1.3; Synergy Software, Reading, PA) for Macintosh.

Statistical methods

The results are given as mean ± SD or range. A comparison of paired values was done by using the Wilcoxon signed-rank test. Unpaired values were evaluated by the Mann-Whitney *U* test. Correlation was tested by the Spearman rank correlation coefficient, and relative spread was obtained by a nonparametric sum of rank procedure for relative spread in unpaired samples (27, 28). Significance levels were always expressed as two-tailed values and the significance level was set at 0.05.

RESULTS

Blood indexes and response to vitamin substitution

Plasma and urinary Hcy; serum MMA, cobalamin, and folate; and other blood indexes before vitamin supplementation are given in Table 1. All subjects had moderate to severe hyperhomocysteinemia (range: 23.5–142.8 μmol Hcy/L) that responded to supplementation with cobalamin and/or folate (**Figure 1**). They were classified as folate-deficient, cobalamin-deficient, or miscellaneous, according to the criteria listed in the Methods section. Metabolic response to vitamin supplementation is shown in Figure 1.

In general, the cobalamin-deficient subjects had higher plasma tHcy concentrations before therapy but lower concentrations 2–4 wk after vitamin supplementation began compared with the folate-deficient subjects and the miscellaneous group. The diagnosis was confirmed by a high serum MMA concentration, which declined in response to cobalamin therapy. In subjects who were categorized as folate-deficient, serum MMA was normal and the hyperhomocysteinemia responded to folate supplementation. The miscellaneous group included hyperhomocysteinemic subjects who responded to either cobalamin or folate with a reduction in plasma tHcy, but their concentrations of vitamins or MMA did not strictly adhere to the criteria defining the cobalamin- or folate-deficient groups (Table 1, Figure 1). There was a significant increase ($\bar{x}$ ± SD: 5.5 ± 5.8 μmol/L, $P = 0.0025$) in fasting plasma methionine after supplementation with the vitamins (Figure 1).

Kinetics of plasma Hcy

Plasma tHcy (**Figure 2**) and fHcy started to rise within 15 min after Hcy administration. The increase in fHcy was more rapid and pronounced than was the increase in tHcy (data not shown). This resulted in a marked increase in the ratio of fHcy to tHcy during the first 2–3 h after the administration of Hcy (Figure 2). Notably, cobalamin-deficient subjects had higher ratios of fHcy to tHcy both before and during the loading than did subjects in the two other groups (Figure 2). This is probably explained by the differences in fasting tHcy, which strongly correlated with the ratio of fHcy to tHcy before vitamin therapy ($r_s = 0.86$, $P < 0.001$).

A maximum increase in plasma tHcy (C_{max}) was reached within 2 h in all subjects (Figure 2). Mean values were 71.0 ± 17.2 μmol/L before vitamin treatment and 76.3 ± 16.7 μmol/L ($P > 0.05$) after treatment. Before treatment, the mean C_{max} was lowest ($\bar{x}$ ± SD: 55.6 ± 14.0 μmol/L) in the cobalamin-deficient subjects, intermediate ($\bar{x}$ ± SD: 75.0 ± 14.7 μmol/L) in the folate-deficient subjects, and highest ($\bar{x}$ ± SD: 80.7 ± 13.3 μmol/L) in the miscellaneous group (**Table 2**). There was an inverse relation between the fasting tHcy concentration and the C_{max} before vitamin treatment ($r_s = -0.68$, $P = 0.004$), which was absent after treatment ($r_s = 0.23$, $P > 0.05$).

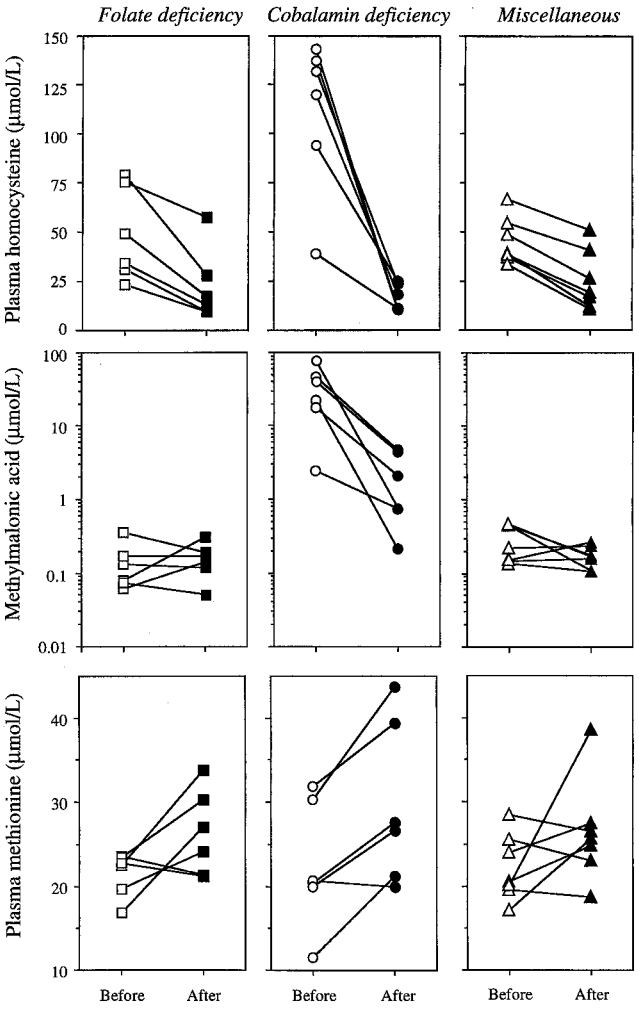

FIGURE 1. Fasting plasma total homocysteine, serum methylmalonic acid, and plasma methionine in subjects with folate and cobalamin deficiency and in subjects belonging to the miscellaneous group before (open symbols) and after (closed symbols) therapy with folic acid or cobalamin.

On the basis of the mean increase in tHcy after Hcy administration, the absorption rate constant for tHcy was calculated. It was lower before treatment ($k_a = 0.033$/min) than after treatment ($k_a = 0.056$/min) (data not shown).

The half-life for plasma tHcy and AUC values before and after vitamin treatment are shown in Table 2 and in **Figure 3**. Before treatment, the mean half-life (4.3 ± 1.9 h) and mean AUC (522 ± 217 μmol · h/L) were only slightly (15–25%) higher than the corresponding values for healthy young subjects (17), and after vitamin supplementation the mean half-life and mean AUC were reduced by only 21% and 8%, respectively.

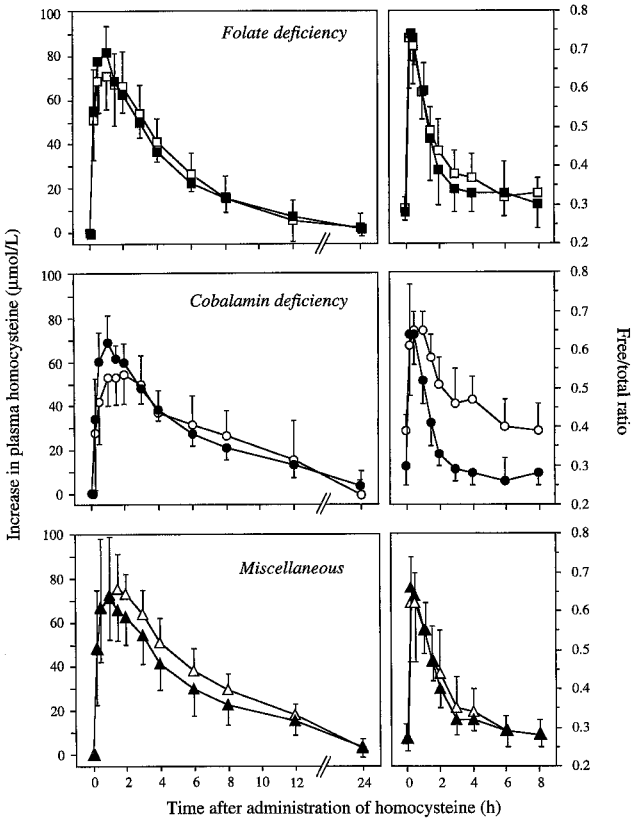

FIGURE 2. Increase in plasma total homocysteine (tHcy) and the ratio of free to tHcy after peroral Hcy loading (65 μmol/kg) before (open symbols) and after (closed symbols) therapy with folic acid or cobalamin. $\bar{x} \pm$ SD.

Notably, the spread was markedly larger in the hyperhomocysteinemic subjects (n = 19) than in the healthy control subjects for both half-life (P = 0.06) and AUC ($P < 0.001$), and vitamin therapy was associated with a marked reduction in spread, which was significant for the AUC ($P < 0.05$). After exclusion of two subjects who had a reduction in plasma tHcy after vitamin therapy of < 25%, the reduction in spread was significant for half-life as well. We therefore plotted the distribution of AUC and half-life values against fasting plasma tHcy (Figure 3). The range of both these indexes was high when the fasting tHcy concentration was high, and the range was progressively reduced when the fasting tHcy concentration approached normal values.

We observed no consistent change in half-life or AUC values after a reduction of fasting tHcy by vitamin supplementation (Figure 3, Table 2). In three cobalamin-deficient subjects with tHcy concentrations > 90 μmol/L and a long half-life (subjects 8, 10, and 12), vitamin therapy was associated with a marked reduction in both half-life and fasting tHcy. However, two other cobalamin-deficient subjects (subjects 7 and 11) with fasting tHcy concentrations in the same range had relatively short half-lives, which did not decrease substantially after vitamin supplementation.

The AUC was inversely correlated with fasting tHcy before treatment ($r_s = -0.516$, P = 0.03) but not after treatment. There was a significant positive relation between AUC and half-life both before (r_s = 0.48, P = 0.04) and after (r_s = 0.59, P = 0.01) vitamin therapy.

We also measured the half-life for plasma fHcy and found that it was consistently lower than the half-life for tHcy (Table 2). Although the decline in tHcy is a measure of elimination from plasma, the reduction in fHcy depends on both the elimination of Hcy from the plasma compartment and on the binding of Hcy to plasma proteins. Generally, in subjects with a long half-life for plasma tHcy (eg, the cobalamin-deficient group), the net binding of Hcy to plasma proteins was responsible for the removal of ≈60% of plasma fHcy. In contrast, in subjects who had short half-lives (eg, folate-deficient subjects after therapy) net binding of Hcy to plasma proteins removed only 17% of plasma fHcy (data not shown).

Plasma methionine

Before therapy, Hcy administration induced a small increase in mean plasma methionine in the folate-deficient and miscellaneous groups, and a decline in mean plasma methionine in the cobalamin-deficient subjects. Notably, after supplementation, mean plasma methionine increased within 2 h by > 10 μmol/L in all three subject categories (**Figure 4**).

There was a significant inverse correlation between the fasting plasma tHcy concentration and the maximal change in methionine both before ($r_s = -0.59$, P = 0.01) and after ($r_s = -0.63$, $P < 0.01$) vitamin therapy. However, a high methionine response was not associated with a low AUC or rapid half-life, indicating that the increase in methionine after vitamin therapy did not reflect improved elimination of Hcy from plasma.

Urinary excretion of Hcy after a peroral load

The urinary Hcy excretion in the hyperhomocysteinemic subjects before vitamin supplementation ranged from 0.8 to 19.1 μmol/h ($\bar{x}$: 4.1 μmol/h) (Table 1) and was positively correlated ($r_s = 0.96$, $P < 0.001$) with fasting concentrations of tHcy. Between 2.9% and 14.3% of the Hcy dose was excreted unchanged into the urine, and the portion excreted was positively related (r_s = 0.70, $P < 0.001$) to fasting plasma tHcy concentrations (**Figure 5**).

DISCUSSION

Study population, study design, and aim

We studied 19 subjects with hyperhomocysteinemia due to impaired Hcy remethylation caused by deficient or borderline concentrations of folate and/or cobalamin (Table 1). Plasma kinetics of Hcy were determined after Hcy loading, both in the deficient state and after metabolic correction by supplementing with the deficient vitamins. The blood-sampling protocol was based on knowledge of plasma kinetics in healthy individuals (17), and allowed the assessment of C_{max}, t_{max} (the time at which C_{max} was reached), AUC, and $t_{1/2}$. From these indexes, plasma kinetics during impaired Hcy remethylation and the kinetic basis of hyperhomocysteinemia in folate- and cobalamin-deficient subjects could be derived.

The relation and interpretation of measured kinetic indexes

The mean absorption rate constant for tHcy was slightly higher after vitamin supplementation (data not shown), and

TABLE 2
Kinetics of plasma homocysteine after peroral administration before and after vitamin therapy[1]

Category	Total plasma Hcy								Free plasma Hcy	
	C_{max}		T_{max}		$AUC_{0-24\ h}$		Half-life[2]		Half-life[2]	
	Before	After	Before	After	Before	After	Before	After	Before	After
	μmol/L		*h*		*μmol · h/L*		*h*		*h*	
Folate deficiency										
1	89.2	80.7	1.5	1.0	401	396	2.4	2.4	2.4	2.6
2	95.6	108.7	0.5	0.5	685	557	3.9	3.0	2.3	2.6
3	64.8	106.1	0.5	0.5	190	475	1.9	2.2	1.3	2.1
4	57.6	77.1	0.5	1.0	275	245	3.5	2.1	1.9	1.9
5	75.1	76.5	1.0	1.0	551	511	3.1	2.9	2.1	2.3
6	67.8	67.1	1.0	0.5	477	403	3.6	3.9	3.8	3.1
$\bar{x}$	75.0	86.0	0.8	0.8	430	431	3.1	2.8	2.3	2.4
SD	14.7	17.2	0.4	0.3	181	110	0.8	0.7	0.8	0.4
Cobalamin deficiency										
7	63.7	58.7	2.0	1.5	337	389	3.6	3.6	2.3	3.2
8	44.1	88.8	2.0	1.0	259	456	7.0	3.1	1.8	0.9
9	54.1	57.9	1.5	1.0	359	385	2.3	2.9	2.1	1.6
10	79.9	69.3	0.5	1.0	1059	596	8.0	3.7	1.7	3.1
11	45.2	65.9	1.0	1.0	398	454	4.3	3.7	2.9	3.2
12	46.3	74.1	1.0	1.0	456	606	8.4	4.2	3.0	3.0
$\bar{x}$	55.6	69.1	1.3	1.1	478	481	5.6	3.5	2.3	2.5
SD	14.0	11.5	0.6	0.2	292	98	2.5	0.5	0.6	1.0
Miscellaneous										
13	83.8	60.5	0.5	1.0	671	443	4.6	3.0	3.5	3.3
14	84.7	90.0	1.5	0.5	654	524	2.8	3.4	2.0	2.2
15	83.5	91.0	0.5	0.5	687	504	4.9	3.7	2.8	3.0
16	100.3	93.9	1.0	1.0	785	685	4.8	4.2	2.3	2.3
17	69.4	74.6	2.0	1.5	618	818	5.5	7.5	1.6	2.0
18	84.9	47.2	1.0	2.0	685	369	5.3	3.7	2.9	2.3
19	58.5	61.3	1.5	0.5	375	342	2.7	2.0	2.4	2.2
$\bar{x}$	80.7	74.1	1.1	1.0	639	526	4.4	3.9	2.5	2.5
SD	13.3	18.3	0.6	0.6	127	171	1.1	1.7	0.6	0.5
All subjects combined										
$\bar{x}$	71.0	76.3	1.1	1.0	522	482	4.3	3.4	2.4	2.5
SD	17.2	16.7	0.5	0.4	217	132	1.9	1.2	0.7	0.6
Healthy subjects[3]										
$\bar{x}$	57.4		1.0		416		3.7			
SD	9.9		0.3		41		0.8			

[1] Hcy, homocysteine and its oxidized species; C_{max}, maximum increase in plasma Hcy above the fasting concentration; T_{max}, the time at which C_{max} was reached; $AUC_{0-24\ h}$, area under the plasma concentration curve in the interval 0–24 h.
[2] Calculated 2–6 h after administration of Hcy.
[3] Data from Guttormsen et al (17).

C_{max} and t_{max} were not significantly different before and after supplementation (Table 2). Based on these findings, we assume that vitamins had essentially no effect on the bioavailability of peroral Hcy. Bioavailability, which depends on absorption and presystemic metabolism (26), probably equals 0.53, the value previously determined in one individual injected with Hcy (17).

The AUC is a measure of the total systemic exposure to administered Hcy, and its relation to total clearance is given by the following formula (26):

$$\text{AUC} = \text{Bioavailability} \times \text{dose/total clearance} \quad (3)$$

Because the dose is constant and bioavailability probably shows small variability, the AUC is inversely related to total clearance. The plasma half-life is also inversely related to total clearance, as shown by the following formula:

$$\text{Half-life} = \ln 2 \times V/\text{total clearance} \quad (4)$$

where V is the distribution volume of tHcy.

The kinetic basis of hyperhomocysteinemia in folate- or cobalamin-deficient subjects

Vitamin supplementation markedly reduced the plasma tHcy concentration (Figure 1), but we observed only slight effects on the mean AUC or mean half-life in the three study groups (Table 2), and the mean values were comparable with those reported previously for young healthy subjects (17). On the basis of the relations discussed in the preceding paragraph, including equations 3 and 4, we suggest that hyperhomocysteinemia in these subjects is not explained by reduced total clearance of plasma tHcy. Hyperhomocysteinemia could be regarded as an increase in the steady state concentration of

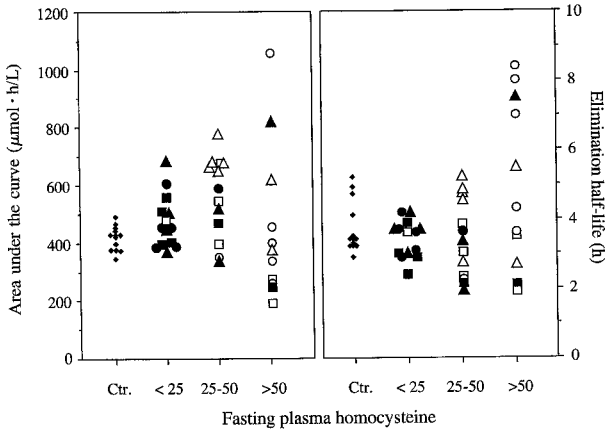

FIGURE 3. Half-life and area under the curve values at different concentrations of fasting plasma total homocysteine in subjects with folate (□, ■) and cobalamin (○, ●) deficiencies and in subjects belonging to the miscellaneous group (△, ▲) before (open symbols) and after (closed symbols) therapy with folic acid or cobalamin.

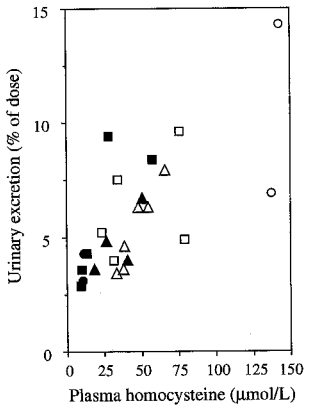

FIGURE 5. Urinary homocysteine (Hcy) excretion after Hcy loading in subjects with folate (□, ■) and cobalamin (○, ●) deficiencies and in subjects belonging to the miscellaneous group (△, ▲) before (open symbols) and after (closed symbols) therapy with folic acid or cobalamin. Hcy was determined in morning urine collected during 1.5–8 h (baseline) and in urine fractions for 24 h after the administration of the Hcy load. The results are expressed as a percent of the administered dose of Hcy. To estimate the amount derived from the administered Hcy, urinary excretion per hour was determined in every fraction and then the baseline urinary excretion per hour was subtracted.

tHcy in plasma (C_{ss}), which is expressed by the following equation (26):

$$C_{SS} = R_0/\text{total clearance} \qquad (5)$$

where R_0 is the rate of influx of Hcy into the plasma compartment. Thus, hyperhomocysteinemia in folate- or cobalamin-deficient patients is probably due to an increased export of Hcy from cells into the extracellular environment, including plasma.

Based on the principles outlined above and equation 5, the following estimations can be made: in a subject with a normal plasma tHcy concentration of 10.8 μmol/L and a normal clearance of 0.081 L/min (17), R_o = 0.87 μmol/min, corresponding to 1260 μmol/24 h. This corresponds to 5–10% of total cellular Hcy production in normal humans (29). For a cobalamin-deficient patient with a plasma tHcy concentration of 140 μmol/L and a normal plasma tHcy clearance (eg, subjects 7 and 11, Table 1), R_0 was calculated to be 16 000 μmol/24 h. Notably, this is a major portion (40–80%) of the total Hcy formed (29).

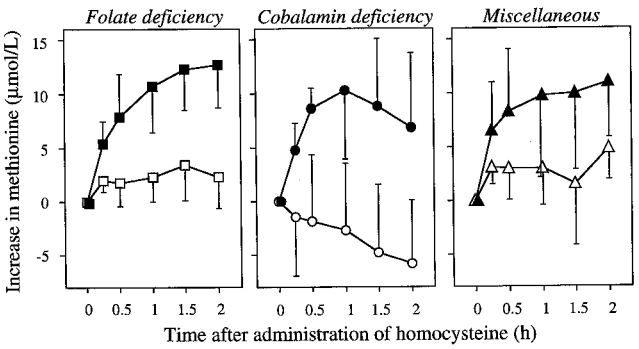

FIGURE 4. Change in plasma methionine after a peroral homocysteine loading (65 μmol/kg) before (open symbols) and after (closed symbols) therapy with folic acid or cobalamin. The subjects were fasting at the time of administration of the loading dose, and for the following 2 h. $\bar{x} \pm$ SD.

Kinetic variability, methionine synthesis, and metabolic adaptation

There was a tendency for subjects with extremely elevated tHcy concentrations to have a long half-life, but most of the subjects with moderate or intermediate hyperhomocysteinemia had half-lives and AUCs within the range observed in healthy subjects (17), and the mean half-lives and mean AUCs after vitamin supplementation were only slightly lower than those before therapy (Table 2). A striking observation was the higher spread of AUC and half-life values before compared with after vitamin therapy (Table 2) or compared with healthy subjects (17); the spread of both indexes was closely related to the fasting concentration of tHcy (Figure 3). Both before and after therapy, the AUCs and half-lives were correlated ($P < 0.05$) with each other and showed a concerted relation to fasting tHcy (Figure 3). Inspection of equations 3 and 4 suggests variation in tHcy clearance as a common denominator. This indicates a large interindividual difference in plasma tHcy clearance in hyperhomocysteinemic subjects.

The large variations in tHcy clearance in cobalamin- or folate-deficient subjects (Figure 3) are consistent with interindividual differences in metabolic adaptation. Conceivably, a low plasma tHcy clearance (long half-life) may be related to impaired function of methionine synthase and could be supported by the inverse correlation between the maximal methionine response to Hcy loading and the fasting plasma tHcy concentration before therapy, and normal methionine response after vitamin substitution (Figure 4). However, neither fasting plasma tHcy nor the methionine response correlated with the half-life. This indicates that the methionine synthase reaction is probably a minor elimination pathway of Hcy in plasma, or that folate and vitamin B-12 deficiency is associated with adaptive mechanisms that compensate for reduced remethylation of Hcy from plasma.

A possible adaptive mechanism is increased flux through alternate Hcy-consuming pathways (2), which may enhance

clearance and lower half-lives for plasma tHcy. This may be related to metabolic regulation or enhanced synthesis of the enzyme. In rat liver extracts, Finkelstein and Martin (30) showed that the absence of 5-methyltetrahydrofolate stimulates betaine-Hcy methyltransferase, whereas a high concentration of Hcy stimulates both cystathionine β-synthase and betaine-Hcy methyltransferase. Notably, cystathionine β-synthase is an Hcy-catabolizing enzyme with a high K_m value (> 1 mmol/L) (2), which is compatible with a dose-independent first-order elimination of supraphysiologic concentrations of Hcy observed in these subjects.

In patients exposed to nitrous oxide (31, 32) or methotrexate (33), there is an acute drop in plasma methionine followed by an increase that may exceed pretreatment concentrations within a few days. Folate-and cobalamin-deficient subjects had a markedly increased serum cystathionine concentration, a slightly lower serum betaine concentration, and a marked spread in serum methionine concentrations compared with control subjects (34). These clinical data indicate variable metabolic adaptation under conditions of disturbed vitamin function. The determination of whether this variability is due to genetic, nutritional, or other factors requires further studies.

Urinary Hcy excretion

The mean daily urinary Hcy excretion in healthy subjects is ≈6 μmol/24 h (14), or only 1% of the calculated amount filtered in the glomeruli (15), indicating almost complete Hcy reabsorption. In the folate- and cobalamin-deficient subjects studied here, the mean urinary Hcy excretion was ≈100 μmol/24 h during fasting and was positively correlated with the plasma tHcy concentration. This value is ≈15 times higher than that in healthy subjects (14), and 3–4 times the Hcy excretion observed in patients receiving methotrexate (35) or nitrous oxide (31). After peroral Hcy administration, the fraction of Hcy excreted in the urine was positively correlated with fasting plasma tHcy concentrations (Figure 5). This should be related to a higher percentage of plasma fHcy at high plasma concentrations (Figure 2, right panel), which is probably due to saturation of binding sites in plasma (36, 37). However, the mean recovery of Hcy in urine was < 7% of the administered dose, demonstrating that the urinary excretion of Hcy is a minor elimination pathway that cannot compensate for impaired Hcy metabolism in folate- and cobalamin-deficient patients.

Conclusion

The main conclusions of this study are as follows:

1) The mean half-lives and AUCs for plasma tHcy were not significantly elevated in folate- and vitamin B-12–deficient subjects compared with healthy control subjects. These findings suggest that clearance of Hcy from plasma through methionine synthase is either quantitatively unimportant or can be compensated by increased flux through alternate pathways.

2) Because most cobalamin- or folate-deficient subjects have normal plasma tHcy clearance, the hyperhomocysteinemia is probably explained by an increased influx of Hcy into the plasma compartment. In patients with severe hyperhomocysteinemia (> 100 μmol/L) a major portion of the total Hcy formed may be exported into the extracellular medium.

3) The methionine response is a metabolic marker that discriminates folate- and cobalamin-deficient subjects from healthy control subjects.

4) Less than 7% of the Hcy loading dose was recovered in the urine, suggesting that urinary excretion of Hcy does not compensate for the increased Hcy export in cobalamin- or folate-deficient subjects.

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