CHANGES IN OXIDATIVE DAMAGE, INFLAMMATION & [NAD(H)] WITH AGE IN HUMAN CEREBROSPINAL FLUID: INFLUENCE OF CAROTENOIDS

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Introduction

Aging is an unavoidable biological process characterized by a progressive decline in physiological and biochemical function resulting in an increased predisposition to disease. The oxidative stress theory of aging suggests that the accumulation of unrepaired oxidative damage results in the typical aging phenotype.[1] The term 'oxidative stress' describes a significant imbalance between antioxidant defenses and the bodies' formation of reactive nitrogen and/or oxygen species (ROS).

The brain is particularly vulnerable to oxidative damage as a consequence of its high oxygen demand, high level of both polyunsaturated fatty acids and transition metals, and poor antioxidant defenses.[2] As we age, the vulnerability of the brain to oxidative damage increases.[3,4] Indeed animal and tissue studies have shown the aging brain to be accompanied by an accumulation of markers of lipid, protein and DNA oxidative damage.[5-7]

Evidence indicates that neuroinflammation is also elevated during normal brain aging.[8] Under normal homeostatic conditions both immune and oxidative activity are largely transitory due to inherent negative feedback mechanisms including increased production of anti-inflammatory cytokines and enhanced endogenous antioxidant activity. During periods of chronic disease however, these processes can be continuously activated amplifying each other in a feed forward cycle, causing cell death, tissue dysfunction and disease.[9] Indeed both neuroinflammation and oxidative activity are increased in several neurodegenerative disorders including Alzheimer's disease.[10]

As inflammation and oxidative damage rise with age a decrease in available nicotinamide adenine dinucleotide (NAD^+) has been observed in multiple organs of the rat, including the brain.[11] NAD^+ is a ubiquitous molecule that is required for a number of vital cellular processes. In addition to its role in cellular energy and metabolism there are several enzymes, including poly(ADP-ribose) polymerase 1 (PARP) and silent information regulators (e.g. SIRT1), that use NAD^+ as their substrate. Importantly PARP requires NAD^+ to facilitate DNA repair.[12] However over-activation of PARP, due to excessive DNA damage, can result in neuronal death as a consequence of decreased ATP production via NAD^+ depletion.[13] In order to preserve cellular energy and concomitantly SIRT1 and PARP activity, adequate levels of NAD^+ must be sustained.

While age is the major risk factor for the development of most neurodegenerative disorders, a number of lifestyle choices have been linked to either promotion or prevention of pathogenesis by increasing or decreasing oxidative stress and inflammation. Carotenoids, a family of phytochemicals synthesised by plants that are responsible for the red, orange, and yellow pigments of fruit and vegetables (Figure 1), have been the subject of increased attention as a result of their anti-inflammatory and anti-oxidant properties. Importantly serum carotenoids have been shown to be positively associated with brain carotenoid levels and can be considered a reliable predictor of brain carotenoid concentrations.[14] Within the brain carotenoids are thought to exert a variety of protective effects, including attenuating Aβ induced ROS formation.[15] Low serum levels of selected carotenoids have also been linked to impaired cognitive function.[16]

Collectively these reports indicate that higher carotenoid levels may mitigate against the development of an age associated oxidative-inflammatory state and thereby reduce tissue damage within the CNS. While evidence from cell culture, animal and limited post-mortem brain tissue studies support this hypothesis, to date no study has investigated this putative association in a healthy human cohort.

Figure 1: Tomatoes are rich in the carotenoid lycopene

Aim

The aim of this study was to investigate whether markers of oxidative and inflammatory activity increase with age in the central nervous system and evaluate the relationship between plasma concentrations of carotenoids and plasma and cerebrospinal fluid (CSF) markers of inflammation, oxidative stress and NAD^+ in an essentially healthy human cohort.

Methods

Participants
Male (n=20) and female (n=50) participants, who required a spinal tap for the administration of anesthetic as part of routine care, were recruited at Sydney Adventist Hospital, Australia. The average age of participants was 53 years (SD=19.9, interquartile range=38).

Participants were excluded from the cohort if they were smokers or had a confirmed diagnosis of a neurological/neurodegenerative disorder or central nervous system infection. In total 70 CSF and 38 matched blood samples were collected from consenting participants considered in general good health.

Methods Cont.

Sample Collection
Fasting (≥10 h) blood and CSF samples were collected by an accredited anesthetist no longer than 30 minutes apart. CSF samples were collected, prior to injection of spinal anesthetics, via standard lumber puncture (Figure 2). Blood samples were collected into heparinized tubes prior to the administration of fluids or anesthetics. Samples were prepared by centrifuging at 1800 rpm for 10 minutes and stored within 1 hour of collection, at -194° C. Samples intended for F2-isoprostane analysis where stored in the presence of a glutathione/butylated hydroxytoluene preservative.

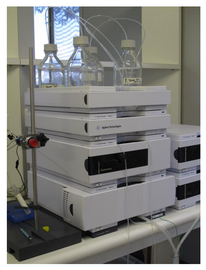


Figure 3: Carotenoid analysis by HPLC

Figure 2: CSF sample collection

Table 1: Biochemical Analysis

Measure	Marker	Sample	Method
Inflammation	Interleukin-6	CSF + Plasma	ELISA
Lipid oxidation	F2-Isoprostanes	CSF + Plasma	GC-MS
DNA oxidation	8-hydroxy-2'-deoxyguanosine	CSF + Plasma	ELISA
Metabolism	Total NAD(H)	CSF + Plasma	Colourimetry
Antioxidant capacity	Total antioxidant capacity	CSF + Plasma	Colourimetry
Diet derived antioxidants	Carotenoids	Plasma	HPLC (Fig 3)

Statistical analysis
Statistical analyses were performed using SPSS version 16.0. Data is presented as means ± standard deviation unless otherwise stated. The Independent T Test or Mann-Whitney U Test was employed to analyze the effect of age on markers of oxidative damage, inflammation and metabolism. The Pearson correlation coefficient and Multiple linear regression, controlling for age and gender, was used to identify significant relationships between carotenoids, [NAD(H)], and markers of oxidative stress and inflammation. Adjusted and non-adjusted *P*-values are provided throughout with test significance set at *P* value ≤ 0.05.

Results

CSF of participants aged >45 years was found to contain increased levels of lipid peroxidation (F2-isoprostanes) (p=0.04) and inflammation (IL-6) (p=0.00) and decreased levels of both total antioxidant capacity (p=0.00) and NAD(H) (p=0.05), compared to their younger counterparts (Table 2).

Table 2: Differences in selected CSF markers according to age

♂♀	≤ 45 years, mean (± SD)	> 45 years, mean (± SD)
(n)	34	36
Average Age (years)	34 (5)	71 (8)
CSF F2-Isoprostanes (pmol/L)	395.1 (34.0)	417.5 (34.4)*
CSF 8-OHdG (ng/mL)	0.4 (0.1)	0.5 (0.1)
CSF IL-6 (pg/mL)	0.7 (0.4)	2.4 (1.9)**
CSF TAC (nmol/mg protein)	1.5 (0.5)	0.9 (0.3)**
CSF [NAD(H)] (μg/mL)	88.6 (21.1)	75.9 (30.1)*

Due to small sample volume, some tests have one or more missing data. Comparisons made using the Independent T Test or Mann-Whitney U Test. *p ≤ 0.05 compared to ≤ 45 years, **p ≤ 0.001 compared to ≤ 45 years.

Assessing these trends in each gender revealed that the CSF of females >45 years contained significantly higher levels of IL-6 (p=0.00, 1.71 ± 1.23 vs. 0.69 ± 0.43 pg/mL) and lower TAC levels (p=0.00, 0.98 ± 0.30 vs. 1.49 ± 0.51 nmol/mg protein) than their younger counterparts (Table 3). Due to low number of male participants ≤45 years, valid comparisons between age group for this gender were not possible.

Table 3: Differences in selected CSF markers according to age in females

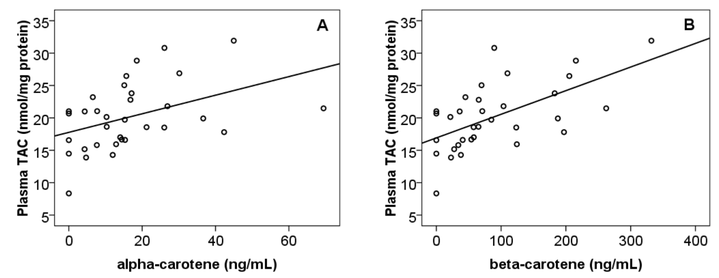
♀	≤ 45 years, mean (± SD)	> 45 years, mean (± SD)
(n)	33	17
Average Age (years)	34 (5)	73 (9)
CSF F2-Isoprostanes (pmol/L)	394.57 (34.79)	413.23 (29.81)
CSF 8-OHdG (ng/mL)	0.45 (0.09)	0.51 (0.12)
CSF IL-6 (pg/mL)	0.69 (0.43)	1.71 (1.23)**
CSF TAC (nmol/mg protein)	1.49 (0.51)	0.98 (0.30)*
CSF [NAD(H)] (μg/mL)	89.98 (19.75)	73.13 (25.99)*

Due to small sample volume, some tests have one or more missing data. Comparisons made using the Independent T Test or Mann-Whitney U Test. *p ≤ 0.05 compared to ≤ 45 years, **p ≤ 0.001 compared to ≤ 45 years.

A significant positive association was observed between both α-carotene (*P* = 0.01, r = 0.43, n = 33) and β-carotene (*P* < 0.001, r = 0.59, n = 33) and the total antioxidant capacity (TAC) in plasma (Figure 4).

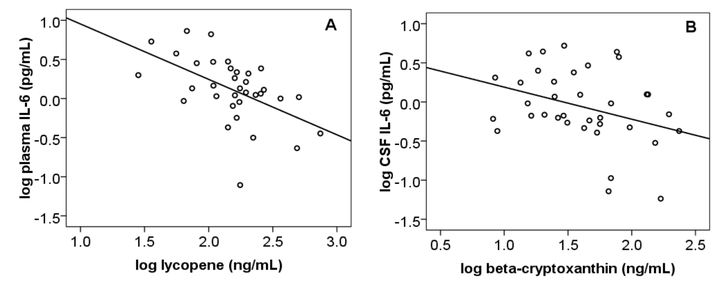
Results Cont.

Figure 4. Positive association between plasma total antioxidant capacity (TAC) and (A) α-carotene (B) β-carotene.



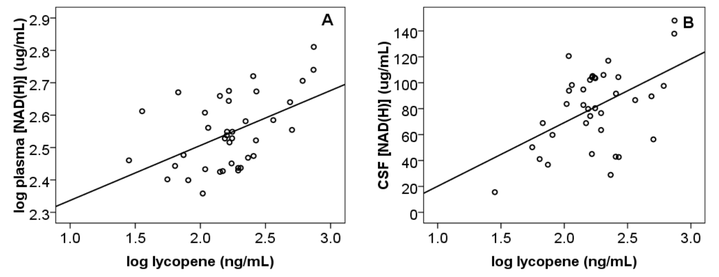
A significant inverse association was observed between plasma levels of lycopene and plasma IL-6 (*P* < 0.001, r = -0.53, n = 34). This association remained even after controlling for age and gender (*P* = 0.02, R^2 = 0.21) (Figure 5A). An increase in plasma β-cryptoxanthin levels was associated with a decrease in CSF IL-6 (*P* = 0.04, r = -0.34, n=36). This association remained even after controlling for age and gender (*P* = 0.04, R^2 = 0.45) (Figure 5B).

Figure 5. Association between carotenoids and reduced levels of central inflammation.



A significant positive association was found between lycopene and both plasma (*P* < 0.01, r = 0.50, n = 38) and CSF (*P* < 0.01, r = 0.50, n = 37) [NAD(H)] (Figure 6). These relationships remained even after controlling for age and gender (*P* < 0.001, R^2 = 0.31; *P* < 0.001, R^2 = 0.21 respectively).

Figure 6. Association between carotenoids and increased levels of peripheral and central [NAD(H)].



Conclusion

This study provides evidence:

- Of a progressive age associated increase in oxidative damage, inflammation and reduced [NAD(H)] in the brain.
- Of a link between increased plasma carotenoid concentrations and reduced central inflammation and oxidative activity.
- That the carotenoid lycopene may influence levels of NAD(H), in both the plasma and the CSF.

Acknowledgments

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