Simultaneous Determination of Total Plasma Glutathione, Homocysteine, Cysteinylglycine, and Methionine by High-Performance Liquid Chromatography With Electrochemical Detection

Pascal Houze,^{1*} Stéphanie Gamra,^{1,2} Isabelle Madelaine,² Bernard Bousquet,¹ and Bernard Gourmel¹

¹Laboratoire de Biochimie A, Hôpital Saint-Louis, Paris, France

²Pharmacie Centrale, Hôpital Saint-Louis, Paris, France

We here describe an ion-exchange high-performance liquid chromatography technique with electrochemical detection for rapid quantification of glutathione, homocysteine, cysteinylglycine, and methionine. The analytical validation of the technique showed within-assay and between-assay coefficients of variation between 3.1 and 4.3%, and 3.7 and 8.6%, respectively. Percentages of recovery for overload and dilution tests were between 87 and 120%. Detection limits were 1 $\mu\text{mol/L}$ for methionine and 0.5 $\mu\text{mol/L}$ for other compounds. There was no interference with any physiological and pharmacological substances possessing a thiol function. Amino thiol concentrations determined in 100 control sub-

jects (50 women and 50 men) showed no age- or sex-rated differences for except for homocysteine which was increased (+28%) in oldest subjects of both sexes. In 60 patients at risk (30 with chronic renal failure, 30 with diabetes), homocysteine concentration was significantly increased. No variation in other amino thiols was observed in diabetic subjects. Methionine was decreased and cysteinylglycine was increased in patients with chronic renal failure. The present technique—rapid, easy to use, and reliable—appears suitable for routine application in the exploration of amino thiol metabolic pathways including mechanisms of hyperhomocysteinemia. *J. Clin. Lab. Anal.* 15:144–153, 2001. © 2001 Wiley-Liss, Inc.

Key words: amino thiols; HPLC; reference values; diabetes; chronic renal failure

INTRODUCTION

Homocysteine (HCYS) is an intermediary amino acid formed during methionine (MET) metabolism. In plasma, 80% of circulating homocysteine is bound to proteins, mainly albumin. The other 20% is present as disulfides, with only traces of free homocysteine (1). Homocysteine is metabolized either by remethylation resulting from the action of methionine synthase, a vitamin B12-dependent enzyme, or by transsulfuration producing cysteine under the action of vitamin B6-dependent cystathionine β -synthase (2). The transsulfuration pathway is secondary, being involved when excess methionine is present or when cysteine synthesis is necessary. These two metabolic pathways explain why hyperhomocysteinemia can be observed during genetic deficits involving methionine synthase or cystathionine β -synthase, or when there is a vitamin B6 or B12 deficiency (1,2). Although infrequent in their homozygous form, such deficits are clinically characterized by an early appearance of thrombosis (3). Various retrospective and prospective stud-

ies have shown that hyperhomocysteinemia is an independent risk factor in cardiovascular diseases, in diabetes, and in renal failure (4,5).

Various techniques for assaying total homocysteine and other thiols (GSH, CYS-GLY, cysteine, and cysteamine) have been reported, based on separation by high-performance liquid chromatography (HPLC) with fluorometric (6–8) or electrochemical (9,10) detection after reduction of disulfide bonds. None of these techniques allows simultaneous determination of homocysteine and methionine, which has only been performed with more complex methods such as mass spectrometry (11) and pulsed integrated amperometry (12). Simultaneous determination is of interest not only for ho-

*Correspondence to: P. Houze, Laboratoire de Biochimie A, Hôpital Saint-Louis, 1 Avenue Claude Vellefaux, 75010 Paris, France.
E-mail: pascal.houze@sls.ap-hop-paris.fr

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homocysteine assays after methionine loading (13), but also for the study of mechanisms involved in hyperhomocysteinemia. Hyperhomocysteinemia due to a deficit in the transsulfuration pathway are characterized by an increased concentration of circulating methionine, whereas those secondary to a methionine synthase deficit show hypomethioninemia (14).

This article reports the development and analytic validation of a rapid technique for simultaneous assays of plasma glutathione, homocysteine, cysteinylglycine, and methionine by HPLC with electrochemical detection. To our knowledge, this is the first report of a simultaneous determination of these four aminothiols. This technique allows simultaneous exploration of homocysteine metabolism and pathological mechanisms involved. The methodology has been applied to the determination of reference values and the study of populations at risk, including patients with chronic renal failure or non-insulin-dependent diabetes.

MATERIALS AND METHODS

Reagents

Homocysteine, DL-cysteinylglycine (reduced form), methionine, DL-N-acetylcysteine, penicillamine, DL-glutathione (reduced form), cysteamine, sodium 1-heptanesulfonate, 1-octanesulfonate sodium salt (OSS), sodium borohydride, dihydrated disodium phosphate, monopotassium phosphate, and human gammaglobulins were supplied by Sigma-Aldrich (St. Quentin Fallavier, France). Perchloric acid, soda, orthophosphoric acid, EDTA, and methanol, all of analytic quality, were purchased from Merck (Nogent-sur-Marne, France). The internal standard was a synthetic peptide (NH₂-Glu-Cys-Cys-Gly-COOH) obtained from Sigma-Aldrich. Water for injectable preparations was used to prepare different reagents and was supplied by Frésenius (Louviers, France).

Chromatographic Conditions

The chromatographic system consisted of an isocratic pump (Waters 515, St Quentin-en-Yvelines, France) and an automatic injector (Autosampler 712, Waters). Separation was performed on an Inertsil 5 ODS 3 250 × 4.6 mm column (i.d) (Chrompack, Les Ulis, France). The mobile phase (1 ml/minute) was composed of 8% methanol and 92% buffer containing disodium phosphate (15 mM), monopotassium phosphate (15 mM), and 1-octanesulfonate sodium salt (1 μM) adjusted to pH 2.6 with orthophosphoric acid (15 M). Detection of the thiol function was ensured by an electrochemical detector composed of a Coulochem 5100A model and a Coulochem 5011 analytic cell (Eurosep, Cergy-Pontoise, France). Measurement cells had respective potentials of +0.65 and +0.90 V, while the potential of the guard cell was +1.40 V. The detector gain was set at 10. Data were collected on an integrator (Data Module 746, Waters) with attenuation set at 32.

Sample Collection

Blood samples were collected in Vacutainer tubes containing EDTA, which were placed on ice for transport to the laboratory. Samples were immediately centrifuged upon arrival (2,000g for 5 minutes). Plasma were stored at -80°C until assay.

Preparation of the Calibration Range and Controls

The calibration range and different controls (low and high) were prepared from pooled plasma obtained from healthy subjects. The pool was stirred on active carbon for 24 h and then filtered (Nalgène 0.45 μm filter, Prolabo) before being fractionated and frozen at -80°C.

A mother solution (1 mmol/l), prepared for each of the four thiols by dissolving each product in an aqueous solution of EDTA (2 mM), was stored at -80°C. The first calibration point at 100 μmol/L in each thiol, obtained by dilution (1:10) of the mother solution in pooled plasma, was then fractionated and frozen at -80°C. The calibration range was prepared extemporaneously by serial 1:2 dilution of the 100 μmol/l point down to the 6.25 μmol/l point. Point 0 was composed of pooled plasma only.

Low (C1) and high (C2) controls were obtained by pooling plasma samples. They were fractionated and frozen at -80°C.

The mother solution of the internal standard (1 mg/ml) was prepared in water, fractionated and frozen at -80°C. In our conditions, all prepared solutions are stable for a maximum of 3 months.

Assay Principle and Performance

The reaction is based on the release of the protein aminothiols and the reduction of oxidated forms by a reducing agent followed by proteins precipitation in an acidic medium. Briefly, 100 μl of plasma (calibration points, controls or samples), 50 μl of internal standard (extemporaneous dilution 1:20 in an aqueous solution of EDTA 1 mM) and 40 μl of an aqueous solution of sodium borohydride (1 M in hydroxyde sodium 0.1 N) prepared extemporaneously were mixed in an Eppendorf tube and incubated for 30 minutes at 50°C. After incubation, the proteins were precipitated by addition of 100 μl of perchloric acid (0.6 M) containing EDTA (1 mM). After a 5-minute centrifugation, the supernatant was 1:5 diluted in the mobile phase, and 50 μl of the mixture were injected in the chromatographic system.

Precision and Recovery Studies

Within-assay precision was determined by measuring the C1 and C2 controls 30 times in the same assay series. Between-assay precision was obtained in 30 different series over a one-month period. The relative recovery study was performed by adding known concentrations of glutathione, homocysteine, cysteinylglycine, and methionine

to plasmas. Different samples prepared were measured five times against the calibration curve. Recovery percentages (measured values/expected values ratio) were calculated for each concentration.

Linearity and the Detection Limit

The linearity of the method was checked by serial 2-fold dilution of the two plasma in a 70 g/l solution of human gammaglobulins. Concentrations were measured 5 times and recovery percentages were calculated. The detection limit of

each thiol was defined as the concentration producing a signal-to-background ratio of 3.

Correlation With the Reference Technique

Homocysteine is the only aminothiols for which a fluorescence technique identical to that described by Dias et al. (15) is available. Plasma homocysteine concentrations were measured a single time with both techniques in 160 samples from 100 subjects considered as normal and in 60 patients at risk (with chronic renal failure or diabetes).

Fig. 1. Chromatograms of a plasma "control" (a); plasma overloaded with 25 µm/l of each amino thiol (b); and of a patient with chronic renal failure

(c). Peaks: 1 = glutathione, 2 = homocysteine, 3 = cysteinylglycine, 4 = internal standard, and 5 = methionine.

Studies of Interference Substances

Retention time was determined for different physiological (cysteine) or medicinal (DL-penicillamine, cysteamine, N-acetyl-cysteine) substances potentially interfering with our detection method.

Reference Values

Reference values of the four aminothiols were determined in a population of 100 healthy subjects (50 women, mean age 39.2 ± 12.5 years; 50 men, mean age 40.1 ± 13.2 years) with normal vitamin B12, folate, and albumin status. All subjects are regular volunteer blood donors. Their written consent was obtained in accordance with recommendations of the ethics committee of St. Louis Hospital.

Study of At-Risk Populations

Concentrations of aminothiols were measured in 30 patients (10 women, mean age 49 ± 8 years; 20 men, mean age 55 ± 15 years) with chronic renal failure ($150 < \text{creatinine} < 280 \mu\text{mol/L}$) and 30 non-insulin-dependent patients (12 women, mean age 58 ± 12 years; 18 men, mean age 56 ± 16 years). Their written consent was obtained and all procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Statistical Analysis

Results are expressed as mean $\pm$ standard deviation. Analysis of variance (ANOVA) was used to compare thiol plasma concentrations as a function of sex, age, and pathology. A P value of < 0.05 was considered significant.

RESULTS

Chromatography Conditions

Chromatograms of a control plasma overloaded with $25 \mu\text{mol/L}$ of each thiol and of a patient with chronic renal failure are shown in Figure 1. In our analytic conditions, peaks were well separated. Chromatograms showed small unidentified peaks that did not interfere with those of the thiols measured. Glutathione, homocysteine, cysteinylglycine, internal standard, and methionine were eluted in that order with the following retention times: 11.2, 13.3, 17.6, 24.2, and 27.7 minutes.

To optimize separation, the influence of two counter-ions (heptane and octanesulfonate sodium salt) was studied at four concentrations (0.5, 1, 2.5, and $5 \mu\text{M}$). The percentage of methanol was also modified (2, 5, 8, and 10%). The use of 1-heptanesulfonate sodium does not allow for a good separation of glutathione and homocysteine, regardless of the contra-ion concentration and the percentage of methanol. Results obtained with 1-octanesulfonate sodium salt

Fig. 2. Influence of the percentage of methanol (a) and 1-octanesulfonate sodium salt concentration (b) on the retention times of the different compounds.

are shown in Figure 2. The best separation within the shortest time (< 30 minutes) was obtained with $1 \mu\text{M}$ 1-octanesulfonate sodium salt and 8% methanol in the mobile phase.

Calibration Curves

A linear relation was found between the height ratio (aminothiols/internal standard) and the concentration of each thiol studied in a range between 0 and $100 \mu\text{mol/L}$. Figure 3 shows the mean $\pm$ two standard deviations of three calibration ranges for each analyte studied. The pooled plasma showed residual concentrations of GSH, HCYS, CYS-GLY, and MET (Fig. 1a). The area ratio for each thiol studied was subtracted from the ratio obtained for each other points in the range. In these conditions, the equations for the regression curves were $y = 0.024x + 0.03$ for glutathione; $y = 0.028x + 0.011$ for homocysteine; $y = 0.021x - 0.012$ for cysteinylglycine; and $y = 0.011x + 0.005$ for methionine (where y corresponds to the area ratio for thiol/internal standard and x to the concentration of the analyte studied). All correlation coefficients were excellent (between 0.9993 and 0.9997).

Fig. 3. Calibration curves of the different thiols. Each point is the mean of three determinations.

Precision and Recovery

Results for intra- and interseries precision are shown in Table 1. For intraseries precision, coefficients of variation (CV) determined for C1 and C2 controls were all below 5% for the four thiols studied. For interseries precision, coefficients obtained for C1 and C2 were higher, but still below 10%. Recovery tests performed gave results between 89 and 109% (Table 2).

Linearity and Detection Limit

Technic linearity was checked in two plasmas. Recovery was between 87 to 120%. These results confirm the good linearity of the method for each of the aminothiols studied. Minimal detectable concentrations were 0.5 $\mu\text{mol/L}$ for glutathione, homocysteine, and cysteinylglycine, and 1 $\mu\text{mol/L}$ for methionine.

Correlation With the Reference Technique

For homocysteine, a good correlation between the fluorescence technique and our technique with electrochemical detection was obtained. Slope of the regression curve was 0.99 with a y intercept of 0.69 and a correlation coefficient of 0.986 (Fig. 4). The same study performed for physiological values of homocysteine ($<15 \mu\text{mol/L}$) yielded similar results, with a slightly lower correlation coefficient of 0.925 (Fig. 4). Using Bland and Altmann analysis (16), we compared the two techniques. The mean difference (d) was $-0.4 \mu\text{mol/L}$, with D of $2.1 \mu\text{mol/L}$ and a distribution of $d - 2\text{SD} - 4.6$ to $d + 2\text{SD} = 3.8 \mu\text{mol/L}$ for homocysteine concentrations of 3 to $62 \mu\text{mol/L}$. The difference plot analysis between the two methods is consistent with the regression analysis showing a homogenous distribution.

Reference Values

Mean values for homocysteine, cysteinylglycine, and methionine are indicated in Table 3. They were obtained for each aminothiol by calculating the mean $\pm$ standard deviation for each age group and sex. Mean values for homocysteine (all ages combined) were $9.6 \pm 2.8 \mu\text{mol/L}$ in men and $8.4 \pm 2.5 \mu\text{mol/L}$ in women. No significant sex-related differences were found, regardless of the age group studied. However, a significant increase with age (+28%) in homocysteine concentration was observed in both sexes for the 50- to 60-year-old group versus the 20- to 30-year-old group (reference). No difference was observed for glutathione, cysteinylglycine, and methionine concentrations (Table 3).

Variations in At-Risk Populations

A significant increase in homocysteine concentrations was found in patients with chronic renal failure (+250% vs. controls) and those with diabetes (+130% vs. controls). No variation in cysteinylglycine and methionine was noted in the diabetic population. In patients with renal failure, we observed a significant decrease in methionine (-23% vs. controls) whereas CYS-GLY was increased (+17% vs. control). Plasma glutathione showed no significant variations. All these modifications were not sex-related (Fig. 5).

TABLE 1. Precision studies

Compounds	Plasma controls	Intra-assays (n = 30)			Inter-assays (n = 30)		
		Mean ($\mu\text{mol/L}$)	SD ($\mu\text{mol/L}$)	CV (%)	Mean ($\mu\text{mol/L}$)	SD ($\mu\text{mol/L}$)	CV (%)
Glutathione	C1	10.2	0.4	3.9	10.6	0.6	5.7
	C2	15.5	0.6	3.9	14.9	0.8	5.4
Homocysteine	C1	11.5	0.4	3.4	11.3	0.6	5.3
	C2	51.6	1.9	3.6	50.7	3.5	6.9
Cysteinylglycine	C1	20.4	0.9	4.4	20.9	1.8	8.6
	C2	47.9	1.5	3.1	48.5	1.8	3.7
Methionine	C1	19.8	0.7	3.5	19.6	1.4	7.3
	C2	41.5	1.8	4.3	42.2	2.9	7.0

TABLE 2. Recovery studies^a

	Aqueous thiol solution (50 $\mu\text{mol/l}$) (μl)	Glutathione			Homocysteine			Cysteinylglycine			Methionine		
		Measured values ($\mu\text{mol/l}$)	Expected values ($\mu\text{mol/l}$)	Recovery (%)	Measured values ($\mu\text{mol/l}$)	Expected values ($\mu\text{mol/l}$)	Recovery (%)	Measured values ($\mu\text{mol/l}$)	Expected values ($\mu\text{mol/l}$)	Recovery (%)	Measured values ($\mu\text{mol/l}$)	Expected values ($\mu\text{mol/l}$)	Recovery (%)
Plasma A		12.8			11.8			22.9			32.2		
	25	19.6	20.2	97	16.9	18.6	91	27	28.4	95	37.3	35.6	105
	50	24.5	25.2	97	21.8	24.5	89	30.3	31.3	97	39.9	38	105
	75	28.9	28.7	101	27.2	28.2	96	32.9	34	97	44.8	42.6	105
	100	32.8	31.4	104	31.4	31.1	101	35.3	36	98	46.9	43.5	108
Plasma B		16.2			22.2			27.5			31.1		
	25	22.5	23.0	98	26.9	27.6	97	31.8	31.6	101	33.9	34.8	97
	50	27.6	27.5	100	29.6	31.3	95	32.8	34.6	95	37.5	37.3	100
	75	29.6	30.1	98	34.6	34	102	35.1	36.8	95	42.5	39.1	109
	100	34.5	33.1	104	36.1	36	100	38.6	38.5	100	43.8	40.5	108

^aEach measured value is the mean of 3 determinations.

Fig. 4. Correlation of the HPLC technique with electrochemical detection and the reference technique for all homocysteine values combined; and for those values below 15 µmol/l (inset).

Interference Substances

Tests were conducted on different compounds (physiological or with a pharmacological action) possessing a thiol function likely to react with our detection system. Among physiological aminothiols, cysteine was eluted very rapidly (a retention time of only 5.4 minutes). Products with a pharmacological action, such as N-acetylcysteine, cysteamine, and DL-penicillamine, were eluted 6.2, 18.1, and 20.2 minutes after injection. Others derivated amithiols (such as methionine sulfoxide) were not detected in our conditions.

DISCUSSION

Most homocysteine assay techniques are based on HPLC with fluorometric detection (17–19). Procedures for reduction of disulfide bonds and derivatization are often long and difficult, even though recent methods have led to some simplifications (15,20). Chromatographic methods with elec-

trochemical detection are less frequently used because of a lack of reproducibility related to the deterioration of the measurement cell (6). However, the major advantage of this system is the direct measurement of the thiol function based on oxidation, thereby requiring no derivatization.

For the first time this study reports the development of an ion-exchange HPLC method with electrochemical detection that allows simultaneous determination of HCYS and other aminothiols such as glutathione, cysteinylglycine, and methionine. The composition of the mobile phase (8% methanol, 1 µM of octanesulfonate sodium salt, pH = 2.6) is based on the study of retention times as a function of methanol and OSS concentrations. Conditions adopted allowed the best resolution within an analysis time compatible with a routine technique (< 30 minutes) and similar to those described in the literature (9,17,21). The effect of OSS on thiol separation was rapid, allowing adequate resolution with a concentration of 1 µM (20). Increased concentrations of methanol

TABLE 3. Distribution of the four aminothiols as a function of age and sex^a

Age (years)	n	Women				n	Men			
		Glutathione	Homocysteine	Cysteinylglycine	Methionine		Glutathione	Homocysteine	Cysteinylglycine	Methionine
20–60	50	9.2 (2.1)	8.4 (2.5)	41.2 (2.5)	22.2 (2.3)	50	9.4 (1.5)	9.6 (2.8)	42.4 (4.1)	22.8 (1.6)
20–30	10	8.8 (1.5)	7.9 (2.1)	42.5 (3.1)	21.5 (1.3)	8	8.9 (2.1)	8.5 (2.1)	43.2 (2.4)	19.5 (0.3)
31–40	10	9.1 (1.8)	8.0 (1.8)	39.5 (1.6)	23.9 (1.1)	10	9.1 (1.8)	9.4 (2.5)	40.4 (1.8)	22.1 (1.0)
41–50	12	9.3 (2.1)	8.1 (2.3)	41.4 (2.5)	19.3 (0.8)	12	9.6 (2.8)	9.3 (1.9)	42.8 (2.1)	23.2 (1.1)
51–60	18	8.9 (2.6)	10.9 (2.1) ^b	43.1 (3.6)	24.6 (2.5)	20	9.7 (3.2)	12.2 (2.8) ^b	39.8 (2.6)	22.8 (1.9)

^aEach age group includes 10 women and 10 men. Results are expressed in µmol/L. The standard deviation value is indicated in parentheses.

^bStatistical significance, $P < 0.05$ vs. the 20- to 30-year-old age group.

Fig. 5. Concentrations of the different aminothiols in control subjects ($n = 100$), patients with chronic renal failure ($n = 30$), and diabetics ($n = 30$). Statistical significance: *, $P < 0.05$; **, $P < 0.01$ vs. controls.

modified the retention time of all four compounds without changing the order of elution (9). The pH of the mobile phase was set at 2.6 after studying the influence of ion hydrogen concentrations on compound separation. At a pH value of 3, separation was not obtained between GSH, HCYS, and CYS-GLY. Lower pH values could not be studied because of a potential risk of column alteration (9). As indicated in different chromatograms (Fig. 1), peaks corresponding to each aminothiol were clearly separated with satisfying resolution. Importantly, there were no major reactive peaks like those seen with fluorescence techniques using bromobimane derivatization (17,19).

The presence of different forms of homocysteine plasma transport requires a reduction step of disulfide bonds followed by protein precipitation. Procedures and reagents used are quite variable thus the problem of result standardization arises (22). Reduction was here achieved by the sodium borohydride, an alkaline mixture close to that described by Fermo et al. (7). The use of phosphine and dithiothreitol derivatives has often been preferred to sodium borohydride because of a better reaction yield (8,9,12). In our experiment, dithiothreitol gave two interference peaks identical to those reported in the literature (9). As the second of these peaks eluted at the same time as glutathione, dithiothreitol was not considered a suitable reduction method. Reduction by phosphines generally

involves tri-*n*-butyl-phosphine in dimethylformamide (23). The preparation of this reagent is difficult and thus not easily applicable for routine. A sodium borohydride reduction method was preferred because it is simple, inexpensive, and has already been used in other methods with electrochemical detection (24).

Different substances have been recommended as an internal standard (N-acetyl-cysteine, cysteamine, and DL-penicillamine) (7–9). N-acetyl-cysteine was not selected here because its elution was too rapid (retention time = 6.2 minutes). The choice of DL-penicillamine has been recommended in techniques with coulometric detection because its structure and electroactivity are similar to that of homocysteine (9). However, penicillamine, and cysteamine are used in the treatment of hereditary diseases for which an aminothiol assay may be necessary (infantile cystinuria and cystinosis) (14–19) even though this frequency is low. Therefore, a synthetic peptide (a homologue superior to glutathione) with an analytical and pre-analytic behavior similar to that of the aminothiols measured was selected.

Analytical characteristics of our method were satisfactory with CV below 5% in intraseries and below 9% in interseries for the four compounds studied. For homocysteine, our technique is correlated with the fluorometric reference method. Aminothiol values are comparable to those reported with other

techniques using fluorometric or electrochemical detection (8,9,21). Except for methionine, the minimal detectable concentration was 0.5 $\mu\text{mol/L}$. Its is similar to that reported before for total amount of amino thiols determination (7,9). The main overall criticism of electrochemical techniques is the lack of reproducibility related to the contamination of the measurement cells (6). The technique developed has been used routinely for six months in our laboratory and no loss of sensitivity has been observed (the detection cell is washed daily with methanol).

Plasma samples from 100 healthy subjects were analyzed with this technique. HCYS values are very comparable to those obtained with the reference fluorescence technique. Regression analysis showed a good correlation between the two techniques over a wide range of concentration. HCYS concentrations were slightly higher in men than women (mean value: 9.6 $\mu\text{mol/L}$ vs. 8.4 $\mu\text{mol/L}$). This sex-related difference, though not significant for all ages combined, was significant for the 51- to 60-year-old age group. These values and variations are in agreement with the literature (7,19,25). For glutathione and cysteinylglycine, concentrations were similar to those reported by others (10,26), but 30% lower than those reported by Pastore et al. (19) who used sodium borohydride reduction but at higher concentrations, which could account for the differences observed. For methionine, mean values were close to 25 $\mu\text{mol/L}$ and similar to those determined by amino-acid analyzers (27).

The study of aminothiol concentrations in patients with chronic renal failure showed that values were significantly increased for HCYS and CYS-GLY, but lower for MET. A mutation of methyltetrahydrofolate reductase is often found in subjects with renal failure. The lower capacity of this enzyme to resynthesize folates, coenzymes essential for the activity of methionine synthase, apparently leads to an homocysteine accumulation and a methionine deficiency (28). CYS-GLY variations have not often been investigated, although a study in 1995 (5) found that this compound was increased in subjects with renal failure. The mechanism involved is poorly understood. CYS-GLY is formed from glutathione metabolism. Chronic renal failure in some way could affect renal or sulfur amino acid and peptide metabolism (29). Within the diabetic population, an isolated increase in HCYS was evident. This suggests that underlying mechanisms of hyperhomocysteinemia are different than those observed during renal failure. This increase, frequently observed in type I and also type II diabetic patients, is considered to be an independent cardiovascular risk factor (13,30).

In conclusion, the HPLC technique developed here for plasma assays of different amino thiols is easy to use, rapid, and suitable for routine practice. Analytical efficiency is comparable to that of fluorometric detection, but the preparation step is easier. This technique determines homocysteine and methionine simultaneously, thereby allowing indirect study of mechanisms of hyperhomocysteinemia.

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