Title

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RESEARCH ARTICLE

Alterations of Brain Metabolites in Adults With HIV

A Systematic Meta-analysis of Magnetic Resonance Spectroscopy Studies

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Abstract

Objective

A meta-analysis of proton magnetic resonance spectroscopy studies to investigate alterations in brain metabolites in people with HIV (PWH), the relationship between metabolite alterations and combination antiretroviral therapy (cART), and the relationship between metabolite alterations and cognitive impairment.

Methods

The PubMed database was searched for studies published from 1997 to 2020. Twenty-seven studies were identified, which included 1255 PWH and 633 controls. Four metabolites (N-acetyl aspartate [NAA], myo-inositol [mI], choline [Cho], and glutamatergic metabolites [Glx]) from 5 brain regions (basal ganglia [BG], frontal gray and white matter [FGM and FWM], and parietal gray and white matter [PGM and PWM]) were pooled separately using random-effects meta-analysis.

Results

During early HIV infection, metabolite alterations were largely limited to the BG, including Cho elevation, a marker of inflammation. cART led to global mI and Cho normalization (i.e., less elevations), but improvement in NAA was negligible. In chronic PWH on cART, there were consistent NAA reductions across brain regions, along with Cho and mI elevations in the FWM and BG, and Glx elevations in the FWM. Cognitive impairment was associated with NAA reduction and to a lesser degree mI elevation.

Conclusions

The BG are the primary region affected during early infection. cART is successful in partially controlling neuroinflammation (global mI and Cho normalization). However, neuronal dysfunction (NAA reductions) and neuroinflammation (mI and Cho elevations) persist and contribute to cognitive impairment in chronic PWH. Novel compounds targeting NAA signal pathways, along with better neuroinflammation control, may help to reduce cognitive impairment in PWH.

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Glossary

BG = basal ganglia; **cART** = combination antiretroviral therapy; **Cho** = choline; **FDR** = false discovery rate; **FGM** = frontal gray matter; **FWM** = frontal white matter; **Gln** = Glutamine; **Glu** = glutamate; **Glx** = glutamatergic metabolites; **HAND** = HIV-associated neurocognitive disorder; **mI** = myo-inositol; **MRS** = magnetic resonance spectroscopy; **NAA** = N-acetyl aspartate; **PGM** = parietal gray matter; **PWH** = people with HIV; **PWM** = parietal white matter.

Despite successful peripheral viral suppression with combination antiretroviral therapy (cART), the brain can be a reservoir for HIV,[1] and neurologic complications are common in people with HIV (PWH). It is estimated that HIV-associated neurocognitive disorders (HAND) may affect up to 30%–50% of PWH in the cART era.[2-4] The precise neural mechanisms underlying HIV brain disease, however, remain to be elucidated.[5]

Magnetic resonance spectroscopy (MRS) offers a noninvasive way to estimate biochemical shifts linked to pathologic processes. The most common form of MRS is 1H-MRS or proton MRS, which has been widely used to study HIV disease.[6] However, there is a lack of consensus in relating metabolite alterations to HIV disease, cART, and neurocognitive impairment. Here, we performed meta-analyses on MRS studies published in the cART era and with adults living with HIV.

This meta-analysis study had 4 aims. The first and primary aim was to assess alterations in brain metabolites in chronic PWH (compared with healthy controls) in the cART era. The second aim was to investigate metabolite alterations associated with cognitive impairment in PWH. The third aim was to assess alterations in brain metabolites in acute/early infection PWH who were cART naive (compared with controls). The fourth aim was to evaluate the effect of antiretroviral treatment in cART-naive patients (before vs after cART).

Three hypotheses were tested: (1) neuroinflammation is present during acute/early infection; (2) cART reduces neuroinflammation; and (3) mild neuronal dysfunction and neuroinflammation persist in chronic PWH in the cART era and are associated with cognitive impairment.

Methods

Literature Search

A total of 376 studies were identified through an online search of PubMed on July 17th, 2019, using the following search parameters: "((HIV [Title/Abstract] OR HIV-1 [Title/Abstract] OR HIV1 [Title/Abstract] OR HIV+[Title/Abstract] OR human immunodeficiency virus [Title/Abstract])) AND (MRS [Title/Abstract] OR MR spectroscopy [Title/Abstract] OR magnetic resonance spectroscopy [Title/Abstract])". Sixteen additional publications were identified through additional literature review and another PubMed search on February 3, 2020. Only studies that reported the means and SDs of metabolites in the article or supplementary material were included. When deciding between 2 or more publications from the same cohort, studies were favored in the following order: study with a larger sample size; study published more recently.

Selection of Metabolites and Brain Regions

The most commonly examined metabolites in neuroHIV are N-acetyl aspartate (NAA), choline (Cho), myo-Inositol (mI), creatine (Cr), and glutamate (Glu). NAA is the second most abundant amino acid in the human brain, and a reduction in NAA concentration is believed to reflect either permanent neuronal loss or reversible neuronal/axonal dysfunction.[7] mI has a higher concentration in glial cells than neurons; thus, an elevation in mI is recognized as a potential marker for inflammation and gliosis.[8] The Cho signal mainly comes from phosphorylcholine and glycerophosphorylcholine, which have a higher concentration in glial cells than neurons. The Cho level is often regarded as a membrane marker[9] as well as a potential inflammation and gliosis marker.[10] Glu is the most abundant amino acid and the dominant excitatory neurotransmitter in the human brain. Glutamine (Gln) is the main precursor for neuronal Glu and has a concentration at ~50% of Glu. Depending on the MRI field strength and MRS approach, Glu and Gln may be reported separately or in combination as glutamatergic metabolites (Glx) to assess the dysfunction of the glutamatergic system; thus, reductions may indicate hypometabolism,[11] whereas increases may represent excitotoxicity.[12] The Cr signal comes from creatine and phosphocreatine, which are generally in dynamic equilibrium and linked to energy metabolism and neuronal plasticity. Generally, the Cr signal is relatively stable over time and is often used as an internal reference to quantify other metabolites (but also see references 13 and 14).

This meta-analysis focused on the Cho, glutamate (Glu/Glx), mI, and NAA metabolites in the 5 most commonly investigated regions: basal ganglia (BG), frontal white matter (FWM), parietal white matter (PWM), medial frontal gray matter (FGM), and medial occipital/parietal gray matter (PGM). Data from other metabolites (e.g., lactate) as well as other brain regions (e.g., hippocampus) were excluded due to an insufficient number of studies. More than half of the studies did not report creatine concentration but instead used it as a denominator; thus, creatine was not included in this study. However, as HIV disease may affect the levels of creatine in the brain,[13] we conducted post hoc analyses to confirm that using subsets of studies with absolute values or metabolites/Cr ratios alone produced similar results.

Data Extraction and Data Analysis

The mean and SD were extracted from each study and used in the corresponding meta-analysis. Significant heterogeneity was found in all regions and many metabolites (Table 1), so a random-effect approach was used.[15]

The Review Manager (RevMan) software (version 5.4.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) (cochrane.org) was used to perform statistical analyses, create forest plots, and conduct sensitivity analyses. Moderator analyses and publication bias analyses were conducted using the Excel package Meta-Essentials (erim.eur.nl/research-support/meta-essentials/),[16] which uses a weighted variance method that is slightly different from the RevMan. We verified that both software packages produced equivalent results that led to the same conclusions. Correction for multiple comparisons was performed using Benjamini and Hochberg false discovery rate (FDR) correction as implemented by the online calculator (sdmproject.com/utilities/?show=FDR). Publication bias was examined using the Egger test and funnel plot.[17] The presence of between-study heterogeneity was tested using the Cochran Q statistic, and its magnitude was estimated using the I^2 statistic.[18]

The robustness of findings was first investigated using the standard leave-one-study-out sensitivity analysis, in which each study was excluded once from each meta-analysis. In addition, as several research groups had 2 or more of their publications (although with different subject groups) that were included in the same analysis, we conducted an additional sensitivity analysis to examine whether some of the results were biased by these overrepresented research groups. In this additional leave-one-team-out sensitivity analysis, we excluded all publications from each team once.

Qualitative Analysis of the Relationship Between Metabolite Alterations and Cognitive Impairment in PWH

In addition to the quantitative analysis, we conducted a qualitative data analysis to further evaluate the associations between metabolite alterations and cognitive impairment in PWH. In this analysis, we first identified studies that investigated the effect of cognitive impairment in PWH on brain metabolites (including studies that were excluded from the quantitative meta-analysis due to a lack of data to calculate effect size); then for each metabolite, we summarized the number of studies that examined this metabolite (at any region) and the number of studies that identified a significant effect of cognitive impairment on the metabolite levels (at 1 or more brain regions). A total of 17 studies and 1,585 PWH were included in this qualitative analysis (vs 6 studies and 358 PWH in the quantitative meta-analysis).

Results

Additional data are available from Dryad (Figures e1 to e7, Tables e-1 to e-8, and e-references, doi.org/10.5061/dryad.2280gb5rq).

Study Selection

The literature search identified 27 studies that were included in this study, with a total of 1255 PWH and 633 controls (Figure 1).

Twenty-two studies examined the difference between chronic PWH and controls. Six studies examined the difference between cognitively impaired PWH vs cognitively normal PWH. Four studies examined the difference between patients with acute/early HIV infection and controls. Nine studies examined the effect of antiretroviral treatment in cART-naive patients. The MRS protocols for the studies included in the quantitative meta-analyses are presented in Table e-1 (doi.org/10.5061/dryad.2280gb5rq). The demographic and clinical data are presented in Tables e-2 to e-5. A metabolite in a brain region was examined if the data were available from at least 3 studies.

In addition, 17 studies (including 11 additional studies that were not included in the primary quantitative meta-analysis) were reviewed and entered into a qualitative analysis to further evaluate the association between metabolite alterations and cognitive impairment in PWH (Table e-6).

Primary Meta-Analysis: Alterations of Metabolites at Individual Regions

Compared with healthy controls, chronic PWH had the following (Table 1, Figures 2 and 3, Figure e-1, doi.org/10.5061/dryad.2280gb5rq):

i. Lower NAA levels in the FGM (Hedges g = −0.42, 95% CI: −0.64 to −0.20, $p_{uncorrected} = 0.0002$, $p_{FDR} = 0.002$), PGM (g = −0.30, 95% CI: −0.49 to −0.10, $p_{uncorrected} = 0.003$, $p_{FDR} = 0.019$), and PWM (g = −0.50, 95% CI: −0.71 to −0.30, $p_{uncorrected} < 0.00001$, $p_{FDR} < 0.00001$);
ii. Higher Cho levels in the FWM (g = 0.20, 95% CI: 0.06 to 0.35, $p_{uncorrected} = 0.005$, $p_{FDR} = 0.024$) and BG (g = 0.18, 95% CI: 0.02 to 0.33, $p_{uncorrected} = 0.020$, $p_{FDR} = 0.063$);
iii. Marginally higher mI levels in the BG (g = 0.22, 95% CI: 0.02 to 0.42, $p_{uncorrected} = 0.030$, $p_{FDR} = 0.081$) and FWM (g = 0.18, 95% CI: 0.00 to 0.37, $p_{uncorrected} = 0.050$, $p_{FDR} = 0.119$);
iv. Marginally higher Glu/Glx levels in the FWM (g = 0.25, 95% CI: 0.05 to 0.46, $p_{uncorrected} = 0.020$, $p_{FDR} = 0.063$).

Compared with cognitively normal PWH, cognitively impaired PWH had the following (Fig. 3A, Figure e-2):

i. Marginally higher mI levels in the FWM (g = 0.37, 95% CI: 0.01 to 0.74, $p_{uncorrected} = 0.040$, $p_{FDR} = 0.330$) and BG (g = 0.35, 95% CI: −0.03 to 0.73, $p_{uncorrected} = 0.070$, $p_{FDR} = 0.330$);
ii. A weak and nonsignificant trend with lower NAA levels in the PGM (g = −0.32, 95% CI: −0.69 to 0.05, $p_{uncorrected} = 0.090$, $p_{FDR} = 0.330$).

However, none of the metabolite alterations at any region reached significance after correction for multiple comparisons.

Table 1 Primary Meta-analysis Result Summary at Each Individual Brain Region

					Effect size			Heterogeneity	
	Brain region by metabolite	Studies	Cases	Controls	95% CI	*p* Value	P-FDR	I^2, %	*p* Value
Chronic PWH vs controls									
BG	NAA	17	766	441	−0.11 (−0.26 to 0.03)	0.130	0.242	24.00	0.180
	Cho	17	766	441	0.18 (0.02 to 0.33)	**0.020**	0.063	30.00	0.120
	mI	15	701	401	0.22 (0.02 to 0.42)	**0.030**	0.081	55.00	0.006
	Glx	7	230	146	0.06 (−0.16 to 0.28)	0.590	0.659	0.00	0.550
FWM	NAA	16	702	478	−0.14 (−0.31 to 0.04)	0.140	0.242	49.00	0.020
	Cho	16	702	478	0.20 (0.06 to 0.35)	**0.005**	**0.024**	23.00	0.200
	mI	15	682	448	0.18 (−0.00 to 0.37)	**0.050**	0.119	48.00	0.020
	Glx	8	337	209	0.25 (0.05 to 0.46)	**0.020**	0.063	20.00	0.270
PWM	NAA	6	212	179	−0.50 (−0.71 to −0.30)	**< 0.00001**	**<0.00001**	0.00	0.900
	Cho	6	212	179	−0.00 (−0.21 to 0.20)	0.960	0.960	0.00	0.610
	mI	4	105	86	0.19 (−0.11 to 0.49)	0.210	0.285	0.00	0.480
FGM	NAA	10	355	285	−0.42 (−0.64 to −0.20)	**0.0002**	**0.002**	35.00	0.120
	Cho	10	355	285	−0.10 (−0.26 to 0.06)	0.200	0.285	0.00	0.640
	mI	10	357	285	−0.02 (−0.27 to 0.23)	0.870	0.918	50.00	0.030
	Glx	4	127	90	−0.40 (−0.91 to 0.11)	0.120	0.242	62.00	0.050
PGM	NAA	12	529	309	−0.30 (−0.49 to −0.10)	**0.003**	**0.019**	40.00	0.080
	Cho	12	529	309	0.10 (−0.13 to 0.33)	0.390	0.463	58.00	0.006
	mI	12	529	309	0.10 (−0.10 to 0.29)	0.330	0.418	42.00	0.060
	Glx	6	285	168	−0.13 (−0.32 to 0.07)	0.200	0.285	0.00	0.930
CI PWH vs CN PWH									
BG	NAA	6	205	153	−0.11 (−0.35 to 0.14)	0.400	0.562	13.00	0.330
	Cho	6	205	153	−0.26 (−0.96 to 0.43)	0.460	0.562	88.00	<0.00001
	mI	4	166	97	0.35 (−0.03 to 0.73)	0.070	0.330	49.00	0.120
	Glx	3	105	58	0.18 (−0.21 to 0.57)	0.360	0.562	21.00	0.280
FWM	NAA	4	153	128	−0.35 (−0.81 to 0.11)	0.140	0.385	65.00	0.030
	Cho	4	153	128	0.05 (−0.24 to 0.35)	0.720	0.792	20.00	0.290
	mI	4	153	128	0.37 (0.01 to 0.74)	**0.040**	0.330	45.00	0.140
	Glx	3	92	89	−0.38 (−1.07 to 0.30)	0.270	0.562	74.00	0.020
PGM	NAA	3	80	97	−0.32 (−0.69 to 0.05)	0.090	0.330	16.00	0.300
	Cho	3	80	97	0.27 (−0.44 to 0.97)	0.460	0.562	73.00	0.030
	mI	3	80	97	−0.02 (−0.34 to 0.30)	0.910	0.910	0.00	0.400
Early infection PWH vs controls									
BG	NAA	3	120	62	0.35 (0.04 to 0.67)	**0.030**	0.120	0.00	0.390
	Cho	3	120	62	0.63 (0.21 to 1.06)	**0.004**	**0.048**	42.00	0.180
	mI	3	120	62	0.07 (−0.31 to 0.44)	0.730	0.740	30.00	0.240
	Glx	3	120	62	0.37 (0.06 to 0.69)	**0.020**	0.120	0.00	0.950

Continued

Table 1 Primary Meta-analysis Result Summary at Each Individual Brain Region *(continued)*

					Effect size			Heterogeneity	
	Brain region by metabolite	Studies	Cases	Controls	95% CI	*p* Value	P-FDR	I^2, %	*p* Value
FWM	NAA	3	97	62	−0.17 (−0.68 to 0.34)	0.510	0.733	53.00	0.120
	Cho	3	97	62	0.12 (−0.50 to 0.74)	0.710	0.740	68.00	0.040
	mI	3	97	62	0.11 (−0.53 to 0.75)	0.740	0.740	70.00	0.030
	Glx	3	97	62	−0.12 (−0.45 to 0.20)	0.450	0.733	0.00	0.430
FGM	NAA	3	97	62	−0.57 (−1.24 to 0.11)	0.100	0.240	71.00	0.030
	Cho	3	97	62	−0.22 (−0.81 to 0.37)	0.460	0.733	65.00	0.060
	mI	3	97	62	−0.27 (−1.15 to 0.61)	0.550	0.733	84.00	0.002
	Glx	3	97	62	−0.57 (−1.23 to 0.08)	0.090	0.240	69.00	0.040
After cART vs before cART									
BG	NAA	8	183	194	0.07 (−0.25 to 0.38)	0.690	0.863	51.00	0.050
	Cho	8	183	194	−0.37 (−0.68 to −0.07)	**0.020**	0.075	45.00	0.080
	mI	4	106	108	−0.17 (−0.45 to 0.11)	0.240	0.600	7.00	0.360
FWM	NAA	9	192	211	−0.03 (−0.22 to 0.17)	0.790	0.912	0.00	0.710
	Cho	9	192	211	−0.10 (−0.29 to 0.10)	0.330	0.619	0.00	0.810
	mI	9	191	211	−0.29 (−0.49 to −0.10)	**0.004**	**0.020**	0.00	0.520
	Glx	3	90	103	−0.01 (−0.30 to 0.27)	0.930	0.930	0.00	0.880
FGM	NAA	8	184	194	0.19 (−0.01 to 0.39)	0.070	0.210	0.00	0.450
	Cho	8	184	194	0.11 (−0.09 to 0.31)	0.290	0.619	0.00	0.770
	mI	8	182	192	0.01 (−0.19 to 0.21)	0.930	0.930	0.00	0.820
	Glx	3	92	103	−0.12 (−0.41 to 0.16)	0.390	0.650	0.00	0.530
PGM	NAA	3	90	103	0.11 (−0.20 to 0.42)	0.480	0.720	12.00	0.320
	Cho	3	90	103	−0.50 (−0.82 to −0.17)	**0.003**	**0.020**	16.00	0.300
	mI	3	90	103	−0.49 (−0.78 to −0.20)	**0.0009**	**0.014**	0.00	0.510
	Glx	3	90	103	0.67 (−2.03 to 3.36)	0.630	0.859	98.00	<0.00001

Abbreviations: Brain regions: BG = basal ganglia; FGM = medial frontal gray matter; FWM = frontal white matter; PGM = medial parietal gray matter; PWM = parietal white matter. Metabolites: Cho = choline; Glx = glutamate (Glu) or a combination of Glu and glutamine (Gln); mI = myo-Inositol; NAA = N-acetyl aspartate. Cognitive status: CI = cognitively impaired; CN = cognitively normal. Significance in effect size: P = uncorrected *p* values; P-FDR = *p* values after FDR correction for multiple comparison; *p* values less than 0.05 are shown in bold font. Cases vs controls: chronic or early infection PWH vs controls; cognitively impaired PWH vs cognitively normal PWH; after vs before cART.

Compared with healthy controls, acute/early infection and cART-naive PWH had the following (Fig. 3B, Figure e-3):

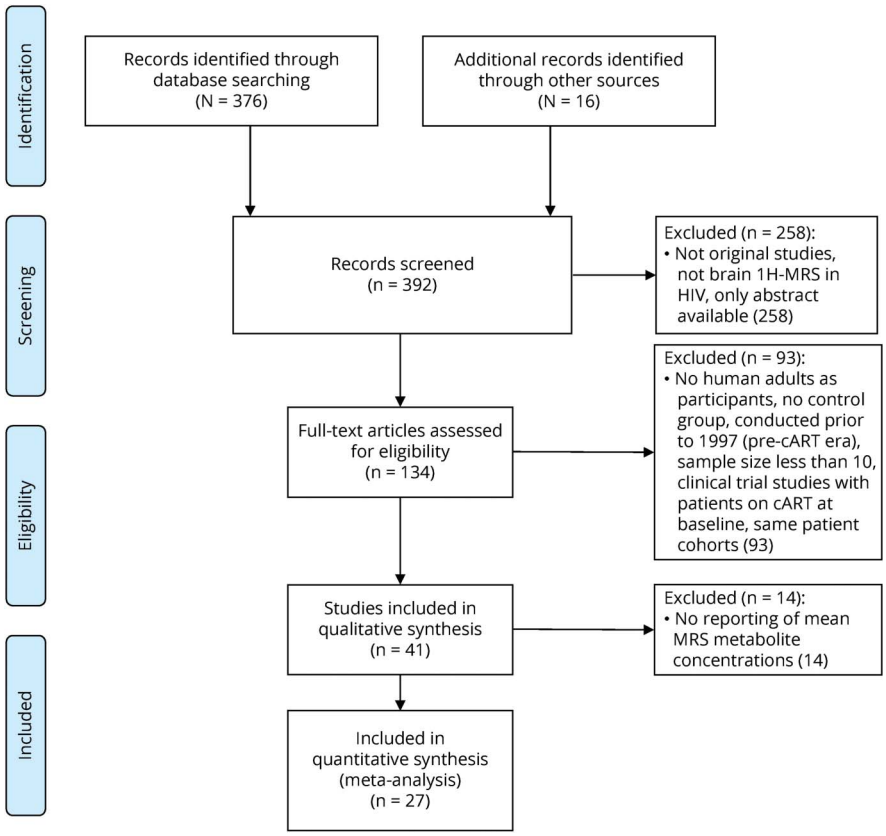
i. Higher Cho levels in the BG (g = 0.63, 95% CI: 0.21 to 1.06, $p_{uncorrected}$ = 0.004, p_{FDR} = 0.048);
ii. Marginally higher NAA in the BG (g = 0.35, 95% CI: 0.04 to 0.67, $p_{uncorrected}$ = 0.030, p_{FDR} = 0.120);
iii. Marginally higher Glu/Glx levels in the BG (g = 0.37, 95% CI: 0.06 to 0.69, $p_{uncorrected}$ = 0.020, p_{FDR} = 0.120).

The marginally higher NAA in the BG was unexpected and should be taken with caution as the effect size was small and did not survive correction for multiple comparison. Nevertheless, one of the studies[19] reported an initial NAA elevation in the BG at the baseline visit, which was followed by an NAA reduction after 24 weeks on cART. Of interest, this pattern (an initial NAA elevation) was also found in animal MRS studies[20,21] (but also see reference 22). This unexpected and puzzling finding warrants future longitudinal studies with both human and nonhuman participants with acute infection.

In cART-naive PWH, ~6–12 months on cART led to the following (Fig. 3C, Figure e-4):

i. A reduction in mI in the FWM (g = −0.29, 95% CI: −0.49 to −0.10, $p_{uncorrected}$ = 0.004, p_{FDR} = 0.020) and PGM (g =

Figure 1 PRISMA Flow Diagram of Literature Search and Study Selection



Studies were selected for the meta-analyses of 4 comparisons: (1) chronic PWH vs controls; (2) cognitively impaired PWH vs cognitively normal PWH; (3) acute/early HIV infection patients vs controls; and (4) the effect of antiretroviral treatment in cART-naive patients (pre- vs post-cART initiation). For the comparison of chronic PWH vs controls, studies were selected after reviewing the methods section of each publication to ensure that the PWH groups were not patients with acute/early infection, except 1 study,[19] which included patients with a disease duration of approximately 6 months. However, excluding this particular study[19] did not change the conclusion of chronic PWH vs controls meta-analysis (see sensitivity analysis). cART = combination antiretroviral therapy; PWH = people with HIV.

−0.49, 95% CI: −0.78 to −0.20, $p_{uncorrected} = 0.0009$, $p_{FDR} = 0.014$);

ii. A reduction in Cho in the PGM (g = −0.50, 95% CI: −0.82 to −0.17, $p_{uncorrected} = 0.003$, $p_{FDR} = 0.020$) and a weak trend in the BG (g = −0.37, 95% CI: −0.68 to −0.07, $p_{uncorrected} = 0.020$, $p_{FDR} = 0.075$).

Secondary Meta-Analysis: Alterations of Metabolites in the Brain

In addition to the primary analysis that examined metabolite alterations at each brain region separately, we conducted a secondary analysis, in which the data from each region were treated as an independent study (Table 2, Figure e-5, doi.org/10.5061/dryad.2280gb5rq).[23] The purpose of this secondary analysis was to estimate global metabolite alterations. As the metabolites from different regions within 1 study are not independent from each other, this secondary analysis was supplemented by a tertiary analysis, in which the data of each metabolite were first averaged across regions within each study before being entered into the meta-analysis.[23] Similar results were obtained in the tertiary analysis (Table e-7 and Figure e-6).

Compared with healthy controls, chronic PWH had the following:

i. Lower NAA levels (g = −0.24, 95% CI: −0.33 to −0.16, $p_{uncorrected} < 0.00001$, $p_{FDR} < 0.0001$);

ii. Higher mI levels (g = 0.14, 95% CI: 0.04 to 0.24, $p_{uncorrected} = 0.004$, $p_{FDR} = 0.008$);

iii. Higher Cho levels (g = 0.11, 95% CI: 0.03 to 0.20, $p_{uncorrected} = 0.006$, $p_{FDR} = 0.008$).

Compared with cognitively normal PWH, cognitively impaired PWH had the following:

i. Lower NAA levels (g = −0.27, 95% CI: −0.46 to −0.08, $p_{uncorrected} = 0.005$, $p_{FDR} = 0.020$);

ii. Marginally higher mI levels (g = 0.20, 95% CI: 0.00 to 0.41, $p_{uncorrected} = 0.050$, $p_{FDR} = 0.100$)

Compared with healthy controls, acute/early infection and cART-naive PWH had the following:

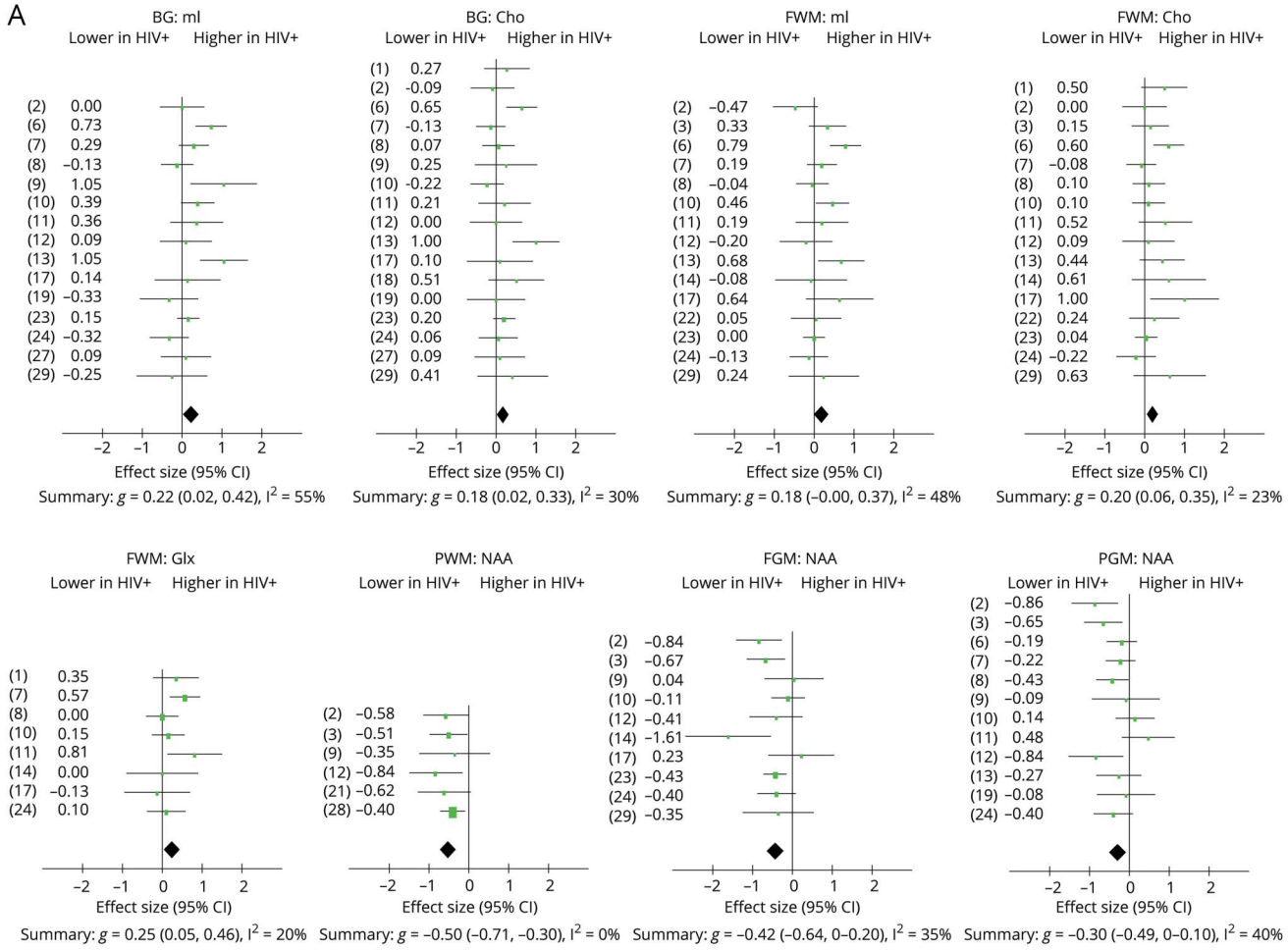
i. No significant alteration for any metabolite, suggesting metabolite alterations during acute/early infection may be largely limited to the BG, but also see reference 24.

In cART-naive PWH, ~6–12 months on cART led to the following:

i. A reduction in mI (g = −0.21, 95% CI: −0.33 to −0.09, $p_{uncorrected} = 0.0007$, $p_{FDR} = 0.003$);

ii. A reduction in Cho (g = −0.18, 95% CI: −0.31 to −0.04, $p_{uncorrected} = 0.010$, $p_{FDR} = 0.02$).

Figure 2 Study Effect Sizes of Chronic PWH vs Healthy Controls in the Primary Meta-Analysis



B

(1) Bairwa et al., 2016e-5
(2) Bladowska et al., 2013e-6
(3) Boban et al., 2017e-7
(4) Chang et al., 1999e-29
(5) Chang et al., 2002e-28
(6) Chang et al., 2004e-8
(7) Cysique et al., 2018 BLe-9
(8) Cysique et al., 2018 FUe-9
(9) Ernst et al., 2000e-10
(10) Ernst et al., 2010e-11
(11) Kallianpur et al., 2013e-12
(12) Kołtowska et al., 2010e-13
(13) Lee et al., 2003e-14
(14) Lentz et al., 2009e-15
(15) Mohamed et al., 2010e-26
(16) Mora-Peris et al., 2018e-30
(17) Pulliam et al., 2011e-16
(18) Roc et al., 2007e-17
(19) Sailasuta et al., 2012e-18
(20) Sailasuta et al., 2016e-27
(21) Salvan et al., 1997e-19
(22) Tarasow et al., 2004e-20
(23) Taylor et al., 2007e-21
(24) Valcour et al., 2015e-22
(25) Valcour et al., 2015 cARTe-22
(26) Valcour et al., 2015 cART+e-22
(27) VonGiesen et al., 2001e-23
(28) Wilkinson et al., 1997e-24
(29) Winston et al., 2009e-25
(30) Winston et al., 2010 Arm 1e-31
(31) Winston et al., 2010 Arm 2e-31
(32) Winston et al., 2010 Arm 3e-31

(A) Chronic PWH vs healthy controls. (B) The list of studies included in the primary meta-analysis. The results that reached $p \leq 0.05$ (uncorrected) are listed here, and the study effect sizes of all metabolites and all regions from the RevMan 5 software (cochrane.org) are presented in figure e-1. The significance of difference is presented in Table 1. Note: (#) ## in (A) represents Hedges g (##) from the study (#) listed in (B); the 32 studies were extracted from 27 publications. BG = basal ganglia; FWM = frontal white matter; PWH = people with HIV; PWM = parietal white matter; FGM = frontal gray matter; PGM = parietal gray matter.

Qualitative Analysis of the Association Between Metabolite Alterations and Cognitive Impairment in PWH

The additional qualitative data analysis provided additional support for an important role of NAA reduction in regard to cognitive impairment in PWH (Figure 4).

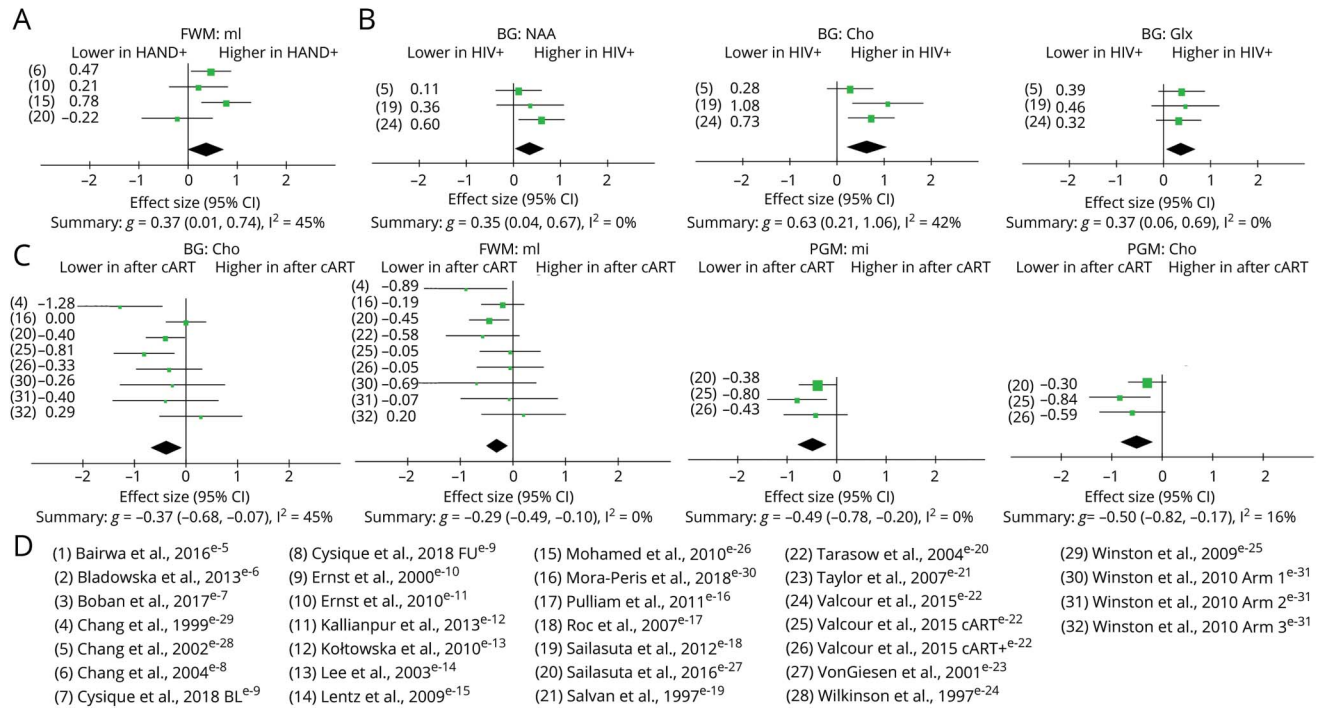
Alterations in Metabolites: From Controls to Chronic PWH to Cognitively Impaired Chronic PWH

To illustrate the alterations in metabolites related to HIV disease and cognitive impairment, we created a waterfall plot using the effect sizes from 2 secondary analyses: chronic PWH vs controls and cognitively impaired vs cognitively normal PWH. As shown in Figure 5, the data suggested a continuous decrease in the NAA levels and increase in the mI levels from controls to PWH and then from cognitively normal PWH to cognitively impaired PWH.

Moderator Analysis

We investigated the association between current CD4 and metabolite alterations in chronic PWH (compared with controls). There was not a sufficient number of studies for other comparisons.[25] Overall, higher current $CD4^+$ counts tended to

Figure 3 Study Effect Sizes of Other Comparisons in the Primary Meta-Analysis



(A) Cognitively impaired PWH vs cognitively normal PWH. (B) Patients with acute/early HIV infection vs healthy controls. (C) After vs before cART in cART-naive PWH. (D) The list of studies included in the primary meta-analysis (also see figure 1B). The results that reached $p \leq 0.05$ (uncorrected) are listed here, and the study effect sizes of all metabolites and all regions from the RevMan 5 software (cochrane.org) are presented in figures e-2, e-3, and e-4. The significance of difference is presented in Table 1. Note: (#) ## in (A) to (C) represents Hedges g (##) from the study (#) listed in (D). BG = basal ganglia; CI = cognitively impaired; CN = cognitively normal; FGM = frontal gray matter; FWM = frontal white matter; PGM = parietal gray matter; PWH = people with HIV; PWM = parietal white matter.

be associated with a smaller effect size for Cho elevations in PWH.

There was no association between effect size of any metabolites at any brain region and mean age, sex, or voxel size, except Cho at PGM, which did not differ between chronic PWH and controls.

Heterogeneity, Small Study Publication Bias, and Sensitivity Analysis

Publication bias was only identified in 2 comparisons: FWM Cho in chronic PWH vs healthy controls and PGM mI in cognitively impaired PWH vs cognitively normal PWH (Figure e-7).

Both the leave-one-study-out and the leave-one-team-out sensitivity analyses found that the significant results were generally robust, and there was no change in conclusions of significance (after correction for multiple comparisons) (Table e-8, doi.org/10.5061/dryad.2280gb5rq).

Discussion

In this systematic review and meta-analysis study, we reviewed HIV MRS studies published after 1997 and included 27 published works into quantitative data analyses. We found that metabolite alterations during acute/early infection might be largely limited to the BG, including an elevation in Cho. For cART-naive patients, approximately 6–12 months on cART led to a reduction in mI and Cho, indicating that cART was successful in reducing neuroinflammation. However, mild neuroinflammation (elevations in Cho and to a lesser degree, mI) persisted, and neuronal dysfunction (NAA reductions) was evident in chronic PWH, and both were associated with cognitive impairment. In addition, there was a trend for an elevation in Glx in the FWM in chronic PWH.

NAA is located exclusively in neurons and their processes; a decrease in NAA is detected in many diseases characterized by neuronal damage, such as stroke and dementia,[11,26] and by axonal injury, such as multiple sclerosis.[27] We found that in the cART era, the NAA across brain regions was consistently lower in chronic PWH than healthy controls, indicating widespread neuronal injury. Many factors may contribute to this reduction.[28,29] First, persisting or even irreversible neuronal injury from the past (i.e., a history of severe immunosuppression before ARV treatment) may result in a significant decrease in NAA concentrations that cannot be completely restored with cART.[19] This is consistent with the positive association between NAA levels and CD4 nadir in chronic PWH who are on cART and have largely successful viral suppression.[30-32] Second, progressive or transient neuronal/axonal injury may contribute to additional NAA reduction,

Table 2 Secondary Meta-Analysis Result Summary (Regardless of Brain Regions)

					Effect size			Heterogeneity	
	Metabolite	Studies	Cases	Controls	95% CI	*p* Value	P-FDR	I^2, %	*p* Value
Chronic PWH vs controls	NAA	61	2,564	1,692	−0.24 (−0.33 to −0.16)	**<0.00001**	**<0.00001**	41.00	0.001
	Cho	61	2,564	1,692	0.11 (0.03 to 0.20)	**0.006**	**0.008**	33.00	0.008
	mI	56	2,374	1,529	0.14 (0.04 to 0.24)	**0.004**	**0.008**	48.00	<0.0001
	Glx	25	979	613	0.01 (−0.13 to 0.14)	0.920	0.920	37.00	0.030
CI PWH vs CN PWH	NAA	15	465	446	−0.27 (−0.46 to −0.08)	**0.005**	**0.020**	36.00	0.080
	Cho	15	465	446	−0.07 (−0.38 to 0.23)	0.630	0.630	76.00	<0.00001
	mI	13	426	390	0.20 (−0.00 to 0.41)	**0.050**	0.100	43.00	0.050
	Glx	8	224	215	−0.17 (−0.50 to 0.17)	0.330	0.440	58.00	0.020
Early infection PWH vs controls	NAA	9	314	186	−0.11 (−0.46 to 0.23)	0.520	0.910	68.00	0.001
	Cho	9	314	186	0.20 (−0.15 to 0.54)	0.270	0.910	69.00	0.001
	mI	9	314	186	−0.02 (−0.36 to 0.32)	0.910	0.910	68.00	0.002
	Glx	9	314	186	−0.06 (−0.37 to 0.24)	0.690	0.910	60.00	0.010
After vs before cART	NAA	28	649	702	0.06 (−0.06 to 0.18)	0.330	0.440	16.00	0.230
	Cho	28	649	702	−0.18 (−0.31 to −0.04)	**0.010**	**0.020**	30.00	0.070
	mI	24	569	614	−0.21 (−0.33 to −0.09)	**0.0007**	**0.003**	5.00	0.390
	Glx	9	272	309	0.16 (−0.57 to 0.88)	0.670	0.670	94.00	<0.00001

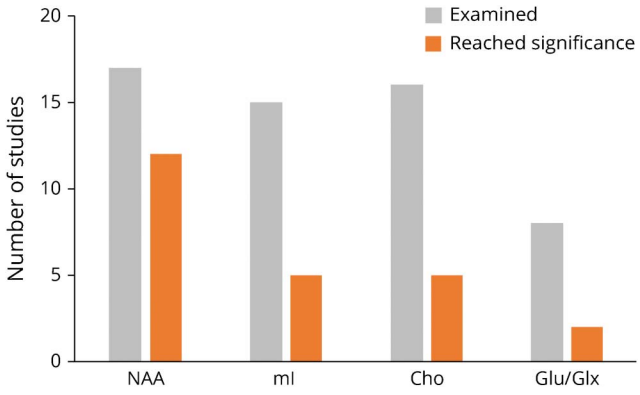
Abbreviations: cART = combination antiretroviral therapy; PWH = people with HIV. Metabolites: Cho = choline; Glx = glutamate (Glu) or a combination of Glu and glutamine (Gln); mI = myo-Inositol; NAA = N-acetyl aspartate. Cognitive status: CI = cognitively impaired; CN = cognitively normal. Significance in effect size: P = uncorrected *p* values; *p*-FDR = *p* values after FDR correction for multiple comparison. Cases vs controls: chronic or early infection PWH vs controls; cognitively impaired PWH vs cognitively normal PWH; after vs before cART.

including ongoing neuroinflammation (such as correlations with CSF/plasma inflammation markers[13,33]), current immunosuppression,[34] viral load,[35] and common comorbidities in PWH (e.g., substance abuse).[35] Progressive decline in NAA concentrations is also evident in longitudinal HIV studies with human[36] and nonhuman subjects.[21]

In both quantitative and qualitative data analyses, we found that worse cognitive function was associated with lower NAA, suggesting that as in other neurodegenerative disorders,[11] NAA reductions or the mechanisms behind NAA reductions may play an important role in HAND. This association could come from past and/or ongoing neuronal injury. For instance, the NAA levels in cART-naive PWH predict future cognitive impairment even after they receive cART.[37] We found that the recovery of NAA after starting cART was largely negligible, suggesting the inefficiency of current ARV to completely restore neuronal function, as evidenced by the persistent NAA reductions in PWH on cART,[19,37] as well as the association between low $CD4^+$ nadir counts and other types of neural injury (including cortical thinning[38]). Together, these findings support the need for novel compounds specifically targeting the NAA signal pathways, which—unfortunately—are still poorly understood[7] but may be related to neuronal mitochondria[28] that are affected in HIV.[39] A recent postmortem study found that HIV disease exacerbates age-associated mitochondrial DNA damage, which correlates cognitive performance in PWH,[40] providing direct evidence supporting a link between cognitive impairment and mitochondrial injury in PWH. In MRS, lactate levels—a marker of mitochondrial dysfunction[41]—may help to test this hypothesis. Indeed, it has been shown that lactate concentrations were higher in PWH than controls,[42,43] and the elevations increase with more severe cognitive impairment in PWH.[43] However, there are few MRS studies of lactate in HIV, and many of these focus solely on progressive multifocal leukoencephalopathy. This is probably due to the low concentration of lactate (<1 mM) in the normal brain and the strong overlap with the lipids that in general require special sequences to be effectively suppressed.[44] With recent developments in MRI (including 7 T human scanners) and MRS techniques,[45] it is of great interest to measure lactate concentrations in future HIV MRS studies.

mI is considered to be a marker of glial cells sensitive to anti-inflammatory treatments, based on comprehensive NMR studies in cell culture.[8] We found a decrease in mI across brain regions after cART, especially in the FWM

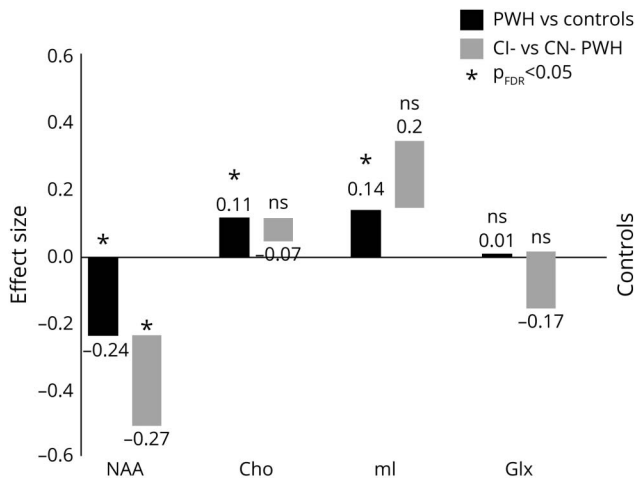
Figure 4 Qualitative Analysis of the Association Between Alterations in Metabolites and Cognitive Impairment in PWH



A total of 17 studies were selected, including 1,585 PWH (723 cognitively impaired, 755 cognitively normal, and 107 unknown). The cognitive status was unknown in 2 studies, which conducted a correlation analysis between cognitive performance and metabolites. Gray bar represents the number of studies that examined the association between cognitive impairment/performance and metabolites at least in 1 brain region; red bar represents the number of studies that identified a significant association between cognitive impairment/performance and metabolite concentrations in at least 1 brain region. Cognitive impairment is always associated with lower NAA (100% of studies) and Glx (100% of studies) levels and more likely with higher mI (70% of studies) and Cho (57% of studies) levels. See Table e-6 (doi.org/10.5061/dryad.2280gb5rq) for a detailed list of the 17 studies. Glx = glutamatergic metabolites; NAA = N-acetyl aspartate; PWH = people with HIV.

and PGM, indicating that cART successfully reduced neuroinflammation.[46] However, we found that the mI levels in the BG and FWM were marginally higher in chronic PWH than controls, suggesting that mild neuroinflammation might persist in these chronic patients in the cART era. In addition, the mI levels were marginally higher in the BG and FWM in cognitively impaired PWH than cognitively normal PWH. This association suggests that worsening neuroinflammation might contribute to cognitive impairment in chronic PWH[47] and is a potential target for HAND treatment,[48-50] for example, enhancing cART.[51]

Figure 5 Waterfall of Effect Sizes of Alterations in Metabolites



This is for illustration purposes only, and 0 represents controls. Black bar represents the effect size of metabolite alterations from controls to chronic PWH; gray bar represents the effect size of metabolite alterations from cognitively normal PWH to cognitively impaired PWH. "*" represents significant changes (after correction for multiple comparisons); "ns" represents nonsignificant changes. See Table 2 and Figure e-5 for the original results from the secondary meta-analysis. CI = cognitively impaired; CN = cognitively normal; PWH = people with HIV.

In addition to serving as a membrane marker, elevations in Cho (especially with a concomitant increase in mI) may reflect gliosis and neuroinflammation.[10] Indeed, we found an elevation in Cho in the BG of acute/primary infection patients, which may be indicative of ongoing neuroinflammation and is consistent with HIV studies of human[19,46] and nonhuman subjects.[52,53] Similar to mI, cART led to a decrease in Cho in the PGM (and to a lesser degree, the BG) —indicating successful inflammation reduction, but Cho elevations persisted in chronic PWH (with a significant elevation in the FGM and a marginal elevation in the BG). However, there were no significant associations between Cho concentration and cognitive impairment in chronic PWH. A previous study suggested a probable nonlinear (i.e., inversed U shape) relationship between HAND severity and Cho levels,[54] as neuronal death/loss at advanced disease stages could result in Cho reductions,[55] but this hypothesis remains to be tested.

Compared with NAA, mI, or Cho, the concentrations of glutamatergic metabolites (Glu/Gln or Glx) were only examined in approximately half of the HIV MRS studies (Table 1). Nevertheless, across brain regions, we found that the glutamatergic metabolite levels were higher in the FWM in PWH than controls, although it did not survive the FDR correction. In multiple sclerosis, elevated Glx are found in both acute lesions and normal appearing white matter (WM)[56] and are attributed to reduced Glu uptake and ineffective Glu removal resulting from oligodendrocyte dysfunction[57] and astrocyte damage[58]—both may also underlie glutamatergic metabolite elevations in WM and contribute to the highly prevalent WM injury as well as cognitive impairment in PWH.[59,60]

There are some limitations of this meta-analytic study. First, the number of studies for some comparisons was small, especially for certain brain regions (e.g., PWM) and metabolites (e.g., Glx). This could reduce the power to detect additional metabolite alterations in HIV (such as injury to the cortex during acute/early infection[24]) and limit the feasibility for a comprehensive meta-regression analysis to examine the effect of clinical factors. Second, although we carefully excluded studies that had the same patient populations, it is possible that some of the patients might still be included in multiple studies of the same meta-analysis. This possible overrepresentation was investigated using 2 separate sensitivity analyses. Third, there were not enough longitudinal studies with data for meta-

analysis; thus, we could not investigate longitudinal metabolite alterations in chronic PWH or the long-term trajectory of metabolite alterations after starting cART.

In summary, this comprehensive meta-analysis reveals several important findings that are consistent with and/or add to the existing literature: BG may be the primary region affected during early infection, with Cho elevations that are indicative of neuroinflammation; antiretroviral treatment is effective in reducing inflammation, resulting in a global decrease in mI and Cho elevations; but mild neuroinflammation (i.e., mI and Cho elevations) and widespread neuronal dysfunction (i.e., NAA reductions) are evident in chronic PWH and may play important roles in cognitive impairment in the cART era, suggesting that NAA and mI may serve as potential surrogate markers or even therapeutic targets for HAND.

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Disclosure

The authors report no disclosures. Go to Neurology.org/N for full disclosures.

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Sophia Dahmani	Georgetown University Medical Center, Washington, DC	Systematic review; data collection; data analysis; and write-up
Nicholas Kaliss	Georgetown University Medical Center, Washington, DC	Data collection; data analysis; and write-up
John W. VanMeter, PhD	Georgetown University Medical Center, Washington, DC	Critical revisions
David J. Moore, PhD	University of California, San Diego, San Diego, CA	Critical revisions
Ronald J. Ellis, MD, PhD	University of California, San Diego, San Diego, CA	Critical revisions
Xiong Jiang, PhD	Georgetown University Medical Center, Washington, DC	Study concept and design; systematic review; and write-up

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