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Plasma F₂-isoprostane level and cognitive function over eight years in non-demented older adults: Findings from the Health ABC Study

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Summary

F₂-isoprostanes (F₂-IsoP) are reportedly increased in dementia patients and are considered a reliable biomarker of oxidation. However, few studies have examined the predictive value of peripheral F₂-IsoP levels in non-demented older adults. This study assesses the association between plasma F₂-IsoP and change in cognitive function in non-demented elderly over eight years. Plasma F₂-IsoP was measured by gas chromatography-mass spectrometry in a biracial cohort of 726 elderly men and women. Digit Symbol Substitution test and the Modified Mini-Mental State Exam were administered over time. No association was found between F₂-IsoP tertile and baseline or change (slope) in cognitive function over eight years. Plasma F₂-IsoP is not a valuable biomarker in predicting cognitive change over years in non-demented older adults.

INTRODUCTION

Oxidative stress, defined as an imbalance between exposure to free radicals and antioxidant defenses, plays an important role in the development of neurological disease, including Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD)[1]. Animal studies show age-related increases in oxidative damage in the hippocampus and frontal cortex homogenates

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due to products of lipid peroxidation [2-4]. Lipid peroxidation is particularly toxic to the brain as it alters properties of the cell membrane and the function of membrane-bound receptors and enzymes [5]. Over the past few years, F₂-isoprostanes (F₂-IsoP) have gained attention as a reliable marker of oxidation. F₂-IsoPs are prostoglandin-like compounds produced through the auto-oxidation of arachidonic acid *in vivo* that provide a valuable indicator of lipid peroxidation in humans. F₂-IsoPs are relatively stable molecules that can be detected in measurable quantities in all body tissues and fluids, and are considered to be a reliable non-invasive method to assess oxidation *in vivo* (for review see [6,7]).

It is suggested that oxidant stress and the production of reactive oxygen species in the brain plays an important role in the pathogenesis of Alzheimer's disease [8]. Thus, recent studies have examined the relationship between F₂-IsoP levels and cognitive impairment. The majority of research has entailed case-control studies, examining F₂-IsoP levels in postmortem brains and in cerebral spinal fluid (CSF). Using gas chromatography-mass spectrometry assay, some postmortem studies have reported evidence of increased lipid peroxidation in AD brains compared to healthy controls [9,10], while others have reported negative findings [11,12]. Enhanced levels have been noted in frontal, temporal, and parietal cortex, and in the hippocampus of postmortem AD brains.

Studies that have analyzed F₂-IsoP in CSF consistently report that levels are increased in AD patients compared to healthy controls [13-15]. Furthermore, F₂-IsoP levels are reported to increase with an increase in severity of dementia [11,15] and are also reported to be higher in apolipoprotein E (APOE) e4 carriers compared to those without the e4 allele, suggesting that APOE status may influence how the brain responds to oxidative injury [15]. In a longitudinal study that assessed the value of CSF F₂-IsoP as a biological markers in the progression from mild cognitive impairment (MCI) to AD, it was found that individuals who declined over time, either from normal to MCI and MCI to AD (n=8) displayed higher CSF F₂-IsoP levels at baseline compared to stable normal controls [13]. It is reported that F₂-IsoP measured in CSF significantly improves the diagnostic accuracy and predictive outcomes over memory testing and quantitative magnetic resonance imaging (MRI) measurement [13]. To this end, it has been suggested that F₂-IsoP may serve as a valuable biomarker in the early detection of AD pathology.

Although CSF closely corresponds to brain composition and is thus likely to provide the most consistent and reliable biomarker in the evaluation for dementia, CSF is an invasive procedure that is not routinely performed in practice. Identification of biomarker molecules in peripheral measures, such as blood, would provide a means for less invasive, expensive and time-consuming testing.

Studies that have evaluated peripheral F₂-IsoP markers are less consistent. One group reported that AD and MCI patients [16] display elevated F₂-IsoP in urine and in blood, compared to healthy controls. Conversely, negative findings have also been reported for both peripheral F₂-IsoP measures [17,18]. In a study that assessed plasma F₂-IsoP in normal controls, MCI, AD, and Parkinson patients, no group differences were observed. Further, authors reported that levels did not correlated with duration of disease or cognitive performance [18]. However, the high frequency of anti-oxidant use among MCI and AD patients, which could potentially decrease F₂-IsoP levels, may have dampened any detectable group differences.

Despite growing evidence that oxidative stress is implicated in the development of dementia and cognitive impairment [13,15], little research has assessed the relationship between less invasive peripheral measures of F₂-IsoP and prospective cognitive function in non-demented older adults. Although differences in biological markers are often found to differ between

groups in case-control studies, biomarkers that can predict decline in cognitive function in the preclinical phase, years before dementia diagnosis, are of great value as they 1) add to the biology of the disease, 2) may help identify those at risk for AD or other dementias, and 3) emphasize that these diseases are a continuum. Furthermore, the elucidation of biomarkers that are non-invasive can have significant clinical implications for the detection and prevention of adverse cognitive decline.

Our goal was to examine the association between plasma F₂-IsoP levels and change in cognitive function over eight years in a large biracial cohort of non-demented elderly men and women. Given previous findings in the dementia literature, we hypothesized that higher F₂-IsoP levels in plasma would be observed in elderly displaying greater decline in cognitive function over eight years.

MATERIALS AND METHODS

Study Population

The present study involved a sub-cohort random sample of participants from the Health, Aging and Body Composition (Health ABC) study with available plasma F₂-IsoP measurement. The sub-cohort was randomly selected within strata by race, sex, and prevalent diabetes status. The subsample consisted of 726 non-demented community-dwelling black and white men and women aged 70 to 79 upon recruitment in 1997. Participants from the original cohort (N=3075) were randomly selected within designated zip code areas in Memphis, TN, and Pittsburgh, PA. Eligibility requirements included no reported difficulty with activities of daily living, walking a quarter of a mile, or climbing 10 steps without resting. Participants also had to be free of life-threatening cancer diagnoses and did not plan to move out of the study area within 3 years. All eligible participants signed a written informed consent, approved by the institutional review boards (IRB) at the clinical sites and the coordinating center (University of California, San Francisco). For details of entire cohort, see [19]. Participants with at least one time point were included in the present analyses.

Measurements

Baseline Demographic and Health Factors—Demographic factors assessed at baseline include: age, race (white, black), sex, and self reported education level (categorized as < or ≥ high-school). Baseline health characteristics included self-reported health (categorized as good, very good, or excellent versus fair or poor), and current smoking status. Depressive symptoms were assessed with the 20-item Center for Epidemiologic Studies-Depression Scale (CES-D; [20] with participants scoring above 15 categorized as having high depressive symptoms. Body mass index (BMI: kg/m²) was calculated from direct height and weight measurements. Hypertension was defined by self-report of a diagnosis, use of anti-hypertensive medications, or measured systolic blood pressure exceeding 140 mmHg or diastolic blood pressure exceeding 90 mmHg. Diabetes was defined by self-report of diabetes diagnosis, use of diabetes drug or fasting plasma glucose ≥126 mg/dl or 2-hour post-challenge glucose ≥ 200 mg/dl. History of myocardial infarction, stroke or transient ischemic attack (TIA) was determined from self-report of physician diagnoses. Apolipoprotein E (APOE) genotype was determined using standard single nucleotide polymorphism analyses (Bioserve.com, Laurel, MD).

Cognitive Tests—The Modified Mini-Mental State Examination (3MS) was administered at Years 1 (baseline), 3, 5 and 8. The 3MS is an expanded, and more sensitive, version [21,22] of Folstein's Mini-Mental State Examination (MMS), with possible scores ranging

from 0 to 100. It is a measure of global cognitive performance with components for orientation, concentration, language, praxis, and immediate and delayed memory [23].

The Digit Symbol Substitution Test (DSST) was administered at Years 1 (baseline), 5 and 8. The DSST measures response speed, sustained attention, visual spatial skills and set shifting, all of which reflect executive cognitive function [24,25]. The test is reported to distinguish mild dementia from healthy aging [26]. The DSST score is calculated as the total number of items correctly coded in 90 seconds, with a higher score indicating better cognitive function [25].

F₂-Isoprostane Measurement—Baseline (Year 1) F₂-IsoP samples were taken from 726 randomly selected Health ABC participants (23% of originally sample), stratified by sex and race, resulting in equal representation of black and white men and women. Venous blood specimens were collected in the morning after an overnight fast and plasma was separated by centrifugation, transferred to 2.0 ml vials, and stored at –80°C at the Health ABC core laboratory. Unthawed specimens for the study sample were shipped to the Molecular Epidemiology and Biomarker Research Laboratory (MEBRL) at the University of Minnesota, Minneapolis, MN for plasma F₂-IsoP analysis. Plasma free F₂-IsoPs were stable under various collection procedures and were measured by a gas chromatography-mass spectrometry (GC-MS)-based method [27], using an Agilent 6890 Series GC and an Agilent 5973N Mass Selective Detector. The column was manufactured by J&B Scientific (BD-1701). Sample and control sizes were 1 mL when sufficient quality was available. The method used for purification and derivitization is described elsewhere [27], with the following modifications: 1. Conversion to pentafluorobenzyl (PFB) esters took place at 37°C for 20 minutes and then dried under N₂ at room temp; 2. TLC plates were scraped 1.0 cm above and 1.0 cm below methyl ester of PGF₂α band; 3. The TLCs were developed using 7.5 % ethanol, and; 4. The standard line was forced through zero. The assay has an analytical variation of less than 10% for control pools at three concentrations, tested numerous times over a 16-month period by MEBRL. Blind duplicates were measured from approximately 5% of subjects with a coefficient of variation of 6.96%. For this study, whole blood samples were processed, plasma samples were stored at 4°C for less than 30 minutes and then transferred to a –70°C freezer. We anticipate stable F₂-IsoP levels with this collection process.

Statistical Analysis—Due to the skewed distribution, which did not normalize following transformation, tertiles were created for plasma F₂-IsoP level. Associations between F₂-IsoP tertile and baseline characteristics were determined using chi-square for categorical variables and analysis of variance (ANOVA) for continuous variables. Baseline characteristics significantly associated with F₂-IsoP tertile and cognitive score on 3MS and DSST were included as covariates in subsequent analyses. The statistical threshold for selection of covariates was set at p<0.1.

Repeated measures mixed effects models were conducted to determine if F₂-IsoP tertile was associated with baseline and rate of change over 8 years (i.e. slope) on the 3MS and DSST. Models accounted for baseline performance on cognitive tasks and included random intercepts and slopes for change in cognitive score over years. Specifically, F₂-IsoP tertile (low tertile as referent category) and covariates (in adjusted models) were entered as fixed effect predictors and random intercept and slope were introduced to account for inter-individual variability. A variable indicating time between first and subsequent visit was created and entered into the model to specify that the slopes over time are associated with an individual and are also random. Tests were considered statistically significant at p<0.05.

Interactions between F₂-IsoP tertile and APOE genotype, sex, race, and hypertension on cognitive scores were examined for intercept and slope. Interactions were considered significant at $p < 0.1$. Analyses were conducted in Stata 10.1 (StataCorp LP, College Station, TX).

RESULTS

F₂-IsoP tertile and baseline characteristics

Bivariate analyses showed that F₂-IsoP tertile groups differed on the following baseline factors: race, sex, education, hypertension, and BMI (all $p < 0.1$) (Table 1). Specifically, individuals in the high F₂-IsoP tertile group were less likely to be black, more likely to be female, less likely to have an education level below high school, more likely to be hypertensive, and tended to have a higher BMI than the other tertile groups. These factors were also significantly associated with cognitive function and therefore were included as covariates in subsequent analyses.

Participants with at least one time point were included in the analyses; 720 participants contributed to the present analysis. At each follow-up year, the number of participants with available cognitive data included 594, 606 and 413, for years 3, 5, and 8, respectively. Participants lost to follow-up were more likely to be black, have an education level below high school, were more likely to report general health as fair/poor, and were more likely to display lower baseline cognitive scores ($p < 0.05$). No differences were found for baseline F₂-IsoP level.

Association between F₂-IsoP tertile and baseline cognitive score in global and executive function

In unadjusted models, baseline 3MS scores were found to differ between F₂-IsoP tertile group ($p = 0.006$), with the High tertile group performing at a higher level than the Mid and Low tertile groups. However, after adjusting for baseline age, race, sex, education, BMI and hypertension, no association was found between F₂-IsoP tertile and baseline 3MS score ($p = 0.94$) (Table 2). Similarly, unadjusted baseline DSST scores were found to differ between F₂-IsoP tertile group ($p < 0.001$), again with the High tertile group performing at a higher level. However, this association was no longer statistically significant after adjusting for baseline age, race, sex, education, BMI and hypertension ($p = 0.31$) (Table 3).

Association between F₂-IsoP tertile and 7-year change in global and executive function

No significant association was found between F₂-IsoP tertile and 7-year change on the 3MS for either the unadjusted ($p = 0.54$) or the adjusted model ($p = 0.60$) (Table 2). A trend was found in the relationship between F₂-IsoP tertile and 7-year change on the DSST both before adjusting ($p = 0.14$) and after adjusting for baseline age, race, sex, education, BMI and hypertension ($p = 0.16$), with the High tertile group showing greater decline over 7-years compared to the Mid and Low tertile groups (Table 3).

F₂-IsoP tertile interactions on global and executive cognitive function

No interactions between F₂-IsoP tertile and APOE genotype, gender, race, or hypertension were found for either baseline or change score on the 3MS and DSST (all $p > 0.1$).

DISCUSSION AND CONCLUSION

Overall, no statistically significant association was found between plasma levels of F₂-IsoP and baseline or longitudinal change of global and executive cognitive function in generally healthy non-demented community residents. It is interesting to note, however, that there was

a trend for participants in the highest F₂IsoP tertile group to experience greater decline in executive function compared to the lower tertile groups. This trend is consistent with the hypothesis that high F₂-IsoP levels are related to greater cognitive decline in late life.

These findings bring into question the suitability of plasma F₂-IsoP as a biomarker for cognitive aging in the general population of older adults. The majority of studies that have reported a significant association have assessed F₂-IsoP levels in CSF, either in postmortem tissue or through lumbar puncture, in patient populations [10,14,28]. It is questionable whether peripheral measures of F₂-IsoP provide accurate or direct levels of oxidation in the brain, considering that they also include F₂-IsoPs produced from non-CNS cells in the body. Indeed, plasma provides a biochemically complex channel that may dilute brain proteins and metabolites. The fact that peripheral measures may not accurately reflect CNS disease may explain why findings have been conflicting, with some groups reporting an association [15,16,29] and other groups reporting no association [18,30,31] between F₂-IsoP in urine and/or blood and cognitive impairment. Similar to the present findings, a recent longitudinal study of community-dwelling elderly men reported no association between urinary F₂-IsoP and risk of AD or dementia over 12 years [32]. Also, it has been shown that F₂-IsoP measures taken in urine and plasma simultaneously may render divergent results [33], further illustrating the unreliability of peripheral measures as a predictive marker of cognitive decline.

An alternative explanation is that elevations in F₂-IsoP reflect disease presence (i.e. present dementia) or late-stage consequence of the disease. Elevated oxidation levels in blood and urine have mostly been reported in patients with MCI and AD [15,16,29]. Indeed, it may be suggested that elevated F₂-IsoP is consequence of the disease and not a diagnostic marker that can be assessed years before disease onset [15].

Strengths of this study included the diverse nature of the cohort, which allowed for tests of interaction. However, no significant interactions were found for APOE, sex, race, or hypertension. Although no significant interactions were found, the present findings are in agreement with previous studies reporting significant confounding effects of sex, race and hypertension (for review, see [6]). However, unlike past reports, smoking was not significantly related to F₂-IsoP and thus did not act as a classic confounder in the relationship between F₂-IsoP and cognitive score on the MMSE and DSST. The confounding effects of gender and race are especially noteworthy when contrasting the unadjusted and adjusted models for baseline cognitive function. For both cognitive tasks in the unadjusted model, the high F₂-IsoP group exhibits better performance scores than the other two groups. It is likely that this relationship is driven by the positive confounding effect of gender and race, as being female and white are both associated with better cognitive performance on the 3MS and DSST (results not shown).

A limitation to this study is that we only assessed plasma F₂-IsoP at baseline. It is highly plausible that static measurements of oxidation are unsuitable in the context of cognitive aging and that time-dependent changes in plasma F₂-IsoP should be assessed. Future large prospective studies should assess F₂-IsoP levels annually and correlate these measures with cognitive trajectory. It is postulated that elevations may occur closer to time of cognitive decline rather than several years prior. Another potential study limitation is the presence of selection bias. The cohort under investigation represents relatively well-functioning persons aged 70 to 79 years, and thus the proportion of individuals who experienced significant decline in cognitive function over time may have been lower than what would be observed in a less well-functioning sample.

In conclusion, high plasma F₂-IsoP levels measured in healthy non-demented elderly at baseline do not predict poorer baseline performance or greater decline in cognitive function over eight years. Future studies are warranted to assess longitudinal change in central and peripheral oxidation measures in tandem with changes in cognitive function in non-demented elderly in order to re-evaluate plasma F₂-IsoP as a potential biomarker for risk of AD and dementia in late life.

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Table 1Baseline Characteristics Across F₂-Isoprostane Level Tertile

Baseline Characteristics	F ₂ -Isoprostane Level Tertile			P-value [†]
	Low (n = 242)	Mid (n= 242)	High (n= 242)	
Mean (SE) or % [*]				
F ₂ -Isoprostane	36.9 (5.8)	54.7 (5.3)	95.6 (33.0)	<0.001
Age	73.5 (0.2)	73.8 (0.2)	73.8 (0.2)	0.216
Black	49.5	43.7	32.2	0.001
Female	35.0	50.3	68.4	<0.001
Education < HS	26.4	29.0	17.6	0.010
Current smoker	11.8	8.2	8.6	0.331
Exercise kcal	1273 (144)	1125 (121)	918 (104)	0.102 [‡]
BMI (kg/m ²)	26.9 (0.3)	27.4 (0.3)	27.9 (0.3)	0.028
Hypertension	54.9	66.9	67.5	0.009
Diabetes	27.7	23.8	21.7	0.383
Myocardial infarction	10.9	12.4	13.4	0.718
Stroke	10.4	8.4	10.5	0.695
APOE e4 carrier	33.9	29.2	29.2	0.484
Depression score ≥16	3.4	4.4	3.9	0.868
Self-reported health (poor/fair)	13.7	14.7	13.2	0.887

* All statistics incorporate sampling weights.

[†] P value for ANOVA of continuous variables and χ^2 for dichotomous variables.

[‡] Log-transformed for statistical comparison across tertiles

Table 2Mean (Standard Error) Scores on 3MS by F₂-Isoprostane Tertile

	F ₂ -Isoprostane Tertile			
Test Score	Low (n = 241)	Mid (n = 240)	High (n = 239)	P-value
Unadjusted (Mean, SE)				
3MS Year 1	89.1 (0.5)	90.1 (0.5)	91.4 (0.4)	0.006
Year 8 – Year 1	–3.0 (0.1)	–2.7 (0.1)	–2.9 (0.1)	0.545
Adjusted* (Mean, SE)				
3MS Year 1	89.1 (0.5)	89.9 (0.5)	91.4 (0.4)	0.941
Year 8 – Year 1	–2.7 (0.2)	–2.6 (0.2)	–2.6 (0.2)	0.597

Note: p-values were estimated from linear mixed models. Year 8 – Year 1 change score based on n=413 participants.

* Adjusted for baseline age, race, sex, education, BMI, hypertension

Table 3Mean (Standard Error) Scores on the DSST by F₂-Isoprostane Tertile

F₂-Isoprostane Tertile				
Test Score	Low (n = 241)	Mid (n = 240)	High (n = 239)	P-value
Unadjusted (Mean, SE)				
DSST Year 1	31.7 (0.9)	33.2 (0.8)	37.2 (0.8)	<0.001
Year 8 – Year 1	–2.8 (0.2)	–3.3 (0.2)	–4.4 (0.2)	0.141
Adjusted* (Mean, SE)				
DSST Year 1	31.8 (0.9)	33.1 (0.9)	37.4 (0.8)	0.307
Year 8 – Year 1	–2.9 (0.2)	–3.5 (0.2)	–4.5 (0.2)	0.160

Note: p-values were estimated from linear mixed models. Year 8 – Year 1 change score based on n=413 participants.

* Adjusted for baseline age, race, sex, education, BMI, hypertension.