Kinetics of Plasma Homocysteine in Healthy Subjects after Peroral Homocysteine Loading
Anne Berit Guttmersen,1 Azam M. Mansoor, Toruna Fiskerstrand, Per M. Ueland, and Helga Refsum

The kinetics of plasma homocysteine were determined in 13 healthy subjects after peroral administration and in one person after intravenous injection. Various forms of homocysteine completely dissolved in an aqueous solution were rapidly absorbed after peroral administration, and the bioavailability was estimated to be 0.53. The volume of distribution was 0.66 L/kg. The area under the plasma concentration curve (AUC<sub>0-48 h</sub>) was proportional to the administered dose (33.5–134 μmol/kg body wt), and showed small interindividual variations. Plasma homocysteine showed first-order elimination kinetics for at least 6 h. The half-life (t<sub>1/2</sub>) was 223 ± 45 min, and there was a significant correlation between t<sub>1/2</sub> values determined on two different occasions in the same individual. The transient hyperhomocysteinemia was associated with an increase in plasma methionine, which probably reflects intracellular remethylation of homocysteine. Less than 2% of the administered homocysteine dose was recovered in the urine. These findings may form the basis for future studies on the regulation of plasma homocysteine in health and disease, and should motivate the evaluation of a homocysteine loading test as a diagnostic tool.

Indexing Terms: amino acids • metabolism • methionine • urine

Homocysteine is a sulfur amino acid formed from methionine as a product of transmethylation. Intracellular homocysteine is either salvaged to methionine or degraded to cysteine. The former reaction is in most tissues catalyzed by the enzyme 5-methyltetrahydrofolate–homocysteine S-methyltransferase (methionine synthase; EC 2.1.1.13), which requires 5-methyltetrahydrofolate as methyl donor and cobalamin as cofactor. The first step in homocysteine degradation is catalyzed by the vitamin B<sub>6</sub>-dependent enzyme cystathionine β-synthase (1). Homocysteine export into the extracellular medium is an alternative route of homocysteine disposal and, under conditions of impaired metabolism, the extracellular homocysteine concentration, like that in plasma and urine, becomes markedly increased (2). The export into the extracellular medium is the biochemical basis for plasma homocysteine as a marker for several disease states (2).

Plasma homocysteine is markedly increased during folate (3) or cobalamin deficiency (4). It is also increased to high amounts in patients with the rare inborn error called homocystinuria, which is most commonly caused by deficiency of the enzyme cystathionine β-synthase. These patients have a high morbidity of cardiovascular disease, which may cause death in early adolescence and even in childhood (5). Recent clinical studies including more than 1800 patients have amply demonstrated that a moderate increase of plasma homocysteine, so-called hyperhomocysteinemia, is a common and independent risk factor of premature cardiovascular disease in the general population. Hyperhomocysteinemia is caused by both nutritional and genetic factors, including heterozygosity for cystathionine β-synthase deficiency (6, 7).

The plasma homocysteine concentration in fasting subjects heterozygous for cystathionine β-synthase deficiency is usually within the normal range. To identify such subjects, the methionine loading test has been used in several clinical studies (6, 7). The test is based on peroral administration of a standard dose of methionine and measurement of the plasma homocysteine concentration 4–6 h after intake. The test improved the discrimination of heterozygotes from controls in some studies, but discontinuous segregation between the two groups was not obtained (8). This may reflect the interindividual variation in response to a standard methionine load (9), which may be due to, among other factors (9), the fact that plasma homocysteine is a function of both homocysteine formation and metabolism.

Homocysteine loading has been attempted in patients with homocystinuria, but the results were not interpretable because of the low solubility and absorption of this compound (10). In another attempt, free base of racemic DL-homocysteine was given to eight homocystinurics and four healthy subjects, and the increase in the free homocysteine mixed and symmetric disulfides was measured in plasma (11).

No data exist on the plasma kinetics of total homocysteine in normal subjects given peroral doses of L-homocysteine. Such data may provide information of homocysteine disposition and metabolism in healthy subjects. In addition, the kinetic features should be decisive regarding further evaluation of a homocysteine loading as a test to reveal defects in homocysteine metabolism. In the present work, we characterized the plasma kinetics of L-homocysteine in 13 healthy subjects.

Materials and Methods
Materials and Subjects
Sources of most reagents used have been given previously (12). L-Homocysteine thiolactone and L-homocysteine were purchased from Sigma Chemical Co., St. Louis, MO.

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Thirteen healthy volunteers, six women and seven men (ages 19–36 years), participated in the study. The participants had provided their written informed consent, and the protocol was approved by the regional ethical committee of western Norway.

All subjects had plasma concentrations of folate, cobalamin, total homocysteine, total cysteine, and methionine within the normal range (Table 1).

Homocyst(e)ine Solutions

All homocyst(e)ine solutions were prepared immediately before administration.

Peroral administration. Reduced homocysteine was the administered form, unless otherwise stated. Powdered L-homocysteine thiolactone (300–1200 mg, 33.5–134 μmol/kg of body wt) was dissolved in 5 mL of 5 mol/L NaOH and allowed to stand for 5 min to open the thiolactone ring. We then added 5 mL of 5 mol/L HCl for neutralization, and carefully adjusted the pH to between 4 and 5. A mixture of water and apple cider (to mask the unpleasant taste of homocysteine) was added, to give a total volume of 200 mL.

L-Homocysteine (526 mg; 67 μmol/kg) and L-homocysteine thiolactone (600 mg; 67 μmol/kg) for peroral use were dissolved in 25 mL of 1 mol/L HCl and in 25 mL of 1 mol/L NaCl, respectively. The former solution was adjusted to pH between 4 and 5 by adding 1 mol/L NaOH. Both solutions were diluted with water and apple cider to a final volume of 200 mL.

Intravenous injection. Reduced homocysteine was given in these experiments. L-Homocysteine thiolactone powder (720 mg) was dissolved in 4.4 mL of 2.7 mol/L NaOH and allowed to stand for 5 min. This solution was then carefully adjusted to pH 6 with HCl. Distilled water was added to give a volume of 60 mL and an osmolarity of about 500 mosmol/kg. The solution was sterilized by filtration (0.22-μm pore size), and 50 mL was injected into the subject.

Protocol

Peroral loading. After an overnight fast, 13 persons received a standard peroral dose of 67 μmol of reduced homocysteine per kilogram of body weight. This corresponds to a dose of L-homocysteine thiolactone of 10 mg/kg body weight. Ten of these subjects (five men and five women) were given a second loading.

To obtain blood samples, we inserted a 1.7-mm-diameter cannula into a cubital vein. Immediately before the administration of homocysteine, blood was collected for determination of plasma folate and cobalamin and of preload values for the plasma aminothiols and methionine.

The homocysteine solution (200 mL) was swallowed in <2 min. Each person was allowed to freely drink water and apple cider, but remained fasting for at least 60 min after the administration. During the next 48 h, 12 blood samples were routinely collected (at 15, 30, 60, 90 min, and 2, 3, 4, 6, 8, 12, 24, and 48 h after the administration). From one person, urine was collected in fractions for 24 h before and for 48 h after the administration.

One subject (HH) was loaded three times with equal doses (67 μmol/kg body weight) of homocysteine, homocystine, and homocysteine thiolactone, to compare the bioavailability of these forms. Blood sampling was performed according to the above protocol.

Another volunteer (BB) was loaded three times with increasing peroral doses (33.5, 67, and 134 μmol/kg body weight) of homocysteine. The protocol was as described above.

Intravenous administration. Volunteer BB also received homocysteine as an intravenous dose, injected over a period of 20 s through a 1.4-mm-diameter cannula mounted in the cubital vein. The homocysteine dose was 67 μmol/kg body weight. Blood samples were collected from the 1.7-mm cannula in the cubital vein of

Table 1. Characteristics of Subjects

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<th>Sex</th>
<th>Age, years</th>
<th>Weight, kg</th>
<th>Height, cm</th>
<th>Cysteine, μmol/L</th>
<th>Homocysteine, μmol/L</th>
<th>Methionine, μmol/L</th>
<th>Folate, nmol/L</th>
<th>Cobalamin, pmol/L</th>
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Mean    | 29   | 64         | 172        | 248.4       | 9.0             | 26.5                | 13.3              |              | 414              |

SD      | 5    | 8          | 8          | 19.7        | 2.5             | 4.2                 | 4.7               |              | 160              |

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the opposite arm. During the first 2 h after injection, frequent blood samples were drawn, but then sampling followed the protocol described above.

Analytical Methods

Whole blood was collected in Vacutainer Tubes (Becton Dickinson, Rutherford, NJ) containing EDTA, immediately placed on ice, and centrifuged within 30 min at 3000 × g for 5 min at 0–2 °C. Plasma was stored at −20 °C until analysis.

Total plasma homocysteine and cysteine were determined by a modification (12) of an automated procedure developed for the determination of total homocysteine in plasma (13).

Plasma methionine was determined in deproteinized plasma with an assay based on derivatization with o-phthalaldehyde and fluorescence detection (14). Plasma cobalamin was determined with a microparticle enzyme assay of intrinsic factor run on an IMx system from Abbott (Abbott Park, IL), and plasma folate was assayed with the Quantaphase folate radioassay produced by Bio-Rad (Hercules, CA). Plasma S-adenosylhomocysteine was determined in deproteinized plasma by reversed-phase chromatography and ultraviolet absorbance detection (15).

Calculation of Kinetic Parameters

The decline in plasma homocysteine concentration after peroral administration of homocystine is consistent with a one-compartment, open pharmacokinetic model. The elimination of homocysteine after peroral loading obeys first-order kinetics between 90 and 480 min in most subjects, and in all subjects between 120 and 360 min. The half-life (\(t_{1/2}\)) was calculated by log linear least-squares curve-fitting in the interval 120–360 min, by use of the following equations (16):

\[
C = C_0 e^{-kt}\quad\text{and}\quad t_{1/2} = \ln 2/k
\]

where \(C\) is the plasma concentration at time \(t\), \(C_0\) the extrapolated plasma concentration at \(t = 0\), and \(k\) the rate constant of the elimination.

To calculate the absorption rate constant, \(k_a\), we used these modifications of the above equations (16):

\[
C' = C_0 e^{-kt}
\]

\[
R = C' - C
\]

\[
R = R_0 e^{-k_d}
\]

where \(C'\) is the extrapolated plasma concentration at times \(t\) during the absorption and distribution phase, \(R\) is the residual value, and \(R_0\) is the residual value at \(t = 0\).

\(\text{AUC}_{0-\infty}\) is the area under the plasma concentration–time curve from zero to infinite time, as measured by the trapezoidal rule. Because plasma homocysteine returned to the basal level (<2.5 μmol/L different from the pretreatment level) within 48 h in all subjects receiving the standard dose, \(\text{AUC}_{0-48}\) was taken as identical to \(\text{AUC}_{0-\infty}\).

When homocysteine was administered intravenously, the elimination curve showed multiple-compartment characteristics. Therefore, the volume of distribution (\(V_d\)) was calculated as a model-independent parameter (17):

\[
V_d = D(\text{AUMC}_{0-\infty})/(\text{AUC}_{0-\infty})^2
\]

where \(D\) is dose, and \(\text{AUMC}_{0-\infty}\) is the area under the curve of product of time and plasma concentration from zero to infinite time.

Clearance (Cl) is calculated according to the equation (16):

\[
\text{Cl} = D/(\text{AUC}_{0-\infty})
\]

Statistical Methods

The results are given as mean and SD. Paired values were compared by using the Wilcoxon signed rank test. Unpaired values were evaluated by the Mann–Whitney U-test. Correlation was tested by using the Spearman rank correlation coefficient (18). Significance levels were always expressed as two-tailed.

Results

In the present work, 13 volunteers received a total of 30 peroral homocystine loadings. None experienced adverse effects except those subjects responding with dizziness during insertion of the cannula. The subject receiving intravenous administration of homocysteine reported an immediate (within 2–4 min) but temporary (12 h) relief of a headache.

Absorption

In a preliminary experiment, 135 mg of L-homocysteine (the symmetric disulfide) suspended in 100 mL of water was administered to one volunteer. Plasma homocysteine increased gradually over 4 h, from 10 to 18 μmol/L, and remained at a plateau for the following 3–4 h (data not shown). The delayed absorption is probably due to low solubility of homocystine in water.

Figure 1 shows the estimation of the absorption rate constant from the first part of the mean plasma concentration curve for homocysteine obtained by peroral administration of homocystine, 67 μmol/kg. An absorption rate, \(k_a\), of 3.6 h⁻¹ was obtained from the data from the 13 subjects, and 4.6 h⁻¹ when the loading was repeated in 10 subjects. The maximal increase in plasma homocysteine concentration (\(C_{\max}\)) was obtained after about 60 min (\(t_{\max}\)) (Table 2). Thus, reduced homocysteine is rapidly absorbed after peroral administration.
Fig. 1. Absorption rate of homocysteine after peroral administration
Thirteen volunteers were subjected to a peroral homocysteine loading (67 
μmol/kg body weight) (open symbols); 10 of these repeated the loading a second 
time (closed symbols). The mean plasma concentration curves were used for 
the calculation of the absorption rate constants by equations 3–5 given in 
Materials and Methods. The residual values (Δ, Δ) are the extrapolated 
plasma concentrations (□, □) minus the observed concentrations (○, ○). The 
absorption rate constants for the first (ka1) and second (ka2) loading are 
obtained from a semilogarithmic plot of the residual values vs time

Kinetics after Intravenous Administration: Bioavailability

One volunteer (BB) was given reduced homocysteine, 67 μmol/kg body weight, once as an oral dose and once as an intravenous injection.

After intravenous administration, the increase in total plasma homocysteine reached a Cmax of 250 μmol/L within 10 s after the end of injection. This was followed by a short distribution phase and then a plateau (Figure 2). A monoexponential decay curve was observed between 90 and 720 min, corresponding to a half-life of 186 min. The weight-corrected volume of distribution (Vd) was calculated as 0.66 L/kg, and total body clearance (Cl) was 81 mL/min.

After the absorption phase was completed, the elimination curve of peroral homocysteine (t1/2 = 199 min) paralleled the curve obtained after intravenous admin-

istration (Figure 2). The bioavailability (F), obtained by dividing the AUC0-48 h obtained after peroral administration with the value obtained after intravenous administration, was 0.53.

We evaluated the bioavailability of different forms of homocysteine (reduced, oxidized, and thiolactone) by giving a peroral dose of 67 μmol/kg body weight of each form to one volunteer (HH). All species were completely dissolved in the aqueous medium (see Materials and Methods). Under these conditions, all forms were rapidly absorbed (tmax between 30 and 60 min) and the AUC0-48 h values were essentially equal (25.55–27.65 mmol·L⁻¹·min⁻¹), suggesting that the bioavailability of different forms of dissolved homocyst(e)ine is the same.

Kinetics at Different Peroral Doses

To test whether the kinetics were dependent on the loading dose, we gave one person (BB) increasing doses of homocysteine (33.5, 67, and 134 μmol/kg) perorally. There was a linear increase in AUC0-48 h with dose (Figure 3). Clearance (assuming a value for F of 0.53) was 63, 80, and 75 mL/min, and t1/2 was 209, 199, and 217 min, respectively. These data show that homocysteine given perorally obeys dose-independent linear kinetics within the dose range investigated.

Interrelations and Statistics of Kinetic Parameters

A standard homocysteine dose (67 μmol/kg) was given perorally to 13 subjects, and the loading was repeated in 10 of these subjects. The time interval between the two loading experiments was 2–3 weeks. The mean plasma concentration curves for the first and second loadings are shown in Figure 4, and the corresponding kinetic parameters for each individual in Table 2.

There was no significant difference between the kinetic parameters obtained for the first and second loadings. There was a rapid absorption phase for the two loadings, corresponding to tmax of 59 ± 20 and 54 ± 13

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Mean       | 24.95     | 223  | 57.4  | 59   |
SD         | 2.48      | 45   | 9.9   | 20   |
Subject BB was given the same dose of reduced homocysteine (67 μmol/kg body wt) intravenously and perorally. The plasma concentration curves are shown in the main panel; the inset shows the first 90 min of the intravenous curve. The AUC values were 26.52 and 49.80 mmol · L⁻¹ · min after the peroral and intravenous loading, respectively; the bioavailability (F), calculated as the ratio between these two values, was 0.53.

**Fig. 3. Kinetics of plasma homocysteine at different peroral doses**
Subject BB received three doses of homocysteine (33.5, 67, and 134 μmol/kg body wt), and the plasma concentration curves are shown. Inset: corresponding AUC values plotted against the homocysteine dose.

**Fig. 4. Kinetics of plasma homocysteine in healthy subjects**
Thirteen subjects underwent a peroral homocysteine loading (67 μmol/kg) (open symbols); 10 of these repeated the loading a second time (closed symbols). The main panel shows the mean plasma concentrations with standard deviations (bars) plotted vs time. Inset: semilogarithmic plot of these data in the time interval (120–360 min) during which homocysteine is eliminated according to first-order kinetics.

**Fig. 5. Correlation between t₁/₂ values obtained from two homocysteine loadings of the same individual**
Ten subjects underwent two peroral homocysteine loadings. The t₁/₂ values are calculated (according to equations 1 and 2 in Materials and Methods) from four time points in the time interval 120–360 min. t₁/₂ values from the first loading are plotted against the t₁/₂ values from the corresponding subjects during the second loading. There is a significant correlation (r = 0.66, P < 0.05) between the two sets of values.

min and C₅₀ values of 57.4 ± 9.9 and 63.2 ± 10.2 μmol/L, respectively. t₁/₂ calculated for the first and second loadings were 224 ± 44 and 216 ± 43 min, and the AUC values were 24.95 ± 2.48 and 24.19 ± 4.28 mmol · L⁻¹ · min, respectively (Figure 4, Table 2).

Because the t₁/₂ showed some interindividual variations (Table 2), we compared the value obtained for the first and second loadings in 10 subjects; the correlation was significant (P < 0.05) (Figure 5).

We also compared 23 values for t₁/₂ when the calculations were based on two time points (y: 217 ± 42 min) and four time points (x: 221 ± 43 min) within the period 120–360 min after loading. Essentially the same values were obtained: y = 0.97x + 4.43 (r = 0.99). This observation may justify restricting sample collection to times 120 and 360 min after loading for obtaining t₁/₂ values.

The AUC₅₀ values showed less interindividual variation (24.95 ± 2.48 mmol · L⁻¹ · min) than the other kinetic parameters (Table 2), and there was no significant correlation between the values observed for the first and second loadings in 10 subjects.

There was no correlation between AUC₅₀ and t₁/₂, and neither parameter was correlated to the fasting basal concentration of homocysteine (data not shown).

**Metabolic Effects**

The transient hyperhomocysteinemia after homocysteine loading was accompanied by a decline in total plasma cysteine (t₅₀ of 60–90 min) (Figure 6). The maximal decrease was 75.9 ± 20.3 μmol/L, which slightly exceeds C₅₀ for homocysteine (Table 2). The cysteine response may at least partly be explained by displacement of cysteine from plasma protein(s) (19).

Plasma methionine increased within 15 min after peroral homocysteine administration; after 60 min, the
increase in mean plasma methionine was ~9 \mu mol/L (Figure 6). The mean increase in plasma homocysteine was ~60 \mu mol/L at this time point. The subjects were allowed to eat after 60 min, which may have influenced the plasma methionine concentration in samples collected after this time.

The increase in plasma methionine showed no correlation to preload concentrations of homocysteine or methionine. It was also unrelated to \( t_{1/2} \) or \( C_{\text{max}} \) for increases in plasma homocysteine. There was a significant \( (P < 0.05) \) positive correlation between AUC\(_{0-48 \text{ h}} \) and increase in plasma methionine in the interval 15–90 min (data not shown).

We investigated the methionine response in the subject (BB) who received three different doses of peroral homocysteine. The methionine concentration increased as a function of the homocysteine dose (Figure 7). When the same subject received intravenous homocysteine, a methionine response was observed 2.5 min after injection, and the methionine concentration increased to a somewhat higher amount \( C_{\text{max}} = 27.4 \mu \text{mol/L} \), \( t_{\text{max}} = 75 \text{ min} \) (data not shown) than observed after peroral administration of the same dose (Figure 7).

Both after the peroral and the intravenous homocysteine loads, we observed no change in \( S \)-adenosylhomocysteine concentration in plasma (data not shown).

**Urinary Excretion of Homocysteine and Cysteine**

In one person (HH), the urine fractions were collected before and for 48 h after a peroral load of homocysteine.

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**Discussion**

**Homocysteine Form and Dose**

Reduced homocysteine is rapidly (Figure 1) and efficiently absorbed from the gastrointestinal tract, and shares this property with homocysteine thiolactone. Homocysteine is insoluble in water, and is slowly absorbed when administered as a suspension in water. This is in accordance with the observation of Brenton et al.
al. (10). When homocysteine is dissolved in dilute acid, its bioavailability seems equal to that of reduced homocysteine.

We chose reduced homocysteine in these experiments, especially because homocysteine may precipitate in solution after the neutralization required for intravenous administration. Homocysteine thiolactone also has some unfavorable properties, including its ability to acylate biogenic amines and proteins, which can possibly change their function or antigenic properties (20). Furthermore, the fact that enzymic (21) or chemical hydrolysis of the thiolactone is required before homocysteine can enter the homocysteine-metabolizing pathways can result in variations in kinetics unrelated to homocysteine metabolism.

The homocysteine dose administered was based on the assumption that homocysteine is distributed in total body water (about 60% of total body weight). We wanted to obtain a maximum increase of 100 μmol/L in plasma homocysteine. With rapid absorption and a 100% bioavailability of the administered dose, the calculated amount of homocysteine is about 60 μmol/kg body weight, equivalent to 9 mg of L-homocysteine thiolactone per kilogram of body weight. This was for simplicity increased to 10 mg/kg body weight.

Disposition and Kinetics of Plasma Homocysteine

The kinetic data we obtained provide new information on the disposition of homocysteine during transient hyperhomocysteinemia, but also identify processes important for the regulation of plasma homocysteine under physiological conditions.

The distribution volume of plasma homocysteine (0.66 L/kg) equals total body water (16). This suggests that plasma homocysteine is distributed into both extravascular fluids and the intracellular aqueous phase. Furthermore, this process probably occurs rapidly, as demonstrated by the first part of the distribution curve after intravenous injection (Figure 2, inset). Because distribution is not the rate-limiting factor, the monoeponential decay curve (Figures 2–4, 8) probably reflects the rate of elimination.

Urinary excretion of homocysteine after homocysteine administration is closely related to the increase in plasma homocysteine (Figure 8). However, urinary excretion accounts for <2% of the administered dose. Thus, the elimination rate constant may be a measure of the activity of homocysteine-metabolizing enzyme(s). Dose-independent, first-order kinetics (Figures 2–4, 8) indicate that the homocysteine-metabolizing enzyme(s) operate(s) at substrate (homocysteine) concentrations far below the $K_m$ for homocysteine.

The bioavailability of 50% indicates a substantial presystemic metabolism, and the rapid increase of methionine in plasma could suggest that homocysteine remethylation was considerable during its first pass through the liver. However, both methionine synthase and betaine-homocysteine methyltransferase have relatively low $K_m$ values (1), and the positive correlation between the methionine response and AUC$_{0-48}$ h for homocysteine in the individual subjects argues against the possibility that first-pass remethylation in the liver is a major determinant of reduced bioavailability. Thus, it is possible that the alternative pathway, i.e., catabolism via cystathionine $\beta$-synthase, contributes substantially to the presystemic metabolism. This is in accordance with the $K_m$ value of this enzyme, which is in the millimolar range (1).

Homocysteine is metabolized to cysteine via the transsulfuration pathway (1), but the increased plasma homocysteine was associated with a decline in plasma cysteine (Figure 6) and no increase in urinary cysteine excretion was observed. This may be explained by displacement of cysteine from its binding sites in plasma, as has previously been suggested for other conditions associated with hyperhomocysteinemia (19, 22, 23).

Homocysteine Loading vs Methionine Loading

In a standard methionine loading test, 100 mg/kg (670 μmol/kg) of methionine is administered perorally, i.e., 10 times higher than the homocysteine dose used in the present work. The kinetics of plasma homocysteine after methionine and homocysteine loadings are quite different. After methionine intake, the increase in homocysteine reaches $C_{\text{max}}$ after 4–8 h, the $C_{\text{max}}$ is somewhat lower than after homocysteine loading, and the concentration is reduced to 50% after about 12 h (19, 24). Recalculation of published data (19, 24) shows that the AUC values for homocysteine in plasma after methionine loading are of similar magnitude or slightly higher than after homocysteine loading.

The slow kinetics of the homocysteine response after methionine intake may be explained by a continuous formation of homocysteine from methionine, which occasionally remains markedly high in plasma even after 12 h (19). Similar AUC values after both methionine and homocysteine loadings add support to the conclusion that the capacity of the homocysteine-metabolizing enzymes far exceeds that required to handle a standard homocysteine loading dose. Assuming that most of the administered methionine is directed into the transmethylation/transsulfuration pathway, similar AUC values also suggest that only a small fraction (<10%) of the homocysteine formed from methionine is equilibrated with plasma homocysteine, or that administered and endogenously formed homocysteine do not have equal distribution properties.

Kinetic Features and Clinical Applicability

Conceivably, the plasma kinetics of homocysteine after peroral homocysteine loading could reflect the activity of homocysteine-metabolizing enzyme, whereas the homocysteine response after methionine loading (19, 24) may be influenced by additional factors such as methionine incorporation into proteins, the activity of methionine adenosyltransferase, and the overall transmethylation rate of $S$-adenosylmethionine (25).

The kinetics of plasma homocysteine after peroral loading have some features that should motivate a future evaluation of homocysteine loading as a clinical
test. Homocysteine is rapidly absorbed, and the amount entering the systemic circulation, determined as AUC_{0-48 h}, shows small interindividual variability (Table 2). After absorption, a monoeXponential elimination phase was identified between 90 and 480 min, and the linearity of this phase allowed the exact assessment of the t_{1/2} from a two-point determination (120 and 360 min). t_{1/2} shows some interindividual variation, and comparison of values from the same individual tested on two different occasions showed a positive significant correlation between the two sets of values. This suggests that t_{1/2} may be a measure of the metabolic phenotype.

In conclusion: rapid absorption and high bioavailability of homocysteine allow the determination of plasma kinetics of homocysteine during the transient hyperhomocysteinemia that follows peroral administration. Plasma homocysteine is eliminated according to dose-independent, first-order kinetics at plasma concentrations of homocysteine within the concentration range tested, i.e., up to about 100 μmol/L during the elimination phase. This suggests that the enzyme system(s) responsible for elimination operates far below the K_m for homocysteine. The kinetics parameters obtained showed small variations in healthy subjects, and t_{1/2} values obtained in two loading experiments of the same individual were significantly correlated. This opens up the evaluation of a homocysteine loading test as a tool to diagnose acquired and genetic defects of homocysteine metabolism. Such investigations are under way.

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References

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