JOGGING THEIR MEMORY: DEVELOPMENTAL EFFECTS OF FITNESS, BODY COMPOSITION, AND NUTRITION ON RELATIONAL MEMORY

BY

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DISSERTATION

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ABSTRACT

Increasingly prevalent in the media and public awareness, the growing problem of obesity, particularly childhood obesity, has become a significant concern in the United States and many developed countries around the world. Current longevity estimates predict that if trends continue, children born today may die younger than their parents’ generation for the first time in history. Many factors have contributed to the rising incidence of childhood obesity including increasingly sedentary and unfit lifestyle, as well as poor diet resulting from an ethos that taste and convenience are the paramount concerns in deciding what to consume.

Sedentary lifestyle, poor diet, and the weight gain that accompanies those choices have been repeatedly shown to cause adverse consequences for physical health, including markedly increasing the risk for stroke, type 2 diabetes, and cardiovascular disease. More recently these unhealthy lifestyle choices are beginning to be linked to negative outcomes in brain health as well, including increasing risk of premature cognitive aging and Alzheimer’s disease.

How these health and fitness factors affect the developing brain is even more sparsely studied in the literature to date. It may be the case that health factors do not affect the brain and its functionality until their effects have accumulated for many decades, but it is equally possible that early adoption of healthy or unhealthy habits may set the developmental trajectory for the long-term early in life and also manifest in the short-term.

Although aspects of the whole brain may be vulnerable to these health habits, one region of the brain, the hippocampus, has been shown to be particularly susceptible to these factors due in part to its high metabolic demand and extraordinary capabilities of plasticity including
neurogenesis. The hippocampus is necessary for a type of memory, relational memory, which is critical for many types of learning, and thus for successful scholastic achievement. Thus, it may be expected that changes to the functionality of the hippocampus may strongly impact learning capabilities. Another type of memory, item memory, relies not on the hippocampus, but on surrounding medial temporal lobe cortices. These cortical structures are neither as plastic nor metabolically demanding as the hippocampus and thus may be expected to be less functionally or structurally susceptible to changes in physical health, although they may still potentially be vulnerable to broad effects such as global inflammation.

The current investigation thus sought to extend our understanding of the ways in which various lifestyle factors including physical fitness and nutrition, along with health factors that may result from lifestyle choices including body mass and body composition, interact with the developmental time courses of relational and item memory as they unfold over the first several decades of life.

Using a mixed-approach combining cross-sectional and longitudinal studies, the current investigation sampled across three age groups: prepubertal children (ages 7-9), young adolescents (ages 11-13), and young adults (ages 18-34). In sampling across these three time points in development, we aimed to begin to shed light on how these various lifestyle factors relate to these various types of memory across development, and also to probe how early in time these effects can be seen to emerge. In measuring this, we combined behavioral and eye movement measures of memory to allow for a rich representation of how these health and fitness factors may interact with memory function across the age groups.
Aerobic fitness was found to relate positively to relational memory function across all time points sampled, with the relationship becoming stronger in the young adults than either of the younger groups. The relationship between aerobic fitness and item memory showed a different pattern, with fitness predicting behavioral memory performance in the children but not in the young adults. Potential mechanisms for these relationships within age group are discussed in the appropriate chapter discussions and how they relate across development is discussed in the General Discussion.

Body mass index was also assessed across all three developmental time points and showed a negative relationship with memory performance in the prepubertal children but not the adolescents or young adults, suggesting that body size may be more important to the developing brain than the more mature brain.

Interestingly, body composition was shown to be a stronger predictor of relational memory performance than body size alone, in children, such that children who stored a greater amount of fat in the central abdominal region showed poorer relational memory performance than those who preferentially stored fat peripherally.

Finally, nutritional intake, particularly of dietary fats, was found to correlate with memory performance in prepubertal children. Specifically, higher intake of saturated fats was found to negatively correlate with both types of memory performance and higher intake of omega-3 fatty acids was found to positively correlate with relational memory performance, even after adjusting for body mass.
Taken together the results of the current investigation demonstrate that health and fitness factors are related to memory function in differing ways across the first several decades of life and begins to suggest a developmental trajectory by which these effects unfold. Furthermore, the current investigation suggests that these effects emerge early in development, after less than one decade of life.

The public health implications of these findings and the findings that will follow stand to be potentially enormous as compromised brain health may interfere with learning and achievement during the school years and beyond, and set children on a lower developmental trajectory from which it is difficult or impossible to recover. As such, the current findings underscore the importance of early adoption and continued maintenance of a healthy, active lifestyle.
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# TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION ............................................................................................................. 2

CHAPTER 2: AEROBIC FITNESS SPECIFICALLY PREDICTS RELATIONAL MEMORY PERFORMANCE IN HEALTHY YOUNG ADULTS ................................................................. 25

CHAPTER 3: LONGITUDINAL ASSESSMENT OF RELATIONAL MEMORY, PHYSICAL FITNESS, AND SCHOLASTIC ACHIEVEMENT IN 11-13 YEAR OLDS ........................................ 48

CHAPTER 4: CROSS-SECTIONAL ASSESSMENT OF THE RELATIONSHIPS BETWEEN AEROBIC FITNESS AND BODY MASS ON RELATIONAL AND ITEM MEMORY IN PREPUBERTAL CHILDREN ........................................................................................................... 69

CHAPTER 5: CENTRAL ADIPOSITY IS NEGATIVELY ASSOCIATED WITH HIPPOCAMPAL MEMORY IN CHILDREN ......................................................................................................................... 100

CHAPTER 6: DIFFERENTIAL EFFECTS OF OMEGA-3 AND SATURATED FATTY ACIDS ON RELATIONAL AND ITEM MEMORY IN PREPUBERTAL CHILDREN ....................................... 127

CHAPTER 7: GENERAL DISCUSSION .................................................................................................... 158
CHAPTER 1: INTRODUCTION
**Motivation**

Converging evidence from human and animal work provides compelling support for the influence of aerobic fitness, diet, and other health factors on cognition. Indeed, given the rising incidence of obesity in the United States and worldwide, the public health and financial burdens of an unhealthy lifestyle may extend beyond physical health to encompass cognitive and brain health as well. With the prevalence of childhood obesity (currently 17%; Centers for Disease Control and Prevention, 2011) approaching that of adult obesity (35.7% in 2008; Centers for Disease Control and Prevention, 2012), this growing health issue stands to interfere with education, workplace productivity, and military service for years to come.

While unhealthy lifestyle factors such as low physical fitness and energy-dense but nutrient-poor diet that lead to overweight and obese individuals have well understood physical consequences, their influences on particular facets of cognitive development have yet to be fully understood. Healthy lifestyle factors can also affect cognitive development but the beneficial effects of aerobic fitness on memory in children have yet been studied by only a handful of researchers (e.g., Chaddock et al., 2010; Kamijo et al., 2011; Monti, Hillman, & Cohen, 2012). Furthermore, the multiplicity of effects of diet on memory in children has only fairly recently begun to be characterized but results show that various nutritional markers can influence the structure and function of the hippocampus (Osendarp et al., 2007; McNamara et al., 2010). In many cases hippocampal volume has been shown to be a general index of brain health, due to its metabolic demands as well as epidemiological evidence that shows hippocampal atrophy in many neurological and psychiatric disorders including anxiety,
depression, Alzheimer’s disease, Tourette syndrome, and post-traumatic stress disorder, among others. Given the known effects from human and animal models of aerobic fitness and nutrition on the function of the hippocampus in particular (Chaddock et al., 2010; Erickson et al., 2011; Monti, Hillman, & Cohen, 2012; van Praag et al., 2007, Wu, Ying, & Gómez-Pinilla, 2008), this investigation focuses primarily on relational memory, an ability uniquely subserved by the hippocampus (Eichenbaum & Cohen, 2001; Ryan, Althoff, Whitlow, & Cohen, 2000).

Relational memory is an exceptionally important cognitive function for accumulating information about the world, particularly declarative information such as facts and events, because it is critical for representing the arbitrary associations between components of events and between pieces of information (Eichenbaum & Cohen, 2001). With this functionality it is necessary for encoding not only day to day happenings as we experience life, but also for many aspects of learning including reading comprehension, in which relational memory allows us to integrate ideas contained in the text. As such, early and rapid relational memory development is particularly important for children and adolescents as they form understanding of the world and learn information in school. If relational memory is slow to develop due to factors such as poor nutrition or sedentary lifestyle, this could delay learning and impair scholastic performance, potentially setting the course for more distant life outcomes. Thus, a more detailed understanding of how lifestyle factors affect relational memory development is critical for nurturing learning and development in childhood through young adulthood.

Lifestyle, Health Factors, and Relational Memory Development
**Fitness.** Physical fitness, particularly aerobic fitness, has been shown in a variety of human and animal studies to be associated with better functioning in several aspects of cognitive performance including executive function and hippocampal-dependent memory (Buck, Hillman, & Castelli, 2008; Castelli, Hillman, Buck, & Erwin, 2007; Chaddock et al., 2010; Colcombe & Kramer, 2003; Erickson et al., 2011; Hillman, Castelli, & Buck, 2005; Sibley & Etnier, 2003; van Praag et al, 2007). Furthermore, in school-aged children aerobic fitness has been correlated with overall scholastic achievement based on standardized testing, while other fitness measures (e.g., muscle strength as measured by push-ups and curl-ups) were unrelated to achievement scores (Castelli, Hillman, Buck, & Erwin, 2007). While the relationship between fitness and relational memory has been previously examined sparsely at either end of the developmental spectrum, the current investigation aimed to characterize how these relationships between fitness and different forms of memory function develop or change across the first several decades of life by examining fitness across three age ranges: prepubertal children, early adolescents, and young adults.

**Body Mass and Body Composition.** Evidence is accumulating that obesity may be associated with cognitive impairments in adulthood, with obesity in middle adulthood being an independent risk factor for development of Alzheimer’s disease and dementia in older adulthood (Fitzpatrick et al., 2009; Burkhalter & Hillman, 2011; Whitmer et al., 2005). Further emphasizing the inverse relationship between obesity and cognitive function, patients who had undergone bariatric surgery 12 weeks prior performed better on memory tests than obese controls (Gunstad et al., 2011). Among children, obesity has begun to be associated with
impairments in cognitive control as well as scholastic performance (Castelli, Hillman, Buck, & Erwin, 2007; Kamijo et al., 2012a; Li, 2008), but the relationship between body mass and memory in children has not yet been examined to our knowledge.

Beyond general obesity or body size, it may further be the case that adiposity in the abdominal region may be particularly associated with memory function, given potential metabolic links between visceral fat and hippocampal structure or function. In older adults, measures of central adiposity, including waist-to-hip ratio, have been shown to be negatively correlated with hippocampal volume and behavioral memory performance (Dore et al., 2008; Jagust, Harvey, Mungas, & Haan, 2005), however these relationships have not yet been examined in children.

**Nutrition.** Nutrient intake has been shown to affect various aspects of cognition in humans and animals with particular emphasis in the animal literature on saturated fat, omega-3 fatty acids, micronutrients, and flavonoids as beneficial or harmful to cognition and neural structure (Agrawal & Gómez-Pinilla, 2012; Casadesus et al 2004; Georgieff, 2007; Gómez-Pinilla, 2008; Molteni et al., 2002; Stangl & Thuret, 2009; Vauzour et al., 2008). Potential mechanisms mediating these relationships include their roles in neurogenesis and oxidative stress (Gómez-Pinilla, 2008).

While most of the world’s traditional diets have a fairly good balance of these nutrients, the convenience-based Western diet does not. Instead the Western diet is high in sugar and saturated fat and low in many essential nutrients. As the popularity of the Western diet increases, individuals are consuming higher levels of unhealthy fats and refined sugars, as well
as lower levels of neuroprotective healthy fats, flavonoids, and micronutrients. When consumed regularly, the Western diet can lead to nutrient imbalance and deficiency. Why is it important to address nutritional deficiencies early in life? In some cases, undernutrition not only has been shown to have a profound effect on cognition in young rats, but these impairments have been shown to continue through adulthood (Castro, Tracy, & Rudy, 1989), essentially setting maturing rats on a lower developmental trajectory from which it is difficult if not impossible to recover. In many cases, intake of an energy-dense but nutrient-poor diet such as the Western diet could mirror the effects of under-nutrition, as in both cases the body is not attaining sufficient essential nutrients.

Assessing Interactions Across Lifestyle and Health Factors. Many of these fitness and health variables are tightly intertwined, not only with each other, but with other factors such as genetics, IQ, SES, and physical activity, making independent analysis of their effects on cognition nearly impossible. Thus it is difficult to conduct controlled studies in humans isolating any of these factors given the high degree of collinearity and interaction between them. Humans who are healthier in one area simply often tend to also pay attention to health in other arenas. To make strides toward understanding these factors independently and relative to one another, we turn to the animal literature, which is able to more tightly regulate the influence of each of these factors on cognition and brain structure.

Indeed much of the cleanest work done on the direct influence of health and lifestyle factors to date has been in animal models. Aerobic fitness has been shown in rodents to be causally
inducive of an increase in proliferation and survival of new neurons in the dentate gyrus of the hippocampus, as well as enhancements in behavioral performance (van Praag, Christie, Sejnowski, & Gage, 1999; van Praag, Kempermann, & Gage, 1999; Wu, Ying, & Gómez-Pinilla, 2008). Further, rodent models of overweight and obesity have shown causal deficits in hippocampal-dependent memory relative to normal weight animals (Winocur et al., 2005). Perhaps the most difficult work to conduct in humans and also the most difficult to translate from animal models to humans is in the area of nutrition, in which much of the research has focused on individual or sets of nutrients or food extracts; this work has shown important causal relationships between nutrient intake and anatomical or physiological changes in the hippocampus, as well as in behavioral performance, although to what degree they translate to human brain function is not clear (e.g., blueberry; Casadesus et al., 2004; Gómez-Pinilla, 2008; Molteni et al., 2002; van Praag et al., 2007).

The picture becomes even more complicated when considering more than one of these factors in the same organism. This too, however, can often be nicely controlled in animal models. For example, above and beyond the independent beneficial effects of fitness and certain nutrients, several studies have examined the combined effects of exercise and nutrition and discovered a synergistic or protective relationship between the two (Wu, Ying, & Gómez-Pinilla, 2008). One study demonstrated that intake of flavonoids in combination with voluntary exercise induces neurogenesis in the adult rat hippocampus as measured by BDNF-mediated synaptic plasticity and enhanced spatial learning (van Praag et al., 2007). Exercise has also been shown to be
protective against impairments caused by a diet high in saturated fat and refined sugar (Molteni et al., 2004).

Even within these lifestyle factors, it can be difficult to assess the individual contributions of their components. For example, the effects of diet become even more complex when considering simultaneous intake of multiple nutrients. In one study, Wu, Ying, and Gómez-Pinilla (2004) showed that vitamin E supplementation reversed the oxidative stress and corresponding DNA damage caused by a diet high in saturated fat. Surprisingly, the incorporation of antioxidants with the high fat diet restored levels of hippocampal BDNF to that of control rats, as measured by post-mortem stains, as well as improving in vivo Morris water maze performance (Wu, Ying, & Gómez-Pinilla, 2004). Another antioxidant, curcumin, was shown to have similar restorative effects (Wu, Ying, & Gómez-Pinilla, 2006).

Even in tightly controlled animal work, however, the story is not complete, given the limited generalizability to the anatomy and physiology of the human brain. One fortunate feature of the hippocampus, however, is that this structure is one of the evolutionarily oldest structures in the brain, and thus there is great deal of structural and functional overlap across species, allowing for a large degree of translation from animal to human models. To make meaningful conclusions in humans, we must of course also conduct human studies, which although more confounded almost by necessity, are directly applicable when attempting to understand how these relationships manifest across human development.
In humans, several approaches have provided valuable findings that converge with the animal work to elucidate our understanding of these lifestyle and memory relationships. The most tightly controlled and illuminative human work involves randomized controlled trials (RCTs) in which treatment and control groups are randomly assigned and in theory differ in only the one respect of interest (see Erickson et al., 2011; Kamijo et al., 2011). RCTs in this health and fitness arena are notoriously difficult to conduct, however, particularly if attempting to tease apart multiple lifestyle factors as humans may, for example upon feeling subjectively better after adding an exercise routine, change their other health behaviors as well. They may further bring to bear demand characteristics in which, for example, knowing they were in a control group, may not perform as well on cognitive tasks simply for this reason. Due to these limitations in combination with the immense financial and time costs of conducting RCTs, they are relatively less common than cross-sectional studies in the literature. Cross-sectional studies have the advantage that they are easier and cheaper to conduct, and often allow for examination of a larger population, but they come with the drawback that they are by definition less well controlled and must rely on statistical adjustment for confounding factors rather than controlling these factors through random assignment (e.g., Chaddock et al., 2010; Hillman et al., 2006). Third, longitudinal or repeated measures studies may be particularly useful in characterizing how these lifestyle, health, and memory interactions develop over time, but these too can be costly and cannot directly control potential confounding variables (e.g., Yaffe et al., 2001).
Ultimately no single one of these approaches, including the animal work, can offer the final word on these health-memory relationships. To more fully characterize these relationships we must take together converging evidence from studies performed across multiple modalities with the goal in mind of understanding how these relationships manifest across human development, with a focus on hippocampal and relational memory development.

**Developmental aspects of relational memory**

While relational memory performance appears to depend on an intact hippocampus in adults (Eichenbaum & Cohen, 2001; Ryan, Althoff, Whitlow, & Cohen, 2000), the trajectory of relational memory development is not well understood. Simple forms of relational memory have been shown to emerge early in development, with certain relational memory abilities appearing as early as 9 months of age (Richmond & Nelson, 2009). While that study demonstrated a strikingly similar pattern of viewing to that of adults in a relational memory paradigm at 9 months of age (Richmond & Nelson, 2009), most other studies in the literature start to focus on emerging relational memory abilities toward the latter half of the second year of life, in the period between 18-24 months (Newcombe, Huttenlocher, Drummey, & Wiley, 1998; Sluzenski, Newcombe, & Satlow, 2004).

Turning to the brain, the hippocampus is one of the brain structures most rapid to develop during the first years of life (Gilmore et al., 2012). The hippocampus has been shown to mature to adult size prior to age 4, although structural and functional changes appear to continue to occur through young adulthood, due in part to the processes of cellular and synaptic pruning.
and neurogenesis (Gogtay et al., 2006). There are thus reasons to expect changes in
hippocampal function across development, which may be modulated by lifestyle factors.
Furthermore, changes in the connectivity between the hippocampus and related structures
such as the medial temporal lobe cortex and lateral prefrontal cortex continue to develop
across the first several decades of life. As such, it is reasonable to predict that lifestyle-induced
changes in connectivity or communication between these structures may further play a role in
how health factors affect relational memory performance. Although relational memory was our
primary target, the functional and anatomical connectivity among memory structures in the
brain suggests that it may also be illuminating to assess whether there may be broader memory
effects of lifestyle. To test this we additionally measured item memory, which is known to rely
on the parahippocampal cortex in the medial temporal lobe. In so doing, we were able to test
the selectivity of these health effects to the function of the hippocampus in children and young
adults.

Whether and which lifestyle choices may then translate to and effect changes in these
maturational processes of the hippocampus and its connections and to alterations in relational
memory performance as early as middle childhood remains largely an open question.
Additionally, what are the developmental trends across the first few decades of life and to what
degree these various lifestyle factors affect hippocampal function relative to one another in the
same group of individuals has yet to be characterized. As such, beginning to address these
questions is the overarching theme of the current investigation.
Measuring Lifestyle Factors & Relational Memory in Children

How then do we best go about measuring the developmental trajectory of relational memory across childhood and early adulthood in humans and how it is influenced by fitness, health, and nutrition? Behavioral responses can certainly be useful in measuring relational memory, used to recall facts and events, but in some cases may not be the best index of memory. For example, in certain cases, such as with young children, the complexity of a relational memory judgment may be overwhelmed by additional processing that must take place to make a correct behavioral response (e.g., executive functions such as inhibition, response selection, and motor planning). Previous research has shown that young children are able to succeed in tasks when the dependent measure is spontaneous viewing rather than a directly elicited response (Scott & Baillargeon, 2009; Scott, He, Baillargeon, & Cummins, 2012; He, Bolz, & Baillargeon, 2011). In these instances, spontaneous looking behavior may be a better reflection of past experience. This has also been shown to be the case in prepubertal children and young adults through eye tracking studies in which eye movements can be a better index of prior exposure than behavioral response and have been further been shown to correspond to hippocampal activity (Hannula & Ranganath, 2009; Hannula, Baym, Warren, & Cohen, 2012; Monti, Hillman, & Cohen, 2012).

We then combine these cognitive measures with a variety of externally valid measures of scholastic achievement including standardized test scores, physical health including VO2 max and Fitnessgram to assess aerobic and overall fitness, dual-energy X-ray absorptiometry (DXA) to assess body composition through fat distribution, body mass index (BMI) as a general
measure of body size and health, and food frequency questionnaires to assess dietary patterns over the preceding year. Each of these measures has been used in the literature in service of drawing connections between health variables and cognitive performance. For example, in schools, Castelli and colleagues (2007) found that aerobic fitness and BMI as measured using the Fitnessgram were correlated with standardized test scores (Castelli, Hillman, Buck, & Erwin, 2007). In laboratory settings, VO₂max measures of aerobic fitness have been found to correlate with executive function in children and adults (e.g., Themanson, Pontifex, and Hillman, 2008; see also Hillman, Erickson, & Kramer, 2008), as well as spatial memory in older adults (Erickson, et al., 2011). Furthermore, laboratory-based DXA measures of adiposity have been shown to negatively correlate with inhibition (Kamijo, et al., 2012b). Finally, food frequency questionnaires have typically been used to quantify how components of diet relate to standardized neuropsychological tests and cognitive decline (e.g., Okereke, et al., 2012).

**General Overview**

The current investigation consists of five parallel studies each designed to assess a different facet of the developmental time course of the interactions between fitness, body mass and composition, and nutrition on relational memory development. First, Chapter 2 provides a foundation in young adulthood (ages 18-35) with which to compare studies in young adolescents and prepubertal children. This chapter focuses on bridging the gap between previous studies of relational memory in adults with the current investigation as a touchstone for characterizing the specificity of the relationship between the fitness effects and brain function across development. Chapter 3 goes on to describe a longitudinal study aimed to specifically address how overall physical fitness (including aerobic fitness) is related to
relational memory performance across the middle school years (ages 11-13). Chapter 4 describes a cross-sectional eye tracking study investigating the differential effects of aerobic fitness and body size on relational and item memory in prepubertal children (ages 7-9) with and without adjustment for various demographic variables. That study is designed to evaluate how aerobic fitness and BMI relate to both behavioral responses and spontaneous preferential viewing of arrays of previously studied items and associations between stimuli. Chapter 5 then aims to further characterize effects of physical factors on relational and item memory in prepubertal children (ages 7-9) by assessing how body composition, particularly central adiposity, may be related to memory performance as measured behaviorally and with eye-movement measures, after adjustment for demographic factors and aerobic fitness. Chapter 6 then asks the question of whether components of the Western diet, namely high intake of saturated fats and refined sugars and low intake of omega-3 fatty acids and monounsaturated fatty acids, relate to relational and item memory performance in prepubertal children. In attempting to answer this question, we gathered self-reported food frequency questionnaire data from a subset of the children in Chapters 4 and 5, and adjusted for other health and demographic factors that may affect these relationships, including BMI. Finally, Chapter 7 provides a discussion of how the results of this investigation inform our understanding of the dynamic relationships between fitness, nutrition, and body composition across development. That chapter considers how our findings at each developmental time point begin to inform our understanding of how these individual lifestyle-memory relationships wax or wane across the first decades of life and how the measured lifestyle factors compared to one another in explanatory power in the current experiments. Finally, that chapter discusses several novel
cognitive findings and limitations of the current set of studies, and suggests avenues for future research.
References


CHAPTER 2: AEROBIC FITNESS SPECIFICALLY PREDICTS RELATIONAL MEMORY PERFORMANCE IN HEALTHY YOUNG ADULTS

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\textit{Keywords}: Memory, relational memory, aerobic fitness, eye movements
Abstract

Health factors such as maintaining an active lifestyle and high aerobic fitness have long been linked to decreased risk of cardiovascular disease, stroke, and other adverse health outcomes. Only more recently have researchers begun to investigate the relationship between aerobic fitness and memory function. The current study examined this link in a population of healthy young adults with the goal of examining the relationships between aerobic fitness and hippocampal-dependent relational memory and hippocampal-independent item memory. Since these memory functions have been shown to be reliant on distinct neural substrates, a dissociation between them in this context would demonstrate that aerobic fitness has selective effects on different neural subregions in the healthy, young adult brain. Aerobic fitness was assessed using a graded exercise test to measure maximal oxygen consumption during exercise (VO₂max) and relational and item memory were assessed using an eye-tracking task. Behavioral results show that aerobic fitness was positively correlated with relational memory performance but not item memory performance, suggesting that the beneficial effects of aerobic fitness selectively affect the hippocampus and not the surrounding medial temporal lobe cortex. Eye-movement results further supported the specificity of this fitness effect to hippocampal function, in that aerobic fitness predicted disproportionate preferential viewing of previously studied relational associations but not of previously viewed items. Potential mechanisms underlying this pattern of results are discussed.
Introduction

The incidence of low cardiovascular health in the United States has risen dramatically over the past two decades in large part due to unhealthy lifestyle factors such as decrease in fitness, physical activity, and diet quality. Indeed, the top two causes of death in the United States, cardiovascular disease and cancer, can be in many cases linked to these types of lifestyle choices. So while a sedentary lifestyle and corresponding low aerobic capacity have well understood physical consequences, their influence on cognition has only recently gained attention.

Previous research has linked aerobic fitness to cognition across the human lifespan (Åberg et al., 2009; Chaddock et al., 2010; Erickson et al., 2011; Hillman et al., 2006), however the relationship between aerobic fitness and cognitive function has been only sparsely examined in healthy young adults. One reason for this preferential focus on the ends of the maturational spectrum concerns variability, in that children and older adults show more cognitive and neuroarchitectural variability, maximizing opportunity to observe strong fitness effects (Voss et al., 2011). Despite this potential power problem in young adults, several studies have investigated the relationship between aerobic fitness or physical activity and cognition in this age range. In one cohort study of over 1 million men, aerobic fitness at age 18 significantly predicted full-scale intelligence as well as specific subscales of intelligence (Åberg et al., 2009). Showing greater cognitive specificity to executive function, Hillman and colleagues (2006) demonstrated a positive relationship between physical activity and measures of inhibitory control, such that adults of all ages who were more physically active showed shorter response
times while maintaining a high level of accuracy than those who were less physically active (Hillman et al. 2006). Similarly, physical activity has been shown to enhance task-switching abilities (Hillman, Kramer, Belopolsky, & Smith, 2006). Further in the scope of executive function, aerobic fitness has been shown to improve ability to flexibly employ cognitive control in a task requiring inhibition and action monitoring (Themanson, Pontifex, and Hillman, 2008).

Moving into the broad realm of memory, one cohort study showed that physical activity in a middle-aged sample predicted rate of verbal memory decline in later adulthood (Richards, Hardy, & Wadsworth, 2003). To our knowledge, however, the relationship between aerobic fitness and hippocampal memory in healthy young adults has not yet been examined.

In animal models, however, as well as in several studies at either end of the human lifespan aerobic fitness and physical activity have been selectively linked to hippocampal structure or function (Erickson et al., 2011; Monti, Hillman, & Cohen, 2012; van Praag, Christie, Sejnowski, & Gage, 1999). The current study thus sought to investigate 1) whether aerobic fitness is related to memory function in young adults, and 2) the specificity of the relationship between aerobic fitness and hippocampal function relative to surrounding medial temporal lobe (MTL) cortex (Davachi & Wagner, 2002), by assessing hippocampal-dependent relational memory and hippocampal-independent item memory. Given that relational and item memory are known to rely on different neural subregions with different metabolic and cytoarchitectural profiles, it is reasonable to predict that the two types of memory may be differentially affected by health and lifestyle factors.
In addition to measuring behavioral response, we measured relational and item memory using both declarative behavioral and non-declarative eye movement measures, which have been shown to be a more sensitive measure of previous experience than declarative behavioral response (Hannula, Baym, Warren, & Cohen, 2012). Additionally, we measured aerobic fitness using VO_{2}max and hypothesized that aerobic fitness would be positively correlated with behavioral relational, but not item, memory performance and with greater proportion of time spent viewing correctly selected relational associations than incorrectly selected relational associations.

**Methods**

**Participants**

Sixty-eight neurologically-intact young adults age 18-34 (21.79 +/- 0.4; 32 males) were recruited from the University of Illinois community to participate in this study. One additional participant was excluded for failure to reach two or more testing criteria on the aerobic fitness assessment. Two participants were excluded from eye movement analyses due to data file corruption or watering eyes, leaving a total of 66 participants in those analyses. All participants provided written informed consent in accordance with the regulations of the University of Illinois Institutional Review Board and all procedures were in accordance with the regulations of the University of Illinois Institutional Review Board.

**Aerobic Fitness Assessment**
Aerobic fitness was evaluated using a graded exercise test designed to measure maximal oxygen consumption (VO₂ max) during physical activity. Higher scores on this test indicate increased aerobic capacity. VO₂ max scores were assessed using an indirect computerized calorimetry system (ParvoMedics True Max 2400) during a modified Balke treadmill test (American College of Sports Medicine [ACSM], 2010). During the test, participants’ heart rate was constantly monitored using a Polar heart rate monitor (Polar WearLink® +31, Polar Electro, Finland) and individual subjective rate of perceived exertion was assessed every 2 minutes using the Borg scales of perceived exertion (ACSM, 2010).

The VO₂ max test included an initial warm-up period in which the treadmill gradually increased in speed while the participant walked on it. After this warm-up period, treadmill speed remained constant while incline was increased 2-3% every 2 minutes. Average oxygen consumption and respiratory exchange ratio (RER) were sampled every 20 seconds during the test using a mouthpiece and maximum oxygen consumption (VO₂ max) was measured in milliliters per kilogram per minute and based on maximum effort, which was defined using two or more of the following criteria: 1) age-defined maximum heart rate norms (i.e., heart rate > 85% of predicted maximum heart rate), 2) RER (CO₂:O₂) greater than 1.1, 3) subjective rate of perceived exertion greater than 17 out of 20, and 4) leveling of VO₂ despite increasing aerobic demand (ACSM, 2010). Reported VO₂ max scores are measured as oxygen usage relative to body weight across time (ml/kg/min).

**Memory Task**
Several days following fitness testing (3.15 +/- 0.29), participants completed a memory task designed to measure both relational and item memory (Monti, Hillman, & Cohen, 2012). This task was divided into four study-test blocks each designed to measure either relational memory or item memory.

Stimuli included 216 novel creatures created in Spore Creature Creator (Electronic Arts Inc., California; see Figure 2.1 for examples) and were presented using Presentation software (Neurobehavioral Systems, http://nbs.neuro-bs.com) on a 21” color monitor. Creatures were presented on black backgrounds and resized to 480 × 480 pixels. One-hundred ten color images of real world scenes measuring 1280 × 1280 pixels and taken by Brand X Photography were used as scene backgrounds.

![Figure 2.1: Example stimuli and displays from the memory task. The left panel describes the task progression. The right panel describes the relational condition in the top row, and the item block in the An Eyelink 1000 eye-tracker (SR Research, Ontario, Canada) was used to remotely record eye-movements. The eye-tracker was calibrated immediately prior to the start of the test phase of each block. A desk-mounted gel-padded chin rest was used to minimize head movement during eye-tracking data collection.](image)
Each block of the task followed the same format (see Figure 2.1). During the study phase, participants studied 36 creature-scene pairings in which a scene preview was presented for 2000 ms at which time a creature was superimposed in the center of the scene. The creature-scene pairing then remained on the screen for 3000 ms. A fixation cross was then presented for 2000 ms prior to the onset of the next scene preview. Following the study trials, a test phase used behavioral response and eye movement measures to assess encoding success. During each trial, a 3000 ms scene preview was shown after which time three creatures were superimposed atop the scene for 6000 ms (see Figure 2.1 for an example). To minimize eye movement away from the test display during behavioral response, participants were instructed to press a single button when they saw the creature that was originally paired with that scene (Relational condition) or that had been previously studied (Item condition). Following the probe, the test array was replaced with a black screen with three white boxes where the creatures had been. Each box was numbered and participants were told to voice to the experimenter which number corresponded to the creature they had selected. Confidence in that decision was then assessed using a three-point scale (1 = “not sure,” 2 = “kind of sure,” and 3 = “very sure”).

In each block of the Relational condition participants were explicitly encouraged to encode the relations between unique creature-scene pairings (as in Hannula, Ryan, Tranel, & Cohen, 2007) by pressing one of two buttons to indicate whether they supposed each creature “lived” in the paired habitat “alone” or “with others.” Following each study block, participants were tested on 12 probe trials in which one of the scenes from the study phase was shown then superimposed with three creatures that had been studied in that block, only one of which had been studied
with that scene. Familiarity across probe creatures was thus matched necessitating the use of
hippocampal-dependent relational memory. Participants were instructed to make a single
button press when they felt they knew which creature had been studied with that scene.

In the Item condition, participants studied creature-scene pairings with unique creatures each
paired with the same scene. To encourage encoding of individual items, participants were
instructed to press one of two buttons to indicate whether they judged that creature to look
more like a “boy” or a “girl.” During the test phase, probe displays showed one studied creature
and two novel creatures paired with the same scene that each of the items had been studied
with. Participants were instructed to make a single button press when they felt they knew
which creature had been studied. Given that two creatures were novel and one studied, the
Item condition could be solved using familiarity alone, an ability that has been shown to be
independent of the hippocampus (Davachi & Wagner, 2002)

The order of blocks was counterbalanced such that half of participants completed the Item
condition first and the other half the Relational condition first. To familiarize participants with
the task demands and to ensure that the participants who completed the item block first were
aware of the memory demands of the task, an abbreviated practice study-test block was
provided prior to the start of each condition. Finally, lists of stimuli presented in the Item or
Relational conditions and target location on test trials were counterbalanced across
participants.
Prior to the memory testing session, participants were instructed to refrain from any moderate-to-vigorous physical activity for minimum one hour prior to their arrival, including walking or biking to the appointment.

**Data Preprocessing and Analysis**

**Behavioral Data.** Pearson’s correlations were performed to assess bivariate relationships between VO\textsubscript{2}max and memory outcomes. Next, to determine which covariates were associated with VO\textsubscript{2}max, and therefore may influence our outcome of interest, we performed bivariate correlations between VO\textsubscript{2}max and age, sex, and body mass index (BMI). Finally, partial correlations included sex and body mass index (BMI) as control variables. Statistics were performed using SPSS 19 (IBM, Somers, NY).

**Eye movement data.** Eye movement analyses were performed on regions-of-interest in the three-creature test arrays. Each test array contained three regions-of-interest that corresponded to the locations of each of the three creatures. Timecourses of viewing were plotted both time-locked to the onset of the three-creature display and time-locked to behavioral response (Figure 2.3). Eye movement measures were quantified using preferential disproportionate viewing (PDV), which was defined as the difference in proportion of time spent viewing correctly-selected matching creatures relative to proportion of time spent viewing incorrectly-selected creatures prior to behavioral response (Figure 2.3). This measure was designed to account for the phenomenon in which eye movements are directed toward the creature that the participants will behaviorally select. Indeed this difference between correctly selected creatures and incorrectly selected creatures reflects the magnitude to which previous
exposure to the stimuli is layered atop this effect (Hannula, Ryan, Tranel, Cohen, 2007). Trials were excluded from further analysis if the participant failed to look at any of the three regions-of-interest for >30% of total viewing time. Eye movement preprocessing and analysis was performed using Matlab 10.0 (Mathworks, Natick, Massachusetts).

Results

Behavioral Data

Unadjusted bivariate correlations between VO₂max and behavioral accuracy yielded a significant positive correlation in the Relational condition \((r = 0.473, p < 0.001)\) but no significant correlation in the Item condition \((r = 0.188, p < 0.124; \text{Figure 2.2})\). Demographic variables sex and BMI were found to be significantly related to VO₂max \((t_{66} = 6.87, p < 0.001\) and \(r = -0.254, p < 0.037, \text{respectively}\), but age was not \((p>0.05)\). Finally then, adjusting for sex and BMI, the partial correlation between VO₂max and Relational accuracy remained significant \((r = 0.320, p < 0.009)\).

Figure 2.2: Scatterplots depicting the relationships between aerobic fitness and behavioral accuracy in the Relational and Item conditions

\[ R^2 = 0.226 \]

\[ R^2 = 0.026 \]
Eye movement Data

Onset-locked time-courses. Relative to the onset of the three-creature display, PDV became significant during the first 1000 ms of viewing in the Relational condition, replicating previous studies using a similar paradigm ($t_{65} = 5.17, p < 0.001$; Hannula, Ryan, Tranel, & Cohen, 2007), and during the first 2000 ms in the Item condition ($t_{65} = 2.78, p < 0.007$; Figure 2.3).

Response-locked time-courses. Eye-movement data again replicated previous findings.

Figure 2.3: Eye movement timecourses. Top panel depicts Relational condition. Bottom panel depicts Item Condition. Horizontal dashed lines indicate chance viewing of 33%. *<0.05, Bonferroni corrected. Error bars indicate S.E.M.
in that approximately 500-1000 ms prior to behavioral response, participants showed
significant PDV of stimuli that were previously paired with a scene background in the Relational
condition ($t_{65} = 4.35, p < 0.001$; Hannula, Ryan, Tranel, & Cohen, 2007). A similar pattern of PDV
was also observed in the Item condition ($t_{65} = 5.62, p < 0.001$; Figure 2.3).

**Correlations with aerobic fitness.** The bivariate correlation between VO$_2$max and the
magnitude of PDV prior to response yielded a positive relationship in the Relational condition ($r$
= 0.273, $p < 0.029$), but not in the Item condition ($r = 0.164, p < 0.191$; Figure 2.4), further
supporting the specificity of the fitness effect to relational memory. A partial correlation
including sex and BMI as control variables continued to show a marginal positive relationship
between VO$_2$max and PDV in the Relational condition ($r = 0.247, p < 0.053$).

![Figure 2.4: Scatterplots depicting the relationships between aerobic fitness and disproportionate viewing of correct selections greater than incorrect selections prior to response in the Relational and Item conditions](image)

**Discussion**

The current study yielded several novel findings, including showing for the first time a positive
relationship between aerobic fitness and memory function in healthy young adults. Moreover,
we show that this effect is specific to hippocampal-dependent relational memory, without a corresponding effect on hippocampal-independent item memory (Davachi & Wagner, 2002; Ryan, Althoff, Whitlow, & Cohen, 2000). The observed specificity between aerobic fitness and hippocampal function mirrors the results of a large animal literature investigating the effects of physical activity on hippocampal structure and function. In rodents, aerobic exercise has been demonstrated to increase angiogenesis and synaptogenesis in the whole brain and angiogenesis, synaptogenesis, and neurogenesis in the hippocampus (van Praag, Christie, Sejnowski, & Gage, 1999; van Praag, Kempermann, & Gage, 1999; van Praag et al. 2002; Wu, Ying, & Gómez-Pinilla, 2008). Additionally, aerobic exercise has been shown to increase production and secretion of brain derived neurotrophic factor (BDNF), a marker of neurogenesis, in the hippocampus and to decrease inflammation in the brain (Cotman, Berchtold, & Christie, 2007; Wu, Ying, Gómez-Pinilla, 2008). On the behavioral side, rodent models have shown improvements in learning and memory following aerobic exercise (van Praag, Shubert, Zhao, & Gage, 2005). Mirroring this in humans, aerobic exercise has been shown to increase cerebral blood volume in the hippocampus, which has been linked to neurogenesis (Pereira et al., 2007). This exercise-related neurogenesis has been shown to be associated with improved memory performance in older adults (Erickson et al., 2011).

There are several possible explanations for the observed relationship between aerobic fitness and memory function in young adults stemming from the known effects of physical activity in supporting angiogenesis, synaptogenesis, and neurogenesis, as well as its role in decreasing neural inflammation (Cotman, Berchtold, & Christie 2007; van Praag, Christie, Sejnowski, & Gage, 1999; van Praag et al. 2002; Wu, Ying, & Gómez-Pinilla, 2008). Adjudicating between
these possibilities, however, is the observed specificity of aerobic fitness on hippocampal memory relative to cortical dependent memory. With convergence from the animal literature one major factor that differentiates hippocampus and MTL cortex is the presence of neurogenesis in the hippocampus. Many rodent studies demonstrate a positive relationship between aerobic exercise and neurogenesis in the hippocampus, which is also reflected in enhanced performance on hippocampal tasks such as spatial memory and pattern separation (Creer et al., 2010; van Praag, Christie, Sejnowski, & Gage, 1999; van Praag, Shubert, Zhao, & Gage, 2005). Indeed these effects have been shown to be present in hippocampal tasks but notably absent in non-hippocampal tasks such as motor tasks (Clark et al., 2008).

Next, the current study bolsters the literature regarding the utility of eye movements in assessing memory. Indeed the eye movement results replicate previous studies using a similar paradigm in that preferential viewing to the previously viewed relation was apparent up to 1000 ms prior to behavioral response (Hannula, Ryan, Tranel, & Cohen, 2007). The current study extended these results, however, to show a similar pattern of preferential viewing for previously viewed items as well. That preferential viewing emerges significantly earlier in time than behavioral response in both the Relational and Item conditions is consistent with the idea that eye movements are a veridical index of previous experience (Hannula et al., 2010).

Additionally replicating previous studies using this paradigm, time-courses plotted relative to the onset of the three creature display showed early divergence in viewing between correctly selected relations relative to merely selected relations (Figure 2.3; Hannula, Ryan, Tranel, &
Cohen, 2007). Interestingly, however, comparing across conditions this effect emerges earlier in the Relational condition than the Item condition, in the first 1000 ms in the Relational condition and not until the the 1500-2000 ms window in the Item condition. Converging with previous work showing that removing the scene preview delays preferential disproportionate viewing by approximately 1000 ms (Hannula, Ryan, Tranel, & Cohen, 2007), this effect is likely due to the lack of diagnostic scene preview in the Item condition, given that each scene background is identical in that condition. In other words, the scene background in the Item condition carries no information about which set of creatures will appear with it, whereas the scene background in the Relational condition allows for pattern completion with the target creature. Indeed, the rapidly emerging preferential viewing in the time-course of this effect in the Relational condition suggests reactivation of the relational association during the scene preview, allowing for preferential viewing to emerge sooner when the creatures appear, as well as allowing for faster behavioral responses relative to the Item condition. Connecting these eye movement effects to fitness, results showed that aerobic fitness was positively correlated with the magnitude of preferential viewing prior to response in the Relational condition, but not the Item condition, demonstrating that fitness relates to assessments of memory that emerge even prior to behavioral response.

One potential limitation of the current study is that we did not match task difficulty between Item and Relational conditions, leaving open the possibility that the observed specificity was merely a task difficulty effect, such that aerobic fitness simply enhances performance of difficult tasks, rather than it being a neurally specific effect. Given the large body of work on
physical activity increasing both neurogenesis and hippocampal memory performance in rodents (Pereira et al., 2007; van Praag, Christie, Sejnowski, & Gage, 1999; Wu, Ying, & Gómez-Pinilla, 2008), as well as the finding in older adults that aerobic exercise has a causal relationship with hippocampal volume (Erickson et al., 2011), it is unlikely that the observed effect is simply due to task difficulty. Furthermore, by necessity the two conditions differ on a variety of dimensions, such as list length, prior exposure to stimulus components, and need to engage various task processes, all of which may lead to variable performance across individuals across condition. In the meantime, even if one were to attribute the observed fitness effects merely to task difficulty, an interesting dichotomy still remains whereby increased aerobic fitness selectively enhances performance on a more challenging task, despite accuracy on the easier task being below ceiling. Regardless, future studies may account for this task difficulty explanation by matching behavioral task performance across these two types of memory.

**Conclusion**

In conclusion, the current study extends the literature in several meaningful ways. First, we showed that the pre-response preferential viewing eye movement effects previously observed for relational memory extend to item memory, further reinforcing the utility of eye movements as a sensitive measure of prior experience. Second, we demonstrated a selective effect of aerobic fitness on hippocampal-dependent relational memory measured both behaviorally and using eye movements, with no corresponding effects on hippocampal-independent item memory. Taking into consideration the increasingly sedentary lifestyle so prevalent in the
modern world, the current results highlight the importance of aerobic fitness to maximize hippocampal function even during the cognitive peak of young adulthood.
References


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CHAPTER 3: LONGITUDINAL ASSESSMENT OF RELATIONAL MEMORY, PHYSICAL FITNESS, AND SCHOLASTIC ACHIEVEMENT IN 11-13 YEAR OLDS

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Abstract

Various forms of physical health and fitness have been linked to cognition across the lifespan from preadolescence through the geriatric years. Much of the research in this area in both the human and animal work focuses on aerobic fitness or physical activity, and indeed aerobic fitness and physical activity have been linked with cognition across development. In the brain, the hippocampus is one region that has been shown to be particularly sensitive to fitness at several points across the lifespan. The longitudinal time-course of when these relationships between overall physical fitness and hippocampal memory appear in late childhood, however, has not been previously studied. The current study longitudinally assessed 99 middle school children at the beginning and end of sixth grade, sampling hippocampal memory function, scholastic achievement, and components of physical fitness (aerobic fitness, muscular strength, endurance, flexibility, body mass index). Results show that overall physical fitness was positively related to memory performance at both time points. Aerobic fitness only significantly predicted memory performance at the end of sixth grade. Furthermore, overall physical fitness, particularly aerobic fitness, and memory scores at the end of sixth grade were positively related to scholastic achievement scores, demonstrating the ecological validity of the memory measure. The relationships between each of the factors and implications for academic performance are discussed.
Introduction

From eating breakfast to smaller class sizes (Angrist et al., 1999; Rampersaud et al., 2005), many factors influence performance in school. One that is often ignored, however, is the effect of physical fitness. Despite recommendations for 60 minutes of physical activity per day, current estimates of childhood activity put just 42% of children age 6-11 as meeting this standard, and only 8% of adolescents age 12-19 years (Trioano et al., 2008). Given the growing literature connecting physical activity and resulting aerobic fitness with cognition and achievement in school, this raises concerns about the cognitive health of our children, particularly as they cross this threshold between childhood and into adolescence and become an increasingly sedentary group (Castelli, Hillman, Buck, & Erwin, 2007; Trioano et al., 2008). An understanding of the interaction of physical fitness and scholastic performance is particularly pressing following the No Child Left Behind act, which encourages achievement through longer hours in the classroom at the expense of physical education and other “extracurricular” activities. While appealing on the surface, this approach overlooks the growing literature relating physical fitness (and other “extracurricular” activities) in childhood to cognition and academic achievement. Indeed several health and fitness factors have been shown to correlate with cognitive performance and scholastic achievement (Tomporowski, Davis, Miller, & Naglieri, 2008).

Notable among these health and fitness factors is aerobic fitness, which has been correlated with improvements in overall scholastic achievement in school-aged children and cognitive performance in both children and adults (Buck, Hillman, & Castelli, 2008; Burkhalter & Hillman, 2011; Castelli, Hillman, Buck, & Erwin, 2007; Colcombe & Kramer, 2003; Hillman, Castelli, &
Buck, 2005; Monti, Hillman, & Cohen, 2012). One study demonstrated a positive relationship between aerobic fitness and overall scholastic achievement as well as subscores of reading and mathematics achievement based on standardized testing, and a negative relationship between body mass index (BMI) and overall achievement test performance (Castelli, Hillman, Buck, & Erwin, 2007). Related, a meta-analysis examining the relationship between physical activity and cognitive performance in school-aged children found a significant positive relationship between the two, with the largest effect size in middle school and elementary school, suggesting that the middle school years are a prime target for examining the developmental timecourse of the relationship between physical activity and cognitive aptitude. Interestingly, in that meta-analysis, positive effects were observed for aerobic training, resistance training, and motor skill training (Sibley & Etnier, 2003). It should be noted however that of the eight measures of cognition examined in that study, “memory” was the only one that was not significantly related to physical fitness (Sibley & Etnier, 2003). Human and animal work has shown, however, that certain forms of memory are extraordinarily important for learning (Eichenbaum & Cohen, 2001). Chief among these is relational or associative memory, which is involved in representing the relationships between multiple elements in a scene or event (Eichenbaum & Cohen, 2001; Konkel & Cohen, 2009). This type of memory is supported by the hippocampus, which has been shown to be particularly sensitive to structural and functional modulation by multiple health and fitness factors (Chaddock et al., 2010; Erickson et al., 2011; van Praag et al., 1999; Wu, Ying, & Gomez Pinilla 2008). What form or forms of memory were used in Sibley & Etnier’s (2003) meta-analysis was not explained in their methods, and no other studies have examined this to
our knowledge, which leaves open the question of whether relational memory specifically is related to physical fitness in this age group.

The relationship between aerobic fitness and cognitive performance is likely due to a combination of factors including enhanced angiogenesis and synaptogenesis as well as decreased neural inflammation in higher fit individuals, in addition to increased neurogenesis specifically in the hippocampus (Cotman, Berchtold, & Christie, 2007; van Praag, Christie, Sejnowski, & Gage, 1999; van Praag et al., 2002). In older adults, exercise-related neurogenesis has been shown to be causally associated with structural changes in the hippocampus as well as improved memory performance (Erickson et al., 2011). As all of these fitness-related anatomical and physiological changes have been shown to occur in the hippocampus, the current experiment targets the functionality of this region, relational memory, as a possible faculty that may be particularly susceptible to the effects of aerobic fitness and other health and fitness measures. Since aerobic fitness is the fitness measure most closely related to hippocampal function, likely through its role in neurogenesis and plasticity, we separately examined aerobic fitness in addition to overall physical fitness, in which other aspects of physical fitness may benefit cognition in children through other mechanisms. Concordant with Sibley & Etnier’s (2003) finding that middle school children show the largest relationship between physical fitness and cognition, adolescence is known to be a period of notable reorganization of the brain (Dahl, 2004; Ghetti & Bunge, 2012), thus potentially leading to an increased period of susceptibility to external factors such as overall health and fitness. The current study investigated these relationships with relational memory in a longitudinal study using two cognitive testing sessions spanning the first year of middle school.
During the course of this study, children completed two relational memory assessments, two fitness assessments, and a standardized scholastic achievement test. Physical fitness was assessed in school through the Illinois state-mandated Fitnessgram program and included measures of aerobic fitness, muscular strength, endurance and flexibility, and body composition (measured through body mass index [BMI]). Scholastic achievement was measured using the Illinois Scholastic Achievement Test (ISAT), an annual state-mandated test given to all students grades three through eight in Illinois.

**Methods**

**Participants**

Ninety-nine participants were recruited through Jefferson Middle School in Champaign, Illinois. Students were tested once at the start of sixth grade (age 11-12, 11.66 +/- 0.04) and once at the end of sixth grade (age 11-13, 12.09 +/- 0.04). Two additional enrolled participants were excluded for failing to complete either cognitive or fitness testing during the first session. Participants’ parents signed consent forms during a pre-study registration session and students were compensated $5 for each participation session, which took place during one Physical Education class per testing session. All participants provided written assent and legal guardians provided written informed consent in accordance with the regulations of the University of Illinois Institutional Review Board.

**Memory Task**
Stimuli for each testing session of the relational memory task consisted of 90 creatures approximately 150 × 150 pixels each (see Figure 3.1) created in Spore Creature Creator (Electronic Arts Inc., California). Creatures were unique to each testing session. Relational memory function was assessed using a child-friendly version of the iPosition task (Watson et al., 2013). In the version of this task used in the current study, five creatures appeared pseudorandomly spaced on a laptop monitor such that creatures were never aligned in either the x or y dimension (see Figure 3.1). Creatures remained on the screen for ten seconds. Following a four second delay, the same five creatures reappeared lined up across the top of the screen. In this self-paced portion of the study, participants were directed to click-and-drag each creature back to the location where they had studied it. After they placed all the creatures and were satisfied with their positions, a space-bar button press advanced the experiment to the next trial.

Figure 3.1: Task example

Memory data were analyzed following the methods used by Watson and colleagues (2013).

Four measures of item placement were assessed: magnitude of Item Misplacement, a measure
of distance in pixels between the original object location and the placed object location, magnitude of Edge Resizing, a measure of placed distance between each pair of objects relative to original distance between them in Cartesian space, incidence of Rearrangement, a measure of displacement between pairs of objects in which either the x or y relation has changed, and incidence of Swaps, a measure of whether pairs of placed items have changed locations on both x and y dimensions. These four scores were normalized within each sample of participants. Normalized scores were then averaged within individual for a composite memory score, in which a lower score indicated better memory (i.e., item placements were more similar to originally studied arrays).

**Fitness Assessment**

Fitness data were collected twice over the course of the study each roughly corresponding in time to the cognitive testing session, through the Illinois state-mandated Fitnessgram program. Fitness measures include the PACER test, a measure of aerobic capacity, Pushups, a measure of upper body muscular strength, Curlups, a measure of abdominal strength, Trunklift, a measure of back strength, Back-saver sit-and-reach, a measure of flexibility, and Body Mass Index (BMI), a ratio of height to weight. To capture overall fitness, these six individual scores were normalized within each sample of participants. Normalized scores were then averaged within individual participant for a composite fitness score, in which a higher score indicated a higher level of fitness.

**Results**
Baseline Testing (T1)

**Overall Fitness is Correlated with Memory.** As described in Watson et al. (2013), measures of item misplacement, edge resizing, and rearrangement were cleaned of influence of swaps (see Watson et al., 2013). Pearson’s correlation was used to correlate composite memory scores with composite fitness scores, producing a significant negative correlation ($r = -0.24$, $p < 0.02$), indicating a significant positive relationship between increased fitness and improved relational memory performance. BMI explained no additional variance beyond the fitness measures (no BMI: $r = -0.24$, $p < 0.02$). Possible confounding variables of age and sex were not associated with composite fitness score at T1 and were therefore not used in any analyses of T1 ($p > 0.05$).

**Memory is Significantly Correlated with Curl-ups, Marginally Correlated with Aerobic Fitness, and Not Correlated with Other Fitness Measures.** For the T1 time-point, examination of individual measures of fitness and memory show marginal correlations. The aggregate memory score was significantly correlated with curl-ups ($r = -0.26$, $p < 0.01$), marginally correlated with the PACER test ($r = -0.17$, $p < 0.08$), but not significantly related to any of the other fitness measures ($-0.123 \leq r \leq 0.000$, $p > 0.226$).

![Figure 3.2: Memory and fitness correlation from T1 & T2. T1 N = 99. T2 N = 97.](image)
Second Phase of Testing (T2)

The second phase of testing (T2) included 97 of the 99 participants included in the first testing session. Data were analyzed using the same procedure as in T1.

**Overall Fitness is Correlated with Memory.** A Pearson’s correlation was used to assess the linear relationship between aggregate fitness and aggregate memory scores. The resulting correlation showed a significant negative correlation ($r = -0.25$, $p < 0.014$), again indicating a significant positive relationship between increased fitness and improved relational memory performance. Again, BMI explained no additional variance beyond the fitness measures (no BMI: $r = -0.25$, $p < 0.014$). Possible confounding variables of age and sex were again not associated with composite fitness score at T2 and were therefore not used in any analyses of T2 ($p > 0.05$).

**Memory is Correlated with Aerobic Fitness But Not Other Fitness Measures.** For the T2 time-point only, examination of individual measures of fitness and memory show significant correlations. The aggregate memory score was significantly correlated with the PACER test ($r = -0.20$, $p < 0.046$; see Figure 3.3), but none of the other fitness measures (-0.149 ≤ $r$ ≤ 0.029, $p > 0.146$).
Individual Relational Memory Measures are Correlated with Aerobic Fitness.

Examining the relationship between aerobic fitness and memory more closely, bivariate correlations between PACER scores and individual sub-measures of memory were performed. PACER was significantly correlated with edge resizing and rearrangement ($r = -0.21, p < 0.044$ and $r = -0.21, p < 0.040$ respectively), marginally correlated with misplacement ($r = -0.196, p < 0.054$) and not correlated with swaps ($r = -0.148, p < 0.149$).

Memory Performance and Fitness are Correlated with Achievement Test Performance.

Standardized test scores include Total score and subscores for Reading and Math. Aggregate memory score was negatively correlated with Total score, Reading subscore, and Mathematics subscore (indicating better performance on reading and math is correlated with better memory performance; Total: $r = -0.447, p < 0.001$; Reading: $r = -0.413, p < 0.001$; Mathematics: $r = -0.425, p < 0.001$). Aggregate fitness score is positively correlated with the Total score ($r = 0.277, p < 0.007$) and Mathematics subscore ($r = 0.343, p < 0.001$) but not with the Reading subscore ($r = 0.154, p < 0.137$). PACER was positively correlated with all three ISAT measures (Total: $r = 0.386, p < 0.001$; Reading: $r = 0.284, p < 0.005$; Mathematics: $r = 0.423, p < 0.001$; Figure 3.4).

Figure 3.4: Scatterplots depicting relationships between aerobic fitness scores and standardized testing scores
Longitudinal Testing Results

We next examined whether changes in aggregate memory score were associated with changes in aggregate fitness score and PACER across the two time points. Results showed that improvement in aggregate fitness or PACER across the two time points was not significantly correlated with change in memory performance (aggregate fitness: $r = 0.003$, $p < 0.973$; PACER: $r = -0.040$, $p < 0.697$). This is likely due to the small degree of within participant variation in aggregate memory scores between T1 and T2.

Discussion

The current study demonstrated that overall physical fitness and specifically aerobic fitness were related to relational memory performance and scholastic achievement in sixth graders. Interestingly, aerobic fitness only significantly predicted memory performance at the end of sixth grade, but not at the beginning of sixth grade. Each of these findings will be discussed in turn.

First, overall physical fitness was found to be correlated with memory score at both time points such that higher overall physical fitness predicted more accurate placements on the spatial relational memory task (Figure 3.2). This is consistent with a large animal literature showing a positive relationship between physical activity and hippocampal memory task performance (van Praag, Christie, Sejnowski, & Gage, 1999; Wu, Ying, & Gómez-Pinilla, 2008). Furthermore, it adds to an emerging literature showing a positive relationship between health and fitness measures with hippocampal function in children (Chapter 5; Chaddock et al., 2010; Monti,
Hillman, & Cohen, 2012). Examining the individual measures that compose the overall fitness score, we found no predictive value of BMI on relational memory performance. Excluding BMI, overall fitness continued to predict relational memory performance. Why muscular strength, endurance, and flexibility add to the predictive value of fitness on relational memory over aerobic fitness during the first timepoint is not clear from a mechanistic perspective. One possible reason for this connection, however, is the relationship between physical activity and improvement in these fitness measures. By this account, the predictive value of overall physical fitness would lie with physical activity rather than anything inherent to these fitness measures themselves, which would be consistent with the animal literature relating physical activity to hippocampal structure and function (van Praag, Christie, Sejnowski, & Gage, 1999; Wu, Ying, & Gómez-Pinilla, 2008).

The second significant finding, and arguably the most interesting one from a developmental standpoint as it shows a difference across time points, was that performance on the aerobic fitness subscale, the PACER test, significantly predicted memory score at the end of sixth grade, but only marginally predicted memory score at the start of sixth grade. To our knowledge no previous studies have examined the relationship between any measures of physical fitness with hippocampal relational memory in this age range, but our study with younger children age 7-9 showed a similar pattern (see Chapter 4), by which relational memory was marginally predicted by aerobic fitness. If the significance of the relationship between aerobic fitness and relational memory remains through future assessments at the end of seventh and eighth grades, this would lend support to the idea that this relationship strengthens across middle childhood to
early adolescence. As discussed in the introduction, there are clear relationships between physical activity and aerobic fitness and between physical activity and hippocampal neurogenesis (Morrow & Freedson, 1994; Pereira et al., 2007; van Praag, Christie, Sejnowski, & Gage, 1999), providing a mechanistic link between aerobic fitness and relational memory performance. Why this relationship unfolds over development in this manner across late childhood to early adolescence is not clear from a hippocampal development perspective. One possibility for this developmental shift concerns the strengthening in long-range connectivity between the hippocampus and prefrontal cortex (PFC) across childhood into early adulthood, particularly given that participants are directed to intentionally encode the spatial locations of the stimuli (Ghetti & Bunge, 2012). Stronger hippocampal-PFC interaction may increase task performance via top-down control of intentional encoding and retrieval, management of encoding strategy, or enhancement in allocation of attentional resources.

Third, we observed that measures of relational memory and physical fitness were correlated with standardized achievement test scores. Specifically, better relational memory performance was correlated with higher Total ISAT score, as well as Reading and Mathematics subtest scores, indicating a general relationship in which children who perform better on one cognitive test also perform better on other cognitive tests. More interestingly, overall physical fitness was found to predict Total ISAT score and the Mathematics subscore, but not the Reading subscore, suggesting that certain aspects of scholastic achievement may be more sensitive to overall physical fitness and health, but that there is an overall positive relationship between them. A similar finding in a cross-section of over one million California children in grades 5, 7,
and 9 showed a positive relationship between overall physical fitness with achievement test scores, in that case including total score and subscores of both language and mathematics (California Department of Education, 2005). Additionally, the current study found that aerobic fitness significantly predicted each of the achievement test scores (Total, Math, and Reading). Contrasting this with the predictive value of overall fitness on achievement test scores suggests that much of the explanatory power of the overall fitness score lies within the aerobic fitness component, and that the other measures of physical fitness may actually in some cases detract from the predictive power of aerobic fitness on scholastic achievement.

In terms of practical application, the current findings further underscore the position that physical education during the school day does not detract from academic performance, but rather may actually serve to support learning and scholastic achievement. Specifically, the current results suggest that aerobic fitness should be a primary focus of physical education classes in sixth grade in order to maximize scholastic achievement, but that including other training in aspects of physical fitness may also contribute to certain forms of cognition, namely relational memory which is critical for many types of learning (Eichenbaum & Cohen, 2001).

Conclusions

The current study found positive relationships between overall physical fitness, aerobic fitness, hippocampal relational memory performance, and scholastic achievement test performance in sixth graders. While the relationship between overall physical fitness and relational memory performance remained significant across sixth grade, the relationship between aerobic fitness
and relational memory performance strengthened across the year from the start to the end of sixth grade. The finding that aerobic and overall physical fitness predicted relational memory performance, a form of memory critical for learning, has implications for the scholastic achievement of our children. Indeed this result was confirmed in an ecologically valid manner in that overall physical fitness and aerobic fitness measured near in time to children completing a standardized scholastic achievement test demonstrated positive relationships across measures. This further highlights the importance of physical activity and resulting better health and fitness to maximize learning and scholastic achievement in middle school children. These results have important public policy implications for the future of physical education in public schools.
References


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CHAPTER 4: CROSS-SECTIONAL ASSESSMENT OF THE RELATIONSHIPS BETWEEN AEROBIC
FITNESS AND BODY MASS ON RELATIONAL AND ITEM MEMORY IN PREPUBERTAL CHILDREN

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Abstract

High aerobic fitness and healthy body weight are linked to lower risk for disorders such as cardiovascular disease, type 2 diabetes, and metabolic syndrome. Recently these health and fitness factors are also being connected to brain structure and function. The current study examined the relationships between aerobic fitness and body mass index (BMI) with measures of hippocampal-dependent relational memory and non-hippocampal-dependent item memory in a sample of 98 prepubertal children aged 7-9. Aerobic fitness was measured using a progressive exercise test to assess maximal oxygen consumption during exercise (VO₂max). BMI was calculated using the standard weight-to-height ratio (kg/m²). Relational and item memory were assessed using behavioral and eye-movement measures while children completed a computerized memory paradigm. Results show that both relational and item memory accuracy were positively correlated with aerobic fitness, although this relationship was attenuated by inclusion of sex and socioeconomic status (SES) in the model. BMI was negatively correlated with relational and item memory accuracy after adjusting for age, IQ, and SES. This effect was driven by children classified as overweight or obese (≥85th percentile of BMI-for-age), with no relationship between BMI and memory performance within the group of children who were classified as normal or underweight (<85th percentile of BMI-for-age). No significant relationships were observed between eye-movement data and fitness or health factors. Additional novel cognitive findings and implications of the health findings are discussed.
Introduction

Converging evidence from human and animal work provides compelling support for the influence of physical health on scholastic achievement and cognition across the lifespan (Buck, Hillman, & Castelli, 2008; Burkhalter & Hillman, 2011; Castelli, Hillman, Buck, & Erwin, 2007; Colcombe & Kramer, 2003; Hillman, Castelli, & Buck, 2005; Monti, Hillman, & Cohen, 2012). Neuroimaging and electrophysiological evidence lend additional support for the idea that aerobic fitness and brain function are positively related (for a review see Voss, Nagamatsu, Liu-Ambrose, & Kramer, 2011). In addition to a large body of work examining the effects of physical activity and aerobic fitness on prefrontal executive function (see Hillman, Erickson, & Kramer, 2008), much of the work done is this area has focused the hippocampus, possibly due to its metabolic profile, connectivity, and importance for learning. In this vein, the hippocampus has been shown to be critical for relational or associative memory, which is used for representing relationships between elements in a scene or event (Eichenbaum & Cohen, 2001; Konkel & Cohen, 2009). This type of memory is in turn essential for many types of learning and thus for children’s successful performance in school. Given its remarkable plasticity, the hippocampus has been shown to be particularly sensitive to structural and functional modulation by multiple health and fitness factors (Chaddock et al., 2010; Erickson, et; al., 2011; Hillman, Erickson, & Kramer, 2008; van Praag et al., 1999a, 1999b).

In rodents, aerobic exercise supports neurogenesis, angiogenesis, and synaptogenesis in the hippocampus (van Praag et al. 1999a, 1999b; van Praag et al. 2002). Additionally, aerobic exercise has been shown to decrease inflammation in the brain and increase production and
secretion of brain derived neurotrophic factor (BDNF), a marker of neurogenesis that has been shown to be necessary for long-term potentiation (Cotman, Berchtold, & Christie, 2007; Wu, Ying, Gómez-Pinilla, 2008). On the behavioral side, rodent models have shown improvements in learning and memory following exercise (van Praag et al., 2005). In humans, aerobic exercise has been shown to increase cerebral blood volume in the hippocampus, which has been linked to neurogenesis (Pereira et al., 2007). This exercise-related neurogenesis has been shown to be associated with improved memory performance in older adults (Erickson et al., 2011).

In children, Chaddock and colleagues (2010) tested the relationship between aerobic fitness and hippocampal volume in 9- and 10-year-olds. Results showed that a group of children who were more aerobically fit had significantly larger hippocampal volumes than a group of less aerobically fit children. These structural differences were accompanied by corresponding differences in relational but not item memory (Chaddock et al., 2010), adding to a prospering literature that the hippocampus is well suited and selective for relational memory (Eichenbaum & Cohen, 2001; Davachi & Wagner, 2002). More recently, Monti, Hillman, and Cohen (2012) found that relative to children in a wait-list control group, children who had participated in a 9-month fitness intervention allocated a significantly higher proportion of viewing time to previously viewed faces in context. Furthermore, consonant with the proposed specificity of the role of aerobic fitness in hippocampal function, the effect was selective to relational memory, with no such effect on item memory (Monti, Hillman, & Cohen, 2012). As all of these fitness-related enhancements have been shown to occur in the hippocampus, the current experiment targets the functionality of this region as a possible faculty that may be particularly susceptible to the effects of aerobic fitness and other health measures.
Another measure of health, body mass index (BMI), has also been shown to be associated with cognitive performance and scholastic achievement, although the literature is more mixed, perhaps due to the coarseness of BMI in predicting health outcomes. On the positive side, Castelli and colleagues (2007) showed that among third- and fifth-graders BMI was negatively related to academic achievement, including subscores of both mathematics and reading (Castelli, Hillman, Buck, & Erwin, 2007). Kamijo and colleagues (2012) observed that BMI was negatively associated with scholastic achievement and laboratory measures of inhibitory control (Kamijo et al., 2012). Other studies have additionally shown significant decrements in performance on standardized intelligence or neuropsychological tests for obese children and adolescents relative to their normal-weight peers (Campos et al., 1996; Datar, Sturm, & Magnabosco, 2004; Li, Dai, Jackson, & Zhang, 2008; Shore et al., 2008). Complicating the story, however, is that in some cases these effects have been explained by other factors such as SES or level of physical activity (Datar, Sturm, & Magnabosco, 2004; Shore et al., 2008). On the negative side, other studies have found no evidence that BMI status is related to performance on neuropsychological tasks across groups of children and adolescents between 6 and 19 years of age (Barrigas & Fragoso, 2012; Gunstad et al., 2008; Veldwijk, Scholtens, Hornstra, & Bemelmans, 2011). It should be noted, however, that many of these studies collapsed across a broad age range, potentially obscuring any developmental trends across this time period.

Indeed, in contrast to the current study, our longitudinal study of relational memory development across the middle school years found no predictive power of BMI on relational memory accuracy (see Chapter 3). Within the realm of memory, although several studies have found negative associations in adults between BMI and memory performance, hippocampal
volume, or medial temporal lobe gray matter volume (Cournot et al., 2006; Gunstad et al., 2006; Raji et al., 2010; Taki et al., 2008), to our knowledge no studies have examined the relationship between BMI and memory performance in prepubertal children. Given the immense developmental reorganization that occurs across childhood through adolescence (Ghetti & Bunge, 2012), it remains an open question whether and what kinds of memory may be related to body mass during the prepubertal years.

In addition to behavioral performance, the current study assessed these memory processes using eye-movement measures which are an indirect measure of past experience (Hannula, Ryan, Tranel, & Cohen, 2007; Ryan, Althoff, Whitlow, & Cohen, 2000). Eye movements have been used as an implicit measure of relational memory in preadolescent children and infants as young as 9 months of age. In these studies, eye movements have been shown to be a veridical index of prior exposure (Monti, Hillman, & Cohen, 2012; Richmond & Nelson, 2009), with children and infants showing strikingly similar viewing patterns to adults (Hannula, Ryan, Tranel, & Cohen, 2007).

Methods

Participants

As part of the larger Fitness Improves Thinking (FITKids) study (see Castelli, Hillman, Hirsch, & Drollette, 2011; Kamijo et al., 2012; Monti, Hillman, & Cohen, 2012), 98 pediatric participants aged 7-9 (45 males; aged 8.71 +/- 0.06 years) were recruited from the community to participate in this study (Table 4.1). An additional 9 participants were excluded based on poor calibration (N = 3), computer malfunction (N = 1), failure to follow task directions (N = 4), and failure to
complete the task due to headache (N = 1). All participants provided written assent and legal guardians provided written informed consent in accordance with the regulations of the University of Illinois Institutional Review Board. Children were screened for serious medical conditions, neurological or attentional disorders, physical disabilities, psychoactive medication status, and normal or corrected-to-normal vision using a parental report health history questionnaire. Data were also collected on several additional characteristics that have been shown to be related to physical activity or aerobic fitness: IQ, socioeconomic status (SES), and pubertal status. Fluid and crystallized intelligence were assessed using the Kaufman Brief Intelligence Test (K-BIT; Kaufman & Kaufman, 1990) or the Woodcock-Johnson Tests of Cognitive Abilities (Woodcock, McGrew, & Mather, 2001). SES was estimated based on household income, participation in a school meal-assistance program, maternal and paternal education levels, and how many parents work full-time (Birnbaum et al., 2002). Pubertal status was estimated using the Tanner Staging Scales (Tanner, 1962; Taylor et al., 2001).

**Aerobic Fitness Assessment**

Aerobic fitness was evaluated using an exercise test designed to measure maximal oxygen consumption (VO₂ max) during physical activity. Higher scores on this test indicate increased aerobic capacity. VO₂ max scores were assessed using a computerized indirect calorimetry system (ParvoMedics True Max 2400) during a modified Balke treadmill test (American College of Sports Medicine, 2010). During the test, participants’ heart rate was constantly monitored using a Polar heart rate monitor (Polar WearLink® +31, Polar Electro, Finland) and individual
subjective rate of perceived exertion (RPE) was assessed every 2 minutes using the children’s OMNI scale of perceived exertion (Utter et al., 2002).

The VO\textsubscript{2} max test included an initial warm-up period in which the treadmill gradually increased in speed while the children walked on it. After this warm-up period, treadmill speed remained constant while incline was increased 2.5% every 2 minutes. Average oxygen consumption and respiratory exchange ratio (RER) were measured every 20 seconds during the test using a mouthpiece. Maximum oxygen consumption (VO\textsubscript{2} max) was measured and is reported in milliliters per kilogram per minute (ml/kg/min; relative VO\textsubscript{2} max) and based on maximal effort, which was defined using two or more of the following criteria: 1) age-defined maximum heart rate norms (i.e., heart rate > 185 bpm), 2) RER (CO\textsubscript{2}:O\textsubscript{2}) greater than 1.0 (Bar-Or, 1983), 3) subjective RPE greater than 8, and 4) leveling of VO\textsubscript{2} despite increasing aerobic demand (American College of Sports Medicine, 2010).

**Memory Task**

On a separate day from the VO\textsubscript{2} max test, children participated in a memory task designed to assess both hippocampal-dependent relational memory and hippocampal-independent item memory (Figure 4.1). The task was divided into eight study-test blocks each designed to test either relational or item memory. Stimuli included 216 novel creatures created in Spore Creature Creator (Electronic Arts Inc., California; see Figure 4.1 for examples) and were presented using Presentation software (Neurobehavioral Systems, http://nbs.neuro-bs.com) on a 21” color monitor. Creatures were presented on black backgrounds and resized to 480 × 480
pixels. One-hundred ten color images of real world scenes measuring $1280 \times 1280$ pixels and taken by Brand X photography were used as scene backgrounds.

An Eyelink 1000 eye-tracker (SR Research, Ontario, Canada) was used to remotely record eye-movements. The eye-tracker was calibrated immediately prior to each test block of the memory task. A desk-mounted gel-padded chin rest was used to minimize head movement during eye-tracking data collection.

Item memory was assessed in two study-test blocks (Figure 4.1). In each block, participants were shown 18 creature-scene pairings in which a scene preview was shown for 3000 ms at which time a creature was superimposed atop the scene. The background scene was the same for all 18 study trials in each item block. The creature-scene pairing then remained on the screen for 5000 ms. During this period, and to encourage item encoding of each creature, participants were instructed to press one of two buttons in response to whether they judged the creature to look more like a “boy” or a “girl.” A fixation cross was presented for 2000 ms.
between each study pairing. Following the study period, a test phase was presented in which three creatures, one studied and two novel, were simultaneously presented atop the studied scene for 6000 ms, following a 3000 ms scene preview. Participants were instructed to press a single response button when they felt they knew which creature had been studied. After the 6000 ms test display, a display containing three white boxes where the creatures had been prompted participants to voice to the experimenter which creature they had selected. Confidence in that decision was then assessed using a three-point scale (1 = “not sure,” 2 = “kind of sure,” and 3 = “very sure”). Given that two creatures were novel and one studied, the item condition could be solved using familiarity alone, an ability shown to be independent of the hippocampus (Davachi & Wagner, 2002; Davachi, 2006).

Relational memory was evaluated in separate blocks (Figure 4.1). In the six Relational study-test blocks, participants studied 18 unique creature-scene pairings. To encourage relational encoding, participants were asked to press one of two buttons to indicate whether they judged each creature to live in that habitat (scene) “alone” or “with others.” Following each study block, participants were tested on six probe trials in which they were instructed to find the creature originally studied with that scene. Each test trial consisted of one of the 18 studied backgrounds superimposed with three of the creatures studied in that block. One of the creatures had been studied with that scene and two had been studied with other scenes; familiarity across the three creatures was thus matched, necessitating the employment of relational memory (Hannula, Ryan, Tranel, & Cohen, 2007).
Target location on test trials was counterbalanced within condition within participant. Lists of stimuli presented in the Item or Relational conditions were counterbalanced across participants. Additionally, order of study-test blocks was counterbalanced across participants in the following manner: “Item, Rel, Rel, Rel, Item, Rel, Rel” or “Rel, Rel, Rel, Item, Rel, Rel, Rel, Item.”

Data Analysis Approach

Eye-movement data were divided into trials in which participants correctly selected the matching target and trials in which participants incorrectly selected a competitor, and were analyzed using both onset-locked and response-locked time courses. Onset-locked time courses examined patterns of viewing time-locked to the onset of the three creature probe array. Response-locked time courses examined viewing patterns in the 2500 ms windows prior to and following button press responses (see Figure 4.2). Onset-locked time courses of eye-movement data were then analyzed using Accuracy (Correctly Selected, Incorrectly Selected) × TimeWindow (0-500, 500-1000, 1000-1500, 1500-2000, 2000-2500, 2500-3000, 3000-3500) repeated measures ANOVAs. Response-locked time courses of eye-movement data were analyzed using Accuracy (Correct, Incorrect) × TimeWindow ((-2500) to (-2000), (-2000) to (-1500), (-1500) to (-1000), (-1000) to (-500), (-500) to Response, Response-500, 500-1000, 1000-1500, 1500-2000, 2000-2500) repeated measures ANOVAs. Greenhouse-Geisser correction was used where appropriate in the ANOVAs, and Bonferroni correction was used to correct for multiple comparisons in t-tests comparing individual time bins in the eye-movement time courses.
Eye movement measures were quantified using preferential disproportionate viewing (PDV), which was defined as the difference in proportion of time prior to behavioral response spent viewing correctly-selected matching creatures relative to proportion of time spent viewing incorrectly-selected creatures (gray shading in Figure 4.2). In both cases the participant has a subjective feeling of selecting correctly, but PDV distinguishes between these two situations based on actual previous experience, thus providing a more sensitive measure than behavioral report alone. As such, this was the eye-movement measure used to examine relationships between memory and health and fitness factors.

Results

Behavioral and eye-movement memory measures collapsed across all participants are reported below, followed by relationships with fitness and BMI.

Behavioral Performance

Behavioral proportion correct was 0.86 ± 0.01 (Mean ± SEM) for the Item condition and 0.63 ± 0.01 for the Relational condition. Response time was 2680.56 ± 51.75 for correct Item trials and 2502.08 ± 49.17 for correct Relational trials. Two-tailed paired-sample t-tests show a significant difference in behavioral accuracy ($t_{97} = 18.89$, $p < 0.001$) and response time ($t_{97} = 3.80$, $p < 0.001$) between conditions such that participants were significantly more accurate on Item trials but responded significantly faster on Relational trials.
Behavioral confidence for correct trials in the Item condition was correlated with accuracy in both the Item condition ($r = 0.31, p = 0.002$) and the Relational condition ($r = 0.33, p < 0.001$). In contrast, behavioral confidence in the Relational condition for correct trials was positively correlated with accuracy in the Relational condition ($r = 0.31, p = 0.002$) but not the Item condition ($r = -0.04, p = 0.709$). Confidence was not significantly related to VO$_2$max or BMI measures ($ps > 0.44$).

**Eye-Movement Time Courses**

Onset-locked time courses in the Relational condition show significant main effects of Accuracy ($F_{1,97} = 111.49, p < 0.001$) and TimeWindow ($F_{4.56,441.96} = 31.04, p < 0.001$) with no Accuracy × TimeWindow interaction ($F_{4.78,463.50} = 0.68, p = 0.64$; Figure 4.2). Significant PDV emerged during the 0-500 ms time bin and sustained through the 3000-3500 ms time bin (all $ps < 0.005$). Onset-locked time courses in the Item condition show significant main effects of Accuracy ($F_{1,97} = 82.03, p < 0.001$) and TimeWindow ($F_{4.64,449.94} = 27.62, p < 0.001$) with a significant Accuracy × TimeWindow interaction ($F_{4.54,440.09} = 10.39, p < 0.001$). Post-hoc comparisons of individual time points show significant PDV emerging during the 1000-1500 ms time bin and sustaining through the 3000-3500 ms time bin (all $ps < 0.001$; Figure 4.2).
Figure 4.2. Left panel depicts onset-locked time courses. Right panel depicts response-locked time courses. Top panel depicts the Relational condition and bottom panel depicts the Item condition. Error bars indicate standard error of the mean and are obscured by the markers for many time points. *p < 0.05, Bonferroni corrected.

Response-locked time courses in the Relational memory condition show significant main effects of Accuracy ($F_{1,95} = 50.22$, $p < 0.001$) and TimeWindow ($F_{4,92,467.32} = 30.37$, $p < 0.001$) and significant Accuracy × TimeWindow interaction ($F_{6,23,591.34} = 3.00$, $p = 0.006$). The Relational condition shows significant PDV emerging prior to response, during the -1500 to -1000 ms time bin and sustaining through the 500-1000 ms time bin after response (all $p$s < 0.001; Figure 4.2).

Response-locked time courses in the Item condition show significant main effects of Accuracy ($F_{1,81} = 57.69$, $p < 0.001$) and TimeWindow ($F_{5,58,452.53} = 18.49$, $p < 0.001$) and a significant Accuracy × TimeWindow interaction ($F_{6,485.98} = 3.11$, $p = 0.005$). Post-hoc comparisons of
individual time points show significant PDV emerging during the -1500 to -1000 ms time bin and sustaining through the 2000-2500 ms time bin (all $ps < 0.001$; Figure 4.2).

Aerobic Fitness Effects on Relational and Item Memory

Unadjusted Pearson’s correlations show significant positive correlations between VO$_2$max and behavioral accuracy in both the Relational ($r = 0.26$, $p = 0.01$) and Item ($r = 0.24$, $p = 0.02$) conditions. Next, to determine which demographic factors were correlated with VO$_2$max, we performed bivariate correlations, independent samples t-tests, or one-way ANOVAs, where appropriate, between age, sex, SES, and IQ, with VO$_2$max ($ps \leq 0.05$). Results showed significant relationships between sex and SES with VO$_2$max. Partial correlations adjusting for these factors showed a marginal or no significant relationship between VO$_2$max and relational ($r = 0.16$, $p = 0.11$) or item ($r = 0.17$, $p = 0.10$) memory accuracy. Next, bivariate correlations showed a marginal correlation between VO$_2$max and PDV in the Relational condition ($r = 0.17$, $p = 0.09$), and no significant relationship between VO$_2$max and PDV in the Item condition ($r = 0.06$, $p = 0.54$). Both correlations were non-significant after adjusting for sex and SES ($ps > 0.37$).
BMI is Negatively Correlated with Behavioral Memory Accuracy

Results show significant negative correlations between BMI and behavioral accuracy in both the Relational ($r = -0.22, p = 0.03$) and Item ($r = -0.28, p = 0.005$) conditions. Next, we observed significant relationships between age, IQ, and SES with BMI. After adjusting for these factors, partial correlations remained significant (Relational: $r = -0.22, p = 0.03$; Item: $r = -0.27, p = 0.009$). A marginal bivariate correlation was observed between BMI and PDV in the Relational condition ($r = -0.18, p = 0.07$), with no significant correlation between BMI and PDV in the Item condition ($r = -0.07, p = 0.53$). These relationships both became non-significant after adjusting for age, IQ, and SES ($ps > 0.24$).

To further investigate the specificity of the relationship between BMI and behavioral measures of relational and item memory, participants were divided into an Overweight/Obese group (BMI-for-age percentile ≥ 85; N = 41) and a Normal/Underweight group (BMI-for-age percentile < 85; N = 57) based on norms from the U.S. Centers for Disease Control and Prevention (Kuczmarski et al., 2002). Results showed no relationships between BMI and behavioral accuracy on the Relational ($r = 0.02, p = 0.91$) or
Item ($r = 0.06, p = 0.68$) conditions for the Normal/Underweight group, but significant negative relationships between BMI and behavioral accuracy for the Relational ($r = -0.45, p = 0.003$) and Item ($r = -0.34, p = 0.03$) conditions for the Overweight/Obese group (Figure 4.4).

**Discussion**

The current study was one of the first to explore the relationship between aerobic fitness and relational and item memory in prepubertal children, and found that aerobic fitness was positively related to memory performance, although this effect was attenuated by the addition of demographic covariates. Furthermore, this was the first study to our knowledge on the relationship between BMI and memory in prepubertal children. We observed a negative relationship between BMI and both forms of memory tested, which persisted after adjustment for demographic factors. This effect was carried by children who were classified as overweight or obese based on BMI-for-age normative values. Additionally, the current study yielded several novel cognitive findings for this age group on viewing in sampling previously studied items and relations.

**Aerobic Fitness**

While unadjusted correlations between VO$_2$max and behavioral measures of relational and item memory were significant (Figure 4.3), adjustment for sex and SES rendered these correlations non-significant. Eye-movement measures prior to response were also not significantly correlated with VO$_2$max. These findings then partially replicate studies that have found a relationship between aerobic fitness and hippocampal structure or function in
prepubertal children (Chaddock et al., 2010; Monti, Hillman, & Cohen, 2012). Chaddock and colleagues (2010) matched their low-fit and high-fit groups on several demographic factors but did not match groups on sex, which was found to be significantly related to relative VO2 max in the current study, so it is not clear whether the current study replicates that study in this respect. Integrating across the current and previous studies suggests that aerobic fitness may have some relationship with hippocampal and MTL cortical function at this age, but that the effects are small. It should also be noted that the range of aerobic fitness (relative VO2 max) was fairly restricted due to the generally low fitness level of the population sampled, which may obscure potentially stronger memory effects that may take place across a broader range of fitness levels. Taken together with work in older children, adolescents, and adults, however, it seems likely that the relationship between aerobic fitness and hippocampal function strengthens over development. For example, in the current investigation, significant effects of aerobic fitness relative to hippocampal function were observed around age 12 (see Chapter 3) and strengthen into young adulthood (see Chapter 2). Other findings in the literature support the strength of this relationship during adolescence, as adolescents who were more aerobically fit have shown more evidence of learning on a human analogue of the Morris Water Maze, as well as larger hippocampal volumes (Herting & Nagel, 2012).

**BMI**

Body mass index was negatively correlated with behavioral measures of both relational and item memory, even after adjusting for age, IQ, and SES. Adding specificity to these effects, however, was that these effects were found to be carried entirely by the children who were in
the 85th or greater percentile of BMI-for-age, classified as overweight or obese (Figure 4.4). This suggests that body size is not related to memory function within the normal range for this age group, but that there may be a steady drop in behavioral performance once body size surpasses a certain level. This adds to the mixed literature regarding the relationship between BMI and cognition in childhood (e.g., Castelli, Hillman, Buck, & Erwin, 2007, Datar, Sturm, & Magnabosco, 2004; Gunstad et al., 2008; Li, Dai, Jackson, & Zhang, 2008), in that the current results converge with previous research showing a significant negative relationship between BMI and cognition, even after adjusting for multiple covariate factors. Turning to the animal literature, the current results mirror work in rodent models showing deficits in delayed hippocampal-dependent memory in obese rats relative to lean rats (Winocur et al., 2005). Despite the mixed findings in the literature, the current study adds to our understanding of these relationships by demonstrating not only a difference across BMI status groups with regard to memory performance, but also a difference in the relationship between BMI and memory performance within an overweight/obese group compared to a normal/underweight group of children.

**Novel Cognitive Findings**

Behaviorally, participants were significantly more accurate on the Item condition than the Relational condition, but responded significantly faster on the Relational condition than the Item condition. One explanation for these findings is a speed-accuracy tradeoff, wherein participants took longer to respond to Item trials than Relational trials and were thus more accurate for that reason. Another possibility, however, is based on previous research using a
similar paradigm in adults (Hannula, Ryan, Tranel, Cohen & 2007). In that study, Hannula and colleagues determined that the presence of a scene preview, which in the current study was unique to the Relational condition, was critical in allowing for preferential viewing approximately 1000 ms earlier than for trials without a scene preview (Hannula, Ryan, Tranel, & Cohen, 2007). Extrapolating this result to the current study, the faster response time in the Relational condition than the Item condition is likely due to the availability of reactivation of the relational binding during the scene preview in that condition, but the absence of reactivation in the Item condition, as the scene was non-diagnostic of which creatures would appear on each Item trial. Eye-movement data from the current study further support the interpretation that the behavioral findings are likely not simply due to a speed-accuracy tradeoff. Similar to the findings of Hannula and colleagues (2007), in the current study PDV emerged more than 1000 ms earlier in time in Relational condition than the Item condition relative to the onset of the three-creature display, possibly explaining the relatively modest 180 ms difference in behavioral response times between conditions.

The second novel cognitive finding in the current study was that response-locked time courses showed that significant PDV emerged during the 1000-1500 ms time bin prior to behavioral response in both the Relational and Item conditions. This is a similar time course, or even further in advance of behavioral response, than that observed in healthy young adults (see Chapter 2; Hannula, Ryan, Tranel, Cohen, 2007), suggesting that similar processing preceding conscious awareness may be occurring in prepubertal children as in young adults (Hannula & Ranganath, 2009). Furthermore, along with our investigation in young adults (see Chapter 2)
this was the first study to examine the time course of this effect in an item memory task. The finding that significant PDV emerged on a similar time scale to that in the Relational condition suggests a generality in the relationship between viewing and conscious access to behavioral response.

Third, subjective confidence regarding the accuracy of behavioral memory decisions in the Item condition predicted accuracy on both the Item condition and the Relational condition. Given that the Item and Relational conditions were independent from one another apart from the individual completing them (indeed due to counterbalancing the participants were not even aware of the existence of the other condition until completing half of the trials of the first condition), the significant relationship between confidence in the Item condition and accuracy in the Relational condition can be taken as evidence of a trait ability of the participant. This can thus be taken as evidence that making a confidence decision that reflects actual performance may be in itself a relational ability.

**Conclusions**

The current study examined relationships between aerobic fitness, body mass index, and two forms of memory known to rely on different neural substrates, in a group of prepubertal children. Results showed that aerobic fitness was positively related to Relational and Item memory accuracy, but that these effects were attenuated by the inclusion of sex and SES as covariate factors. Furthermore, results demonstrated a negative relationship between BMI and both Relational and Item accuracy, even after adjusting for age, IQ, and SES. Examining
separately children who were classified as overweight or obese from children classified as Normal or Underweight, we observed a strong negative relationship between BMI and memory performance in the overweight/obese group, but no relationship in the Normal/Underweight group. Taken together these results suggest that body size may be an important predictor of memory performance in prepubertal children, particularly when it exceeds the healthy range. Finally, the absence of an observable dissociation between memory type with either of the health measures tested (aerobic fitness or BMI) suggests that these health effects have broad effects in the developing brain, extending across both cortical and subcortical brain structures. These results demonstrating a positive relationship between aerobic fitness and memory performance and a negative relationship between BMI and memory performance in prepubertal children provide another incentive to keep a healthy body weight through maintaining an active lifestyle.
References


Acknowledgments

The authors would like to thank and Bonnie Hemrick for recruitment and scheduling of participants and Teresa Borowski, Becky Delgado, Inge Karosevica, Ari Pence, Jackie Rodriguez, Grace Song, and Sebastian Wraight for assistance with data collection. This research was funded by NICHD HD055352 (CHH).
### Table 4.1. Participant demographics, weight status, and fitness

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<th>Characteristic</th>
<th>Females (n = 52)</th>
<th>Males (n = 46)</th>
<th>All children (n = 98)</th>
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<tr>
<td>Age, years</td>
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<td>8.74 (0.08)</td>
<td>8.71 (0.06)</td>
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<td>SES (n [%])</td>
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<tr>
<td>Low</td>
<td>19 (37)</td>
<td>17 (37)</td>
<td>36 (37)</td>
</tr>
<tr>
<td>Middle</td>
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<td>29 (30)</td>
</tr>
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<td>High</td>
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<td>15 (33)</td>
<td>33 (34)</td>
</tr>
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</tr>
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<td>68.09 (2.93)</td>
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<td>BMI status</td>
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<tr>
<td>Underweight</td>
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</tr>
<tr>
<td>Normal Weight</td>
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<tr>
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<td>7 (15)</td>
<td>14 (14)</td>
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<tr>
<td>Obese</td>
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<td>13 (28)</td>
<td>26 (27)</td>
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<tr>
<td>VO₂max, ml/kg/min</td>
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<td>42.24 (0.90)*</td>
<td>40.33 (0.64)</td>
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<tr>
<td>Relational memory accuracy (%)</td>
<td>60.57 (1.19)</td>
<td>66.68 (1.82)*</td>
<td>63.44 (1.35)</td>
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<tr>
<td>Item memory accuracy (%)</td>
<td>85.10 (1.36)</td>
<td>87.62 (1.27)</td>
<td>86.28 (0.94)</td>
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<td>2574.37 (71.62)*</td>
<td>2502.08 (49.17)</td>
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<td>Item memory response time, correct trials only</td>
<td>2542.98 (68.08)</td>
<td>2836.09 (73.12)*</td>
<td>2680.56 (51.75)</td>
</tr>
</tbody>
</table>

Data values indicate mean (S.E.M.) unless otherwise specified. BMI status was based on the Centers for Disease Control and Prevention BMI-for-age growth charts.

IQ, intelligence quotient; SES, socioeconomic status; BMI, body mass index

*p < 0.05 as calculated by t-tests
CHAPTER 5: CENTRAL ADIPOSITY IS NEGATIVELY ASSOCIATED WITH HIPPOCAMPAL MEMORY IN CHILDREN

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Abstract

**Context** Central adiposity has long been associated with an increased risk of cardiovascular disease and diabetes. Recent literature suggests a link to adverse cognitive outcomes as well. The current study examined the effects of central adiposity on memory, testing whether such effects emerge as early as middle childhood, decades earlier than previously shown.

**Objective** To investigate whether total and central adiposity are associated with different aspects of memory performance with distinct neural substrates, testing whether such effects emerge as early as middle childhood, decades earlier than previously shown.

**Design, Setting, and Participants** Cross-sectional study of 95 prepubertal children aged 7-9 years observed during 2011 and 2012. Volunteers were recruited from the general community and tested at the University of Illinois at Urbana-Champaign using dual-x-ray-absorptiometry (DXA) to quantify central (FM-abd) and total adiposity (%FM), weight-to-height ratio to assess body-mass index (BMI), a treadmill test to measure aerobic fitness (VO₂max), and a memory task using behavioral and eye-movement measures. To assess whether fat distribution was related to memory performance independent of total adiposity, we calculated an index of central adiposity that accounted for total fat mass (CAI).

**Main Outcome Measures** Behavioral measures (choice accuracy) and eye-movement measures (proportion of total viewing time (PTVT) directed to the correct stimulus in the test display) of hippocampal-dependent relational and non-hippocampal-dependent item memory performance.
Results After adjusting for IQ, age, sex, socioeconomic status, birthweight status, and VO$_2$max, while BMI was not related to behavioral memory performance ($ps > 0.12$), %FM was negatively associated with item memory accuracy ($p < 0.03$), and CAI was negatively related to behavioral relational memory accuracy ($p < 0.03$) and item memory PTVT ($p < 0.005$).

Conclusions and Relevance Regional fat deposition in prepubertal children was associated with distinct types of memory with different neural substrates. Given the known and critical role of the hippocampus in relational memory, these data relate central adiposity to hippocampal function in prepubertal children. That such early lifestyle choices may affect the integrity of the hippocampus, so critical to learning and successful scholastic achievement, has substantial public health implications and may be used as evidence for encouraging healthy weight status in pediatric patients.
Introduction

Ever since its sharp rise in the 1980s, the incidence of obesity has steadily increased to epidemic proportions. Currently in the United States, over 30% of adults are obese, and estimates of childhood overweight (≥85th percentile BMI-for-age) and obesity (≥95th percentile BMI-for-age) prevalence stand at approximately 32% and 17%, respectively\(^1\). Some of the major causes of mortality in the modern world including type 2 diabetes, cardiovascular diseases (CVD), and cancers are complications that arise due to obesity\(^2\). Of further concern is that pediatric obesity is implicated in the early onset of what have been historically considered adult disorders, including type 2 diabetes, polycystic ovarian syndrome, and nonalcoholic fatty liver disease\(^3\).

Converging evidence now suggests that poor cognitive function may be another complication associated with obesity in adults\(^4,5\). Obesity in middle adulthood is an independent risk factor for developing dementia and Alzheimer’s disease later in life\(^6,7\). Obesity in children is known to be associated with impaired cognitive control and scholastic performance\(^8,9\), but whether obesity in children might also be associated with memory impairment remains unknown.

Work in this area has been limited methodologically by relying on BMI as a proxy measure of obesity. Given that obesity is a condition defined by excess fat mass rather than overall body weight\(^10\), measuring only BMI is less than ideal. Furthermore, relying on BMI alone neglects the physiological importance of fat distribution. Specifically, central adiposity is mechanistically implicated in CVD and type 2 diabetes\(^11\). Whether central adiposity also
negatively affects specific cognitive or memory processes, and their brain substrates, during childhood has yet to be determined. But, in work with adults, waist-to-hip ratio, utilized as a surrogate measure of central adiposity, was shown to be negatively correlated with behavioral, verbal, and working memory performance as well as hippocampal volume\textsuperscript{12,13}.

The possible connection with the hippocampus may be especially promising. The hippocampus is critical for a particular form of memory, called relational or associative memory, which supports representations of the relations among multiple items, such as the relations among the constituent elements of scenes or events, and their subsequent flexible expression\textsuperscript{14}. The acquisition of relational knowledge provides a strong foundation for scholastic achievement and its flexible expression provides a basis for success in handling novel challenges. Accordingly, changes in the development of this system in childhood would have wide-ranging impact. In contrast, item memory is known to rely on perirhinal cortex, the anterior region of the parahippocampal gyrus, which surrounds the hippocampus in the medial temporal lobe\textsuperscript{14}. Accordingly, it is reasonable that these two forms or aspects of memory might be affected differentially by separate physiological factors.

Additionally distinctive is that the hippocampus is one of just two brain regions that undergo neurogenesis, the generation of new neurons, throughout the lifetime\textsuperscript{15}. This extraordinary plasticity provides the opportunity for modification of the developmental trend of this structure, positively through certain interventions, such as an exercise program\textsuperscript{16}, or negatively by increased risk for obesogenic detriments in growth. Hippocampal memory performance has been positively related to aerobic fitness across the lifespan\textsuperscript{16,17}, demonstrating that this tissue is susceptible to health behaviors. Taken together with the
known inverse relationship between aerobic fitness and obesity, it is reasonable to predict that fat mass may be negatively related to hippocampal function in prepubertal children.

In the current work we assessed the relationship between obesity and memory function, using measures of both hippocampal-dependent relational memory and non-hippocampal-dependent memory for individual items. In addition to standard behavioral measures we also employed eye-movement methods that provide an especially sensitive measure of memory\textsuperscript{17}, sometimes more veridical than that revealed by behavioral report\textsuperscript{18}, and that have been shown to be tied specifically to hippocampal relational memory function\textsuperscript{19}. The measure of interest derived from the eye-movements, proportion of total viewing time (PTVT), provides information on memory representation of previous experience even prior to behavioral awareness, with greater PTVT to previously target stimuli indicating more veridical memory\textsuperscript{18}.

To our knowledge, no previous study has examined the effects of whole body or central adiposity on distinct forms of memory in prepubertal children. Accordingly, our study aimed to determine in prepubertal children: 1) whether relational and/or item memory are negatively related to whole-body percentage fat mass (%FM), and 2) which measure of adiposity (whole body or central) best explains the variability in hippocampal-dependent memory. Given the correlation between abdominal fat mass (FM-abd) and whole body fat mass (FM), we developed a central adiposity index (CAI) based on the ratio of FM-abd to FM to assess the independent contribution of each adiposity variable on memory function. Considering that pediatric obesity in the United States is projected to double in the next 20 years, delineating
the impact of adiposity on distinct forms of memory is of crucial importance for intervention studies targeting the pediatric population.\textsuperscript{20}

Methods

Participants

Ninety-five prepubertal children (7-9-year-olds) provided written assent and legal guardians provided written informed consent in accordance with the regulations of the University of Illinois Institutional Review Board. Prior to participation, children were screened for serious medical conditions, neurological or attentional disorders, physical disabilities, psychoactive medication status, and normal or corrected-to-normal vision using a parental report health history questionnaire. Data were also collected on 1) IQ, using the Kaufman Brief Intelligence Test\textsuperscript{21} or the Woodcock-Johnson Tests of Cognitive Abilities\textsuperscript{22}, 2) socioeconomic status (SES) as estimated based on household income, participation in a school meal-assistance program, maternal and paternal education levels, and how many parents work full-time, 3) pubertal status\textsuperscript{23} and 4) birthweight (for detailed methods see REF. 8).

BMI, Body Composition, and Fitness Assessments

BMI and BMI-for-age percentile and z-score were calculated as recommended by the U.S. Centers for Disease Control and Prevention\textsuperscript{24}. Adiposity was assessed by DXA (Hologic QDR 4500A, software version 11.2, Bedford, Massachusetts). FM-abd was defined as fat mass located between lumbar vertebral bodies L1 and L4\textsuperscript{25,26}. CAI was calculated by dividing FM-abd by total body fat.
Aerobic fitness (VO$_2$max) was measured using a modified Balke protocol on a motor-driven treadmill $^{8,27}$. Since previous findings have demonstrated that the effect of body weight on VO$_2$max is primarily due to lean mass and not fat mass$^{28}$, we used DXA data to derive relative VO$_2$max (ml/kg/min) based on fat-free mass instead of total body weight.

**Memory Tasks**

On a separate day, children completed a task that was adapted from Monti and colleagues$^{17}$, however the current version used child-friendly creatures (Electronic Arts Inc., California) rather than faces (Figure 5.1). In separate study-test blocks, children studied individual creatures (Item condition) or uniquely paired associations between creatures and backgrounds (“habitats”; Relational condition).

![Figure 5.1. Memory task. Left Panel: Progression of study and test phase trials. Right panel: Example study and test phase displays for Relational and Item conditions. Each test trial consisted of one studied background superimposed with three of the creatures that had been studied in that block (Relational condition), or with one of the creatures that had been studied in that block and two novel creatures (Item condition).](image)

In order to encourage relational encoding in the Relational condition, participants were instructed to press one of two buttons to indicate whether they judged each creature to live
alone in their habitat or with others. At test, participants were instructed to find the creature originally studied with that scene. In this condition, one of the creatures had been studied with that scene (target) and two had been studied with other scenes (foils). Familiarity across the three creatures was thus matched, necessitating the employment of hippocampal-dependent relational memory$^{17,29}$.

In the Item condition, the background scene was the same for all creature-scene pairings within each block. To encourage encoding of each creature, participants were instructed to press one of two buttons indicating whether they judged each creature to be a “boy” or “girl.” At test, participants were instructed to find the previously viewed creature. In each test display two creatures were novel and one studied, allowing the discrimination to be made on the basis of familiarity, an ability that has been shown to be independent of the hippocampus$^{30}$.

Lists of stimuli were counterbalanced across conditions between participants, and target location on test trials was counterbalanced within participant such that the target was equally likely to appear in any of the three possible locations. Order of study-test blocks was counterbalanced across participants such that half the participants began with the Relational condition and half with the Item condition.

An Eyelink 1000 eye-tracker (SR Research, Ontario, Canada) was used to remotely record eye-movements at 500 Hz. A desk-mounted gel-padded chin rest was used to minimize head movement during eye-movement data collection.
Statistical Analyses

Initial Pearson’s correlations were performed to assess bivariate relationships between obesity measures and memory outcomes. A second round of analyses used multiple hierarchical linear regression to examine which measures of obesity (BMI z-score, %FM, CAI) were associated with memory. Control variables age, sex, birthweight status, IQ, VO₂max, and SES were included in the initial model. Obesity measures were added individually in the second step. The significance of the change in $R^2$ value between steps was used to test the explanatory power of each obesity measure for relational and item memory accuracy. The α-level was set at 0.05. Statistics were performed using SPSS 19 (IBM, Somers, NY). Post-hoc power analysis was conducted using a β of 0.8 using G*Power 3.1 (REF. 31).

Results

Participant characteristics are presented in Table 5.1.

Behavioral Performance

Unadjusted correlations between participant characteristics, obesity measures, and memory accuracy are summarized in Table 5.2. Relational memory accuracy was positively correlated with VO₂max and males outperformed females on this condition. However, the gender difference was mediated by birthweight status in subsequent regression modeling. In contrast, none of the demographic or fitness variables correlated with item memory accuracy.
Relational memory was negatively correlated with %FM and CAI, but not related to BMI z-scores. Item memory was negatively related to all measures of obesity.

Next, multiple hierarchical linear regression analyses examined which measures of obesity (BMI z-score, %FM, CAI) were associated with memory (Table 5.3). Adding BMI z-score at the second step yielded non-significant changes in $R^2$ for relational ($\Delta F_{1,88} = 0.80, p = 0.38$) and item memory accuracy ($\Delta F_{1,88} = 2.48, p = 0.12$), indicating that higher BMI z-score was unrelated to either aspect of memory. %FM did not significantly improve $R^2$ at the second step for relational memory ($\Delta F_{1,88} = 2.35, p = 0.13$) but did improve $R^2$ for item memory ($\Delta F_{1,88} = 4.72, p = 0.03$), indicating that higher %FM was related to item, but not relational, memory accuracy. Addition of CAI yielded significant improvement at the second step for relational memory accuracy ($\Delta F_{1,88} = 5.03, p = 0.03$; Figure 5.2) but only marginal improvement for item memory accuracy ($\Delta F_{1,88} = 3.63, p = 0.06$), indicating that higher CAI was significantly related to poorer performance on the relational, but not item, memory condition. A sample size of 95 was used to assess post hoc statistical power using a 7 predictor variable equation. The statistical power for detecting a small ($f^2 = 0.02$), medium ($f^2 = 0.15$) and large ($f^2 = 0.35$) effect was 0.28, 0.96, and 0.99, respectively. Thus, we had adequate power to detect medium-to-large, but not small effect sizes.
Figure 5.2. Mean-centered scatterplots adjusted for age, sex, birthweight status, IQ, SES, and VO₂max.

Eye-movements

Eye-movement analyses were performed on three regions-of-interest in the test arrays corresponding to the locations of each of the three creatures. PTVT was defined as proportion of time spent viewing a given region-of-interest for the duration of the probe array. Trials were excluded from further analysis if the participant failed to look at any region-of-interest for >30% of total viewing time.

Unadjusted Pearson’s correlations showed no significant relationship between BMI z-score, %FM, or CAI and PTVT in the Relational condition (all rs ≤ 0.08, all ps > 0.2, Table 5.2). In
the Item condition, unadjusted correlations showed a significant positive relationship between CAI and PTVT \( r = 0.26, p = 0.005 \), but no significant relationship between BMI z-score or %FM and PTVT \( r = 0.07, p = 0.25 \) and \( r = 0.12, p = 0.13 \) respectively.

The next analysis was a series of multiple linear regression models including age, sex, birthweight status, IQ, SES, and VO\(_2\)max in the first step, and BMI z-score, %FM, or CAI in the second step (Table 5.3). Results mirrored the correlational analyses showing that the addition of CAI in the second step significantly improved the model for PTVT in the Item condition only \( (\Delta F_{1,88} = 8.40, p = 0.005) \). Post-hoc analyses showed that this effect was not specific to viewing of correctly selected items, however. Indeed there was a positive correlation between CAI and PTVT of selected items overall \( (r = 0.19, p < 0.03) \), such that children with higher CAI simply spent more time viewing the item they would select whether it was the one they had previously studied or not. This remained significant as the second step of a multiple linear regression model \( (\Delta F_{1,88} = 5.85, p = 0.02) \).

**Discussion**

In this report we document for the first time an inverse relationship between adiposity and both hippocampal-dependent relational and hippocampal-independent item memory in a diverse cross-section of prepubertal children. We found further specificity of these effects, showing that central adiposity is strongly associated with poorer hippocampal-dependent relational memory function but is only marginally related to hippocampal-independent item memory. This negative relationship persists following adjustment for demographic and fitness covariates suggesting that the observed effects cannot be attributed to age, sex, birthweight
status, IQ, SES, or aerobic fitness. The novel finding that central adiposity negatively affects relational memory performance in prepubertal children may suggest early dysregulation in one or more aspects of regional or systemic metabolic function such as insulin resistance, inflammation, or cortisol levels\textsuperscript{33}.

First, intra-abdominal/visceral fat tissue, in contrast to peripheral fat tissue, is implicated in insulin resistance and subsequent alterations in glucose homeostasis\textsuperscript{34}. Insulin resistance is an established characteristic feature of dementia associated with Alzheimer’s disease\textsuperscript{35}. Furthermore, insulin may be involved with hippocampal-dependent memory processes, as the hippocampus contains an abundance of insulin receptors and expresses large amounts of GLUT4, the translocational target for insulin-mediated glucose uptake\textsuperscript{36}. Insulin resistance and hippocampal function have been linked more directly in animal studies that show that delivering insulin to the hippocampus strengthens spatial memory while blocking insulin signaling impairs memory function\textsuperscript{37}.

A second potential mechanism relates to inflammation. Visceral adipose tissue secretes several proinflammatory adipokines, such as interleukin (IL)-6, tumor necrosis factor (TNF-\(\alpha\)), macrophage chemoattractant protein-1 (MCP-1), and resistin, which cause insulin resistance and are neurotoxic when released from microglia in the brain\textsuperscript{38}. Systemic inflammation has been shown to increase inflammation in the central nervous system and predict cognitive decline\textsuperscript{5}. Additionally, inflammation has been shown to impair adult hippocampal neurogenesis\textsuperscript{39}. Inflammation has also been linked to depression, which is another possible mediator of the observed relationship between central adiposity and hippocampal function\textsuperscript{40}. 
Finally, prolonged cortisol release caused by chronic stimulation of the hypothalamic-pituitary-adrenal (HPA) axis may affect central more than peripheral fat mass due to increased number of glucocorticoid receptors in abdominal fat mass. Further aggravating the cycle, visceral fat has been shown to release cytokines that stimulate the HPA axis to release even more glucocorticoids\(^{41}\). Activation of this cycle results in increased central adiposity, insulin resistance and, critically, hippocampal atrophy\(^{42}\). Other potential mediators of this relationship may include diet, sleep, physical activity, and the weight-regulation hormone leptin\(^{43}\).

The finding that %FM was inversely related to item but not relational memory accuracy was unexpected. However, given that the neural substrates of relational and item memory are known to have distinct connectivity and metabolic profiles, it is not surprising that they were found to be related to different physiological factors. Further examination of these relationships is warranted.

The findings presented here are the first to relate distinct forms of memory with quantitative measures of whole-body adiposity and fat distribution among prepubertal children. These relationships survived analyses that controlled for many possible mediating factors. Very low birthweight (<1,500g) is related to moderate-to-severe deficits in cognitive flexibility and verbal and working memory in later childhood\(^{44}\). Here, birthweight status mediated the observed gender differences in memory performance, but not the negative relationship between adiposity and memory. Finally, while unadjusted correlations showed a negative relationship between BMI and item memory, regression modeling showed that BMI z-scores were not reliable predictors of memory performance. The increased sensitivity of the
obesity measures used in the present study over BMI highlights the importance of assessing fat mass and distribution when studying the cognitive consequences of obesity.

Obtaining both behavioral and eye-movement results here was also illuminating. Central adiposity was positively related to the proportion of time that children directed to the item they would behaviorally select, whether or not they had previously viewed it, in the Item condition. As a result, children with higher central adiposity sampled the display less overall than their lower central adiposity counterparts. Such a strategy may not be detrimental to item memory performance, but would necessarily impair performance in the Relational condition, given the inherent need in that condition to sample multiple regions of the display (at minimum the scene and the correct creature, but more effectively the scene and each creature, probably more than once).

Finally, two caveats should be noted. First, DXA-measured central adiposity cannot distinguish between subcutaneous and visceral fat tissue, preventing us from being able to directly attribute inferior memory performance with higher CAI to the compartmentalization of fat tissue within the abdominal region. Second, although our analyses accounted for many possible factors, future studies should consider such additional variables as diet or maternal BMI and cortisol levels.

**Conclusion**

The current study provides the first evidence in children connecting central adiposity, the most clinically relevant fat deposition in the human body, to the hippocampus and relational or associative memory. The results reported here are consistent with recent findings
connecting obesity and central adiposity with cognitive dysfunction\textsuperscript{5,13,45}, but critically extend the literature by demonstrating a relationship between these factors significantly earlier in life than previously known. That obesity-related impairment is already manifest during the school years and involves a type of memory critical for successful scholastic achievement has considerable implications for the cognitive health of our population. Furthermore, the current study revealed associations between different distributions of adipose tissue and different forms of memory with distinct neural substrates. Particularly in light of the currently increasing rates of childhood obesity, these findings raise troubling public health concerns. Moreover, the current results contribute yet another piece of evidence on the importance of developing a healthy, active lifestyle even early in life.
Acknowledgments

CLB, NAK, and LBR collected the data. CLB and NAK analyzed the data and prepared the first draft of the manuscript. CLB had full access to all the data in the study takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the experimental design, manuscript preparation, and all authors approved the final manuscript. The authors declare no conflicts of interest. The authors would like to thank Bonnie Hemrick, MPH, for participant recruitment, Eric Drollette, BS, Inge Karosevica, Ari Pence, BS, Mark Scudder, BS, and Sebastian Wraight, BA, for assistance with data collection, Patrick Watson, PhD for assistance data analysis, and Nicole Boniquit, MD and Sarah Kinsella, MD for helpful comments on the manuscript. This research was funded by NICHD HD055352 (CHH). The sponsoring agency had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.
References


42. Sapolsky R. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000;57:925-935.


### Tables

#### Table 5.1. Participant demographics, weight status, fitness

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>All children (n=95)</th>
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<td>109.63 (13.9)</td>
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<td>Birthweight status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (&lt;2500g), [n (%)]</td>
<td>5 (10)</td>
<td>5 (11)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Normal (2,500-4000g), [n (%)]</td>
<td>42 (82)</td>
<td>28 (64)</td>
<td>70 (74)</td>
</tr>
<tr>
<td>High (&gt;4,000g), [n (%)]</td>
<td>4 (8)</td>
<td>11 (25)</td>
<td>15 (16)</td>
</tr>
<tr>
<td>SES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low, [n (%)]</td>
<td>18 (35)</td>
<td>16 (36.0)</td>
<td>34 (36)</td>
</tr>
<tr>
<td>Middle, [n (%)]</td>
<td>15 (30)</td>
<td>13 (30.0)</td>
<td>28 (29)</td>
</tr>
<tr>
<td>High, [n (%)]</td>
<td>18 (35)</td>
<td>15 (34.0)</td>
<td>33 (35)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>19.3 (4.8)</td>
<td>18.8 (4.2)</td>
<td>19.05 (4.5)</td>
</tr>
</tbody>
</table>

**BMI-for-age Percentile**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Females (n=51)</th>
<th>Males (n=44)</th>
<th>All children (n=95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI z-score</td>
<td>0.68 (1.15)</td>
<td>0.70 (1.14)</td>
<td>0.69 (1.14)</td>
</tr>
<tr>
<td>Underweight, [n (%)]</td>
<td>2 (4)</td>
<td>2 (5)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Normal Weight, [n (%)]</td>
<td>29 (57)</td>
<td>23 (52)</td>
<td>52 (55)</td>
</tr>
<tr>
<td>Overweight, [n (%)]</td>
<td>7 (14.0)</td>
<td>7 (16)</td>
<td>14 (15)</td>
</tr>
<tr>
<td>Obese, [n (%)]</td>
<td>13 (25.0)</td>
<td>12 (27)</td>
<td>25 (26)</td>
</tr>
<tr>
<td>VO₂max</td>
<td>55.2 (6.01)</td>
<td>57.6 (6.5)</td>
<td>56.3 (6.3)</td>
</tr>
<tr>
<td>FM, kg</td>
<td>12.3 (6.7)</td>
<td>10.2 (5.5)</td>
<td>11.3 (6.2)</td>
</tr>
<tr>
<td>%FM</td>
<td>31.3 (7.6)*</td>
<td>26.8 (5.5)</td>
<td>29.22 (7.6)</td>
</tr>
<tr>
<td>FM-abd, kg</td>
<td>1.1 (0.9)</td>
<td>0.9 (0.6)</td>
<td>1 (0.78)</td>
</tr>
<tr>
<td>CAI</td>
<td>0.08 (0.02)</td>
<td>0.08 (0.02)</td>
<td>0.08 (0.02)</td>
</tr>
<tr>
<td>Relational memory accuracy (%)</td>
<td>0.61 (0.14)</td>
<td>0.67 (0.13)</td>
<td>0.64 (0.14)</td>
</tr>
<tr>
<td>Item memory accuracy (%)</td>
<td>0.85 (0.10)</td>
<td>0.88 (0.08)*</td>
<td>0.87 (0.09)</td>
</tr>
</tbody>
</table>

Data expressed as mean (SD) unless otherwise specified. Weight status was based on the Centers for Disease Control and Prevention BMI-for-age growth charts. IQ, intelligence quotient; SES, socioeconomic status; BMI, body mass index; FM, whole body fat mass; %FM, whole body %fat mass, FM-abd, abdominal fat mass; CAI, Central Adiposity Index calculated as a ratio of FM to FM-abd. *p < 0.05 as calculated by one-way ANOVAs
Table 5.2. Unadjusted correlations between participant characteristics and memory

<table>
<thead>
<tr>
<th></th>
<th>Relational Memory Accuracy</th>
<th>Item Memory Accuracy</th>
<th>Relational Memory PTVT</th>
<th>Item Memory PTVT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.17</td>
<td>0.06</td>
<td>0.13</td>
<td>0.17*</td>
</tr>
<tr>
<td>Sex</td>
<td>0.24*</td>
<td>0.15</td>
<td>0.03</td>
<td>-0.05</td>
</tr>
<tr>
<td>Birthweight Status</td>
<td>0.17*</td>
<td>0.01</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>IQ</td>
<td>0.14</td>
<td>0.14</td>
<td>0.08</td>
<td>-0.02</td>
</tr>
<tr>
<td>SES</td>
<td>0.17*</td>
<td>0.12</td>
<td>0.24**</td>
<td>0.17*</td>
</tr>
<tr>
<td>VO₂max</td>
<td>0.18*</td>
<td>0.10</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>-0.08</td>
<td>-0.18*</td>
<td>0.00</td>
<td>0.07</td>
</tr>
<tr>
<td>%FM</td>
<td>-0.21*</td>
<td>-0.27**</td>
<td>0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>CAI</td>
<td>-0.24**</td>
<td>-0.21*</td>
<td>0.08</td>
<td>0.26**</td>
</tr>
</tbody>
</table>

IQ, intelligence quotient; SES, socioeconomic status; BMI, body mass index; FM, whole body fat mass; %FM, whole body %fat mass, FM-abd, abdominal fat mass; CAI, Central Adiposity Index; PTVT, Proportion of Total Viewing Time; Sex was arbitrarily weighted: Females = 0, Males = 1.

** Pearson correlation is significant at the 0.01 level (one-tailed)

* Pearson correlation is significant at the 0.05 level (one-tailed)
Table 5.3. Summary of regression analyses for predicting relational and item memory behavioral accuracy and viewing patterns

| Step and variable | Behavioral Accuracy | Eye movements |  |
|-------------------|---------------------|---------------|  |
|                   | Relational Memory   | Item Memory   | Relational Memory | Item Memory   |
|                   | \( \beta \) \( \Delta R^2 \) | \( \beta \) \( \Delta R^2 \) | \( \beta \) \( \Delta R^2 \) | \( \beta \) \( \Delta R^2 \) |
| Step 1            |                     |               |  |
| Age               | 0.14                | 0.05          | 0.14                | 0.08                | 0.18                |
| Sex               | 0.18                | 0.14          | 0.04                | -0.04               |
| Birthweight Status| 0.16                | 0.01          | 0.07                | 0.004               |
| IQ                | 0.11                | 0.11          | 0.01                | -0.06               |
| SES               | 0.12                | 0.08          | 0.24                | 0.15                |
| \( VO_{2} \text{max} \) | 0.09                | 0.03          | -0.04               | 0.02                |
| Step 2            |                     |               | 0.000               | 0.004               |
| BMI z-score       | -0.09               | -0.17         | 0.02                | 0.06                |
| Step 2            | 0.02                | 0.05*         | 0.002               | 0.01                |
| %FM               | -0.17               | -0.24*        | 0.05                | 0.11                |
| Step 2            | 0.05*               | 0.04          | 0.01                | 0.08*               |
| CAI               | -0.22*              | -0.20         | 0.12                | 0.30**              |

IQ, intelligence quotient; SES, socioeconomic status; BMI, body mass index; FM, whole body fat mass; %FM, whole body %fat mass, FM-abd, abdominal fat mass; CAI, Central Adiposity Index calculated as a ratio of FM-abd to FM; Sex was arbitrarily weighted: Females = 0, Males = 1.; *\( p < 0.05 \) as calculated using a multiple linear regression model **\( p < 0.005 \) as calculated using a multiple linear regression model
CHAPTER 6: DIFFERENTIAL EFFECTS OF OMEGA-3 AND SATURATED FATTY ACIDS ON RELATIONAL AND ITEM MEMORY IN PREPUBERTAL CHILDREN

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\textsuperscript{1}Department of Psychology, \textsuperscript{2}Beckman Institute for Advanced Science and Technology, \textsuperscript{3}Department of Kinesiology and Community Health, \textsuperscript{4}Center for Nutrition, Learning, and Memory, \textsuperscript{5}Neuroscience Program, University of Illinois, Urbana, IL

Keywords: Relational memory, prepubertal children, nutrition, diet, cognition, development
Abstract

The increasing popularity of the Western diet raises concerns about the future physical and cognitive health of our nation’s citizens. Already shown to drastically increase the risk of obesity, cardiovascular disease, stroke, and diabetes, this saturated fat, trans fat, and sugar rich diet has been shown to accelerate cognitive decline among the elderly and impair neural development in animal models. Poor in the healthy fats that promote neural development, it is an open question as to whether the Western diet is related to the integrity of hippocampal relational memory and cortical item memory in the developing human brain. In the current study, we assessed dietary intake of markers of the Western diet including saturated fat, trans fat, added sugar, and total sugar, as well as fats that are known to be deficient in the Western diet including omega-3 fatty acids and monounsaturated fatty acids in 52 prepubertal children aged 7-9 years. Participants completed two memory tasks designed to assess relational and item memory, which are known to rely on different neural substrates. Potential confounding factors of age, sex, IQ, socioeconomic status, and aerobic fitness (VO₂max) were not significantly related to any of the dietary intake measures. Partial correlations adjusting for body mass index show a positive relationship between relational memory accuracy and intake of omega-3 fatty acids, and negative relationships between both relational and item memory accuracy with intake of saturated fat. Eye movement measures reveal a negative relationship between preferential viewing to the target stimulus in the Relational condition with intake of added sugar. Potential mechanisms that may mediate these relationships and public health implications of these findings are discussed.
Introduction

Converging evidence from human and animal work provides compelling support for the influence of diet on cognition. In the United States, and increasingly around the globe, people are eschewing more traditional diets such as the Mediterranean diet in favor of the fast, easy, convenience foods that comprise the Western diet. With a focus on taste, shelf-life, and ease of access rather than fresh, natural foods, components of this diet have proven negative consequences for health including increasing the risk for obesity, type 2 diabetes, cardiovascular disease, and stroke (Cordain et al., 2005). Evidence is further emerging that the highly processed foods of the Western diet, rich in saturated and trans fatty acids, added sugars, and sodium while low in healthy fats such as omega-3 and monounsaturated fats may deleteriously influence brain structure and function across the lifespan (Gómez-Pinilla, 2008). These effects may be particularly important early in life, when the brain is developing neural structures and establishing connectivity (Boitard et al., 2012; Cheatham, Colombo, & Carlson, 2006).

One neural subregion that appears particularly susceptible to both the beneficial and deleterious effects of diet is the hippocampus, possibly due to a combination of its high metabolic demand and capability for neurogenesis beyond the gestational period (Gómez-Pinilla, 2008; Kanoski & Davidson, 2011; Kaplan & Hinds, 1977). Human and animal studies have shown that various nutrients have been associated with increase or decrease in hippocampal neurogenesis (Molteni et al., 2004; Wu, Ying, & Gómez-Pinilla, 2008). Modeling the Western diet, a diet high in refined sugar and saturated fats has been shown to increase neural oxidative
stress, resulting in a decrease in hippocampal BDNF and spatial and non-spatial hippocampal memory performance (Kanoski, Meisel, Mullins, & Davidson, 2007; Wu, Ying, & Gómez-Pinilla, 2004, 2006, 2008).

Compounding these effects in the typical Western diet is the relatively low quantities of other essential nutrients such as antioxidants and omega-3 fatty acids that would otherwise serve to mitigate these harmful processes (Wu, Ying, & Gómez-Pinilla, 2004a, 2006). Animal models have shown omega-3 fatty acids, particularly docosahexaenoic acid (DHA) a long-chain polyunsaturated omega-3 fatty acid, to be important for myriad aspects of neural and cognitive health including roles in regulation of gene expression, increasing BDNF levels in the hippocampus, enhancing behavioral memory performance, upregulating genes important for synaptic plasticity, enhancing cell membrane fluidity, stimulating glucose utilization, and enhancing mitochondrial function (Agrawal & Gómez-Pinilla, 2012; Bhatia et al., 2011; Gómez-Pinilla, 2008; Innis, 2007; Wu, Ying, Gómez-Pinilla, 2004a). Furthermore, omega-3 fatty acids can be converted to neuroprotective metabolites (Innis, 2007). Indeed dietary deficiency of omega-3 fatty acids impairs hippocampal and prefrontal reliant memory and learning, and gene expression related to synaptic plasticity, cellular assembly, signal transduction and ion channel formation (Agrawal & Gómez-Pinilla, 2012; Cheatham, Colombo, & Carlson, 2006; Innis, 2007). Moreover, insufficient dietary intake of omega-3 fatty acids decreases cell body size in the hippocampus and reduces the complexity of dendritic arborization in cortical neurons (see Innis, 2007). Since the human body is inefficient at synthesizing DHA, we are largely dependent on dietary sources of DHA and its precursors α-linolenic acid (ALA) and eicosapentaenoic acid (EPA; Cheatham, Colombo, & Carlson, 2006). Given this potential for synthesis of DHA, although
inefficient, the current study examined summed intake of all three of these omega-3 fatty acids (Cheatham, Colombo, & Carlson, 2006; Innis, 2007).

These nutritional effects have also been reflected in behavioral performance in humans, with various nutrients being correlated with improvements or impairments in behavioral learning and memory. For example, saturated fats and refined sugar have been associated with impairments in human learning and memory, and omega-3 fatty acids, flavonoids, blueberry, curcumin, and multivitamin supplementation have all been associated with enhancements in human learning and memory (see Stangl & Thuret 2009). Moreover, in adults high intake of saturated fat has been associated with global cognitive impairments as well as impairments in verbal and prospective memory (Kanoski & Davidson, 2011; Okereke et al., 2012). In children, higher plasma DHA in the umbilical cord at birth and intake of dietary DHA near in time to test have been shown to relate to electrophysiological measures of continuous recognition during middle childhood (Boucher et al., 2011). Given that memory, particularly hippocampal-dependent relational memory, is critical for learning, the integrity of the neural architecture that supports various forms of memory is important for maximizing learning across the lifespan and scholastic achievement among children. Following, if nutritional intake affects the structural integrity and function of these brain structures, the Western diet may be shown to impair learning ability, which would have serious consequences for the future cognitive and economic health of the nation. We additionally included a measure of item memory, known to be reliant on medial temporal lobe cortex, rather than hippocampus, to assess effects of diet on cortical memory systems (Davachi & Wagner, 2002; Davachi, 2006).
Thus the current study aimed to examine how intake of various nutrients available in the Western diet such as saturated fat and refined sugar, as well as essential nutrients that are low in the Western diet such as omega-3 fatty acids and monounsaturated fatty acids relate to relational and item memory function using both direct behavioral and indirect eye movement measures (Hannula et al., 2010), which have been shown to be a veridical index of prior exposure in children (Richmond & Nelson, 2009; Monti, Hillman, & Cohen, 2012).

**Methods**

**Participants**

As part of the larger Fitness Improves Thinking (FITKids) study (see Castelli, Hillman, Hirsch, & Drollette, 2011; Kamijo et al, 2011; Monti, Hillman, & Cohen, 2012), fifty-two pediatric participants age 7-9 (27 males; aged 8.66 +/- 0.08 years) were recruited from the community to participate in this study (see Table 6.1 for additional demographic information). All participants provided written assent and legal guardians provided written informed consent in accordance with the regulations of the University of Illinois Institutional Review Board. Children were screened for serious medical conditions, neurological or attentional disorders, physical disabilities, psychoactive medication status, and normal or corrected-to-normal vision using a parental report health history questionnaire. Data were also collected on several additional characteristics that may be related to dietary intake: IQ, socioeconomic status (SES), body mass index (BMI), and pubertal status. BMI values were converted to z-scores as recommended by the U.S. Centers for Disease Control and Prevention (Kuczmarzski et al. 2002). Fluid and crystallized intelligence were assessed using the Kaufman Brief Intelligence Test K-BIT; Kaufman
& Kaufman, 1990) or the Woodcock-Johnson Tests of Cognitive Abilities (Woodcock, McGrew, & Mather, 2001). SES was estimated based on household income, participation in a school meal-assistance program, maternal and paternal education levels, and how many parents work full-time (Birnbaum et al., 2002). Finally, pubertal status was assessed using the Tanner Staging Scales (Tanner, 1962; Taylor et al., 2001). All participants were in Tanner stages 1 or 2, indicating that they had not yet, or had only just begun puberty.

**Memory Task**

Children participated in a memory task designed to assess both hippocampal-dependent relational memory and hippocampal-independent item memory. The task was divided into eight study-test blocks each designed to test either relational or item memory. Stimuli included 216 novel creatures created in Spore Creature Creator (Electronic Arts Inc., California; see Figure 6.1 for examples) and were presented using Presentation software (Neurobehavioral Systems, http://nbs.neuro-bs.com) on a 21” color monitor. Creatures were presented on black backgrounds and resized to 480 × 480 pixels. One-hundred ten color images of real world scenes measuring 1280 × 1280 pixels and taken by Brand X photography were used as scene backgrounds.
Figure 6.1: Task. Left panel depicts single trial progression in the study and test phases. Right panel depicts example study and test trials from the Relational condition (top) and Item condition (bottom).

An Eyelink 1000 eye-tracker (SR Research, Ontario, Canada) was used to remotely record eye-movements. The eye-tracker was calibrated immediately prior to each test block of the memory task. A desk-mounted gel-padded chin rest was used to minimize head movement during eye-tracking data collection.

Item memory was assessed in two study-test blocks. In each block, participants were shown 18 creature-scene pairings in which a scene preview was shown for 3000 ms at which time a creature will be superimposed atop the scene. The background scene was the same for all 18 study trials during each Item block. The creature-scene pairing then remained on the screen for 5000 ms. During this period, and to encourage item encoding of each creature, participants were instructed to press one of two buttons in response to whether they judged the creature to look more like a “boy” or a “girl.” A fixation cross was presented for 2000 ms between each study pairing. Following the study period, a test phase was presented in which three creatures, one studied and two novel, were simultaneously presented atop the studied scene for 6000 ms, following a 3000 ms scene preview. Participants were instructed to press a single response
button when they felt they knew which creature had been studied. After the 6000 ms test display, a display showing three white boxes where the creatures had appeared prompted participants to voice to the experimenter which creature they selected. Confidence in that decision was then assessed using a three-point scale (1 = “not sure,” 2 = “kind of sure,” and 3 = “very sure”). Given that two creatures were novel and one studied, the Item condition could be solved using familiarity alone, an ability that has been shown to be independent of the hippocampus (Davachi, 2006; Davachi & Wagner, 2002).

Relational memory was evaluated in separate blocks. In Relational memory blocks, participants studied 18 unique creature-scene pairings. To encourage relational encoding, participants were asked to press one of two buttons to indicate whether they judged each creature to live in that habitat (scene) “alone” or “with others.” Following each study block, participants were tested on six probe trials in which they were instructed to find the creature originally studied with that scene. Each test trial consisted of one of the 18 studied backgrounds superimposed with three of the creatures studied in that block. One of the creatures had been studied with that scene and two had been studied with other scenes; familiarity across the three creatures was thus matched, necessitating the employment of relational memory.

Lists of stimuli presented in the Item or Relational conditions and target location on test trials were counterbalanced across participants. Additionally, order of study-test blocks was counterbalanced across participants in the following manner: “Item, Rel, Rel, Rel, Item, Rel, Rel, Rel” or “Rel, Rel, Rel, Item, Rel, Rel, Rel, Item.”

**Nutritional Intake Assessment**
Intake of various foods was assessed using both the Youth and Adolescent Food Frequency Questionnaire (YAQ), which has been validated for use in 9-18 year olds (Rockett et al., 1997). This questionnaire, adapted from an adult food frequency questionnaire, contains 152 questions pertaining to various foods and dietary habits over the preceding one year. Questionnaires were analyzed by the Harvard School of Public Health, and outcome variables included servings of each measured food per day, estimated total caloric intake per day, and total intake of various nutrients per day. Participating families were given instructions for the child and parent to complete the questionnaire together.

**Aerobic Fitness Assessment**

Potential confounding factor of aerobic fitness was evaluated using an exercise test that measures maximal oxygen consumption (VO₂ max) during physical activity. VO₂ max scores were assessed using a computerized indirect calorimetry system (ParvoMedics True Max 2400) during a modified Balke treadmill test (American College of Sports Medicine, 2010). During the test, participants’ heart rate was constantly monitored using a Polar heart rate monitor (Polar WearLink® +31, Polar Electro, Finland) and individual subjective rate of perceived exertion (RPE) was assessed every 2 minutes using the children’s OMNI scale of perceived exertion (Utter et al., 2002).

The VO₂ max test included an initial warm-up period in which the treadmill gradually increased in speed while the children walked on it. After this warm-up period, treadmill speed remained constant while incline was increased 2.5% every 2 minutes. Average oxygen consumption and respiratory exchange ratio (RER) were measured every 20 seconds during the test using a
mouthpiece. Maximum oxygen consumption (VO₂ max) was measured in milliliters per kilogram per minute and based on maximal effort, which was defined using two or more of the following criteria: 1) age-defined maximum heart rate norms (i.e., heart rate > 185 bpm), 2) RER (CO₂:O₂) greater than 1.0 (Bar-Or, 1983), 3) subjective RPE greater than 8, and 4) leveling of VO₂ despite increasing aerobic demand.

**Data Analysis Approach**

Eye-movement data were divided into trials in which participants correctly selected the matching target and trials in which participants incorrectly selected a competitor and were analyzed using response-locked time courses. Response-locked time courses of eye-movement data were analyzed using Accuracy (Correct, Incorrect) × TimeWindow ((-2500)-(-2000), (-2000)-(-1500), (-1500)-(-1000), (-1000)-(-500), (-500)-Response, Response-500, 500-1000, 1000-1500, 1500-2000, 2000-2500) repeated measures ANOVAs using Greenhouse-Geisser correction where appropriate.

Eye movement time courses were quantified using preferential disproportionate viewing (PDV), which was defined as the difference in proportion of time spent viewing correctly-selected matching creatures relative to proportion of time spent viewing incorrectly-selected creatures, prior to behavioral response (Figure 6.2). A priori t-tests comparing viewing to correctly-selected matching creatures relative to incorrectly-selected creatures were corrected for multiple comparisons using Bonferroni correction.
Nutrition questionnaires were analyzed by an algorithm implemented by the Harvard School of Public Health, with an output measure that provides information on intake of individual macro- and micronutrients (Rockett et al., 1997). Nutrient intake was normalized by average total daily calorie consumption within participant (Willett, Howe, & Kushi, 1997).

**Results**

Participant characteristics are presented in Table 6.1.

**Behavioral Results**

Behavioral proportion correct was $0.635 \pm 0.017$ for the Relational condition and $0.865 \pm 0.012$ (Mean ± SEM) for the item condition. Mean response time was $2504.19 \pm 67.88$ for Relational trials and $2713.78 \pm 69.30$ for correct Item trials. Two-tailed paired-sample t-tests show a significant difference in behavioral accuracy ($t_{51} = 15.17, p < 0.001$) and response time ($t_{51} = 3.21, p < 0.002$) between conditions such that participants were significantly more accurate on Item trials but responded significantly faster on Relational trials. These significant differences can be explained by a speed-accuracy tradeoff, but based on previous research using the same paradigm in adults (Hannula, Ryan, Tranel, Cohen, 2007), it is more likely that the scene preview, which was unique to the Relational condition, allowed for faster response times in the Relational condition than in the Item condition. This is likely due to the possibility of reactivation of the relational binding during the scene preview in that condition (Hannula, Ryan, Tranel, & Cohen, 2007) but the absence of reactivation in the Item condition, as the scene was non-diagnostic of which creatures would appear on each Item trial.
Eye Movement Results

Response-locked time courses in the Relational memory condition show a significant main effect of Accuracy ($F_{1,51} = 22.57, p < 0.001$), significant main effect of Time Window ($F_{4,56,232.97} = 19.342, p < 0.001$), an no Accuracy × Time Window interaction ($F_{5.59,284.98} = 1.35, p < 0.24$). The Relational condition shows significant PDV emerging prior to response, during the -1000 to -500 ms time bin and sustaining through response, Bonferroni corrected for multiple comparisons.

Response-locked time courses in the Item condition show a significant main effect of Accuracy

![Graph of Item and Relational PDV](image)

Figure 6.2: Response locked eye-movement time courses depicting viewing to selected creatures. Vertical dotted line indicates behavioral response. PDV, preferential disproportionate viewing, which was assessed prior to behavioral response. $(F_{1,41} = 24.95, p < 0.001)$, main effect of Time Window $(F_{5.29,216.92} = 10.03, p < 0.001)$ but no Accuracy × Time Window interaction $(F_{6.05,247.94} = 0.979, p < 0.44)$. Comparisons of individual time points show significant PDV in the Item condition emerging 1500-1000 ms prior to response, continuing through the 1000-500 ms prior to response, then becoming non-
significant in the 500 ms leading up to response, Bonferroni corrected for multiple comparisons.

**Correlations Between Nutritional Intake and Relational and Item Memory**

Bivariate correlations show a significant positive correlation between behavioral accuracy on the Relational condition and omega-3 fatty acid intake ($r = 0.281, p = 0.044$). Behavioral accuracy on both the Relational and Item conditions was negatively correlated with saturated fat intake ($r = -0.315, p < 0.023$ and $r = -0.323, p < 0.019$, respectively). Furthermore, PDV in the Item condition was marginally correlated with saturated fat intake ($r = -0.255, p < 0.068$; Figure 6.3). Measures of total sugar, added sugar, trans fatty acids, and monounsaturated fatty acids were not significantly related to performance (all $p$s $> 0.05$).
Figure 6.3: Scatterplots depicting relationships between intake of Omega-3 fatty acids and Saturated Fats with Item and Relational Accuracy. Each dietary intake measure is expressed as milligrams per kilocalorie consumed per day.

Next, to determine whether measures of age, sex, SES, IQ, VO$_2$max, and BMI z-score may explain these correlations, we performed bivariate correlations, one-way ANOVAs, or t-tests, where appropriate, comparing each of those measures relative to the dietary intake values of interest. Results showed that BMI z-score was positively related to intake of trans fatty acids ($r = 0.281, p < 0.044$), but that all other relationships failed to reach significance (all $ps > 0.05$), indicating that they did not account for a significant portion of the variance observed in dietary
intake. Partial correlations thus included BMI z-score as a factor, but not age, sex, SES, IQ, or VO₂max.

Partial correlations of the dietary factors with behavioral memory accuracy measures adjusting for BMI z-score showed the same pattern of results as the uncorrected correlations, such that omega-3 intake was positively correlated with relational accuracy \((r = 0.292, p < 0.037)\), and that saturated fat intake was negatively correlated with both Relational \((r = -0.305, p < 0.030)\) and Item \((r = -0.302, p < 0.031)\) accuracy. Interestingly, adjusting for BMI z-score changed the eye-movement results such that PDV in the Relational condition was negatively correlated with intake of added sugar \((r = -0.336, p < 0.016)\). All other relationships failed to reach significance (all \(ps > 0.05\)).

**Discussion**

The major findings in the current study were that intake of omega-3 fatty acids was positively correlated with behavioral accuracy on a hippocampal-dependent relational memory task, and that intake of saturated fat was negatively correlated with behavioral accuracy on both the relational memory task and a non-hippocampal-dependent item memory task. These relationships remained significant even after adjusting for BMI z-score. Furthermore, eye movement results showed that viewing prior to response in the Item condition was related to intake of saturated fat such that individuals who had lower intake of saturated fat showed greater preferential viewing of previously viewed stimuli. After adjustment for BMI z-score, this
relationship became non-significant, but a significant negative relationship emerged between preferential viewing in the Relational condition with intake of added sugar.

That the behavioral correlations with diet remained significant after adjusting for BMI z-score indicates that the observed relationships between dietary intake and memory performance were not simply due to greater body weight. Studies in rodents have observed a similar effect, such that dietary intake of fats or sugars affected cognition even before weight gain or accumulation of adipose tissue was evident, or in which intake of a particular nutrient was associated with memory impairment in only one of two groups of weight-matched experimental animals (Kanoski & Davidson, 2010; Lindqvist et al., 2006; Jurdak & Kanarek, 2009).

The current results further converge with the animal literature in that omega-3 and saturated fat have been shown to facilitate or impair behavioral measures of hippocampal memory, respectively (Innis, 2007; Gómez-Pinilla, 2008; Wu, Ying, Gómez-Pinilla, 2008). For example, Wu, Ying, & Gómez-Pinilla (2008) showed that rats fed chow supplemented with omega-3 fatty acids displayed enhanced spatial memory performance relative to animals fed standard chow (Wu, Ying, Gómez-Pinilla, 2008). In contrast, rodents consuming diets high in saturated fat and refined sugar have been shown to be impaired in hippocampal-dependent spatial memory relative to rats fed control diets (Boitard et al, 2012; Kanoski & Davidson, 2010; Kosari et al., 2012; Lindqvist et al., 2006; Molteni et al., 2002, 2004; Park et al., 2010). Similarly in human adults, high intake of saturated fat has been associated with global cognitive impairments as
well as impairments in verbal and prospective memory (Kanoski & Davidson, 2011; Okereke et al., 2012)

Several possible factors may mediate the observed relationships between dietary intake of omega-3 fatty acids, saturated fatty acids, and memory performance. Indeed the Western diet has been shown to cause neurophysiological changes in neurogenesis, reduced levels of neurotrophins including brain-derived neurotrophic factor (BDNF), interference with glucose regulation, increased oxidative stress and neuroinflammation, and to have deleterious effects on the blood-brain barrier (BBB). Each of these has been shown to affect hippocampal structure or function (see Kanoski & Davidson, 2011).

First, the low levels of omega-3 fatty acids in a typical Western diet may interfere with neurogenesis, as omega-3 fatty acids are important for the creation and maintenance of neuronal plasma membranes (Wu, Ying, Gómez-Pinilla, 2008; Innis, 2007). Compounding this effect, high intake of saturated fats can further reduce the formation of new neurons potentially though its role in inhibiting the formation of BDNF (Molteni et al., 2002).

BDNF is not only important for neurogenesis, however, and the reduced levels of neurotrophins including BDNF, may affect mature hippocampal neuronal communication as well. BDNF is critical for synapse function, and also has a role in protecting neurons from stress and disease (Molteni et al., 2002; McAllister, Katz, & Lo, 1999; Radecki, Brown, Martinez, & Teyler, 2005). Specifically related to hippocampus, a diet rich in omega-3 fatty acids promotes BDNF synthesis
(Wu, Ying, Gómez-Pinilla, 2008). In contrast, high dietary intake of saturated fats has been shown to reduce hippocampal BDNF secretion, thereby reducing the efficacy of BDNF in promoting cognitive function (Molteni et al., 2002).

A third factor that may mediate the observed relationships between dietary fat intake and memory performance is glucose regulation and related insulin resistance. Consumption of a high fat diet may increase insulin resistance in the brain (Alsaif & Duwaihy, 2004). Moreover, intake of saturated fats in particular has been shown to interfere with hippocampal insulin signaling (Kanoski & Davidson, 2011). Further demonstrating the role of insulin in hippocampal function, one study showed that direct hippocampal infusion of insulin enhanced spatial memory in control rats but not in animals fed a high fat diet (McNay et al., 2010). Conversely, sufficient dietary consumption of omega-3 fatty acids has been shown to be necessary for proper insulin receptor signaling in the hippocampus, at least in the presence of high fructose intake (Agrawal & Gómez-Pinilla, 2012).

Fourth, saturated fatty acids have been demonstrated to increase oxidative damage to neurons through increased production of free radicals in the brain, causing cell death and impairing cellular communication (Wu, Ying, Gómez-Pinilla, 2004b). Supplementation with antioxidants such as vitamin E or curcumin, found in higher levels in traditional diets, apparently reverses this damage and the spatial memory impairment that accompanies it, thus supporting the likelihood of this mechanism playing an important role in mediating the relationship between dietary intake of saturated fat and cognitive impairment (Wu, Ying, Gómez-Pinilla, 2004b).
Similarly, omega-3 fatty acids have been shown to ameliorate neuronal oxidative stress (Bazan, 2006).

Accompanying this increase in oxidative stress comes an increase in neuroinflammation secondary to dietary intake of saturated fats. Saturated fats have been shown to increase neural inflammation as marked by increased expression of inflammatory cytokines IL-6 and TNF-α, and chemokine MCP-1 (Pistell et al., 2010). This is particularly worrisome for cognitive health as brain inflammation has been related to cognitive deficits later in life such as increased risk of developing Alzheimer’s disease (Akiyama et al., 2000). Intake of omega-3 fatty acids, however, has been shown to reduce expression of proinflammatory genes (Bazan, 2006).

Finally, a sixth potential mediator of the observed relationship between Western diet and memory function is that intake of saturated fats has been shown to reduce the integrity of the BBB, another risk factor for developing Alzheimer’s disease (Kanoski & Davidson, 2011). Reduced integrity of the BBB may allow heavy metals access to the brain or allow β-amyloid to enter the brain. Interestingly these effects may affect the hippocampus and resulting functionality earlier than other cortical memory systems (Kanoski & Davidson, 2011). It is not yet known whether omega-3 fatty acids may be protective against dietary fat induced BBB damage (Kanoski & Davidson, 2011).

Dietary saturated fat intake was further shown in the current study to be related to decreased preferential viewing in the Item condition (Figure 6.3). Why this pattern of viewing
accompanied behavioral deficits only in the Item condition, and not the Relational condition is not clear. One possible reason is the size of the observed effect, with greater PDV in the Item condition separating viewing to correctly versus incorrectly selected creatures (Figure 6.2). This would allow for a larger range with which to observe more subtle relationships with diet. Additional examination of this effect is warranted.

In addition to an unbalanced ratio of dietary fat intake, another hallmark of the Western diet is high intake of refined sugars, which were found in the current study to relate to reduced PDV in the Relational condition, after adjusting for body size using BMI z-score. While it is not clear why this effect emerged only after accounting for body size, diet high in refined sugars has been shown in animal models to cause impairments in learning and memory independent of body weight (Jurdak & Kanarek, 2011), and in healthy children to cause impairments in immediate memory after ingestion of sugars (Benton, Maconie, & Williams, 2007). When taken together with a diet high in saturated fat, as in the Western diet, diet high in refined sugar causes impairments in hippocampal spatial memory (Jurdak, Lichtenstein, & Kanarek, 2008). Like saturated fat, the deleterious effects of high fructose consumption on memory performance can be counteracted by adequate levels of omega-3 fatty acids in the diet (Agrawal & Gómez-Pinilla, 2012).

**Conclusion**
The increasing prevalence worldwide of the Western diet, rich in saturated fats and refined sugars, while low in omega-3 fatty acids is beginning to cause concern about brain health and cognitive function, in addition to the well-established negative consequences on the cardiovascular system. The current study found that prepubertal children who consumed higher amounts of saturated fats showed impairments in hippocampal-dependent relational memory and cortical-dependent item memory relative to children who consumed lower quantities of saturated fats and that children who consumed greater quantities of omega-3 fatty acids showed better relational memory than children who consumed lower amounts of omega-3 fatty acids. The low quantities of omega-3 fatty acids in a typical Western diet simply may not be sufficient to support proper neuronal growth and communication. This would then compounded in the hippocampus where creation and integration of new neurons would be impaired. That these effects can be observed after just a few years of these dietary patterns among prepubertal children raises concerns not only for brain health, but particularly in regard to the learning capabilities of children consuming a typical Western diet, in that children who are less able to learn both relational and item information will likely have worse life outcomes than children who consume a diet higher in healthy fats and antioxidants. The public health consequences of early intervention to improve these dietary intake patterns in children are far reaching, and may include cognitive health, mental health, workplace productivity, economic welfare, and life satisfaction. Thus the current findings underscore the importance of developing healthy eating habits early in life.
References


Wu, A., Ying, Z., & Gómez-Pinilla, F. (2004b). The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. European Journal of Neuroscience, 19, 1699-1707.


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### Tables

**Table 6.1. Participant demographics, weight status, and fitness**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Females (n = 25)</th>
<th>Males (n = 27)</th>
<th>All children (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>8.61 (0.13)</td>
<td>8.70 (0.11)</td>
<td>8.66 (0.08)</td>
</tr>
<tr>
<td>IQ</td>
<td>114.92 (2.34)</td>
<td>111.33 (2.85)</td>
<td>113.06 (1.86)</td>
</tr>
<tr>
<td>SES (n [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>5 (20)</td>
<td>9 (33)</td>
<td>14 (27)</td>
</tr>
<tr>
<td>Middle</td>
<td>7 (28)</td>
<td>8 (30)</td>
<td>15 (29)</td>
</tr>
<tr>
<td>High</td>
<td>13 (52)</td>
<td>10 (37)</td>
<td>23 (44)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>17.59 (0.58)</td>
<td>19.35 (0.80)</td>
<td>18.50 (0.51)</td>
</tr>
<tr>
<td>BMI-for-age percentile</td>
<td>61.00 (5.16)</td>
<td>73.75 (5.32)</td>
<td>67.62 (3.79)</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>0.38 (0.19)</td>
<td>0.91 (.21)</td>
<td>0.66 (0.14)</td>
</tr>
<tr>
<td>Underweight</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Normal Weight</td>
<td>19 (76)</td>
<td>12 (44)</td>
<td>31 (60)</td>
</tr>
<tr>
<td>Overweight</td>
<td>2 (8)</td>
<td>6 (22)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>Obese</td>
<td>3 (12)</td>
<td>8 (30)</td>
<td>11 (21)</td>
</tr>
<tr>
<td>VO₂ max, ml/kg/min</td>
<td>40.26 (1.15)</td>
<td>41.83 (1.22)</td>
<td>41.08 (0.84)</td>
</tr>
<tr>
<td>Relational memory accuracy (%)</td>
<td>62.09 (2.65)</td>
<td>64.84 (2.22)</td>
<td>63.51 (1.71)</td>
</tr>
<tr>
<td>Item memory accuracy (%)</td>
<td>85.56 (1.97)</td>
<td>87.55 (1.57)</td>
<td>86.59 (1.25)</td>
</tr>
<tr>
<td>Relational memory response time, correct trials only</td>
<td>2304.41 (100.50)</td>
<td>2689.17 (77.68)*</td>
<td>2504.19 (67.88)</td>
</tr>
<tr>
<td>Item memory response time, correct trials only</td>
<td>2472.28 (94.75)</td>
<td>2937.40 (80.44)*</td>
<td>2713.78 (69.30)</td>
</tr>
</tbody>
</table>

Data values indicate mean (S.E.M.) unless otherwise specified. Weight stats was based on the Centers for Disease Control and Prevention BMI-for-age growth charts.

IQ, intelligence quotient; SES, socioeconomic status BMI, body mass index

*p < 0.05 as calculated by t-tests
CHAPTER 7: GENERAL DISCUSSION
General Overview

The current investigation aimed to examine the sensitivity of memory, particularly relational and item memory, to health factors such as fitness, body size and composition, and nutrient intake across several time points in the first few decades of life. Furthermore, in so doing, we aimed to examine how early in development these effects may be evident. The public health and economic implications of understanding these relationships between physical health and fitness with the developmental time course of memory abilities have potential to be enormous, particularly given the increasing rates of obesity in both children and adults. The current set of experiments specifically targeted the functionality of the hippocampus due to the known roles of fitness and nutrition on this brain region. So as the function of the hippocampus was expected to be particularly sensitive to fitness and nutritional intake, all but one of the individual studies tested the specificity of behavioral and eye-movement effects to hippocampal-dependent relational memory as contrasted with non-hippocampal-dependent item memory. This discussion first considers how these effects unfold across time points in development, specifically middle childhood, early adolescence, and young adulthood. It then examines the relative strength of these relationships across health factors within age group. Finally, it closes with a discussion of observed novel cognitive findings across sampled age groups and several directions for future research in this area.

Major Findings

Significant relationships between fitness, health, and nutritional factors were evident in each sampled age group in the current investigation. Interestingly not all tested effects were
apparent for each age group, beginning to hint at a developmental trajectory for the influence of each of these lifestyle factors across childhood to young adulthood. Moreover it is of interest that these effects were shown to emerge as young as preadolescence (ages 7-9), the youngest developmental time point assessed. That these effects were not only present by this age, but that they were already fairly robust, suggests that health and fitness factors may begin to interact with memory function early in development, after less than a decade of postnatal brain and body development. Furthermore this suggests that the known positive and negative effects on the brain of these lifestyle factors decades later in adulthood and old age may begin to take root as early as childhood, potentially setting children on particular developmental trajectories at or before this age. Specifically, aerobic fitness, healthy body weight, and high quality nutritional intake may enhance potential for growth and protect against eventual cognitive decline while poor nutrition, low aerobic fitness, and large body mass, particularly caused by high central adiposity, may do the opposite in setting the course for arrested cognitive development and hastened cognitive decline later in life.

**Cross-Sections Across Age Groups**

**Aerobic Fitness Across Age Groups.** Vis-à-vis aerobic fitness, relational and item memory showed opposite developmental trends with the strength of the relationship increasing across time points for relational memory and diminishing for item memory. Specifically, we observed a positive relationship between aerobic fitness and relational memory among prepubertal children, although that relationship was attenuated by adjustment for sex and SES (Chapter 4). Into the middle school years, we found no significant relationship between aerobic fitness (or,
at least a proxy measure of aerobic fitness) at the start of sixth grade, but a significant relationship emerged at the end of sixth grade, suggesting that this relationship may strengthen across this period of development (Chapter 3). Further reinforcing the idea that this relationship strengthens over development was the finding that aerobic fitness was strongly positively correlated with relational memory performance in young adulthood (Chapter 2). Interestingly, a different pattern emerged across time for cortical-dependent item memory. In the youngest group, the prepubertal children, item memory was indistinguishable from relational memory in that both showed significant positive relationships with aerobic fitness before adjustment for demographic factors (Chapter 4), however this relationship extinguished by young adulthood with no discernable correlation between VO$_2$max and item memory performance (Chapter 2).

That these two types of memory that rely on different neural substrates showed differing patterns of connection with fitness across development suggests that fitness may differentially affect different neural structures. The question then becomes what might explain these differences at these two developmental time points. In adults, one major difference between the hippocampus and medial temporal lobe cortex is that the hippocampus is known to undergo neurogenesis (Kaplan & Hinds, 1977). Indeed studies in both the human and animal literature link this hippocampal neurogenesis to physical activity, which is in turn strongly correlated with aerobic fitness (van Praag, Christie, Sejnowski, & Gage, 1999; Wu, Ying, & Gómez-Pinilla, 2008). Thus it seems likely that one of the major underlying mechanisms of this relationship in adults is that fitness is linked to enhancement of neurogenesis, which would affect the hippocampus but not the surrounding cortical memory structures. We would expect
then that aerobic fitness would play a larger role in modulations in hippocampal memory performance relative to cortical memory performance in the mature brain, and that is what we observed in the current investigation (Chapter 2).

In prepubertal children, we observed a different pattern of results, in which there were broader effects of fitness, connecting it with both relational and item memory performance (Chapter 4). This is consistent with the idea that neurogenesis may be the link between fitness and relational memory performance, but it does not explain why item memory may also be related to fitness in children. One possibility for this effect is that fitness might have more global effects across the developing brain as it establishes local and long-range connections, simultaneously impacting the effectiveness of both cortical memory mechanisms and hippocampal memory mechanisms.

**Body Mass Index Across Age Groups.** In contrast to the effects observed for aerobic fitness, body mass index (BMI) effects diminished across developmental time points for both relational and item memory. BMI predicted relational memory among prepubertal children, with the effect largely due to a strong negative relationship within the group of children classified as overweight or obese (Chapter 4). By middle school, however, BMI was no longer predictive of relational memory performance (Chapter 3), and this effect remained null through the young adult time point (Chapter 2). Similarly, BMI was negatively correlated with item memory performance in prepubertal children, again largely due to the overweight and obese children (Chapter 4). We did not assess item memory in the middle school sample, but by young adulthood BMI was no longer predictive of item memory performance (Chapter 2).
Given that the relationships between BMI and memory did not differ between memory types across development, we can infer that BMI has a different means of action on the brain than does aerobic fitness, for which relational and item memory showed different patterns across age groups. Mechanisms for this health factor are likely then related to broader processes such as increasing inflammation or dysregulating glucose homeostasis that may take action across the whole brain, or at minimum throughout the medial temporal lobes (see Chapter 5 for additional discussion).

Taken together, these findings nod toward the idea that different health factors may act on the brain via different mechanisms including neurogenesis and neuroinflammation. What specific mechanisms underlying these effects are, and to what degree they are related to cognition remains to be determined. One common thread among these mechanisms that may be particularly important is neuroinflammation, in which systemic inflammation activates microglia in the brain to release proinflammatory and neurotoxic cytokines as well as reactive oxygen species, which in turn increase oxidative damage to neurons. These processes seem to be exacerbated by large body size, high intake of saturated fatty acids, and low intake of omega-3 fatty acids, and ameliorated by physical activity and the oft accompanying higher aerobic fitness, although the relative weightings of these health factors on neuroinflammation is not known.

**Cross-Sections Across Health and Lifestyle Factors**

In addition to providing the basis for initial characterization of the developmental trends of these health and fitness effects, examination of multiple effects within the same group of
prepubertal children allowed for direct comparison of the contribution of each factor to
relational and item memory performance (at least within the confines of the current
experimental design). Specifically, we were able to compare the effects of aerobic fitness, body
mass, body composition, and nutritional intake within our sample.

**BMI Was Found To Be a Better Predictor of Memory Performance than Aerobic Fitness.** In
Chapter 4, we examined aerobic fitness and BMI in the same group of prepubertal children. By
doing so, we were able to examine the relative contributions of these factors in explaining the
variance within the current sample in relational and item memory performance. We observed
that BMI was a stronger predictor of memory performance than aerobic fitness, although these
effects may be explained by the relatively restricted fitness range sampled and the reduced
variability then across which to observe effects. Regardless of the relationship between these
two health factors, however, these results suggest that maintaining a healthy body weight in
childhood may be important for optimizing brain function and for multiple types of learning
abilities.

**Body Composition Was Found To Be a Better Predictor of Memory Performance than BMI.**
Next, in Chapter 5, we examined not only BMI (converted to z-scores), but further measured to
what degree adiposity was localized to the central region of the body. Separate regression
models adjusting for age, sex, birthweight status, IQ, SES, and VO_{2max} showed that central
adiposity was a significant predictor of relational memory performance, but that BMI z-score
did not explain additional variance beyond those demographic factors\(^1\). Neither BMI z-score nor

\(^1\) Seemingly at odds with Chapter 4, the second step of this model was significant using BMI instead of BMI z-score. A discussion of which of these two measures is more appropriate for this purpose is warranted.
central adiposity explained behavioral item memory performance, although central adiposity was a significant predictor of viewing patterns in the item memory condition, whereas BMI z-score was not. Taken together these results suggest that the interaction with memory performance is more nuanced than simply body size, at least across a broad cross-section of children, and that where fat is stored may be a more meaningful index of how body fat relates to relational memory performance than simply how much fat is stored. In other words, children showing a more android body shape may be more susceptible to negative effects on hippocampal function than children with a more gynoid body shape.

**Intake of Dietary Fat Was Found To Be a Better Predictor of Memory Performance than BMI.**

In Chapter 6, we examined how nutrients that are generally deficient (omega-3 fatty acids and monounsaturated fatty acids) or abundant (saturated fats and refined sugars) in the Western diet may be related to memory function in prepubertal children, as they have been in animal models. After adjusting for total caloric intake (after all children who eat more overall will be expected to have higher intake of all nutrients), consumption of omega-3 fatty acids was found to be positively correlated with relational memory performance, but not item memory performance, and saturated fat was negatively correlated with both types of memory performance. To investigate whether these nutrition effects were simply mirroring the BMI effect and to answer the question of whether BMI is simply the cumulative effect of what one eats, we adjusted the nutritional intake model for BMI and found that these effects of dietary fat consumption remained significant after adjusting for BMI. Similarly, preferential

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2 Amount of fat stored was also found to be an important predictor of brain function, with percent fat mass being correlated with item memory function in Chapter 5. It is simply the case here that it was not found to be related to hippocampal memory function.
disproportionate viewing to the matching creature in the Relational condition was negatively correlated with intake of refined sugars after adjustment for BMI. Taken together these results suggest that in terms of memory function what a child eats may be more important than how much he eats.

**Novel Cognitive Findings Across Age Groups**

Several novel cognitive findings stemmed from the current line of research. First, the cross-sectional eye-tracking study of relational and item memory (Chapter 4) showed that prepubertal children, like young adults (Chapter 2), were able to successfully retrieve prior relational memories when one part of a relational binding was provided (Hannula, Ryan, Tranel, & Cohen, 2007). This was indexed by the fact that both age groups showed preferential viewing to studied relations significantly earlier in time (relative to the onset of the complete binding on Relational trials) than studied items (see Figures 2.3 and 4.2). This is likely due to the fact that when pattern completion was possible, relational retrieval could guide early viewing of probe arrays. That preferential viewing appeared to emerge over a similar timeframe in both prepubertal children and adults (i.e., within the first 500-1000 ms), or even earlier in children than adults, suggests that this aspect of relational memory ability has already begun to mature prior to adolescence. Furthermore, that hippocampal-prefrontal cortex connections via the uncinate fasciculus are not yet mature at this age speaks to the necessity of those connections in driving this effect (Ghetti & Bunge, 2012).
Second, relative to behavioral response, preferential disproportionate viewing emerged similarly across age groups, with children showing a significant effect up to 1500 ms before and adults up to 1000 ms before response. Again this suggests that the processes underlying this effect have begun to mature prior to adolescence. Third, we observed similar viewing effects relative to behavioral response across both Relational and Item conditions for both adults and children (Chapters 2 and 4). This suggests that these response-locked preferential viewing effects may be due to processing of the displays that precedes conscious awareness or identification of the target (Hannula, Ryan, Tranel, & Cohen, 2007).

Limitations & Future Research

While the current investigation was illuminative in an early exploration of the interactions between health and fitness with memory across several developmental time points and relative to one other within developmental time points, it is just the first step in understanding the complexity of these effects across the first decades of life. One limitation of the current investigation was the cross-sectional nature of each experiment. While important for characterizing inter-individual differences, cross-sectional designs cannot address causality, which is critical for understanding the underlying mechanisms of the observed health-memory connections. We aim to speak to this issue of causality in an ongoing randomized controlled trial in which prepubertal children participate in a 9-month intervention designed to increase aerobic fitness. In that trial, the FITKids trial, each school-day children participate in at least 70 minutes of intermittent moderate to vigorous exercise while maintaining their heart rates in a specified aerobic zone. When compared with a wait-listed control group we predict that
children who increase their level of aerobic fitness through this intervention will show enhanced relational memory relative to the wait-listed control group when tested after the intervention. In light of the cross-sectional findings regarding fitness and item memory in this age group (Chapter 4), one possibility is that their item memory performance will also exceed that of the control group potentially by decreasing neural inflammation or increasing synaptogenesis or angiogenesis across the brain (Cotman, Berchtold, & Christie, 2007; van Praag, 2009). Another possibility, however, which would be more in line with previous work (e.g., Chaddock, et al., 2010; Monti, Hillman, & Cohen, 2012), is that the intervention will specifically increase relational memory performance potentially through specific effect on neurogenesis. Which, if either, of these two outcomes we observe will help to guide our understanding of the mechanisms of action of aerobic fitness on memory function in the brain in children.

Second, through diet assessment in the young adult sample, using an adult-specific analog of the food frequency questionnaire used in the child sample, we endeavor to show that similar nutrients and overlapping or related classes of foods (e.g., tuna fish & salmon; fruit punch & sugar-sweetened soda) are similarly correlated with relational and item memory in prepubertal children and young adults. This would offer additional credence to the idea that there are sets of nutrients and foods that are beneficial or detrimental to human cognition as there are in animals (see Gómez-Pinilla, 2008) and would allow us to further speculate on how they might affect the trajectory of development of different brain structures and the different types of memory they support. Another open question here concerns the magnitude of the effects
across the different age groups. On one hand it is possible that nutritional intake matters more in adulthood as young adults have more cumulative effects of nutrition (having simply eaten for more years), but we predict it is more likely that nutritional intake will affect early development to a greater extent as the brain begins to establish regional and network function. We will further examine the relationships between relational memory and dietary intake in toddlers in the third and fourth years of life (aged 32-45 months) to assess whether dietary intake affects how early in development toddlers can perform transitive inference, a form of relational memory that requires flexible use of learned relations.

Third, through assessment of aerobic fitness and nutritional intake in the same young adult sample, we expect that aerobic fitness and nutritional intake will interact in a synergistic manner, such that individuals who eat healthier diets and show higher levels of aerobic fitness will perform significantly better on relational memory tests than either low-fit/healthy diet or high-fit/unhealthy diet groups, which in turn will perform better than low-fit/unhealthy diet individuals. This result would mirror results in the animal literature (van Praag et al., 2007; Wu, Ying, Gómez-Pinilla, 2008) in showing that aerobic exercise can combine with intake of various nutrients to enhance expression of hippocampal neurogenesis and behavioral performance and can buffer the detrimental effects of other nutrients on the same and suggest that similar processes are occurring in the human brain.

Conclusions
As the incidence of childhood obesity continues to grow in the United States and worldwide, an understanding of how lifestyle factors that contribute to this problem affect cognition is of utmost importance in projecting how this problem will manifest in scholastic performance, long-term health and financial success, longevity, and myriad other life outcomes. Based on research in children showing that lower fitness is associated with (Chaddock et al., 2010) and indeed may cause (Monti, Hillman, & Cohen, 2012) impairments in memory development relative to higher fitness, combined with an understanding of our evolutionary history, we can infer that children who are less fit show stunted development of memory abilities relative to what may be otherwise considered normal. Similar claims may be made about nutritional intake as undernourishment (i.e., lack of essential nutrients) has been shown to impair cognitive development in animal models. The current investigation used a mixed approach, sampling in middle childhood, early adolescence, and young adulthood to begin to understand in what ways fitness, nutrition, body mass, and body composition relate to memory across these points in development. In so doing, we aimed to start to elucidate how these lifestyle factors may be associated with the trajectory of memory development over the first several decades of life such that this understanding may be applied to set the foundation for improving dietary and exercise recommendations and policies for children. These recommendations and policies may in turn begin to ameliorate and even reverse the detrimental cognitive effects of the public health epidemic of childhood obesity.
References


