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Relationships among Brain Metabolites, Cognitive Function, and Viral Loads in Antiretroviral-Naïve HIV Patients

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This study aims to determine the relationship among cerebral metabolite concentrations (on proton magnetic resonance spectroscopy or ^{1}H MRS), cognitive function, and clinical variables (CD4, plasma and CSF viral loads, and lipids) in antiretroviral medication-naïve HIV patients. We hypothesized that the probable glial markers *myo*-inositol [MI] and choline compounds [CHO] would correlate with cognitive function, CD4 count, and viral loads, but not with serum lipids. Forty-five antiretroviral-drug-naïve HIV patients and 25 control subjects were evaluated. Frontal lobe [MI], [CHO], and total creatine [CR] were elevated, while basal ganglia [CR] were decreased, with increasing dementia severity. As a group, HIV patients showed slowing on fine motor (Grooved Pegboard) and psychomotor function (Trails A & B), and deficits on executive function (Stroop tasks). Lower CD4 counts and elevated plasma viral loads were associated with elevated frontal white matter [MI], which in turn correlated with the Stroop tasks. These findings suggest that systemic factors (resulting from suppressed immune function and higher plasma viral load) may lead to glial proliferation (elevated [MI], [CHO], and [CR]) in the frontal white matter, which in turn may contribute to deficits on executive function in HIV. Studying antiretroviral-naïve patients minimized the confounding effects of antiretroviral treatment on the clinical, MRS, and neuropsychological variables, and allowed for a more accurate assessment of the relationships among these measurements. Metabolite concentrations, rather than metabolite ratios, should be measured since [CR], a commonly used reference for metabolite ratios, varies with disease severity in both frontal lobe and basal ganglia. © 2002 Elsevier Science (USA)

Key Words: **brain; HIV; magnetic resonance spectroscopy; MRS; neuropsychological tests.**

INTRODUCTION

Prior to potent combination therapy, approximately 20–40% of patients with AIDS developed a dementia termed HIV-cognitive motor complex (HIV-CMC) (McArthur *et al.*, 1989, 1993; Miller *et al.*, 1990; Navia *et al.*, 1986). Nuclear medicine studies demonstrated that early HIV-CMC is associated with metabolic rather than structural brain changes (Masdeu *et al.*, 1991; Rottenberg *et al.*, 1996; Tatsch *et al.*, 1990). Magnetic resonance (MR) studies, including MR spectroscopy (Barker *et al.*, 1995; Chang *et al.*, 1999a; Chong *et al.*, 1993; Jarvik *et al.*, 1993; Laubenberger *et al.*, 1996; Meyerhoff *et al.*, 1999; Paley *et al.*, 1996; Tracey *et al.*, 1996), perfusion MRI (Chang *et al.*, 2000a; Tracey *et al.*, 1998), and functional MRI (Chang *et al.*, 2001), concurred with nuclear medicine studies showing abnormalities in brain metabolism, perfusion, and activation in patients who had early stages of HIV dementia or were neurologically asymptomatic. Therefore, *in vivo* neuroimaging may provide clinically accessible surrogate markers to assess HIV-associated brain injury and monitor treatment in the early stages when these abnormalities may be reversible. Evaluation of the relationships between the various objective noninvasive markers (plasma and CSF viral loads, neuropsychological tests, and neuroimaging studies) will further validate the neuroimaging measures.

Previous reports have shown strong relationships between cerebral metabolites measured by ^{1}H MRS (particularly the neuronal marker *N*-acetyl compounds [NA] and putative glial markers, *myo*-inositol [MI] and choline compounds [CHO]) and CD4 count or HIV dementia stage (Chong *et al.*, 1993; Paley *et al.*, 1996; Tracey *et al.*, 1996; Chang *et al.*, 1999a). Abnormalities in neuronal and glial cells have been well described in HIV-associated dementia (Wiley *et al.*, 1991; Power *et al.*, 1993). Several reports also demonstrate that MRS might be useful for monitoring the effect of antiretroviral treatments (Wilkinson *et al.*, 1997b; Chang *et al.*, 1999b; Stankoff *et al.*, 2001). One report found improvement of NA/creatine [CR] in HIV dementia pa-

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tients treated with zidovudine (Wilkinson *et al.*, 1997b); another demonstrated that the initially elevated choline compounds [CHO] and [MI] in patients with mild HIV dementia normalized after highly active antiretroviral therapy (HAART) (Chang *et al.*, 1999b). Similarly, abnormalities on neuropsychological tests in HIV-infected patients improved after HAART (Sacktor *et al.*, 1998) or during HAART (Stankoff *et al.*, 2001). However, the relationship between neuropsychological function and brain metabolism is not well understood, for a number of reasons: Prior studies involved subjects who were treated with antiretroviral medications; in addition, the response to treatment may depend on numerous factors, such as treatment regimen and duration, the patients' immune responses, degree of viral suppression, or the presence of virally mediated neurotoxic proteins.

The goal of this study was to determine the relationships between cerebral metabolite abnormalities (^{1}H MRS), cognitive function (neuropsychological tests), and other clinical variables (CD4, plasma viral load, CSF viral loads, and lipids) in antiretroviral medication-naïve HIV patients. Evaluating patients prior to initiation of antiretroviral treatment minimized variables influenced by treatment. We hypothesized that in treatment-naïve HIV patients, cerebral metabolites, especially the glial markers [MI] and [CHO], would correlate with cognitive function, CD4 count, plasma and CSF viral loads, but not with serum lipids (triglycerides and cholesterols).

METHODS

Subjects

Forty-five HIV patients naïve to antiretroviral medications (38 men and 4 women, 3 transsexuals, aged 35.7 ± 1.4 years) were evaluated. Each subject fulfilled the following inclusion criteria: (1) age 18 to 65 years; (2) seropositive for HIV-1; (3) CD4 $< 500/mm^3$; (4) naïve to antiretroviral medications; (5) negative urine toxicology screen (cocaine, amphetamine, marijuana, benzodiazepine, barbiturates, and opiates); (6) willingness and ability to give informed consent or have it given by a valid representative. In addition, subjects were excluded if they fulfilled any of the following exclusion criteria: (1) history of psychiatric illness which might confound the analysis of the study (e.g., schizophrenia, major depression); (2) presence of opportunistic brain lesions (e.g., toxoplasmosis, lymphoma, and progressive multifocal leukoencephalopathy); (3) confounding neurological disorder (e.g., multiple sclerosis, Parkinson's disease, degenerative brain diseases, any brain infections or neoplasms); (4) severe hepatic or renal dysfunction; (5) current or history of drug dependence (including cocaine, methamphetamine, alcohol, opiates, inhalants, and barbiturates); (6) head trauma with loss of consciousness for more than 30 min; (7) for women, pregnant or breast feeding; (8) metallic or electronic implants in the body (e.g., pacemaker, surgical clips, pumps, etc.); (9) inability to read English at 8th grade level. Twenty-five seronegative comparison subjects (13 men and 12 women, ages 32.2 ± 2.1 years) who were on no medications, except for oral contraceptives or vitamins, were evaluated using the same exclusion criteria as above. All subjects signed an informed consent approved by our Institutional Review Board.

MR Studies

The MR studies were performed on a 1.5-T scanner (General Electric Signa, Milwaukee, WI). Three imaging sequences were performed: (1) sagittal T1-weighted localizer (TE/TR 11/500, 4-mm slice thickness, 1-mm gap, 24 cm FOV); (2) axial fast-inversion recovery (TE/TI/TR 32/120/4000, 3.5-mm slices, 24 cm FOV); (3) coronal T2-weighted fast spin-echo (TE/TR 102/4000 ms, 5-mm slices, no gap, 24 cm FOV). Three volumes-of-interest (or voxels) were selected for MRS: frontal gray matter, frontal white matter, and basal ganglia (Fig. 1). Data were acquired using a point-resolved spectroscopy (PRESS) sequence (Bottomley, 1987), which provided optimal parameters for assessing frontal and subcortical brain regions (Ernst and Chang, 1996) (TE 30 ms, TR 3 s, 64 averages, 2048 acquisitions, and 2.5-kHz bandwidth). Metabolite concentrations of NA, CR, CHO, MI, and GLX were determined using a previously described method (Ernst *et al.*, 1993; Kreis *et al.*, 1993), which corrects for the partial volume of CSF (% CSF) in each voxel. A semiautomatic program was used to analyze the spectra (Ernst *et al.*, 1993; Kreis *et al.*, 1993).

Neuropsychological Tests

All subjects underwent a neuropsychological test battery that included measures sensitive to functional deficits associated with the frontal lobe and the striatum, except for working memory which was evaluated by California Computerized Assessment Package (CalCAP). The battery included measures of: (1) fine motor speed: Grooved Pegboard (KlØve, 1963); (2) gross motor functioning: Timed Gait; (3) psychomotor speed: Trail Making Tests (Anonymous, 1944) and Symbol Digit Modalities (SDM) (Smith, 1982); (4) verbal memory: Rey Auditory Verbal Learning Test (AVLT) (Rey, 1941); (5) executive function: Stroop Color Interference Test (Stroop, 1935); (6) mood: Center for Epidemiologic Studies—Depression Scale (CES-D) (Radloff, 1977); (7) intellectual function: New Adult Reading Test-Revised (NART) (Blair and Spreen, 1989).

To evaluate psychomotor speed and reaction times, all participants also performed a battery of customized computerized tests, the CalCAP, expanded version

(Miller, 1990). The CalCAP consists of nine tests (see Table 4) that first measure simple and choice reaction times; four of the tests involve sequential letters or numbers and require the use of working memory [one-back cued response (push space bar whenever "X" follows "A"), one-back sequential letters, two-back sequential letters, one-increment sequential numbers], and three tests require visual discrimination (degraded words with distracters, response reversal, and form discrimination). Both the standard and the computerized neuropsychological tests were performed either on the same day as, or within one week of, the MRI studies.

Statistical Analyses

The overall effects of HIV serostatus and ADC stage on cerebral metabolite concentrations were analyzed with two-way repeated measures analysis of variance (ANOVA), separately for each metabolite of interest (NA, CR, CHO, MI, and GLX). HIV serostatus and ADC stage were between variables, and brain region (frontal gray matter, frontal white matter, and basal ganglia) was a within variable. To identify variables for the correlation analyses, the metabolite concentrations and neuropsychological test scores were compared between seropositive and seronegative subjects via the independent samples *t* test (for normally distributed data) or the Wilcoxon rank-sum test (for data that did not follow a normal distribution). The normality assumption was examined via the Shapiro-Wilk test. The *P* value (two-sided) is reported whenever it is less than 0.05.

Multiple correlation analyses were performed to evaluate correlations between cerebral metabolites and both neuropsychological tests (including the reaction times on CalCAP) and clinical variables (CD4, plasma, and CSF viral loads). Only variables that showed a significant effect of serostatus were included in these analyses; in particular, only the frontal white matter CR, CHO, and MI and the frontal cortex CHO and GLX were included in the analysis. Statistical significance for the correlations was defined as $P \leq 0.05$ (double-sided); correlations with $P < 0.1$ were reported as trends.

RESULTS

Clinical Characteristics

The average ADC stage (Aronow *et al.,* 1988) of the seropositive subjects was 0.99 ± 0.13 (10 were ADC 0; 9 were ADC 0.5; 15 were ADC stage 1; 8 were ADC stage 2; and 3 were ADC stage 3). The average HIV dementia scale was 12.2 ± 0.61 (range 0.5–16, maximum 16) and the average Karnofsky score was 83.1 ± 2.3 (range: 30–100, 100 being normal and maximum).

TABLE 1

Laboratory Studies in Antiretroviral-Naïve HIV Patients[a]

	Mean ± SE	Range
CD4 (cells/mm3)	184.4 ± 22.3	3–500
Plasma viral load (copies/mL)	196,187.1 ± 39725.5	66–1,066,140
Log plasma viral load (copies/mL)	4.7 ± 0.96	1.8–6.0
CSF viral load (copies/mL)	8,128.3 ± 2198.2	34–75,000
Log CSF viral load (copies/mL)	3.3 ± 0.14	1.5–4.9
CSF red blood cells (#/mm^3)	34.4 ± 12.7	1–420
CSF white blood cells (#/mm^3)	4.4 ± 1.0	1–33
CSF glucose (mg/dL)	53.7 ± 2.0	30–106
CSF protein (mg/dL)	42.1 ± 2.4	6–80
Cholesterol (mg/dL)	163.2 ± 6.2	94–265
Triglycerides (mg/dL)	155.2 ± 11.9	45–404

[a] CSF values are from 42 subjects. All other values are from all 45 patients.

The HIV patients' mean CD4 count was less than 200 cells/mm^3, and their plasma and CSF viral loads were relatively high (Table 1). The patients also demonstrated mild pleocytosis in the CSF, although their CSF glucose and protein were within normal limits. In these antiretroviral–naïve patients, the serum lipids, cholesterol, and triglycerides were within normal limits. The average duration since HIV diagnosis was 22.2 ± 6.9 months (range: 0.25–248 months). As a group, the majority of the patients were mildly depressed, probably due to the recent diagnosis of HIV. The education levels were not significantly different between HIV patients and control subjects (HIV-positive, 12.1 ± 0.54 years, range 8–23 years; HIV-negative, 13.1 ± 0.5 years).

Cerebral Metabolite Concentrations

Table 2 shows the cerebral metabolite concentrations in the two subject groups. Relative to the control subjects, the HIV patients showed significantly increased CHO and MI concentrations ($P = 0.02$ for both metabolites; effect of serostatus on ANOVA, averaged across brain regions), as well as a trend for increased CR concentration ($P = 0.1$). For the GLX concentration, there was a statistically significant interaction between HIV serostatus and brain region ($P = 0.04$, interaction effect on ANOVA). Post hoc tests demonstrated elevated concentrations of CR (+8.6%, $P = 0.03$), CHO (+12.5%, $P < 0.01$), and MI (+16.4%, $P < 0.01$) in the frontal white matter of the patients, and mildly elevated choline compounds (+5.3%, $P < 0.04$) and decreased GLX (−4.8%, $P < 0.05$) in the frontal cortex (Fig. 1). As a group, no abnormalities in individ-

TABLE 2

Metabolite Concentrations of Controls and HIV Patients (Mean ± SE)

	Normal controls (n = 25)	HIV patients (n = 45)	% Relative to controls	P values
Frontal cortex (gray matter)				
N-acetylaspartate	8.2 ± 0.16	8.2 ± 0.09	0%	n.s.
Total creatine	7.8 ± 0.12	7.7 ± 0.16	−1.3%	n.s.
Choline compounds	1.9 ± 0.05	2.0 ± 0.06	+5.3%	0.04
myo-Inositol	7.0 ± 0.18	7.7 ± 0.26	+10%	n.s.
GLX	18.7 ± 0.32	17.8 ± 0.29	−4.8%	0.05
Frontal white matter				
N-acetylaspartate	7.4 ± 0.14	7.2 ± 0.11	−2.7%	n.s.
Total creatine	5.8 ± 0.16	6.3 ± 0.13	+8.6%	0.03
Choline compounds	1.6 ± 0.04	1.8 ± 0.05	+12.5%	0.008
myo-Inositol	6.7 ± 0.27	7.8 ± 0.26	+16.4%	0.008
GLX	11.8 ± 0.43	11.1 ± 0.25	−5.9%	n.s.
Basal ganglia				
N-acetylaspartate	8.4 ± 0.16	8.5 ± 0.14	+1.2%	n.s.
Total creatine	8.6 ± 0.20	8.9 ± 0.17	+3.5%	n.s.
Choline compounds	2.1 ± 0.05	2.2 ± 0.06	+4.8%	n.s.
myo-Inositol	6.4 ± 0.21	7.0 ± 0.25	+9.4%	n.s.
GLX	17.6 ± 0.59	18.8 ± 0.47	+6.8%	n.s.

ual metabolites were found in the basal ganglia of the HIV patients.

Figure 2 displays the concentrations of the four major metabolites by ADC stage. The ANOVA showed main effects of ADC stage on the [NA] (P = 0.005; averaged across the three brain regions), CHO (P = 0.0003), and MI ($P < 0.0001$). The neuronal marker [NA] showed a trend to decrease with increasing ADC stage in the frontal white and gray matter, but was significantly decreased only during the late or severe stage (ADC stage 3) in the basal ganglia. With increasing ADC stage, [CR] was elevated in the frontal lobe

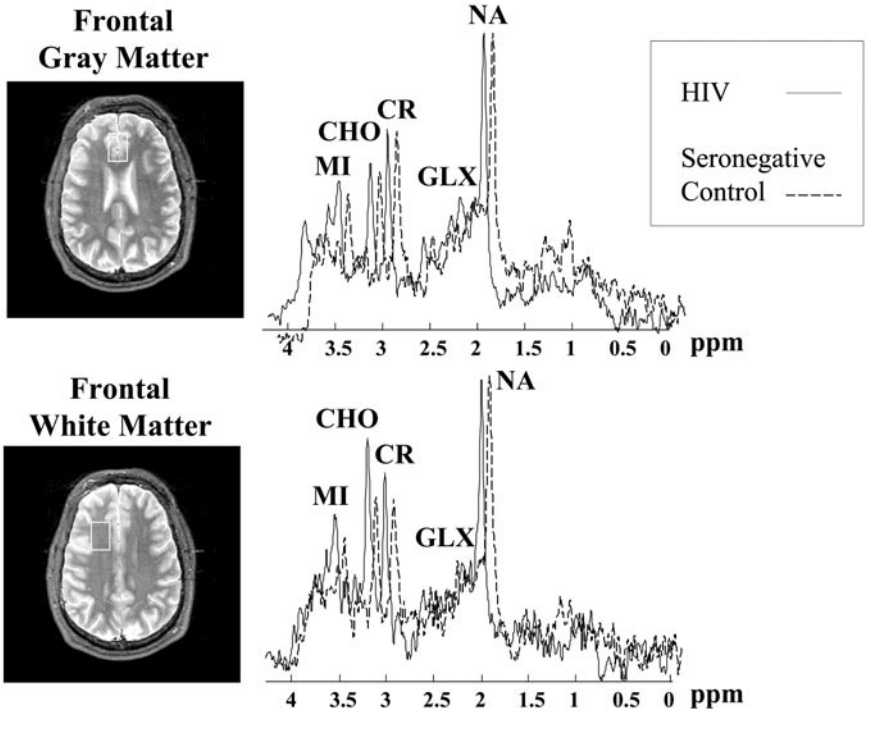

FIG. 1. T2-weighted coronal MRI showing the typical VOIs in the frontal gray matter and the right frontal white matter (left column); VOI from the right basal ganglia is not shown. Representative MR spectra from the corresponding regions of an HIV patient compared to a control subject (right column).

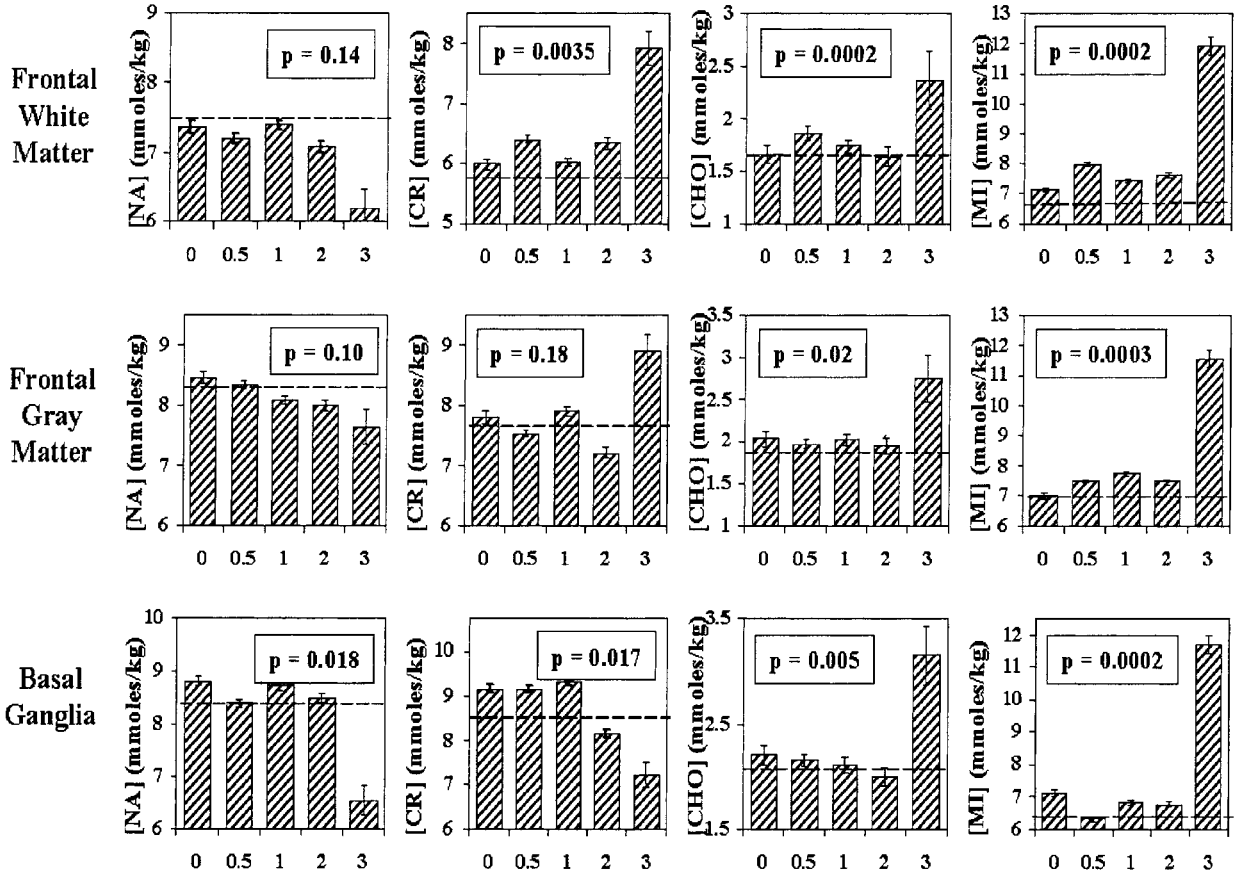

FIG. 2. Bar graphs illustrating the four major metabolite concentrations in the HIV patients according to ADC stage and brain regions: frontal white matter (top row), medial frontal cortex (middle row), and basal ganglia (bottom row). The dashed line in each graph represents the mean value for the corresponding metabolite concentration in the control subjects.

but decreased in the basal ganglia. [CHO], however, was minimally elevated in those with mild to moderate stages of dementia and was elevated during the severe stage. Similarly, the glial marker [MI] was mildly elevated in the early stages, but significantly elevated in stage 3 in all three brain regions. Therefore, a shift from neuronal to glial content in the brain regions according to ADC was explored with MI/NA, and significant correlations were found in all three brain regions (midfrontal gray matter, $r = 0.56$, $P < 0.0001$; frontal white matter, $r = 0.49$, $P = 0.0006$; basal ganglia, $r = 0.43$, $P = 0.003$).

Neuropsychological Test Scores

Tables 3 and 4 show the neuropsychological tests scores. Although the two subject groups had comparable estimated verbal intelligence quotients (VIQ), derived from the NART (Table 3), HIV patients were slower on measures of fine motor function (Grooved Pegboard, 17–22% slower) and psychomotor speed (Symbol Digit Modalities, 20% slower; Trail A & B, 34% slower), but not on gross motor function (Timed Gait). Although the HIV patients' verbal memory on immediate recall was poorer (AVLT, −23%) compared to the control subjects, they showed no significant difference in repeated AVLT trials, with interference recall or delayed recall. The HIV patients also had lower performance on all three Stroop tasks compared to the control subjects (12–18%).

Correlations between Metabolite Concentrations and Neuropsychological Tests

In HIV patients, metabolite concentrations in the frontal white matter showed good correlations mostly with the Stroop test scores: [MI] strongly correlated with all three Stroop tests (overall multiple correlation, $r = 0.59$, $P = 0.0006$; individual correlations, Word, $r = 0.46$, $P = 0.002$; Color, $r = 0.32$; $P = 0.04$; Interference, $r = 0.51$, $P = 0.0004$, see Fig. 3); [CHO] correlated with Stroop Word ($r = 0.29$, $P = 0.05$), whereas [CR] correlated with Stroop Interference ($r = 0.30$, $P = 0.05$). In the frontal cortex, only [GLX] showed trends for correlation with a fine motor task (pegboard, non-dominant hand, $r = -0.32$, $P = 0.06$) and with the Stroop color test ($r = 0.29$, $P = 0.08$).

TABLE 3

Neuropsychological Tests Scores of HIV Patients (Mean ± SE)

	Normal controls	HIV patients	% Relative to controls	*P* values
Timed Gait (s)	10.0 ± 0.32	9.0 ± 0.21	−10%	0.01
Grooved Pegboard				
Dominant hand (s)	61.1 ± 1.65	74.5 ± 2.29	+21.9%	0.0001
Non-dominant hand (s)	74.4 ± 3.62	87.3 ± 3.29	+17.3%	0.006
Symbol Digit Modalities	58.0 ± 1.81	46.3 ± 1.92	−20.2%	0.0002
Auditory Verbal Learning Test				
Immediate (# words)	6.5 ± 0.39	5.0 ± 0.24	−23.1%	0.003
After 5 trials (# words)	12.4 ± 0.39	11.4 ± 0.33	−8.1%	n.s.
Following interference (# words)	10.7 ± 0.56	9.8 ± 0.38	−8.4%	n.s.
Delayed (# words)	10.6 ± 0.48	9.6 ± 0.46	−9.4%	n.s.
Trail Making A (s)	23.9 ± 1.03	32.0 ± 2.21	+33.9%	0.01
Trail Making B (s)	60.6 ± 4.8	81.1 ± 6.0	+33.8%	0.009
Stroop Color Interference Test				
Word (s)	43.2 ± 1.74	48.5 ± 1.40	+12.3%	0.01
Color (s)	58.6 ± 2.15	66.8 ± 2.15	+14%	0.02
Interference (s)	108.0 ± 5.18	127.1 ± 5.1	+17.7%	0.008
NART estimated VIQ	102.4 ± 2.2	99.3 ± 2.3	−3.0%	n.s.
CES–depression scale	Norm ≤ 16	17 ± 1.5		

* *P* values are from unpaired *t* tests (for normally distributed data) or Wilcoxon rank-sum tests (for nonnormally distributed data).

In the control subjects, the frontal white matter [MI] did not correlate with any neuropsychological test scores. In contrast, the frontal white matter [CHO] had a negative correlation with AVLT1 ($r = -0.46$, $P = 0.02$), while [CR] correlated with all three Stroop measures (Word, $r = 0.45$, $P = 0.03$; Color, $r = 0.46$, $P = 0.02$; Interference, $r = 0.40$, $P = 0.05$).

Correlations between Metabolite Concentrations and Reaction Times on CalCAP

In the HIV patients, only a visual discrimination task (degraded word with distractors) correlated with the frontal white matter [MI] ($r = 0.32$, $P = 0.03$) and [CHO] ($r = 0.34$, $P = 0.03$). Another visual discrimination task, response reversal, also showed a tendency to correlate with the frontal white matter [MI] ($r = 0.26$, $P = 0.09$) and [CR] ($r = 0.27$, $P = 0.08$). Frontal gray matter [CHO] correlated with the two-back task on CalCAP ($r = 0.3$, $P = 0.05$). In the control subjects, the only significant correlation was between the frontal white matter [MI] and response reversal ($r = 0.47$, $P = 0.02$).

Correlations between Metabolite Concentrations and Clinical Variables

To understand the relationship between cerebral metabolites and systemic measures of HIV infection, we evaluated correlations between abnormal metabolites and CD4 count and plasma and CSF viral loads (log transformed). The three elevated frontal white

TABLE 4

Reaction Times (Mean ± SE) on CalCAP in HIV Patients and Controls

	Reaction times (ms)			
CalCAP tasks	Normal controls	HIV patients	% Relative to controls	*P* values[a]
Simple reaction time	308.4 ± 11.5	416.1 ± 27.1	+34.9%	0.0023
Choice reaction time–single digit (“7”) recognition	386.2 ± 7.2	410.9 ± 8.8	+6.4%	n.s.
One-back cued response (“X” only after “A”)	387.8 ± 23.4	422.5 ± 19.0	+8.9%	n.s.
Sequential numbers (one-back)	483.6 ± 15.5	567.2 ± 17.8	+17.3%	0.0051
Sequential numbers (one-increment)	581.4 ± 24.8	632.7 ± 17.4	+8.8%	n.s.
Sequential numbers (two-back)	743.7 ± 36.9	887.3 ± 27.2	+19.3%	0.0017
Degraded words with distracters	483.9 ± 12.4	574.1 ± 21.0	+18.6%	0.0039
Response reversal/visual scanning	614.0 ± 15.0	712.5 ± 21.8	+16.0%	0.0045
Form discrimination	716.0 ± 18.9	790.7 ± 20.6	+10.4%	0.0206

[a] *P* values are from unpaired *t* tests (for normally distributed data) or Wilcoxon rank-sum tests (for nonnormally distributed data).

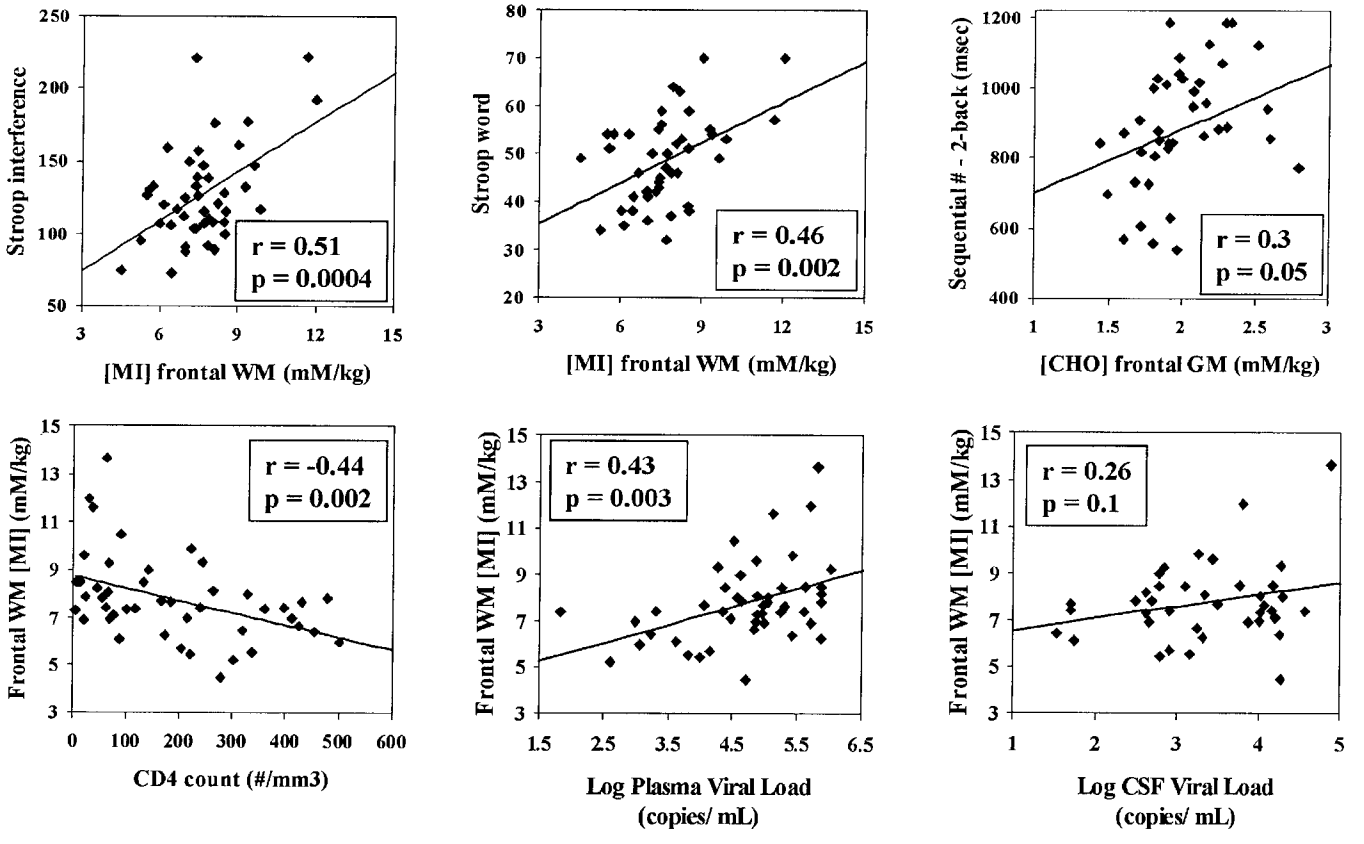

FIG. 3. The graphs in the top row show the associations between two of the cerebral metabolites in the frontal lobe and two of the tests that reflect frontal lobe function. The graphs in the bottom row show the associations between the glial marker [MI] with the CD4 count and the log plasma viral load, but not with the log CSF viral load.

matter metabolites ([CR], [CHO], and [MI]) strongly correlated with CD4 counts (multiple correlation, $r = 0.44$, $P = 0.03$; individual correlations, [MI], $r = -0.44$, $P = 0.002$; [CHO], $r = -0.29$, $P = 0.06$; and [CHO], $r = -0.27$, $P = 0.08$). Additionally, the frontal white matter [MI] correlated with the log plasma viral load ($r = 0.43$, $P = 0.003$) but not with the log CSF viral load. Conversely, frontal gray matter [CHO] correlated with Log CSF viral load ($r = 0.35$, $P = 0.04$) but not with plasma viral load or CD4 counts. No significant correlations were observed between metabolites and serum lipids.

Correlations between Clinical Variables and Cognitive Tests

In HIV patients, the CD4 count correlated with the three Stroop tests (multiple correlation, $r = 0.42$, $P = 0.05$; individual correlations, Word, $r = -0.34$, $P = 0.03$; Color, $r = -0.38$, $P = 0.01$; Interference, $r = -0.36$, $P = 0.01$) and with the three visual discrimination tests (multiple correlation, $r = 0.52$, $P = 0.006$; degraded word with distractors, $r = -0.36$, $P = 0.02$; response reversal, $r = -0.52$, $P = 0.0004$; form discrimination, $r = -0.31$, $P = 0.04$).

DISCUSSION

Advantages of Antiretroviral-Naïve Subjects and Measuring Metabolite Concentrations

A unique aspect of this study is that all of our HIV subjects were naïve to antiretroviral drugs. Evaluating antiretroviral-naïve subjects is important, since these drugs may affect brain metabolism directly or indirectly due to effective treatment of HIV (Chang *et al.*, 1999b; Stankoff *et al.*, 2001; Wilkinson *et al.*, 1997b). For example, zidovudine (AZT) and other nucleoside reverse-transcriptase inhibitors may cause significant mitochondrial toxicity (Lewis and Dalakas, 1995), which may lead to decreased *N*-acetylaspartate (Dautry *et al.*, 2000). Independently, treatment with antiretroviral medications also may distort the relationships among cerebral metabolite concentrations, neuropsychological tests, and clinical variables of HIV infection. Systemic measures of HIV infection typically improve rapidly after initiation of HAART, whereas cognitive deficits may not improve until 6 months or longer (Sacktor *et al.*, 1998; Stankoff *et al.*, 2001). Resistance to antiretroviral medications also may cause similar discordance between systemic HIV measures

and brain metabolism or cognitive function. Finally, HAART may lead to discordant effects even on systemic HIV measures, in that some HIV patients treated with HAART experience a rebound in plasma viral load despite persistent elevation in CD4 (Cohen, 1998). Altogether, the multitude of confounding effects of antiretroviral treatment demonstrates the advantage of studying a population that is naïve to antiretroviral medications.

The second advantage of this study is that metabolite concentrations were measured, whereas many previous studies determined metabolite ratios only, typically using CR as an internal reference (Chong *et al.*, 1993; Jarvik *et al.*, 1993; Laubenberger *et al.*, 1996; Marcus *et al.*, 1998; Moller *et al.*, 1999; Simone *et al.*, 1998; Stankoff *et al.*, 2001; Tracey *et al.*, 1996; von Giesen *et al.*, 2001). However, it is difficult to interpret metabolite ratios, because CR concentration may vary with disease severity and across brain regions (Chang *et al.*, 1999a; Chang *et al.*, 1999b). For example, many investigators have observed decreased NA/CR (Barker *et al.*, 1995; Jarvik *et al.*, 1993; Meyerhoff *et al.*, 1996; Paley *et al.*, 1995), even in HIV patients who were neurologically asymptomatic (Suwanwelaa *et al.*, 2000), and interpreted this as decreased neuronal marker NA due to neuronal damage. In contrast, this and earlier studies demonstrate normal [NA] (Chang *et al.*, 1999a,b; Meyerhoff *et al.*, 1999), but elevated [CR], especially in the basal ganglia, during the cognitively asymptomatic stage. Normal [NA] suggests minimal or no neuronal injury or loss in early HIV infection, which is consistent with prior neuropathological studies (Gray *et al.*, 1996). In the later stages of ADC, [CR] is significantly elevated in the frontal lobe, but decreased in the basal ganglia, along with decreased [NA]. This demonstrates that abnormal NA/CR ratios are difficult to interpret since both NA and CR may change depending on disease severity.

Cerebral Metabolite Abnormalities and Their Dependence on ADC Stage

HIV patients naïve to antiretroviral drugs showed several metabolite abnormalities, including elevated frontal white matter [MI], [CHO], and [CR], and to a lesser extent elevated [CHO] and decreased [GLX] in the frontal cortex. These findings are similar to the previously reported elevated CHO/CR (Chong *et al.*, 1993; Jarvik *et al.*, 1993; Marcus *et al.*, 1998; Moller *et al.*, 1999; Simone *et al.*, 1998; Tracey *et al.*, 1996), MI/CR (Chang *et al.*, 1999a; Laubenberger *et al.*, 1996; Stankoff *et al.*, 2001; von Giesen *et al.*, 2001), and decreased "marker peak"/CR (Jarvik *et al.*, 1996) (which corresponds to the current GLX region), in HIV patients on various regimens of antiretroviral medications. Therefore, our results provide further evidence that cerebral metabolite concentrations on ^{1}H MRS, especially the probable glial marker [MI] and the cell membrane marker [CHO], might serve as good *in vivo* surrogate markers for detecting the severity of brain injury. Significantly elevated [CHO], [MI], and decreased [NA] are most likely to be observed during the later stages of dementia. Probable increases in glia-to-neuron ratio, either from glial proliferation, glial hypertrophy secondary to systemic HIV disease, or decreased neuron density, are also supported by the positive correlations of MI/NA and ADC stage.

Our seropositive subjects also showed abnormal [CR], which represents the sum of metabolites involved in high-energy metabolism, although it is unknown whether creatine or phosphocreatine, or both are abnormal. In the basal ganglia, the variation of [CR] with HIV dementia stage (increased during early stages but decreased during later stages) parallels that of glucose metabolism. Fluorodeoxyglucose positron emission tomography (PET) found that the basal ganglia are hypermetabolic during the early stages of HIV (Rottenberg *et al.*, 1987) and hypometabolic at later stages when motor slowing occurs (Rottenberg *et al.*, 1996; von Giesen *et al.*, 2000). However, concurrent PET and MRS measurements in the same subjects would be necessary to evaluate this possible relationship.

The relationship between cerebral choline and HIV dementia stage is inconsistent among MRS studies. In some studies, HIV patients who were neurologically or cognitively asymptomatic, but were treated with various antiretroviral medications, had elevated [CHO] or CHO/CR (Chang *et al.*, 1999a,b; Meyerhoff *et al.*, 1999; Salvan *et al.*, 1997; Stankoff *et al.*, 2001). In contrast, this study showed relatively normal [CHO] not only in antiretroviral drug-naïve patients with ADC 0 but also in those with mild to moderate HIV dementia, whereas elevated [CHO] was observed only in patients with severe dementia (ADC = 3). Normal CHO/CR ratios were also reported in HIV patients who were systemically asymptomatic (Wilkinson *et al.*, 1997a), CDC groups A & B (Moller *et al.*, 1999) or neurologically asymptomatic (Marcus *et al.*, 1998; Suwanwelaa *et al.*, 2000). Because we have found elevated [CHO] 3 months after initiation of HAART (Chang *et al.*, 2000b), it is possible that antiretroviral medication treatment affects the cerebral metabolite concentration of [CHO].

Cerebral Metabolites Are Related to Cognitive Function, CD4, and Plasma Viral Load

Prior studies in patients treated with antiretroviral drugs demonstrated that metabolites measured with MRS correlated with clinical variables, despite possible confounding effects of antiretroviral medications as discussed above. Specifically, CHO and MI inversely correlated with CD4 (Chang *et al.*, 1999a; Chong *et al.*, 1993; Meyerhoff *et al.*, 1999), and [MI] also correlated

with ADC stage (Chang *et al.*, 1999a; Tracey *et al.*, 1996) and to a lesser extent with plasma and CSF viral loads (Chang *et al.*, 1999a). In antiretroviral-naïve patients, [MI] showed similar relationships with CD4 count and ADC stage. In contrast to a pilot study of treated HIV patients (Chang *et al.*, 1999b), which demonstrated a correlation with CSF viral load, our antiretroviral-naïve patients showed a strong correlation of white matter [MI] with plasma viral load but not with CSF viral load. This finding suggests that glial proliferation may be due to systemic rather than centrally derived factors.

The current study also demonstrates an association between cognitive deficits and neurochemical abnormalities in antiretroviral medication-naïve patients. In particular, executive function (performance on Stroop test) appears to be related to frontal white matter [MI] in the HIV patients. In addition, both frontal white matter [MI] and [CHO] were related to performance on a visual discrimination test (degraded words with distracters), which also requires decision-making. Therefore, the elevated frontal white matter [MI], and to a lesser extent [CHO], may contribute to executive dysfunction in patients with HIV. Only one study, prior to the HAART era, evaluated the relationship between MRS findings and cognitive performance in HIV patients, and found that decreased subcortical gray matter NA was weakly related to poorer performance on a variety of neuropsychological tests (abstraction, memory, and fine motor function) but not to CDC stage (Meyerhoff *et al.*, 1999). However, neither metabolites in the frontal lobe nor [MI] were measured in that study.

While several of the metabolites correlate with some of the neuropsychological test scores and clinical variables, they do not represent the same phenomenon. Metabolite concentrations measured with MRS are a direct, biological measure reflecting brain injury. In contrast, neuropsychological tests, while sensitive for detecting brain dysfunction, do not directly reflect brain injury, and may be affected by education, socioeconomic or cultural influences, fatigue, or depression, all of which are common confounding variables in patients with HIV infection. For example, depressive symptoms in many of our patients (due to recent HIV diagnosis) may have contributed to psychomotor slowing and poorer attention, which would affect primarily the immediate recall on the AVLT and possibly some of the CalCAP tasks. Such confounding effects on neuropsychological, but not spectroscopic, variables might explain a lack of correlation between these variables.

In summary, studying antiretroviral-naïve patients minimized the confounding effects of antiretroviral treatment on MRS and neuropsychological measurements, and allowed for a more accurate assessment of the relationships among cerebral metabolite abnormalities, cognitive function, and systemic measures of HIV infection. Metabolite concentrations, rather than metabolite ratios, should be measured since the CR concentration varies with disease severity. Lower CD4 and elevated plasma viral load were associated with elevated frontal white matter [MI], which in turn correlated with the Stroop tasks. These findings suggest that systemic factors (resulting from suppressed immune function and higher plasma viral load) lead to glial proliferation (elevated [MI]) in the frontal white matter, which in turn may contribute to the deficits on executive function in HIV.

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