

# Selegiline and oxidative stress in HIV-associated cognitive impairment



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## ABSTRACT

**Objective:** To assess the effectiveness of the selegiline transdermal system (STS) in reversing HIV-induced metabolic brain injury (as measured by proton magnetic resonance spectroscopy [MRS]) and in decreasing oxidative stress, measured by CSF protein carbonyl concentration.

**Methods:** Sixty-two subjects with HIV-associated cognitive impairment were enrolled in a 24-week placebo-controlled study (AIDS Clinical Trial Group protocol A5090) and were randomly assigned to receive STS 3 mg/24 h, STS 6 mg/24 h, or matching placebo. Cognitive performance was evaluated using the neuropsychological z score (NPZ)-8 and NPZ-6, as well as cognitive domain scores. Subjects underwent proton MRS at study entry and weeks 12 and 24. CSF protein carbonyl was measured at baseline and week 24.

**Results:** A slight increase in *N*-acetyl aspartate/creatine from baseline to week 24 was found in the basal ganglia ( $p = 0.023$ ) and centrum semiovale ( $p = 0.072$ ) of the placebo group compared with the STS groups; however, there were no significant changes when the absolute metabolite concentrations were analyzed. The levels of choline/creatine in the midfrontal cortex were also significantly higher during the week 12 visit in the combined STS groups. This persisted to the week 24 visit ( $p = 0.002$ ). Evaluation of the change in NPZ-8, NPZ-6, and cognitive domain scores from baseline to weeks 12 and 24 revealed no significant differences between treatment arms. Protein carbonyl analysis revealed no significant changes among the groups.

**Conclusion:** In this 24-week study, the selegiline transdermal system (STS) had no effect on either magnetic resonance spectroscopy (MRS) metabolites or oxidative stress, as measured by CSF protein carbonyl concentration. The lack of effect on these biomarkers is also reflected in the lack of cognitive improvement in the STS groups compared to placebo.

**Level of evidence:** This study provides Class II evidence that STS had no effect on either MRS metabolites or oxidative stress, as measured by CSF protein carbonyl concentration over a period of 24 weeks. *Neurology*® 2009;73:1975-1981

## GLOSSARY

**ADC** = dementia complex; **Cho** = choline; **Cr** = creatine; **DNP** = 2,4-dinitrophenylhydrazine; **FOV** = field of view; **Glx** = glutamate/glutamine; **HAART** = highly active antiretroviral therapy; **MI** = myoinositol; **MRS** = magnetic resonance spectroscopy; **NAA** = *N*-acetylaspartate; **NEX** = number of excitations; **NPZ** = neuropsychological z score; **PBS** = phosphate-buffered saline; **SNR** = signal-to-noise ratio; **STS** = selegiline transdermal system; **TE** = echo time; **TR** = repetition time.

Proinflammatory products and reactive oxygen species secreted by infected or activated microglia and macrophages have been implicated in the pathogenesis of HIV-associated neurologic disorders.<sup>1</sup> Additionally, elevated markers of oxidative stress have been reported in the CSF of subjects with HIV-associated cognitive impairment.<sup>2-5</sup> Despite highly active antiretroviral therapy (HAART), levels of oxidative stress can remain elevated in HIV-infected subjects with cognitive impairment,<sup>3</sup> and HAART alone does not fully prevent the occurrence of cognitive impairment.<sup>6</sup> Antioxidant intervention may provide a useful adjunctive therapy to HAART in the treatment of HIV-

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associated cognitive impairment. The AIDS Clinical Trial Group has conducted a phase 2, placebo-controlled, double-blind study of the selegiline transdermal system (STS) in the treatment of HIV-associated cognitive impairment (study A5090).<sup>7</sup> Selegiline is a monoamine oxidase B inhibitor capable of decreasing oxygen-free radicals, increasing the formation of the antioxidant enzymes superoxide dismutase and catalase, and providing additional neuroprotection by enhancing the synthesis of neurotrophic factors.<sup>8-13</sup> Results of 2 prior placebo-controlled pilot studies of oral selegiline and STS in subjects with HIV-associated cognitive impairment suggested improvement in several tests of psychomotor speed.<sup>14,15</sup>

A5090 results demonstrated no significant benefit in either cognitive or functional outcome in subjects treated with selegiline compared with those who received placebo.<sup>7</sup> However, it is possible that the exposure to STS in A5090 was too short to translate in a measurable cognitive or functional effect. The open-label extension of A5090<sup>16</sup> did not fully address this issue. We have conducted a substudy of A5090 using magnetic resonance spectroscopy (MRS) and CSF protein carbonyl concentration as biomarkers of CNS injury, which we hypothesized would be able to measure response to selegiline before observable clinical or functional changes.

**METHODS Standard protocol approvals, registrations, and patient consents.** The study was reviewed and approved by the institutional review board at each participating institution. All subjects signed a written informed consent before enrollment. This study was registered with clinicaltrials.gov under identifier NCT00027040.

**Subjects.** One hundred twenty-eight HIV-infected subjects with cognitive impairment were enrolled in A5090 and were randomly assigned to receive STS 3 mg/24 h, STS 6 mg/24 h, or matching placebo for 24 weeks.<sup>7</sup> Randomization was stratified by plasma HIV viral load (undetectable, <200 copies/mL, vs detectable,  $\geq$ 200 copies/mL) and clinical stage of the AIDS dementia complex (ADC; 0.5 vs 1.0 or higher). Sixty-two subjects were coenrolled in this substudy at sites with MRS expertise. Cognitive impairment was defined as performance at least 1 SD below the mean on 2 or more independent neuropsychological tests, or at least 2 SDs below the mean on 1 neuropsychological test using a standard neuropsychological battery. Cognitive performance was evaluated using the NPZ-8 (average *z* score of 8 neuropsychological tests) and NPZ-6 (average *z*-score of 6 neuropsychological tests), as well as cognitive domain scores (average *z* score of neuropsychological tests corresponding to cognitive domain).

**Imaging.** MRI was performed at study entry and weeks 12 and 24 on commercial GE Signa (Harvey, IL) 1.5-T scanners. Sagittal T1-weighted images were collected with the following parameters: 5-mm slice thickness, echo time (TE)/repetition time (TR) = 20/600, field of view (FOV) = 24 cm, and matrix size = 256  $\times$  256, and number of excitations (NEX) = 1. Axial fast spin echo images were collected according to the following parameters: 5-mm slice thickness, TE1/TE2/TR = 30/80/2,500, FOV = 24 cm, matrix size = 245  $\times$  192, echo train length = 8, bandwidth = 32 KHz, and NEX = 1.

Single-voxel proton spectra were acquired from 3 regions of interest using the commercially available GE PROBE-P PRESS sequence. Voxels 20  $\times$  20 mm<sup>2</sup> (slice thickness = 15 mm) were prescribed graphically from axial MR images in the midline of the frontal lobes (gray matter), right (or left) midfrontal centrum semiovale (white matter), and right (or left) basal ganglia (deep gray matter). Shimming was performed on each voxel using the automated FID-based algorithm from GE. Water suppressed spectra were collected according to the following parameters: TE/TR = 35/3,000, bandwidth = 2,500 Hz, 128 averages, and NEX = 8. Additionally, single-scan, fully relaxed water spectra were collected from each voxel with 8 different echo times. LCModel spectral analysis software<sup>17</sup> was used to calculate the metabolite ratios of *N*-acetylaspartate (NAA) to creatine (Cr), choline (Cho) to Cr, myoinositol (MI) to Cr, and the combined peak of glutamate/glutamine (Glx) to Cr. Absolute quantitation of these metabolites was performed using the technique described by Kreis et al.<sup>18</sup> and yielded metabolite concentrations corrected for the percentage of CSF in each voxel.

**CSF protein carbonyl.** CSF was obtained at baseline and week 24. The level of protein oxidation was determined by an Oxidized Protein Detection Kit (Oxyblot, Chemicon, Temecula, CA). Equal amounts of protein (1  $\mu$ g) from each CSF sample were loaded on the blot, and the intensity of the bands was quantitated by densitometry. Samples were incubated for 20 minutes with 6% (wt/vol) sodium dodecyl sulfate and 2,4-dinitrophenylhydrazine (DNPH) in 10% (vol/vol) trifluoroacetic acid, vortexing every 5 minutes, and then neutralized in Oxyblot neutralization solution. Samples were transferred to nitrocellulose membrane by slot-blotting technique. After the transfer, membranes were blocked with 5% (wt/vol) skim milk (in phosphate-buffered saline [PBS] with 0.1% (vol/vol) Tween-20) overnight at 2° to 8°C. The nitrocellulose membrane was exposed to a primary rabbit anti-DNPH protein antibody from Chemicon Oxyblot (1:150 working dilution) for 1 hour, and then to a secondary antibody (anti-rabbit immunoglobulin G coupled to horseradish peroxidase, Sigma, St. Louis, MO) diluted in the blocking solution 1:5,000 for 60 minutes at room temperature. Membranes were washed after every step in washing buffer (PBS with 0.1% Tween-20). The nitrocellulose membrane was then developed by an EHP plus kit (GE Healthcare) and exposed to film, until the bands of oxidized proteins appeared. Blots were analyzed using BioRad (Hercules, CA) Quantity One software.

**Statistical analysis.** Power calculations were based on our previous cross-sectional work<sup>19</sup> and longitudinal work.<sup>20</sup> Assumptions were based on the correlation of successive MRS evaluations obtained on the same individuals. Under most scenarios, we determined that with a minimum of 48 subjects, there would be adequate power (i.e., >80%) to detect similar differences between baseline and week 24 as we had seen cross-sectionally in the study by Chang et al.<sup>19</sup> between neurologically impaired and nonimpaired HIV-infected patient groups.

<b>Table 1 Baseline demographics and clinical characteristics</b>				
	<b>STS 3 mg/24 h (n = 19)</b>	<b>STS 6 mg/24 h (n = 18)</b>	<b>Placebo (n = 25)</b>	<b>p Value*</b>
Age, median (IQR), y	45 (41 to 50)	45 (43 to 50)	47 (44 to 52)	0.474
Men/women	17/2	15/3	22/3	0.801
White/black/Hispanic	9/10/0	7/10/1	8/14/3	0.614
Years of education, median (IQR)	12 (12 to 14)	13 (10 to 14)	12 (11 to 14)	0.755
CD4 <sup>+</sup> count, median (IQR), mm <sup>3</sup>	361 (276 to 703)	366 (260 to 744)	384 (263 to 765)	0.878
Plasma HIV RNA, median (IQR), copies/mL	<50 (<50 to 108)	<50 (<50 to 2,913)	<50 (<50 to 700)	0.252
% <50 copies/mL	69	41	50	
% 50–10,000 copies/mL	25	42	28	
% ≥10,000 copies/mL	6	18	23	
CSF HIV RNA copies/mL, median (IQR)	<50 (<50 to <50)	<50 (<50 to <50)	<50 (<50 to 52)	0.550
% <50 copies/mL	79	77	75	>0.999
% 50–10,000 copies/mL	21	23	25	
% of subjects on antiretroviral therapy	94.7	88.9	92	0.857
Weight, median (IQR), lb	153.35 (120.0 to 181.5)	135.50 (80.4 to 158.0)	153.7 (94.0 to 171.0)	0.399
CES-D score, median (IQR)	23.0 (18.0 to 34.0)	17.5 (13.0 to 21.0)	14.0 (9.0 to 25.0)	0.089
Karnofsky Scale Score ≥ 80, %	63.2	66.7	75.8	0.038
(WAIS-R) Vocabulary Scale Score	10 (9 to 12)	8 (7 to 11)	9 (7 to 11)	0.133
AIDS dementia complex stage 0.5/1/2	8/9/2	6/10/2	11/13/1	0.301
NPZ-6, median (IQR)	−1.09 (−1.86 to −0.45)	−0.85 (−1.29 to −0.47)	−1.15 (−1.59 to −0.63)	0.615
NPZ-8, median (IQR)	−0.91 (−1.75 to −0.55)	−0.78 (−1.19 to −0.47)	−0.76 (−1.42 to −0.51)	0.403

\*Frequencies compared by Fisher exact test; continuous factors compared by Kruskal-Wallis test.

STS = selegiline transdermal system; IQR = interquartile range; CES-D = Center for Epidemiologic Studies Depression Scale; WAIS-R = Wechsler Adult Intelligence Scale-Revised; NPZ = neuropsychological z score.

Comparisons of continuous measures between groups were performed using the Kruskal-Wallis test, and comparisons of frequencies between groups were performed using the Fisher exact test.

To evaluate changes in MRS metabolite ratios over time, analysis of covariance models were applied to the 2 postbaseline MRS evaluations adjusted for baseline metabolite ratio levels using Box-Cox transformed measures for the longitudinal models of the metabolite levels. An autoregressive order 1, variance/covariance matrix was used to model the covariance structure of the model. In all resulting comparisons, the 2 selegiline arms have been pooled because there was never a significant difference observed between the 3-mg/24 h and 6-mg/24 h doses. Similar models were applied to assess absolute MRS metabolite changes over time.

Comparisons of the median protein carbonyl concentrations between groups were performed using the Kruskal-Wallis test.

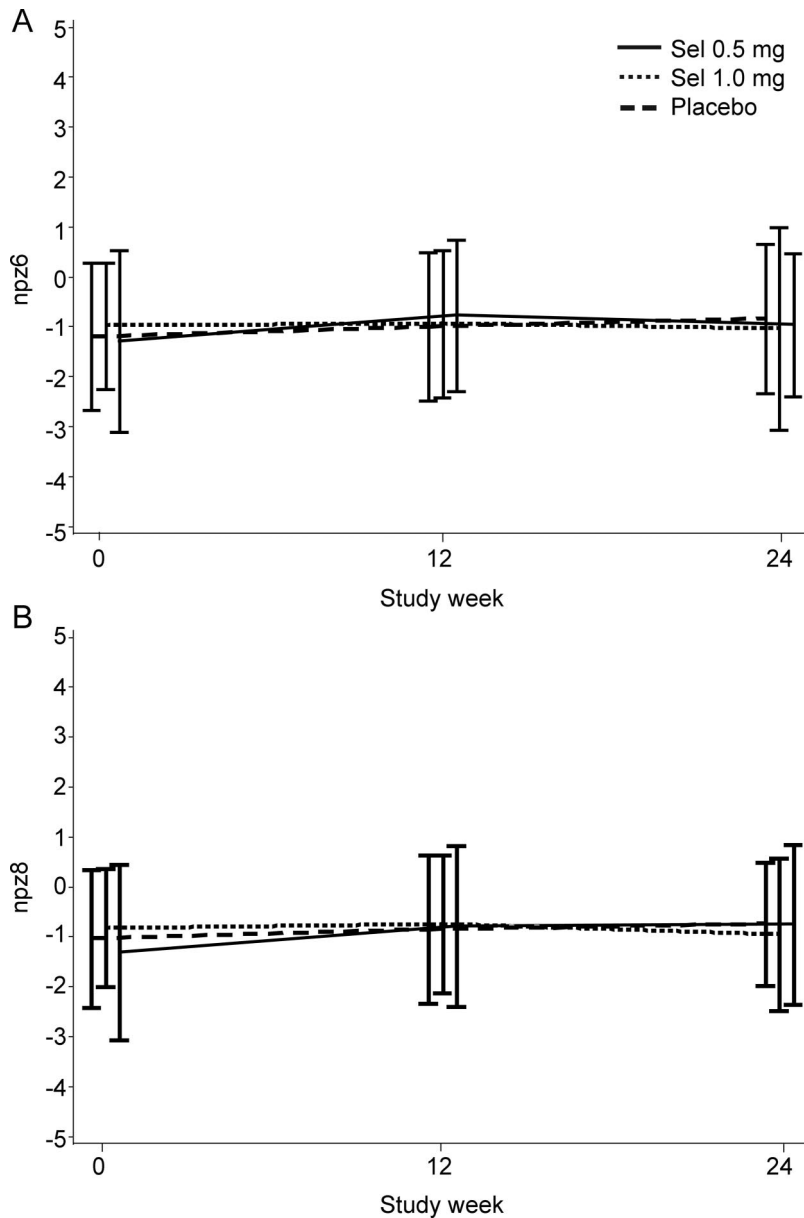
**RESULTS** The 62 subjects enrolled in this substudy were predominantly male (87%) and African American (55%) (table 1). The median age at baseline was 46 years, with a median education level of 12.5 years. The 3 treatment groups did not differ significantly at baseline with respect to these demographic characteristics, ADC stage, HIV viral load, CD4<sup>+</sup> count, NPZ scores (table 1), or cognitive domain scores (data not shown). No significant differences in proton MRS metabolite ratios were detected between

treatment arms at baseline, with the exception of Glx/Cr in the centrum semiovale (table e-1 on the *Neurology*<sup>®</sup> Web site at [www.neurology.org](http://www.neurology.org)).

As observed in the parent study, evaluation of the change in NPZ-8 and NPZ-6 scores (figure 1), as well as cognitive domain scores (data not shown) from baseline to weeks 12 and 24 revealed no significant differences between treatment arms.

Changes in MRS metabolite ratios from baseline to weeks 12 and 24 are reported in table e-2. There was a slight increase in NAA/Cr in the basal ganglia (figure 2A) and in the centrum semiovale (figure 2B) of the placebo group compared with the STS groups (basal ganglia,  $p = 0.023$ ; centrum semiovale,  $p = 0.072$ ), although the change in the absolute concentration on NAA was not significant. The combined STS groups also demonstrated higher levels of Cho/Cr (figure 2C) in the midfrontal cortex ( $p = 0.002$ ) when compared with the placebo group after adjusting for baseline levels of the metabolite. This difference in Cho was still present when the concentration of Cho was analyzed, although the significance was reduced ( $p = 0.046$ ). As shown in table

**Figure 1** Changes in NPZ scores by treatment arm



(A) Neuropsychological z score (NPZ)-6. (B) NPZ-8.

e-2, no other comparison results were significantly different among the groups. Analyses using absolute metabolite concentrations (data not shown) were similar to using their ratio to Cr, with the exception of those reported above.

Protein carbonyl concentration in CSF (available in 47 subjects) decreased from baseline to week 24 in both placebo and STS groups; however, there was no significant difference among the groups (table 2). Only a small subset of subjects had both protein carbonyl CSF concentrations and MRS data available at baseline (8 were on placebo, and 11 were on STS). There were no significant correlations between protein carbonyl concentrations and MRS measures. Lower baseline protein carbonyl concentrations were

associated with a greater increase in NAA/Cr in the centrum semiovale at weeks 12 and 24 ( $p = 0.005$ ). A trend was also observed between higher concentrations of protein carbonyl at baseline and a greater increase of MI/Cr in the basal ganglia at weeks 12 and 24 ( $p = 0.063$ ).

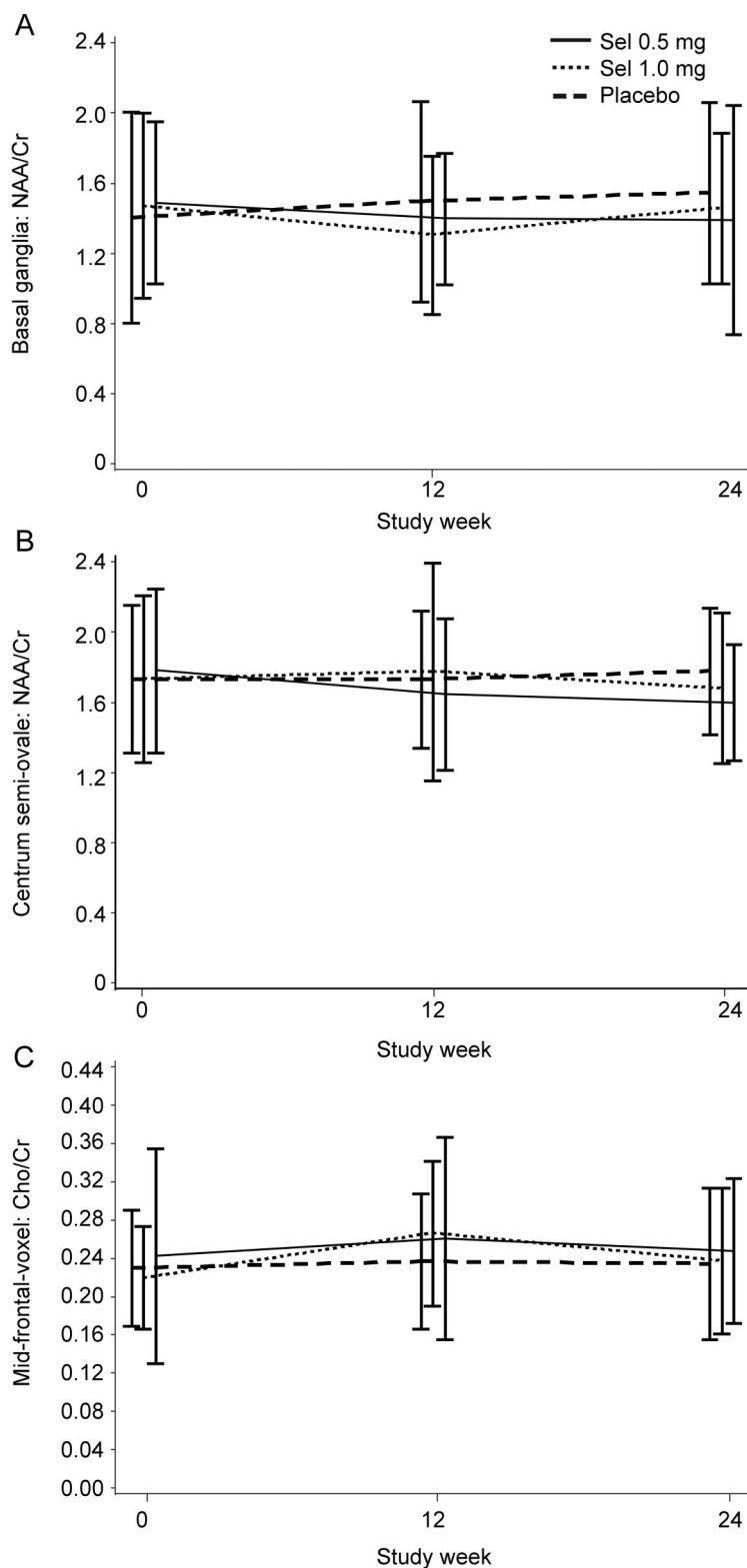
**DISCUSSION** The use of the selegiline transdermal system (STS) in this cohort of HIV-infected individuals with cognitive impairment was not associated with either improvement in brain metabolites (as measured by MRS) or decreases in oxidative stress levels (as measured by CSF protein carbonyl concentrations) when compared with placebo. These results are complementary to the lack of cognitive improvement in the STS groups compared with placebo observed in this substudy and the larger parent study.<sup>7</sup> The trend toward a decrease in NAA/Cr in the basal ganglia and centrum semiovale and increase in Cho/Cr in the frontal cortex in the STS groups compared with the placebo group raises additional questions regarding the neuroprotective role of STS in HIV-infected individuals. Although most in vitro and animal investigations have suggested that selegiline is neuroprotective,<sup>21</sup> including some models of HIV-associated neurotoxicity,<sup>2,22</sup> there have been reports in the simian immunodeficiency model that selegiline may worsen CNS pathology, possibly mediated by the resulting increase in dopaminergic activity.<sup>23,24</sup>

To our knowledge, the combination of MRS and protein carbonyl as the outcome of a clinical trial has not been reported before. MRS is a noninvasive technique that allows in vivo assessment of brain metabolites, including NAA (a marker of neuronal integrity), Cho (a marker of membrane damage), and MI (an osmolyte and astrocyte marker elevated in inflammatory and neoplastic disorders).<sup>25</sup> Several studies indicate that MRS provides a sensitive and robust in vivo method to monitor the pattern and course of CNS injury in HIV infection.<sup>26-30</sup> Particularly relevant to the use of MRS as a biomarker is the evidence that increases in NAA/Cr concentration and decreases in MI/Cr concentration occur in response to antiretroviral therapy and are associated with improved cognitive performance.<sup>26,31</sup> Furthermore, MRS sensitivity in monitoring CNS injury is underscored by its ability to detect changes even before clinical manifestations of diseases.<sup>28,32,33</sup> The usefulness of MRS in measuring CNS injury has been demonstrated in several other neurodegenerative diseases, including Parkinson disease,<sup>34</sup> multiple system atrophy,<sup>35</sup> multiple sclerosis,<sup>36</sup> Huntington disease,<sup>32</sup> and cortical dementias,<sup>33</sup> as well as in toxic or neoplastic CNS injury.<sup>25</sup>

The MRS protocol used in this study reflected the imaging capability of the sites involved at the time of



**Figure 2** Means  $\pm$  2 SDs of metabolite ratios with between-group differences in change from baseline to week 24



(A and B) Slight increases in *N*-acetylaspartate (NAA)/creatinine (Cr) in the basal ganglia and in the centrum semiovale of the placebo group compared with the selegiline transdermal system (STS) groups ( $p = 0.023$  and  $p = 0.072$ , respectively). Change in the absolute concentration of NAA was not significant. (C) Combined STS groups had higher levels of choline (Cho)/Cr in the midfrontal cortex ( $p = 0.002$ ) compared with the placebo group after adjusting for baseline levels of the metabolite. A smaller difference in Cho ( $p = 0.046$ ) was present when the absolute concentration of Cho was analyzed.

the study. Having 3-T data might have been beneficial, but there were not enough 3-T systems available when the study was designed. Furthermore, although higher field strength probably would have afforded some increases in the signal-to-noise ratio (SNR) compared with 1.5 T, gains in SNR do not necessarily translate into improvements in metabolite ratio reliability. For example, whole-brain NAA levels were not more reproducible at 3.0 T compared with 1.5 T.<sup>37</sup> Finally, we used a single-voxel approach to maximize the detection of changes in brain regions known to be affected by HIV neuropathology.

The second biomarker, protein carbonyl, is a marker of protein oxidation and has been used to assess the impact of oxidative stress in aging and neurodegeneration.<sup>38-41</sup> Furthermore, recent animal studies suggest that protein carbonyl concentrations may be responsive to antioxidant treatment.<sup>41</sup> We have previously shown that CSF concentration of protein carbonyl is increased in HIV-infected individuals with cognitive impairment compared with HIV-infected subjects without cognitive impairment and compared with HIV-negative controls.<sup>2</sup> It should be emphasized that CSF represents a limited window for assessing oxidative stress in the brain; however, it constitutes the closest compartment to the brain that we can easily and safely access.

We hypothesized that protein carbonyl concentration would have an inverse correlation with NAA/Cr and a positive correlation with Cho/Cr and MI/Cr, but we did not find significant correlations at baseline. However, lower CSF protein carbonyl concentration at baseline was associated with subsequent significant increase of NAA/Cr in the frontal white matter. One possible interpretation of this finding is that changes in levels of oxidative stress predate changes measured via MRS. Unfortunately, only a small number of subjects had both MRS and CSF protein carbonyl measured, because the MRS and lumbar puncture were optional in the parent study, thereby limiting the power of assessing the relationship between CSF protein carbonyl and MRS-measured metabolites.

Overall, the concordance between the lack of clinical response and lack of improvement in MRS-measured brain metabolites in this study and the concordance between trends in cognitive improvement and significant increases in NAA/Cr in 2 previous clinical trials in HIV-associated cognitive impairment<sup>20,42</sup> reinforce the usefulness of MRS as a biomarker in HIV-associated cognitive impairment. The responsiveness of CSF protein carbonyl concentrations in predicting clinical outcomes merits further evaluation.

We are cognizant that a larger sample size would have provided greater power in assessing the effect of selegiline on brain metabolites and protein carbonyl.

<b>Table 2 Protein carbonyl changes by treatment arm</b>			
	<b>Placebo (n = 19)</b>	<b>Combined STS arms (n = 28)</b>	<b>p Value*</b>
<b>Baseline optical density</b>			
<b>Median (IQR)</b>	119,720 (52,510 to 328,550)	164,473 (86,410 to 344,826)	0.260
<b>Week 24-baseline optical density</b>			
<b>Median (IQR)</b>	54,818 (-19,413 to 123,298)	22,321.5 (-46,781.5 to 88,017.0)	0.260

\*Kruskal-Wallis test.

STS = selegiline transdermal system; IQR = interquartile range.

However, based on the results of this study that found no improvement in brain metabolites or in markers of oxidative stress and combined with the lack of clinical improvement observed in the larger parent study,<sup>7</sup> we conclude that there are no strong bases for future development of STS as a neuroprotective compound for the treatment of HIV-associated cognitive impairment.

## AUTHOR CONTRIBUTIONS

Statistical analyses were conducted by Constantin T. Yiannoutsos, PhD.

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## APPENDIX

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