Impaired Verbal Episodic Memory in Healthy Older Adults is Marked by Increased F_2-Isoprostanes.

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Abstract

Age-associated cognitive decline amongst otherwise healthy older individuals is a multifaceted characteristic of ageing. The role of oxidative stress biomarkers has been increasingly examined in the context of pathological aging conditions that affect cognition. Plasma F_2-Isoprostane levels are a reliable index of systemic oxidative stress (specifically lipid peroxidation) and are elevated in dementia patients. Less is known about their role in healthy cognitive ageing. This study evaluated the relationship between F_2-Isoprostanes and cognitive functioning in a cohort of 211 healthy elderly adults (60-75 years: Male; 88, Female; 123). Cognitive assessment included the Cognitive Drug Research (CDR) computerised assessment battery, which produces five validated factor scores (corresponding to 'Quality of Episodic Memory', 'Speed of Memory', Quality of Working Memory', Power of Attention' and 'Continuity of Attention'). Participants with higher F_2-Isoprostane levels had significantly lower Quality of Episodic Memory scores (suggesting inferior abilities in retaining and retrieving verbal information in episodic memory). This is, to our knowledge, the first report of compromised verbal episodic memory in healthy ageing humans being linked to increased levels of F_2-Isoprostanes. These results have relevance for interventions aimed at improving cognitive performance in the healthy elderly.

KEYWORDS: Cognition; Isoprostane; Memory; Aging; Oxidative Stress; Verbal Memory

1. INTRODUCTION

Normal ageing is associated with a gradual and variable rate of decline of mental functions and cognitive processes (Bishop, Lu, & Yankner, 2010). This decline in cognitive abilities affects attention, speed of information processing, as well as specific aspects of memory and intelligence (Simen, Bordner, Martin, Moy, & Barry, 2011). The biological mechanisms underpinning this decrease in cognitive ability are yet to be completely understood. Processes such as oxidative stress, can accelerate the ageing process. Oxidative damage affects phospholipids, proteins and nucleic acids, and is considered a major contributor to pathological ageing, co-occurring cognitive decline, and age related cognitive decline (Dimopoulos et al., 2007; Finkel & Holbrook, 2000; Harman, 1956; Insel, Moore, Vidrine, & Montgomery, 2012; Li, et al., 2014; Padurariu et al., 2013; Syslová et al., 2014). Therefore, oxidative phospholipid levels may be a reliable biomarker with predictive validity for cognitive changes associated with ageing in older adults.

Prolific metabolic activity within cellular mitochondria produces unstable endogenous oxygen radicals called reactive oxygen species (ROS) or free radicals as a result of cellular respiration, which over time causes cumulative damage to cell components (Floyd, 1999; Harman, 2003; Rahman, 2007). Under normal conditions, an endogenous antioxidant system such as superoxide dismutase (SOD) catalyses superoxide radicals and reduces their harmful affect into oxygen and hydrogen peroxide which are less harmful than the original radical state (Pauling, 1979). A different endogenous antioxidant, Catalase (CAT), then reduces hydrogen peroxide into water and oxygen. This occurs to maintain beneficial levels of ROS and prevent damage to molecular bonds, cell membranes and DNA (Basu, 2010). An imbalance between the oxidant scavenging systems, antioxidant defence mechanism and ROS levels, in favour of the latter, results in oxidative stress. Oxidative stress produces

destructive consequences including lipid peroxidation. Phospholipids are highly vulnerable to ROS, especially within the brain where there is a high concentration of polyunsaturated fatty acids (PUFA). For example, excessive free radical mediated peroxidation of PUFA is a biochemical process with a destructive impact on cell membranes, structures and function (Syslová et al., 2014).

The peroxidation of lipids produces a number of compounds. For instance, F_2-Isoprostanes are a group of prostaglandin compounds that are non-enzymatically produced as a by-product of the peroxidation of arachidonic acid (Milne, Musiek, & Morrow, 2005). F_2-Isoprostanes are widely studied, and measuring the level of this compound is considered to be the most reliable marker to investigate the in vivo oxidative damage to lipids and more broadly, oxidative stress (Montuschi, Barnes, & Roberts, 2004; Niki, 2014). In particular, F_2-Isoprostanes are markers of the rate of lipid peroxidation and are known to be relatively stable in bodily fluids, including blood (Tacconelli, Capone, & Patrignani, 2010). Peripheral measurements from blood plasma or urine are favourable over cerebral spinal fluid (CSF) measurements as they are less invasive and more cost effective (Milne, Dai, & Roberts Ii, 2015; Roberts & Morrow, 2000).

Increased isoprostane levels are associated with various disorders associated with elevated oxidative stress such as diabetes (Sampson, Gopaul, Davies, Hughes, & Carrier, 2002), atherosclerosis (Praticò et al., 1997), as well as disorders that produce marked deficits in cognition such as Alzheimer's disease (Montine et al., 2005), and mild cognitive impairment (Praticò, Clark, Liun, Lee, & Trojanowski, 2002). Animal studies investigating oxidant injury have also found significant increases in isoprostanes (Morrow et al., 1990), but they seem unchanged by the lipid content of the diet (Richelle et al., 1999). Recently, F_2-Isoprostanes

have been observed to increase with age, as determined through levels detected in CSF (Guest, Grant, Mori, & Croft, 2014). However, few studies exist regarding general age associated changes of F_2-Isoprostanes and its relationship with cognition in healthy ageing.

There are somewhat inconsistent findings regarding the relationship between oxidative stress biomarkers and cognitive performance in healthy elderly cohorts (see Baierle et al., 2015; Fiocco et al., 2011; Sánchez-Rodríguez, Arronte-Rosales, & Mendoza-Núñez, 2009). Additionally, there is a paucity of studies that have explicitly investigated the relationship between peripheral measures of F_2-Isoprostane concentrations and cognition in healthy non-demented cohorts. Li and colleagues investigated whether the decline in cognitive abilities in a cohort of more than 300 cognitively normal adults (21-110 yrs) was related to CSF biomarkers of oxidative injury as well as AD biomarkers. They observed that higher levels of F_2-Isoprostanes were associated with poorer executive function (Li et al., 2014). A study conducted by Polidori and colleagues investigated the relationship between the consumption of fruits and vegetables, antioxidant levels and cognitive ability with 193 cognitively healthy participants (45 - 102 yrs). Compared to those whose diets consisted of low levels of fruits and vegetables, those whose intake was higher had lower plasma F_2-Isoprostane levels, higher plasma antioxidants, and scored better on neuropsychological tests (MMSE, Clock Drawing task and Dem Tec) indicators of greater cognitive ability (Polidori et al., 2009).

Fiocco and colleagues (2011), however, observed no significant relationship between plasma F_2-Isoprostane levels and cognitive performance using two tasks: the 3MS, a modified version of the Mini Mental State Examination, and the Digit Symbol Substitution Task from the Wechsler Adult Intelligence Scale. Interestingly, the healthy cohort of this study included participants with reported diagnoses of diabetes, stroke and myocardial infarction; all

conditions where elevated F_2-Isoprostane levels have been identified, yet no significant differences were found between F_2-Isoprostane levels and cognitive performance. To potentially address the inconsistencies in the findings related to F_2-Isoprostanes and cognitive performance, we employed a validated, highly sensitive, computerised cognitive assessment system to test participants' attention, working memory, episodic memory and information processing abilities. The Cognitive Drug Research system was designed to assess major aspects of human cognitive function (Wesnes, McNamara & Annas, 2016), and has been repeatedly shown to be sensitive to non-clinical ageing (e.g., Ryan, et al., 2008) as well as a wide variety of age-related clinical conditions (e.g., including Alzheimer's disease; Galvin et al., 2008) and changes in cognitive performance in more than 1000 clinical trials (e.g., Wesnes & Brooker, 2011).

Therefore, the purpose of the current study was to examine the influence of oxidative phospholipid concentrations as measured in vivo by plasma F_2-Isoprostanes on cognitive performance, measured using the CDR battery, in a group of healthy adults without diagnosed cardiovascular, neurological or psychiatric illness. It was hypothesised that higher F_2-Isoprostane concentrations would be associated with poorer cognitive performance scores across the cognitive tasks.

2. MATERIALS AND METHOD

2.1 *Participants*

The sample comprised 211 healthy volunteers (Male; 88: Female 123) aged 60 -75years 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 natural supplements 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 932pmol/L: see Figure 1); the demographics of the two groups appear in Table 1.

2.2 Participant Recruitment

Participants were recruited through radio, newspaper articles as well as poster and flyer distribution. Participants were excluded if they were taking cognitive enhancing supplements (e.g. Ginkgo biloba), were current smokers, had a history of drug and/or alcohol abuse or 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. Eligibility was initially determined from telephone screening by an experienced research assistant relying on the interested participants' self-report. During a face-to-face interview, general demographic information was collected and, further eligibility screening was conducted using the Mini Mental State Examination (MMSE: Folstein, Folstein & McHugh, 1975) excluding those with a score of 23 or less; and excluding those with a score of 20 or more using the 30-item self-report Geriatric Depression Scale (GDS: Yesavage et al., 1982) to measure depressive symptomatology.

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).

2.3 Cognitive Outcomes: The Cognitive Drug Research battery

Multiple cognitive domains were assessed using a validated computerised cognitive test battery, the Cognitive Drug Research® (CDR), due to the range of tasks that capture cognitive processes that decline with age. The CDR test battery takes approximately 30 minutes to complete. It assesses information processing speed, reasoning, attention, working memory and episodic memory (Wesnes, Ward, McGinty, & Petrini, 2000). The CDR battery consists of 11 tasks, three of which measure aspects of attention, vigilance and information processing (Simple Reaction Time, Choice Reaction Time and Digit Vigilance), two of the tests assess working memory (Numeric and Spatial Working Memory), and four of the tests assess episodic Memory (Immediate and Delayed Word Recall, Word Recognition, and Picture Recognition). As such, the CDR system provides validated factor scores derived from performance on these tests measures that can be used to evaluate specific aspects of cognitive performance (for a full description of the tests and normative data, see; Wesnes, McNamara, & Annas, 2016).

The 'Power of Attention' factor score is produced by summing the reaction times of the three tasks involving attention: Simple Reaction Time, Choice Reaction Time, and Digit Vigilance. The measure reflects the ability to focus attention and process information while ignoring distraction. The 'Continuity of Attention' factor is derived by combining the percentage accuracy of the Choice Reaction Time and the Digit Vigilance Task and adjusting for false alarms from the Digit Vigilance Task. This score reflects the ability to sustain attention. The 'Quality of Working Memory' factor is produced via combining the sensitivity indices from the Numeric and Spatial Working Memory tasks, and reflects how much information can be held in short term memory. The 'Quality of Episodic Secondary Memory' factor is

derived through combining the overall percentage accuracy scores from the memory tests: Immediate Word Recall, Delayed Word Recall, Delayed Word Recognition, and Delayed Picture Recognition. This measure reflects the amount of information that can be retrieved in episodic memory. The final factor, ‘Speed of Memory’, is represented by the sum of the reaction times of the Spatial Working Memory, Numeric Working Memory, Word Reaction, and Picture Recognition tasks. This measure reflects how quickly information can be retrieved from episodic and working memory. All tasks are computer-controlled, with the information being presented on the screen of a computer and the responses recorded via a response module containing two buttons, one marked 'NO' and the other 'YES'. The tests take approximately 20 minutes to perform. The outcome measures from these tasks are presented in Table 2.

2.4 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.

2.5 Self-reported Memory Complaints

In addition to the MMSE screening tool, participants were asked “Do you have problems with your memory?”.

2.6 General intelligence (IQ)

Two tests from the Wechsler Abbreviated Scale of Intelligence (WASI) were administered to all participants to provide an IQ estimate. Participants completed the vocabulary and matrix

reasoning subsets of the WASI. The vocabulary subtest is a 42-itemtask, which requires the examinee to orally define words that are presented visually and orally. The matrix reasoning subtest shows a series of 35 incomplete grid patterns, which the examinee is asked to complete by pointing to, or stating, the correct pattern from five possible choices. The WASI is a reliable measure of intelligence for use in clinical and research settings.

2.7 *Geriatric Depression Scale (GDS)*

The GDS (Yesavage et al. 1982) is a basic screening measure for depression suitable for elderly populations. Although the GDS cannot be used to diagnose clinical depression it provides insight into the severity of depressive symptoms experienced by the rater. 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.

2.8 Biochemical Measure: F_2-Isoprostanes

Plasma concentrations of F_2-Isoprostanes were collected as the measurement of oxidative stress in the current study, with the levels of 8-iso-PGF2a being quantified by gas chromatograph mass spectroscopy (GC-MS). 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 vials prior to storage at -80°C at Swinburne University of Technology. Frozen samples were shipped to the University of Western Australia where they were quantified for F_2-Isoprostanes using a standard assay kit and employing negative ion chemical ionisation-GC-MS as described by Mori and colleagues (1999). Briefly, after the addition of an

internal standard (15-F_2t-IsoP-d4, 5 ng), plasma samples (200mL) were hydrolyzed with KOH in methanol, acidified, and applied to prewashed Certify II cartridges (Varian). Following washing with methanol:water (1:1) and hexane:ethyl acetate (75:25) the F_2-Isoprostanes were eluted with ethyl acetate:methanol (90:10), dried, and derivatized. The F_2-isoprostanes were detected by selected ion monitoring using m/z 569 and m/z 573 for endogenous and tetra-deuterated internal standards, respectively.

2.9 Procedure

Participants attended Swinburne University of Technology in Melbourne for two sessions with the visits being up to a 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. Current intellectual status was assessed using the WASI. General demographic information was also collected such as; age, gender and BMI. Participants completed a preliminary run through of the cognitive tasks. Blood samples were collected at the second visit, prior to participants completing the cognitive test batteries already described.

2.10 Statistical Analysis

All analyses were conducted using the SAS statistical package Version 9.2. Data analysis was conducted using SAS MIXED procedure, utilising ANCOVA analysis with a Main effect of

Type (Low or High F_2-Isoprostanes groupings). Covariates included were; age, gender, depression scores and years of education. Cohen's d effect sizes were calculated for significant group differences to illustrate the magnitude of the differences identified by the analyses (Cohen, 1992).

3. RESULTS

The median split (932pmol/L) of the participants resulted in "Low" and "High" groupings based upon F_2-Isoprostane levels (F_2-Isoprostane levels: Low group, M = 721.33pmol/L, SD = 120.11; High group, M = 1466.97pmol/L, SD = 527.86). Table 1 shows that the groups were well matched on age, MMSE scores, years of education and reported memory problems. Interestingly, the High F_2-Isoprostane group reported marginally (half a point) higher levels of depression. There was a notable imbalance in both groups for gender, with females being overly represented. Expected associations between task performance and the proposed covariates of age and years of education also confirmed the rationale for including them as covariates. The level of task performance on the individual CDR tasks were broadly consistent with the norms generated for the CDR System (Wesnes, McNamara & Annas, 2016).

"Insert Table 1"

The results concerning the influence of F_2-Isoprostane levels on cognitive performance are presented in Table 2. The Quality of Episodic Memory factor score and the Immediate and Delayed Recall measures (number of words recalled) showed a significant main effect of F_2-Isoprostane group.

"Insert Table 2"

Significant differences between the low F_2-isoprostane and high F_2-isoprostane groups were noted for the Factor Score; Quality of Episodic Memory [$F_{(1, 194)} = 6.83$, $p = 0.0097$; $d=0.37$]. Examination of individual tasks contributing to this factor revealed significant effects on Immediate Recall performance [$F_{(1, 197)} = 7.11$, $p = .0083$; $d=0.38$], and Delayed Recall performance [$F_{(1, 197)} = 10.64$, $p =.0013$; $d=0.46$).

"Insert Figure 1"

4. DISCUSSION

Our results indicate that otherwise healthy older adults who present with lower F_2-Isoprostane levels are superior in retaining information in episodic memory, compared to those older adults with relatively raised F_2-Isoprostane levels. The effect sizes for these differences in episodic memory and verbal recall were in the small to medium range (Cohen, 1988), bordering on what is considered 'clinically significant' (Harvey, 2012). To our knowledge, this is the first report of impaired episodic memory and verbal recall in healthy older adults, which highlights the value of F_2-Isoprostanes as a biomarker that could be used to predict cognitive change in non-demented older adults. All participants age, gender, depression scores and years of education were controlled for in these analyses, and no significant differences were observed in the measures of attention, or the speed at which information was retrieved from episodic or working memory. Together with previous reports of the association between F_2-Isoprostane levels and diet quality (Polidori et al., 2009), executive function (Li et al., 2014), and short screening measures of cognitive function in non-clinical older adults (MMSE scores; Polidori et al., 2009), the results of the current study highlight the utility of the oxidative stress biomarker, F_2-Isoprostanes, as an index of age-associated changes in cognitive function.

These results are consistent with the findings of Li et al (2014), who observed that F_2-Isoprostane concentrations were significantly related to completion of the Trail-making B (an assessment of executive functioning) in their cross-sectional analyses of their healthy middle aged sample. Prior to correction for multiple comparisons, Li et al (2014) also reported a significant relationship between a composite score composed of the average performances on the Immediate Recall, Delayed Recall, Trails A, Trails B and Category Fluency scores with

F_2-Isoprostane concentrations. The involvement of the Immediate Recall and Delayed Recall scores in this composite score more closely align with the findings of the current study, where lower F_2-Isoprostane concentrations were associated with increased verbal episodic memory performance. Given the well-established age deficits in episodic memory, the potential use of the F_2-Isoprostane as a biomarker for the advanced indication or potential acceleration of age-associated changes in episodic memory appears promising. These results also highlight the potential for interventions (e.g., improved diet - Polidori et al., 2009; antioxidant interventions – Ryan et al., 2008) that concurrently lower F_2-Isoprostane levels and therefore may also result in improved cognitive function (specifically episodic memory) in relatively healthy older adults as potential strategies for slowing age-associated declines in specific cognitive capacities across clinical groups (e.g., mild cognitive impairment).

Polidori et al (2009) previously reported upon the relationship between plasma levels of F_2-Isoprostanes and cognitive performance in groups of 'high' and 'low' fruit and vegetable intake. They observed that the 'high' intake group performed significantly better on the three cognitive measures (MMSE, DemTect and Clock Drawing) than the 'low' intake group, and expectedly, displayed higher plasma levels of the majority of micronutrient and vitamins sampled. Importantly in the context of the current study, MMSE performance was significantly correlated with F_2-Isoprostanes, F_2-Isoprostane levels were further significantly related to the levels of lipophilic antioxidants (α-tocopherol and β-carotene), and the 'high' intake group also displayed significantly lower levels of F_2-Isoprostanes. Given the relatively consistent finding that antioxidant and micronutrient levels are depleted in dementia patients and sufferers of mild cognitive impairment, our results provide further evidence of the role of increased oxidative stress status in the pathophysiology of age-associated cognitive decline (specifically episodic memory in the current study). As highlighted in the Li et al (2014)

findings, the concentrations of F_2-Isoprostanes were significantly correlated with executive function at baseline, but the magnitude of this association diminished with the increasing age of participants (> 60 years) and when examining changes in the longitudinal changes in executive function. This finding suggests that a diet rich in antioxidants can provide some protection against the development of oxidative stress markers such as F_2-Isoprostanes and their associated cognitive deficits, but as people age further, biomarkers such as β-amyloid become more relevant for conversion to mild cognitive impairment or Alzheimer's dementia.

A further implication of our findings that greater F_2-Isoprostane concentrations are associated with reduced episodic memory capacity is that the levels of F_2-Isoprostanes seem to be amenable to reduction by antioxidant intervention (Ryan et al., 2008). In a stringently controlled 3-month intervention trial (controlling for age and IQ), Ryan et al (2008) administered the potent antioxidant, Pycnogenol (and a matched placebo), for 3-months in a healthy elderly cohort (aged 65-80 years). They reported significant reductions in the Pycnogenol treatment for plasma levels of F_2-Isoprostanes, and a significant improvement in the 'quality of working memory' factor score (comprised of the combined performances on tasks for numeric working memory and spatial working memory) from the same CDR battery utilised in the current study. The authors attributed the reductions in the plasma concentrations of F_2-Isoprostanes in the Pycnogenol group to the biological efficacy of Pycnogenol as an antioxidant capable of terminating in-progress oxidative reactions. This may have resulted in decreased lipid peroxidation, which in turn leads to decreased arachidonic acid concentrations, and increased membrane fluidity, which may have improved neural mechanisms of memory consolidation via long-term potentiation processes (including the resultant improvement in the quality of working memory factor).

As our data is drawn from a baseline too, we can only speculate whether the relationship observed between episodic memory performance would remain significant over the course of the ageing process. We can, however, highlight the necessity in utilising highly sensitive measures of cognitive performance to map cognitive capacity in carefully selected human populations. Measure such as the MMSE, or modified versions (e.g., Modified Mini-Mental State Examination: 3MS), are sufficient for use as screening tools, or to classify groups (e.g., mild cognitive impairment versus severe cognitive impairment as seen in demented patients), rather than as a criterion variable, as it is relatively insensitive to changes to specific aspects of cognition. Notably MMSE did not differ between our high and low F_2-Isoprostane groups.

In conclusion, the present study identified that lower plasma levels of F_2-Isoprostanes are associated with superior performance on measures of episodic memory. Together with the findings of Li et al (2014) and Polidori et al (2009), this indicates that the relationship between F_2-Isoprostane levels and cognitive function are worthy of further investigation in healthy elderly cohorts and other clinical conditions associated with increased oxidative stress profiles and compromised cognition.

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Competing Interest Statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The CDR System is proprietary to Bracket (www.bracketglobal.com) who provide it as a service to the clinical trial industry. KW holds stock in Bracket and was employed by the company until February 2014. KW also currently consults for various companies involved in clinical trials, including Bracket.

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