

Effect of aging on brain metabolism in antiretroviral-naive HIV patients

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Objective: Normal aging as well as HIV infection may lead to inflammatory changes and injury to the brain; however, it is unclear if and how these processes interact. The goal of this pilot study was to evaluate the interaction between aging and HIV infection in the brain using proton magnetic resonance spectroscopy (¹H-MRS).

Design: Analyses of covariance (ANCOVA) were performed to determine the effects of HIV and age, and their interaction, on MRS variables.

Methods: Forty-six HIV patients naive to antiretroviral medications and 58 seronegative control subjects were examined using localized ¹H-MRS in the frontal gray matter, frontal white matter and basal ganglia, and metabolite concentrations were determined.

Results: Compared with seronegative controls, HIV-positive subjects showed additional and marked increases in the concentration of glial markers, choline-containing compounds (seronegative controls +2%/decade; HIV-positive subjects +10%/decade) and myoinositol (seronegative controls +3%/decade; HIV-positive subjects +12%/decade), with aging in the frontal white matter. In the basal ganglia, *N*-acetyl compounds and total creatine decreased with age only in HIV patients (*N*-acetyl compounds -3.7%/decade; creatine -4%/decade). ANCOVA showed significant interaction effects between HIV and aging on the metabolites in the basal ganglia (*N*-acetyl peak $P = 0.03$; creatine $P = 0.04$) and in the frontal white matter (interaction: choline-containing compounds $P = 0.002$; myoinositol $P = 0.007$).

Conclusion: In the basal ganglia, HIV infection appeared to induce neuronal damage or loss beyond that observed in normal aging. In the frontal white matter, HIV infection seemed to exacerbate glial activation beyond that observed in normal aging.

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Introduction

Modern non-invasive neuroimaging techniques are increasingly being used to assess brain function, physiology and chemistry. Cerebral metabolite concentrations measured by proton magnetic resonance spectroscopy (¹H-MRS) provides well-validated surrogate markers for the severity of brain injury in HIV dementia. Two of these metabolites (the glial marker myoinositol and the neuronal marker *N*-acetyl aspartate) showed good

correlation with clinical assessments [CD4 cell count, dementia stage, plasma and cerebrospinal fluid (CSF) viral loads] [1–3] and cognitive performance [3]. Furthermore, 6–9 months of highly active antiretroviral therapy led to cognitive improvement [4] and partial reversal of cerebral metabolite abnormalities [5,6] in HIV cerebral metabolite concentrations.

Similarly, ¹H-MRS has been applied to evaluate changes in cerebral metabolism associated with normal

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aging. These studies demonstrated age-dependent increases in the glial marker myoinositol [7], and in metabolites that are found in higher concentrations in glial cells, total creatine [7–11] and soluble choline compounds [7–10,12]. Some studies, however, found no age-related changes in choline compounds [11,13] and creatine [14]. Whereas most of the studies observed either no change in *N*-acetyl aspartate [7,9,12] or decreased *N*-acetyl aspartate [10,14] with advancing age, one study observed increased *N*-acetyl aspartate with aging instead [11]. The discrepancies between these studies may be partly caused by differences in the brain regions studied, sex or age differences in the populations, or the different techniques used.

In summary, aging and HIV infection are both associated with increases, albeit to different degrees, in the metabolites associated with glial cell activation (myoinositol, choline compounds and creatine), probably as a result of inflammation. In contrast, decreases in the neuronal marker *N*-acetyl aspartate are much more common and pronounced in HIV patients, especially in those with more severe brain injury. However, very little is known about how aging and HIV might interact on brain metabolism. Therefore, the goal of this study was to determine the potential interaction between HIV and aging on brain metabolism, as assessed non-invasively using ¹H-MRS. The study was performed in antiretroviral medication-naïve patients, in order to avoid the potential confound of partial treatment effects.

Methods

The study involved 46 HIV-positive patients aged 18–58 years (mean ± standard deviation 36.1 ± 9.8 years; 42 men and four women). All HIV patients fulfilled the following inclusion criteria: (i) aged 18–65 years; (ii) seropositive for HIV-1; (iii) CD4 cell count less than 500 cells/mm³; (iv) naïve to antiretroviral medications; and (v) willingness and ability to give informed consent or to have it given by a valid representative. In addition, individuals were excluded if they fulfilled any of the following criteria: (i) a history of psychiatric illness that might confound the analysis of the study (e.g. schizophrenia, major depression); (ii) the presence of opportunistic brain lesions (e.g. toxoplasmosis, lymphoma, or progressive multifocal leukoencephalopathy); (iii) confounding neurological disorders (e.g. multiple sclerosis, stroke, Parkinson's disease, Alzheimer's disease, other degenerative brain diseases, any brain infections or neoplasms); (iv) chronic medical illnesses that might affect cognition (e.g. uncontrolled hypertension, abnormal thyroid function, or diabetes); (v) severe cardiac, hepatic or renal dysfunction; (vi) current or a history of drug dependence, or positive

urine toxicology screen (including cocaine, methamphetamine, alcohol, opiates, inhalants and barbiturates); (vii) head trauma with loss of consciousness for more than 30 min; (viii) for women: pregnancy or breastfeeding; and (ix) metallic or electronic implants in the body that would be contraindicated for magnetic resonance studies. Age-related changes in cerebral metabolites of the HIV-positive subjects were compared with those in 58 healthy seronegative individuals aged 19–78 years (51.5 ± 20.8 years; 16 men, 42 women). The control subjects fulfilled the same exclusion criteria as HIV-positive subjects, and were on no medications (not even hormonal replacement therapy for women), except for vitamins. Before enrollment, all subjects signed an informed consent approved by the Institutional Review Board. All subjects completed the ¹H-MRS and a battery of neuropsychological tests [3]. Seropositive subjects also had CD4 cell count, plasma and CSF viral load measurements, and assessment by Karnofsky score [15], HIV dementia scale [16], and ADC staging [17,18] by a neurologist experienced in the evaluation of HIV patients.

Magnetic resonance imaging and proton magnetic resonance spectroscopy

Magnetic resonance imaging and MRS were performed on a clinical 1.5-Tesla scanner (General Electric, Milwaukee, WI, USA) using a previously described protocol [5]. Magnetic resonance spectra were acquired in normal-appearing brain regions in the mid-frontal gray matter, right frontal white matter, and right basal ganglia (Fig. 1). ¹H-MRS was performed using a well-validated and optimized double-spin echo sequence [19,20], with an echo time of 30 ms and a recovery time of 3 s. Metabolite concentrations of *N*-acetyl aspartate, creatine, choline compounds, and myoinositol, corrected for the CSF partial volume within each voxel, were determined as previously described [21,22]. The data were processed by investigators who were blinded to the clinical status of the subjects.

Statistical analyses

Analyses of covariance (ANCOVA) were performed to evaluate the effects of age and HIV serostatus on brain metabolite concentrations. In this model, interactions between age and serostatus demonstrated differential effects of HIV serostatus on age-related changes in brain metabolism. To determine the slope and significance of age-related metabolite changes separately for each group, post-hoc analyses were performed (using simple linear regression analyses) only for metabolites that showed a significant interaction on the ANCOVA. Statistical significance was defined as $P \leq 0.05$ (double-sided) for all statistical tests.

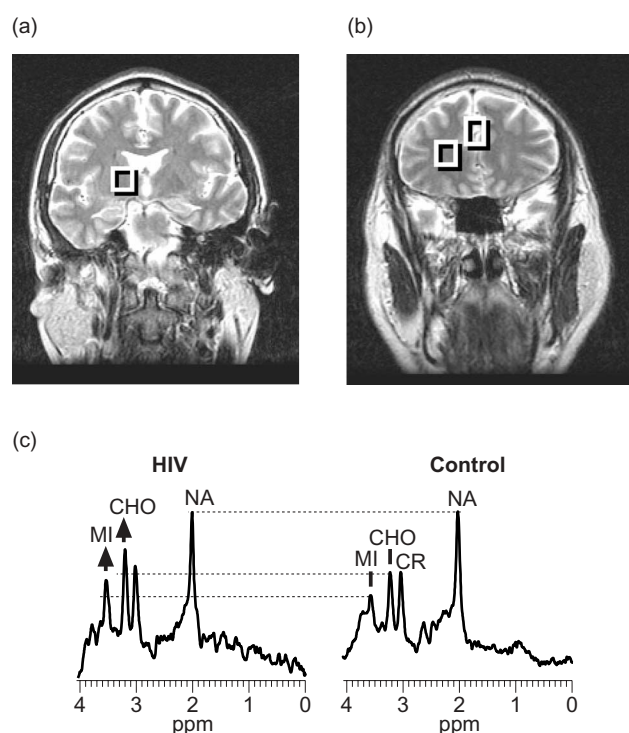


Fig. 1. T2-weighted coronal magnetic resonance images showing the voxel locations, and representative magnetic resonance spectra from an HIV patient. Three voxel locations in (a) the right basal ganglia, and (b) the right frontal white matter and the mid-frontal gray matter. (c) Representative magnetic resonance spectra showing elevated choline compound and myo-inositol levels (ADC stage 1). Horizontal lines indicate normal levels. NA, *N*-acetyl compounds (primarily *N*-acetyl aspartate); CR, total creatine; CHO, water-soluble choline compounds; MI, myo-inositol.

Results

Clinical assessments

The average ADC stage [17] of the seropositive subjects was 0.99 ± 0.13 (10 were ADC stage 0; nine were ADC stage 0.5; 16 were ADC stage 1; eight were ADC stage 2; and three 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). The mean CD4 cell count of the HIV patients was 184 ± 22 cells/mm³, and they had relatively high plasma ($196\,321 \pm 39\,718$ copies/ml) as well as CSF (8128 ± 2198 copies/ml) viral loads. The patients also demonstrated mild pleocytosis in the CSF (4.4 ± 1.0 cells/mm³), although their CSF glucose and protein levels were within normal limits. In these antiretroviral-naïve patients, the serum lipid levels were within normal limits (cholesterol 162.6 ± 6.2 mg/dl; triglycerides 158.7 ± 12.3 mg/dl). The average duration since HIV diagnosis was 22.2 ± 6.9 months (range 0.25–248). As a group, the majority of patients were

mildly depressed (Clinical Epidemiological Scale for Depression 17 ± 1.5), possibly as a result of the recent HIV diagnosis. Education levels were not significantly different between HIV patients and control subjects (HIV-positive 12.1 ± 0.54 years, range 8–23; HIV-negative 13.1 ± 0.5 years).

Cerebral metabolites

Abnormalities in brain metabolites in these seropositive subjects ($n = 45$ in comparison with 25 control subjects who were matched for age), and their relationships with cognitive and clinical disease markers, have been described previously [3]. Briefly, the HIV patients as a group had elevated creatine (+8.6%, $P = 0.03$), choline compounds (+12.5%, $P < 0.01$) and myo-inositol (+16.4%, $P < 0.01$) levels in the frontal white matter, and mildly elevated choline compounds (+5.3%, $P < 0.04$) and decreased glutamate plus glutamine (−4.8%, $P < 0.05$) in the frontal cortex. The severity of the ADC stage was associated with decreased *N*-acetyl aspartate ($P = 0.005$; averaged across the three brain regions), and increased choline compounds ($P = 0.0003$) and myo-inositol ($P < 0.0001$). For the current analysis of aging effects, an additional group of 33 healthy seronegative subjects, including elderly subjects, was included. The ANCOVA demonstrated significant age-related increases in metabolite concentrations (both groups combined) for myo-inositol in all three brain regions (frontal white matter $P < 0.0001$; frontal gray matter $P = 0.01$; basal ganglia $P = 0.01$), for creatine in the frontal gray and white matter (white matter $P = 0.0005$; frontal gray matter $P = 0.004$), and for choline compounds in the frontal white matter ($P < 0.0001$).

Interactions between HIV serostatus and age were observed for four MRS variables. In the frontal white matter, increases in choline compounds and myo-inositol with age were much larger in the HIV patients than in the control subjects (choline compounds $P < 0.002$; myo-inositol $P = 0.007$; interaction between age and serostatus on ANCOVA) (Fig. 2). In particular, the age-related increase in the frontal white matter choline compounds of control subjects was 0.033 mM/decade (approximately 2%/decade), but five times higher in HIV patients (0.165 mM/decade, or 10%/decade). Similarly, the increase in frontal white matter myo-inositol with age was four times larger in seropositive subjects (seronegative 0.21 mM/decade or 3%/decade; seropositive 0.85 mM/decade or 12%/decade).

The findings were qualitatively different in the basal ganglia, in that the HIV patients showed age-dependent metabolic changes whereas the control subjects showed no changes (Fig. 3). In particular, the patients showed decreasing basal ganglia *N*-acetyl aspartate ($P = 0.03$ for age by serostatus interaction on ANCOVA, slope in patients -0.32 mM/decade, or

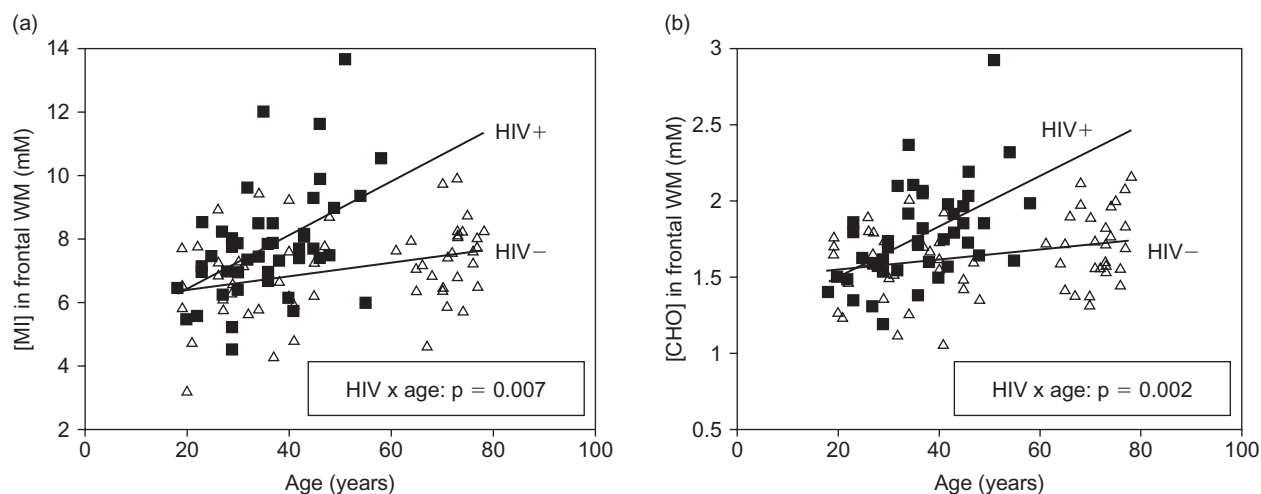


Fig. 2. Interaction plots showing marked and additional age-related increases in frontal white matter myoinositol and frontal white matter choline compounds in HIV patients compared with seronegative controls. The age-related increases in the frontal white matter (WM) (a) myoinositol (MI) and (b) choline compounds (CHO) were four to five times greater in HIV patients compared with control subjects (myoinositol: seronegative 0.21 mM/decade, 3%/decade; seropositive 0.85 mM/decade, 12%/decade; choline compounds: seronegative 0.033 mM/decade, 2%/decade; seropositive 0.165 mM/decade, 10%/decade).

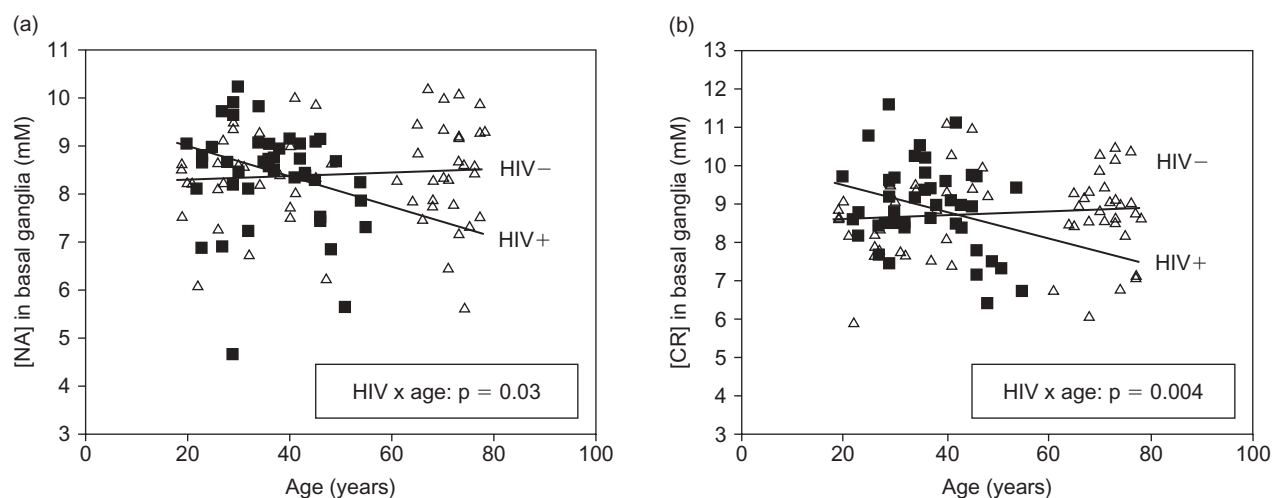


Fig. 3. Interaction plots showing age-related decrement in *N*-acetyl aspartate and creatine in HIV patients but not in seronegative control subjects. The patients, but not the control subjects, showed age-related decreases in basal ganglia (a) *N*-acetyl aspartate (NA; slope in patients -0.32 mM/decade, or -3.7% /decade) and (b) creatine (CR; slope in patients -0.34 mM/decade, or -4% /decade).

-3.7% /decade) and creatine with age ($P = 0.04$ for interaction, slope -0.34 mM/decade, or -4% /decade).

Discussion

The results demonstrate that, of the three brain regions evaluated, the interactive effect of HIV on brain aging occurs primarily in the frontal white matter and the basal ganglia, but not in the medial frontal cortex. Because the same two regions (frontal white matter and basal ganglia) show much more pronounced ^1H -MRS

abnormalities in patients with early HIV dementia [2], the finding of interaction with aging indicates that HIV infection has injurious effects beyond those observed with normal aging in these regions.

In the control subjects, frontal white matter choline compounds and myoinositol increased with age. Similar age-related increases in frontal cortical myoinositol in another cohort of 19–78-year-old healthy volunteers were previously reported [7]. The increased myoinositol concentration probably reflects increased glial cell content in the aging brain, because studies of postmortem brains have shown increased glial fibrillary acidic

protein and increased glial cell numbers with aging [23]. Likewise, the age-related increase in white matter choline compounds is in agreement with the results of several other studies [8,10,12], and might reflect the well-described inflammatory changes and glial cell (astrocytes and microglia) activation associated with aging [24–26]. As increases in choline compounds and myoinositol in the frontal white matter are also among the earliest changes detectable in HIV brain disease [2,27], and probably reflect glial activation caused by the infection, these changes might exacerbate those already observed with normal aging. Whereas the combined effects of HIV infection and aging on cerebral metabolism might be expected to be additive, HIV caused a pronounced (fivefold) acceleration of aging effects on inflammatory and glial markers in the frontal white matter. This compounded glial reaction in HIV patients might be caused by the excess upregulation of microglial and astrocytic activity that is induced by neurotoxic proteins (e.g. gp120, tat, cytokines and chemokines) in the infected brains [28–30].

The basal ganglia showed distinct patterns of brain aging with and without HIV infection. First, age by HIV interactions were observed for *N*-acetyl aspartate and creatine, but not for choline compounds and myoinositol. Second, age-related increases in basal ganglia *N*-acetyl aspartate and creatine only occurred in the HIV patients, but not in the control subjects. This differential effect in the basal ganglia is particularly remarkable because the greater age range in the seronegative group (aged up to 78 years versus 58 years in the HIV group) would have allowed the easier detection of age-related changes in the control group. As *N*-acetyl aspartate is considered to be a marker for neuronal viability, these findings suggest minimal or no neuronal damage or loss in the seronegative control subjects, despite their substantially greater age (> 20 years older) than the HIV patients. This observation is also consistent with postmortem analyses that found primarily a shrinkage of large neurons and constant neuronal density with normal aging [23]. In contrast, postmortem studies demonstrated that HIV dementia is associated with neuronal apoptosis, especially in the basal ganglia of patients with HIV encephalitis [31]. Furthermore, morphometric analyses found atrophy of the basal ganglia in HIV patients [32]. Taken together, the basal ganglia appear to be particularly vulnerable to the neurotoxicity associated with HIV infection. The findings indicate that HIV infection may exacerbate the mild neuronal loss or damage associated with aging. It is currently unknown whether these neuronal abnormalities suggested by lower *N*-acetyl aspartate levels are reversible with treatment, and how aging might affect the ability of neurons to recover.

In the elderly population, increased oxidative damage to proteins and lipids, irreversible protein glycation,

and damage to DNA, have all been proposed to cause the increased incidence of 'degenerative' conditions such as Alzheimer's disease and Parkinson's disease [33]. In addition, normal aging is associated with a decline in presynaptic dopaminergic transporters (−6%/decade) [34] and postsynaptic dopamine D₂ receptors (−4 to −6%/decade) [35]. Both the dopamine transporters and receptors have the highest density in the basal ganglia, and decreased D₂ receptors are associated with cognitive and motor decline in normal aging [36]. As both oxidative stress-mediated neuronal apoptosis [37] and dopaminergic deficits [38] have been implicated in HIV-associated dementia, aging would probably further exacerbate the psychomotor slowing and other cognitive signs associated with HIV dementia.

This study has several limitations, mostly because the study was not originally designed to evaluate aging effects. First, the maximum age in the HIV group (58 years) was 20 years below that in the control group (78 years). This difference reflects the low prevalence of HIV infection in elderly individuals, and limits the ability to generalize these findings to seropositive individuals over 60 years of age. However, some of the interactions between age and HIV status were very robust, especially in the frontal white matter, where HIV-positive subjects showed a significantly greater (approximately five times) increase in choline compounds and myoinositol with age compared with control subjects. A second potential limitation of the study was a difference in the sex composition between the two groups (most of the HIV patients were men, and most of the control subjects were women). It has been speculated that women may enjoy some protection from brain aging, possibly because of the neuroprotective influence of oestrogen [39–41]. However, the rates of metabolite changes with age in the seronegative subjects were similar to those previously observed in the frontal gray and white matter of both men and women [7], as well as to those in other aging studies of both men and women in the parietal white matter [12] and many other brain regions [8,13]. In addition, none of the elderly women in the study were on hormone replacement therapy. Finally, the cross-sectional design of the study is another potential concern because group differences might be caused by unknown factors specific to one of the two populations. Therefore, future studies should use a longitudinal design (over a 5–10 year period) to determine the differential aging effects in HIV patients compared with seronegative healthy controls. However, the robustness of some of the interactions between age and serostatus in this study makes it unlikely that the observed effects are erroneous.

One advantage of this study is that metabolite concentrations, rather than metabolite ratios, were measured,

which allows a clear interpretation of metabolite abnormalities. The concentration measurement also included a correction for the partial volume effects of CSF, which is particularly important in measuring cortical brain regions where atrophy and increased CSF might 'dilute' the metabolite concentrations in HIV patients or elderly individuals. Another unique aspect of this study is that all of the HIV-positive subjects were naive to antiretroviral medications. The exclusion of patients treated with antiretroviral medications is advantageous in eliminating some of the potential confounds, such as differences in treatment regimens, partial treatment effects, treatment duration, drug resistance, etc. Similarly, it is possible that some antiretroviral drugs might be neurotoxic, especially during long-term treatment, and it is desirable to separate such drug-related side-effects from those of HIV infection. However, as the majority of HIV-positive patients have to be maintained on highly active antiretroviral therapy for life, it may ultimately be more important to evaluate the effect of HIV on aging in treated individuals.

In summary, these data suggest that HIV infection causes additional injury to the aging brain as assessed non-invasively by ¹H-MRS. In particular, HIV infection caused an additional elevation of glial markers in the frontal white matter with age (a fivefold acceleration in the rate of choline compounds and myoinositol increases with age). Conversely, in the basal ganglia, HIV appeared to induce age-associated neuronal damage or loss that was not detectable in healthy control subjects up to 80 years of age. However, a better matching of subject populations as well as longitudinal studies are needed to corroborate these findings. Furthermore, as some of the metabolite abnormalities (in particular creatine, choline compounds and myoinositol) might be reversible with treatment, it remains to be determined how much these metabolite abnormalities will impact on aging, and whether the apparent neuronal damage in the basal ganglia will improve with treatment.

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