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Neurometabolite Alterations Associated With Cognitive Performance in Perinatally HIV-Infected Children

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Abstract: Despite treatment with combination antiretroviral therapy (cART), cognitive impairment is still observed in perinatally HIV-infected children. We aimed to evaluate potential underlying cerebral injury by comparing neurometabolite levels between perinatally HIV-infected children and healthy controls. This cross-sectional study evaluated neurometabolites, as measured by Magnetic Resonance Spectroscopy (MRS), in perinatally HIV-infected children stable on cART (n = 26) and healthy controls (n = 36).

Participants were included from a cohort of perinatally HIV-infected children and healthy controls, matched group-wise for age, gender, ethnicity, and socio-economic status. N-acetylaspartate (NAA), glutamate (Glu), *myo*-inositol (mI), and choline (Cho) levels were studied as ratios over creatine (Cre). Group differences and associations with HIV-related parameters, cognitive functioning, and neuronal damage markers (neurofilament and total Tau proteins) were determined using age-adjusted linear regression analyses.

HIV-infected children had increased Cho:Cre in white matter (HIV-infected = 0.29 ± 0.03; controls = 0.27 ± 0.03; *P* value = 0.045). Lower nadir CD4+ T-cell Z-scores were associated with reduced neuronal integrity markers NAA:Cre and Glu:Cre. A Centers for Disease Control and Prevention (CDC) stage C diagnosis was associated with higher glial markers Cho:Cre and mI:Cre. Poorer cognitive performance was mainly associated with higher Cho:Cre in HIV-infected children, and with lower NAA:Cre and Glu:Cre in healthy controls. There were no associations between neurometabolites and neuronal damage markers in blood or CSF.

Compared to controls, perinatally HIV-infected children had increased Cho:Cre in white matter, suggestive of ongoing glial proliferation. Levels of several neurometabolites were associated with cognitive performance, suggesting that MRS may be a useful method to assess cerebral changes potentially linked to cognitive outcomes.

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Abbreviations: cART = combination antiretroviral therapy, CDC = Centers for Disease Control and Prevention, Cho = choline, Cre = creatine, CSF = cerebrospinal fluid, CSI = chemical shift imaging, Glu = glutamate, GM = gray matter, HIV = human immunodeficiency virus, LP = lumbar puncture, mI = *myo*-inositol, MRS = magnetic resonance spectroscopy, NAA = N-acetylaspartate, NfH = neurofilament heavy, NfL = neurofilament light, NPA = neuropsychological assessment, tTau = total Tau protein, VL = viral load, VOI = volume of interest, WM = white matter.

KEY FINDINGS

- HIV-infected children had higher white matter levels of Cho:Cre as compared to healthy controls.
- Lower nadir CD4 T-cell Z-scores were associated with lower levels of neuronal metabolites NAA:Cre and Glu:Cre, and a history of CDC stage C was associated with higher levels of glial metabolites mI:Cre and Cho:Cre.
- Higher Cho:Cre was associated with poorer cognitive performance in HIV-infected children, whereas in healthy controls, higher neuronal metabolite levels were associated with better cognitive performance.
- Neurometabolites were not associated with neuronal damage markers in blood or CSF.

INTRODUCTION

With the introduction of combination antiretroviral therapy (cART), the incidence of HIV-encephalopathy in perinatally HIV-infected children declined profoundly.[1] However, milder cognitive impairments remain widely prevalent in this patient group, and HIV-related cerebral injury may underlie these impairments.[2]

Magnetic resonance spectroscopy (MRS) has been suggested as a valuable tool to assess neurocognitive functioning through measuring brain metabolite levels.[3] Neurometabolite levels have been widely studied in cART-treated HIV-infected adults[4–6] and in children with HIV-encephalopathy.[7] Yet findings from small studies in cART-treated, HIV-infected children with milder cognitive impairment are inconsistent. Whereas cerebral levels of N-acetyl aspartate (NAA) and glutamate (Glu)—presumed to reflect neuronal integrity—were comparable between HIV-infected and healthy children,[8–10] glial proliferation markers *myo*-inositol (mI) and choline (Cho) have been reported as both increased or decreased in specific cerebral regions.[11–13]

Increased cerebrospinal fluid (CSF) levels of neurodegeneration markers total Tau (tTau) and neurofilament proteins have been associated with cognitive impairment,[14,15] and recent evidence suggests cognitive deficits are associated with decreased cerebral NAA:Cre and Glu:Cre ratios in HIV-infected adults.[16,17] It remains unknown whether these biomarkers are related to cognitive outcomes in HIV-infected children.

In this study we aimed to assess neuronal integrity and glial markers in a cohort of perinatally HIV-infected children that were stable on cART, as compared to matched healthy controls. As we previously detected poorer cognitive functioning and structural cerebral injury in these HIV-infected children,[18,19] we expected that neurometabolite levels in this group differ from those in matched healthy controls and will be associated with HIV-related characteristics, cognitive functioning and neurodegenerative markers.

METHODS

Participants

Study participants were included from the NOVICE cohort, which evaluated neurological, cognitive and visual performance in perinatally HIV-infected children as compared to healthy controls. Perinatally HIV-infected children between 8 and 18 years of age, were recruited via the outpatient clinic of the Emma Children's Hospital in Amsterdam, and healthy children from the community, group-wise matched for gender, age, ethnicity and SES as previously described.[18] Participants were excluded if they had non-HIV related chronic neurological diseases, seizure disorders, intracerebral neoplasms or psychiatric disorders. The ethics committee of the Academic Medical Center in Amsterdam approved the study protocol. Written informed consent was obtained from participants' parents, as well as from children above 12 years of age.

HIV Disease and Treatment Characteristics

HIV and cART assessments were obtained as previously described.[18] Historical HIV viral loads (VL), CD4+ T-cell counts, Centers for Disease Control and Prevention (CDC) clinical staging and cART treatment history were derived from the Dutch HIV Monitoring Foundation database. CD4+ T-cell counts were transformed into Z-scores to correct for age and gender related differences. The nadir CD4+ T-cell Z-score was determined by the lowest Z-score before cART or up to 3 months after cART initiation. Additionally, for each participant, the duration living with a detectable VL (years) and with CD4+ T-cell counts below 500×10^6/L (months) were calculated.

MRS

Chemical Shift Imaging (CSI) H^+-MRS was performed on a 3.0 Tesla (3T) MRI scanner at the Academic Medical Center (Intera, Philips Healthcare, Best, the Netherlands), equipped with a 16-channel phased array head coil. Structural 3D echo gradient T1 with multiplanar reconstruction was conducted as previously reported.[19] MR spectra were acquired from 1 axial T1 slice positioned directly above the corpus callosum. Water was suppressed by applying 3 chemical shift-selective excitation pulses, using a Point Resolved Spectroscopy sequence (TE/TR = 37/2000 milliseconds). Using the advantage of CSI to cover large brain areas while accounting for varying metabolite levels across brain regions,[20] we selected total grey matter (GM) and total white matter (WM) within our slice as volumes of interest (VOI). The segmentation of GM and WM was performed using a T1-weighted scan. Figure 1 illustrates the acquisition and analysis workflow. LC Model (Stephen Provencher, Oakville, Canada) was used for the quantification of the metabolite spectra.[21] LC Model validated the concentration of each metabolite, adjusted the phase and ppm shift of the spectra, estimated the baseline and performed eddy current correction. Peak registration in LC Model used the most prominent peaks of NAA, Cho, and Cre for initial referencing. Spectra were generated for 33 neurometabolites. We excluded spectra with poor signal-to-noise ratios, a disproportionate water signal or other significant artifacts. Based on previous literature, we selected NAA, Glu, mI, Cho, and Cre as neurometabolites of interest, which were all good quality spectra

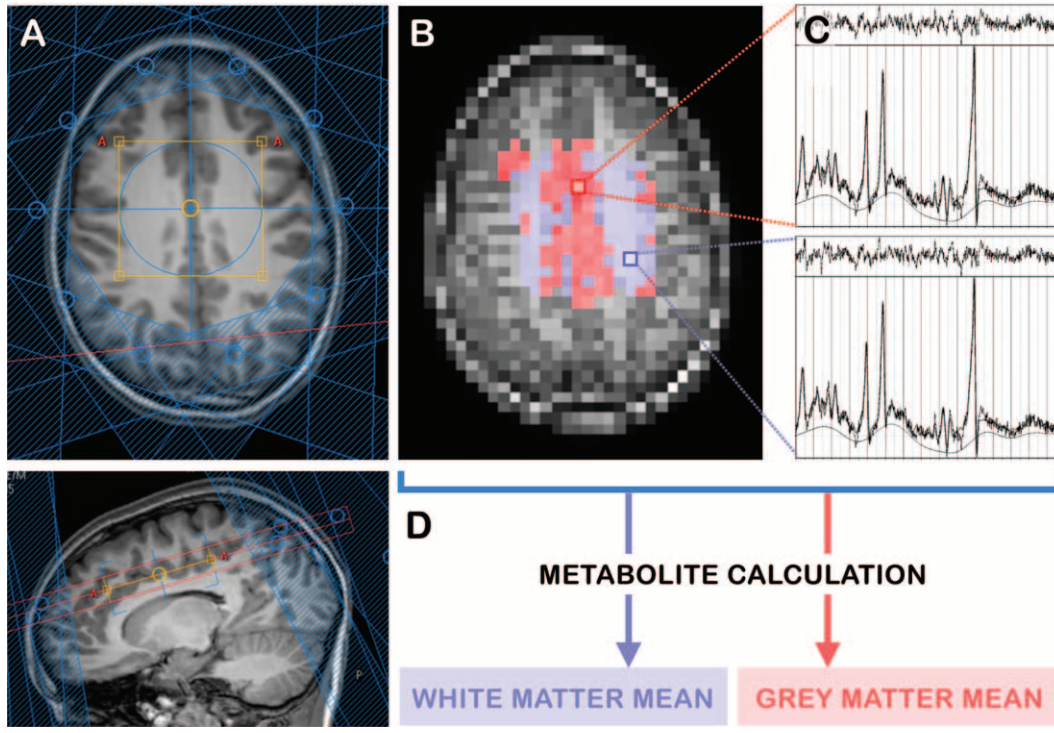

FIGURE 1. Exemplar planning of chemical shift imaging (CSI), superimposed on a 3D-T1-weighted image. Based on the 3D-T1-weighted scan (A), gray matter (GM), and white matter (WM) segmentation was performed within the CSI field-of-view (B). Estimated metabolite levels (C) were averaged within all GM and WM voxels to obtain mean GM and WM values (D). CSI = chemical shift imaging, GM = gray matter, WM = white matter.

(SD < 20%). As spectral peak areas were not directly proportional to metabolite concentrations, metabolite levels were measured as a ratio over Cre, a commonly used, relatively stable marker for energy potential in brain tissue.[22] Measuring metabolites as ratios to Cre enabled correction for differences between imaging and localization methods, as well as different contributions of CSF to the VOIs.

Neuropsychological Assessment

A single neuropsychologist (JAtS) performed all neuropsychological assessments (NPA) as described in detail before.[18] We evaluated various cognitive domains including (i) intelligence; (ii) processing speed; (iii) attention/working memory; (iv) visual-motor function; (v) memory; (vi) executive functioning. As processing speed and memory were measured by multiple subtests, results were combined into a mean variable per domain for further analyses.

CSF and Blood Markers

Blood samples for all participants were obtained using venipuncture. In a subset of HIV-infected children for whom a lumbar puncture was relevant as part of patient care, CSF was collected. Samples were centrifuged within 2 hours at 1700× g for 10 minutes and the supernatant was transferred into a polypropylene tube (Sarstedt, Numbrecht, Germany) and stored at −80°C until biomarker analysis. Neurofilament light (NfL) in serum was analyzed by a highly sensitive electrochemiluminescence-based immunoassay (Meso Scale Discovery, MD).[23] NfL in CSF was measured by an enzyme-linked immunosorbent assay (Uman Diagnostics, Umeå, Sweden). Neurofilament heavy (NfH) in CSF was analyzed by an in-house developed Luminex assay.[24] CSF tTau was analyzed using the Innotest (Fujirebio, Gent, Belgium).[25]

Statistical Analysis

Data analysis was conducted using Stata Statistical Software, Version 13 (StataCorp, College Station, TX). We compared demographic characteristics and serum NfL levels between cases and controls using Mann–Whitney *U* tests for continuous data and X^2 tests for categorical data. With linear regression analyses, we compared neurometabolite ratios between groups, and evaluated associations between the different types of neurometabolites, and between neurometabolites and HIV parameters, cognitive functioning, and neuronal damage markers. We adjusted all regression analyses for age, as age has been shown to exert an effect on brain metabolite levels.[26] The analysis evaluating associations between different types of neurometabolites included all study participants and was therefore additionally adjusted for HIV status. Associations with HIV parameters were further assessed in a multivariable model, including only variables with a *P* value <0.20 in the age-adjusted regression analysis. Variables that were not normally distributed were transformed by base-10 log (zenith VL, serum NfL) or square root (duration of detectable VL) before regression analysis.

RESULTS

Participants

A total of 72 participants were included, consisting of 35 HIV-infected children and 37 controls. A detailed demographic

TABLE 1. Participant Characteristics

		HIV-Infected (n = 26)	Healthy (n = 36)	*P* Value
Demographics				
Gender (male)		15 (58)	18 (50)	0.549
Age (y)		13.2 (11.4–15.8)	12.1 (11.5–15.8)	0.429
Ethnicity	Black	19 (73)	26 (72)	0.403
	Mixed black	4 (15)	5 (14)	
	White	0 (0)	3 (8)	
	Other	3 (12)	2 (6)	
Intelligence, IQ		75 (68–83)	88 (79–95)	0.004*
ISCED educational level of parent†		5 (3–6)	5 (5–6)	0.042*
Parents employed	0	13 (52)	12 (33)	0.306
	1	9 (36)	16 (44)	
	2	3 (12)	8 (22)	
HIV- and cART related characteristics				
CDC stage	N/A	9 (35)	–	–
	B	10 (38)	–	–
	C	7 (27)	–	–
Undetectable blood HIV VL		22 (85)	–	–
Undetectable CSF HIV VL Undetectable		17 (85)	–	–
Zenith HIV VL (log copies/mL)		5.5 (5.1–5.8)	–	–
Duration VL detectable (y)		2.6 (1.5–6.4)	–	–
$CD4^+$ T-cell count ($\times 10^6$/L)		695 (580–1030)	–	–
$CD4^+$ T-cell Z-score		−0.11 (−0.28–0.16)	–	–
Nadir $CD4^+$ T-cell Z-score		−0.75 (−1.56– −0.44)	–	–
Duration $CD4^+$ T-cell count $<500 \times 10^6$/L (mo)		2.8 (0–18.3)	–	–
Currently using cART		22 (85)	–	–
Age at cART initiation (yrs)		2.6 (0.9–5.9)	–	–

Values are displayed as median (IQR) or in N (%).

cART = combination antiretroviral therapy, CDC category = Centers for Disease Control and Prevention stage (N/A = no to minimal symptoms. B = moderate symptoms. C = severe symptoms or AIDS), CSF = cerebrospinal fluid, HIV = human immunodeficiency virus, ISCED = International Standard Classification of Education, IQ = intelligence quotient, Mo = months, N = number, Nadir = lowest CD4+ T-cell count, VL = viral load, Y = years, Zenith = highest HIV viral load.

* *P* value <0.05.

† Most educated parent.

table of this cohort has been published previously.[18] We were able to obtain good quality spectra (SD < 20%) in 26 HIV-infected children (74%) and 36 healthy controls (97%). Four HIV-infected children were excluded from MRI scanning due to dental braces and claustrophobia. Five HIV-infected and 1 healthy participants were excluded because the acquired spectra were of insufficient quality (SD > 20%). The MRS inclusion ratio differed significantly between HIV-infected and healthy children (*P*-value = 0.003), but demographic, clinical or HIV-related characteristics did not differ between included and excluded children (data not shown). Demographic and clinical characteristics of the included children are summarized in Table 1. Children were matched for gender, age, and ethnicity. HIV-infected children had a lower IQ score (*P* value = 0.004). Also, HIV-infected children showed a slightly lower parental educational level (*P* value = 0.042) as compared to healthy controls, which may influence cognitive functioning. However, when adjusting for this factor, the IQ of HIV-infected children was still significantly lower than that of healthy controls (coefficient: −8.8; *P* value = 0.024). At time of inclusion, 22 HIV-infected children (85%) were using cART with undetectable HIV VL; of the remaining children, 3 were previously on cART and 1 was cART-naive. Seven HIV-infected children (27%) had a CDC stage C diagnosis, 3 of which had cerebral HIV involvement (i.e., 2 had a clinical diagnosis of HIV-encephalopathy and 1 cytomegalovirus encephalitis).

Neurometabolite Levels

Table 2 shows neurometabolite levels across groups. HIV-infected children had higher levels of Cho:Cre in WM (*P* value = 0.045) and a trend toward higher Cho:Cre levels in GM (*P* value = 0.080) as compared to controls. No differences were found in NAA:Cre, Glu:Cre and mI:Cre levels. To evaluate a potential distinction between metabolites associated with neuronal integrity (NAA and Glu) and those associated with glial proliferation (mI and Cho), we assessed associations between the different types of neurometabolites (Table 3). For all individual neurometabolites, GM levels correlated strongly with WM levels (*P* values <0.001). Neuronal metabolites NAA:Cre and Glu:Cre were associated within GM and WM (*P* values <0.001) and between WM and GM (*P* values <0.001). Glial metabolites mI:Cre and Cho:Cre were associated within WM only (*P* value <0.001). Furthermore, we detected several associations between WM glial metabolites and neuronal metabolites in both GM and WM. WM mI:Cre and Cho:Cre were associated with NAA:Cre in both WM (*P* values = 0.012 and 0.021, respectively) and in GM (*P* values = 0.038 and 0.032, respectively). WM mI:Cre was also

TABLE 2. Neurometabolite Levels in HIV-Infected Children and Healthy Controls

	Gray Matter			White Matter		
	HIV-infected	Healthy	*P* Value	HIV-infected	Healthy	*P* Value
NAA:Cre	1.17 (0.10)	1.17 (0.12)	0.839	1.24 (0.10)	1.26 (0.12)	0.468
Glu:Cre	1.26 (0.12)	1.25 (0.15)	0.586	1.20 (0.12)	1.20 (0.16)	0.910
mI:Cre	0.63 (0.06)	0.61 (0.05)	0.130	0.59 (0.06)	0.58 (0.05)	0.282
Cho:Cre	0.25 (0.03)	0.24 (0.03)	0.080	0.29 (0.03)	0.27 (0.03)	0.045*

Results of the age-adjusted linear regression analysis comparing neurometabolites between HIV-infected children and controls. Neurometabolites are displayed as mean (SD).

Cho = choline, Cre = creatine, Glu = glutamate, mI = *myo*-inositol, NAA = N-acetylaspartate.

* *P* value <0.05.

associated with WM Glu:Cre (*P* value = 0.020). No associations were found between GM glial metabolites and any neuronal metabolites.

Associations With HIV Parameters

In multivariable regression analyses (Table 4), lower nadir CD4+ Z-scores were associated with lower neuronal markers NAA:Cre and Glu:Cre in GM, and lower NAA:Cre in WM, whereas CDC stage C was associated with higher glial markers mI:Cre (*P* value = 0.009) and Cho:Cre in GM (*P* value = 0.017). An association was also found between longer duration of CD4 T-cell count $<500 \times 10^6$/L and lower GM mI:Cre (*P* value = 0.004). Neurometabolites were not associated with lifetime years with or without cART, nor with HIV VL or CD4+ Z-scores at time of inclusion (*P* values >0.20; data not shown).

Associations With Cognitive Functioning

Results of the linear regression analysis evaluating associations between neurometabolites and cognitive functioning are shown in Table 5. In HIV-infected children, higher Cho:Cre levels in both GM and WM were associated with poorer attention/working memory (*P* values = 0.035 and 0.003, respectively) and poorer executive functioning (*P* values = 0.012 and 0.027, respectively). GM Cho:Cre levels were also associated with poorer memory (*P* value = 0.047). In healthy children, higher WM levels of NAA:Cre and Glu:Cre were associated with better performance on executive functioning (*P* values = 0.042 and 0.008, respectively). Furthermore, higher Glu:Cre in GM was associated with better executive functioning (*P* value = 0.006), and in WM with better performance on information processing speed (*P* value = 0.013). Higher GM mI:Cre was associated with poorer visual-motor function (*P* value = 0.039).

Associations With Neuronal Damage Markers

Serum NfL levels did not differ between HIV-infected children and controls (HIV-infected: median = 7.0 [IQR = 4.8–13.0]; controls: median = 6.6 [IQR = 4.3–13.0]; *P* value = 0.57). We obtained CSF from 22 HIV-infected children (85%). Children who underwent LP had a lower plasma HIV VL at study inclusion than those who did not undergo LP (LP: plasma HIV VL = 3.20 log copies/mL; no LP: plasma HIV VL = 6.88 log copies/mL; *P* value = 0.016), but these groups did not differ in neurometabolite levels or other demographic, clinical or HIV-related characteristics (data not shown). As we only acquired CSF samples from HIV-infected children, no group differences of CSF neuronal damage markers were analyzed. Cerebral metabolite levels were not associated with neuronal damage markers in serum or CSF (Table 6).

DISCUSSION

This study evaluated the potential of MRS to detect HIV-related brain injury in children. We found that HIV-infected children had similar levels of neuronal integrity marker levels NAA:Cre and Glu:Cre, and glial marker mI:Cre, and increased levels of glial marker Cho:Cre as compared to matched healthy controls. Reduced neuronal integrity marker and increased glial marker levels were associated with a more severe disease history as assessed by nadir CD4+ Z-score and CDC stage. Furthermore, increased neuronal integrity markers were associated with better cognitive functioning in healthy controls, whereas increased Cho:Cre levels were associated with poorer cognitive functioning in HIV-infected children.

Higher levels of Cho and mI have previously been described in HIV-infected children that were stable on cART.[27] As Cho levels mainly reflect cell membrane turnover, increased Cho:Cre levels together with normal neuronal marker levels may indicate increased glial cell density. Elevations in Cho:Cre have been found in HIV-infected children with severe HIV-associated cognitive impairment,[8] but findings in children without encephalopathy have been less consistent. Elevated Cho levels in HIV-infected children suggest that glial proliferation may play a role in HIV-related cerebral injury, despite clinical stability. This glial proliferation is possibly due to glial activation in response to ongoing neuroinflammation, but the role of lifelong cART use still needs to be elucidated.

We could not detect differences in NAA:Cre levels between HIV-infected children and controls, suggesting that neuronal integrity was not strongly affected in our group of perinatally HIV-infected children that were stable on cART. Indeed, NAA:Cre levels have consistently been reported to be normal in HIV-infected children without encephalopathy,[3,9,10] whereas children with encephalopathy show decreased NAA:Cre levels.[7,8] Our study could not replicate the latter finding, likely due to the small number of children with a historical diagnosis of HIV encephalopathy.

Glu mediates most of the excitatory neurotransmission in the CNS and is considered a potent indicator of neuronal integrity.[28] The Glu peak in metabolite spectra can only accurately be distinguished from glutamine since the introduction of 3T MRI scanners, therefore reports evaluating cerebral Glu levels are scarce. Studies in HIV-infected adults detected elevated Glu:Cre before treatment initiation, followed by

TABLE 3. Associations Between Different Types of Neurometabolites

		Neuronal Markers				Glial Markers			
		Gray Matter		White Matter		Gray Matter		White Matter	
		NAA:Cre	Glu:Cre	NAA:Cre	Glu:Cre	mI:Cre	Cho:Cre	mI:Cre	Cho:Cre
Gray matter	NAA:Cre								
	Glu:Cre	0.545 (<0.001*)							
White matter	NAA:Cre	0.742 (<0.001*)	0.647 (<0.001*)						
	Glu:Cre	0.402 (<0.001*)	0.640 (<0.001*)	0.560 (<0.001*)					
Gray matter	mI:Cre	−0.123 (0.649)	0.172 (0.592)	0.045 (0.874)	−0.356 (0.314)				
	Cho:Cre	0.626 (0.231)	−0.230 (0.713)	0.241 (0.663)	0.175 (0.799)	0.101 (0.694)			
White matter	mI:Cre	0.533 (0.038*)	0.327 (0.289)	0.669 (0.012*)	0.774 (0.020*)	0.432 (<0.001*)	0.114 (0.078)		
	Cho:Cre	0.960 (0.032*)	0.275 (0.610)	1.079 (0.021*)	0.790 (0.181)	−0.090 (0.685)	0.718 (<0.001*)	0.561 (0.012*)	

Results of the linear regression analysis evaluating associations between neuronal metabolites N-acetylaspartate and glutamate, and glial metabolites *myo*-inositol and choline. Analyses were adjusted for age and HIV status and results are displayed as coefficient (*P*-value).

Cho = choline, Cre = creatine, Glu = glutamate, mI = *myo*-inositol, NAA = N-acetylaspartate.

*P value <0.05.

normalized or even decreased levels after cART initiation.[29,30] In this study we report on comparable Glu:Cre levels between perinatally HIV-infected children stable on cART and healthy children for the first time. Elevated Glu:Cre levels might be a reflection of disturbed glutamate re-uptake by activated glial cells, leading to overstimulation of neuronal N-methyl-D-aspartate receptors.[31] The resulting neuronal apoptosis may subsequently reduce Glu:Cre levels. Even though Glu has been proposed as an early marker for brain injury,[29] the exact role of Glu in HIV-related cerebral injury remains to be elucidated.

No differences were found in mI:Cre levels between cases and controls. MI is a key messenger involved in cellular signal transduction and is mainly used as a glial marker. Increased levels of mI:Cre have been related to increased gliosis resulting from chronic inflammation.[4] However, mI is not confined to glial cells and is also detected in neuronal cell lines.[32] This could be the reason that we detected unaltered mI:Cre levels in HIV-infected children, despite increased glial proliferation as suggested by increased Cho:Cre levels.

The correlation patterns between metabolites with HIV-related parameters and cognitive functioning show a limited distinction between neuronal and glial metabolites. Indeed, NAA:Cre and Glu:Cre show distinct correlation patterns as compared to mI:Cre and Cho:Cre. Moreover, we found significant correlations between metabolites within these 2 subsets of markers, consistent with a previous study in adults.[16] However, some correlations were also found between subsets, reflecting the close relation between different neurometabolites, and the difficulty of distinguishing the exact underlying (patho)physiological mechanisms.

Reduced levels of neuronal markers NAA:Cre and Glu:Cre were associated with lower nadir CD4 Z-scores, and a CDC-C diagnosis was associated with higher levels of glial markers mI:Cre and Cho:Cre. Both nadir CD4+ Z-score and CDC stage are variables that indicate disease severity before cART, indicating that accrued cerebral damage as a result of severe HIV disease may be persistently detected in perinatally HIV-infected children even after years on potent cART.[33] Previously, lower mI levels were reported in HIV-infected children that had started treatment early as compared to those that started treatment at an older age,[3] but our study did not replicate this specific association between mI:Cre and age at cART initiation. No associations were found between metabolite levels and HIV disease and treatment characteristics at study inclusion, in line with previous evidence.[7,8,13]

In our cohort of HIV-infected children, higher WM Cho:Cre levels correlated with poorer results in 3 cognitive domains (i.e. attention/working memory, memory and executive functioning), suggesting that increased glial activation in both GM and WM may contribute to poorer cognitive functioning.[34] Glial cells regulate the neuronal environment, and can greatly affect neuronal activity.[35] In HIV-infected patients, glial cells release inflammatory cytokines and chemokines, factors that often show stronger associations with cognitive performance than disease severity markers.[36,37] In agreement with this association between Cho:Cre and cognition, a previous study in our cohort detected increased WM diffusivity in HIV-infected children, which was associated with poorer working memory,[19] and might also be due to increased glial activation. In healthy participants, poorer performance on processing speed and executive functioning were related to lower NAA:Cre and Glu:Cre levels NAA enhances neuronal mitochondrial energy production from Glu, making it a marker for neuronal health.[38] Glu is an excitatroy neurotransmitter. Therefore, lower levels of

TABLE 4. Associations Between Neurometabolites and HIV Characteristics

			Gray Matter							
			NAA:Cre		Glu:Cre		mI:Cre		Cho:Cre	
		N	Univariable Analysis	Multivariable Analysis	Univariable Analysis	Multivariable Analysis	Univariable Analysis	Multivariable Analysis	Univariable Analysis	Multivariable Analysis
Nadir CD4+ T-cell Z-score		24	0.0905 (<0.001)	0.0855 (0.001*)	0.0977 (0.010)	0.0869 (0.025*)	−0.0014 (0.946)	–	−0.0096 (0.343)	–
Zenith HIV VL (log copies/mL)		24	−0.0525 (0.154)	−0.0233 (0.430)	−0.0933 (0.063)	−0.0283 (0.575)	0.0281 (0.270)	–	0.0204 (0.105)	0.0051 (0.684)
Duration detectable VL ($\sqrt{y}$)		26	−0.0145 (0.618)	–	−0.0150 (0.682)	–	−0.0009 (0.958)	–	0.0056 (0.532)	–
Duration $CD4^+$ T-cell count $<500\times10^6$/L (mo)		26	−0.0003 (0.434)	–	−0.0003 (0.634)	–	−0.0007 (0.004)	−0.0007 (0.004*)	$4.00^{*}10^{-5}$ (0.767)	–
CDC stage	N/A	9	–	–	–	–	–	–	–	–
	B	10	−0.0106 (0.816)	–	0.0223 (0.672)	0.0440 (0.396)	0.0238 (0.317)	0.0420 (0.073)	0.0058 (0.608)	0.0041 (0.749)
	C	7	−0.0444 (0.394)	–	−0.1058 (0.088)	−0.0529 (0.386)	0.0796 (0.007)	0.0707 (0.009*)	0.0426 (0.003)	0.0389 (0.017*)
Age at cART initation (y)		23	−0.0013 (0.815)	–	0.0039 (0.620)	–	−0.0078 (0.038)	−0.0025 (0.411)	−0.0022 (0.257)	–
			White Matter							
			NAA:Cre		Glu:Cre		mI:Cre		Cho:Cre	
		N	Univariable Analysis	Multivariable Analysis	Univariable Analysis	Multivariable Analysis	Univariable Analysis	Multivariable Analysis	Univariable Analysis	Multivariable Analysis
Nadir $CD4^+$ T-cell Z-score		24	0.1129 (<0.001)	0.1050 (<0.001*)	0.0954 (0.021)	0.0820 (0.052)	0.0163 (0.428)	–	0.0076 (0.495)	–
Zenith HIV VL (log copies/mL)		24	−0.0715 (0.093)	−0.0354 (0.270)	−0.1136 (0.033)	−0.0535 (0.375)	−0.0172 (0.510)	–	0.0066 (0.643)	–
Duration detectable VL ($\sqrt{yrs}$)		26	0.0157 (0.598)	–	0.0089 (0.817)	–	0.0045 (0.802)	–	0.0188 (0.050)	0.0196 (0.070)
Duration $CD4^+$ T-cell count $<500^{*}10^6$/L (mo)		26	−0.0004 (0.351)	–	0.0005 (0.388)	–	−0.0005 (0.073)	−0.0005 (0.065)	−0.0002 (0.314)	–
CDC stage	N/A	9	–	–	–	–	–	–	–	–
	B	10	−0.0061 (0.897)	0.0203 (0.714)	0.0628 (0.284)	0.0231 (0.383)	0.0337 (0.193)	−0.0013 (0.930)	0.0011 (0.944)	0.0011 (0.944)
	C	7	−0.0459 (0.392)	−0.1088 (0.095)	−0.0239 (0.724)	0.0580 (0.062)	0.0552 (0.061)	0.0321 (0.057)	0.0191 (0.245)	0.0191 (0.245)
Age at cART initation (y)		23	−0.0044 (0.514)	–	0.0112 (0.174)	0.0062 (0.448)	−0.0031 (0.440)	–	−0.0041 (0.047)	−0.0028 (0.160)

Results of the age-adjusted linear regression analysis evaluating associations between neurometabolites and HIV disease and treatment characteristics, displayed as coefficient (*P* value).

cART = combination antiretroviral therapy, CDC category = Centers for Disease Control and Prevention stage (N/A = no to minimal symptoms. B = moderate symptoms. C = severe symptoms or AIDS), Cho = choline, Cre = creatine, Glu = glutamate, mI = *myo*-inositol, Mo = months, N = number, NAA = N-acetylaspartate, Nadir = lowest CD4+ T-cell count, Y = years, Zenith = highest HIV viral load.

* *P* value <0.05 in multivariable analysis.

both NAA:Cre and Glu:Cre indicate a poorer neuronal signal transduction, which could lead to poorer cognitive functioning. In our study, these associations were more pronounced in healthy participants and may therefore not be useful to specifically detect HIV-related brain injury. Notably, associations between cognition and neuronal integrity markers were found almost exclusively in healthy participants, whereas associations between cognition and glial markers were mainly found in HIV-infected participants. This suggests that HIV-induced glial proliferation may negatively impact cognitive functioning even

TABLE 5. Associations Between Neurometabolites and Cognitive Functioning

	Gray matter							
	NAA:Cre		Glu:Cre		mI:Cre		Cho:Cre	
	HIV-infected	Healthy	HIV-infected	Healthy	HIV-infected	Healthy	HIV-infected	Healthy
Intelligence	−11.22 (0.692)	3.88 (0.851)	−2.04 (0.928)	12.65 (0.486)	22.63 (0.627)	−30.43 (0.533)	−113.9 (0.210)	−41.47 (0.661)
Information processing speed	−19.85 (0.385)	12.48 (0.436)	−2.33 (0.898)	23.33 (0.093)	19.38 (0.608)	18.17 (0.634)	−108.3 (0.140)	33.48 (0.650)
Attention/working memory	−0.075 (0.990)	4.59 (0.201)	−2.11 (0.671)	6.06 (0.052)	9.02 (0.380)	−5.14 (0.550)	−41.13 (0.035*)	14.53 (0.381)
Visual-motor function	−61.73 (0.067)	−19.51 (0.308)	−38.92 (0.149)	−7.34 (0.666)	99.64 (0.073)	−91.5 (0.039*)	−37.89 (0.739)	89.18 (0.311)
Memory	−12.53 (0.647)	15.29 (0.207)	12.97 (0.548)	0.725 (0.947)	25.83 (0.566)	−21.37 (0.462)	−169.8 (0.047*)	37.07 (0.509)
Executive functioning	31.19 (0.225)	24.18 (0.118)	30.27 (0.159)	36.24 (0.006*)	5.10 (0.911)	45.05 (0.223)	−212.8 (0.012*)	65.72 (0.361)
	White matter							
	NAA:Cre		Glu:Cre		mI:Cre		Cho:Cre	
	HIV-infected	Healthy	HIV-infected	Healthy	HIV-infected	Healthy	HIV-infected	Healthy
Intelligence	9.59 (0.727)	13.6 (0.496)	−11.80 (0.582)	15.40 (0.327)	−83.42 (0.059)	23.46 (0.613)	−135.24 (0.091)	−5.64 (0.943)
Information processing speed	−5.85 (0.793)	29.37 (0.053)	8.17 (0.639)	29.37 (0.013*)	−13.64 (0.715)	35.13 (0.328)	−117.6 (0.069)	10.51 (0.865)
Attention/working memory	−3.16 (0.604)	3.83 (0.274)	−6.11 (0.192)	3.93 (0.153)	6.52 (0.522)	5.37 (0.510)	−48.91 (0.003*)	15.21 (0.272)
Visual-motor function	−21.81 (0.518)	4.92 (0.793)	−43.76 (0.088)	17.90 (0.221)	−61.91 (0.268)	30.48 (0.481)	−70.94 (0.484)	128.2 (0.077)
Memory	5.93 (0.824)	7.53 (0.526)	2.58 (0.901)	6.76 (0.471)	−44.99 (0.307)	12.14 (0.660)	−121.8 (0.118)	22.96 (0.626)
Executive functioning	34.13 (0.198)	30.14 (0.042*)	33.51 (0.101)	30.24 (0.008*)	−32.37 (0.470)	32.47 (0.357)	−169.4 (0.027*)	24.28 (0.688)

Results of the age-adjusted linear regression analysis evaluating associations between neurometabolites and cognitive functioning, displayed as coefficient (*P*-value).

Cho = choline, Cre = creatine, Glu = glutamate, mI = *myo*-inositol, NAA = N-acetylaspartate.

* *P* value <0.05.

when neuronal integrity is not affected. This is consistent with the findings of normal NAA:Cre and Glu:Cre levels in the HIV-infected group despite poorer cognitive functioning, as well as the lack of associations between metabolite levels and neuronal damage markers.

The neurodegeneration markers examined in this study (i.e. CSF and serum NfL, CSF NfH and tTau) did not correlate with any neurometabolite levels in HIV-infected children. Serum NfL has not been previously associated with neuronal injury in the HIV-infected population, but elevations have been observed in other neurological diseases.[23] Neurodegeneration markers in CSF have previously been associated with neurocognitive impairment in adult HIV infection.[39–41] NfL, an axonal damage marker, was suggested to be the most sensitive indicator of HIV-related neuroinflammation and a predictor for neurologic disease progression.[15] Previous MRS studies in HIV-infected adults reported correlations between elevated NfL and decreased NAA:Cre and Glu:Cre.[16,17] However, these studies included patients with acute or primary HIV-infections, whose metabolite levels differ from those in chronically HIV-infected patients.[42] The lack of associations between metabolites and CSF NfL could also be explained by the significantly lower plasma HIV VL at study inclusion in children who underwent LP, which resulted in CSF data of a subgroup of HIV-infected children with potentially milder disease. Additionally, serum NfL levels were not elevated in HIV-infected children as compared to healthy controls, which may explain the lack of association with MRS cerebral metabolites. Our results imply that MRS is a more sensitive tool to detect early pediatric HIV-induced neuropathology.

To our knowledge, this is the first study that reports on cerebral metabolites in areas encompassing the whole GM and WM separately in an HIV-infected population. Using 2D CSI MRS on a 3T MRI scanner enabled us to reliably measure metabolite levels over large VOIs. However, a downside of assessing larger areas is the risk of masking regional variations. As several neurometabolites have been reported to vary across regions,[43] future studies should include both small and large regions to control for regional differences. Another limitation of our study is that we did not include deep GM in our slice. Deep

TABLE 6. Associations Between Neurometabolite Levels and Neuronal Damage Markers

			Gray matter							
			NAA:Cre		Glu:Cre		mI:Cre		Cho:Cre	
		N	HIV+	Healthy	HIV+	Healthy	HIV+	Healthy	HIV+	Healthy
CSF	tTau (pg/mL)	18	0.07 (0.897)	–	−0.06 (0.907)	–	0.27 (0.768)	–	1.51 (0.574)	–
	NfL (pg/mL)	17	197.8 (0.335)	–	151.6 (0.427)	–	−76.9 (0.823)	–	−453.4 (0.653)	–
	NfH (pg/mL)	19	0.36 (0.400)	–	0.24 (0.555)	–	−0.57 (0.441)	–	2.12 (0.317)	–
Blood	NfL (log pg/mL)	60	1.53 (0.378)	0.16 (0.850)	0.661 (0.631)	−0.43 (0.567)	3.68 (0.203)	−1.53 (0.450)	−0.12 (0.984)	4.10 (0.292)
			White Matter							
			NAA:Cre		Glu:Cre		mI:Cre		Cho:Cre	
		N	HIV-infected	Healthy	HIV-infected	Healthy	HIV-infected	Healthy	HIV-infected	Healthy
CSF	tTau (pg/mL)	18	0.07 (0.888)	–	−0.23 (0.651)	–	−1.73 (0.089)	–	−1.83 (0.394)	–
	NfL (pg/mL)	17	86.1 (0.687)	–	188.8 (0.317)	–	332.3 (0.398)	–	502.6 (0.535)	–
	NfH (pg/mL)	19	0.17 (0.675)	–	0.46 (0.257)	–	−0.33 (0.702)	–	1.58 (0.358)	–
Blood	NfL (log pg/mL)	60	1.53 (0.378)	−0.34 (0.684)	−1.79 (0.191)	−0.13 (0.841)	−3.20 (0.273)	−1.89 (0.322)	−0.01 (0.999)	2.68 (0.413)

Results of the age-adjusted linear regression analysis evaluating associations between neurometabolites and neuronal damage markers, displayed as coefficient (*P* value).

Cho = choline, Cre = creatine, CSF = cerebrospinal fluid, Glu = glutamate, mI = *myo*-inositol, NAA = N-acetylaspartate, NfL = neurofilament light, NfH = neurofilament heavy, pg/mL = picogram per milliliter, tTau = total Tau.

GM plays an important role in HIV-related cerebral injury.[34] However, this area is susceptible to artifacts (e.g. iron accumulation), impeding the acquirement of reliable metabolite spectra. Also, even though our study is among the larger MRS studies in HIV-infected children, our small sample size may have resulted in fewer detectable correlations. Moreover, we attempted to match study participants group wise, but due to the selection criteria for MRS, groups were not matched for parental education level, country of birth, age arrived in the Netherlands, adoption, and language variables. Finally, this is a cross-sectional study and therefore illustrating metabolite changes over time was impossible.

In conclusion, our cohort of perinatally HIV-infected children showed unaltered NAA:Cre, Glu:Cre, and mI:Cre levels, and increased levels of Cho:Cre as compared to healthy controls. Metabolite levels were associated with higher severity of HIV disease before cART initiation. Increased Cho:Cre levels—but not neuronal metabolite alterations—were associated with poorer cognitive functioning in HIV-infected children, implying ongoing glial proliferation despite effective virological suppression with cART. These findings suggest that Cho:Cre as measured by MRS could serve as a potential marker for assessing HIV-related cerebral injury in the pediatric population. Larger longitudinal studies are necessary to substantiate these findings and to better understand the underlying pathophysiology. This could lead towards earlier detection of pediatric HIV-related neuropathology and contribute to improvement of treatment strategies for perinatally HIV-infected children as they survive into adulthood.

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