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Active Cigarette Smoking in Cognitively-Normal Elders and Probable Alzheimer's Disease is Associated with Elevated Cerebrospinal Fluid Oxidative Stress Biomarkers

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Abstract

Neurodegenerative diseases and chronic cigarette smoking are associated with increased cerebral oxidative stress (OxS). Elevated F_2-isoprostane levels in biological fluid is a recognized marker of OxS. This study assessed the association of active cigarette smoking with F_2-isoprostane in concentrations in cognitively-normal elders (CN), and those with mild cognitive impairment (MCI) and probable Alzheimer's disease (AD). Smoking and non-smoking CN (n = 83), MCI (n = 164), and probable AD (n = 101) were compared on cerebrospinal fluid (CSF) $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI F_2-isoprostane concentrations. Associations between F_2-isoprostane levels and hippocampal volumes were also evaluated. In CN and AD, smokers had higher $iPF_{2\alpha}$-III concentration; overall, smoking AD showed the highest $iPF_{2\alpha}$-III concentration across groups. Smoking and non-smoking MCI did not differ on $iPF_{2\alpha}$-III concentration. No group differences were apparent on 8,12, *iso*-$iPF_{2\alpha}$-VI concentration, but across AD, higher 8,12, *iso*-$iPF_{2\alpha}$-VI level was related to smaller left and total hippocampal volumes. Results indicate that active cigarette smoking in CN and probable AD is associated with increased central nervous system OxS. Further investigation of factors mediating/moderating the absence of smoking effects on CSF F_2-isoprostane levels in MCI is warranted. In AD, increasing magnitude of OxS appeared to be related to smaller hippocampal volume. This study contributes additional novel information to the

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mounting body of evidence that cigarette smoking is associated with adverse effects on the human central nervous system across the lifespan.

Keywords

Alzheimer's disease; cigarette smoking; F_2-isoprostanes; hippocampus; mild cognitive impairment

INTRODUCTION

The adverse effects of chronic cigarette smoking extend well beyond cardiovascular disease, chronic obstructive pulmonary diseases, and cancers, and includes neurobiological and neurocognitive deficits, some of which are progressive over time, and are not directly attributable to the foregoing biomedical conditions [1–4]. Specifically, active cigarette smoking in young-to-elder adults, without a history of clinically significant biomedical or psychiatric conditions, is associated with significant abnormalities in brain morphology, blood flow, biochemistry, microstructural integrity, as well as in multiple neurocognitive domains of functioning [5–7]. Additionally, we observed that a history of cigarette smoking in cognitively-normal elders was associated with a markedly increased *in vivo* cortical amyloid deposition, and decreased cortical glucose metabolism [8]. Correspondingly, in postmortem studies, cigarette smoking in both cognitively-normal elders and those with pathologically confirmed Alzheimer's disease (AD), is associated with greater amyloid-β and/or hyperphosphorylated tau levels (see [6] for review). Taken together, the foregoing neurobiological abnormalities observed in chronic cigarette smokers indicate potential mechanisms that link smoking to the significantly increased risk for AD reported in large scale epidemiological studies (see [6] for review). It is suggested that an international decrease in the prevalence of smoking (and its associated biomedical morbidities) would promote a decrease in the prevalence of AD worldwide [9, 10].

Cigarette smoke is a complex admixture of approximately 5000 combustion products that contains extremely high concentrations of short-and-long-lived free radicals [11, 12], and smoking inhibits synthesis of essential endogenous intracellular anti-oxidants, such as glutathione [13, 14]. Therefore, cigarette smoke appears to serve as a major contributor to amplified oxidative stress (OxS) in multiple organ systems in humans [6]. Cerebral OxS is operationalized as the detection of biomarkers of brain tissue damage (e.g., lipid peroxidation, proteolysis) subsequent to exposure to reactive oxygen species (ROS), or more broadly by damage from ROS, reactive nitrogen species (RNS), and other oxidizing agents [15–17]. The human brain is exceedingly vulnerable to OxS-related damage due to its high metabolism, low levels of antioxidant enzymes (e.g., glutathione peroxidase, catalase) and susceptibility of membrane phospholipids to radical attack and oxidizing agents [18]. Hippocampal neurons are particularly predisposed to OxS-related injury [17]. Correspondingly, smoking is associated with smaller hippocampal volume in middle-aged adults [19], and long-term, low intensity exposure to cigarette smoke inhibits neurogenesis in the dentate gyrus of the hippocampus in adult mice [20]. OxS in the central and peripheral nervous system that is induced by cigarette smoke is suggested as a mechanism initiating the multiple neurobiological abnormalities observed in smokers [6, 21]. OxS is also implicated

in the initiation of increased brain amyloid deposition observed in mild cognitive impairment (MCI) and AD (see [6] for review).

Elevated F_2-isoprostane level in biological fluid is a recognized biomarker of radical-induced OxS [22], and has been employed to assess OxS-related tissue damage in neurodegenerative diseases, atherosclerosis, pulmonary diseases, and chronic smoking [23–28]. F_2-isoprostanes are prostaglandin-like compounds derived from free radical-mediated peroxidation of arachidonic acid, a highly abundant polyunsaturated fatty acid in brain neuronal and glial tissue [25, 26]. Multiple studies have reported significantly elevated cerebrospinal fluid (CSF) F_2-isoprostane levels in those with MCI and AD (see [24, 29] for review). We recently reported that cognitively normal elders with a history of cigarette smoking demonstrated significantly higher CSF F_2-isoprostane concentrations relative to never-smokers [21]. However, it is unknown if cigarette smoking in MCI and AD is associated with increased CSF F_2-isoprostane levels.

Here, we assessed the effects of cigarette smoking on CSF $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI F_2-isoprostane concentrations in cognitively-normal elders (CN), MCI, and AD. In this report, we adhere to the F_2-isoprostane nomenclature suggested by Rokach and colleagues [30]. $iPF_{2\alpha}$-III (alternate nomenclatures: 8-*iso*-$PGF_{2\alpha}$; 15-F_{2t}-IsoP) and 8,12, *iso*-$iPF_{2\alpha}$-VI (alternate nomenclature: 5-F_{2c}-IsoP) are most studied of the 64 constitutional F_2-isoprostanes isomers, with the majority of research focused on $iPF_{2\alpha}$-III [24, 26]. Smoking serves as an exogenous source of OxS [6]; therefore, we predicted that cigarette smoking status (active smoker, non-smoker) interacts with diagnostic group (CN, MCI, and AD), where non-smoking CN demonstrate lowest and smoking AD show the highest CSF F_2-isoprostane levels. Given the vulnerability of the hippocampus to OxS [17], we predicted that higher CSF $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI concentrations, across diagnostic groups, are associated with smaller hippocampal volumes.

MATERIALS AND METHODS

Participants and study design

Participants were 83 CN, 164 MCI, and 101 AD from the Alzheimer's Disease Neuroimaging Initiative (ADNI) project, Phase 1 (PI: Michael W. Weiner). Phase 1 of ADNI (ADNI1) was a multisite study supported by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the FDA, private pharmaceutical companies, and non-profit organizations, as a 5-year public-private partnership. The primary goal of ADNI1 was to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biomarkers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD [31, 32]. Written informed consent was obtained from all participants before procedures were performed. The study was conducted according to the Declaration of Helsinki, and U.S. 21 CFR Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards. Inclusion criteria for CN participants were mini-mental state examination (MMSE) scores of 24–30, Clinical Dementia Rating (CDR) 0. MCI participants had MMSE scores from 24–30, subjective memory complaints, and objective memory dysfunction (adjusted for education) as measured by Wechsler Memory Scale Logical Memory II, CDR of 0.5, absence of

significant levels of impairment in other cognitive domains, essentially preserved activities of daily living, and did not meet diagnostic criteria for dementia. AD subjects had MMSE scores from ≤26, CDR from 0.5–1, and met the NINCDS-ADRDA criteria for probable AD. See http://www.adni-info.org for additional details on ADNI1 CN, MCI and AD inclusion/exclusion criteria. Participants were designated as non-smokers if they responded "no" and as smokers if they responded "yes" to the smoking status question at screening. Detailed information on smoking history (e.g., duration of lifetime smoking, cigarettes consumed per day) was not collected for ADNI1. Individuals who indicated they were previous smokers were excluded. Six groups were formed: non-smoking CN (nsCN), smoking CN (sCN), non-smoking MCI (nsMCI), smoking MCI (sMCI), non-smoking AD (nsAD), smoking AD (sAD). Antioxidant (vitamins C and E, omega 3), antihypertensive, antidepressant (primarily serotonergic specific reuptake inhibitors), and statin/cholesterol absorption blocking agents (statin/CAB) usage was recorded (binary variable – yes, no) and body mass index (BMI) calculated for all participants. See Table 1 for group demographic and clinical information.

CSF iPF$_{2\alpha}$-III and 8,12, iso-iPF$_{2\alpha}$-VI acquisition and quantitation

Procedures for CSF lumbar puncture sampling, transport, and storage are detailed in Shaw and colleagues [33]. The iPF$_{2\alpha}$-III and 8,12, *iso*-iPF$_{2\alpha}$-VI quantitation was accomplished with a HPLC-atmospheric pressure chemical ionization-tandem mass spectrometry method that demonstrates high sensitivity and selectivity for these F_2-isoprostanes [26].

MRI acquisition and processing

Participants completed a 1.5 Tesla magnetic resonance (MR) scan within approximately 3 weeks of collection of CSF. T1-weighted MR imaging scans using 3D volumetric magnetization prepared rapid gradient echo (MPRAGE) and 3D T2-weighted sequences were acquired for morphological analyses (see [34] for MR acquisition parameter details). All images were calibrated with phantom-based geometric corrections to ensure consistency among different study sites [35]. Volumetric segmentation and cortical surface reconstruction methods [36–39] was conducted with Freesurfer (v4.5) to obtain regional cortical and subcortical brain volumes (mm^3) from T1-weighted images, including the bilateral hippocampus, which was the target region of interest in this study. Total hippocampal volume was calculated from the sum of the left and right hippocampi. Total white matter hyperintensity volume was calculated from T2-weighted images [40].

Statistical analyses

Group comparisons demographic and clinical variables were conducted with univariate analysis of variance, Kruskal-Wallis H test or Fisher's exact test, where appropriate. Square root transformations of iPF$_{2\alpha}$-III and 8,12, *iso*-iPF$_{2\alpha}$-VI concentrations were performed due to their skewed distributions, and the transformations produced symmetrical distributions for both F_2-isoprostanes. Group comparisons on iPF$_{2\alpha}$-III and 8,12, *iso*-iPF$_{2\alpha}$-VI concentrations were conducted with generalized linear modeling. Diagnostic group (CN, MCI, AD), smoking status (smoker, non-smoker), age, sex, APOE4 carrier status, BMI, vitamin E and omega 3 supplementation, antidepressant and statin/CAB use, and the diagnostic group × smoking status interaction were used as predictors. BMI, antioxidant supplementation (i.e., vitamin E and omega 3), and statin/CAB use were used as predictors

because they were associated with F_2-isoprostanes levels in previous research [21, 23, 25]. Follow-up pairwise *t*-tests comparing nsCN, sCN, nsMCI, sMCI, nsAD, and sAD were conducted if main effects for diagnostic group, smoking status and/or the group × smoking status interaction were statistically significant. T-tests evaluating the hypothesis of lowest mean F_2-isoprostanes levels in nsCN and highest in sAD relative to the other groups were considered statistically significant at $p < 0.05$. All other group comparisons were corrected for multiplicity of comparisons with a standard Bonferroni correction (.05/6 possible pairwise comparisons; adjusted $p < 0.008$). Effect sizes for statistically significant mean F_2-isoprostanes differences between groups were calculated with Cohen's d. Associations between $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI concentrations, left, right and total hippocampal volumes were examined with linear regression (part coefficients are reported) separately for CN, MCI, and AD. Covariates for these analyses were age, sex, antioxidant supplementation and statin/CAB use, intracranial volume, smoking status, and APOE ε4 (APOE4) carrier status (carrier, non-carrier). APOE4 carrier status was included as a covariate because APOE4 carriers demonstrated increased hippocampal volume loss in CN, MCI, and AD [41–43]. *P*-values <0.05 were considered statistically significant for the a priori predicted associations between $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI levels and hippocampal volumes in CN, MCI, and AD.

RESULTS

Participant characteristics

See Table 1 for demographic and clinical characteristics and group comparisons on these variables.

Group comparisons on CSF $iPF_{2\alpha}$-III and 8,12, iso-$iPF_{2\alpha}$-VI concentration

$iPF_{2\alpha}$-III concentration—A diagnostic group × smoking status interaction [($\chi^2(2) = 7.6$, $p = 0.022$] and main effects for smoking status [($\chi^2(1) = 9.9$, $p = 0.002$], vitamin E supplementation [($\chi^2(1) = 5.4$, $p = 0.021$], antidepressant usage [($\chi^2(1) = 14.1$, $p < 0.001$], BMI [($\chi^2(1) = 13.7$, $p < 0.001$], and education [($\chi^2(1) = 7.1$, $p = 0.008$] were observed for $iPF_{2\alpha}$-III level (see Fig. 1). Sex, age, and APOE4 carrier status were not significant predictors (all $p > 0.20$). Across diagnostic groups, smokers showed higher $iPF_{2\alpha}$-III level than non-smokers. Participants who took vitamin E had a lower level, and those who took antidepressants had a higher $iPF_{2\alpha}$-III level, across diagnostic groups. Higher BMI was associated with a higher $iPF_{2\alpha}$-III concentration and higher education was related to a lower $iPF_{2\alpha}$-III level. Pairwise comparisons indicated sCN [$p = 0.014$, effect size (ES) = 0.60], nsMCI ($p = 0.007$, ES = 0.42), sMCI ($p = 0.032$, ES = 0.42), and sAD ($p < 0.001$, ES = 0.84) had higher $iPF_{2\alpha}$-III level than nsCN. sAD had a higher $iPF_{2\alpha}$-III concentration than nsAD ($p = 0.005$, ES = 0.64). sAD showed trends for higher $iPF_{2\alpha}$-III level than nsMCI ($p = 0.08$) and sMCI ($p = 0.07$). There were no significant differences between nsMCI and sMCI ($p = 0.92$).

8,12, iso-$iPF_{2\alpha}$-VI concentration—A diagnostic group × smoking status interaction [($\chi^2(2) = 9.1$, $p = 0.011$] and main effects for vitamin E [($\chi^2(1) = 28.0$, $p < 0.001$] and omega 3 supplementation [($\chi^2(1) = 5.4$, $p = 0.021$], antidepressant usage [($\chi^2(1) = 11.5$, $p <$

0.001], age [($\chi^2(1) = 12.9$, $p = 0.002$], BMI [($\chi^2(1) = 8.8$, $p = 0.003$] and education [($\chi^2(1) = 12.8$, $p < 0.001$] were observed for 8,12, *iso*-iPF$_{2\alpha}$-VI concentration. Sex and APOE4 carrier status were not significant predictors (both $p > 0.10$). Across diagnostic groups, participants who took vitamin E and omega 3 supplements had a lower 8,12, *iso*-iPF$_{2\alpha}$-VI level, and those who used antidepressants had a higher 8,12, *iso*-iPF$_{2\alpha}$-VI concentration. Higher age and BMI were both related to higher 8,12, *iso*-iPF$_{2\alpha}$-VI level, while greater education was associated with lower 8,12 *iso*-iPF$_{2\alpha}$-VI concentration. Despite the significant diagnostic group *x* smoking status interaction, follow-up pairwise comparisons yielded no significant group differences.

Associations of CSF iPF$_{2\alpha}$-III and 8,12, iso-iPF$_{2\alpha}$-VI concentrations with hippocampal volumes—In AD, smaller left (r = −0.27, $p = 0.008$) and total hippocampal (r = −0.21, $p = 0.038$) volumes were associated with higher 8,12, *iso*-iPF$_{2\alpha}$-VI concentration (see Fig. 2); no significant associations were found for the right hippocampus (r = –0.12, $p = 0.24$). Both nsAD and sAD showed similar magnitude relationships between 8,12, *iso*-iPF$_{2\alpha}$-VI concentration and left, right and total hippocampal volumes (data not shown). No significant associations between left, right, or total hippocampal volumes and 8,12, *iso*-iPF$_{2\alpha}$-VI level were observed in CN or MCI. No significant relationships between left, right or total hippocampal volumes and iPF$_{2\alpha}$-III concentration were found in any group.

DISCUSSION

In this study, active smokers in the CN and AD groups showed significantly higher iPF$_{2\alpha}$-III levels than their non-smoking counterparts, and the largest magnitude difference in iPF$_{2\alpha}$-III concentration was observed between nsCN and sAD. No significant differences in iPF$_{2\alpha}$-III concentration were observed between actively smoking and non-smoking MCI. A previous study [44] reported higher CSF 8,12, *iso*-iPF$_{2\alpha}$-VI concentration in both MCI and AD relative to CN, but in the present report, diagnostic group (i.e., CN, MCI, AD) and smoking status were unrelated to 8,12, *iso*-iPF$_{2\alpha}$-VI level. The absence of significant differences between nsCN versus nsAD and nsMCI versus sMCI on iPF$_{2\alpha}$-III concentration, as well as the lack of group differences on 8,12, *iso*-iPF$_{2\alpha}$-VI level, cannot be specifically attributed to group disparities on APOE4 carrier frequency, salient demographic (e.g., age, education, sex) or clinical variables (e.g., antioxidant supplementation, antidepressant usage, BMI) because groups were equivalent on these variables and/or models comparing groups on the F$_2$-isoprostanes levels included these predictors as covariates. While differences among MCI, CN and AD on relevant genetic, clinical and demographic variables included in this study do not appear to account for the lack of smoking effects in MCI, information of smoking history (e.g., lifetime years of smoking, cigarettes smoked per day) was not specifically collected in for the majority of ADNI1 participants. Some studies reported that greater smoking severity (e.g., greater number of cigarettes smoked/day) were associated with increased urine F$_2$-isoprostane levels (see [45] and references therein). While speculative, it is possible that sMCI may have a less severe smoking history compared to sCN and sAD, which may, at least partially, explain the lack of differences between sMCI and nsMCI on iPF$_{2\alpha}$-III concentration.

The association between F_2-isoprostane levels and hippocampal volume was examined because of the high vulnerability of hippocampal tissue to OxS [17]. Although there were no significant group differences on 8,12, *iso*-$iPF_{2\alpha}$-VI level, a higher concentration was associated with smaller left and total hippocampal volume across AD participants. No significant associations were observed between 8,12, *iso*-$iPF_{2\alpha}$-VI level and hippocampal volumes for CN or MCI. The lack of a significant relationship between 8,12, *iso*-$iPF_{2\alpha}$-VI concentration and hippocampal volume in CN and MCI was not due to a restriction of ranges in these groups because they showed statistically equivalent variances to AD on 8,12, *iso*-$iPF_{2\alpha}$-VI concentration and hippocampal volume (data not shown). Previously [21], we found that higher 8,12, *iso*-$iPF_{2\alpha}$-VI level was related to smaller total hippocampal volume in sCN; however, that smoking sample was composed of both active and former smokers. In the current study, we did not include self-identified former smokers, which reduced the sCN sample size by approximately 50%, and, correspondingly, diminished the power to detect significant associations in this group. Consistent with our previous study with CN [21], $iPF_{2\alpha}$-III level was not related to hippocampal volumes in any group. $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI are two of 64 F_2-isoprostane constitutional isomers [22]. $iPF_{2\alpha}$-III may possess bronchoconstrictive and vasoconstrictive properties, while 8,12, *iso*-$iPF_{2\alpha}$-VI is reported to have no biological effects [46]. However, the relationship between 8,12, *iso*-$iPF_{2\alpha}$-VI level and hippocampal volume in AD and CN may indicate that this isomer is a unique proxy for OxS-related damage to hippocampal neuronal and/or glial tissue. Elevated OxS biomarkers indicate that the endogenous antioxidant system is compromised and/or overwhelmed, which leaves cellular components susceptible to damage by ROS/RNS and other oxidizing agents [6]. Since most central nervous system neurons are post-mitotic (i.e., do not proliferate), the cumulative structural damage secondary to chronic OxS may be enduring [21, 28]. This stresses the relevance of the association between higher 8,12, *iso*-$iPF_{2\alpha}$-VI and smaller hippocampal volumes in AD in the current study and in sCN in our previous report.

The increased cerebral amyloid deposition in MCI and AD is suggested to serve as a secondary endogenous source of cerebral OxS. Alternately, exogenously induced OxS is associated with increased β-and-y-secretase cleavage of amyloid-β protein precursor that results in elevated extracellular fibrillar amyloid deposition (see [6] for review). It is well established that smoking is associated with elevated central nervous system OxS; therefore, smoking-related OxS may directly facilitate or amplify neurodegeneration and the proteolytic pathway that promotes cerebral amyloid-β deposition (see [6] for review). Previously, we found that sCN, adjusting for APOE genotype, demonstrated significantly increased cortical amyloid deposition via ^{18}F florbetapir positron emission tomography than nsCN [8]. The higher $iPF_{2\alpha}$-III level in actively smoking sCN and sAD, compared to nsCN, in this report suggests that smoking-related OxS may serve as an exogenous factor promoting the markedly elevated cortical amyloid levels observed in these groups. Correspondingly, postmortem studies found smoking in CN and AD was associated with increased cerebral amyloid and phosphorylated tau levels (see [6] for review).

Antioxidant supplementation, particularly vitamin E, was associated with lower $iPF_{2\alpha}$-III and 8,12, *iso*-$iPF_{2\alpha}$-VI levels across CN, MCI, and AD, which is consistent with previous animal and human studies [25, 28, 47]. In this study, antidepressant use (SSRIs were the

predominant antidepressant) was associated with increased iPF$_{2\alpha}$-III and 8,12, *iso*-iPF$_{2\alpha}$-VI concentrations across groups, and an earlier investigation found depressed middle-aged smokers showed higher plasma biomarkers of oxidative stress than depressed non-smokers [48]. However, contrary to our findings, antidepressant use was related to decreased OxS biomarkers in those with major depressive disorders [49]. SSRIs and other antidepressants may decrease cerebral OxS through suppression of proinflammatory cytokines and ROS/RNS production or by enhancing enzyme-based antioxidant defense such as superoxide dismutase or catalase [49]. The factors promoting the higher F_2-isoprostanes levels in those taking antidepressants in this study require further investigation.

This study has other limitations that may influence the generalizability of the findings. The participants were predominately well-educated elder Caucasians. Detailed smoking information (e.g., duration of lifetime smoking, cigarettes consumed per day) was not available for these ADNI1 participants. Smoking in the US promotes at least a 10-year reduction in life expectancy [50], which may create a survivor bias due to premature death. Therefore, assessment of the effects of smoking in elders may be biased toward the healthiest smokers—those individuals who survived or did not experience significant smoking-related morbidity [51] that would have excluded them from participation in ADNI. Consequently, the effects of smoking on F_2-isoprostane levels in this elder sample may be underestimated due to survivor bias.

Results indicate that current cigarette smoking in cognitively-normal elders and individuals with probable AD is associated with increased central nervous system OxS. The greatest magnitude differences in iPF$_{2\alpha}$-III level was between nsCN and sAD. To our knowledge, this is the first *in vivo* study to demonstrate cigarette smoking in AD was related to increased CSF F_2-isoprostane biomarkers of OxS, and that higher 8,12, *iso*-iPF$_{2\alpha}$-VI level in AD was associated with smaller hippocampal volume. Smoking status in MCI was unrelated to F_2-isoprostane levels, and further investigation of factors mediating/moderating the absence of smoking-related effects in this group is warranted and may assist in identifying additional vulnerability/resiliency factors related to the neurobiological consequences of smoking for the human brain. Additionally, since smoking-related OxS is proposed as a potential mechanism promoting AD neuropathology (see [6] for review), additional longitudinal research is necessary on the potential unique characteristics of CSF iPF$_{2\alpha}$-III and 8,12, *iso*-iPF$_{2\alpha}$-VI as biomarkers of smoking-related OxS in the central nervous system and their association biomarkers of AD-related neuropathology (e.g., CSF or PET measurements of amyloid-β and tau levels) in smokers. This study contributes additional novel information to the mounting body of evidence that cigarette smoking is associated with adverse effects on the human central nervous system across the lifespan.

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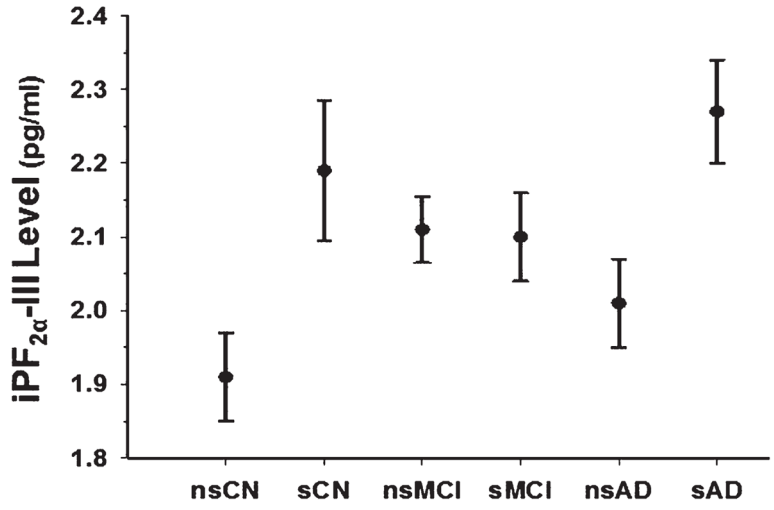

Fig. 1.

Group CSF iPF$_{2\alpha}$-III levels (square root transformed; mean ± standard error of the mean).

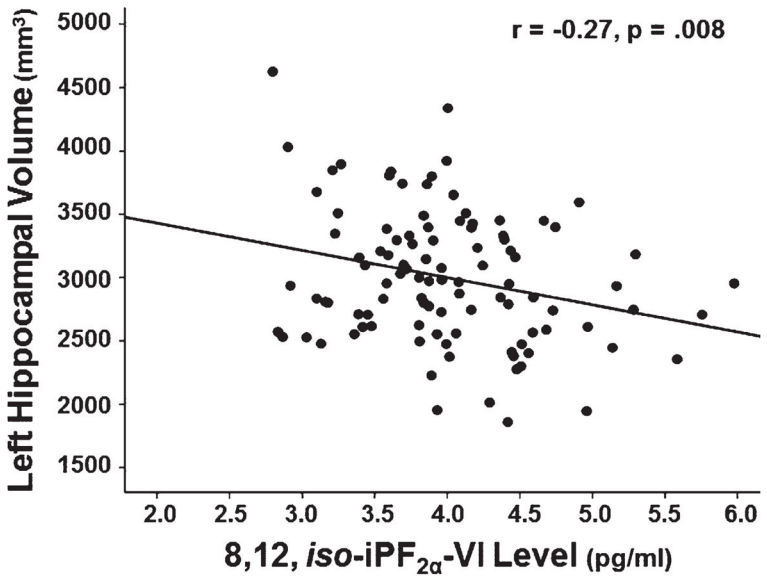

Fig. 2.

Association between CSF 8,12, *iso*-iPF$_{2\alpha}$-VI level (square root transformed) and left hippocampal volume in AD.

Table 1

Group demographics and clinical variables

Measures	**nsCN (*n* = 60)**	**sCN (*n* = 23)**	**nsMC (*n* = 108)**	**sMCI (*n* = 56)**	**nsAD (*n* = 59)**	**sAD (*n* = 42)**
Age	76.2 ± 5.5	75.6 ± 5.3	75.6 ± 5.3	76.1 ± 6.9	74.2 ± 8.0	75.0 ± 7.3
Education	15.9 ± 2.9	15.3 ± 2.5	15.9 ± 3.0	15.1 ± 3.1	15.0 ± 3.5	15.3 ± 3.0
Male (%)[a]	40	65	60	71	47	71
MMSE[b]	29.3 ± 0.90	28.8 ± 1.3	27.0 ± 1.8	26.6 ± 1.7	23.5 ± 1.8	23.4 ± 2.7
GDS[c]	0.7 ± 1.0	0.9 ± 1.0	1.5 ± 1.4	1.7 ± 1.2	1.8 ± 1.5	1.5 ± 1.2
BMI	25.7 ± 4.1	26.0 ± 3.3	25.8 ± 4.0	25.9 ± 3.2	25.3 ± 4.0	25.8 ± 3.5
Log Triglycerides (mg/dl)	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.3	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.3
Vitamin E use (%)	27	17	32	25	32	28
Omega 3 use (%)[d]	19	4	17	11	5	12
Antihypertensive use (%)	37	21	33	37	41	37
Statin/cholesterol absorption inhibitor use (%)	37	41	33	42	39	40
Antidepressant use (%)[e]	11	4	27	16	29	16
APOE 4 carriers (%)[f]	16	8	54	34	44	27
Log white matter hyperintensity (cc)	−0.7 ± 0.8	−0.7 ± 0.8	−0.6 ± 0.7	−0.6 ± 0.7	−0.5 ± 0.7	−0.5 ± 0.8

MMSE, Mini-Mental Status Examination; GDS, Geriatric Depression Scale; BMI, body mass index;

[a] sMCI and sAD > nsCN;

[b] nsCN & sCN > nsMCI & sMCI > sAD & nsAD;

[c] nsCN & sCN > nsMCI & sMCI = sAD & nsAD;

[d] nsCN > sCN & nsAD;

[e] nsMCI & nsAD > sCN;

[f] nsMCI, sMCI & nsAD > nsCN & sCN (all listed group comparisons $p < 0.05$).