Higher plasma levels of F_2-isoprostanes are associated with slower psychomotor speed in Healthy Older Adults

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Abstract

Oxidative stress has been identified as a process which is detrimental to brain health, and associated with age-related cognitive declines. Few studies to-date have examined the relationship between *in vivo* oxidative stress biomarkers and cognitive performance within healthy elderly populations. The current study investigated the relationship between reaction time and oxidative stress, as measured by blood plasma concentrations of F_2-isoprostanes using a sample of 251 healthy, non-demented, elderly volunteers (Male; 111: Female 140) aged 60 -75 years from the Australian Research Council Longevity Intervention (ARCLI) study cohort (Stough et al., 2012). A Jensen Box was used in conjunction with the Hick paradigm in order to differentiate simple from choice reaction time (2, 4 and 8-choice conditions) as well as movement (MT) and decision times (DT). MT, but not DT, was found to be significantly slower for participants in the high F_2-isoprostane group compared to the low F_2-isoprostane group, across all stimulus choices. F_2-isoprostanes, age and WASI full scale IQ were found to be significant predictors of average MT in the sample as a whole. These findings provide preliminary evidence to suggest that higher levels of oxidative stress may be associated with impaired psychomotor speed in the healthy elderly population.

Keywords: *Processing speed, Psychomotor speed; Isoprostanes; Aging; Oxidative Stress.*

Introduction

Declines in cognitive abilities with aging have been found to occur across a range of abilities including processing speed, episodic memory, spatial ability and reasoning (Hedden & Gabrieli, 2004; Simen, Bordner, Martin, Moy, & Barry, 2011). Oxidative stress is one of the key processes to be implicated in aging and cognitive decline, whereby reactive oxygen species (ROS) cause cumulative damage to components including proteins, lipids and DNA (Harman, 1992; Insel, Moore, Vidrine, & Montgomery, 2012). In comparison to other organs, the human brain is particularly vulnerable to oxidative stress due to its large consumption of oxygen and polyunsaturated fatty acids (Halliwell, 1992). One of the main mechanisms by which ROS cause damage to neurons is via lipid peroxidation (Simonian & Coyle, 1996). Higher concentrations of certain end-products of lipid peroxidation in plasma are being increasingly linked to dementia type pathologies and age-associated declines in cognitive capacity.

Isoprostanes are a group of prostaglandin-like compounds which are one of the major end products of lipid peroxidation (Milne, Musiek, & Morrow, 2005). F_2-isoprostanes are produced by the non-enzymatic peroxidation of arachidonic acid and are stable molecules which are detectable in all human tissues and biological fluids. F_2-isoprostanes have been well validated as markers of oxidative stress in animal and human studies (Milne, Dai, & Roberts, 2015; Milne et al., 2005), and have been used to provide the most accurate and reliable measure of *in vivo* oxidative stress status when compared to other biomarkers (Kadiiska et al., 2005; Montuschi, Barnes, & Roberts Ii, 2004; Niki, 2014). There is some evidence to suggest that the measurement of F_2-isoprostanes from cerebrospinal fluid (CSF) may represent a more direct measure of oxidation in the brain, due to the fact that urine or plasma may contain F_2-isoprostanes that have also been produced by non-CNS cells in the body (Fiocco et al., 2011). However, peripheral measures (e.g. urine or blood plasma) may be considered favourable over CSF measures due to being less invasive and more cost effective alternatives (Milne et al., 2015). In regards to the most accurate method for quantifying F_2-isoprostane concentrations in peripheral fluids, gas chromatography-mass spectrometry (GC/MS) is currently the "gold standard" of measurement (Griendling et al., 2016).

Previous clinical research has demonstrated a strong association between F_2-isoprostanes and health conditions known to be associated with increased oxidative stress, including; diabetes (Sampson, Gopaul, Davies, Hughes, & Carrier, 2002), cardiovascular

disease (Griendling et al., 2016; Praticò et al., 1997), Alzheimer's disease (Montine et al., 2005), anxiety (Steenkamp et al., 2017) and mild cognitive impairment (Praticò, Clark, Liun, Lee, & Trojanowski, 2002). Similarly, rats exposed to oxidant injury have also been found to display elevated levels of F_2-isoprostanes (Morrow et al., 1990), and studies by both Montine et al. (2011) and Guest et al. (2014) have provided evidence of a positive association between F_2-isoprostane levels and aging more generally.

Decrements in cognitive abilities would be predicted to be a consequence of higher levels of oxidative stress in the brain, in consideration of the molecular evidence of damage to lipids and DNA within neurons. However, only a handful of preliminary studies to-date have provided evidence of associations between F_2-isoprostanes and cognitive function. For example, interventions using an antioxidant pine-bark extract or high levels of fruit and vegetable dietary intake were both found to result in concurrent improvement to cognitive function and oxidative stress, as measured by F_2-isoprostane levels (Polidori et al., 2009; Ryan et al., 2008). Similarly, a large cross-sectional study of healthy participants aged 45 years or older also found greater concentrations of F_2-isoprostanes to be associated with poorer cognitive performance (Li et al., 2014). This is in contrast to a longitudinal study by Fiocco et al. (2011) whereby changes in cognitive function over an 8 year period were found to be unrelated to F_2-isoprostane levels.

An important limitation of these previous studies is that, with the exception of the study by Ryan et al. (2008), these studies relied on traditional paper and pencil tests of cognitive function. For example, the Mini Mental State Examination (MMSE; Folstein, Folstein, & McHugh, 1975) as utilized in the study by Fiocco et al. (2011) and Polidori et al. (2009) is an excellent screening measure for dementia, but lacks the sensitivity to detect more subtle changes in cognitive function (O'Bryant et al., 2008). For example, processing speed, which is postulated to explain a high degree of common variance in age-related cognitive decline (Salthouse, 1985, 1996, 2000), is more accurately measured using computerized testing with millisecond accuracy. Since processing speed tasks often involve motor responses (typically a key press), it is also desirable to differentiate processing speed, or decision time (DT), from movement time (MT).

In the 1970s Jensen developed an apparatus which differentiated simple from choice and DT from MT in terms of a reaction time (Jensen, 1982). The importance of this distinction is that, DT and MT have been found to be uncorrelated variables which load onto separate factors of cognitive ability and psychomotor ability, respectively (Jensen, 2011). This apparatus, also known as the 'Jensen Box' or 'Hick Box' has subsequently been used in

a large number of mental chronometry (MC) studies, with both DT as well as the rate of change of DT with stimulus choice found to be related to general intelligence (IQ) (Bates & Stough, 1998; Jensen, 1987). In contrast, MT is typically found to be unrelated to general intelligence, although some authors have argued that this is due to individual differences in the guidance of motor responses (see Buckhalt, Reeve, & Dornier, 1990).

In relation to the effects of aging, declines in psychomotor speed have long been reported in the literature (e.g. Salthouse, 1985). For example, Houx and Jolles (1993) reported a greater degree of age-related slowing for MT when compared to DT when analysing data from a sample of 247 participants aged 20 to 80 years old. Similarly, in a large study of over 7000 participants aged 30 years or over Era et al. (2011) reported systematic and significant declines in MT, as well as DT, when proceeding from the age of 30 through to 80+ years old. Sleimen-Malkoun et al. (2013) also reported age-related slowing in both DT and MT, as well as the slope as a function of stimulus choice, when comparing older and younger adults. These declines in MT may be attributable to age-related changes in brain regions involved in motor functioning, such as the left deep anterior central sulcus (Ward & Frackowiak, 2003). In contrast, decreases in processing speed, as measured by DT, have been linked to more global reductions in grey matter, loss of myelination, reduced frontal white matter volume and connectivity, as well as decreased cerebellar grey matter volume (Eckert, 2011).

To the best of our knowledge, the current study is the first of its kind to investigate the relationship between oxidative stress, as measured using blood plasma F_2-isoprostane concentration determined by mass spectrometry, and processing speed and psychomotor speed in a healthy, non-demented, elderly population. It was hypothesised that participants with higher levels of oxidative stress would have slower processing speeds and psychomotor speeds in comparison to participants with lower levels of oxidative stress. The findings of the study may have important implications in regards to better understanding the extent to which cognitive processes are impacted by oxidative stress during normal ageing.

Methods

Participants

The sample comprised 251 healthy volunteers (Male; 111: Female 140) aged 60 -75 years from the Australian Research Council Longevity Intervention (ARCLI) study cohort (Stough et al., 2012). The data for this analysis was drawn from baseline data from the

ARCLI study (a large intervention study aimed at examining the effects of two compounds on cognitive performance). For the purpose of this sub-study, the participants were divided into two groups (low and high F_2-isoprostanes), defined through a median split of F_2-isoprostanes (occurring at 817pmol/L); the demographics of the two groups appear in Table 1.

Participant Recruitment

Participants were recruited through radio, newspaper articles as well as poster and flyer distribution. Volunteers were excluded if they were taking cognitive enhancing supplements (e.g. Ginkgo biloba), were current smokers, had a history of drug and/or alcohol misuse or were currently taking prescribed antidepressant, anxiolytic or antipsychotic medication. Participants were eligible if they did not have a diagnosis of diabetes, dementia, neurological or psychiatric disorder or cardiovascular disease. Eligibility also included not having a recent history (defined as a period longer than 6 weeks, over the past 5 years), of a chronic or severe illness. Initial telephone screening was conducted by an experienced research assistant using participant self-reports. A second round of face-to-face interviewing was conducted to confirm eligibility according to the following screening measures: the Mini Mental State Examination (MMSE; Folstein et al., 1975) excluding those with a score of 23 or less which indicates cognitive impairment; and the Geriatric Depression Scale (GDS; Yesavage et al., 1982) excluding those with a score of 20 or more, which is indicative of depressive symptoms in the severe range. All participants provided written and informed consent, and the Swinburne University Human Research Ethics Committee approved the study. The ARCLI trial was registered with the Australian and New Zealand Clinical Trials Registry (ANZCTR12611000487910).

Jensen Box Task

The Jensen Box is an apparatus that distinguishes simple and choice decision time and movement time from total reaction time (Jensen, 1982, 1987). The box consists of eight lights which are arranged in a semi-circular configuration, with square response buttons. Participants were presented with 64 trials in total, consisting of four blocks of 16 trials from each of the following conditions: 1-choice, 2-choice, 4-choice and 8-choice conditions. When each target light appeared they were required to release the home button (located in the centre of the semi-circular configuration) as quickly as possible and press the response button adjacent to the stimulus light. Decision time (DT) was defined as the time from stimulus

onset to the release of the home button, and movement time (MT) was defined as the time from the release of the home button until the depression of the stimulus button. For a more detailed description please refer to the Supplementary materials.

Memory and Cognition Screening Tools

The Mini-Mental State Examination (MMSE; Folstein et al., 1975) is a brief 30-point test that is commonly used to screen for dementia. The MMSE evaluates six areas of cognitive function: orientation, attention, immediate recall, short-term recall, language, and the ability to follow simple verbal and written commands. Scores $\geq$ 24 points indicate normal cognition, whereas scores below this level indicate some level of cognitive impairment (Folstein et al., 1975).

General intelligence (IQ)

Two tests from the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) were administered in order to provide an estimate of the full scale intelligence quotient (IQ). The vocabulary and matrix reasoning subtests of the WASI were administered to all participants. The vocabulary subtest is a 42-item task, which requires the participant to orally define words that are presented visually and orally. The matrix reasoning subtest involves the presentation of a series of 35 incomplete grid patterns, which the participant is asked to complete by selecting the correct pattern from five possible choices. The WASI is a reliable and valid measure of intelligence for use in research settings (Canivez, Konold, Collins, & Wilson, 2009).

Geriatric Depression Scale (GDS)

The GDS (Yesavage et al., 1982) is a basic screening measure for depressive symptom severity which is suitable for use in elderly populations. The GDS sums 30 questions each of which are answered “yes” or “no”. A total score of 0-9 is considered normal, a score of 10-19 indicates mild depressive symptoms and a score of 20-30 indicates severe depressive symptoms.

Oxidative Stress Measure: F_2-isoprostanes

Plasma concentrations of F_2-isoprostanes were used in the current study as a measure of oxidative stress. Blood samples were collected from the forearm of each participant via the

cephalic vein by a registered nurse or qualified phlebotomist into a 10mL vial containing ethylenediaminetetraacetic acid and then centrifuged at 3000 x *g* for 10 minutes at 4°C. To protect the samples from oxidation, 10µl of butylated hydroxytoluene was added to the 10mL vials (0.1% by volume) prior to storage at -80°C. Frozen samples were then shipped to the University of Western Australia where they were quantified for F_2-isoprostanes by negative ion chemical ionisation-gas chromatograph mass spectrometry as described by Mori and colleagues (Mori, Croft, Puddey, & Beilin, 1999; Proudfoot et al., 1999).

Procedure

Participants attended Swinburne University of Technology in Melbourne for two sessions with the visits being up to one week apart. Eligible participants could attend during the morning or afternoon with the allocated time of day remaining consistent, minimizing confounding effects of fatigue and diurnal variation on cognitive and blood parameters. Morning participants attended after fasting overnight while afternoon participants were able to consume a light breakfast and lunch. At the beginning of the first visit, participants provided written and informed consent prior to engaging in any screening questionnaires or undergoing blood tests or cognitive test batteries. Participants were screened for depressive symptoms and any possible memory impairments using the GDS and the MMSE, respectively. General cognitive ability (IQ) was assessed using the WASI. General demographic information was also collected at the time of the first visit, including age, gender, body mass index (BMI) and total years of education (including primary school). Blood samples were collected at the second visit, prior to participants completing the Jensen box task.

Statistical Analysis

All analyses were conducted using IBM SPSS Statistics Version 25. Repeated measures analysis of covariance (ANCOVA) was chosen as the primary analysis in order to explore the main effect for plasma F_2-isoprostane group (divided on the basis of median split) on Jensen MT and DT variables, as well as the interaction between F_2-isoprostane group and the number of stimulus choices in the Jensen task. A series of linear regressions were included in secondary analyses in order to explore the degree of linear association between F_2-isoprostane levels and Jensen MT and DT variables. For the repeated measures ANCOVA analysis the SPSS MIXED procedure was used, with F_2-isoprostane group (Low, High) as the between-subject variable and number of stimuli choices (1, 2, 4, 8) as the repeated measures

variable. The Residual Maximum Likelihood estimation method (REML) was used with an unstructured (UN) covariance matrix. Fixed terms were fitted for F_2-isoprostane group, number of stimuli choices, and their interaction. The following covariates were also included in the analysis on the basis of forming significant correlation ($p<.05$) with MT or DT measures: age and WASI estimate of full scale IQ. MT and DT slopes were calculated for each individual by calculating the least squares regression coefficients across 1-choice, 2-choice, 4-choice and 8-choice values. Univariate ANOVA was used to test for significant between-group differences for F_2-isoprostane group according to both MT and DT slopes using the same covariates as above. Follow-up regression analysis was conducted on average DT and MT, or MT and DT slope, in the case where more than one predictor was found to form significant correlations with the outcome measure ($p<.05$). All statistical tests were conducted using a two-tailed alpha level of .05 and 95% confidence intervals.

Results

Demographic details for high and low F_2-isoprostane groups are displayed in Table 1, with values provided for F_2-isoprostane concentration (pmol/L), age, MMSE, years of education (including primary school), body mass index (BMI), geriatric depression scale (GDS) and WASI full scale IQ estimate. Mean plasma F_2-isoprostane concentrations in the current study was found to be comparable to those published in previous studies using healthy elderly, non-demented, populations. The mean F_2-isoprostane concentration in the current study was 979.96 pmol/L. This value is lower than the baseline value reported for the placebo group in the study by Ryan et al. (2008): (M=1417.887 pmol/L), yet higher than the average values reported for high and low fruit and vegetable intake groups in the study by Polidori (2009): (M=275.70, and M=399.65 pmol/L respectively). Note that 1 pg/mL = 0.284 pmol/L when converting values from these studies.

A higher number of females were found in the high F_2-isoprostane group compared to the low F_2-isoprostane group: Low F_2-isoprostane group: 66 male and 60 female, compared with High F_2-isoprostane group: 45 male and 80 female, ($\chi^2(1) = 6.826$, $p=.009$). However, repeated measures ANOVA revealed that neither the main effect of gender or the Gender × F_2-isoprostane group interaction were significant when using MT or DT as dependent variables, and for this reason, gender was excluded from subsequent analyses. Age and WASI full scale IQ estimates were found to correlate significantly with MT and DT ($p<.05$), and were included as covariates in subsequent analyses.

* INSERT TABLE 1 HERE *

F_2-isoprostane group ANOVAs

For MT, significant main effects were found for both number of stimulus choices $F(3,247.97)=144.72$, $p<.001$) and F_2-isoprostane group $F(1, 246.10)=9.70$, $p=.002$). MT was found to be significantly faster in the Low F_2-isoprostane group when compared to the High F_2-isoprostane group, across all stimulus choices with the exception of the 8-choice condition: 1-choice condition: $F(1, 246.80)=11.57$, $p=.001$, 2-choice condition: $F(1, 246.59)=10.73$, $p=.001$, 4-choice condition: $F(1, 246.12)=10.43$, $p=.001$). For DT, no significant main effects or interactions were found for F_2-isoprostane group, although a significant main effect was identified for stimulus choices ($F(3,247.15)=255.33$, $p<.001$). In univariate analysis of MT and DT slopes, no significant main effects were identified for F_2-isoprostane group. Means and Standard errors of estimated marginal means for MT and DT, together with slopes are displayed in Table 2 and Figure 1.

* INSERT TABLE 2 AND FIGURE 1 HERE *

F_2-isoprostane Linear Regression

F_2-isoprostane values were found to be positively skewed, and for this reason were transformed using natural logarithm for all subsequent correlational and regression analyses. Zero-order correlations between demographic variables and Jensen MT and DT variables are displayed in Table 3.

* INSERT TABLE 3 HERE *

Average MT, across all stimulus choices, was found to be significantly correlated with both F_2-isoprostane values ($r=.168$, $p=.008$), age ($r=.156$, $p=.015$) and WASI full scale IQ estimate ($r=-.174$, $p=.006$). Average DT, across all stimulus choices, was found to only be significantly correlated with age ($r=.163$, $p=.011$). Gender was significantly correlated with F_2-isoprostane values ($r=.182$, $p=.004$). DT and MT slopes were not observed to be correlated with any demographic variables at the $p<.05$ level. Linear regression analysis on average MT

demonstrated that both F_2-isoprostane concentration (β=.173, t=2.850, p=.005), age (β=.174, t=2.850, p=.005) and WASI full scale IQ estimate (β=-.179, t=-2.934, p=.004) were significant predictors of MT. The overall model with the three predictors was found to explain 8.9% (adjusted R^2=7.8%) of the variance in average MT, and was significant [R^2 = .089, F(3,246)=7.987, p<.001]. A summary of the regression output is displayed in Table 3, and a scatterplot of log transformed F_2-isoprostane concentrations versus average MT is displayed in Figure 2.

* INSERT TABLE 4 AND FIGURE 2 HERE *

Discussion

The primary finding of the current study was that healthy older, non-demented, adults with a higher plasma F_2-isoprostane concentration, displayed decreased MT in the Jensen box task. Further, F_2-isoprostane concentration was found to be a significant predictor of MT after controlling for both age and intelligence in older adults. The finding of age-related declines in psychomotor speed, as measured by MT, is consistent with a large number of previous studies and similarly, the finding that processing speed, as measured by average DT in the Jensen task, was also correlated with age is consistent with previous research (Era et al., 2011; Houx & Jolles, 1993; Salthouse, 1985).

A potential neurobiological explanation for the finding of an association between oxidative stress and decreased psychomotor speed may be that age-related damage is occurring within the cerebellum. The cerebellum has been identified as an important brain region for the control of eye and hand movements (Nitschke, Arp, Stavrou, Erdmann, & Heide, 2004). Previous animal research suggests that due to the high metabolic demands of Purkinje cells within the cerebellum, this region may be particularly vulnerable to damage from oxidative stress (Andersen, Gundersen, & Pakkenberg, 2003; Eckert, 2011; Lee, Weindruch, & Prolla, 2000). Age-related loss of motor coordination, using behavioural measures in rats, have also been correlated with oxidative molecular damage within the cerebellum in a number of previous studies (Cui, Hofer, Rani, Leeuwenburgh, & Foster, 2009; Forster et al., 1996; Hu, Serrano, Oury, & Klann, 2006; Shukitt-Hale, 1999). Hence, the decreased average MT observed in the high F_2-isoprostanes group may be attributable to

impaired cerebellar functioning within this group, a possibility that could be explore in future studies using structural and functional imaging.

A number of underlying molecular mechanisms may be used to explain how oxidative stress damages the cerebellum. Firstly, oxidative damage to DNA and RNA proteins within this region has been found to increase during aging (Cui, Hofer, Rani, Leeuwenburgh, & Foster, 2009; Dorszewska & Adamczewska-Goncerzewicz, 2004), and damage to DNA and RNA may result in the gradual accumulation of mutations within these cells, and eventual cell death (Barja, 2004). Considering that there is currently no evidence to suggest the cerebellum is capable of neurogenesis (Grimaldi & Rossi, 2006), these cells are not able to be replaced. Secondly, with specific reference to F_2-isoprostanes, it is noteworthy that these molecules are not only markers of oxidative stress, but also exert detrimental effects on brain function themselves, most notably by acting as vasoconstrictors within cerebral arterioles (Comporti et al., 2008; Hoffman, Moore, & Ellis, 1997). Finally, oxidative stress has also been associated with increased neuro-inflammatory processes during aging, another well documented contributor to impaired cognitive functioning across a number of brain regions in the elderly (Baierle et al., 2015; Lucas, Rothwell, & Gibson, 2006).

It is noteworthy that no significant between-group differences for F_2-isoprostanes were observed for the rate of change in DT or MT as the number of stimulus choices increased. This finding suggests that oxidative stress did not differentially effect performance in the more difficult conditions involving multiple stimuli choices. Also contrary to previous findings by Bates and Stough (1998), the rate of change of DT and MT with stimulus choice was found to be unrelated to intelligence. However, this latter finding may be due to restriction in range of IQ scores within this sample (i.e. average IQ of 120).

Contrary to our hypotheses, processing speed, as measured by DT, was not found to be significantly related to F_2-isoprostane concentration in either between-groups or correlational analyses. It is noteworthy that in previous research by Li et al. (2014), higher levels of F_2-isoprostanes were associated with impaired Trail Making Test A completion time, which has traditionally been interpreted as a measure of processing speed (Bowie & Harvey, 2006). For this reason it could be argued that the current findings in relation to DT are inconsistent with the findings of Li et al. (2014). More recent research by Sanchez-Cubillo et al. (2009) provides evidence to suggest that the Trail Making Test A is more closely associated with speed of visual search and perceptual speed, while the Trail Making Test B has been found to be associated most strongly with working memory function. In light of this interpretation, the current DT measure can be considered to be more closely

aligned with the processing speed construct when compared to Trail Making Test A completion time. In this regard the lack of a significant effect for oxidative stress on DT is surprising considering that speed of processing is considered by a number of authors to be a core ability that declines with aging and explains similar declines across other more heterogeneous cognitive tests (Deary & Der, 2005; Salthouse, 1996).

Certain limitations of the current study need to be acknowledged. Firstly, the current elderly adult group represented a high-functioning cohort who were both highly educated and of above average intelligence (average IQ ~ 120). For this reason, it could be argued that MT and DT as well as F_2-isoprostane concentrations may have been range-limited, hence reducing the power to detect significant relationships between the variables. In future studies investigating these outcomes it would be advantageous to also include participants who were of below average intelligence and education level as well as a greater number of male participants, due to the disproportionate number of females included in the current sample. The use of a blood plasma measure of F_2-isoprostanes is also a limitation of the study, as the use of CSF for the measurement of F_2-isoprostanes has been argued to be a more direct measure of brain oxidation, and greater consistency in regards to the association between F_2-isoprostanes and cognitive decline has been reported in the literature when using this measure (Fiocco et al., 2011). Similarly, F_4-neuroprostanes which are formed by free radical non-enzymatic peroxidation of docosahexaenoic acid (DHA) may also have been used as a more specific marker of brain oxidative stress (Galano et al., 2013), although the use of this biomarker is a more recent addition to the literature in this area. In order to further explore hypotheses relating to the neurobiological underpinnings of changes to psychomotor and processing speeds associated with oxidative stress then brain imaging data such as fMRI would be of value.

It is also important to acknowledge that the interpretation of the current findings are limited by the current lack of a cut-off point for what constitutes clinically significant "healthy" F_2-isoprostane levels, in comparison to those which are "unhealthy". For this reason, future studies are required in order to determine whether the current finding of differences in psychomotor speed are similarly observed in samples which consist of either lower or higher upper ranges of F_2-isoprostanes. However, the fact that regression analysis revealed a linear relationship between these variables in the current healthy elderly sample raises the possibility that even low levels of oxidative stress, which may be below the level of clinical significance, may still have a detrimental effect on psychomotor speeds. Finally, in future studies the inclusion of other clinical or nutritional information, such as C-reactive

protein or dietary lipids, that are known to have an influence on F_2-isoprostane levels, would greatly aid in the interpretation of the findings.

In summary, the current study found MT, but not DT to be significantly slower for participants with higher concentrations of F_2-isoprostane after controlling for age and general intelligence. These findings provide preliminary evidence to suggest that higher levels of oxidative stress may be associated with impaired psychomotor speed in the healthy elderly population. A possible explanation for this psychomotor impairment is the vulnerability of the cerebellum (an area of the brain that contributes to programming of movement) to cell death and genetic mutation due to oxidative stress, a possibility that may be further explored by the use of additional oxidative stress biomarkers and the use of functional neuroimaging in future studies.

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Competing Interest Statement

The authors have no actual or potential conflict of interests to declare with respect to the research, authorship, and/or publication of this article.

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Table 1: *Demographic sample characteristics according to high and low F2-isoprostane groups. Values displayed are means and standard deviations.*

	Low F_2-isoprostane M (SD)	High F_2-isoprostane M (SD)
Age	66.03 (4.13)	65.83 (4.00)
Body Mass Index	25.67 (4.30)	25.99 (4.31)
Years of Education	16.15 (3.81)	15.77 (4.27)
MMSE	28.73 (1.08)	28.62 (1.33)
GDS	3.66 (3.42)	3.38 (3.38)
WASI Fullscale IQ	120.06 (10.67)	119.21 (11.51)
Plasma F_2-isoprostane concentration (pmol/L)	633.52 (112.167) median = 643.5	1329.17 (527.802) median = 1122.00

GDS: Geriatric Depression Scale, MMSE: Mini Mental State Examination, WASI: Wechsler Abreviated Scale of Intelligence, estimate of full-scale Intelligence Quotient.

Table 2: *Movement time (MT) and decision time (DT) mean values in the Jensen box task, according to F2-isoprostane group and stimulus choice.*

	Stimulus choice					
F_2-isoprostane group	1-choice†	2-choice†	4-choice†	8-choice†	Average††	Slope††
	M (SE)	M (SE)	M (SE)	M (SE)	M (SE)	M (SE)
Decision Time (DT)						
Low F_2-isoprostane	335.53 (4.91)	369.28 (4.46)	390.02 (4.18)	427.79 (5.43)	381.02 (4.23)	36.17 (4.93)
High F_2-isoprostane	345.59 (4.91)	373.26 (4.46)	397.33 (4.18)	433.84 (5.43)	387.70 (4.23)	35.40 (4.93)
Movement Time (MT)						
Low F_2-isoprostane	265.64 (6.04)	286.67 (6.09)	297.56 (5.92)	339.48 (6.31)	297.52 (5.70)	23.24 (1.54)
High F_2-isoprostane	294.73 (6.04)	314.92 (6.09)	324.60 (5.92)	355.63 (6.31)	322.62 (5.70)	19.16 (1.54)

† *Covariates appearing in the repeated measures model (1, 2, 4 and 8-choice) are evaluated at the following values: Age in years = 65.92, WASI full scale IQ = 119.79.* †† *Covariates for univariate analyses of average MT, DT and slopes are as follows: Age in years = 65.91, WASI full scale IQ = 119.77.*

Table 3: *Zero-order correlations between demographic and Jensen Movement time and decision time variables*

		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.	F2-isoprostanes†	1									
2.	Age	-.012	1								
3.	Gender	.182**	-.049	1							
4.	BMI	.098	-.078	-.152*	1						
5.	Years of education	-.092	-.205**	-.053	-.064	1					
6.	Intelligence	-.007	.057	-.032	.020	.280**	1				
7.	MT average	.168**	.156*	.081	-.016	-.080	-.174**	1			
8.	MT slope	-.059	-.035	-.028	-.118	.023	.007	-.053	1		
9.	DT average	-.009	.163*	-.030	-.051	.028	-.061	.343**	-.189**	1	
10.	DT slope	-.026	.087	.032	-.056	.045	.119	-.247**	-.131*	.045	1

†*Transformed using natural logarithm. * Value is significant at the p<.05 level (two-tailed), ** Value is significant at the p<.01 level (two-tailed)*

Table 4: *Linear Regression Model for Average Movement Time as Predicted by F_2-isoprostane level, Age (years) and WASI full scale IQ estimate.*

	Unstandardized coefficients		Standardized coefficients						
	B	SE	β	t	p	Adj. R^2	F	df	p
F_2-isoprostanes†	25.950	9.105	.173	2.850	.005	.078	7.987	(3,246)	<.001
Age (years)	2.854	1.001	.174	2.850	.005				
Full scale IQ††	-1.073	.366	-.179	-2.934	.004				

† Transformed using natural log (LN) ††Estimate based on Wechsler Abbreviated Scale of Intelligence (WASI).

Figure 1: *Mean movement times (MT) in the Jensen Box task. Displayed for high and low plasma F_2-isoprostane groups after co-varying for age and intelligence (WASI IQ estimate). Error bars represent 95% confidence intervals for the marginal means. ** difference is significant with at the p<.01 level (two-tailed).*

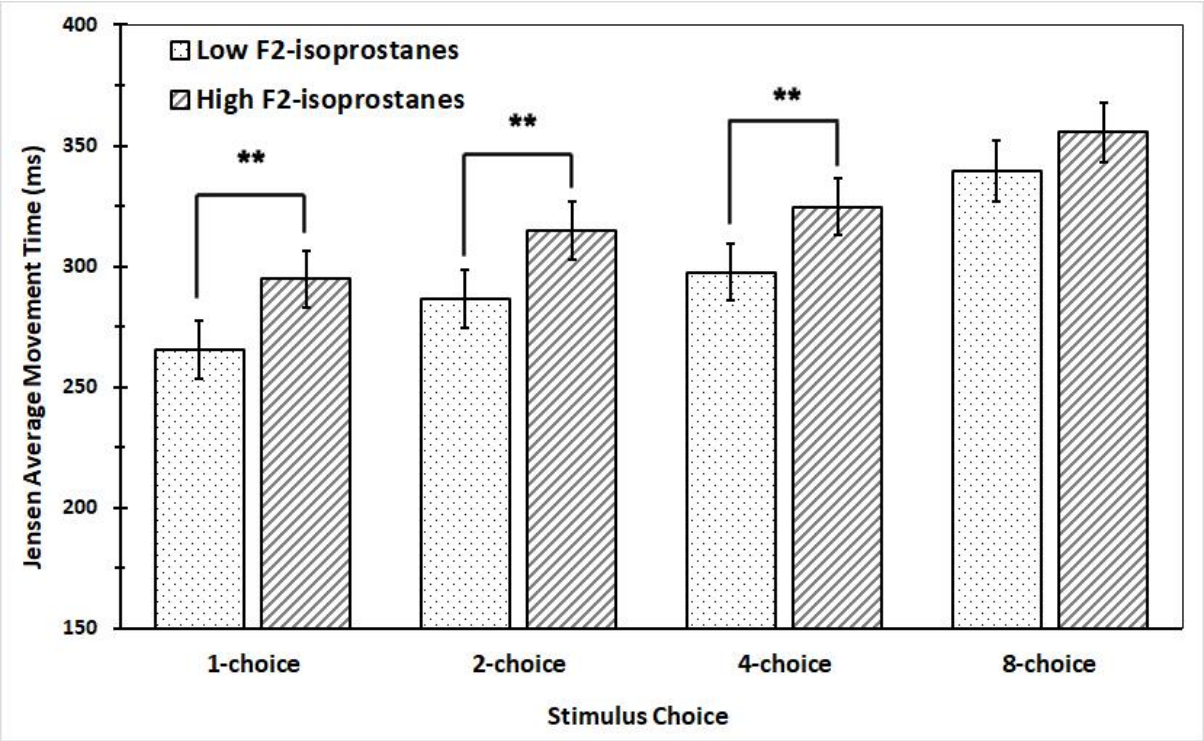

Figure 2: *Scatterplot of plasma F_2-isoprostane concentration (pmol/L) versus average movement time (MT) in the Jensen Box task.*