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# Healthy dietary intake moderates the effects of age on brain iron concentration and working memory performance

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ABSTRACT

Age-related brain iron accumulation is linked with oxidative stress, neurodegeneration and cognitive decline. Certain nutrients can reduce brain iron concentration in animal models, however, this association is not well established in humans. Moreover, it remains unknown if nutrition can moderate the effects of age on brain iron concentration and/or cognition. Here, we explored these issues in a sample of 73 healthy older adults (61-86 years old), while controlling for several factors such as age, gender, years of education, physical fitness and alcohol-intake. Quantitative susceptibility mapping was used for assessment of brain iron concentration and participants performed an N-Back paradigm to evaluate working memory performance. Nutritional-intake was assessed via a validated questionnaire. Nutrients were grouped into nutrition factors based on previous literature and factor analysis. One factor, comprised of vitamin E, lysine, DHA omega-3 and LA omega-6 PUFA, representing food groups such as nuts, healthy oils and fish, moderated the effects of age on both brain iron concentration and working memory performance, suggesting that these nutrients may slow the rate of brain iron accumulation and working memory declines in aging.



## 1. Introduction

Aging is associated with accumulation of non-heme brain iron, which has been linked with oxidative stress, neurodegeneration and cognitive decline (Lauffer, 1992; Martin et al., 1998; Zecca et al., 2004a,b; Daugherty et al., 2015; Daugherty and Raz, 2016). While non-heme brain iron is essential for neuronal function, and is involved in multiple beneficial cellular processes, such as adenosine triphosphate (ATP) generation in mitochondria, neurotransmitter synthesis and myelin generation, it is also a potent oxidizer (Mills et al., 2010; Todorich et al., 2009; Ward et al. 2014; Raz and Daugherty 2018). As such, non-heme brain iron is typically sequestered in iron storage complexes like ferritin, which release it in a tightly regulated manner (Hentze et al., 2004; Moos et al., 2007).

Normal aging perturbs the iron sequestration process, allowing non-heme iron to accumulate outside of storage complexes (Hallgren and Sourander, 1958; Lauffer, 1992; Martin et al. 1998; Zecca et al., 2004a; Zecca et al., 2004b). This age-related accumulation of non-heme iron can contribute to the endogenous production of reactive oxygen species (ROS; Chakravarti and Chakravarti, 2007), which can react with cellular structures (e.g., lipids, proteins and nucleic acids), damaging neurons, glia and myelin, leading to cognitive impairment (Zecca et al., 2004a; Zecca et al., 2004b; Chakravarti and Chakravarti, 2007; Ke and Qian, 2007; Butterfield and Halliwell, 2019). For instance, excess

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non-heme brain iron has been repeatedly linked with poorer working memory performance (Bartzokis et al., 2011; Daugherty et al., 2015; Darki et al., 2016; Zachariou et al. 2020) as well as declines in other cognitive and motor domains (Ayton et al., 2017; Penke et al., 2012; Rodrigue et al., 2013; Sullivan et al., 2009)

Evidence from animal models suggests that certain nutrients can reduce brain iron concentration, slow age-related brain iron accumulation and/or protect against oxidative stress (e.g., Hagen et al., 2002; Zaidi et al., 2004; Suh et al., 2005; Ahmed 2012; Abadi 2013). Protective nutrients may include, but are not limited to vitamins (e.g., vitamins C and E; Pappert et al., 1996; Lan & Jiang, 1997; Butterfield et al., 2002; Terpstra & Gruetter 2004; Ahmed 2012), polyunsaturated fatty acids (PUFAs; Yehuda et al. 2005; Simopoulos 2006; Freund et al., 2014; Stavrinou et al., 2020; Ogłuszka et al., 2020), flavonoids (e.g., epigallocatechin 3-gallate, quercetin (Butterfield et al., 2002; Mandel et al., 2008; Ansari et al., 2009; Faria et al., 2014; Imam et al., 2017) and iron-chelators such as alpha-lipoic acid (Lovell et al., 2003; Maczurek et al., 2008; Shay et al., 2009; Domenico and Giudetti, 2017; Fernandez-Moriano et al., 2015; Molinari et al., 2019; Farr et al., 2003; Joshi et al., 2006).

Despite evidence from animal models, the impact of nutrition on age-related accumulation of brain iron in humans is not well-established (e.g., Hagemeier et al., 2015). In particular, it remains unknown if the nutrients studied in animal models can moderate the effects of age on brain iron concentration and cognition in humans. To address these issues, we explored the association between age, brain iron concentration, nutrition and working memory performance in a cohort of 73 healthy, older adults ranging in age from 61 to 86 years.

MRI-based quantitative susceptibility mapping (QSM) was performed for assessment of non-heme brain iron concentration which provides at least 2 benefits compared to more traditional susceptibility weighted imaging (SWI), T2* or R2* measures: (1) QSM can compute the underlying susceptibility of each voxel as a scalar quantity while more traditional methods can only provide regional susceptibility due to blooming artifacts (Wang et al., 2017); (2) QSM susceptibility values, unlike those from SWI, T2* etc., do not vary based on the orientation of the head in the scanner (Li et al., 2012).

We focused on specific groups of vitamins, antioxidants, flavonoids, iron chelating nutrients and polyunsaturated fatty acids (PUFA) due to prior results from animal models suggesting these nutrients can cross the blood brain barrier (BBB) and either reduce oxidative stress, chelate brain iron or protect against age-related ferroptosis (Zaidi et al., 2004; Suh et al., 2005; Dixon et al., 2012; Domenico and Giudetti, 2017; Fernandez-Moriano et al., 2015; Molinari et al., 2019; Ogluszka, et al., 2020). Thus, all selected nutrients have the potential to reduce the impact of excess brain iron on cognition and positive associations between dietary intake of these nutrients and cognitive function have been identified in previous studies (e.g., Butterfield et al., 2002; Youdim and Josepth, 2001; Hager et al., 2001; Yehuda et al., 2005; Kuriyama et al., 2006; Carmichael et al., 2018; Stavrinou et al. 2020).

Dietary information was obtained via a self-administered online nutrition questionnaire, which catalogs participants' diet for the preceding month and has been validated against plasma micronutrient levels of carotenoids, retinols, tocopherols, cholesterol, and vitamin C (Satia et al., 2009). Considering cognition, the domain of working memory was tested due to its established negative associations between both aging and brain iron concentration (Bartzokis et al., 2011; Daugherty et al., 2015; Darki et al., 2016; Zachariou et al., 2020).

**Table 1**
Group demographics and mean cognitive measures

| | |
|---|---|
| n | 73 |
| M:F | 33:40 |
| Age range | 61-86 |
| Age (years) | 70.83±5.78 |
| Education (years) | 16.90±2.28 |
| PASE[a] | 127.31±63.31 |
| Alcohol drinking frequency (drinks/day) | 0.34±0.71 |
| MoCA[b] | 26.62±2.73 |

Group demographics and mean cognitive measures. The table lists the number of participants included in analyses, male/female distribution, the age range of the participant cohort as well as mean (± sd) for participants' age, years of education, Physical Activity Scale for the Elderly scores (PASE), alcohol drinking frequency (drinks per day) and Montreal Cognitive Assessment (MoCA) scores.
Mean ± standard deviation is shown for participants.
[a] PASE: Physical Activity Scale for the Elderly (scale 0-793; higher = more physical activity).
[b] MoCA: Montreal Cognitive Assessment (Scale 0-30; 26 or higher = cognitively normal).

## 2. Materials and methods

### 2.1. Participants

Seventy-seven older adults were recruited for the experiment (42 women, age range 61-86 years old) from an existing longitudinal cohort at the Sanders-Brown Center on Aging (Schmitt et al., 2012) and the broader Lexington, KY, community. All participants provided informed consent under a protocol approved by the Institutional Review Board of the University of Kentucky. All participants were cognitively intact based on clinical consensus diagnosis and scores from the Uniform Data Set (UDS3) used by US ADCs (procedure outlined in Morris et al., 2006; Besser et al, 2018) or a score of 26 or higher on the Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005). The UDS3 includes a comprehensive battery of neuropsychological tests assessing global cognition, memory encoding, memory retrieval, semantic memory, working memory, attention, executive function, processing speed, and verbal retrieval.

In addition to the neuropsychological assessment, information about participants' physical activity (using the Physical Activity Scale for the Elderly (PASE); Washburn et al., 1993, 1999), years of education, smoking and alcoholic drink frequency (drinks/packs of cigarettes per day) was collected during a post-experiment telephone interview, and was used to assess/control for lifestyle factors which could affect nutritional habits and brain iron concentrations.

Study exclusionary criteria were self-reported significant head injury (defined as loss of consciousness for more than 5 minutes), heart disease, neurological or psychiatric disorders, claustrophobia, pacemakers, the presence of metal fragments or any metal implants that are incompatible with MRI, diseases affecting the blood (anemia, kidney/heart disease etc.) or significant brain abnormalities detected during imaging.

Four participants were excluded from analyses. Two participants were excluded on the basis of MRI abnormalities. Two additional participants were excluded due to smoking. We excluded participants who smoke from our analyses rather than control for it (as we did for education, alcohol consumption and exercise frequency) because only 2 participants (out of 77) were smokers, making it impossible to control for smoking or evaluate its impact on our findings. Characteristics of the participant cohort included in analyses are shown in Table 1.

### 2.2. Imaging protocol

Participants were scanned with a Siemens 3T PRISMA Fit scanner, using a 64-channel head-coil, at the University of Kentucky

Magnetic Resonance Imaging and Spectroscopy Center (MRISC). The following sequences were collected for this study: a 3D multi-echo, T1-weighted anatomical image (MEMPR) and a 3D, multi-echo, gradient-recalled echo (GRE) sequence used for Quantitative Susceptibility Mapping (QSM). Several other sequences were collected during the scanning session related to other scientific questions and are not discussed further here. The MEMPR sequence had 4 echoes [repetition time (TR) = 2530 ms; first echo time (TE1) = 1.69 ms echo time spacing ($\Delta$TE) = 1.86 ms, flip angle (FA) = 7°] and covered the entire brain [176 slices, field of view = 256 mm, parallel imaging (GRAPPA), acceleration factor = 2, 1mm isotropic voxels, scan duration =5.53 min]. The MEMPR sequence was used to optimize the Freesurfer cortical segmentation and improve the accuracy of the gray matter lobar masks (van der Kouwe et al., 2008). The sequence used for QSM was a flow compensated, multi-echo, 3D spoiled GRE sequence in the sagittal plane with 8 echoes (TR/TE1/$\Delta$TE/FA= 24ms/2.98ms/2.53ms/15°). The entire brain was covered [acquisition matrix = 224 × 224 × 144, parallel imaging (GRAPPA), acceleration= 2, 1.2 mm isotropic voxels and scan duration = 6.18 min].

### 2.3. Quantitative susceptibility mapping (QSM) processing

GRE images were processed in MATLAB using the Morphology Enabled Dipole Inversion toolbox (MEDI toolbox, release of 11/06/2017; Liu et al., 2011a,b, 2012). This approach generates QSM images by inverting an estimate of the magnetic field that is structurally consistent with anatomy in order to generate a distribution of local magnetic susceptibility values. The required scans for the MEDI analyses are a phase image and a magnitude image, which were collected as part of the same GRE sequence.

The following steps were performed in MEDI: (1) nonlinear fitting to the multi-echo data to estimate the magnetic field inhomogeneity; (2) phase unwrapping using the magnitude image as a guide (Liu et al., 2013); (3) removal of the background field by applying a projection onto the dipole field (see Liu, Khalidov et al., 2011); (4) inversion of the remaining field to calculate the quantitative susceptibility map. Finally, local magnetic susceptibility within cerebrospinal fluid (CSF; specifically within the lateral ventricles) was used to scale the QSM maps such that positive values corresponded to local magnetic susceptibility greater than that of CSF and negative values corresponded to local magnetic susceptibility less than that of CSF. CSF within the lateral ventricles was selected as the reference for the QSM analyses because CSF susceptibility is fairly uniform and does not scale with participant demographic variables such as age and gender. For this last step, ventricular reference masks were created separately for each participant as follows: The MEMPR was first aligned to the QSM magnitude image using the AFNI function align_epi_anat.py. Lateral ventricle masks were then created for each participant using this magnitude-aligned MEMPR scan in conjunction with ALVIN (see Kempton et al., 2011) and SPM12. Lateral ventricle masks were then eroded by one voxel to prevent partial volume effects with the surrounding subcortical gray and white matter, resampled to the QSM voxel resolution (1.2 mm isotropic) and used in the MEDI toolbox as the CSF reference mask for each participant.

### 2.4. Subcortical and cortical GM masks for QSM

Freesurfer 6.0 was used with the recon-all option (all available parcellations) to segment each participant's MEMPR scan. Masks corresponding to the caudate, putamen and pallidum were created from the corresponding Freesurfer segmented structures of the basal ganglia (Figs. 1C; 1D). Next, lobar cortical gray matter (GM) masks, corresponding to frontal, parietal, occipital and temporal lobe GM, were created as recommended by Freesurfer (https://surfer.nmr.mgh.harvard.edu/fswiki/CorticalParcellation) by joining together the relevant GM segmented cortical structures/masks associated with each of the 4 lobes (Figs. 1A; 1B).

The transformation matrices resulting from the alignment of the MEMPR to the QSM magnitude image (described in the QSM processing section) were then applied to each participants' lobar GM/basal ganglia masks using the AFNI function 3dAllineate with a nearest neighbor interpolation method. Lastly, each mask was eroded by one voxel to prevent partial volume effects and then resampled to the QSM voxel resolution (i.e., from 1 mm isotropic to 1.2 mm isotropic voxels).

Values from QSM maps were then extracted from each of the magnitude-aligned and resampled masks for each participant. Positive QSM values (susceptibility greater than that of CSF in the lateral ventricles) were extracted, representing QSM signal associated with iron (e.g., Wisnieff et al., 2015; Hametner et al., 2018). Normalized QSM values were then created for each participant by dividing the sum of QSM values from each mask by the total number of voxels of their corresponding mask, resulting in a final unit measure of iron concentration in parts per billion by $mm^3$ ($ppb/mm^3$).

### 2.5. Working memory task

The visual working memory paradigm, described in detail in Zachariou et al., (2020), was performed during an fMRI scan and collected during the same scanning session as the QSM scan. In the present study, relationships between behavioral performance on the working memory task and QSM results are explored. Relationships between behavioral performance on the working memory task and fMRI results were considered in our previous study, (see Zachariou et al., 2020), which included a subset of 56 participants from the present study.

#### 2.5.1. Behavioral data analyses

Behavioral data collected during the scans were imported to Excel in order to calculate D-prime (Stanislaw and Todorov, 1999) for each of the N-Back task conditions. D-prime is a measure of discrimination performance corrected for response bias. That is, the participants' tendency to respond with either "same" or "different" in a trial of the N-back task when they are uncertain or guessing. For this reason, D-prime is a more optimal measure of task performance in forced-choice discrimination tasks than accuracy which does not account for response bias. D-prime was log transformed in all analyses involving MRI-based measures under the assumption that large differences in D-prime are typically associated with smaller differences in MRI-based measures. Log D-prime was then used in SPSS to conduct subsequent analyses in conjunction with the QSM and nutrition measures of interest.

### 2.6. Nutrition data acquisition and analyses

The 92-item, Newly Developed Antioxidant Nutrient Questionnaire (NDANQ; Satia et al., 2009) was used to obtain information on dietary-intake for the preceding month. The NDANQ has been validated against plasma antioxidant levels of carotenoids, retinols, tocopherols, cholesterol, and vitamin C, as well as with recall-derived measures of nutrition, obtained via unannounced telephone-administered, 24-hour dietary recalls (see Satia et al., 2009 for more details). The questionnaire required participants to report amount consumed per day/week and portion size for 92 food items and 15 common, off-the-shelf nutrition supplements/multivitamins (e.g., Centrum Performance, GNC

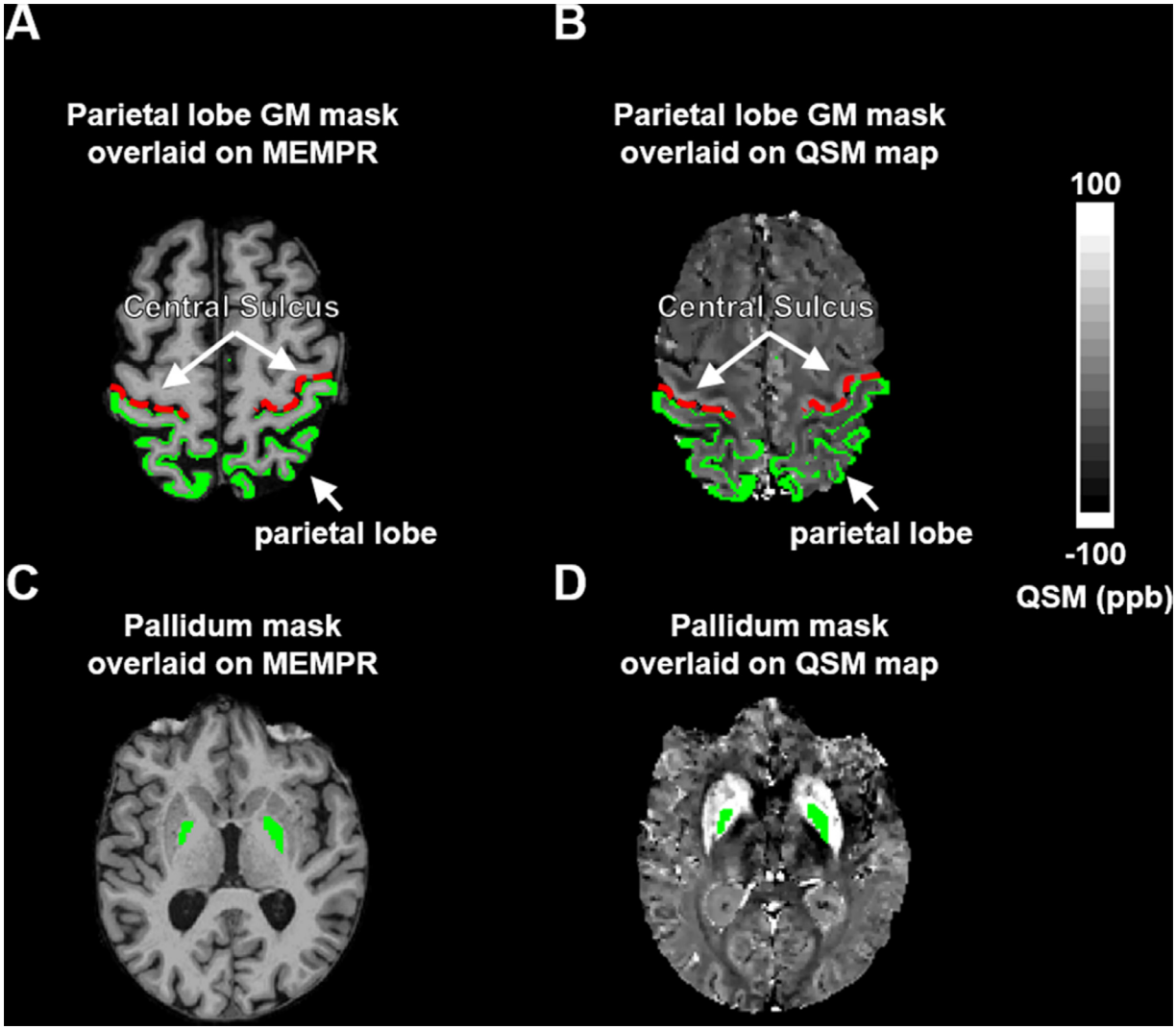


**Fig. 1.** QSM region of interest masks. A representative example of a single participant's Freesurfer segmented parietal lobe GM (A & B; in green) and pallidum masks (C & D; in green) overlaid on their MEMPR image (A & C) and their QSM map (B & D). The QSM map depicts iron concentration in parts per billion (ppb) relative to CSF. Brighter areas on the QSM map indicate regions of higher iron concentration than CSF while darker areas indicate regions of lower iron concentration than CSF (scaled between -100 to 100 ppb). The rostral boundary of the parietal lobe mask (central sulcus) is indicated with a dashed red line to highlight the accuracy of mask registration (i.e., the anatomical correspondence of GM structures captured by the mask across image modalities).

for women/men etc.). Participants were also allowed to add-in brands of supplements/multivitamins that were not included in the questionnaire. To facilitate data collection and analyses, an online version of NDANQ was created in Qualtrics XM (Qualtrics, Provo, UT) and delivered to participants via e-mail with completion instructions. This online version of NDANQ allowed participants to work on the questionnaire at their own pace with the option to automatically save and resume their session by clicking on the link provided in the e-mail.

Data from each completed survey was then processed using in-house developed software (coded in Visual Basic for Applications in Excel) in order to convert foods consumed per day into mg of nutrients per day, per participant, for a total of 122 nutrients. For this conversion, the VBA software used nutrition information from the following U.S. Department of Agriculture, Research Service databases: The food and nutrient database for dietary studies (FNDDS 2015-2016); the flavonoid values for USDA survey foods and beverages (Flavonoid 2007-2010; Sebastian et al., 2016) and the National Nutrient Database for Standard Reference (release 28; May 2016). Nutrition information related to supplements/multivitamins was acquired directly from the manufacturer (either via their website or by contacting the company).

A literature review was used to select a subset of these nutrients of highest relevance to our study goals/analyses based on previous evidence indicating a nutrient can cross the BBB and either reduce oxidative stress, chelate brain iron and/or protect against ferroptosis. Using these criteria, the following nutrients were selected for inclusion in subsequent analyses: vitamin C (Butterfield et al. 2002; Terpstra & Gruetter 2004), vitamin E (Pappert et al., 1996; Lan & Jiang 1997; Ahmed 2012), epigallocatechin 3-gallate (Mandel et al., 2008; Imam et al. 2017), quercetin (Butterfield et al., 2002; Ansari et al., 2009; Faria et al., 2014), lysine used as a proxy for alpha-lipoic acid (Lovell et al., 2003; Maczurek et al., 2008; Shay et al., 2009; Ahmed 2012), $\beta$-cryptoxanthin (Lorenzo et al., 2009; Tanprasertsuk et al., 2019), $\beta$-carotene (Lee et al., 2004; Tanprasertsuk et al., 2019), DHA omega-3 and LA omega-6 polyunsaturated fatty acids (PUFA; Yehuda et al., 2005; Simopoulos 2006; Freund et al., 2014; Stavrinou et al., 2020; Ogłuszka et al., 2020). The nutrients selected, and questionnaire

**Table 2**
Nutrients selected based on literature review and questionnaire food items containing high concentrations of those nutrients

| Nutrient | Questionnaire food item |
|---|---|
| Vitamin C | Orange, grapefruit, kiwi, mango, cabbage, tomato, broccoli, multivitamin supplements |
| Vitamin E | Safflower, soybean, and olive oils, almonds, peanuts, multivitamin supplements |
| Epigallocatechin 3-gallate | Tea, cranberry juice, peaches |
| Quercetin | Onion, apple, cooked greens, cranberry juice, hot peppers |
| Lysine | Chicken and beef liver, fish, cheese |
| $\beta$-carotene | Carrots, sweet potato, cooked greens, cooked spinach, cantaloupe, multivitamin supplements |
| DHA omega-3 | Fish, eggs, mayonnaise, wheat germ, soybean, safflower and olive oils |
| LA omega-6 | Wheat germ, soybean, safflower and olive oils, almonds |
| $\beta$-cryptoxanthin | Tangerine, orange, watermelon, carrots, papaya, multivitamin supplements |

Literature-review selected nutrients with examples of food items from the nutrition questionnaire that contain them. The questionnaire food items listed in the table are those with the highest amount of the nutrient they represent.

food items with the highest concentrations of those nutrients, are listed in Table 2.

### 2.7. Factor analysis methodology

Exploratory factor analysis was conducted in SPSS 27 (IBM, Chicago, IL, USA) to facilitate subsequent linear regressions on the relationships between nutrition, susceptibility-based iron concentrations and age. Specifically, exploratory factor analysis was conducted to (1) group nutrients that co-vary into largely independent nutrition factors and (2) reduce the number of dimensions and number of statistical comparisons required in subsequent analyses. This analysis used principal components as the extraction method (based on eigenvalues $>$ (1) and an oblique rotation for the factors (direct oblimin with Kaiser Normalization), under the assumption that factors may not be independent and could be correlated (food items contain multiple nutrients, e.g., carrots contain high amounts of both $\beta$-carotene and $\beta$-cryptoxanthin). The factor analysis used vitamin C, vitamin E, epigallocatechin 3-gallate, quercetin, lysine, $\beta$-cryptoxanthin, $\beta$-carotene, DHA omega-3, and LA omega-6 PUFAs as inputs.

Factor scores were created for the output nutrition factors using the regression method (DiStefano et al., 2009). Regression factor scores predict the location of each participant on each nutrition factor. As opposed to a simple weighted sum of the nutrients that comprise a factor, the regression method is a multivariate procedure, which takes into account the correlation between the factors and between factors and nutrients (via item loadings) and also the correlation among nutrients.

### 2.8. Statistical analyses

Statistical analyses were performed using SPSS 27 (IBM, Chicago, IL, USA). Analyses included bootstrapped (10,000 samples) univariate, multivariate, repeated measures ANOVAs and linear regression models, with bias-corrected accelerated (BCa) 95% confidence intervals (CI). In all ANOVAs, age, gender, years of education, PASE scores and alcohol drinking frequency (drinks per day) were added as covariates whereas in linear regression models these were added as predictors (added in the same model as all other independent variables). Years of education, PASE scores and alcohol drinking frequency were used to control for participants lifestyle factors which could affect their nutrition habits and brain iron concentrations. Years of education included primary, secondary, university level, postgraduate and time spent towards specializations. All multiple comparisons are reported using the Sidak correction.

Moderation effects were explored in linear regression models by mean centering predictors of interest and then multiplying them together to create interaction terms. In all linear regression models with moderators (interaction terms), predictors, covariates and interaction terms were all added in the same model. Variance inflation factors (VIF) are provided in all linear regression analyses in order to evaluate the degree of collinearity between independent variables. All repeated measures ANOVAs include partial $\eta^2$ calculations as a measure of effect size. Analyses on QSM-based iron concentrations in subcortical structures were conducted separately from those in lobar GM, because iron concentration in subcortical brain regions is substantially higher than in cortical brain regions (see results: *Subcortical and Cortical QSM-Based Iron Concentrations*).

To simplify the regression models and to reduce the number of multiple comparisons in the analyses between QSM measures and nutrition factors (3 nutrition factor scores + 5 covariates {age, gender, years of education, PASE score, alcohol drinking frequency} + 3 moderation terms {between age and nutrition factor scores} * 7 brain regions), these analyses focused only on brain regions in which susceptibility (1) correlated positively with age and (2) correlated negatively with task performance (working memory) as these relationships represent key foci of the present study.

## 3. Results

### 3.1. Subcortical and cortical QSM-based iron concentrations

In the analysis on subcortical susceptibility values (results summarized in Table 3), "subcortical structure", comprised of 3 levels (caudate, putamen and pallidum) was a significant main effect. Pairwise comparisons between subcortical structures (adjusted for multiple comparisons using Sidak) revealed that all differed significantly from each other. The analysis also revealed age as a significant between subjects effect. The parameter estimates from the analysis indicated positive correlations between age and subcortical susceptibility in the caudate and putamen. Age did not predict susceptibility values in the pallidum.

In the analysis on cortical/lobar GM susceptibility values (results summarized in Table 4), "lobe," comprised of 4 levels (frontal, parietal, occipital and temporal) was not a significant main effect. That is, susceptibility values between lobes were not significantly different. The analysis further revealed age as a significant between-subjects effect. The parameter estimates from the analysis indicated positive correlations between age and cortical susceptibility values in the frontal and parietal lobes. Age did not predict susceptibility values in the occipital and temporal lobes.

### 3.2. N-back task performance

This analysis used a repeated measures ANOVA with N-Back task performance (D-prime) as the dependent variable and task

**Table 3**
Repeated measures ANOVA results: Subcortical susceptibility

| Within-subjects effects | F-stat | *p*-value | partial $\eta^2$ | | | |
|---|---|---|---|---|---|---|
| Subcortical structure[a,b] | 20.756 | <0.0001[d] | 0.234 | | | |
| Between-Subjects effects | | | | | | |
| Age | 13.42 | <0.0001[d] | 0.165 | | | |
| *Parameter Estimates for Age* | | | | | | |
| Subcortical structure | Average susceptibility (ppb/mm[c]) | t-stat | *p*-value | partial $\eta^2$ | $\beta$ | SE |
| Caudate | 66.95 | 3.17 | 0.002[d] | 0.129 | 1.17 | 0.367 |
| Putamen | 84.22 | 4.99 | 0.0001[d] | 0.268 | 2.94 | 0.588 |
| Pallidum | 132.36 | 0.54 | 0.590 | 0.004 | 0.28 | 0.509 |

The results of the repeated measures ANOVA on QSM-based iron concentration differences between subcortical brain structures while controlling for age, gender, years of education, PASE score and alcohol drinking frequency (drinks/day).
[a] Greenhouse-Geisser corrected results. (Mauchly's test of sphericity: $p = 0.001$).
[b] All levels significantly differed from each other: $p_s < 0.0001$, Sidak corrected.
[c] $\beta$ = beta value: rate of susceptibility change as a factor of age.
[d] $p < 0.01$

**Table 4**
Repeated measures ANOVA results: Cortical susceptibility

| Within-Subjects effects | F-stat | *p*-value | partial $\eta^2$ | | | |
|---|---|---|---|---|---|---|
| Lobe[a] | 0.070 | 0.926 | 0.001 | | | |
| Between-Subjects Effects | | | | | | |
| Age | 8.02 | 0.006[c] | 0.107 | | | |
| *Parameter Estimates for Age* | | | | | | |
| Lobe | Average susceptibility (ppb/mm$^3$) | t-stat | *p*-value | partial $\eta^2$ | $\beta$ | SE |
| Frontal | 3.25 | 3.00 | 0.004[c] | 0.119 | 0.088 | 0.029 |
| Parietal | 2.44 | 3.37 | 0.001[c] | 0.145 | 0.097 | 0.029 |
| Occipital | 4.71 | 1.69 | 0.096 | 0.041 | 0.084 | 0.050 |
| Temporal | 5.45 | 1.83 | 0.072 | 0.047 | 0.098 | 0.054 |

The results of the repeated measures ANOVA on QSM-based iron concentration differences between lobes (cortical gray matter) while controlling for age, gender, years of education, PASE score and alcohol drinking frequency (drinks/day).
[a] Greenhouse-Geisser corrected results (Mauchly's test of sphericity: $p < 0.0001$).
[b] $\beta$ = beta value: rate of susceptibility change as a factor of age.
[c] $p < 0.01$

**Table 5**
Repeated measures ANOVA results: N-back task

| Within-Subjects effects | F-stat | p-value | partial $\eta^2$ | | | |
|---|---|---|---|---|---|---|
| Task condition[a,b] | 6.46 | 0.002[d] | 0.619 | | | |
| Between-Subjects effects | | | | | | |
| Age | 10.33 | 0.002[d] | 0.135 | | | |
| *Parameter estimates for age* | | | | | | |
| Task condition | Average D-prime score | t-stat | *p*-value | partial $\eta^2$ | $\beta$ | SE |
| Compare | 6.16 | -0.85 | 0.397 | 0.011 | -0.03 | 0.040 |
| 1-Back | 4.42 | -3.43 | 0.001[d] | 0.152 | -0.15 | 0.043 |
| 2-Back | 2.27 | -2.11 | 0.039[e] | 0.063 | -0.08 | 0.036 |

The results of the repeated measures ANOVA on N-Back task performance, while controlling for age, gender, years of education, PASE score and alcohol drinking frequency (drinks/day).
[a] Sphericity assumed (Mauchly's test of sphericity: $p = 0.835$).
[b] All levels significantly differed from each other: $p < 0.0001$, Sidak corrected.
[c] $\beta$ = beta value: rate of D-prime change as a factor of age.
[d] $p < 0.01$.
[e] $p < 0.05$.

condition (Compare, 1-Back or 2-Back) as a within-subjects independent variable (results summarized in Table 5).

There was a main effect of N-Back task condition on D-prime. Pair-wise comparisons between N-Back task conditions (Sidak corrected) indicated that all differed significantly from each other on D-prime. Participants' had the highest D-prime score on the Compare condition, followed by the 1-Back and then the 2-Back conditions.

The repeated measures ANOVA further revealed age as a significant between-subjects effect. The parameter estimates from the analysis indicated significant negative correlations between participant age and D-prime for the 1-Back and 2-Back task conditions. Age did not predict D-prime for the control Compare condition. In sum, the participants' performance on the N-Back task decreases significantly with task difficulty and with age.

### 3.3. Relationship between QSM and N-back task performance

N-Back Task performance was expressed here as average log D-prime (averaged across the 1-Back and 2-Back conditions of the N-Back task). The analysis of subcortical susceptibility values (caudate, putamen and pallidum; results summarized in Table 6) indicated a significant negative correlation between susceptibility values in the putamen and average log D-prime (Fig. 2A). Susceptibility values within the caudate and pallidum did not correlate with N-Back task performance. The analysis on subcortical susceptibility values further revealed a significant interaction between age and susceptibility values from the putamen on log D-prime. That is, the negative relationship between putamen susceptibility values and log D-prime becomes stronger as a function of age. There was no significant interaction between age and caudate

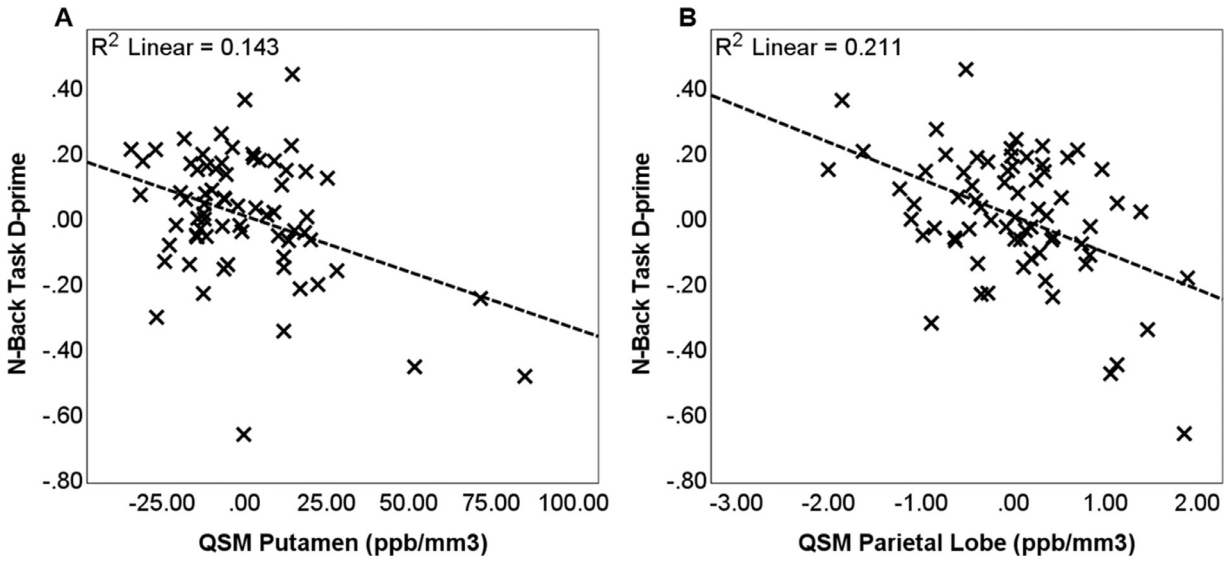


**Fig. 2.** Relationships between susceptibility values and working memory performance. The scatter plot shows susceptibility values (iron concentration in ppb/mm$^3$) from (A) the putamen and (B) the parietal lobe against N-Back task performance (log D-prime, averaged across the 1-Back and 2-Back conditions). Values are standardized residuals after controlling for age, gender, years of education, PASE score and alcohol drinking frequency (drinks/day). The dashed line represents the linear best fit.

**Table 6**
Bootstrapped linear regression results: Subcortical susceptibility and N-back task performance

| Effect | [a]$\beta$ | *p*-value | SE | BCa 95% CI | | VIF |
|---|---|---|---|---|---|---|
| | | | | LL | UL | |
| Caudate | 0.003 | 0.093 | 0.002 | 0.000 | 0.006 | 2.71 |
| Putamen | -0.003 | 0.014[c] | 0.001 | -0.004 | -0.001 | 2.83 |
| Pallidum | 0.000 | 0.850 | 0.001 | -0.002 | 0.002 | 1.59 |
| Caudate*age | 0.013 | 0.566 | 0.028 | -0.049 | 0.072 | 2.25 |
| Putamen*age | -0.065 | 0.004[b] | 0.035 | -0.139 | -0.031 | 2.23 |
| Pallidum*age | 0.048 | 0.149 | 0.036 | -0.017 | 0.095 | 1.84 |
| Age | -0.013 | 0.002[b] | 0.004 | -0.020 | -0.006 | 1.70 |
| Gender | -0.082 | 0.074 | 0.046 | -0.173 | 0.002 | 1.40 |
| Education | 0.025 | 0.012[c] | 0.009 | 0.004 | 0.048 | 1.17 |
| PASE score | 0.000 | 0.623 | 0.000 | -0.001 | 0.001 | 1.12 |
| Alcohol freq. | -0.034 | 0.107 | 0.026 | -0.078 | 0.051 | 1.13 |

The results of the bootstrapped linear regression between N-Back task performance (log D-prime, averaged across the 1-Back and 2-Back conditions of the N-Back task) and subcortical susceptibility values, while controlling for age, gender, years of education, PASE score and alcohol drinking frequency (drinks/day).
Key: BCa, bias-corrected accelerated; CI, confidence interval; LL, lower limit; UL, upper limit.
[a] Bootstrapped (10,000 samples).
[b] $p < 0.01$.
[c] $p < 0.05$.

**Table 7**
Bootstrapped linear regression results: Lobar susceptibility and N-back task performance

| Effect | [a]$\beta$ | *p*-value | SE | BCa 95% CI | | VIF |
|---|---|---|---|---|---|---|
| | | | | LL | UL | |
| Frontal | 0.060 | 0.076 | 0.034 | -0.002 | 0.124 | 3.07 |
| Parietal | -0.063 | 0.048[c] | 0.024 | -0.128 | -0.013 | 2.52 |
| Occipital | 0.011 | 0.286 | 0.012 | -0.014 | 0.033 | 2.77 |
| Temporal | -0.008 | 0.584 | 0.015 | -0.040 | 0.024 | 2.00 |
| Frontal*age | 0.032 | 0.598 | 0.076 | -0.120 | 0.138 | 3.08 |
| Parietal*age | -0.102 | 0.017[c] | 0.051 | -0.181 | -0.011 | 2.73 |
| Occipital*age | -0.011 | 0.698 | 0.045 | -0.102 | 0.094 | 2.62 |
| Temporal*age | 0.065 | 0.109 | 0.058 | -0.023 | 0.228 | 3.68 |
| Age | -0.017 | 0.001[b] | 0.004 | -0.026 | -0.007 | 1.44 |
| Gender | -0.085 | 0.073 | 0.046 | -0.168 | -0.022 | 1.58 |
| Education | 0.022 | 0.015[c] | 0.009 | 0.003 | 0.046 | 1.33 |
| PASE score | 0.000 | 0.609 | 0.000 | -0.001 | 0.001 | 1.38 |
| Alcohol freq, | -0.022 | 0.318 | 0.028 | -0.076 | 0.067 | 1.28 |

The results of the bootstrapped linear regression between N-Back task performance (log D-prime, averaged across the 1-Back and 2-Back conditions of the N-Back task) and lobar GM susceptibility values, while controlling for age, gender, years of education, PASE score and alcohol drinking frequency (drinks/day).
Note. BCa, bias-corrected accelerated; CI, confidence interval; LL, lower limit; UL, upper limit.
[a] Bootstrapped (10,000 samples).
[b] $p < 0.01$.
[c] $p < 0.05$.

susceptibility values or between age and pallidum susceptibility values.

The analysis on cortical/lobar GM susceptibility values (frontal, occipital, parietal, temporal lobe GM; results summarized in Table 7) revealed parietal susceptibility as a significant predictor of log D-prime (Fig. 2B). Susceptibility in the other lobes did not correlate with working memory performance. Additionally, there was a significant interaction between age and parietal lobe susceptibility. That is, parallel to the findings on the putamen susceptibility values, the negative relationship between parietal susceptibility values and log D-prime also increased as a function of age. No significant interactions were observed between age and susceptibility values in the other lobes (Table 7).

In sum, greater iron concentration in the putamen and the parietal lobe were each associated with poorer working memory performance and this effect increased as a function of age. Thus, subsequent analyses between nutrition factor scores and susceptibility focus only on the putamen and parietal lobe brain regions to reduce the number of comparisons and focus on regions in which iron accumulation increases with age and negatively affect cognitive performance.

### 3.4. SNR associated with GM ROIs

An additional analysis was conducted in order to evaluate the signal-to-noise ratio (SNR) in cortical and subcortical GM ROIs (Supplementary Figure 1) that could influence susceptibility in these regions. The GRE magnitude image (root mean square of the 8 echos/volumes of each GRE magnitude image) was used to compute SNR rather than the QSM map because the latter tends to mix

**Table 8**
Output pattern matrix from the factor analysis on nutrition

| | Factor | | |
|---|---|---|---|
| Nutrient | 1 | 2 | 3 |
| Vitamin C | 0.83 | | |
| Vitamin E | | 0.68 | |
| Epigallocatechin 3-gallate | | | 0.98 |
| Quercetin | 0.81 | | |
| Lysine | | 0.75 | |
| $\beta$-carotene | 0.78 | | |
| DHA omega-3 | | 0.79 | |
| LA omega-6 | | 0.82 | |
| $\beta$-cryptoxanthin | 0.90 | | |

The output pattern matrix from the factor analysis conducted on nutrition. The table lists the partial standardized regression coefficients of each nutrition item (vitamin C and E, epigallocatechin 3-gallate, quercetin, lysine, $\beta$-carotene, DHA omega-3, LA omega-6 and $\beta$-cryptoxanthin) with a particular factor. Regression coefficients less than 0.35 are not displayed for clarity.
Extraction method: Principal component analysis.
Rotation method: Oblimin with Kaiser Normalization.

noise spatially due to spatial deconvolution. SNR was calculated by dividing the mean image intensity value within a GM ROI (frontal, parietal, occipital, temporal, caudate, pallidum and putamen ROIs), by the standard deviation of the intensity values outside the head (image background). The noise distribution outside the head, however, is not Gaussian but instead follows the Rayleigh distribution. The SD of a Rayleigh distribution is related to the SD of a Gaussian distribution by a factor of $\sqrt{(2-\pi/2)}$ (Edelstein et al., 1984), also known as the Rayleigh distribution correction factor. As such, the result of the previous step needs to be multiplied by this Rayleigh distribution correction factor to yield true, per GM ROI SNR values for each participant.

A repeated measures ANOVA was conducted with anatomical structure as an independent variable (with 7 levels, corresponding to each GM ROI) and SNR as the dependent variable. Age and gender were controlled in this analysis. The results indicated anatomical structure as a significant main effect $F(6,71) = 2.15$, $p = 0.047$. Pairwise comparisons, adjusted for multiple comparisons using Sidak, indicated the following: SNR in the parietal lobe was significantly stronger compared to SNR in all GM ROIs ($p_s < 0.0001$) except for the pallidum GM ROI and occipital lobe GM ROI. SNR in the parietal lobe was comparable to that of the pallidum ($p = 0.906$) and lower compared to the occipital lobe ($p < 0.0001$). Additionally, SNR in the frontal lobe was significantly lower in comparison to all other ROIs ($p_s < 0.0001$). Lastly, SNR in the occipital lobe was significantly higher than in all other ROIs ($p_s < 0.0001$). In summary, with the exception of the frontal lobe, SNR was generally comparable or higher in cortex than in subcortical ROIs.

### 3.5. Factor analysis for nutrient groupings

Three factors were identified using this analysis: Vitamin C, quercetin, $\beta$-carotene and $\beta$-cryptoxanthin loaded primarily on factor one (Cronbach's $\alpha = 0.89$, smallest corrected item-total correlation = 0.68 for $\beta$-cryptoxanthin). Vitamin E, lysine, DHA omega-3, and LA omega-6 loaded primarily on factor 2 (Cronbach's $\alpha = 0.86$, smallest corrected item-total correlation = 0.5 for DHA omega-3). Lastly epigallocatechin 3-gallate loaded primarily on factor 3 (Table 8).

Factor one appears to represent nutrients derived from common fruits and vegetables, factor 2 represents nutrients primarily derived from nuts, healthy oils and fish, and factor 3 represents epigallocatechin 3-gallate which is primarily found in certain herbs/teas (mainly in green tea) and in less amounts in fruit such as cranberries and peaches. This factoring is largely consistent with established National Institute on Aging food groups (https://www.nia.nih.gov/health/know-your-food-groups) such as vegetables and fruit, protein and oils (nuts, fish etc.) and herbs (tea). Factor scores were created for these factors using the regression method, since this method tends to maximize the correlation between the factor scores and the underlying factors (DiStefano et al. 2009).

**Table 9**
Bootstrapped linear regression results: Nutrition factor scores and N-back task performance

| Effect | $\beta$[a] | $p$-value | SE | BCa 95% CI | | VIF |
|---|---|---|---|---|---|---|
| | | | | LL | UL | |
| Factor 1 | -0.007 | 0.770 | 0.025 | -0.055 | 0.029 | 1.72 |
| Factor 2 | 0.057 | 0.023[c] | 0.024 | 0.015 | 0.094 | 1.93 |
| Factor 3 | -0.006 | 0.844 | 0.032 | -0.072 | 0.064 | 1.86 |
| Factor 1 * age | -0.043 | 0.225 | 0.039 | -0.113 | 0.041 | 2.07 |
| Factor 2 * age | 0.071 | 0.040[c] | 0.037 | 0.001 | 0.137 | 2.60 |
| Factor 3 * age | -0.011 | 0.774 | 0.040 | -0.080 | 0.028 | 1.30 |
| Age | -0.016 | 0.005[b] | 0.005 | -0.025 | -0.003 | 1.40 |
| Gender | -0.080 | 0.085 | 0.044 | -0.166 | -0.001 | 1.37 |
| Education | 0.019 | 0.054 | 0.009 | -0.001 | 0.041 | 1.38 |
| PASE score | 0.000 | 0.434 | 0.000 | -0.001 | 0.001 | 1.22 |
| Alcohol freq. | -0.038 | 0.123 | 0.031 | -0.091 | 0.066 | 1.24 |

The results of the bootstrapped linear regression between N-Back task performance (log D-prime, averaged across the 1-Back and 2-Back conditions of the N-Back task) and nutrition factor scores while controlling for age, gender, years of education, PASE score and alcohol drinking frequency (drinks/day).
Key: BCa, bias-corrected accelerated; CI, confidence interval; LL, lower limit; UL, upper limit;
[a] Bootstrapped (10,000 samples).
[b] $p < 0.01$.
[c] $p < 0.05$.

### 3.6. Relationships between factor scores and N-back task performance

There was a significant positive correlation between factor 2 scores, but not factor 1 or factor 3 scores, and log D-prime (Table 9 and Fig. 3). There was also a significant interaction between factor 2 scores and age such that the positive relationship between factor 2 scores and log D-prime increased as a function of age. No significant interactions were observed between factor one or 3 scores and age.

### 3.7. Relationship between factor scores and QSM

Separate bootstrapped linear regression analyses were conducted for the putamen and parietal susceptibility values (see methods for details). Interaction terms between age and factor scores were added in each regression model in order to evaluate moderation effects of age on the relationship between factor scores and susceptibility values. Relationships between factor scores and susceptibility values in the putamen are summarized in Table 10. Nutrition factor scores did not predict susceptibility values in the putamen as main effects. However, there was a significant interaction between factor 2 scores and age such that the relationship between factor 2 scores and susceptibility values in the putamen increased as a function of age. The interaction between age and factor one scores and the interaction between age and factor 3 scores were not significant.

Relationships between factor scores and susceptibility values in the parietal lobe are summarized in Table 11. There was a significant negative correlation between factor 3 scores and susceptibility. The interaction between factor 3 scores and age were not significant. Factors 1 and 2 did not correlate significantly with

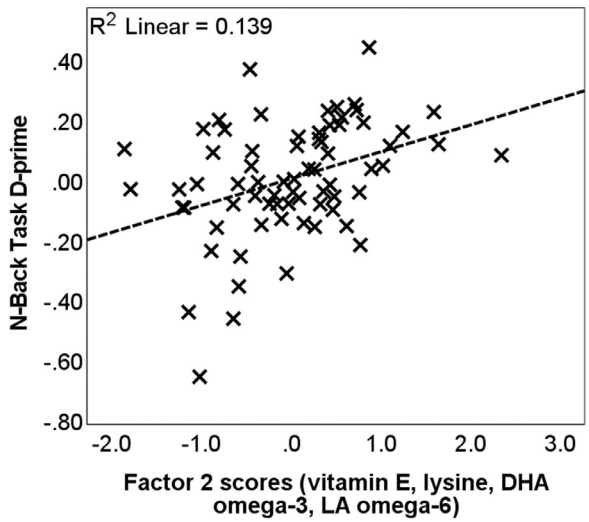


**Fig. 3.** The relationship between task performance and factor 2 scores. The scatter plot shows the factor 2 scores (comprised of vitamin E, lysine, DHA omega-3 and LA omega-6 nutrients) against N-back task performance (log D-prime, averaged across the 1-Back and 2-Back conditions). Values are standardized residuals after controlling for age, gender, years of education, PASE score and alcohol drinking frequency (drinks/day). The dashed line represents the linear best fit.

**Table 10**
Bootstrapped linear regression results: nutrition factor scores versus putamen susceptibility values

| Effect | $\beta$[a] | p-value | SE | BCa 95% CI | | VIF |
|---|---|---|---|---|---|---|
| | | | | LL | UL | |
| Factor 1 | -4.919 | 0.265 | 4.43 | -15.51 | 4.15 | 1.59 |
| Factor 2 | 1.584 | 0.749 | 4.76 | -8.86 | 16.97 | 1.79 |
| Factor 3 | -1.615 | 0.670 | 3.88 | -8.77 | 6.58 | 1.74 |
| Factor 1·age | 14.557 | 0.070 | 8.24 | -0.19 | 31.22 | 2.09 |
| Factor 2·age | -17.863 | 0.006[b] | 6.38 | -30.87 | -7.29 | 2.62 |
| Factor 3·age | -1.963 | 0.607 | 4.18 | -10.45 | 8.97 | 1.35 |
| Age | 2.443 | 0.002[b] | 0.70 | 0.868 | 3.53 | 1.35 |
| Gender | -14.649 | 0.054 | 7.22 | -30.11 | 3.25 | 1.43 |
| Education | 2.443 | 0.118 | 1.52 | -0.338 | 4.535 | 1.34 |
| PASE score | -0.012 | 0.849 | 0.07 | -0.135 | 0.110 | 1.19 |
| Alcohol freq. | 2.145 | 0.526 | 4.12 | -5.38 | 10.69 | 1.20 |

The results of the bootstrapped linear regression between susceptibility values in the putamen (ppb/mm$^3$) and nutrition factor scores.
Key: BCa, bias-corrected accelerated; CI, confidence interval; LL, lower limit; UL, upper limit.
[a] Bootstrapped (10,000 samples).
[b] $p < 0.01$.

parietal susceptibility values. However, there was a significant interaction between age and factor 2 scores such that the relationship between factor 2 scores and parietal susceptibility increased as a function of age. The interaction between age and factor one scores was not significant.

To decompose and visualize the significant interactions between age and factor 2 scores on putamen and parietal susceptibility values, a median split was conducted. The median split divided participants into younger and older age groups (median split at 70.22 years; age range 61-86 years). Separate bootstrapped linear regressions were then conducted for each age group, between (1) susceptibility values in the putamen and factor 2 scores and (2) parietal lobe susceptibility values and factor 2 scores.

For the putamen susceptibility values, the analysis on the older age group (median age > 70.22 years) revealed a significant negative correlation between factor 2 scores and susceptibility values [bootstrapped Beta = -13.98, $p = 0.030$; SE = 6.16; 95% BCa CI= -26.53 to -1.43; VIF = 1.22; Fig. 4A]. The analysis on the younger age group (median age < 70.22) did not reveal a significant correlation between factor 2 scores and susceptibility [bootstrapped Beta = 5.84, $p = 0.161$; SE = 4.06; 95% BCa CI= -2.45 to 14.14; VIF = 1.14; Fig. 4A].

**Table 11**
Bootstrapped linear regression results: nutrition factor scores versus parietal lobe susceptibility values

| Effect | $\beta$[a] | p-value | SE | BCa 95% CI | | VIF |
|---|---|---|---|---|---|---|
| | | | | LL | UL | |
| Factor 1 | -0.208 | 0.280 | 0.20 | -0.596 | 0.135 | 1.60 |
| Factor 2 | 0.175 | 0.318 | 0.19 | -0.133 | 0.593 | 1.82 |
| Factor 3 | -0.594 | 0.001[b] | 0.16 | -0.908 | -0.272 | 1.74 |
| Factor 1·age | 0.281 | 0.179 | 0.23 | -0.162 | 0.821 | 2.09 |
| Factor 2·age | -0.596 | 0.003[b] | 0.20 | -0.987 | -0.328 | 2.62 |
| Factor 3·age | 0.121 | 0.385 | 0.16 | -0.220 | 0.376 | 1.35 |
| Age | 0.065 | 0.014[c] | 0.03 | 0.010 | 0.119 | 1.35 |
| Gender | -0.067 | 0.835 | 0.33 | -0.712 | 0.711 | 1.42 |
| Education | 0.082 | 0.283 | 0.08 | -0.076 | 0.199 | 1.34 |
| PASE score | 0.005 | 0.028[c] | 0.002 | -0.001 | 0.010 | 1.21 |
| Alcohol freq. | -0.313 | 0.087 | 0.22 | -0.770 | -0.042 | 1.20 |

The results of the bootstrapped linear regression between parietal lobe susceptibility (ppb/mm$^3$) and nutrition factor scores.
Key: BCa, bias-corrected accelerated; CI, confidence interval; LL, lower limit; UL, upper limit.
[a] Bootstrapped (10,000 samples).
[b] $p < 0.01$.
[c] $p < 0.05$.

For the parietal susceptibility values, the analysis on the older age group (median age > 70.22 years) revealed a significant negative correlation between factor 2 scores and parietal susceptibility [bootstrapped Beta = -0.605, $p = 0.020$; SE = 0.25; 95% BCa CI= -1.11 to -0.101; VIF = 1.22; Fig. 4B]. The analysis on the younger age group (median age < 70.22) did not reveal a significant correlation between factor 2 scores and parietal susceptibility [bootstrapped Beta = 0.190, $p = 0.445$; SE= 0.24; 95% BCa CI= -0.312 to 0.692; VIF = 1.16; Fig. 4B].

## 4. Discussion

Our results indicated that age was positively associated with cerebral iron concentration and negatively associated with working memory performance in older adults, in accord with previous findings. However, the effects of age on both brain iron concentration (in the putamen and parietal lobe) and on working memory performance were diminished by the dietary intake of specific nutrients (nutrition factor 2: vitamin E, lysine, DHA omega-3 and LA omega-6 PUFA). In addition, consumption of the iron chelator epigallocatechin 3-gallate (nutrition factor 3) was associated with lower iron concentration in the parietal lobe, regardless of age. Our results suggest that specific nutrients may offer protection against brain iron accumulation and cognitive decline in older adults.

The links between age, brain iron accumulation and cognitive decline make it important to identify potential factors that may attenuate brain iron accumulation. Findings from previous animal studies show that certain nutrients, such as vitamins, polyunsaturated fatty acids (PUFA), flavonoids and iron-chelators, can help reduce the impact of excess brain iron on cognitive function due to their antioxidant, anti-inflammatory, iron-concentration-reducing properties and ability to block/moderate ferroptosis (Zaidi et al., 2004; Suh et al., 2005; Domenico & Giudetti, 2017; Fernandez-Moriano et al. 2015; Molinari et al., 2019; Ogluszka, Lipinski & Starzynski, 2020). Similarly, a large body of literature demonstrates a positive association between dietary intake of these nutrients and cognitive function (e.g., Butterfield et al., 2002; Youdim and Joseph, 2001; Hager et al. 2001; Yehuda et al. 2005; Kuriyama et al. 2006; Carmichael et al. 2018; Stavrinou et al. 2020).

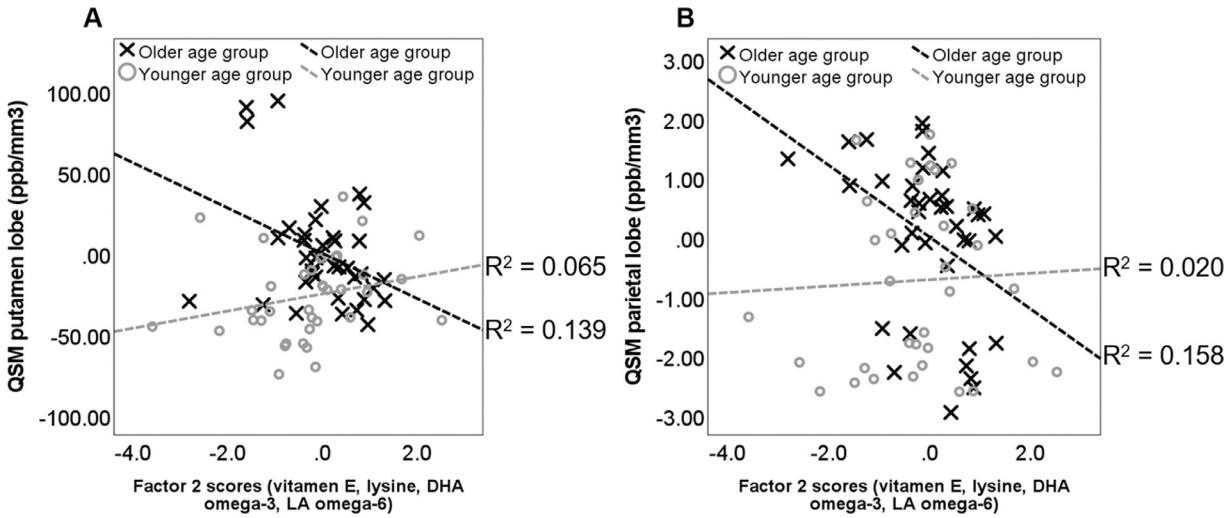


**Fig. 4.** The interaction between age and factor 2 scores on (A) putamen and (B) parietal lobe susceptibility values. The scatter plot shows susceptibility values (iron concentration in ppb/mm$^3$) from (A) the putamen and (B) the parietal lobe, against factor 2 scores (comprised of vitamin E, lysine, DHA omega-3 and LA omega-6 nutrients). Separate regression lines are depicted for the older (X / ––) and younger (O /……) age groups (median age = 70.22 years). Values are standardized residuals after controlling for gender, years of education, PASE score and alcohol drinking frequency (drinks/day). The dashed lines represent the linear best fit.

However, the impact of these nutrients on the relationships between age and both brain iron concentration and cognition are not well-established. Here, we addressed these issues by exploring the relationship between groups of nutrients known to cross the BBB, brain iron concentration and cognition in older adults ranging from 61 to 86 years of age. Our results controlled for age, gender and other potentially confounding health factors such as years of education, alcohol consumption and physical activity. Further, participants who smoke were excluded from analyses. Iron concentration in cortical and subcortical regions was assessed using QSM and working memory performance was assessed via an N-Back task paradigm. We sought to determine whether specific nutrient groups statistically moderate the well-established relationship between age and brain iron concentration and/or between age and working memory performance.

The main finding of our study was that factor 2, consisting of nutrients primarily derived from nuts, healthy oils and fish (vitamin E, lysine, DHA omega-3 and LA omega-6 PUFA), moderated the effects of age on both iron concentration (in the putamen and parietal lobe) and working memory performance. That is, the strength of association between increasing age and higher iron concentration was reduced via high consumption of factor 2 nutrients. Similarly, the strength of association between increasing age and lower working memory performance was reduced via high consumption of factor 2 nutrients. These findings suggest that dietary intake of foods high in vitamin E, lysine, DHA omega-3 and LA omega-6 PUFA may attenuate age-related accumulation of brain iron and working memory declines.

Our finding that high intake of nutrients from nuts, healthy oils and fish moderated the effects of age on brain iron concentration appears consistent with the background aging literature. For example, PUFAs such as omega-3 fatty acids have been previously associated with blocking age-related ferroptosis, the process by which cells die through the interaction between ROS and iron (Ogluszka, et al., 2020), although, the exact mechanism by which this blocking occurs is largely unknown. Further, endogenous defense mechanisms, specifically ones that depend on nonenzymatic antioxidants, become less effective with aging (Harman 1998; Jovanovic 2014; Molinari et al., 2019). Given established age-related reductions in combating oxidative stress with endogenous nonenzymatic antioxidants (e.g., glutathione, melatonin, vitamins A, C and E and flavonoids; Jovanovic 2014), increased intake of exogenous antioxidants (such as vitamin E and lysine) could become more important with increasing age.

The moderation effects we report involving factor 2 nutrients on working memory are broadly consistent with literature on the effects of Mediterranean dietary patterns on cognition in older adults (e.g., Tangney et al. 2014, Petersson and Philippou, 2016; Loughrey et al., 2017). Specifically, adherence to Mediterranean dietary patterns, which overlap with factor 2 food groups (nuts, healthy oils and fish), has been associated with slower rates of global cognitive decline in older adults (Tangney et al. 2014). As such, it is possible that our findings relating to specific nutrients (factor 2) and those reported in Mediterranean diet studies may have a common basis in their potential effects on brain iron concentration. Our moderation findings are also consistent with the age-dependent hypothesis, which suggests that reserve factors tend to have greater protective effects in older age, when neurocognitive declines become more apparent (Gold et al., 2013; Hötting and Röder, 2013; Hayes et al., 2015; Stern et al., 2018).

A secondary issue explored in our study is whether nutrient groups may show beneficial effects across older adults, regardless of age. Factor 2 was associated with better working memory performance across older adults whereas factor 3 was associated with lower iron concentration in the parietal lobe only. The main effects related to factors 2 and 3 appear consistent with previous literature demonstrating that high intake of antioxidants and iron-chelators are associated with better cognitive performance (e.g., Lovell et al., 2003; Maczurek et al., 2008; Nurk et al., 2009; Shay et al., 2009; Ahmed 2012; Stavrinou et al., 2020). Further, the lack of significant correlations between factor one scores and either brain iron or working memory performance, does not deviate from the literature in that vitamin C and vitamin A precursors ($\beta$-carotene and $\beta$-cryptoxanthin) are typically only related to cognition when deficient in the human body (e.g., Nourhashémi et al., 2000). Lastly, in addition to its antioxidant properties, vitamin C has been shown to regulate iron metabolism and homeostasis in mammals (Lane & Richardson, 2014). However, these benefits do

not appear to scale linearly with consumption of vitamin C and consuming amounts greater than the recommended dietary allowance (75 mg for women and 90 mg for men) does not appear to be particularly beneficial (Jacob & Sotoudeh, 2002).

The regional specificity of the factor 3 main effect across the age-range we observed in the parietal lobe, but not the putamen, may relate to regional brain differences in gene expression, antioxidant capacity, mechanisms for response to oxidative stress and/or immune response (Wang and Michaelis, 2010). For example, in response to oxidative stress, cortical neurons show less drop in ATP levels than non-cortical neurons, which may enable more effective defense against oxidative stress in cortical neurons (Chung et al., 2005; Wang et al., 2009; Butterfield and Halliwell, 2019). Alternatively, the regional effects we observed may be more 'statistical' in nature, possibly related to large baseline differences in iron concentrations between cortical and subcortical structures. In other words, the small effect of epigallocatechin 3-gallate (factor 3) on iron concentration may be insufficient to measurably alter the high iron content in subcortical structures. The effects of nutrition on regional brain iron concentration and oxidative stress response is an important topic for future studies.

It should be noted that the main effects we report across the age-range differ from those reported in Hagemeier et al., (2015). These authors reported positive associations between both vegetables and dairy products and iron concentration in subcortical brain regions and specifically for men. However, our results are not directly comparable with those of Hagemeier et al., (2015) for several reasons. First, we explored groups of specific nutrients known to cross the BBB, rather than single food categories like vegetables or dairy products. Second, participant samples in the 2 studies differed significantly in age, with our study focusing on older adults (mean age = 70 years old, range 61-86 years old) and the Hagemeier et al., (2015) study focusing on young-to-middle aged adults (mean age = 43 years old). Finally, we used a multi-echo GRE sequence for QSM analyses, while Hagemeier et al., (2015) used a single-echo GRE sequence and SWI-based phase analyses. These 2 methodologies differ on how they assess brain iron. In particular, QSM results are more reflective of local tissue susceptibilities, allowing for computation of voxel-level iron concentration values (Liu et al. 2012).

Certain limitations of our study should be acknowledged: this is not a nutritional intervention study and causal inferences cannot be made between nutrition, QSM-based iron concentration and working memory performance. A longitudinal, dietary-intervention study will be required to address potential causality. QSM is not a direct measure of iron concentration and can be affected to a lesser extent by the presence of other metals, as well as calcification. In addition, QSM cannot differentiate between non-heme and heme iron bound to deoxygenated hemoglobin in blood (e.g. Wang and Liu 2015). Consequently, differences in cerebral blood volume may contribute to the QSM signal (e.g. Bianciardi, Van Gelderen and Duyn 2014; Balla et al., 2014). However, we did not observe a relationship between cerebral blood volume and QSM signal in our recent work (Zachariou et al., 2020).

The current study also had a modest sample size which might have affected our ability to detect smaller, regional effects between nutrition, QSM-based iron concentration, working memory performance and age. Future studies with a larger sample size should be used to identify possible subtler effects. It should also be noted that the specific finding of parietal, but not frontal lobe, susceptibility predicting working memory performance should be considered preliminary: SNR was significantly lower in the frontal lobe, compared to other lobes (supplementary Figure 1), which may contribute to the lack of significant correlations between susceptibility in this region and working memory performance. We suspect that the relatively lower SNR in the frontal lobe compared to other cortical regions may result from more signal dropout near the tissue-sinus interface (as seen in gradient-echo fMRI imaging).

Lastly, participants' nutritional intake, as well as key control factors such as physical activity levels were evaluated via self-report questionnaires. These self-report measures do not provide direct biochemical or physiological assessments. Our findings would benefit from replication with more direct biochemical and physiological measures.

## 5. Conclusions

In conclusion, our results suggest that incorporating oxidative-stress reducing antioxidants and vitamins, iron chelating nutrients and polyunsaturated fatty acids in daily dietary-intake may slow age-related accumulation of non-heme brain iron concentration and cognitive declines. Future longitudinal dietary-intervention studies will be required to test this possibility.

## Disclosure statement

The authors declare no competing financial interests.

## CRediT authorship contribution statement

**Valentinos Zachariou:** Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Christopher E. Bauer:** Methodology, Formal analysis, Data curation, Investigation, Writing – review & editing. **Elayna R. Seago:** Formal analysis, Data curation, Visualization. **Georgia Panayiotou:** Formal analysis, Resources, Writing – review & editing. **Edward D. Hall:** Conceptualization. **D. Allan Butterfield:** Conceptualization. **Brian T. Gold:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2021.06.016.

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