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Absence of systemic oxidative stress and increased CSF prostaglandin F$_{2\alpha}$ in progressive MS

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Supplemental data at Neurology.org/nn

ABSTRACT

Objective: We aimed to investigate the role of oxidative stress in the progression of multiple sclerosis (MS).

Methods: We determined by liquid chromatography–tandem mass spectrometry nonenzymatic (F$_2$-isoprostanes) and enzymatic oxidation products of arachidonic acid (prostaglandin F$_{2\alpha}$) in plasma and CSF of 45 controls (other neurologic disease [OND] with no signs of inflammation) and 62 patients with MS. Oxidation products were correlated with disease severity and validated biomarkers of inflammation (chemokine ligand 13; matrix metalloproteinase-9; osteopontin) and axonal damage (neurofilament light protein).

Results: Compared with OND controls, plasma concentrations of F$_2$-isoprostanes and PGF$_{2\alpha}$ were significantly lower in patients with progressive disease, and decreased with increasing disability score (Expanded Disability Status Scale). In contrast, CSF concentrations of PGF$_{2\alpha}$, but not F$_2$-isoprostanes, were significantly higher in patients with progressive disease than OND controls ($p < 0.01$). The content of PGF$_{2\alpha}$ in CSF increased with disease severity ($p = 0.044$) and patient age ($p = 0.022$), although this increase could not be explained by age. CSF PGF$_{2\alpha}$ decreased with natalizumab and methylprednisolone treatment and was unaffected by the use of nonsteroidal anti-inflammatory drug in secondary progressive MS. CSF PGF$_{2\alpha}$ did not associate with validated CSF markers of inflammation and axonal damage that themselves did not associate with the Expanded Disability Status Scale.

Conclusions: Our data suggest that MS progression is associated with low systemic oxidative activity. This may contribute to immune dysregulation with CNS inflammation accompanied by increased local cyclooxygenase-dependent lipid oxidation.

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GLOSSARY

F$_2$-IP = F$_2$-isoprostane; MS = multiple sclerosis; OND = other neurologic disease; PGF$_{2\alpha}$ = prostaglandin F; ROS = reactive oxygen species; RRMS = relapsing-remitting multiple sclerosis.

Multiple sclerosis (MS) is usually relapsing-remitting at onset, but, with time, a majority of patients convert to a secondary progressive disease course, for which current therapies are ineffective. Recently, increased oxidative stress has been proposed as a pathogenic mechanism leading to progressive MS. However, a decrease in reactive oxygen species (ROS) derived from NADPH oxidase has been associated with more severe experimental autoimmune encephalomyelitis, a model of MS. Moreover, disease progression correlates with altered activity of ROS-producing immune cells. Thus, changes in local and systemic oxidative stress are of interest for the transition into progressive MS, and we hypothesize that low oxidative stress may promote such progression.

F$_2$-isoprostanes (F$_2$-IPs) are considered the gold-standard biomarker of in vivo oxidative stress. They are formed predominantly via nonenzymatic oxidation of arachidonic acid

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(20:4). However, the most frequently determined F₂-IP (8-iso-PGF₂α), can also be generated during enzymatic oxidation of 20:4 to prostaglandin F₂α (PGF₂α), involving cyclooxygenase.⁸ As cyclooxygenase is significantly induced during inflammation, it can lead to incorrect biomarker assignment and interpretation.⁸ Therefore, we quantified the ROS-derived F₂-IP and enzyme-derived PGF₂α in plasma and CSF of patients with MS. We correlated these oxidation markers with disease severity, patient age, and other clinical measures. To explore whether CNS 20:4 oxidation changes with treatment, we also analyzed samples from 2 intervention studies of patients with progressive MS treated with natalizumab or methylprednisolone.

**METHODS**

**Materials.** Standards of F₂-IP (5(S)-iPF₂α-VI, 5-[S]-iPF₂α-VI-d₁, 5-iso-15-[S]-PGF₂α, 8-iso-15-[R]-PGF₂α, 8-iso-15-[S]-PGF₂α-d₁, and 15[R]-PGF₂α-d₄), were from Cayman Chemicals (Ann Arbor, MI). Artificial CSF was from Tocris Bioscience (Bristol, UK). Methanol (Fisher Scientific Inc.), hexane, and ethyl acetate (Lab Scan) were of high-performance liquid chromatography grade, and Bond Elut CertifyII SPE columns from Agilent Technologies (Santa Clara, CA). Other chemicals were from Sigma, unless indicated otherwise.

**Standards of F₂-IP** (5(S)-iPF₂α-VI, 5-[S]-iPF₂α-VI-d₁, 8-iso-15-[S]-PGF₂α, 8-iso-15-[R]-PGF₂α, 8-iso-15-[S]-PGF₂α-d₁, and 15[R]-PGF₂α-d₄) were from Cayman Chemicals (Ann Arbor, MI). Artificial CSF was from Tocris Bioscience (Bristol, UK). Methanol (Fisher Scientific Inc.), hexane, and ethyl acetate (Lab Scan) were of high-performance liquid chromatography grade, and Bond Elut CertifyII SPE columns were from Agilent Technologies (Santa Clara, CA). Other chemicals were from Sigma, unless indicated otherwise.

**Table**

| Clinical/paraclinical measures | RRMS | SPMS | PPMS | OND |
|-------------------------------|------|------|------|-----|
| No. of participants          | 23   | 24   | 15   | 45  |
| Mean age, y (range)          | 35.9 (21-52) | 49.4 (33-62) | 55 (35-70) | 40.3 (19-82) |
| Female/male                  | 19/4 | 15/9 | 7/8  | 30/15 |
| Mean disease duration, y (range) | 2.7 (1-12) | 18.5 (9-37) | 6.5 (1-17) | NA  |
| Median EDSS score (range)    | 1.5 (0-4.5) | 5.75 (3.0-8.0) | 4.0 (2.0-6.5) | NA  |
| Annualized relapse rate (range) | 1.17 (0-4) | 0.17 (0-1) | 0 (0-0) | NA  |
| Previous DMT, %              | 13.0 | 50.0 | 6.7  | 2.2* |
| DMT at sample time point, %  | 13.0 | 20.8 | 0    | 2.2* |
| Mean IgG index (range)       | 0.93 (0.50-2.35) | 0.85 (0.46-1.77) | 0.92 (0.48-1.9) | 0.50 (0.43-0.62) |
| Oligoclonal IgG bands (+/-/NA) | 20/3/0 | 21/3/0 | 12/3/0 | 2/4/2 |
| Mean CSF albumin quotient (range) | 4.4 (2.6-7.6) | 6.1 (2.4-12.0) | 7.1 (3.1-12.2) | 4.5 (2.5-11.2) |
| No. of brain MRI lesions, %  | 0-2  | 13   | 0    | 7    | NA  |
|                               | 3-5  | 13   | 0    | 0    | NA  |
|                               | 6-8  | 17   | 4    | 21   | NA  |
|                               | ≥9   | 57   | 96   | 72   | NA  |
| Median contrast-enhancing lesions (range) | 1.0 (0-4) | 0 (0-0) | 0 (0-0) | NA |

**Abbreviations:** DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; IgG = immunoglobulin G; NA = not available; OND = other neurologic disease; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

*One individual in the OND group used corticosteroid at the sampling time point and before.

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Sample preparation. Frozen CSF and plasma samples were thawed for 4 minutes at 25°C, then kept on ice at all times. A solution of internal standards (1 ng 8-iso-15(S)-PGF_{2α}-d_4, 1 ng 5(R/S)-iPF_{3α}-d_4, and 2.5 μg 20:4-d_8, in nitrogen-purged ethanol) was added to 1 mL of plasma or CSF and mixed briefly. Samples were subjected to alkaline hydrolysis by adding 1 mL 1 M KOH in methanol containing 0.005% butylated hydroxyltoluene, purging each tube with nitrogen, followed by incubation at 37°C for 30 minutes. Samples were acidified with 2 mL of sodium acetate (100 mM, pH 4.6) and the pH-adjusted to 4.6 using HCl. Following centrifugation (10 minutes, 800 g, 4°C), the supernate was applied to SPE columns preconditioned with 2 mL of methanol, followed by 2 mL of sodium acetate containing 5% (v/v) methanol, pH 7. Columns were washed with 2 mL of methanol/water (1:1, v/v) followed by 2 mL of ethyl acetate/hexane (1:3, v/v). Analytes were eluted with 2 mL of ethyl acetate/methanol (9:1, v/v), evaporated to dryness under nitrogen, and reconstituted in 50 μL of 50% methanol containing 0.01% acetic acid.

Liquid chromatography–tandem mass spectrometry. PGF_{2α}, F_2-IP, and 20:4 in CSF and plasma were quantified by liquid chromatography–tandem mass spectrometry (QQQ 6490 mass spectrometer; Agilent Technologies) with an ESI source and multiple-reaction monitoring in negative ionization mode. A Zorbax Eclipse C18 column (2.1 × 50 mm, 1.8 μm; Agilent Technologies) was used for separation at a flow rate of 0.35 mL/min, with a gradient of A: 0.01% acetic acid in water, and B: 100% methanol. Solvent B was increased initially from 50% to 60% B in the first 15 minutes, then to 90% at 15.1 minutes, 100% at 20 minutes, and held for 3 minutes before re-equilibration at 50%. For quantification, specific ion pairs were monitored: 5-series F_2-IP m/z 353 → 115; 5-IPF_{2α}-VI-d_1, m/z 364 → 115; 15-series F_2-IP m/z 353 → 193; 15-F_2α-IsoP-d_4, m/z 357 → 197; 20:4 m/z 303 → 205; 20:4-d_8 m/z 311 → 213. Quantification was achieved by peak area comparison with the corresponding internal standard, using Mass Hunter software. Only peaks coeluting with internal standard and with a signal-to-noise ratio of ≥ 3 (defined as limit of detection) were quantified. Results were expressed as amount of oxidized lipid per volume or 20:4 content. Samples in which F_2-IP and PGF_{2α} were below detection limit were not considered for statistical analyses, resulting in variable n-numbers for different F_2-IP and PGF_{2α}. The linearity and reproducibility of the assay was confirmed by spiking plasma or CSF before hydrolysis with authentic standards of 5-IPF_{2α}-VI and 15-F_2α-IsoP (0.05–2.5 ng/mL) or 20:4 (0.1–100 μg/mL). Intra- and interday coefficients of variation (calculated from the responses of the internal standards) were 1.8%–12.6% and 6.4%–15.2% for the 5-series F_2-IPs, and 3.1%–12.2% and 2.9%–13.5% for the 15 series F_2-IPs, respectively.

Statistical analyses. Statistical analyses were performed using GraphPad Prism version 6.0 for Macintosh (San Diego, CA). For comparison of median values between ≥ 2 groups, Kruskal-Wallis test with Dunn posttest was used. Because this was an exploratory study, no adjustment for multiple comparisons was made. Correlation analyses were performed using Spearman ranked correlation at 95% confidence interval. For the intervention studies, statistical significance was determined by the Wilcoxon matched-pairs signed rank test.

RESULTS Plasma F_2-IPs decrease with MS progression. We first examined systemic oxidative stress in progressive MS by measuring the concentrations of 20:4, F_2-IP, and PGF_{2α} in plasma of controls with OND and patients with MS at various disease stages. The method used allowed for quantification of 4 series of F_2-IP (figure 1A). Because of the limited availability of authentic standards required for unambiguous assignment of chromatographic peaks, we restricted our analysis to F_2-IP species for which standards were available commercially (figure 1B).

Plasma concentrations of F_2-IP and PGF_{2α} were significantly lower in patients with progressive MS compared to patients with relapsing-remitting MS (RRMS) and OND controls (figure 2). This was true for individual 5- and 15-series and for the sum of products detected for each IP series. The amount of the oxidation products formed can be affected by the concentration of substrate; however, plasma 20:4 concentrations did not vary between controls with OND and patients with MS (figure 2G). These results indicate that MS progression is not associated with increased systemic oxidative stress.

PGF_{2α} but not F_2-IP is increased in CSF from patients with progressive MS. We next determined 20:4 and 20:4 oxidation products in CSF from patients with MS and OND controls. Concentrations of different F_2-IP species (figure 3, A–E) and 20:4 (figure 3G) were comparable in controls and patients. However, CSF of patients with progressive MS contained significantly higher concentrations of PGF_{2α} than CSF of patients with RRMS and OND controls (figure 3F). Normalizing the F_2-IP and PGF_{2α} data to CSF 20:4 did not change the overall results, consistent with the similar concentrations of 20:4 in controls and patients with MS. These results indicate that MS is associated with an increase in enzymatic oxidation of 20:4 rather than general oxidative stress in the CNS.

Associations among PGF_{2α}, disease severity, CSF biomarkers, and age. In CSF, the concentration of PGF_{2α} increased with disease severity assessed by the Expanded Disability Status Scale score, irrespective of whether PGF_{2α} was standardized to CSF volume (r_s = 0.280, p = 0.044) (not shown) or normalized to 20:4 (figure e-1A at Neurology.org/nn). Such association was not observed for 8-iso-15(S) PGF_{2α} (figure e-1B) and other F_2-IP (not shown).

In contrast to PGF_{2α}, none of the validated CSF biomarkers determined correlated with disease severity (figure e-1, C–I). The CSF concentration of PGF_{2α} (ng/μg 20:4) increased with patient age (figure e-1J), although it remained higher in progressive patients compared with age-matched controls with OND (figure e-1K). Also, CSF PGF_{2α} remained higher in patients with secondary progressive MS taking nonsteroidal anti-inflammatory drugs compared with OND controls (figure e-1L). PGF_{2α}
did not correlate significantly with any of the biomarkers determined (figure e-2).

In plasma, the content of PGF$_{2\alpha}$ and each individual F$_2$-IP species decreased with increasing disease severity (figure e-3, A–F). Plasma PGF$_{2\alpha}$ decreased with patient age (figure e-3G) but it remained significantly lower in progressive patients compared with age-matched OND controls (figure e-3H). As a result, the CSF/plasma ratio of PGF$_{2\alpha}$ and F$_2$-IP (except for 8-iso-15(R)-PGF$_{2\alpha}$) were higher in patients with MS who had progressive disease compared with controls with OND (figure e-3I–N), despite a decrease in the CSF/plasma ratio of 20:4 (figure e-3O). These data suggest that the disease-associated increase in CSF PGF$_{2\alpha}$ originates from increased local enzymatic oxidation of 20:4 rather than circulating PGF$_{2\alpha}$. This interpretation is consistent with the absence of MS-associated increase in the CSF/plasma albumin ratio (figure e-3P).

**Treatment effect on CSF lipid oxidation in progressive MS.** To assess whether lipid oxidation in CNS is affected by current MS therapies, we determined CSF F$_2$-IP from patients with progressive MS treated with natalizumab or methylprednisolone.$^{10,11}$ F$_2$-IPs were affected variably by natalizumab: compared with baseline, the contents of 5(R)-iPF$_{2\alpha}$, 5(S)-iPF$_{2\alpha}$, 8-iso-15(R)-PGF$_{2\alpha}$, and 8-iso-15(S)-PGF$_{2\alpha}$ (figure 4, A–D) decreased significantly following 60 weeks of treatment, while 15(R)-PGF$_{2\alpha}$ remained unchanged ($p = 0.563$; figure 4E). In contrast to nonenzymatic 15(R)-PGF$_{2\alpha}$, its corresponding stereoisomer and enzymatic product, 15(S)-PGF$_{2\alpha}$, was significantly decreased after treatment compared with baseline ($p = 0.002$; figure 4F). CSF concentrations of 20:4 did not vary significantly between baseline and treatment (data not shown).

We repeated the above analyses in a separate set of CSF samples from patients with progressive MS at baseline and 60 weeks after treatment with methylprednisolone.$^{11}$ In this cohort of 23 patients, PGF$_{2\alpha}$ was detected in CSF samples of only 7 patients. In all of these patients, methylprednisolone treatment also significantly decreased PGF$_{2\alpha}$ ($p = 0.031$) without changing the content of 20:4 ($p = 0.819$). Similar to

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**Figure 1** Arachidonic acid (20:4) oxidation pathways

![Diagram of Arachidonic acid (20:4) oxidation pathways](image-url)

(A) General structures of the 4 series of F$_2$-isoprostanes formed from 20:4 via radical (R) mediated oxidation. (B) Chemical structures of the 20:4 oxidation products studied (B.a–B.f) and corresponding heavy isotope-labeled internal standards (B.g and B.h). Compounds B.a–B.e are formed via nonenzymatic oxidation of 20:4, whereas B.f is formed enzymatically and nonenzymatically.
the situation with natalizumab, CSF F2-IPs were generally but not consistently lower at 60 weeks compared with baseline, and this did reach statistical significance only in the case of 8-iso-15(S)-PGF2α (p = 0.031). Together, these results suggest that enzymatic lipid oxidation in CNS of progressive MS is decreased by modulating the enzymatic activity of inflammatory cells (with methylprednisolone) or blocking their migration into the CNS (with natalizumab).

**DISCUSSION** There are 2 main new findings in the present study. First, compared with OND controls, the concentration of PGF2α in CSF is elevated in participants with progressive MS, and it increases with disease progression while it is decreased by treatment with natalizumab and methylprednisolone. The disease-associated increase in PGF2α does not simply reflect the heightened state of inflammation and breakdown of the blood–brain barrier typical of RRMS.14 Rather, it may represent pathologic responses to CNS resident cells, as disease severity did not correlate with CSF inflammatory cells and biomarkers, or the albumin ratio. Together, the data suggest that cyclooxygenase-mediated formation of PGF2α may have a role in the pathogenesis of progressive MS.

Second, plasma concentrations of F2-IP were significantly lower in patients with progressive MS than OND controls and patients with RRMS. This is consistent with progressive MS being a consequence of impaired NADPH oxidase-dependent oxidative
dampening of systemic immune activation. A potential implication of these findings is that the use of antioxidants may be counterproductive, and that instead agents increasing NADPH oxidase activity may be worth considering.

Although not commonly recognized, our observation of increased CSF PGF$_{2\alpha}$ in patients with progressive MS is in agreement with previous reports. Thus, cyclooxygenase-2 is expressed in CD64$^+$ macrophages/microglia in actively demyelinating human lesions and in damaged or apoptotic oligodendrocytes. Cyclooxygenase-derived products in addition to PGF$_{2\alpha}$ (PGE$_2$, PGI$_2$, and PGD$_2$) are elevated in the CSF of patients with MS and animal models of the disease. Moreover, cyclooxygenase inhibition improves MS symptoms in models of the disease.

The synthesis of prostaglandins is complex, with their biological effects mediated via specific cellular receptors. Our findings, combined with previous reports, suggest inhibition of prostaglandin synthesis or their action as a potential drug target(s) for the treatment of MS. Because selective cyclooxygenase inhibitors can have severe side effects, a better understanding of the processes that link elevated CSF PGF$_{2\alpha}$ to MS is required to identify appropriate therapeutic targets to treat MS.

In contrast to previous reports of increased oxidative stress in patients with MS, we found no evidence for MS being associated with an increase in CSF F$_2$-IP, considered the “gold standard” for the assessment of in vivo oxidative stress. Consistent with our findings, others also reported no significant difference between patients with MS and controls in their CSF content of 8,12-iPF$_{2\alpha}$-VI (5-series F$_2$-IP), determined by mass spectrometry. However, an increase in the CSF concentration of 8-iso-15(S)-PGF$_{2\alpha}$ was reported in the CSF of patients with MS with F$_2$-IP determined by ELISA. We attribute
these apparent discrepancies to differences in the methods used to determine F2-IP. It is well known that results from ELISA are not directly comparable to those determined by mass spectrometry–based assays,29 with the former being less specific and commonly yielding higher values for 8-iso-15(S)-PGF2α than mass spectrometry–based assays. Thus, our findings are overall consistent with the literature and strongly suggest that oxidative stress is not increased in the CSF of patients with MS, and it does not appear to have a role in disease progression. While our study did not detect an increase in CSF F2-IP in patients with MS, it does not exclude the possibility that localized oxidative stress and damage to brain tissue contribute to the lesions observed in patients with MS.30

MS lesions are thought to arise as a result of the migration of immune cells through the blood–brain barrier, resulting in an inflammatory cascade that leads to myelin loss, axonal damage, and neuronal death.31 Binding of leukocytes α4-integrin to VCAM-1 on the vascular endothelium facilitates the migration of leukocytes across the blood–brain barrier.32 The humanized α4-integrin monoclonal antibody natalizumab blocks the recruitment of circulating immune cells to the CNS33 and it has recently been shown to reduce intrathecal inflammation and tissue damage in patients with progressive MS.34 The observed decrease in enzymatic and some nonenzymatic markers of 20:4 oxidation in the CSF after natalizumab treatment suggests that intrathecal pathogenic events are triggered by systemic immune cells entering the CNS and this may be amenable to treatment. Consequently, the reduced lipid oxidation following natalizumab treatment is driven by decreased intrathecal inflammation. The finding of decreased CSF PGF2α following treatment with the glucocorticoid methylprednisolone34 is also important since we found that treatment was associated with clinical improvement as well as improvement in magnetization transfer ratio measurements in brain MRI studies.11 This may reflect a direct effect of methylprednisolone on the biosynthesis of PGF2α rather than a general inhibition of intrathecal inflammation since we found little effect of monthly methylprednisolone pulse treatment on a panel of other biomarkers in progressive MS.11 The lack of correlation between CSF PGF2α and validated inflammatory biomarkers, and the differences in their response to treatments, further suggests that multiple pathologic mechanisms coexist in patients with MS contributing to the complexity of this disease.

Our results indicate the potential utility of CSF PGF2α as a biomarker of progressive MS. However, the causal role of any abnormal finding in MS, such as an increase in CSF PGF2α, will need experimental support because it can also represent events secondary to the disease process, for example a reduced level of physical activity resulting from ambulation handicap. Currently used MRI biomarkers are adequate for...
patients with RRMS but do not reflect the complex pathogenesis of progressive MS. Although mass spectrometry–based determination of PGF\(_{2a}\) is labor-intensive, the specificity and reproducibility of the quantitative data for the 6 prostanoids investigated (figure 1B), over several separate batches of analyses and involving 3 independent cohorts of patients from 2 different sites, cannot be achieved with other laboratory methods.

The decrease in plasma concentration of several types of F\(_2\)-IP in patients with MS is important. If replicated, plasma F\(_2\)-IP could serve as a more easily obtainable biomarker for progressive MS. The data are consistent with rodent studies that imply a genetic deficiency in NADPH oxidase–derived ROS to contribute to progressive MS via inadequate oxidative dampening of immune competent cells.\(^{2,3}\) Thus, there is a possible role for regulation of the oxidative burst in humans, and genetic characteristics may be decisive in whether, how soon, and how serious the development of progressive MS ensues. To date, genetic studies have largely been restricted to incidence of disease, while correlation with disease severity and progression remains to be determined.

This study shows that PGF\(_{2a}\) is significantly increased in the CSF of patients with progressive MS and that this is associated with disease severity and amenable to treatment, but independent of age. Since PGF\(_{2a}\) has biological activities, such as modulation of immunologic effector cell functions\(^{35,36}\) and vasoconstriction,\(^{37}\) our results warrant future studies addressing whether cyclooxygenase-derived oxidation products of 20:4, including PGF\(_{2a}\), contribute to MS pathogenesis. In addition, the surprisingly low systemic concentrations of F\(_2\)-IP deserve further attention regarding potential mechanisms of disease progression, its treatment, and in relation to their use as systemic biomarker.

**AUTHOR CONTRIBUTIONS**

M. Lam: study design, analyses or interpretation of data, drafting or revising the manuscript. G. Maghzal: analyses or interpretation of data, drafting or revising the manuscript. M. Khademi: study design or conceptualization, analyses or interpretation of data, drafting or revising the manuscript. F. Piehl: study design or conceptualization, analyses or interpretation of data, drafting or revising the manuscript. R. Ratzer: study design or conceptualization, drafting or revising the manuscript. J. Romme Christensen: study design or conceptualization, analyses or interpretation of data, drafting or revising the manuscript. F. Sellebjerg: study design or conceptualization, analyses or interpretation of data, drafting or revising the manuscript. T. Olsson: study design and conceptualization, analyses or interpretation of data, drafting or revising the manuscript. R. Stocker: served on the editorial board for *Free Radical Biology & Medicine*, Antioxid Redox Signaling, Redox Report, Redox Biology, Archives of Biochemistry and Biophysics, received research support from AstraZeneca, Swedish Research Council, Danish Multiple Sclerosis Society, Lounkær Foundation. T. Olsson served on the scientific advisory board for Merck, Biogen Idec, Genzyme, Sanofi-Aventis, Teva, Novo Nordisk, served on the scientific advisory board for Biogen Idec, received research support for Biogen Idec, Sanofi-Aventis, Novartis, Danish Strategic Research Council, Danish Multiple Sclerosis Society, Lounkær Foundation. T. Olsson served on the scientific advisory board for Merck, Biogen Idec, Genzyme/Sanofi-Aventis, Novartis, received speaker honoraria from Biogen, Bayer Schering, Genzyme, Merck Serono, Sanofi-Aventis, Schering-Plough, Teva, is a section editor for *Multiple Sclerosis and Related Disorders*, consulted for Biogen Idec, received research support from Biogen Idec, Sanofi-Aventis, Novartis, Danish Strategic Research Council, Danish Multiple Sclerosis Society, Lounkær Foundation. T. Olsson served on the scientific advisory board for Merck Serono, Biogen Idec, Genzyme/Sanofi-Aventis, Novartis, received speaker honoraria from Biogen Idec, Genzyme, Sanofi-Aventis, Merck, Genzyme, MedImmune, was coeditor for *Current Opinion in Immunology*, received research support from Merck, Biogen Idec, Sanofi-Aventis, Bayer, Novartis, AstraZeneca; The Swedish Research Council, Eurotrans Neuronox, CombiMS, Swedish Brain Foundation, AFA Foundation, Knut and Alice Wallenberg Foundation, Genzyme, EURATrans. R. Stocker served on the editorial board for *Free Radical Biology & Medicine*, Antioxid Redox Signaling, Redox Report, Redox Biology, Archives of Biochemistry and Biophysics, received research support from AstraZeneca, National Health and Medical Research Council of Australia, Office of Health and Medical Research, NSW State Government, Australia. Go to Neurology.org/nm for full disclosure forms.

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Absence of systemic oxidative stress and increased CSF prostaglandin F$_{2\alpha}$ in progressive MS
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