Decrease of Elevated N,N-Dimethylglycine and N-Methylglycine in Human Immunodeficiency Virus Infection During Short-Term Highly Active Antiretroviral Therapy

Markus P. Look, Reiner Riezler, Heiner K. Berthold, Sally P. Stabler, Kirsten Schliefer, Robert H. Allen, Tilman Sauerbruch, and Jürgen K. Rockstroh

This study investigates fasting serum levels of methionine and related metabolites, vitamin B_6, and folate during highly active antiretroviral therapy in therapy-naive human immunodeficiency virus (HIV)-1–infected outpatients. The research design consisted of before and during therapy measurements with a median treatment period of 100 days (range, 50 to 188) in frozen samples. The subjects included 17 consecutive HIV-1–infected outpatients (15 men and 2 women; 25 to 65-years-old). Controls were 42 healthy individuals (28 men and 14 women; 24- to 82-years-old) without serologic evidence of HIV and/or hepatitis C infection and normal clinical chemistry. Subjects received treatment with the reverse transcriptase inhibitors, azidothymidine (AZT) or stavudine (D4T) plus lamivudine (3TC) and either the protease inhibitors, indinavir (IND), nelfinavir (NELF), ritonavir (RITV), or saquinavir (SAQ) at the standard dosage. Serum concentrations of methionine, total homocysteine (tHcy), cystathionine (CYSTA), N,N-dimethylglycine (DMG), N-methylglycine (MG), methylmalonic acid (MMA), and total cysteine, as well as vitamin B_6, folate, and soluble tumor necrosis factor receptor p75 were taken at baseline and during highly active antiretroviral therapy. Baseline, serum tHcy, MMA, CYSTA, vitamin B_6 concentrations were not significantly different from healthy controls. There was, however, a trend towards lower folate serum concentrations at baseline in HIV-infected patients as compared with healthy controls ($P = .06$). There were no significant correlations between tHcy and vitamin B_6, folate, or MMA. Elevated baseline levels of DMG and MG decreased significantly during antiretroviral therapy ($P = .0019$ and .04, respectively), whereas no significant changes in serum concentrations of CYSTA, MMA, or methionine were detected. tHcy increased in 12 of 17 patients ($P = .09$). HIV-infected patients displayed significant alterations (elevated DMG and MG serum concentrations) in metabolite levels of the betaine pathway in methionine metabolism, which might be positively influenced by newly initiated antiretroviral combination therapy.

INTERMEDIATES OF AMINO acid metabolism are increasingly used as surrogate markers for evaluation of the status of vitamins needed as coenzymes in the respective pathways.[1-3] For example, determination of serum concentrations of intermediates of methionine and propionic acid metabolism (Fig 1), total homocysteine (tHcy), cystathionine (CYSTA), and methylmalonic acid (MMA) provided novel insight that functional availability of vitamin B_6, vitamin B_{12}, and folate in a healthy geriatric population appeared to be reduced despite normal serum vitamin levels.[4] This indicates that determinations of vitamin B_6, vitamin B_{12}, and folate concentrations give only an incomplete picture of their functional availability. The overall integrity of a particular amino acid pathway in different disease states might be better examined by additional metabolite assessment. In human immunodeficiency virus (HIV)-infected outpatients, the prevalence of serum vitamin B_6, vitamin B_{12}, and folate deficiency was not increased compared with the general population[5]; it may, however, increase in advanced disease stages.[6] Moreover, methionine concentrations in serum were decreased in HIV-infected individuals in comparison with healthy persons.[7,8] The transsulfuration pathway provides sulfur from methionine and the carbon skeleton from serine for the biosynthesis of cysteine, which is subsequently used to form glutathione (GSH), a ubiquitous intracellular antioxidant (see Fig 1 for more details).

Myocardial infarction and other vascular events have been reported to occur in HIV disease in association with combination therapy of 2 nucleoside analogues with protease inhibitors (ie, highly active antiretroviral therapy, [HAART]).[9,10] This was possibly related to metabolic abnormalities, such as hypercholesterolemia, hypertriglyceridemia, beta cell dysfunction, peripheral insulin resistance, and alterations in cortisol and dehydroepiandrosterone that are induced by HAART, frequently accompanying the newly defined lipodystrophy syndrome.[11-15] On the other hand, HAART has been shown to significantly improve $CD4^+$-cell count and survival in HIV infection.[16] Therefore, the investigation of all known risk factors for vascular disease in HIV disease is highly recommended. Results of epidemiologic studies strongly suggested that elevated fasting tHcy concentrations are an independent risk factor for vascular disease and venous thrombosis in the general population.[17-20] However, data from prospective studies were less consistent.[20,21] In the absence of genetic alterations,[22,23] homocysteine metabolism depends on the availability of vitamin B_{12}, folate, and vitamin B_6. Accordingly, deficiencies in these vitamins may significantly increase tHcy levels. In a prospective, nested cohort study, it was also shown that higher fasting vitamin B_6 levels offered independent pro-

From the Department of Internal Medicine I, University of Bonn, Bonn; Severi Med, Münster, Germany; and the Department of Internal Medicine, University of Colorado, Denver, CO.

Submitted October 19, 2000; accepted April 6, 2001.

Address reprint requests to M.P. Look, MD, Department of Internal Medicine I, University of Bonn, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany.

0026-0495/01/5011-0008$35.00/0
doi:10.1053/meta.2001.27201

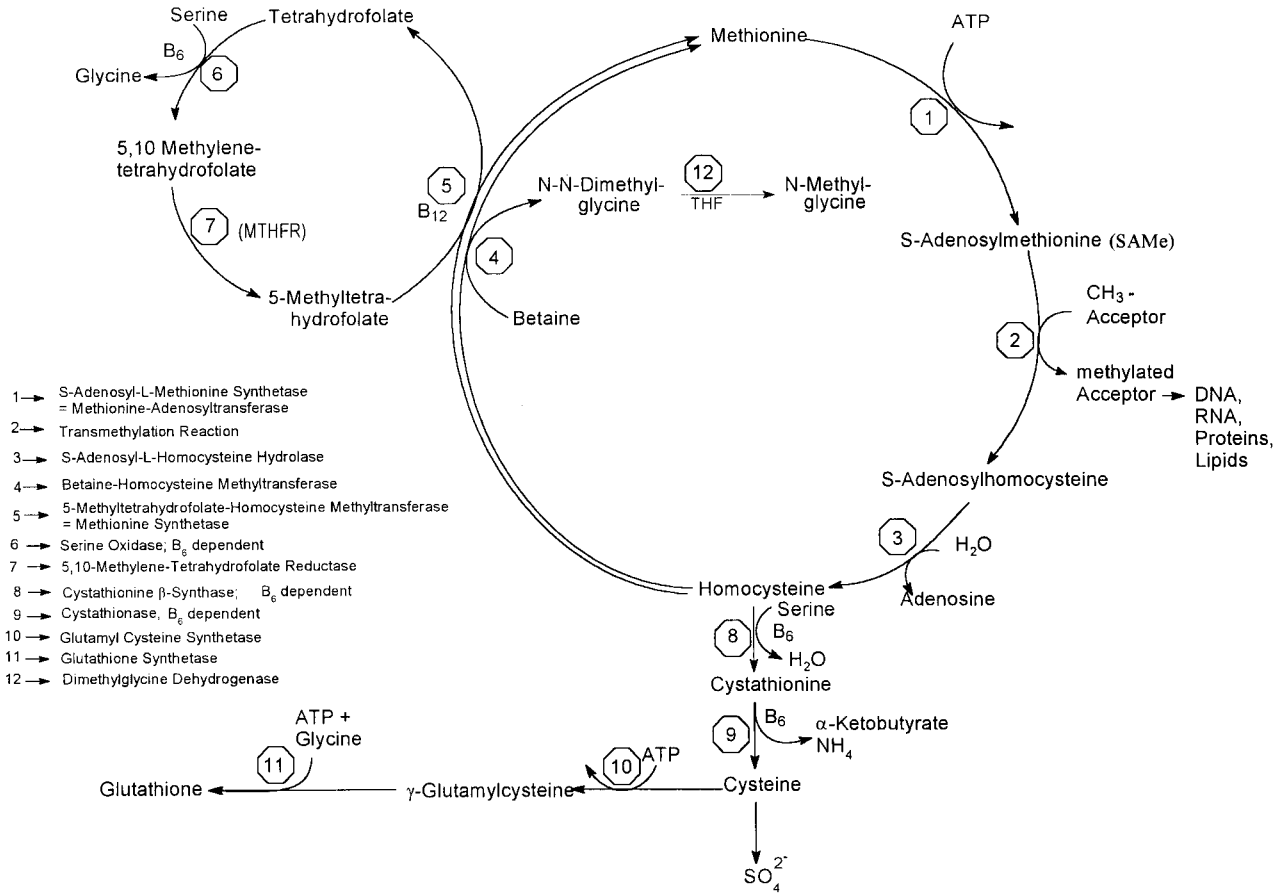

Fig 1. In the first step of transmethylation/transsulfuration, methionine and adenosine triphosphate (ATP) condense to form SAMe, the sole methyl donor in numerous methylation reactions involving DNA, proteins, phospholipids, biogenic amines, as well as creatine and carnitine synthesis. The availability of SAMe is considered to be the key condition for regulating the activity of methionine metabolism.[45,48] High SAMe levels lead to increased transsulfuration, whereas low SAMe levels result in increased remethylation of homocysteine. SAMe is subsequently demethylated to homocysteine, which condenses with serine to CYSTA, a thioether-containing amino acid without known function other than being an intermediate for the biosynthesis of cysteine. In conditions in which methionine is in excess or cysteine synthesis is required, homocysteine enters the transsulfuration pathway, a reaction that depends on vitamin B_6. CYSTA is cleaved by γ-cystathionase to cysteine plus ammonia and α-ketobutyrate. Cysteine is further metabolized to taurine or used for the synthesis of GSH and proteins.

tection against coronary artery disease, after adjustment for other risk factors, when tHcy was no longer associated.[21] In previous studies, fasting tHcy concentrations in HIV-infected individuals were not significantly increased when compared with healthy controls.[24,25]

Fasting serum levels of methionine and related metabolites, ie, tHcy, CYSTA, N,N-dimethylglycine (DMG), and N-methylglycine (MG), as well as vitamins B_6, folate, and the vitamin B_{12} surrogate MMA were measured in the present study to characterize methionine metabolism in therapy-naive HIV-infected outpatients. Furthermore, we measured the changes in serum concentrations of these metabolites and vitamins during therapy to determine whether they are influenced by HAART. A particular aim was to investigate whether HAART increases tHcy and/or decreases vitamin B_6 or folate levels, which may further extend the atherogenic risk profile of HIV-infected individuals receiving HAART. Finally, the changes of methionine-related metabolites during HAART were compared with those of soluble tumor necrosis factor receptors p75 (sTNFR p75), the only immune marker known to increase the prognostic utility of HIV-RNA determination in early-stage HIV disease.[26]

PATIENTS AND METHODS

Patients and Control Subjects

The study group comprised 17 HIV-1–infected individuals (15 men, 2 women; median age, 36; range, 25 to 65 years) sequentially presenting at our outpatient department between December 1997 and May 1998, who were assigned to start protease inhibitor (PI)-containing HAART. The visit when blood was drawn for the first CD4-count analysis and assessment of viral load after starting HAART was designated "treatment visit". This cohort was described in more detail in a previous prospective study of our group.[27] The sera were frozen at −20°C immediately after blood was drawn, and storage time did not exceed 12 months. Seven patients were at stage C3, 3 at stage B3, 1 at stage B2, 4 at stage A3, 1 at stage A2, and 1 at stage A1 according to

the Centers for Disease Control and Prevention (CDC) 1993 classification system for HIV infection[28] (Table 1). Three patients had ongoing treatment of opportunistic infections and 1 had developed a neoplasm. Patient no. 4 was diagnosed with HIV encephalopathy and had evidence of active cytomegalovirus (CMV) infection (early antigen positive, immunoglobulin (Ig)M positive, and isolation of CMV from urine 2 weeks later) at the treatment visit, reflecting CMV reactivation under immune reconstitution. The second patient (no. 5) had positive stool cultures for Salmonella species at baseline and, as well, at the treatment visit. He was also diagnosed with prostate cancer at an early stage. The third patient (no. 6) was being treated for cerebral toxoplasmosis with sulfadiazine and pyrimethamine. No patient or healthy control took vitamin supplements as documented by history. All patients had normal kidney and liver function as indicated by serum creatinine concentrations $\leq$ 1.2 mg/dL and normal serum aminotransferase activities at baseline.

The control group consisted of 42 healthy individuals with no history of metabolic and cardiovascular illnesses (28 men, 14 women) with a median age of 48, range, 24 to 82 years, who had no serologic evidence of HIV and/or hepatitis C infection and had normal laboratory findings (red and white blood cells and thrombocytes, liver transaminases, and serum creatinine). This cohort has already served as part of a control group published in another study of our group.[29] Vitamin B_6 and folate concentrations were determined in a subset of 23 individuals from the control group (15 men, 8 women; median age, 46 years; range, 25 to 64). Normal values for sTNFR p75 in serum were 4.0 μg/mL, 95% confidence interval (CI), 2.9 to 6.6; n = 15.[30] Informed consent was obtained from all participants before blood was drawn. The study was approved by the local ethics committee and followed the guidelines of the 1975 Declaration of Helsinki and its current revision.

Treatment Schedule and Assessment of Clinical Status

All patients were entirely antiretroviral-naive when starting HAART and underwent full clinical evaluation. The indication to apply HAART was based on prevailing therapy-standards[31] (HIV-RNA $>$ 10,000 copies/mL serum, progressive loss of CD4-cells in blood [$<$ 500 cells/μL], or symptomatic HIV infection or acquired immunodeficiency syndrome [AIDS]). All patients received the reverse transcriptase (RT)-inhibitors, azidothymidine (AZT) or stavudine (D4T) plus lamivudine (3TC), and 1 of the protease inhibitors, indinavir (IND), nelfinavir (NELF), ritonavir (RITV), or saquinavir (SAQ) at standard dosage regimens.

Determination of Methionine, tHcy, CYSTA, MMA, DMG, MG, and Total Cysteine in Serum

The concentrations of methionine and its metabolites in serum were measured by capillary gas chromatography-mass spectrometry as previously described.[1,32]

Determination of Vitamin B_6 and Folate

Vitamin B_6 in serum was determined by high-performance liquid chromatography (HPLC) as previously described,[33] and folate in serum was determined by using a commercially available radioassay kit (Becton/Dickinson, Orangeburg, NY).

Determination of the CD4 Count and HIV-RNA

CD4 cells were determined on a Becton/Dickinson-FACScan-flow-cytometer using Simultest IMK-lymphocyte antibodies from Becton/Dickinson (Heidelberg, Germany). The absolute number of the lymphocyte subsets was calculated on the basis of automated white blood cell counts using an autodifferential (H1, Technicon, Tarrytown, NY). Quantitative HIV-(1)-RNA measurements in serum were performed using a commercially available kit, NASBA, by Organon Teknika bv (5281 RM Boxel, The Netherlands). The limit of detection was 8 copies/mL.

Determination of sTNFR p75 in Serum

Serum concentrations of sTNFR p75 were determined on a Multiscan Titertec MCC 340 enzyme-linked immunosorbent assay (ELISA) reader, using a commercially available ELISA kit (synELISA-kit by ELIAS, Freiburg, Germany).

Statistics

The Mann-Whitney *U* test was applied for group comparison of baseline levels of methionine metabolites. Comparison of the effects of treatment between baseline and treatment visit in the same patient was analyzed using the Wilcoxon signed rank test for paired samples. Analysis of correlation was performed using the Spearman coefficient. A P value $< .05$ was considered statistically significant and $.05 < P < .1$ as a trend. The SPSS software package 8.0.0 (SPSS, Chicago IL) was used for all calculations.

RESULTS

Patient characteristics and changes in metabolites, HIV status, and treatment duration are shown in Table 1. There was a median time between baseline and treatment visits of 100 days (range, 50 to 188). A significant decrease in the viral load was observed in all patients. CD4 cell count improved and sTNFR p75 levels decreased in 14 (82.4%) patients. There was no correlation between the changes in CD4 count, viral load, duration of treatment, and the changes seen in methionine-related metabolites. No case of lipodystrophy syndrome has emerged in the patients at the treatment visit. Baseline concentrations of tHcy, MMA, CYSTA, and total cysteine, as well as vitamin B_6 concentrations, were not significantly different from healthy controls. Six of 17 patients (35%) and 6 of 23 healthy controls (26%) had serum folate levels below 2.3 ng/mL, the lower threshold for deficiency,[4] and the difference was not significant. Serum CYSTA concentrations at baseline were increased in only 1 of the patients and in 2 of the healthy individuals, indicating that overall, no functional vitamin B_6 deficiency was present.[2] The mean serum methionine concentration in the patients was significantly lower, and DMG and MG concentrations were significantly higher in the patients as compared with healthy controls at baseline (Table 2).

In univariate analysis, there were no significant correlations between tHcy and vitamin B_6, folate, and MMA, respectively. Six of 17 patients (35%) and 14 of 42 (33%) healthy controls had baseline tHcy levels above 10.3 μmol/L, the upper threshold level indicating increased risk for vascular complications.[34] Although the changes in serum concentrations of tHcy during treatment ($+22\% \pm 34\%$) were not statistically significant, tHcy had increased from baseline in 12 of the 17 patients (70.6%), reaching borderline significance ($P = .09$, Wilcoxon signed rank test; $P = .06$, paired t test). No direct measurement of vitamin B_{12} concentrations in serum was performed, yet no patient displayed vitamin B_{12} deficiency according to their serum MMA levels.[4] Only 1 individual from the group of healthy controls had a slightly elevated MMA level of 492 μmol/L.

Table 1. Patient Characteristics Changes in Metabolites and Folate Between Baseline and the Treatment Visit

Patient No.	CDC	Sex/Age (M/F/yr)	Therapy Combination	Changes in tHcy (μmol/L)* (%)	DMG (μmol/L)	Folate (ng/mL)	sTNFR p75 (ng/mL)	$CD4^+$ cells/μL	HIV-RNA (log 10)	Duration of HAART (days)
1	C3	M/53	AZT, 3TC, NELF	−4.1 (−25)	−5.39	+0.1	−27.2	+179	−3.11	135
2	C3	M/65	D4T, 3TC, NELF	+3.3 (+37)	−4.19	+15.8	−13.8	+135	−3.36	97
3	C3	F/25	D4T, 3TC, NELF	+0.4 (+9)	−3.94	+2	−4.7	nd	−3.47	126
4	C3	M/49	D4T, 3TC, NELF	+3.2 (+30)	−7.41	+1.6	−9.7	+83	−1.89	97
5	B3	M/59	D4T, 3TC, NELF	+7.2 (+47)	−0.34	−2.5	+12.3	+100	−2.5	77
6	C3	M/35	AZT, 3TC, NELF	+2.3 (+77)	+2.37	−3.1	+1.4	+50	−3.31	131
7	A3	M/40	D4T, 3TC, IND	−1 (−16)	+0.33	+0.5	−0.1	+287	−3.58	113
8	B3	M/39	3TC, D4T, IND	+3.7 (−66)	−0.5	−0.2	−4.5	−28	−1.51	86
9	A3	M/30	D4T, 3TC, IND, RITV	+0.7 (+7)	−2.35	−1.6	+0.4	+233	−3	84
10	C3	M/29	IND, RITV, D4T, 3TC	−1.7 (−12)	−1.56	+0.5	−3.2	+92	−2.9	88
11	A1	M/25	AZT, 3TC, RITV, SAQ	+4.6 (+56)	−2.02	+1.6	−0.7	+142	−2.07	98
12	A3	M/34	3TC, IND, RITV, D4T	+0.9 (+19)	−1.4	−0.4	−2.7	+96	−2.90	132
13	A2	M/32	D4T, 3TC, IND	−0.4 (−5)	−0.37	+1.6	−0.2	+103	−1.59	62
14	B3	F/37	D4T, 3TC, NELF	−1.4 (−15)	−1.43	+3.2	−7.1	+326	−2.07	188
15	C3	M/39	3TC, NELF, D4T	+3.3 (+85)	−1.59	+1.2	−13	+269	−2.9	50
16	B2	M/35	D4T, 3TC, IND, RITV	+0.2 (+2)	+0.53	+3	−2.8	−24	−3.15	50
17	A3	M/36	D4T, 3TC, IND	+0.9 (+7)	−1.52	+0.8	−6.8	+37	−2.32	90

Abbreviations: M, male, f, female; AZT, azidothymidine; D4T, stavudine; 3TC, lamivudine; IND, indinavir, NELF, nelfinavir; RITV, ritonavir; SAQ, saquinavir.

*Indicates changes of tHcy in percent compared with baseline.

There were no significant correlations between the changes in folate, vitamin B_6, and homocysteine. Treatment duration also did not influence these parameters. A significant increase in total cysteine and a significant decrease in DMG and MG concentrations in comparison to baseline levels was observed (Table 2). Baseline levels of DMG and MG were significantly correlated (Rho: 0.642, $P = .005$; $r = .761$, $P < .001$). The changes of DMG and sTNFRs p75 from baseline were also significantly correlated ($r = .59$, $P = .003$). There were no significant correlations between methionine, methionine-related metabolites, vitamins, and serum creatinine.

DISCUSSION

This study is the first to show data of serum concentrations of various intermediates of the 1-carbon metabolism of methionine and their changes during HAART in initially therapy-naive HIV-infected patients. Using an improved gas chromatography/mass spectrometry (GC-MS) technique provided sensitive and reliable analytical precision.[32] The data confirm previous findings that serum concentrations of methionine are decreased in the HIV-infected host,[8,35] whereas in contrast to other reports,[35,36] this was not the case for total cysteine.

Table 2. Serum Concentrations of Methionine-Related Metabolites, Vitamine B_6 and Folate in 17 HIV-Infected Patients at Baseline and During HAART (Treatment Visit) and Healthy Controls

Parameter	HIV-Infected Patients (baseline)	HIV-Infected Patients (treatment visit)	*P* Value *v* Baseline	Healthy Controls	*P* Value *v* Patients at Baseline
Methionine (μmol/L)	21.7 (20.1-23.5)	20.5 (19.8-22.6)	NS	25.1 (24-27.3)	.015
Total homocysteine (μmol/L)	8.9 (6.8-11)	10.2 (7.9-12.4)	.09	9.5 (8.3-11)	NS
Cystathionine (nmol/L)	169 (111-286)	217 (142-293)	NS	234 (170-297)	NS
Total cysteine (μmol/L)	263 (235-291)	297 (268-325)	.011	268 (251-283)	NS
N,N,-dimethylglycine (μmol/L)	5.8 (4.6-6.9)	3.94 (3.2-4.7)	.006	3.8 (3.4-4.1)	.0019
N-methylglycine (μmol/L)	2.2 (1.6-2.7)	1.54 (1.2-1.9)	.002	1.8 (1.5-2.0)	.040
Methylmalonic acid (μmol/L)	138 (100-176)	186 (81-291)	NS	160 (138-181)	NS
Vitamine B_6 (ng/mL)	11.9 (10.7-13.2)	15.7 (8.8-22.7)	NS	14.4 (8-25.3)*	NS
Folate (ng/mL)	3.8 (1.0-6.5)	5.2 (1.8-8.5)	NS	6.8 (1.6-15)*	.06
sTNFR p75 (ng/mL)	13.5 (9.3-17.8)	8.7 (5.9-11.4)	.01	4.0 (2.9-6.6)†	.001
Serum creatinine (mg/dL)	0.89 (0.75-1.06)	0.90 (0.81-1.1)	NS	1.0 (0.7-1.1)	NS

NOTE. Comparison between baseline values of HIV-infected patients and healthy controls was performed by the Mann-Whitney U test. Comparisons of the values at baseline and at the treatment visit for each patient was performed using the Wilcoxon signed rank test for paired samples. The values represent mean values. The numbers in parentheses indicate the respective 95% confidence intervals.

Abbreviation: NS, not significant.

*The normal range for sTNFR p75 in serum was obtained from 15 healthy individuals from a previous study of our group.[30]

†n = 23.

Several novel aspects were found: (1) baseline levels of DMG and its catabolic product, MG, were significantly increased in therapy-naive HIV-infected patients compared with the control group and, interestingly, they both normalized during HAART in parallel with the reduction of viral load and of sTNFR p75 and with the increase in CD4 count. (2) The significant increase in total serum cysteine, although within the normal range, should be pointed out, because the role of cysteine as the limiting amino acid for glutathione synthesis has recently been extensively investigated in HIV disease,[30,35,37-40] and it might be interesting to further search for restorative changes in GSH metabolism with sensitive and specific assays during HAART. (3) There was no excessive increase in tHcy and no significant change in serum levels of MMA (vitamin B_{12} surrogate), folate, and vitamin B_6 during short-term HAART.

Metabolism of homocysteine depends on methionine intake in relation to the availability of riboflavin, vitamins B_6, B_{12}, folate, and S-adenosylmethionine (SAMe). Thus, if the drugs used in HAART are responsible for changes in tHcy concentrations, this is supposed to be mediated through modification of one of these parameters. During the short follow-up period, no significant changes in body weight or changes in dietary habits, as documented by history, were reported by the patients. Folate deficiency can cause elevation in serum DMG and MG concentrations in humans,[32] possibly mediated through an increased activity of betaine-homocysteine methyltransferase (reaction no. 4, Fig 1), the enzyme that uses homocysteine as substrate and methyl groups from betaine, in an effort to replenish low methionine levels independently of folate and vitamin B_{12}. Serum methionine concentrations in our patients were indeed decreased, and there was also a trend towards lower folate levels. However, the decreased methionine levels have been found in the absence of vitamin B_{12} deficiency (MMA levels were normal) and were not correlated with low folate concentrations. There was also no particular association between the 6 patients with folate concentrations below the threshold considered to indicate deficiency ($<$2.3 ng/mL) and the highest DMG and MG levels found in the patients. It would also be conceivable that elevated DMG at baseline is a consequence of tissue wasting with loss of phosphatidylcholine from membranes, giving rise to choline that would be metabolized to betaine and DMG.

Overall, the decreases of DMG and MG towards normal values during HAART might be regarded as beneficial changes, indicating amelioration of membrane degradation and improvement of formerly inappropriately functioning metabolic pathways in HIV disease. Yet, this was not accompanied by normalization of methionine concentrations in serum, which is in line with a recent report showing that HAART was not able to overcome considerable alterations of cyst(e)ine metabolism in HIV disease, caused mainly by massive renal loss of sulfur.[41] Low levels of methionine in plasma, even in stable HIV-infected patients, might thus be attributed to increased protein turnover and catabolism of peripheral muscle tissue with cysteine being the limiting amino acid. In addition, concomitant activation of methionine transsulfuration to cyst(e)ine has been presumed.[7] Only little is known about metabolism of sulfur-containing amino acids in HIV infection. Since in healthy humans a sparing effect of cystine on the requirement for methionine is possible, although discussed controversial,[42] this might become relevant in conditions such as extreme protein-calorie malnutrition or HIV infection. Therefore, HIV-infected individuals, irrespectively whether treated or not, might per se have increased requirements of sulfur-containing amino acids and/or vitamin B_6. This is in line with a very recent research letter showing markedly decreased hepatic γ-cystathionase activity (reaction no. 9, Fig 1) measured postmortem in 3 end-stage patients with AIDS compared with healthy persons.[43] A general decrease in γ-cystathionase in HIV-disease, however, should be accompanied by elevated serum cystathionine concentrations. Our data showed normal serum cystathionine levels in 16 of 17 of the HIV-infected patients and also normal vitamin B_6 concentrations, which is not suggestive of impaired de novo cysteine synthesis on the level of γ-cystathionase. Taken together, the disease stage seems to play a crucial role in the changes of transsulfuration metabolism elicted by HIV infection.

Cytokine excess (eg, TNF abundance) and oxidative stress can alter enzyme activities via nucleophilic attack by oxygen radicals of vulnerable sites of the apo-enzymes.[44] The parallel reduction of serum levels of DMG and MG with sTNFRs p75, ie, the significant correlation between the changes of sTNFR p75 and DMG, suggests a connection between the normalization of the pathologic immune activation state in HIV disease (via reduction of the viral load) and the restoration of malfunctioning amino acid metabolic pathways.

We have to address several shortcomings of this study. Unfortunately, it is not possible to reliably determine SAMe or GSH concentrations in frozen samples or in tissues. This would have been important, because SAMe is the key regulator compound of methionine metabolism and exerts inhibitory and stimulatory effects on different enzymatic steps.[45] The metabolism of GSH and of SAMe has been extensively investigated in patients with liver disease.[1,46-50] Published data about SAMe in HIV-infected humans indicate a low-methylation ratio in cerebrospinal fluid[51] and increased erythrocyte levels of SAMe,[25] whereas cerebrospinal fluid levels were decreased compared with healthy controls.[25,51] Furthermore, more formal documentation of the dietary intake of vitamins and protein before and during HAART would have been desirable. At least, it could be excluded that high-potency supplements were consumed.

In summary, HIV-infected outpatients displayed significant alterations in the betaine pathway of methionine metabolism, which were positively influenced by HAART. The results of this study may be clinically relevant, because they show additional and hitherto unknown biologic effects of HAART besides reduction of viral load. It might be speculated whether vitamin B_6, B_{12}, and folate repletion in HIV patients receiving HAART provides additional benefits in terms of risk for cardiovascular disease. Tracer studies using stable isotopes, in addition to measurement of SAMe, GSH, and betaine concentrations and enzyme activities in tissues could provide further insights in the regulation of those pathways and the changes induced by HAART.

REFERENCES

1. Stabler SP, Lindenbaum J, Savage DG, et al: Elevation of serum cystathionine levels in patients with cobalamin and folate deficiency. Blood 81:3404-3413, 1993

2. Ubbink JB, van der Merwe A, Delport R, et al: The effect of a subnormal vitamin B-6 status on homocysteine metabolism. J Clin Invest 98:177-184, 1996

3. Ubbink JB: Metabolite markers of vitamin nutritional status. Am J Clin Nutr 70:789-789, 1999

4. Naurath HJ, Joosten E, Riezler R, et al: Effects of vitamin B12, folate, and vitamin B6 supplements in elderly people with normal serum vitamin concentrations. Lancet 346:85-89, 1995

5. Skurnick JH, Bogden JD, Baker H, et al: Micronutrient profiles in HIV-1-infected heterosexual adults. J Aquir Immune Defic Syndr Hum Retrovirol 12:75-83, 1996

6. Baum MK, Mantero-Atienza E, Shor-Posner G, et al: Association of vitamin B6 status with parameters of immune function in early HIV-1 infection. J Acquir Immune Defic Syndr Hum Retrovirol 4:1122-1132, 1991

7. Laurichesse H, Tauveron I, Gourdon F, et al: Threonine and methionine are limiting amino acids for protein synthesis in patients with AIDS. J Nutr 128:1342-1348, 1998

8. Hortin GL, Landt M, Powderly WG: Changes in amino acid concentrations in response to HIV-1 infection. Clin Chem 40:785-789, 1994

9. Gallet B, Pulik M, Genet P, et al: Vascular complications associated with use of of HIV protease inhibitors. Lancet 351:1958-1959, 1998

10. Henry K, Melroe H, Huebsch J, et al: Severe premature coronary artery disease with protease inhibitors. Lancet 351:1328, 1998

11. Behrens G, Dejam A, Schmidt H, et al: Impaired glucose tolerance, beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. AIDS 13:F:63-70, 1999

12. Carr A, Samaras K, Chisholm DJ, et al: Pathogenesis of HIV-1 protease inhibitor-associated lipodystrophy hyperlipidemia and insulin resistance. Lancet 351:1881-1883, 1998

13. Christeff N, Melchior J-C, de Truchis P, et al: Lipodystrophy defined by a clinical score in HIV-infected men on highly active antiretroviral therapy: Correlation between dyslipidaemia and steroid hormone alterations. AIDS 13:2251-2260, 1999

14. Lo JC, Mulligan K, Tai WW, et al: 'Buffalo hump' in men with HIV-1 infection. Lancet 351:867-870, 1998

15. Berthold HK, Parhofer HG, Ritter MM, et al: Influence of protease inhibitor therapy on lipoprotein metabolism. J Intern Med 246:567-675, 1999

16. Cameron DW, Heath-Chiozzi M, Danner S, et al: Randomized placebo-controlled trial of ritonavir in advanced HIV-1 disease. Lancet 351:543-549, 1998

17. Nygard O, Nordrehaug JH, Refsum H, et al: Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 337:230-236, 1997

18. Selhub J, Jaques PF, Bostom AG, et al: Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med 332:286-291, 1995

19. den Heijer M, Koster T, Blom HJ, et al: Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med 334:759-762, 1996

20. Eikelboom JW, Lonn E, Genest J, et al: Homocysteine and cardiovascular disease: A critical review of the epidemiologic evidence. Ann Intern Med 131:363-375, 1999

21. Folsom AR, Nieto FJ, McGovern PG, et al: Prospective study of coronary heart disease incidence in relation to fasting total homocysteine related genetic polymorphisms, and B vitamins. Circulation 98: 204-210, 1998

22. Mudd SH, Levy HL, Skovby F: Disorders of transsulfuration, in Scriver CR, Beaudet AL, Sly WS, et al (eds): The Metabolic and Molecular Bases of Inherited Diseases. New York, NY, McGraw-Hill, 1995, pp 1279-1327

23. Kang SS, Wong PW, Susamano A, et al: Thermolabile methylenetetrahydrofolate reductase an inherited risk factor for coronary artery disease. Am J Hum Genet 48:536-545, 1991

24. Müller F, Svardal AM, Aukrust P, et al: Elevated plasma concentrations of reduced homocysteine in patients with human immunodeficiency virus infection. Am J Clin Nutr 63:242-248, 1996

25. Castagna A, Le Grazie C, Accordini A, et al: Cerebrospinal fluid S-adenosylmethionine (SAMe) and glutathione concentrations in HIV-infection: Effect of parenteral treatment with SAMe. Neurology 45: 1678-1683, 1995

26. Stein DS, Lyles RH, Graham NM, et al: Predicting clinical progression or death in subjects with early-stage human immunodeficiency virus (HIV) infection: A comparative analysis of quantification of HIV RNA, soluble tumor necrosis factor type II receptors, neopterin, and beta2-microglobulin. Multicenter AIDS Cohort Study. J Infect Dis 175:1161-1167, 1997

27. Look MP, Altfeld M, Kreuzer KA, et al: Parallel decrease of the neurotoxin quinolinic acid and of soluble tumor necrosis factor receptors P 75 in serum during highly active antiretroviral therapy of HIV-1 disease. AIDS Res Hum Retroviruses 16:1215-1221, 2000

28. Centers For Disease Control: 1993 revised classification system for HIV-infection and expanded surveillance case definition among adolescents and adults. MMWR Morb Mortal Wkly Rep 41:1-19, 1993

29. Look MP, Riezler R, Reichel C, et al: Is the increase in serum cystathionine levels in patients with liver cirrhosis a consequence of impaired homocysteine transsulfuration at the level of gamma-cystathionase? Scand J Gastroenterol 35:866-872, 2000

30. Look MP, Rockstroh JK, Rao GS, et al: Serum selenium, plasma glutathione and erythrocyte glutathione peroxidase levels in asmyptomatic versus symptomatic HIV infection. Eur J Clin Nutr 51:266-272, 1997

31. Carpenter CCJ, Fischl MA, Hammer SM, et al: Antiretroviral therapy for HIV infection in 1998. Updated recommendations of the international AIDS Society - USA Panel. JAMA 280:78-86, 1998

32. Allen RH, Stabler SP, Lindenbaum J: Serum betaine, N,N-dimethylglycine and N-methylglycine levels in patients with cobalamin and folate deficiency and related inborn errors of metabolism. Metabolism 42:1448-1460, 1993

33. Ubbink JB, Serfontein WJ, de Villiers LS: Stability of pyridoxal-5-phosphate semicarbazone: Application in plasma vitamin B-6 analysis and population surveys of vitamin B-6 nutritional status. J Chromatogr 342:277-284, 1985

34. Perry IJ, Refsum H, Morris RW, et al: Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged. Lancet 22:1395-1398, 1995

35. Eck HP, Gmünder H, Hartmann M, et al: Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. Biol Chem Hoppe-Seyler 370:101-108, 1989

36. Akerlund B, Jarstrand C, Lindeke B, et al: Effect of N-acetylcysteine (NAC) treatment on HIV-1 infection: A double-blind placebo-controlled trial. Eur J Clin Pharmacol 50:457-461, 1996

37. de Quay B, Malinverni R, Lauterburg BH: Glutathione depletion in HIV-infected: Role of cysteine deficiency and effect of oral N-acetylcysteine. AIDS 6:305-311, 1992

38. Staal FJ, Roederer M, Israelski DM, et al: Intracellular glutathione levels in T-cell subsets decrease in HIV-infected individuals. AIDS Res Hum Retroviruses 8:807-809, 1992

39. van der Ven AJ, Blom HJ, Peters W, et al: Glutathione homeostasis is disturbed in CD4-positive lymphocytes of HIV-seropositive individuals. Eur J Clin Invest 28:187-193, 1998

40. Buhl R, Jaffe HA, Holroyd KJ: Systemic glutathione deficiency in sympto-free HIV-seropositive individuals. Lancet 2:1294-1298, 1989

41. Breitkreutz R, Holm S, Pittack N, et al: Massive loss of sulfur in HIV infection. AIDS Res Hum Retroviruses 16:203-209, 2000

42. Fugakawa NK, Yu YM, Young VR: Methionine and cysteine kinetics at different intakes of methionine and cystine in elderly men and woman. Am J Clin Nutr 68:380-388, 1998

43. Martin JA, Sastre J, de la Ascuncion JG, et al: Hepatic gamma-cystathionase deficiency in patients with AIDS. JAMA 285:1444-1445, 2001

44. Pace GW, Leaf CD: The role of oxidative stress in HIV disease. Free Radic Biol Med 19:523-528, 1995

45. Selhub J, Miller JW: The pathogenesis of hyperhomocysteinemia: Interruption of coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 55:131-138, 1992

46. Meister A, Anderson ME: Glutathione. Annu Rev Biochem 52:711-749, 1983

47. Burgunder JM, Lauterburg BH: Decreased production of glutathione in patients with cirrhosis. Eur J Clin Invest 17:408-414, 1987

48. Corrales F, Ochoa P, Rivas C, et al: Inhibition of glutathione synthesis in the liver leads to S-adenosyl-L-methionine synthetase reduction. Hepatology 14:528-533, 1991

49. Loguerico C, Nardi G, Argenzio C, et al: Effect of S-adenosyl-L-methionine administration on red blood cell cysteine and glutathione levels in alcoholic patients with and without liver disease. Alcohol Alcohol 29:597-604, 1994

50. Mato JM, Camara J, Fernandez de Pas J, et al: S-adenosylmethionine in alcoholic liver cirrhosis: A randomized, placebo-controlled, double-blind, multi-center clinical trial. J Hepatol 30:1081-1089, 1999

51. Keating JN, Trimble KC, Mulcahy F, et al: Evidence of brain methyltransferase inhibition and early brain involvement in HIV-positive patients. Lancet 337:935-939, 1991