



# Macular Carotenoids, Aerobic Fitness, and Central Adiposity Are Associated Differentially with Hippocampal-Dependent Relational Memory in Preadolescent Children

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**Objectives** To examine the associations of macular pigment carotenoids (lutein, *meso*-zeaxanthin, and zeaxanthin), aerobic fitness, and central adiposity with hippocampal-dependent relational memory in prepubescent children.

**Study design** Children between 7 and 10 years of age ( $n = 40$ ) completed a task designed to assess relational memory performance and participated in aerobic fitness, adiposity, and macular pigment optical density (MPOD) assessment. Aerobic fitness was assessed via a modified Balke treadmill protocol designed to measure maximal oxygen volume. Central adiposity was assessed via dual-energy x-ray absorptiometry. MPOD was measured psychophysically by the use of customized heterochromatic flicker photometry. Statistical analyses included correlations and hierarchical linear regression.

**Results** Aerobic fitness and MPOD were associated negatively with relational memory errors ( $P < .01$ ), whereas central adiposity was associated positively with relational memory errors ( $P < .05$ ). Hierarchical regression analysis revealed that MPOD accounted for a significant amount of the variance in relational memory performance even after we accounted for aerobic fitness ( $\beta = -0.388$ ,  $P = .007$ ).

**Conclusions** Even after we adjusted for aerobic fitness and central adiposity, factors known to relate to hippocampal-dependent memory, MPOD positively and significantly predicted hippocampal-dependent memory performance. (*J Pediatr* 2017;183:108-14).

**Trial registration** ClinicalTrials.gov: NCT01619826.

Health behaviors have a known association with physical health across the lifespan, with physical inactivity and poor dietary habits contributing to the prevalence of obesity and metabolic disorders amongst children in the US.<sup>1</sup> Because of its high rate of metabolic activity and plasticity across the lifespan, the hippocampus is one brain region that is particularly susceptible to the effects of health factors such as aerobic fitness, diet, and obesity.<sup>2-5</sup> These effects likely are to be pronounced during childhood, when the hippocampus is still developing and experiences a high degree of plasticity and understanding them is critical, as the hippocampus is necessary for relational memory (the binding and flexible use of elements that make up a scene or event),<sup>6,7</sup> and supports the construction of a flexible knowledge base that is likely critical for children's academic success.

Much of what is understood currently about the mechanisms by which health factors impact hippocampal function is the result of extensive work in rodent models. The mechanisms by which diet and exercise impact hippocampal function suggest these health factors may have interactive, and likely synergistic, effects.

There is growing interest in understanding the role of plant pigments and dietary carotenoids, specifically lutein and zeaxanthin, in cognitive and brain health. These xanthophyll carotenoids are not produced endogenously and thus can only be obtained through diet, specifically through the consumption of dark leafy greens, cruciferous vegetables, eggs, and some fruits.<sup>8</sup> Lutein is also the dominant carotenoid in brain tissue,<sup>9,10</sup> where it may serve antioxidant and anti-inflammatory roles.<sup>11</sup> These carotenoids (along with the lutein metabolite *meso*-zeaxanthin) also preferentially accumulate in the macula of the retina, forming the macular pigment, where they serve as antioxidants, likely protecting against age-related macular degeneration.<sup>12,13</sup> In older adults both macular pigment carotenoids, assessed via macular pigment optical density (MPOD), and brain lutein concentration are associated with cognitive performance.<sup>10,14</sup>

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|         |                                   |                     |                                |
|---------|-----------------------------------|---------------------|--------------------------------|
| DXA     | Dual-energy x-ray absorptiometry  | SES                 | Socioeconomic status           |
| FITKids | Fitness Improves Thinking in Kids | TAAT                | Total abdominal adipose tissue |
| MPOD    | Macular pigment optical density   | VO <sub>2</sub> max | Maximal oxygen consumption     |

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Yet, despite evidence that lutein is the dominant carotenoid in the developing brain,<sup>9</sup> MPOD has rarely been assessed in a pediatric population.<sup>15,16</sup> Given the proposed antioxidant properties of lutein within the brain and the high likelihood of oxidative stress within the hippocampus, lutein may have a pronounced effect on hippocampal function, similar to other dietary and exercise factors that promote hippocampal health. The goal of the present study was to examine the contributions of aerobic fitness, central adiposity, and macular pigment carotenoids to hippocampal-dependent memory performance in preadolescent children. We hypothesized that MPOD would predict a significant proportion of the variance in relational memory performance.

## Methods

Participants were preadolescent children (N = 40, 25 female) between the ages of 7 and 10 years (Table I). Participants were recruited from a larger sample of children who recently completed participation in or were enrolled recently in the Fitness Improves Thinking in Kids (FITKids) Trial ([ClinicalTrials.gov: NCT01619826](http://ClinicalTrials.gov/NCT01619826)). Exclusion criteria for the FITKids Trial included neurologic or attentional disorders, physical disabilities that might prevent participation in physical activity, and psychoactive medication status. Specifically, participants underwent an initial telephone screening with the Attention Deficit/Hyperactivity Disorder Rating Scale-IV to rule out attention deficit hyperactivity disorder, followed by a preparticipation health screening with the Physical Activity Readiness Questionnaire to ensure that participants did not have a family history of early-onset serious heart conditions or pre-existing health conditions (including asthma, high blood pressure, diabetes, epilepsy, fainting) that might put them at risk of an adverse event during exercise. All surveys and questionnaires were com-

pleted by the participant's parent or legal guardian. Participants had normal or corrected-to-normal vision. All participants provided written assent, and each participant's legal guardian provided written informed consent in accordance with the regulations of the University of Illinois institutional review board.

A number of demographic, cognitive, and health measures were gathered as part of the FITKids trial, including measures of age, sex, socioeconomic status (SES),<sup>17,18</sup> IQ (assessed via the Woodcock-Johnson brief intelligence assessment),<sup>19</sup> aerobic fitness (assessed via a maximal oxygen consumption [VO<sub>2</sub>max] test), adiposity (assessed via dual-energy x-ray absorptiometry [DXA]), MPOD, and a cognitive test battery. All participants completed one additional 2-hour cognitive testing session, during which they completed the relational memory task described in this section. For the current sample, this follow-up cognitive testing session took place, on average, 5.5 weeks following the collection of demographic and health data.

Aerobic fitness was assessed with a graded exercise test, during which VO<sub>2</sub>max was measured via an indirect calorimetry system (True Max 2400; ParvoMedics, Sandy, Utah). Participants completed a modified Balke protocol.<sup>20</sup> During testing, the heart rate of each participant was monitored constantly with a Polar heart rate monitor (Polar WearLink1 131; Polar Electro, Vantaa, Finland), and a measure of perceived exertion was attained every 2 minutes with the children's OMNI Scale of Perceived Exertion.<sup>21</sup> VO<sub>2</sub>max was based on accomplishing 2 of the following 4 criteria: (1) a heart rate within 10 beats/min of the age predicted maximum, (2) a respiratory exchange ratio (the ratio between carbon dioxide and oxygen percentage) >1.0, (3) a rating greater than 8 on the children's OMNI scale of perceived exertion, and/or (4) a plateau in VO<sub>2</sub> despite an increase in workload.

Traditional VO<sub>2</sub>max scores are calculated relative to an individual's body weight (relative VO<sub>2</sub>max). The present investigation focused on fat-free VO<sub>2</sub>max, for which scores are calculated relative to an individual's fat-free mass rather than total body weight to more effectively parse out the contributions of muscle and fat mass to the primary outcomes.

Participants' height and weight were measured with a stadiometer (model 240; Seca, Hamburg, Germany) and a digital scale (WB-300 Plus; Tanita, Tokyo, Japan). These measures were used to obtain a body mass index value for each participant.

As previously described by Khan et al,<sup>3</sup> central adiposity was assessed by DXA via a Hologic QDR 4500A bone densitometer (software version 13.4.2; Hologic, Bedford, Massachusetts). Central adiposity was estimated with a measure of total abdominal adipose tissue (TAAT) area. The abdominal region of interest was a 5-cm-wide section placed across the entire abdomen level approximately coinciding with the fourth lumbar vertebrae on the whole-body DXA scan. TAAT was defined as the total adipose tissue (visceral and subcutaneous) area within this region and was selected as the adiposity measure of interest because of previous work demonstrating a relationship between TAAT and relational memory performance in a similar sample of preadolescent children.<sup>3</sup>

MPOD was assessed via customized heterochromatic flicker photometry with a macular densitometer (Macular Metrics

**Table I. Participant demographics and health characteristics (n = 40, except where indicated otherwise)**

| Measures   | N (%) or sample mean ± SE |
|--|---------------------------|
| Sex  |                           |
| Female   | 25 (63%)                  |
| Male   | 15 (37%)                  |
| SES (n = 39)   |                           |
| Low  | 13 (33%)                  |
| Middle   | 16 (41%)                  |
| High   | 10 (26%)                  |
| Age, y (range = 7.9-10.6)  | 8.8 ± 0.11                |
| IQ (Woodcock-Johnson brief intelligence assessment standard score)   | 114.3 ± 2.24              |
| Body mass index, kg/m <sup>2</sup>                                   | 19.0 ± 0.50               |
| Fat-free VO <sub>2</sub> max, mL•kg <sup>-1</sup> •min <sup>-1</sup> | 62.3 ± 1.27               |
| TAAT, cm <sup>2</sup>  | 185.1 ± 15.47             |
| MPOD, n = 39   | 0.66 ± 0.03               |
| Time between health/demographic and cognitive testing, wk            | 5.5 ± 0.54                |

The sample mean column shows the mean ± SEM. SES was measured according to a previously used trichotomous index based on (1) participation of the participant in a free- or reduced-lunch program at school, (2) the number of parents who worked full-time, and (3) the highest level of education obtained by the mother and father.

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