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Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure

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Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. We investigated the elimination of total homocysteine (tHcy) from plasma after peroral homocysteine (Hcy) loading in eight patients with chronic renal failure. Data on bioavailability and distribution volume were obtained from two patients and two healthy controls by performing both intravenous and peroral Hcy loading. Response to high-dose folic acid was studied in six cases. Mean (SD) basal plasma tHcy was 27.4 (11.0) μM at inclusion. The half-life and the area under the curve were about four times higher, and clearance was reduced to 29.8% compared to controls. High-dose folic acid had no influence on half-life for tHcy, but the basal tHcy level declined by 26.8%. The reduction in tHcy was particularly pronounced in three patients with low-normal serum folate, and the enhanced methionine response to Hcy loading after folic acid suggested improved Hcy remethylation in tissues. In conclusion, patients with renal failure had markedly reduced clearance of tHcy from plasma, which probably accounts for their hyperhomocysteinemia. High-dose folic acid reduces fasting tHcy by improving tissue Hcy remethylation without affecting the low renal clearance of tHcy.

A large number of clinical investigations [1–4], including three studies with a prospective design [2–4], demonstrate that elevated levels of the sulphur amino acid homocysteine (Hcy) in plasma is a strong and independent risk factor for cardiovascular disease. In addition to genetic defects or polymorphisms involving Hcy-metabolizing enzymes, several acquired conditions and disease states cause elevated total homocysteine (tHcy), that is, hyperhomocysteinemia. The relation between tHcy and folate and cobalamin status has been studied in great detail [5], and tHcy has been shown to be an early and sensitive marker of impaired function of these two vitamins [6]. The hyperhomocysteinemia in folate or cobalamin deficiencies can be explained by impaired function of the enzyme methionine synthase (5-methyltetrahydrofolate-homocysteine methyltransferase, EC 2.1.1.13), which requires 5-methyltetrahydrofolate as methyldonor and cobalamin as co-factor in a reaction where Hcy is remethylated to methionine [7]. Plasma tHcy is also increased in renal failure and is closely related to serum creatinine [8], but the mechanism behind this relationship is debated. Large amounts of Hcy is formed in conjunction with creatine/creatinine synthesis [9], and recent data suggest that glomerular filtration rate (GFR) is a strong determinant of the plasma tHcy level [10]. Notably, urinary Hcy excretion is normally not a route of Hcy disposal, since only 0.25 μmol/hr of Hcy is excreted unchanged in the urine [11], indicating that more than 99% of filtered Hcy is subjected to tubular reabsorption [12]. A recent study in the rat suggests that substantial metabolism of Hcy may take place in the kidney [13].

The hyperhomocysteinemia in folate/cobalamin deficiency as well as in renal failure is a steady state condition characterized by either increased net transport of Hcy from tissue to plasma and/or decreased Hcy elimination from the central compartment. We recently determined the half-life ($T_{1/2}$) for tHcy in folate and cobalamin-deficient subjects after peroral ingestion of Hcy [12], and observed that it was equal to that found in healthy subjects [14]. This observation together with *in vitro* experiments with cultured cells [15] suggest that the elevation of tHcy in folate/cobalamin deficiency is related to increased net Hcy efflux from tissues to plasma.

In the present study, we used the Hcy loading test [14] to determine the kinetics of tHcy in patients with chronic renal failure. The investigation was performed before and after supplementation with high-dose folic acid, which significantly reduces fasting tHcy in most patients even in the absence of overt folate deficiency [16, 17]. This study shows that the increased tHcy level in renal failure probably is due to a marked reduction of tHcy clearance from plasma, and that clearance is not enhanced by folic acid.

METHODS

Subjects

Eight patients, three females and five males, with a mean ± SD age of 51 ± 12 years, with chronic renal failure (mean ± SD serum creatinine, 477 ± 113 μM) were recruited from the Nephrological Outpatient Clinic at Haukeland University Hospital, Bergen, Norway. Mean ± SD GFR estimated from serum creatinine, age, sex and body wt [18, 19] was 18 ± 6 ml/min. Patient characteristics are shown in Tables 1 and 2.

As controls, we used historical data [14] and one additional healthy male subject, aged 26 years, who received a peroral and intravenous Hcy load.

All individuals had given their written informed consent to the study, which had approval by the ethical committee of Western Norway.

Key words: chronic renal failure, folic acid, clearance, homocysteine, cardiovascular disease.

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Table 1. Characteristics of patients before first and second homocysteine loading

Patient	Sex	Age *years*	Weight *kg*	MTHFR genotype	Hemoglobin *g/liter*		Creatinine *μM*		GFR *ml/min*		S-cobalamin *pM*		S-folate *nM*		E-folate *nM*	
					First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
Group I[a]																
1	M	63	83	CC	133	136	359	395	22	20	373	407	11.9	11.3	801	746
2	F	57	57	CT	129	130	552	454	9	11	668	638	6.7	8.5	432	442
Group II																
3	M	43	102	TT	117	121	582	663	21	18	592	626	5.2	45.0	738	966
4	M	62	82	CC	88	100	418	551	19	14	457	421	12.5	45.0	752	2361
5	F	52	71	TT	113	106	625	623	10	11	417	332	14.7	45.0	718	2012
6	M	46	75	TT	100	119	344	327	25	27	200	268	7.5	43.5	481	1760
7	M	55	75	CT	101	96	556	626	14	13	498	609	13.7	45.0	654	1064
8	F	27	73	TT	139	118	376	436	23	20	499	448	4.7	35.3	634	1495
Mean group II					110	110	484	538	19	17	444	451	9.7	43.1	663	1610
SD					18	11	119	131	6	6	133	144	4.4	3.9	101	543

Abbreviations are: MTHFR, methylenetetrahydrofolate reductase; GFR, glomerular filtration rate; CC, CT and TT refer to normal homozygous, mutant heterozygous and mutant homozygous genotype, respectively; S-cobalamin, serum cobalamin; S-folate, serum folate; E-folate, erythrocyte folate.

[a] Group I consists of two patients undergoing one peroral and one intravenous homocysteine loading, and group II comprises patients receiving two peroral loadings, one before and one after high-dose folic acid supplementation.

Protocol

Plasma tHcy kinetics and folic acid supplementation. The participants had a light breakfast one to two hours before the Hcy loading. Six subjects received two peroral Hcy loadings (65 μmol/kg body wt). The second load was performed after at least 10 days (range 10 to 47 days) of folic acid supplementation (5 mg/day). Assessment of bioavailability and distribution volume (V_d) was performed in two patients with chronic renal failure and in two healthy controls. They were first subjected to one peroral load, and after about three weeks, they received an intravenous load by injecting the same Hcy dose: 65 μmol/kg in the controls and 32.5 μmol/kg in the renal patients. The lower dose in the two patients was chosen to avoid prolonged exposure to high tHcy levels.

The Hcy solution was prepared immediately before administration [14], and was swallowed or injected in less than two minutes. For at least two hours after the administration, the person refrained from intake of food but was allowed to drink water and apple cider.

Blood and urine collections. Before administration of Hcy, a blood sample was collected for the determination of hemoglobin, the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, and serum/plasma levels of creatinine, folate, cobalamin, tHcy, free Hcy (fHcy), total cysteine (tCys), and methionine (Tables 1 and 2). A total of 13 blood samples were routinely collected, that is, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24, 48 and 72 hours after the administration of Hcy. After intravenous injection, more frequent sampling was done during the first 0.5 hours. During the first 8 to 12 hours, blood samples were collected through a 1.7 mm cannula inserted into a cubital vein. Later, blood samples were obtained by venous puncture. In all patients, urine was collected in fractions for 24 hours.

Laboratory procedures

Whole blood was drawn into evacuated tubes without an additive (for preparation of serum) or containing EDTA as the anticoagulant (for preparation of plasma). Blood containing EDTA was immediately transferred to 1.5 ml vials, which were centrifuged at 13,000 × g for 0.5 minutes. This procedure allowed separation of the plasma fraction from the blood cells within three minutes. One milliliter of plasma was immediately deproteinized by adding 100 μl of 2 M sulfosalisylic acid, mixed and centrifuged, and the acid supernatant collected. Plasma and the acid supernatant were stored at −20°C until analyses of total and free plasma aminothiols and methionine. Whole blood without additives was allowed to coagulate for 0.5 to 1 hour at room temperature. Then, the tubes were centrifuged at 1,000 × g for 10 minutes, and the serum fraction transferred to storage vials.

The concentrations of tHcy and tCys in untreated plasma, fHcy in acid-precipitated plasma and Hcy in urine were determined by a modification [20] of an automated procedure developed for the determination of tHcy [21]. The between-day coefficient of variation was about 3%. Plasma methionine was determined with an assay based on derivatization with *o*-phthaldialdehyde and fluorescence detection [22]. Serum cobalamin was determined with a microparticle enzyme intrinsic factor assay run on an IMx system from Abbott (Abbott Park, IL, USA), and serum folate was assayed using the Quantaphase folate radioassay produced by Bio Rad (Hercules, CA, USA). The procedure for detection of the C677T mutation in the methylenetetrahydrofolate reductase gene was based on PCR amplification, restriction cleavage and separation of the DNA fragments by capillary electrophoresis [23].

Kinetic parameters

Kinetic parameters were calculated from the increase in tHcy after Hcy administration, using the parts of the plasma curves with essentially no interference from the basal tHcy level. Two procedures were used for the determination of the kinetic constants. (1) The elimination rate constant was obtained by linear regression of the terminal, linear part of the log-transformed concentration versus time curve. Because of different kinetics in renal patients versus healthy subjects [14], this was in the time interval between three hours and 24 hours for the patients and between two hours and six hours for the healthy controls. The kinetic parameters

Table 2. Plasma and urinary levels of homocysteine, cysteine and methionine before first and second homocysteine loading

	Plasma											
	tHcy μM		fHcy μM		fHcy/tHcy		tCys μM		Methionine μM		Urinary Hcy μmol/hr	
Patient	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
Group I[a]												
1	23.5	22.2	6.4	5.9	0.27	0.27	350.2	331.2	27.2	26.7	0.4	0.6
2	50.2	46.9	14.5	14.3	0.29	0.30	316.5	342.8	15.8	18.7	4.8	3.5
Group II												
3	27.7	17.2	7.8	4.9	0.28	0.28	334.6	334.8	31.1	39.4	2.6	1.1
4	20.7	20.8	5.9	5.5	0.28	0.27	377.1	323.7	39.0	32.3	0.7	1.4
5	24.6	26.0	6.4	7.1	0.26	0.27	424.3	468.7	25.7	18.4	0.8	0.6
6	35.6	15.2	9.4	3.4	0.26	0.22	320.5	245.6	27.9	20.3	0.5	0.4
7	22.2	18.3	6.9	5.7	0.31	0.31	391.1	409.2	26.8	22.3	2.2	5.0
8	14.7	9.0	3.9	2.4	0.27	0.27	227.9	266.6	28.6	15.7	1.7	0.7
Mean group II	24.3	17.8	6.7	4.8	0.28	0.27	345.9	341.5	29.9	24.7	1.4	1.5
SD	7.1	5.7	1.8	1.7	0.02	0.03	69.1	84.8	4.8	9.2	0.9	1.7

Abbreviations are: tHcy, total homocysteine; fHcy, free homocysteine; tCys, total cysteine; Hcy, homocysteine.
[a] Group I is two patients undergoing one peroral and one intravenous homocysteine loading, and group II comprises patients receiving two peroral loadings, one before and one after high-dose folic acid supplementation.

were calculated using KaleidaGraph™, version 2.1.3 for Macintosh (Synergy Software, Reading, PA, USA). (2) The time course for tHcy was also analyzed by the program PCNONLIN version 4.0 (Statistical Consultants Inc., Lexington, KY, USA) based on the Akaike's information criterion [24] for the best curve fit. $T_{1/2}$ obtained by these two methods differed by less than 10% in most patients.

The elimination of tHcy after peroral loading obeys first order kinetics and is consistent with a one-compartment, open pharmacokinetic model. $T_{1/2}$ was obtained by the equations [25]:

$$C = C_0 \cdot e^{-kt} \quad \text{(Eq. 1)}$$

$$T_{1/2} = \ln 2/k \quad \text{(Eq. 2)}$$

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.

$AUC_{0->\infty}$ is the area under the plasma concentration-time curve from zero to infinite time measured by the trapezoidal rule [26]. Because plasma tHcy returned to basal level in most patients within 72 hours, $AUC_{0\text{-}72hr}$ was taken as identical to $AUC_{0->\infty}$.

In the subjects having one intravenous and one peroral Hcy loading, we also calculated bioavailability, F, distribution volume, V_d, and clearance, Cl [25]:

$$F = AUC_{po}/AUC_{iv} \quad \text{(Eq. 3)}$$

$$Cl = F \cdot D/[AUC_{0->\infty}] \quad \text{(Eq. 4)}$$

where D is the dose of Hcy.

V_d was calculated by the area method, which provides the most correct estimate after an intravenous injection [27]:

$$V_d = F \cdot D/[k \cdot AUC_{0->\infty}] = Cl/k \quad \text{(Eq. 5)}$$

Assuming that the kinetics are independent of plasma concentration [14], we can determine the rate of Hcy influx into the plasma compartment (R_0) from the equation:

$$C_{ss} = R_0/Cl \quad \text{(Eq. 6)}$$

where C_{ss} refers to the steady state (basal) concentration of plasma tHcy.

Statistical methods

The results are given as mean and SD or range. Comparison of paired data were performed using the Wilcoxon signed rank test, and unpaired values using the Mann-Whitney *U*-test.

RESULTS

Patient characteristics

The patient characteristics and various biochemical parameters are listed in Tables 1 and 2. All renal failure patients had markedly elevated serum creatinine (> 325 μM), and GFR was reduced. The levels of serum folate, serum cobalamin and plasma methionine were normal, whereas plasma tHcy was elevated (mean ± SD) to 27.4 ± 11.0 μM. The mean ± SD plasma fHcy was 7.7 ± 3.2 μM, which is about twice the level found in normal controls [28], and the plasma tCys was 342.8 ± 59.4 μM, which is higher than in 329 healthy controls (281.9 ± 33.8 μM) randomly selected from the Hordaland Homocysteine Study [29].

Bioavailability, V_d and clearance

Interpretation of tHcy values during fasting and after Hcy loading in renal failure patients requires data on bioavailability and V_d of Hcy. Therefore, we performed both a peroral and intravenous Hcy load in two renal patients and compared the results with two healthy controls. The peroral and intravenous plasma tHcy curves for these four subjects are depicted in Figure 1, and the kinetic parameters are summarized in Table 3. Independent of the route of administration, AUC (corrected for dose) was high and the $T_{1/2}$ was longer (about 4-fold) in the renal patients than in the healthy subjects. Notably, bioavailability (0.64 and 0.88) and V_d (0.42 and 0.45 liter/kg) in two patients with renal failure were similar to these parameters in healthy subjects (bioavailability = 0.70 and 0.53; V_d = 0.42 and 0.35 liter/kg). Based on these values (Table 3), mean plasma tHcy clearances were calculated (equation 4) to 104 ml/min in the controls and 31

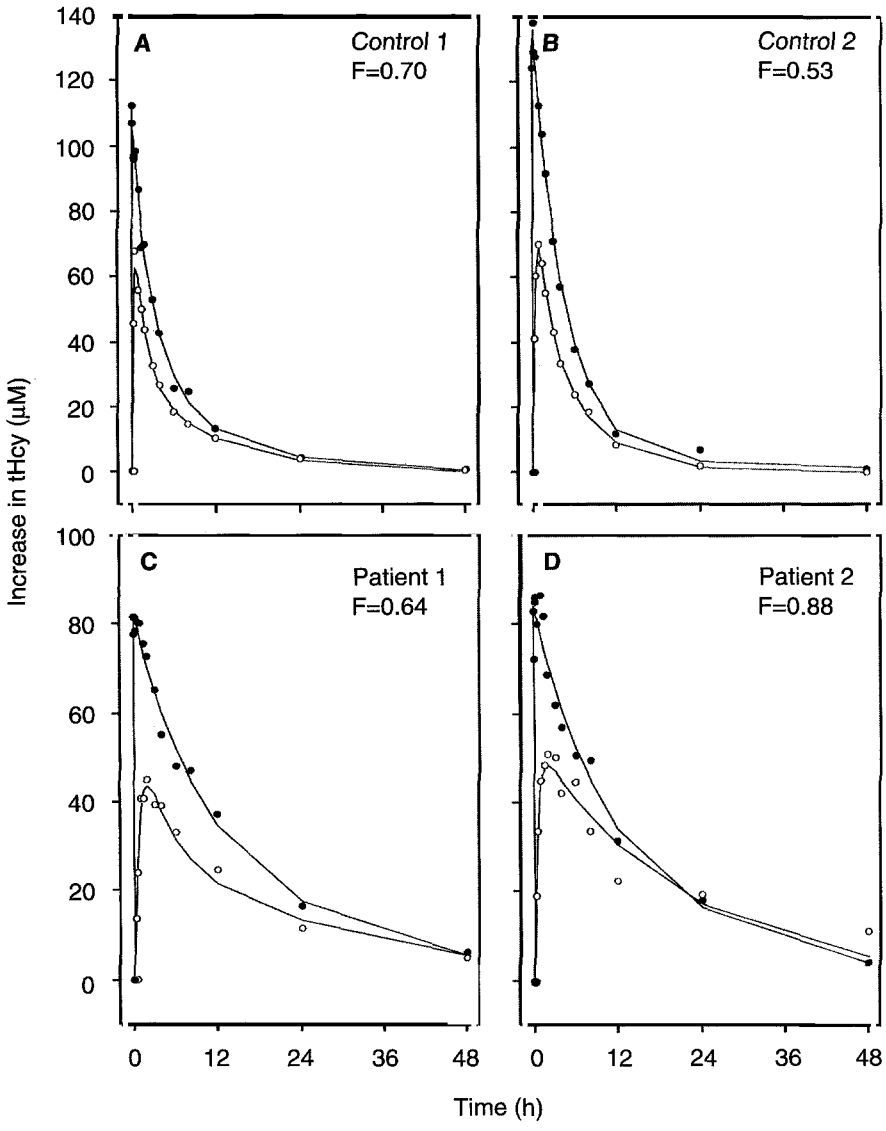


Fig. 1. Bioavailability of peroral homocysteine determined from plasma tHcy curves after peroral (○) and intravenous (●) homocysteine loading of two healthy controls (A, B) and two patients with renal failure (C, D). The program PCNONLIN was used for curve fitting. The area under the plasma concentration curves after peroral (AUC_{po}) and intravenous administration of homocysteine (AUC_{iv}) were calculated by the trapezoidal rule, and the bioavailability, F, was determined as the ratio AUC_{po}/AUC_{iv}. The homocysteine dose was 65 μmol/kg in the healthy controls whereas the dose was reduced to 32.5 μmol/kg in the renal patients.

Table 3. Kinetics of plasma total homocysteine as determined by peroral and intravenous homocysteine loading[a] in two patients and two controls

Subject	C_{max} μM p.o.	T_{max} hr p.o.	$AUC_{0\text{-}48hr}$ p.o.	$AUC_{0\text{-}48hr}$ i.v.	$T_{1/2}$ hr p.o.	$T_{1/2}$ hr i.v.	Bioavailability	V_d liter/kg	Clearance ml/min
Patient 1	45.0	2.0	800	1247	12.0	11.2	0.64	0.42	36
Patient 2	51.1	2.0	1069	1219	15.2	11.6	0.88	0.45	25
Control 1	67.9	0.5	440	626	3.4	2.8	0.70	0.42	130
Control 2	70.2	1.0	442	827	3.3	3.1	0.53	0.35	79

Abbreviations are: C_{max}, maximal increase in plasma total homocysteine (tHcy); T_{max}, time to reach C_{max}; $AUC_{0\text{-}48hr}$; area under the plasma concentration curve for tHcy in the interval 0 to 48 hours; $T_{1/2}$, elimination half-life for tHcy; V_d, distribution volume; p.o., peroral administration; i.v., intravenous administration.

[a] The homocysteine dose was 32.5 μmol/kg in the patients and 65 μmol/kg in the controls.

ml/min in the two renal failure patients; that is, a reduction to 29.8% of the value observed in healthy controls.

Plasma tHcy kinetics before and after folic acid supplementation

The plasma curves for tHcy after peroral Hcy loading in patients with chronic renal failure were described in terms of peak concentration (C_{max}), time to reach peak concentration (T_{max}), $T_{1/2}$ and AUC. AUC was a measure of the systemic exposure to Hcy in the individual. The Hcy loading was performed in six renal patients before and after folic acid supplementation, and the results are summarized in Table 4. In patients not supplemented with folic acid, $T_{1/2}$ (mean ± SD = 12.7 ± 2.8 hr) and AUC (2137 ± 574 μM · hr) were about four times higher than in healthy

Table 4. Kinetics of total plasma homocysteine after peroral homocysteine loading[a]

Subject	C_{max} μM		T_{max} hr		$AUC_{0\text{-}72hr}$ μM·hr		$T_{1/2}$ hr	
	First	Second	First	Second	First	Second	First	Second
Patients, group II								
3	134.7	131.0	1.0	1.5	2129	1964	11.6	13.9
4	102.2	95.9	2.0	2.0	2317	2355	13.0	12.6
5	134.2	134.0	1.5	2.0	2668	3166	11.1	13.7
6	96.7	78.9	3.0	2.0	2715	1949	18.1	16.7
7	98.1	102.7	2.0	0.5	1796	1826	12.3	12.3
8	64.9	50.1	2.0	3.0	1197	1021	10.3	12.8
Mean	105.1	98.8	1.9	1.8	2137	2047	12.7	13.7
SD	26.3	31.8	0.7	0.8	574	702	2.8	1.6
Historical controls[b]								
Mean	57.4		1.0		416		3.7	
SD	9.9		0.3		41		0.8	

Abbreviations are: C_{max}, maximal increase in plasma total homocysteine (tHcy); T_{max}, time to reach C_{max}; $AUC_{0\text{-}72hr}$, area under the plasma concentration curve for tHcy from 0 to 72 hours; $T_{1/2}$, elimination half-life for tHcy.

[a] The homocysteine dose was 65 μmol/kg.

[b] Data are from reference 14.

controls [14]. After supplementation, mean fasting tHcy was decreased by 26.8% (from mean ± SD of 24.3 ± 7.1 μM to 17.8 ± 5.7 μM, $P = 0.22$), but this tHcy response was largely confined to three patients homozygous for the C677T mutation and with serum folate in the low normal range (< 7.5 nM; Table 1). Despite a reduction in their basal tHcy and a reduced C_{max} in two out of three subjects, there was no significant change of $T_{1/2}$ or AUC for tHcy (Table 4).

Assuming that bioavailability is 0.6, the mean ± SD tHcy clearance in the six renal patients was 26 ± 8 ml/min and 28 ± 12 ml/min before and after folic acid, respectively, and 101 ± 15 ml/min in healthy controls [14].

Urinary Hcy excretion

Urinary Hcy excretion was 1.4 ± 0.9 μmol/hr before and 1.5 ± 1.7 μmol/hr after folic acid supplementation. After the first peroral Hcy loading, the fraction of the Hcy dose excreted unchanged into the urine was 2.5 ± 1.6% (mean ± SD) during the first 24 hours, and this was essentially unchanged after folic acid supplementation.

Methionine response

We have previously found that in healthy subjects, plasma methionine increases within the first two hours by about 10 μM in response to a peroral Hcy dose of 65 μmol/kg [14]. In contrast, folate/cobalamin-deficient subjects have a markedly reduced plasma methionine response [12]. In three renal failure patients with normal serum folate, the methionine response was larger (maximal increase in plasma methionine of 18.2, 19.6 and 20.1 μM) than the average response in historical controls [14]. In three renal patients with serum folate < 7.5 nM, we observed a relatively small increase in post-load methionine (maximal response, −4.3, 8.0 and 8.2 μM), which became markedly increased after folic acid therapy (Fig. 2).

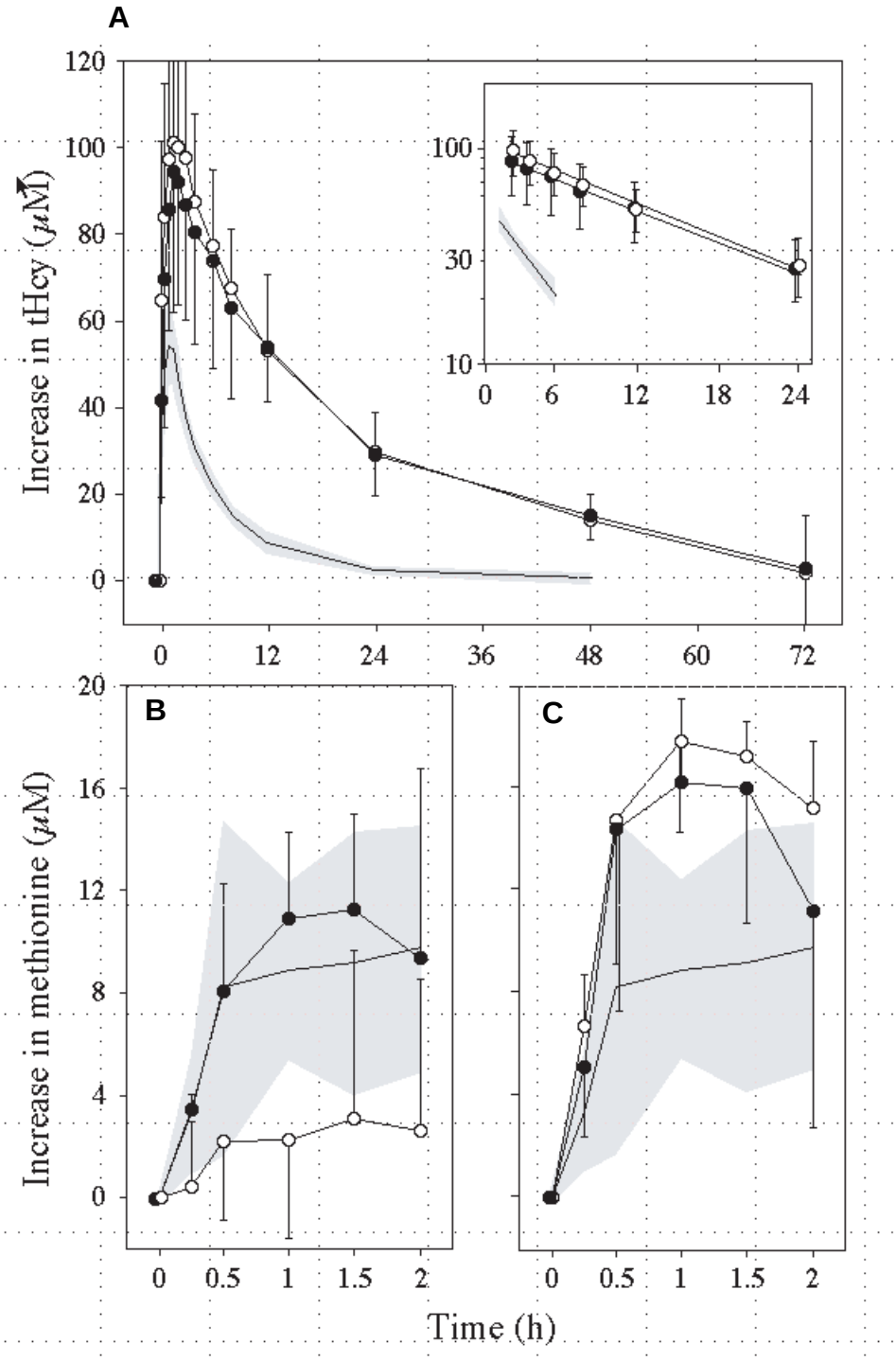
DISCUSSION

We have used a Hcy loading test to investigate the kinetic basis of hyperhomocysteinemia consistently reported in patients with renal failure [30–33]. Notable findings were values for AUC and $T_{1/2}$ that were about four times higher (Table 4) than the values previously reported for healthy controls [14] and patients with folate or cobalamin deficiency [12]. To estimate clearance, we required the data on bioavailability and V_d that were obtained by peroral and intravenous Hcy loading in two patients and two controls. The small difference in these parameters between renal patients and healthy subjects (< 20%) cannot explain the markedly prolonged $T_{1/2}$ in renal patients. Assuming that bioavailability is 0.6 and V_d is similar for patients and healthy subjects, the tHcy clearance was calculated to be 26 ± 8 ml/min, which is only 25.7% of the clearance in healthy controls (101 ± 15 ml/min) [14]. This leads to the key message of this report: that impaired renal function causes a substantial increase in $T_{1/2}$ for tHcy explained by a reduction in total body clearance.

Chronic elevated tHcy represents a steady state concentration (C_{ss}) explained by either enhanced release of Hcy from tissues to plasma and/or decreased total clearance. We have previously demonstrated that hyperhomocysteinemia in folate and cobalamin deficiency is associated with normal tHcy clearance, and that the elevated tHcy level probably is related to increased net Hcy influx into plasma, estimated to about 650 μmol/hr in one subject with cobalamin deficiency [12]. Using equation 6, we determined that the average net influx (R_0) of Hcy into plasma (about 55 μmol/hr) was the same in two healthy controls and the two patients with renal failure. These calculations demonstrate that the markedly reduced tHcy clearance in renal patients accounts for the elevated tHcy level.

The mechanism behind the pronounced reduction of tHcy clearance in renal failure is conjectural. Renal failure seems to have marked and complex effects on amino acid metabolism, and total clearance of some amino acids are increased while that of others are decreased [34], which has partly been attributed to altered hepatic metabolism [35]. However, the changes in plasma level or total clearance of most amino acids in chronic renal failure are usually less than 35% [34, 35], and the marked hyperhomocysteinemia and reduction in clearance by 70% emphasize the importance of kidney function for Hcy homeostasis.

The only known effective tHcy reducing therapy in subjects with renal failure is peroral high doses of folic acid, either alone or in



g. 2. Plasma tHcy curves and methionine sponse after peroral homocysteine loading 5 μmol/kg) in six renal patients before (○) d after (●) folic acid supplementation for at ast 10 days. Upper panel shows the mean crease in plasma tHcy concentration with SD ven as bars, and the data points obeying first der kinetics are replotted in a milogarithmic graph in the inset. Lower left nel shows plasma methionine in three tients with low serum folate and whose basal lcy levels were markedly reduced (−37.9%, 38.8% and −57.3%) following folic acid ministration. Lower right panel shows the ethionine response in three patients with sentially no change (+1.0%, −17.5% and 5.7%) in fasting tHcy after folic acid. For mparison, the tHcy curve (*A and inset*) and e methionine response (*B and C*) after mocysteine loading of 13 healthy historical ntrol [14] are given. These data are depicted thin lines (mean) with a shaded area (SD).

combination with vitamin B_{12} and B_6 [16, 17, 36–38]. We confirmed that there was a reduction in the tHcy level upon folic acid supplementation (Table 2), but the reduction was confined to three renal patients with low-normal serum folate. The increased methionine response suggests improved Hcy remethylation in these patients. Notably, as in folate and cobalamin deficiency [12], folic acid did not influence tHcy clearance (Fig. 2).

We found that in the renal patients the basal urinary excretion of Hcy was 1.4 μmol/hr, which is substantially higher than in healthy subjects (0.25 μmol/h) [11]. With a fHcy concentration of about 3 μM [11], the normal kidneys filter about 20 μmol/hr of Hcy, indicating that only 1% is excreted. The average GFR in the patients was 19 ml/min (Table 1), and the fHcy level was about 8 μM, indicating that about 10 μmol/hr of Hcy will be filtered. This suggests that about 85% of the filtered Hcy is subjected to tubular reabsorption, compared to 99% under normal conditions [12]. However, both in healthy subjects and in the patients with renal failure, the amount of Hcy excreted in the urine is a minor fraction (< 3%) of the estimated supply of Hcy to plasma (55 μmol/hr).

The mechanism for hyperhomocysteinemia in renal failure is

debated in the literature [33, 39]. A preliminary report suggests that there is minimal renal extraction of Hcy in humans [40]. In contrast, Bostom et al [13] have shown that there is a substantial net uptake of Hcy in the normal rat kidney. Our kinetic data show that tHcy clearance is markedly reduced in renal failure, and are in line with the data reported by Bostom et al [13] pointing to the kidney as the major site for elimination of Hcy from plasma.

Conclusion

We have demonstrated that chronic renal failure is associated with a marked (70%) reduction of tHcy clearance, which accounts for the hyperhomocysteinemia in these patients. Experimental and kinetic evidence shows that low clearance is related to impaired uptake and metabolism of Hcy in the kidney. If this is the case, our data suggest that the kidneys are responsible for at least 70% of plasma tHcy clearance under physiological conditions. Folic acid supplementation is an effective means to reduce fasting tHcy in renal failure. However, folic acid enhances Hcy remethylation in tissues, thereby lowering Hcy efflux into the plasma compartment, but does not affect total body clearance of tHcy that remains low in renal patients.

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APPENDIX

Abbreviations used in this paper are: Hcy, homocysteine; tHcy, total homocysteine; GFR, glomerular filtration rate; V_d, distribution volume; fHcy, free homocysteine; tCys, total cysteine; AUC, area under the curve.

REFERENCES

1. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: Probable benefits of increasing folic acid intakes. *JAMA* 274:1049–1057, 1995
2. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, Tishler PV, Hennekens CH: A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in United States physicians. *JAMA* 268:877–881, 1992
3. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE: Serum total homocysteine and coronary heart disease. *Int J Epidemiol* 24:704–709, 1995
4. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG: Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* 346: 1395–1398, 1995
5. Ueland PM, Refsum H, Stabler SP, Malinow MR, Andersson A, Allen RH: Total homocysteine in plasma or serum. Methods and clinical applications. *Clin Chem* 39:1764–1779, 1993
6. Allen LH, Casterline J: Vitamin B-12 deficiency in elderly individuals: Diagnosis and requirements. *Am J Clin Nutr* 60:12–14, 1994
7. Finkelstein JD: Methionine metabolism in mammals. *J Nutr Biochem* 1:228–237, 1990
8. Chauveau P, Chadefaux B, Coudé M, Aupetit J, Hannedouche T, Kamoun P, Jungers P: Hyperhomocysteinemia, a risk factor for atherosclerosis in chronic uremic patients. *Kidney Int* 41(Suppl 43): S72–S77, 1993
9. Brattström L, Lindgren A, Israelsson B, Andersson A, Hultberg B: Homocysteine and cysteine: Determinants of plasma levels in middle-aged and elderly subjects. *J Intern Med* 236:633–641, 1994
10. Arnadottir M, Hultberg B, Nilsson-Ehle P, Thysell H: The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. *Scand J Clin Lab Invest* 56:41–46, 1996
11. Refsum H, Helland S, Ueland PM: Radioenzymic determination of homocysteine in plasma and urine. *Clin Chem* 31:624–628, 1985
12. Guttormsen AB, Schneede J, Ueland PM, Refsum H: Kinetics of total plasma homocysteine in subjects with hyperhomocysteinemia due to folate and cobalamin deficiency. *Am J Clin Nutr* 63:194–202, 1996
13. Bostom A, Brosnan JT, Hall B, Nadeau MR, Selhub J: Net uptake of plasma homocysteine by the rat kidney in vivo. *Atherosclerosis* 116:59–62, 1995
14. Guttormsen AB, Mansoor MA, Fiskerstrand T, Ueland PM, Refsum H: Kinetics of plasma homocysteine in healthy subjects after peroral homocysteine loading. *Clin Chem* 39:1390–1397, 1993
15. Christensen B, Ueland PM: Methionine synthase inactivation by nitrous oxide during methionine loading of normal human fibroblasts. Homocysteine remethylation as determinant of enzyme inactivation and homocysteine export. *J Pharmacol Exp Ther* 267:1298–1303, 1993
16. Friedman JA, Dwyer JT: Hyperhomocysteinemia as a risk factor for cardiovascular disease in patients undergoing hemodialysis. *Nutr Rev* 53:197–201, 1995
17. Wilcken DEL, Dudman NPB, Tyrrell PA, Robertson MR: Folic acid lowers elevated plasma homocysteine in chronic renal insufficiency: Possible implications for prevention of vascular disease. *Metabolism* 37:697–701, 1988
18. Cockroft DW, Gault H: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41, 1976
19. Trollfors B, Alestig K, Jagenburg R: Prediction of glomerular filtration rate from serum creatinine, age, sex and body weight. *Acta Med Scand* 221:495–498, 1987
20. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM: Homocysteine and other thiols in plasma and urine: Automated determination and sample stability. *Clin Chem* 39:263–271, 1993
21. Refsum H, Ueland PM, Svardal AM: Fully automated fluorescence assay for determining total homocysteine in plasma. *Clin Chem* 35:1921–1927, 1989
22. Krishnamurti CR, Heindze AM, Galzy G: 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, 1984
23. Ulvik A, Refsum H, Kluijtmans LAJ, Ueland PM: C677T mutation of methylenetetrahydrofolate reductase gene determined in blood or plasma by multiple-injection capillary electrophoresis and laser-induced fluorescence detection. *Clin Chem* 43:261–272, 1997
24. Yamaoka K, Nakagawa T, Uno T: Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J Pharmacokinet Biopharm* 6:165–175, 1978
25. Welling PG: Pharmacokinetics. Processes and mathematics. *ACS Monographs* 185:290, 1986
26. Rowland M, Tozer TN: Clinical pharmacokinetics. *Concepts and Applications*, Philadelphia, Lea & Febiger, 1989
27. Greenblatt DJ, Koch-Weser J: Drug therapy. Clinical pharmacokinetics (first of two parts). *N Engl J Med* 293:702–705, 1975
28. Ueland PM: Homocysteine species as components of plasma redox thiol status. *Clin Chem* 41:340–342, 1995
29. Nygård O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland PM, Kvåle G: Total plasma homocysteine and cardiovascular risk profile: The Hordaland Homocysteine Study. *JAMA* 274:1526–1533, 1995
30. Robins AJ, Milewczyk EM, Booth EM, Mallick NP: Plasma

amino acid abnormalities in chronic renal failure. *Clin Chim Acta* 42:215–217, 1972
31. SORIA C, CHADEFAUX B, COUDÉ M, GAILLARD O, KAMOUN P: Concentrations of total homocysteine in plasma in chronic renal failure. *Clin Chem* 36:2137–2138, 1990
32. DENNIS VW, ROBINSON K: Homocysteinemia and vascular disease in end-stage renal disease. *Kidney Int* 50(Suppl 57):S11–S17, 1996
33. BOSTOM AG, LATHROP L: Hyperhomocysteinemia in end-stage renal disease (ESRD): Prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* (in press)
34. TIZIANELLO A, FERRARI GD, GARIBOTTO G, GURRERI G, ROBAUDO C: Renal metabolism of amino acids and ammonia in subjects with normal renal function and in patients with chronic renal insufficiency. *J Clin Invest* 65:1162–1173, 1980
35. DRUML W, FISCHER M, LIEBISCH B, LENZ K, ROTH E: Elimination of amino acids in renal failure. *Am J Clin Nutr* 60:418–423, 1994
36. ARNADOTTIR M, BRATTSTRÖM L, SIMONSEN O, THYSELL H, HULTBERG B, ANDERSSON A, NILSSON-EHLE P: The effect of high-dose pyridoxine and folic acid supplementation on serum lipid and plasma homocysteine concentrations in dialysis patients. *Clin Nephrol* 40:236–240, 1993
37. BOSTOM AG, SHEMIN D, LAPANE KL, HUME AL, YOBURN D, NADEAU MR, BENDICH A, SELHUB J, ROSENBERG IH: High dose B-vitamin treatment of hyperhomocysteinemia in dialysis patients. *Kidney Int* 49:147–152, 1996
38. CHAUVEAU P, CHADEFAUX B, COUDÉ M, AUPETIT J, KAMOUN P, JUNGERS P: Long-term folic acid (but not pyridoxine) supplementation lowers elevated plasma homocysteine level in chronic renal failure. *Miner Electrol Metab* 22:106–109, 1996
39. BOCOCK MA, ZLOTKIN SH: Hepatic sulfur amino acid metabolism in rats with chronic renal failure. *J Nutr* 120:691–699, 1990
40. VAN GULDENER C, JANSSEN MJFM, STEHOUWER CDA, DONKER AJM: Estimation of renal homocysteine extraction in patients with normal renal function. (abstract) *Nephrol Dial Transplant* 11:A228, 1996