High Fruit and Vegetable Intake is Positively Correlated with Antioxidant Status and Cognitive Performance in Healthy Subjects

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Abstract. A higher daily intake of fruits and vegetables in healthy elderly is associated with an improved antioxidant status in comparison to subjects consuming diets poor in fruits and vegetables, but the impact on cognitive performance is unclear. Healthy community dwellers (45 to 102 years old, \( n = 193 \)) underwent cognitive testing and blood withdrawal for the measurement of antioxidant micronutrients and biomarkers of oxidative stress as well as administration of a food frequency questionnaire to assess the daily intake of fruits and vegetables (high intake HI, low intake LI). Ninety-four subjects of the HI group had significantly higher cognitive test scores, higher levels of carotenoids, \( \alpha- \) and \( \gamma- \)tocopherol as well as lower levels of F\( \alpha \) isoprostanes than the 99 subjects of the LI group. Cognitive scores were directly correlated with blood levels of \( \alpha- \)tocopherol and lycopene and negatively correlated with F\( \alpha \) isoprostanes and protein carbonyls. The results were independent of age, gender, body mass index, education, total cholesterol, LDL- and HDL-cholesterol, triglycerides, and albumin. Healthy subjects of any age with a high daily intake of fruits and vegetables have higher antioxidant levels, lower levels of biomarkers of oxidative stress, and better cognitive performance than healthy subjects of any age consuming low amounts of fruits and vegetables. Modification of nutritional habits aimed at increasing intake of fruits and vegetables should be encouraged to lower prevalence of cognitive impairment in later life.

Keywords: Antioxidants, cognitive aging, dementia, isoprostanes, micronutrients, neuropsychology, nutrition, oxidative stress, prevention, vitamins

INTRODUCTION

In developed countries, the percentage of subjects aged 60 years and older will increase from 19\% at present to almost 40\% in the year 2050 [1]. Importantly, the number of people aged 80 years and older is projected to grow as much as eight to ten times on the global scale by 2050 [2]. Most neurodegenerative diseases with cognitive impairment occur in these stages of life. Previous epidemiologic studies have consistently shown that diet is implicated in the incidence of 6 of the 10 leading causes of death for Americans. Diet therefore plays a crucial role in prevention of age-related chronic disease [3–8]. Bioactive compounds
like antioxidants, mostly contained in fruits and vegetables, are important for the protection against oxidative and nitrosative stress. These processes have been associated with aging itself and the pathophysiology of some age-related illnesses including cognitive impairment and dementia [9,10]. Epidemiological evidence has linked adequate antioxidant micronutrient status with a decreased risk for dementia [11]. Intervention trials of antioxidant supplementation, however, have demonstrated no major benefit against cognitive impairment [12,13]. There are several reasons explaining this discrepancy (reviewed in [12]). One important unanswered question relates to the largely unexplored relationship between intake of fruits and vegetables, antioxidant micronutrient status, a condition of oxidative stress, and cognitive performance in healthy subjects [14]. The biological interactions between these components in the absence of disease have been poorly evaluated thus far, especially by means of independent measures. Malnutrition and a poor antioxidant status are common in the elderly, but there is a considerable lack of data on the most important factors influencing the micronutrient adequacy in advanced age. In the healthy elderly, we previously reported an age-independent association between a higher daily intake of fruits and vegetables and improved antioxidant status in comparison to subjects consuming diets poor in fruits and vegetables [15]. We demonstrated that dietary habits are major determinants of antioxidant status in healthy elderly subjects. The relationship between nutritional habits and cognitive functions, however, has not been evaluated. Understanding this relationship may also guide strategies for more successful intervention trials. The aim of the present study was to compare two groups of healthy individuals with different dietary habits. We hypothesized 1) that consumption of antioxidant-rich and antioxidant-poor diets is related to the antioxidant status and to cognitive performance; and 2) that subjects with higher plasma levels of antioxidant micronutrients and lower biomarkers of oxidative stress have better cognitive function.

SUBJECTS AND METHODS

Subjects

The investigation conforms to the principles outlined in the Declaration of Helsinki. Healthy German community-dwellers aged 45 to 102 years were included in the study after giving informed consent (n = 193; 93 M, 100 F; 70.8 ± 14.9 years). Subjects underwent full physical/ neurological examination as well as collection of medical history to assess clinical conditions and identify major organ diseases. An ECG and two consecutive measurements of blood pressure, as well as neuropsychological tests including Mini-Mental State Examination (MMSE) [16], Clock Drawing Test [17], and the DemTect Scale [18] were performed. The DemTect Scale is a screening test for dementia comprising five subtests (10-word list repetition, number transcoding, semantic word fluency task, backward digit span, delayed word list recall). Raw scores are transformed to age- and education-independent scores, classified as ‘suspected dementia’ (score < 8), ‘mild cognitive impairment’ (9–12), and ‘appropriate for age’ (13–18). Routine laboratory parameters were also determined on the day of the evaluation including blood cell count, plasma albumin, prealbumin, cholesterol, and triglycerides. Data regarding education and lifestyle habits including physical activity and diet as well as the Body Mass Index (BMI) [weight kg/(height m)²] were collected in all subjects.

The main exclusion criterion was any kind of cognitive impairment as assessed by patient history and MMSE scores < 25, Clock Drawing Test > 2 (range 1–6 points, 1 = normal visuospatial function), and DemTect score < 15. Smokers, subjects with malnutrition, dyslipidemia, BMI < 18 or > 30, as well as subjects living alone, consuming > 1 and < 4 portions of fruit and vegetables per day (see below), and those performing physical activity less than twice a week or more than five times per week were also excluded. Subjects taking medications and/or antioxidant/vitamin/iron supplements were also excluded from the study.

On the day of the evaluation, subjects were asked to complete a qualitative food-frequency questionnaire (FFQ) [19] modified to assess intake of fruits and vegetables in the previous two weeks, in order to identify those individuals consuming a diet rich in fruits and vegetables [4 or more portions/day = > 350 grams/day, group HI (high intake)] and those consuming a diet poor in fruits and vegetables [0–1 portion/day = 0–100 grams/day, group LI (low intake)] [15]. All subjects included in the HI group had a mixed fruit and vegetable intake consisting of equal amounts of fruits and vegetables or higher amounts of vegetables than of fruits by one portion.

Blood sampling and measurements

All subjects underwent blood drawing in a 10 ml heparinized tube between 9 and 10 am, after an overnight
Blood was immediately centrifuged, and plasma was stored frozen at −80°C until analysis. Plasma samples were coded and analysis was carried out in a blind fashion.

Lipophilic antioxidant micronutrients and vitamins including lutein, zeaxanthin, β-cryptoxanthin, lycopene, α- and β-carotene, α- and γ-tocopherol (vitamin E family), and retinol were measured. Carotenoids were analyzed by HPLC with detection at 450 nm according to Stahl et al. [20]. A second UV/vis detector was connected in series and set at 325 nm and at 292 nm for quantitation of retinol (vitamin A) and α-carotene, respectively.

For the measurement of protein oxidation, IgG were separated from plasma and protein content was measured using the bicinchoninic acid (BCA) method as previously described [22]. Protein carbonyls were assessed by ELISA following the method of Carty et al. [22]. Carbonyl content was calculated from a standard curve and expressed as nmol carbonyl per mg of IgG.

### Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS version 16, Chicago, IL). Data are presented as mean ± SD. Differences in cognitive scores were analyzed by two-tailed t-test and were accepted as significant at the p < 0.05 level. For the analysis of the differences of analyte levels between LI and HI groups, raw data were initially analyzed by the One-Sample Kolmogorov-Smirnov Test to verify the distribution pattern. All micronutrient and biomarker values were normalized by log-transformation and analyzed by general linear model to compare the levels of the different analytes in the LI and HI groups. Age, gender, BMI, education, total cholesterol, LDL- and HDL-cholesterol, triglycerides, and albumin were introduced as covariates. Correlations between cognitive scores and log-transformed parameters were examined by Pearson’s correlation. Significance was accepted in case of p-values < 0.05 at the 95% confidence intervals.

### Table 1

Scores at the neuropsychological tests as well as plasma levels of micronutrients and vitamins. IgG content of protein carbonyls and plasma levels of 8,12-IPF2α, VI in the study participants (HI, high intake; LI, low intake). Results are corrected for age, gender, BMI, education, total cholesterol, LDL- and HDL-cholesterol, triglycerides, and albumin differences between LI and HI groups. Age, gender, BMI, education, total cholesterol, LDL- and HDL-cholesterol, triglycerides, and albumin were introduced as covariates. Correlations between cognitive scores and log-transformed parameters were examined by Pearson’s correlation. Significance was accepted in case of p-values < 0.05 at the 95% confidence intervals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HI</th>
<th>LI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 94</td>
<td>n = 99</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMSE</td>
<td>29.4 ± 1.1</td>
<td>28.5 ± 1.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>DemTect</td>
<td>16.8 ± 1.4</td>
<td>16.2 ± 1.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Clock drawing</td>
<td>1.02 ± 0.1</td>
<td>1.38 ± 0.48</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Retinol (µM)</td>
<td>2.02 ± 0.6</td>
<td>1.86 ± 0.58</td>
<td>0.765</td>
</tr>
<tr>
<td>α-Tocopherol (µM)</td>
<td>24.8 ± 5.8</td>
<td>20.4 ± 3.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>γ-Tocopherol (µM)</td>
<td>1.79 ± 0.61</td>
<td>1.38 ± 0.54</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Lutein (µM)</td>
<td>0.56 ± 0.23</td>
<td>0.49 ± 0.25</td>
<td>0.22</td>
</tr>
<tr>
<td>Zeaxanthin (µM)</td>
<td>0.13 ± 0.05</td>
<td>0.09 ± 0.04</td>
<td>&lt; 0.002</td>
</tr>
<tr>
<td>β-Cryptoxanthin (µM)</td>
<td>0.34 ± 0.29</td>
<td>0.20 ± 0.14</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lycopene (µM)</td>
<td>0.97 ± 0.27</td>
<td>0.62 ± 0.27</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>α-Carotene (µM)</td>
<td>0.10 ± 0.07</td>
<td>0.08 ± 0.08</td>
<td>0.037</td>
</tr>
<tr>
<td>β-Carotene (µM)</td>
<td>0.83 ± 0.35</td>
<td>0.53 ± 0.27</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>8,12-IPF2α, VI (pg/mL)</td>
<td>78.3 ± 34.3</td>
<td>113.5 ± 43.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IgG Protein Carbonyls (nmol/mg)</td>
<td>0.78 ± 0.28</td>
<td>0.91 ± 0.24</td>
<td>0.066</td>
</tr>
</tbody>
</table>
RESULTS

Based on the answers to the food-frequency questionnaire, 94 subjects were included in the HI group (44 M, 54 F, 73.7 ± 13.7 years) and 99 subjects in the LI group (49 M, 50 F, 68.1 ± 15.6 years). Subjects did not differ in lifestyle habits or in any of the major variables measured for the assessment of the nutritional status and BMI (not shown).

Table 1 shows results from MMSE, Clock Drawing Test, and DemTect scores in HI and LI subjects. MMSE and DemTect mean scores were significantly lower and those of Clock Drawing Test significantly higher in HI subjects compared to LI subjects \( (p < 0.0001, p = 0.002 \text{ and } p < 0.0001, \text{ respectively}) \). Table 1 also displays the higher plasma levels of micronutrients and vitamins as well as lower levels of 8,12-IPF₂⁻VI \( (p < 0.0001) \) in HI compared to LI subjects.

Significant correlations were observed between MMSE and DemTect \( (r = 0.33, p < 0.0001) \), MMSE and Clock Drawing Test \( (r = -0.25, p = 0.001) \), and DemTect and Clock Drawing Test \( (r = -0.37, p < 0.0001) \). All micronutrients and vitamins measured were correlated with each other, with different levels of strength and significance (not shown). Among micronutrients, the carotenoid lycopene was associated with MMSE \( (r = 0.22, p = 0.003) \) (Fig. 1, panel A), DemTect \( (r = 0.19, p = 0.02) \) (Fig. 1, Panel B), and Clock Drawing Test \( (r = 0.35, p < 0.0001) \) (Fig. 1, Panel C), while α-tocopherol was associated with MMSE \( (r = 0.22, p = 0.005) \) and Clock Drawing Test \( (r = -0.26, p < 0.0001) \). MMSE was also associated with both biomarkers of oxidative stress measured, the association being stronger with 8,12-IPF₂⁻VI \( (r = -0.23, p = 0.007) \) (Fig. 1, Panel D) than with IgG content of protein carbonyls \( (r = -0.17, p = 0.04) \). Weak but significant correlations were also found between biomarkers of oxidative stress and micronutrients (not shown). Among these, α-tocopherol and β-carotene showed the strongest and most consis-
tent correlation to both IgG content of protein carbonyls ($r = -0.28, p < 0.0001$ and $r = -0.23, p = 0.005$, respectively) and to 8,12-IPF$_{2α}$-VI ($r = -0.25, p = 0.004$ and $r = -0.27, p = 0.003$, respectively).

**DISCUSSION**

As a main result the present study provides evidence that healthy subjects consuming a diet rich in fruits and vegetables have higher plasma levels of lipophilic antioxidant micronutrients, lower levels of biomarkers related to oxidative stress, and better scores on neuropsychological evaluation compared to subjects with low intakes of fruits and vegetables. This result is independent of gender, education, BMI, lipid profile, and albumin levels but, most importantly, is independent of age. Micronutrient levels are a good indicator for fruit and vegetable intake and important in influencing the in vivo oxidant/antioxidant balance in healthy subjects [15]. The age- and gender differences of antioxidant profiles shown in previous studies [24,25], therefore, might be epiphenomena of the major influence exerted by fruit and vegetable intake. Importantly, the present study shows that best cognitive performance is reached by subjects with a high daily intake of fruits and vegetables and is associated and interrelated with both good antioxidant micronutrient status and low levels of biomarkers of lipid peroxidation and protein oxidation. This observation also holds true both for young adults in the LI group cognitively scoring worse than young adults in the HI group, and for subjects older than 90 years in the HI group cognitively scoring better than younger ones in the LI group.

Circulating levels of antioxidant micronutrients and vitamins are similarly depleted [26] and F2-isoprostane cerebrospinal fluid and plasma levels are similarly increased [23] in patients with mild cognitive impairment (MCI) and Alzheimer’s disease (AD) as compared to controls, suggesting that oxidative stress is one of the earliest events in the pathophysiology of dementia [27]. Our results provide further evidence of this concept, showing that an incipient condition of increased oxidative stress status and decreased antioxidant defenses is related to cognitive performance even in healthy subjects.

A number of prospective epidemiologic studies and research in animals have found associations between individual dietary components and age-related cognitive change and dementia or between nutritional macronutrients/food components and cognitive performance. However, no study has been performed so far on healthy individuals within a broad age-range, simultaneously undergoing assessment of fruit/vegetable intake, neuropsychological evaluation, measurements of circulating levels of lipophilic antioxidant micronutrients, and biomarkers of oxidative stress. This multi-component approach allowed us the detection of novel correlations which deserve further evaluation in future, prospective and serial studies. While the reciprocal relationships among cognitive tests or among micronutrients is important mainly to verify the validity of the methods used, in fact, the correlation of the carotenoid lycopene but not of other antioxidants with all cognitive tests measured (lycopene / MMSE $p = 0.005$, lycopene / DemTect $p = 0.02$, lycopene / Clock Drawing Test $p < 0.0001$) underlines the preventive potential of this compound. Lycopene has lately gained much attention [28,29], α-Tocopherol, which acts as a peroxy and alkoxyl radical scavenger in lipid environments, thereby preventing lipid peroxidation in lipoproteins and membranes of nervous tissues especially, was associated in our study with both MMSE ($r = 0.22, p = 0.003$) and Clock Drawing Test ($r = -0.26, p < 0.0001$). This might stimulate further studies on the relationship between biomarkers of vitamin E status and clinical markers of cognition, as decreased incidence of chronic disease is associated with lifelong generous dietary vitamin E intakes. Nonetheless, more than 90% of the US-population does not consume the recommended dietary amounts of 15 mg/day [30]. MMSE was also associated with both biomarkers of oxidative stress measured. The association was stronger with 8,12-IPF$_{2α}$-VI ($r = -0.23, p = 0.007$) than with IgG content of protein carbonyls ($r = -0.17, p = 0.04$). This result, together with the correlation observed between F$_{2α}$-isoprostanes and the most powerful lipophilic antioxidants (8,12-IPF$_{2α}$-VI / α-tocopherol $p = 0.004$; 8,12-IPF$_{2α}$-VI / β-carotene $p = 0.003$), strengthens their clinical relevance and reliability as a useful biomarker in the identification of associations with clinical markers of disease [31].

The results presented here also receive support from previous studies conducted on cognition and nutrition. Several significant associations were observed between cognition and concurrent vitamin status, including better abstraction performance with higher levels and dietary intake of thiamine, riboflavin, niacin, and folate in the 137 elderly participants of the New Mexico Aging Process Study [32]. When fruit and vegetable intake in relation to cognitive function and decline among aging women were prospectively followed from 1976 to
2003 with biennial telephone questionnaires, women in the highest quintile of cruciferous vegetables declined slower compared with the lowest quintile [32]. In the same study, women consuming the highest quantity of green leafy vegetables also experienced slower decline than women consuming the least amount [32]. In a subset of subjects participating to the Chicago Health and Aging Project [33], a high vegetable consumption was associated with a slower rate of cognitive decline with older age.

While this cross-sectional study has some advantages related to the number of outcomes and risk factors assessed which help the understanding of disease etiology and the generation of hypotheses in this field, there are a number of limitations which have to be acknowledged. First of all, causality between oxidant/antioxidant balance and cognitive scores cannot be inferred, as we are in possession of one-timepoint biological and clinical assessment. Secondly, although it was inherent in the study design and subject selection that the study population be fully healthy, it is not possible to exclude an ongoing subclinical pathological condition such as an incipient infectious process especially in the oldest-old individuals studied. We finally had to cope with the difficulties linked to the cognitive evaluation of a study sample with a broad age-range. A ceiling effect of neuropsychological tests applied in healthy subjects was observed, i.e., low-cut ranges within normality scores were obtained (for instance, MMSE scores in this study are between 25/30 and 30/30, and not 0–30 as one would expect in a mixed population of demented and cognitively healthy individuals), though this situation did not hinder the achievement of highly significant statistical differences between LI and HI groups.

In summary, we were able to show that healthy subjects consuming the recommended intakes of 5 daily portions of fruits and vegetables have higher circulating levels of antioxidant micronutrients and lower levels of oxidative stress biomarkers as well as better cognitive scores in comparison to healthy subjects consuming one daily portion or less of fruit and vegetables. These results are independent of age. Therefore, nutritional preventive strategies against cognitive impairment should be encouraged at a young age and in advanced age. The relationship between such preventive strategies and cognitive function can only be studied with large prospective trials.

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There are no actual or potential conflicts of interest to disclose (including any financial, personal or other relationships with other people or organizations within three years of beginning the work).

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