Examining the Effect of a High Quality Dietary Intervention on Cognitive Function in Early Adolescence

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ABSTRACT

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Introduction: Excessive consumption of high fat, high sugar foods may precipitate cognitive decline. This effect may be more pronounced during cognitive development. The present single-blind randomized controlled trial was conducted to examine the effect of a moderate fat, low added sugar dietary intervention on cognitive function in 8-11 yr old preadolescents with a pre-established high fat, high sugar dietary pattern. Participants included 17 non-obese (Body Mass Index Percentile: 25.4 - 91.3) low to middle income preadolescents randomized to 2 weeks of their usual diet (control) or the moderate fat, low added sugar intervention diet.

Method: The intervention diet was restricted to 25% of calories/day from total fat and <10% of calories from added sugar while the control diet was maintained at ≥ 40% of calories/day from total fat and ≥15.9% of calories from added sugar. All food served was measured to the tenth of a gram. Any uneaten portion of food was weighed to obtain accurate measures of actual intake. Nutrition Data System for Research dietary analysis software was used to assess macronutrient, micronutrient and added sugar intakes. Participants were weighed weekly to ensure they remained in energy balance throughout the duration of the study. Pre-post cognitive assessment served as the primary outcome measure. A battery of age appropriate tests from the Penn Computerized Neurocognitive Battery as well as the widely used and a previously validated Trail Making task were

selected to assess executive function, speed of processing, working memory, attention and spatial ability.

Results: Both Analysis of covariance and a repeated measures approach were used to evaluate the mean difference of post-intervention scores between conditions, controlling for pre-intervention scores and other covariates including age, gender, sleep and mood. For each statistical approach, 10 tests were run, encompassing each of the cognitive assessments given and, for some, their delayed counterpart. Based on the Analysis of covariance analysis, participants randomized to the intervention had a faster median response time for correct responses on 2 of the 10 tests analyzed, including the initial facial recognition task and its delayed counterpart. Compared to controls, the intervention group displayed 1) a faster total correct response time while controlling for gender ($p = 0.02$), 2) a faster true negative response time when controlling for gender and age ($p = 0.012$), and 3) a faster delayed task median total correct response time when controlling for gender and age ($p = 0.005$). No significant differences between groups were detected for the other assessments. Based on a repeated measures approach, none of the 10 tests analyzed reached statistical significance. Multiple regression analyses revealed a dose response effect on face recognition response time based on % intake of daily calories from total sugar, added sugar, total fat and saturated fat such that a 10% increase in % calories from total sugar, added sugar and saturated fat decreased processing speed for total correct responses on the initial facial recognition task by 0.58 seconds whereas a 10% increase in % total fat decreased processing speed on the same task by 0.44 seconds. The multivariate regression analyses controlled for gender and pretest scores.

Conclusions: A 2-week MF/LS dietary intervention may improve delayed face recognition in low to middle income preadolescents with a pre-established HF/HS dietary pattern. Although the intervention appeared to demonstrate a positive effect on 2 measures of cognitive function (initial and delayed facial recognition), after Bonferroni correction, these results only remained significant for the delayed task median total correct RT when controlling for gender and age ($p = 0.005$). Thus, study results must be interpreted with caution as they may simply be an artifact of chance finding in the ANCOVA statistical analysis. Further investigation of benefits proffered by decreasing % total sugar, % added sugar, % total fat and % saturated fat intake to preadolescent cognition is warranted. Future work should focus on replicating the present study in a larger sample, using hippocampal-dependent specific tasks.

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GLOSSARY OF KEY TERMS

Allostatic Load (AL) - the body's homeostatic response to chronic stress; typically reported as a composite score based on multiple system (cardiovascular, immune etc.) measures. High allostatic load increases morbidity and mortality risk.

Blood-Brain Barrier -a filtering mechanism of the capillaries that carry blood to the brain and spinal cord tissue, blocking the passage of certain substances.

Brain Derived Neurotrophic Factor (BDNF) - a regulator of nerve growth that supports the creation of new neurons and neuron survival. BDNF also plays a significant role in regulating the strengthening and weakening of neuron signaling (see "synaptic plasticity").

Cognitive Dysfunction - the loss of intellectual functions such as thinking, remembering, and reasoning of sufficient severity to interfere with daily functioning. Patients with cognitive dysfunction have trouble with verbal recall, basic arithmetic, and concentration.

Cognitive Flexibility - the brain's ability to transition from thinking about one concept to another

Cognitive Function - a measure, or set of measures assessing mental processes involved in thinking, remembering, reasoning and attending to information

Dendritic Arborization - the process by which dendrites (a component of neural cells that conduct signals toward the cell body) form branches and extend outward to form synapses

Dentate Gyrus - a subregion of the hippocampus believed to be responsible for visuospatial learning and memory as well as pattern separation.

Executive Function (EF) - the ability to hold onto and work with information, focus thinking, filter distractions, and switch gears in thought processes using working memory, mental flexibility, and self-control. EF enables us to plan, focus attention, remember instructions, and juggle multiple tasks simultaneously.

Glycemic Index (GI) - a measurement indicating the speed at which glucose from a dietary carbohydrate source is released into the blood. It is based on 50 g of available carbohydrate.

Glycemic Load (GL) - the GI of the various foods consumed, multiplied by the amount of carbohydrate in grams provided by each food and divided by 100. Thus it accounts for both the quality *and* the quantity of a dietary carbohydrate source.

Highly Processed Food - defined here as food, or food-like, products that are mass produced, packaged and have become so nutrient deplete in the refining and/or cooking process that supplemental nutrients must be added back to them. End products are typically comprised of refined, nutrient stripped ingredients such as white table sugar, HFCS, refined white flour, numerous additives and preservatives as well as supplements with low bioavailability.

Hippocampus - a small region of the brain that forms part of the limbic system and is primarily associated with memory and spatial navigation.

Interleukin 6 (Il-6) - a pro and anti-inflammatory signaling protein (cytokine) involved in inflammation and infection responses as well as the regulation of metabolic, regenerative, and neural processes.

Minimally Processed Food - nutrient replete food that has undergone little chemical alteration and is recognizably derived from "whole" foods grown in the ground, on plants and trees or from an animal. If factory packaged, this food may be labelled as "unrefined" and contains very few (if any) additives or preservatives.

Myelination - the process of forming a myelin sheath (an insulating protein and fat substance) around a nerve axon to allow nerve impulses to move more quickly.

Neural Tube Defect (NTD)- abnormalities that can occur in the brain, spine, or spinal column of a developing embryo and are present at birth

Synapse - a junction between two nerve cells, consisting of a minute gap across which impulses pass by diffusion of a neurotransmitter

Synaptic Transmission - the process by which a neuron communicates with a target cell across a synapse. Chemical synaptic transmission involves the release of a neurotransmitter from the pre-synaptic neuron, and neurotransmitter binding to specific post-synaptic receptors.

Synaptogenesis - a series of processes involved in the formation of new synapses.

Synaptic Plasticity - the process by which specific patterns of neural activity result in changes in synapse signal strength. This process is thought to contribute to learning and memory

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Dedication

This dissertation is dedicated to my beloved son, Nile Woods, and my parents Rikki Smith and Gregory Tate.

CHAPTER 1: INTRODUCTION

Adequate nutrition is essential to normal human brain development. In addition to meeting energy needs requisite for growth, nutrients play a critical role in brain development by 1. supporting glial cell function, 2. altering gene expression (epigenetic effect), 3. altering the course of neural cell differentiation, synaptogenesis, and dendritic arborization, 4. influencing neurotransmitter synthesis, receptor synthesis and neurotransmitter re-uptake mechanisms and 5. affecting neural metabolism and signal propagation.[1] Thus, insufficient intake of even one key nutrient can adversely influence brain morphology and function. Folic acid deficiency provides a prime and well studied example of the correlation between inadequate nutrient intake and aberrant brain development. Lack of sufficient folic acid between 21 and 28 days after conception greatly enhances risk for congenital malformation of the neural tube, an embryonic cluster of cells that will eventually become the brain and spinal cord.[2] Abnormal organization of this early neural structure can lead to development of spina bifida, anencephaly, encephalocele and other neural tube defects (NTDs). At birth, infants with NTDs may present with a number of physiological and neuropsychological deficits that range in severity from cleft lip/palate, poor motor function and tone, bladder/bowel problems and learning delay to severe intellectual disability, cardiovascular irregularity, malformed intestines and still-birth.[3]

Although certain nutrients are critical to brain development at different periods, the brain is especially vulnerable to damage from inadequate dietary intake between 24 and 42 weeks of gestation. During this time, the brain undergoes considerable and rapid change, including an exponential rate of synapse formation and myelination requisite to later learning, language acquisition, spatial navigation and other cognitive skills.[1] By the fourth year of life, the brain weighs 1,200g - just 200g shy of its adult weight, illustrating the magnitude of this accelerated

growth during infancy and early childhood. Albeit far less rapidly, the brain continues to grow well into adolescence, with regions important for reasoning, planning and social communication maturing last. Sufficient nutrient intake is essential to the brain's rapid growth, maturation and function during this time. Given the brain's protracted course of development, early damage can have profound effects on later cognitive function and academic achievement.[2]

In the US, micronutrient deficiency is primarily associated with potassium, vitamin E, calcium, vitamin A, vitamin C, vitamin D and magnesium.[4,5] Folic acid, iodine and zinc deficiencies are generally less common due to national fortification programs. Folate deficiency in particular dropped from 12% to less than 1% as a result of fortification. However, in the CDC's most recent *Second National Report on Biochemical Indicators of Diet and Nutrition,* iodine insufficiency was observed in women of childbearing age and vitamin D deficiency was reported in almost a third of non-hispanic blacks. In the same report, high levels of iron deficiency were observed in Mexican-American children aged 1 to 5, non-Hispanic blacks and Mexican-American women of childbearing age.[5] There is some limited evidence to suggest that micronutrient deficiency may have important implications for brain health, particularly deficiencies in iron and zinc.[6,7]

Although the majority of the US population appears nutrient replete, the primary source of these nutrients often includes highly processed, high sugar and high fat foods.[8] This is concerning not only because it heightens the public's risk for overnutrition/obesity, but because a high fat, high sugar (HF/HS) diet may have an independent, adverse effect on cognitive function. Emergent evidence suggests that a HF/HS dietary pattern may have a deleterious effect on cognitive function across the lifespan through an inflammation related mechanism.

Although animal studies predominate the existing literature on diet and cognition, there is a growing body of work in humans supporting observations in animal models. In particular, research aimed at elucidating connections between diet, metabolic function and Alzheimer's Disease in the elderly suggests that unhealthy eating habits mid-life may precipitate later cognitive dysfunction.[9] Nascent research is also beginning to examine the relationship between diet and cognitive function in younger populations, but this work is limited. In addition to paucity, many of these studies are merely correlational or include a number of other methodological issues that weaken their contribution to the literature. The present study adds to the sparse body of research on diet quality and cognitive function in humans by applying greater methodological rigor and using relevant neurobiological research as a conceptual framework. More specifically, the present research examines whether consumption of a moderate fat, low added sugar (MF/LS) diet improves cognitive function in an 8 to 11 year old early adolescent population with a pre-established HF/HS dietary pattern.

Rationale for Study Parameters

Age

Early adolescence was specifically selected as the focus of the present study for three reasons. First, early adolescence is when most individuals begin to gain autonomy over the foods they consume, making it a key target point for effective intervention. Second, early adolescence marks a critical period when the prefrontal cortex, an area involved in judgment and planning, begins to mature both functionally and morphologically into an adult-like region. This increases the likelihood that an intervention will not only be thoroughly understood, but implemented. Third, during this period of maturation the brain's oxygen demands, glucose utilization and global cerebral blood flow are all heightened compared to adults (1.3, 1.5 and 1.8 times greater,

respectively).[10] As a result of these increased demands as well as a higher ratio of brain weight to liver weight (1.4–1.6 vs. 0.73 in adults) limited muscle mass for gluconeogenesis and longer overnight fasting periods, early adolescent glycogen stores are greatly depleted. [2,10] These considerable metabolic differences may have important implications for the mechanism by which HF/HS foods alter cognitive function in early adolescents as well as the extent of observed effect.

While many recognize the importance of directly assessing the effect a sustained diet has on pediatric cognition, one major impediment to advancing the work is the potential to precipitate even greater damage than predicted by long-term studies in adults and animals. One way to get around this ethical dilemma is to examine response to HF/HS dietary intervention more indirectly. By examining whether a more healthful dietary pattern can improve cognitive function in children with a pre-existing HF/HS diet, the ethical issue posed by providing HF/HS foods can be circumvented. Evidence supporting the hypothesis that a healthier diet can improve cognitive function has been presented in work with adults and more recently, children.[11,12] To examine this hypothesis in the context of a MF/LS dietary intervention, the present study assessed pre-post cognitive function scores in low-income children, ages 8-11 with a pre-established HF/HS diet. The study was a single-blind randomized control trial that randomized participants to an intervention (MF/LS) diet or usual (HF/HS) diet.[11] Brief summary of the rationale and characteristics of the MF/LS intervention diet are provided below. *(For a more detailed description of the control diet, see Chapter 3)*

The Intervention Diet

A MF/LS dietary intervention was specifically selected to ensure nutritional adequacy. As per literature review and national guidelines, a low fat diet was deemed inappropriate for

developing early adolescents. According to the Institute of Medicine (2005), children and adolescents aged 4-18 years should consume 25-35% of their calories from fat.[13] The nutrient dense food components integrated into the MF/LS diet were identified based on similar work by Nilsson et al.[11] In their study, the authors defined these components as foods that have high anti-oxidative capacity, are rich in long chain n-3 PUFA, contain prebiotic carbohydrates rich in viscous fiber, have a low glycemic index (GI) to improve blood lipid profile, and benefit the gut microflora. Participants on the nutrient dense diet demonstrated significant improvement in several aspects of cognitive function compared to control. The present study plans to incorporate several of the same nutrient dense components as Nilsson et al. to the extent that they help meet each preadolescent participant's recommended intake of macronutrients and essential micronutrients. Additional details on the dietary components of the present study are provided in Chapter 3.

Study Duration

Several meal studies in children have detected significant correlation between diet quality and cognition after a single meal intervention.[14–16] Based on this work, it was expected that, at the very least, similar results and level of significance would be found by carrying out a longer-term study. At best, it was proposed that a longer study might yield more robust results, suggesting that chronic exposure may exacerbate cognitive dysfunction in a dose dependent manner. Due to financial and other logistic constraints, it was determined that our study could not exceed 2 weeks. Given that we successfully completed a 2-week feasibility pilot in August of 2014 with zero attrition, 2 weeks seemed logistically and empirically feasible to carry out, as well as of appropriate duration to test acute changes. A more detailed, evidence based rationale for the 2-week study duration is provided in the literature review (Chapter 2). Finding significant

acute changes in cognition following short term improvement in diet could have important implications for child health policy around nutrition as well as best practices among child health care providers.

Cognitive Assessment

Permission to use a validated, computerized neurocognitive battery (CNB) developed by the Neuroimaging and Cognitive Core (NICC) at the University of Pennsylvania's Brain Behavior Laboratory was granted for the duration of the present study. This particular cognitive battery was selected because it assesses cognitive domains considered in the present study's a priori hypothesis based on prior research in this area, has been validated and normed in preadolescents and could be easily administered to several children at once.

In addition to the computerized battery, a paper and pencil task (the Trail-Making Test) was administered to assess speed of cognitive processing and executive functioning. This assessment tool has been previously validated in preadolescents and used in prior research involving short-term dietary manipulation.[17–19]

Selection of a Low SES Study Population

A low-income early adolescent population was chosen for this study for three reasons. First, a pervasive and persistent academic achievement gap exists between low and higher income children.[20] Although a variety of factors are believed to mediate this gap, dietary quality may play a significant role. Second, HF/HS foods implicated in cognitive decline are ubiquitous in many low-income communities. Several studies indicate that HF/HS foods are more readily available in high poverty, urban areas.[21–23] Third, low-income early adolescents are a highly underrepresented group in the literature.

Overview of Study Design

In addition to addressing demographic underrepresentation of vulnerable populations, the present study aimed to fill major gaps in the literature by assessing and controlling for several variables known to affect cognitive function. It is a pre-post, single-blind randomized control design and the first to examine the short-term impact a 2-week MF/LS dietary intervention has on cognitive function in a low-income early adolescent population while controlling for gender, mood, hours of sleep and SES. The study was premised on the hypothesis that a 2-week MF/LS dietary intervention would result in higher cognitive function scores (p-value < 0.05) and shorter reaction time as compared to control. This hypothesis was proposed based on evidence suggesting that a significant change in cognitive function can be easily assessed following acute (single-meal), dietary manipulation.[14,15,17,24–28] Evidence from these studies also informed selection of the specific cognitive tests administered. Further discussion of these measures is provided in Chapters 2 and 3.

Overview of the Neurobiological Framework

The neurobiological mechanism for the primary study hypothesis is based on evidence in both human and animal models, which suggests that a HF/HS diet induces inflammation at the blood brain barrier and dentate gyrus (a region of the hippocampus involved in spatial ability and navigation). Several studies suggest that inflammation in these regions may cause cognitive changes and even adverse brain events (e.g. stroke).[9,29–42] This effect is likely mediated through interleukin (IL)-6 and IL-1β and/or reduced brain-derived neurotrophic factor (BDNF). As further detailed in Chapter 2, there is compelling evidence to suggest that a HF/HS diet may have a deleterious effect on brain architecture, function, and plasticity through increased inflammation, reduced BDNF, and insult to the blood brain barrier.

Primary and Secondary Study Aims

As previously mentioned, the primary aim of the present study was to examine the effect of a 2-week MF/LS dietary intervention on cognitive function in low-income 9-11 year old children with a pre-existing HF/HS dietary pattern. The second aim of this study was to explore the role that select aspects of allostatic load (AL) may play in moderating the diet-cognition link. AL is defined as the "cost" of adaptation to chronic stress that results in multiple system dysregulation and can lead to adverse physiological, psychological, and psychosocial health outcomes.[43] In the literature, assessment of AL varies.[44,45] The measures of AL selected for this study are a proxy for AL and include three 'secondary' AL outcomes (BMI, waist circumference and blood pressure (BP)). These secondary AL measures are implicated in 'tertiary' AL outcomes, including cognitive dysfunction, which makes the AL concept particularly relevant to the present study framework.[46] By examining AL, we hoped to build upon the neurobiological mechanism proposed for the primary study hypothesis. For this secondary aim, it was hypothesized that 1) select aspects of AL would remain stable from T1 (baseline) to T2 (intervention) and 2) select measures of AL would moderate the diet-cognition link. We surmised that if the hypothesis was supported, it could help further explain the relationship between diet and cognitive function in early adolescence, as well as lay groundwork for further elucidating the neurobiological mechanism underpinning this relationship.

Prediction of AL stability for the intervention period was based on its definition as the body's physiological response to chronic stress that 'lasts several weeks to months'. [43] Provided this definition, we thought it unlikely that a 2-week intervention would significantly change AL. The prediction that baseline AL level would moderate the dietary intervention's effect on cognitive function was based on evidence suggesting a link between poor diet and AL dysregulation.[47] High AL has been correlated with cognitive dysfunction, specifically spatial

ability, delayed spatial recognition and delayed story recall scores.[46] The neurobiological mechanism for the second aim was predicated on 1) the proposed mechanism for the first aim implicating a role for inflammatory markers, 2) work by McEwen et al. suggesting that multiple system dysregulation brought on by chronic stressors adversely affects cognitive function, and 3) work by Gianaros et al. suggesting that low SES is independently associated with structural impairment in white brain matter.[43,48] This model is supported by studies that show correlations between economic disadvantage and alterations in brain structure across the lifespan.[49–52]

In sum, the two aims for the present study predict that 1) a 2-week MF/LS dietary intervention would improve cognitive function in participants with an established HF/HS dietary intervention, and 2) select aspects of AL would moderate this effect and lay groundwork for further exploring the neurobiological mechanisms implicated in the complex relationship between AL, diet quality and cognitive function.

Significance

Research on the relationship between dietary pattern and cognitive function is currently in its infancy. To date, much of the research on dietary pattern and cognitive function is limited to older adults and animal models. Very few studies have examined the relationship between diet and cognitive function in early adolescents. Given the key role cognitive function plays in later health, academic and career outcomes, this topic should be of paramount concern to child health researchers, health care professionals and policy makers. The existing literature on dietary pattern and cognitive function establishes clear reason to further explore the relationship between these two, particularly in still developing populations who are most susceptible to irreversible damage. In the animal model, poor dietary quality is associated with deficits in cognitive function, break down of the blood-brain barrier (BBB) that serves to protect the brain from the substances in the periphery, oxidative stress, neuronal cell death and other alterations that appear

to specifically target the hippocampus - a region of the brain essential to learning, memory and navigation. In humans, poor diet quality is associated with increased Alzheimer's disease risk and cognitive function impairment. In particular, this cognitive impairment appears to be most significant in the domains of executive function, attention and memory. Thus, the present study aims to drive further investigation into the effect poor diet quality may have on cognitive function so that the most prudent public health policies, information and guidelines can be disseminated to the public.

Scope & Delimitations

The present research aims to explore the relationship between 2-week dietary allocation (HF/HS or MF/LS) and cognitive functions in a healthy, low-income pre-adolescent population from the greater New York City area with a pre-established HF/HS diet. Thus, the ability to generalize results to the entire population of healthy, US preadolescents is severely limited. Different results may be observed in pre-adolescent children from other areas, socioeconomic backgrounds and baseline diets. Further, given that the intervention includes manipulation of both fat and sugar, this study will not be able to draw conclusions about the contribution of each to observed outcomes. Any cognitive changes observed while controlling/adjusting for confounding, must be attributed to both manipulations (or an unknown confounder). This study is also limited by its assessment of very specific cognitive functions including spatial ability, face recognition, working memory, speed of cognitive processing, executive functioning. Thus, this study can not ascertain the effect of MF/MS intervention on other cognitive functions.

Another limitation to the study design is unrestricted calorie provision. Due to ethical considerations, the study design only allowed for partial control of energy intake. No child was deprived of food if they expressed hunger or coerced into consuming more food than they

wished to eat. To address energy intake as a potential confounder, subjects were weighed each week. If a child gained or lost more than 2.2 lbs (1kg) of weight, we attempted to alter their caloric intake by offering foods of lower or higher caloric density (kcal/gram) or changing initial portions served. Lastly, the study is limited by similarities between diets. To maintain blinding to condition and respect the requests of some parents, both diets were relatively healthful and nutrient dense, providing multiple fresh fruit and vegetable servings throughout the day. It is possible that different results might be observed in a study comparing a diet that is much higher in fat and added sugar and lower in fresh fruits and vegetables to the present MF/LS intervention. This would however require parent compliance, recruitment of a population that consumes virtually no fresh fruits and vegetables, a means of otherwise maintaining equal micronutrient intake between groups and another method of blinding participants to their diets. In summary, the present study is limited to assessing the effect a combination moderate fat, low added sugar intervention has on spatial ability, face recognition, working memory, speed of cognitive processing, executive functioning in healthy, normal to overweight, low-income, NYC preadolescents with a pre-established HF/HS diet.

Research Questions

Q1. Does consuming a moderate fat, low added sugar and nutrient dense (MF/LS) diet for 2 weeks improve certain aspects of cognitive function in low-income pre-adolescents with a pre-existing high fat, high sugar dietary (HF/HS) pattern as compared to control?

Q2. Is there a dose response relationship between pre to post cognitive function performance and % of calories from total sugar intake during the study? % added sugar intake? % total fat? % saturated fat?

Q2. Do select proxy measures of allostatic load (AL) moderate the relationship between dietary pattern and cognition in low-income pre-adolescents with a pre-existing high fat, high sugar (HF/HS) dietary pattern.

CHAPTER 2: LITERATURE REVIEW

Recent literature suggests that excessive consumption of highly processed, high fat, high sugar foods may precipitate cognitive decline in humans (and animals).[2,32,53–58] These findings are especially concerning for younger populations whose cognitive function in infancy, childhood and early adolescence has important implications for literacy and a number of critical life outcomes, including health, academic achievement and socioeconomic status (SES).[59–63]

Given early cognitive dysfunction may result in adverse health and social outcomes, establishing a direct link between a HF/HS diet and children's cognitive performance could have a profound impact on child health policy legislation as well as more local child nutrition initiatives. Schools, already highly motivated to find ways to improve students' tests scores, would likely be among the first sites to institute changes severely limiting children's exposure to HF/HS foods. However, for this to occur, researchers must demonstrate a strong causal relationship between diet quality and pediatric cognition outcomes through rigorous, unbiased and well-controlled investigation. The current study set out to meet these criteria and serve as foundation for larger and longer term randomized control trials. As the literature review below suggests, much of the prior work done in this area is often of too short duration, methodologically weak or merely correlational in scope, establishing no direction for causality.

The design for the present study builds upon prior work by assimilating key strengths and reducing limitations that are either readily apparent or previously identified in systematic review. One of the primary limitations of earlier studies is a failure to sufficiently control for significant confounders. Namely, obesity, allostatic load, SES, physical activity and sleep are all believed to exert a significant, independent influence on cognition that is often unaccounted for in diet and cognition literature.

In the forthcoming literature review, discussion of relevant diet and cognition studies is provided followed by an exploration into possible mechanisms underpinning the diet-cognition link. These mechanisms are primarily examined through the lens of neuro-imaging studies and animal models. Focus is then briefly shifted to discussion of key variables that also affect cognition so that the true contribution of diet can be evaluated and the mechanistic model can be re-examined. Lastly, a summary of the literature findings is provided with particular emphasis on what the present study adds to the current body of pre-adolescent diet and cognition research.

EXAMINING THE EVIDENCE FOR A DIET-COGNITION LINK

Short-term Single Meal Intervention and Cognitive Function

Much of the literature on diet's short-term effect on cognition is focused on infants, adults or the elderly. However, a small set of studies examined this relationship in children, with most focusing on single breakfast meal effect. In one such study, elementary school students (n=30; 15m; 15f; Age range: 9 to 11; BMI range: 14.65 - 37.97; BMI%: not provided) were given either oatmeal (high fiber, minimally processed), ready to eat cereal (low fiber, highly processed) or no breakfast one day a week for four weeks. Authors reported using a counter-balanced cross-over design with participants serving as their own controls. Experimenters were blind to participant condition and cognitive function was assessed one hour after breakfast. Compared to ready to eat cereal, researchers reported that oatmeal consumption significantly improved performance on a backward digit span short term memory task in girls only (M=5.0, SEM=.37 vs M=4.00, SEM=.14). There was no significant difference between the two breakfast types for any of the other cognitive measures.[14]

In a subsequent, virtually identical experiment with 6 to 8 year old participants (n=30; 15m; 15f; Age range: 9 to 11; BMI range: 10.76 - 30.93; BMI%: not provided), Mahoney et al.

reported that compared to ready to eat cereal, oatmeal consumption significantly improved performance on a backward digit span short term memory task in girls (Oatmeal: M=3.6, SEM=.37 vs. Ready to eat: M=2.5, SEM=.32), and a continuous performance auditory vigilance task (Hits: M=36.6, SEM= 2.42 vs. (M=26.8, SEM=3.03); Misses: M=13.4, SEM=2.36 vs (M=24.3, SEM=2.65)).[14]

Given that breakfast meals in both experiments contained equal amounts of fat, sugar and energy, the researchers hypothesized that a difference in level of processing, fiber content, protein content, digestion rate and glycemic score between the cereal and oatmeal may be responsible for the study results.[14] Authors suggested that gender effects may be attributed to mood, which was not assessed. For both experiments, study limitations include lack of BMI range or other weight categorization data, failure to assess significant confounders of cognitive function that may differ from week to week within subjects (eg. sleep, mood and physical activity), use of an assessment that may be poorly suited to test spatial memory (a memorization task requiring subjects to memorize the made up names and locations of countries on a map) and short study duration. Study strengths include a well controlled study design, use of counterbalancing to account for order effect in the provision of meals and cognitive tests, low risk of bias with experimenters were blinded study condition and isocaloric meal comparison.

In a similar 3 day study conducted in adults, subjects (n= 40; Age range: 49-71; BMI range: 20-29) were provided with either white wheat bread (WWB) or white wheat bread enriched with guar gum (G-WWB) in a randomized cross-over design. Guar gum was added to the bread in the second condition to elicit a lower peak blood glucose response and maintain glycemia during the postprandial period.[64] Cognitive function tests of working memory and selective attention were administered in the late potparandial phase (2-4 hrs post-meal), up to

240 minutes after breakfast. Main effects suggested that the group that received the guar gum enriched bread performed significantly better on a test of selective attention ($P<0.01$). No significant differences were observed for selective attention reaction time or working memory. However, authors reported that subjects demonstrating greater glucoregulation prior to intervention performed better on the selective attention task after WWB consumption ($P<0.05$) and had faster reaction times on both tasks ($P<0.05$).[64]

Although the study was not designed to pinpoint a mechanism by which WWB reduced cognitive function, the researchers hypothesized that the WWB may have induced hyperglycemia in subjects who ingested it. They proposed that this hyperglycemic effect could have diminished cognitive function by increasing oxidative stress on the brain, increasing inflammatory marker (IL-6) and cortisol presence or increasing vasoconstriction in the brain. Authors surmised that cognitive benefit proffered by the G-WWB meal may be due to its smoother postparandial blood glucose profile as well as improved brain insulin sensitivity via enhanced GLUT4 receptor presence and activity.[64]

Limitations of this study include incomplete assessment of variables that may vary within subjects over time (eg. sleep and mood), poorly described test selection and short study duration. Merits of the study include its randomized cross-over design, exclusion of overweight and obese participants, exclusion of participants with abnormal fasting blood glucose concentration, assessment of baseline glucoregulation, provision of a standardized meal at the same time across subjects the night before testing and counterbalancing of meal order and test order, provision of meals that were identical in calories and composition except for one ingredient (guar gum) and testing in the late postparandial period based on other studies that have identified significant effect within this window of time.

Another research team found similar results after comparing the effect of a low versus high glycemic index (GI) breakfast cereal (Coco Pops and All Bran, respectively, 35 g portion with 125 ml semi-skimmed milk) on children's cognitive performance (n=64; M:26 F:38; Age range: 6 - 11; BMI range: not provided; BMI%: not provided).[15] Subjects were assessed on nine tasks subdivided for analysis into reports on speed of attention, speed of memory, accuracy of attention, secondary memory and working memory (Cognitive Drug Research (CDR) Computerized Assessment Battery.[65] Authors reported no GI effect on speed of attention, speed of memory, accuracy of attention or working memory. Significant GI effect was only observed for secondary memory in favor of low GI cereal ($p = 0.02$). Authors observed significant effect of assessment time on speed of attention ($p = 0.032$), secondary memory ($p = 0.002$) and working memory ($p = 0.000$) with subjects displaying slower reaction times at 11:40am as compared to 9:40am. Lastly, a significant GI * assessment time interaction was reported as the result of a significant decline in performance at 11:40 am, following the consumption of high GI as compared to low GI cereal ($p = 0.021$).[15]

Based on these findings, Ingwerson et al. posited that the differences observed in cognitive performance between low and high GI meals are not the result of improved cognition, but rather a lessening of natural cognitive decline that occurs over the course of the morning. The authors hypothesized that a low GI breakfast reduces this natural decline in cognition by providing a more steady level of postprandial glucose, as opposed to the oscillating glycemic levels that typically follow a high GI breakfast.[15] A neurobiological mechanism for this effect was not proposed and warrants further investigation.

Significant limitations were noted in the research design employed by Ingwerson et al. Most notably, the study is limited by its short 2-day duration and non-randomized uncontrolled

design, provision of test meals that differed in GI, caloric density, protein, carbohydrate, fat and fiber content, exclusion of details pertaining to GI determination, insufficient BMI data to categorize subjects into a weight category and no assessment of confounding variables associated with cognitive decline within and across subjects (eg. sleep, mood, physical activity, SES and obesity). Further, absence of a no breakfast day condition weakens the study's ability to provide evidence for the morning cognitive decline hypothesis. Strengths of the study design included it's relatively large sample size compared to similar studies, use of counterbalanced meal order presentation and administration of a cognitive battery that previously demonstrated sensitivity to improvements and decrements in cognitive performance following consumption of food components and dietary supplements.

In a systematic review of controlled studies examining the effect of breakfast on children's cognitive function, Hoyland et al. included the work conducted by the Nilsson and Mahoney groups (Ingwerson et al.'s work was also discussed but failed to meet the inclusion criteria for the review).[66] For the review, authors examined forty-five studies published between 1950 and 2008 evaluating the relationship between breakfast intake and composition on cognitive performance in well-nourished and, separately, undernourished and/or stunted children. Only controlled studies that employed objective measures of cognitive function and provided breakfast meals, liquids or snacks of mixed composition (not glucose load) were included.

Based on twenty-one studies of acute breakfast effect in well-nourished children, Hoyland et al. reported that breakfast provision offers some positive cognitive benefits when compared to no breakfast. Further, this effect appears particularly pronounced when cognitive tasks are more demanding and occur later in the morning. With regard to studies examining meals varying in macronutrient composition, GL or GI, the evidence for cognitive benefit is

much less compelling. In part, this finding appears to be due to extensive weaknesses in study design. Lastly, following review of thirteen studies of long-term effects of school breakfast programs and breakfast clubs, Hoyland et al. reported that the positive association between breakfast consumption and academic performance may be an artifact of increased attendance. [66]

On the whole, Hoyland et al. reported that most of the studies lacked scientific rigor with habitual breakfast studies usually receiving the lowest scores. Authors identified numerous methodological flaws in and across the reviewed literature. Across studies Hoyland et al. noted potential for bias from industry funding, insufficient data on diet and cognition in older children and adolescents, little diversity in recruited populations and little attention to cognitive domains outside of attention and memory as weaknesses common to acute diet-cognition literature in well-nourished populations. Within specific studies, reviewers identified the following as significant methodological flaws: 1. a lack of counterbalancing or randomization, 2. unclear blinding procedures, 3. insufficient information on children's nutritional or weight status, 4. reliance on food diaries for dietary assessment, 5. breakfast manipulations that were not matched for energy or volume, 6. lack of palatability assessment, 7. lack of a priori hypotheses about neurobiological mechanism, 8. failure to assess suitability of tests for children of different ages, 9. developmental stages and intellectual level, 10. selection of tests that have not demonstrated sensitivity to nutrient intervention or change over time, 11. omission of accuracy and error rates and 12. inappropriate statistical analysis. [66]

To rectify these issues, Hoyland et al. proposed that, in addition to employing more well controlled study designs, future trials should include measures of motivation (eg. number of trials attempted or frustration tolerance), appetite, mood and repeated measures of glucoregulation. In addition, the reviewers advised that meals be matched for energy but differ

sufficiently in key features (eg macronutrient composition, style or glycaemic response) and assessments span a wide range of cognitive domains with demonstrated sensitivity to nutritional manipulations. Lastly, they emphasized the need for greater examination of the effect of breakfast in older children and adolescents. [66]

In a later study by Micha et al., many of the methodological weaknesses identified by Hoyland et al. were directly addressed. A detailed description of the study method is provided below to underscore its valuable contribution to the literature as well as the development of the current study.

Participants for Micha et al.'s study included seventy-four children matched for age, gender, height, BMI, school and school year (BMI Mean: 19.1). Children were excluded from the study of they were underweight or obese, diagnosed with a medical condition (anaemia or other blood disorders, diabetes or glucose intolerance, other acute or chronic illnesses/diseases, colour blindness, learning disabilities and mood disorders), never consumed breakfast or had allergy or intolerance to any of the components of the breakfast meals.[67]

The study was a randomized cross-over factorial design with 4 conditions: 1. High GI/High GL (55g cornflakes + 7g sugar + 300ml skimmed milk + 200 ml apple juice), 2. High GI/Low GL (66g Alpen Museli + 7g sugar + 200ml skimmed milk + 245 ml apple juice), 3. Low GI/High GL (30g cornflakes + 5g sugar + 300 ml skimmed milk) and 4. Low GI/Low GL (40g Alpen Museli + 5g sugar + 250 ml skimmed milk). Water was used to match meal volume across conditions. Matched children were randomized to High GL or Low GL such that each child was exposed to two conditions and matched pairs were exposed to all four. [67] All four meal conditions were previously tested in a cohort of young adults to assess blood glucose, insulin and cortisol responses in the 0–3 hours following breakfast consumption.[68] Authors reported that

based on calculation from published GI and GL values, there was an estimated 2-fold difference in GL between the high- and the low-GL meals, and a 1.3-fold difference between the low- and the high-GI meals. [67] Details on energy, macronutrient composition, volume, GL and GI were presented for all 4 conditions.

To more clearly delineate neurobiological mechanism, blood glucose and salivary cortisol were measured at baseline and then again before and after cognitive assessment. Cognitive function tests were selected based on their sensitivity to differences in CF following glucose manipulation. Two versions were administered to reduce practice effects. Following CF assessment, participants were asked to complete task demand questionnaires to rate how difficult, effortful and tiring they found the tests to be. Prior to and after breakfast, mood, energy level, hunger and thirst were assessed using a self-rating questionnaire adapted from a prior study involving dietary manipulation. Biomarker, mood and cognitive assessments were completed in the following order: breakfast screening, saliva sample (cortisol), finger prick blood sample, mood scale, breakfast, anthropometric assessment, saliva sample, finger prick blood sample, mood scales, CF testing, task demand questions, saliva sample, finger prick. Mood and cognitive testing occurred 95-140 minutes after breakfast. [67]

Study results suggested that low-GI meals predicted subjective reports of heightened alertness and happiness and reduced nervousness and thirst ($P<0.05$ for each). Micha et al. reported that the high-GL meals predicted subjects feeling more confident, and less sluggish, hungry and thirsty ($P < 0.05$ for each). Biomarker assessment indicated that high-GL ($P < 0.001$) and high-GI ($P = 0.05$) meals increased glucose levels 90 min after breakfast. High-GI meals appeared to increase cortisol levels ($P < 0.01$). After controlling for mood, glucose and cortisol levels, Micha et al. reported that, across all GL groups, low-GI meals predicted better

declarative-verbal memory (P=0·03), whereas high-GI meals predicted better vigilance (P < 0.03). Study authors concluded that the effect of GI breakfast on cognition appears to be domain specific and that a low-GI high-GL breakfast may improve learning. [67]

Micha et al. proposed that the mechanism underlying these observed results may involve interaction between biological response to the GI of the meal and the study setting. For example, they suggested, if blood glucose levels increased in response to a high-GI meal, this event might heighten the body's stress response (via the hypothalamic–pituitary–adrenal axis) and result in higher cortisol levels. Concurrently, cortisol levels might also rise in response to anxiety produced by testing environment. Behaviorally, increased glucose and cortisol levels may manifest as increased anxiety before testing and better performance on vigilance tasks (i.e. faster processing speed), just as the investigators observed in their results. By contrast, investigators expected a low-GI meal to result in lower blood glucose and cortisol levels, reduced feelings of nervousness before the tests and enhanced performance on memory tasks. In sum, Micha et al. posited that a low-GI high-GL breakfast may improve learning by stabilizing glucose and cortisol levels and sustaining normal glycemia in the brain over a prolonged period. [67]

Strengths of the study design include employing randomization, including both GI and GL assessment, isovolumetric meal provision, inclusion of an exceptionally well detailed meal description, careful cognitive testing selection, administration of multiple versions of cognitive measures to reduce practice effects, late postparandial phase cognitive assessment, thoroughly described and validated anthropometric assessment, glucose and cortisol biomarker assessment before and after cognitive testing, mood and task demand assessment and exclusion of underweight, overweight and/or learning disabled participants whose data might confound study results.

The results of the study were limited by the use of participant matching rather than a true randomized cross-over study design, presentation of meals that differed in GI, GL, energy and macronutrient composition rather than by one distinct variable, calculation of GI based on published values for adults which may not accurately capture glucose response in children and a lack of counterbalancing in both meal and test domain presentation.

In a more recent double-blind study by Benton et al., 75 children (M: 28 boys, F: 47; Ages: 5-11 yrs, BMI: not provided, described as "healthy weight") from socially disadvantaged backgrounds were recruited to attend a school breakfast club and on two mornings, at least a week apart.[28] At each study visit participants consumed a meal equivalent in macronutrients, sweetened with either isomaltulose (Palatinose TM) (GL 31.6) or glucose (GL 59.8). Isomaltulose is a fully digestible and absorbable structural isomer of sucrose that provides the same energy as other carbohydrates (4 kcal/g). Compared to sucrose (GI: 65) and glucose (GI:100), Isomaltulose (GI:32) yields a lower postprandial blood glucose and insulin response profile over time. By adding either isomaltulose or glucose to a standard meal, investigators were able to vary GL while keeping energy and macronutrient intake constant.[28]

During meal presentation, participants were encouraged to eat as much as possible but were not forced to consume more than desired. After meal service, any remaining food was weighed to estimate nutritional composition using food tables and information from manufacturers. Meal presentation order was counterbalanced and randomly generated. CF and mood assessment were administered twice following meal intake at 1 and 3 hours. CF tests were administered in the same order both days and included immediate and delayed verbal memory, spatial memory, sustained attention, reaction times, speed of information processing. Mood was

assessed by asking children how they felt 'at this moment' using a non-validated eight-point scale of smiley faces that ranged from very unhappy to very happy. [28]

No significant differences in cognition or mood were observed between meals after 1 hour. However, Benton et al. reported that, compared to the glucose based meal, children's memory ($p<0.01$) and mood significantly improved 3 hours after lower-GL Isomaltose breakfast consumption ($p<0.01$ and $p<0.03$, respectively). Additionally, memory significantly declined between 1 and 3 hours ($p<0.001$) following glucose meal consumption while Isomaltulose intake trended toward improvement at 3 hours. Benton et al. concluded that benefit to memory and mood from lower GI meal intake is due to differences in glycemic properties. Given this effect was more pronounced on the second day compared to the first, the authors hypothesized that the novelty of the task may have worn off and that a lower-GL meal may help an individual persevere with an uninteresting task. [28]

Although a specific biological mechanism wasn't proposed, Benton et al. suggested that, compared with adults, the children's higher rate of glucose utilization may have been an important factor in the study findings.[28] Study strengths include its randomized double blind cross-over design, manipulation of GL while maintaining an identical macronutrient composition between meals, counterbalanced meal presentation, selection of tests that previously demonstrated sensitivity to dietary change in children, examination of CF at specific time points in the early and late postparandial phase, inclusion of an underrepresented group in the literature. Significant study limitations include the use of a rudimentary, non-validated measure of mood, insufficient report of food actually consumed and insufficient details about children's baseline weight.

Although the evidence for acute, low GI associated cognitive benefit is building, some literature supports an opposing hypothesis. More specifically, a number of studies suggest that higher GI/GL meals may improve short-term cognitive performance. In one such study, researchers designed a randomized controlled cross-over design experiment to examine how consumption of an afternoon confectionary snack (likely of high GI but this was not assessed) may affect cognition in 38 boys ages 9 to 11 (Mean BMI: 18.57; BMI range: not provided).[69] The boys were provided either 25g of a "confectionary product" (containing 22g of total carbohydrates (primarily sucrose) and 0g of fat, protein and fiber) or one cup of an artificially sweetened drink matched for sweetness (0g carbohydrate, fat, protein and fiber). The cognitive battery administered 15 minutes later evaluated short and long-term spatial memory as well as attention. Mahoney et al. reported that consumption of the snack generally improved spatial memory (Map task: $p<.05$), short term word recall ($p<.05$) and had mixed effects on attention showing an advantage early in task performance and no differences later ($p<.05$).[69]

The same experiment was conducted in adults with less pronounced effect. To explain this variability, authors proposed that a mid-day snack consisting of dietary sugar may only improve performance on attention and spatial memory tasks when the task imposes a high cognitive demand. They hypothesized that tasks requiring high cognitive demand may deplete extracellular levels of glucose in the brain more than less demanding tasks. Thus, authors asserted, if the experiment was repeated in adults with more demanding tasks, the results may more closely align with their observations in children.[69]

Merits of the study include its randomized cross-over design, control of testing time, consideration of pre-meal effect, assessment of hunger, energy and mood and a detailed description of intervention and placebo ingredients and composition. The study was limited by

the brief 15 minute wait period after meal provision, poorly detailed BMI ranges as well as energy and macronutrient composition of premeal foods, comparison between a liquid placebo meal and a solid food intervention which may have significantly different gastric emptying times.

Two earlier studies of similar duration in adults corroborate Mahoney et al.'s findings. In a set of two discrete non-randomized "partial latin square" design experiments conducted by Kanarek and Swinney, a small sample of college age men (exp 1: n = 10, exp 2: n=8, BMI described as "within 90 to 110% of desirable weight on the 1959 Met Life Tables") were provided breakfast, lunch and an afternoon snack or the same menu with no lunch for 4 consecutive weeks. Breakfast and lunch were standardized whereas the afternoon snack included either 32 oz of a 290 kcal confectionary snack containing 9 g protein, 36 g carbohydrate and 14 g fat, or a 12 oz 4 kcal diet soft drink.[70]

In the second experiment conducted by the same investigators, (n = 8) subjects were provided a 240 kcal 8 oz flavored yogurt containing 9g protein, 43 g carbohydrate and 3 g fat as an afternoon snack. Subjects in both experiments were tested 15 minutes after snack ingestion using a battery of tasks assessing memory (forward and backward digit span tests adapted from the WISC, 1987), arithmetic reasoning (problems modeled after those in the WISC), reading speed (using "vague" stories, modeled after those used by Bransford & Johnson) and attention (examined through a continuous performance task (CPT)).[70,71] Authors reported significant differences in performance favoring the confectionary snack condition as compared to diet soda on assessment of memory (backward digit span; $p<0.05$) and attention (CS: $p<0.05$). Results were reported as more robust in the second experiment comparing the lower calorie, lower fat more carbohydrate rich flavored yogurt to diet soda (backward digit span: $p<0.05$; attention:

$p<0.05$; reading speed: $p<0.05$ and arithmetic assessment: $p<0.01$). From their findings, authors concluded that a late afternoon nutritional snack can have positive effects on cognitive performance.[70]

Study strengths include consideration of pre-meal effect with subjects served standardized breakfast & lunch prior to intervention, a cross-over design with subjects exposed to all 4 conditions and serving as their own control, counterbalancing of the lunch/no lunch conditions and detailed characterization of all study meals including macronutrient composition. Both studies are limited by a number of factors including: small sample size, administration of cognitive measures only 15 mins after meal provision, manipulation of multiple foods variables (calories, fat, carbohydrates, volume), insufficient assessment of variables that may differ significantly within individuals (sleep, mood etc) and insufficient detail on the quantity of food each participant consumed.

Early work by Benton et al. also reported positive effects for word recall in a double blind placebo study comparing 50g glucose or placebo amongst 153 undergraduate subjects.[72] In a second experiment, glucose levels were increased and sustained for 2 hours by having participants drink multiple glucose beverages in series. Again word recall improved with glucose load regardless of baseline blood glucose level. From these results, investigators asserted that the improved cognitive performance was not due to correction of hypoglycaemia, but possibly via a glucose derived increase in acetylcholine synthesis. Authors noted that prior literature suggests that learning is associated with a high demand for acetylcholine.[72]

The effect of high GI/GL dietary manipulation on cognitive performance remains controversial with many inconsistencies across studies. The contradictory findings in the short-term diet-cognition literature may be reconciled if they capture two separate time dependent

effects. For example, it is possible that high GI/GL foods improve cognitive performance immediately after intake, particularly when the task is more demanding. However, when blood glucose levels precipitously drop, mood and cognitive performance may also decline. Thus, over time the beneficial effect of high GI/GL foods may be eclipsed by lower GI/GL foods with a more stable blood glucose profile, especially during the late postparandial phase. There is of course always the possibility that the results observed, particularly right after intake, are simply an artifact of poor study design and/or differences across studies that elude comparison. More rigorous investigation employing a priori hypotheses, randomization, assessment of potential confounders, blinding, detailed descriptions of food composition, GL/GI/other dietary variable manipulation, appropriate cognitive task selection and repeated measures over time is needed to elucidate the veracity of previously observed short term diet-cognition phenomena.

In addition to a number of methodological issues, acute studies of diet and cognition fail to address more recent concerns about effects excessive high sugar snack intake may have on cognitive function over the long term. Given that the brain has a limited reserve of its sole energy source, it makes sense that a brief sustained increase in glucose availability will provide the brain with the energy it needs to temporarily enhance cognitive function. However, in the real world, where Americans are estimated to consume 8,000 teaspoons of sugar every year, the brain may become repeatedly exposed to a hyperglycemic environment that adversely alters its function. For optimal cognitive performance it appears that the foods we consume must 1. ensure that the brain has an adequate supply of glucose from moment to moment and 2. prevent the brain from being acutely swathed in excess of sugar. Solid evidence delineating which specific foods offer these benefits over the short *and* long term is currently lacking but low GI foods appear to fall

into this category in the hours following meal consumption and, as the longer term studies below suggest, possibly beyond.

Long-term Dietary Pattern and Cognitive Function

In contrast to the acute dietary manipulation studies discussed above, several studies in infants and children have examined the relationship between whole dietary intake pattern over several years. In a longitudinal correlation study (N= 3966), researchers found that a 'processed' (defined by authors as high fat, high sugar) dietary pattern at 3 years of age was negatively correlated with IQ assessed at 8.5 years of age (a 1 SD increase in dietary pattern score was associated with a 1.67 point decrease in IQ; $p<0.0001$).[73] A more 'health-conscious' dietary pattern at mealtimes was positively correlated with IQ at 8.5 years of age (a 1 SD increase in pattern score led to a 1.20 point increase in IQ; $p=0.001$). The following factors were taken into consideration in analysis: gender; age at WISC (IQ) assessment; WISC test administrator; the number of stressful life-events experienced by the child; breastfeeding duration (assessed at 6 months of age), estimated energy intake at each time point, a measure of parenting (HOME score) assessed at 18 months of age, 12 maternal education, housing tenure and social class recorded during pregnancy, maternal age at birth of the study child and maternal consumption of oily fish during pregnancy. As with all longitudinal studies included in this section, this work can only detect correlation, not causation. However, Northstone's work, as well as the other studies discussed here, raise important questions about the potential link between diet quality and cognition in pre-adolescent children.

In a similar longitudinal study examining dietary pattern in children (N=241) assessed from 6 months to 4 years, Gale et al. reported that infant dietary pattern characterized by high fruit, vegetable and home-prepared food consumption was associated with better memory

performance, higher full scale IQ and verbal IQ at 4 years of age.[74] This correlation remained significant even after adjustment for maternal education, intelligence, social class, quality of home environment and other potential confounding variables. These findings suggest that dietary intake may significantly alter cognitive function in infancy. It remains unclear whether this early effect stably persists or compounds over time. Further, this study raises questions about which components of an infant's diet (High fruit? High vegetable? Home prepared foods?) later protect and/or enhance their cognitive performance.

A study by Theodore et al. including older children also found an association between intelligence and diet with more specific focus on specific food components.[75] The study assessed 591 children of European descent at 3.5 and 7 years of age. Notably, about half of the children were born small-for-gestational age (SGA, birth weight ≤ 10th percentile). Following multiple regression analyses, authors found that daily margarine consumption was associated with significantly lower IQ scores at 3.5 years in all children and at 7 years in SGA children. In all children, consuming national daily recommended servings of bread and cereal was associated with significantly higher IQ scores at 3.5 years. Authors also reported that those who consumed fish at least weekly had significantly higher IQ scores at 7 years than those who did not. Based on these results, Theodore et al. proposed that meeting nutritional guidelines for fish, breads and cereals consumption may be beneficial to children's cognitive development while consuming margarine daily may precipitate poorer cognitive functioning.[75]

In large observational birth cohort study (n=7,097) aimed at examining correlation between diet and cognition over a longer time span, Smithers et al. found that dietary patterns at 6, 15 and 24 months of age were associated with IQ at 8 years of age.[76] In analysis, authors examined associations between infant dietary pattern and Wechsler Intelligence Scale for

Children at 8 years and concluded that, for all ages, greater "discretionary" intake (characterized by biscuits, chocolate, sweets, soda, crisps) was associated with 1-2 point lower IQ. Breastfeeding at 6 months as well as a "home-made contemporary" dietary pattern (high intake of herbs, legumes, cheese, raw fruit and vegetables) at 15 and 24 months was associated with a 1-to-2 point higher IQ score. By contrast, a dietary pattern characterized by high intake of ready-prepared baby foods at 6 and 15 months was negatively associated with IQ at 8 years of age. However, a positive association was found with a "Ready-to-eat foods pattern" at 24 months. From these results, authors posited that dietary patterns between 6 to 24 months may have a small but significant effect on IQ at 8 years. [76] Like Gale et al.'s work, this study did not examine which specific foods or dietary components may be associated with higher cognitive performance.[76]

In an attempt to further elucidate which dietary components may proffer cognitive benefit in children, Khan et al. assessed cross-sectional correlation between performance on an attentional inhibition task (a modified flanker task) and three aspects of dietary consumption pattern in 65 children ages 7–9 years.[77] Dietary fatty acids (FAs) intake, fiber intake and overall diet quality were assessed using three-day food records. Nutrient-level analyses of this data was then used to calculate Healthy Eating Index (HEI, 2005) scores and assess diet quality. Based on correlational analyses, Khan et al. reported that age, intelligence quotient (IQ), pubertal staging, maximal oxygen uptake ($\dot{V}O_2max$), and percentage of fat mass (% fat mass) were associated with modified flanker task accuracy. After adjustment for these five confounding variables, authors reported that "congruent" task accuracy was positively associated with insoluble fiber ($P = 0.03$) and total dietary fiber ($P = 0.05$), while "incongruent" task accuracy was positively associated with insoluble fiber ($P < 0.01$) pectins ($P = 0.04$) and total dietary fiber ($P < 0.01$). Higher HEI

score (a healthier diet) was correlated with lower accuracy interference (better performance) (P= 0.03), whereas higher total FA consumption was correlated with increased accuracy interference (poorer performance) (P = 0.04). Based on these findings Khan et al. concluded that children's overall diet quality, particularly dietary fiber intake, is correlated with their performance on cognitive control tasks. [77]

In a second, larger study by Khan et al., 150 pre-pubertal children between 7 to 10 years of age were included to assess the association between saturated fat and cholesterol intake and cognitive flexibility (using a task switching paradigm).[78] After adjusting for age, sex, socioeconomic status, IQ, VO_{2max}, and BMI, Khan et al. reported that children who consumed more saturated fats exhibited longer reaction time when demand for cognitive flexibility increased. Additionally, both increased saturated fat and cholesterol intake were associated with impaired ability to task switch (maintain multiple tasks in working memory) and poor cognitive control. Combined, Khan's two studies suggest that poor overall diet quality (low HEI) including high saturated fat and cholesterol intake may have a detrimental effect on cognitive control and flexibility, particularly with increased task demand. Conversely, a high quality diet characterized by high HEI and increased total and insoluble fiber intake may benefit children's cognitive performance.[78]

In addition to IQ and memory, some studies have looked at the role diet may play in psychological/behavioral outcomes. In one such study, authors examined the potential correlation between dietary patterns and attention-deficit hyperactivity disorder.[57] Following dietary pattern analysis, investigators concluded that a "Western" style dietary pattern was associated with ADHD diagnosis in an adolescent cohort (N=1,799) even after adjusting for maternal and adolescent confounding factors from pregnancy to 14 years. The "Western" style

dietary pattern was characterized as including higher intakes of total fat, saturated fat, refined sugars and sodium as well as lower intakes of omega-3 fatty acids, fiber and folate.[57] Given that the cognitive domain of attention has been included in several studies correlating dietary pattern with cognitive function, Howard et al.'s study lends additional support to prior findings and raises new questions about how diet quality may not only affect cognitive performance but potentially, behavior and psychopathology as well.

Taken together, long term correlational studies suggest that thorough investigation into the cumulative effect dietary pattern has on child cognition is warranted. More specifically, the effect high sugar, cholesterol, fiber and fat (total and saturated) intake has on children's cognitive performance needs to be examined more closely in randomized, blinded and well controlled study settings.

Multiple Week Dietary Intervention and Cognitive Function

To date, very few studies in humans have attempted to look at the effect longer term dietary intervention may have on cognitive function. While correlational studies help target areas of inquiry for more rigorous intervention based research and short term intervention trials begin the process of exploring causal relationships in these areas, neither are designed to capture the direct effect chronic dietary intake may have on cognitive performance. Correlational studies do not assess causation and short-term, single meal studies may not be able to detect alterations in some aspects of cognitive function that could take days, weeks or even months to reach significance. Thus, data gathered from multiple-week intervention studies may provide more robust evidence for a direct link between dietary pattern and children's cognitive performance.

Two primary reasons multiple-week intervention trials are scarce in the literature is that 1. they are difficult to control (participant food and liquid intake must be carefully monitored and

controlled over several days/weeks) and 2. they can become quite expensive, particularly if researchers provide participants with food and beverage during the study. However, given the established evidence in this area of research, a number of investigators have sought to carry out longer term dietary intervention trials assessing the effect of chronic intake pattern on cognition. Their work is the focus of this section and serves as the basis for the development of the current study. In particular, Nilsson et al.'s 2013 study served as template for the present study to the extent that this was logistically feasible.[11]

In 2011, Edwards et al. conducted one of the first studies aimed at examining the effect of a multiple day high fat (on average, 74% kcal from fat) dietary intervention on human cognition.[26] Twenty sedentary men, aged 25-45 years, were recruited to participate in the study and asked to consume a diet similar in composition to the one espoused by the late Dr. Atkins. The investigators specifically selected this diet to measurably increase plasma free-fatty acids (FFAs). All 20 subjects served as their own controls. Each participant was provided a daily dietary plan tailored to individual needs and intolerances as well as booklets to record their food and beverage intake. In addition, investigators conducted regular phone interviews to provide subjects with continuous dietary advice and monitor adherence. Following the 7-day intervention period, compliance was also assessed using Forestfield nutritional assessment software. Cognitive function was assessed using a previously validated and computerized testing battery [Cognitive Drug Research Ltd. (CDR), Goring-on-Thames, UK]. As). Assessment included measures of attention, working memory, episodic memory, and self-reported mood and alertness, before and after dietary intervention. A complex attention and working memory task was also included to discriminate above ceiling effect.[26]

Based on analysis, authors reported that the one week high-fat dietary intervention led to a significant increase in simple reaction time (poorer performance) as well as a significant decrease in power of attention. In addition, post-intervention mood scores for calmness and altertness decreased. In contrast to prior work by the same investigators in rats, working memory and other memory parameters appeared unaffected by the intervention. Post-intervention biochemical assessment revealed an increase in plasma FFA by >%40 ($p<0.05$) and 3-hydroxybutyrate by 95% ($p<0.01$), and a decrease in plasma glucose (8%; $p<0.01$) and insulin levels (29%; $p<0.01$). Based on these results, Edwards et al. proposed that although FFAs do not diffuse across the blood brain barrier (BBB), their increased presence during intervention likely resulted in decreased brain glucose levels and subsequent cognitive impairment. They further speculated that cognitive deficit might have been milder than observed in animals because of high circulating levels of 3-hydroxybutyrate. Unlike FFAs, 3-hydroxybutyrate can pass through the BBB and undergo oxidation, potentially offsetting the effect of low carbohydrate intake during the intervention.[26]

Strengths of the study design include cross-over allocation with subjects serving as their own controls, inclusion of free fatty acid (FFA) analysis to assess adherence and quantify effect of the diet on blood profile, assessment of glucose and insulin to help elucidate the biochemical mechanism associated with study results, and inclusion of pre- post- mood and alertness assessment prior to cognitive testing. Limitations include the study's small sample size, lack of pre-study dietary pattern assessment which may confound intervention effect, mismatched duration of standardized low fat control diet (3 days) vs intervention (7 days), use of food records to assess dietary compliance as opposed to pre-weighed diet administration, absence of sleep assessment (unless included in alertness measure, this was not specified) and assessment of

whole body exercise efficiency. Exercise increases BDNF and thus may have influenced cognition results.

More recently, in a 4-week randomized controlled, cross-over dietary manipulation trial aimed at improving cognitive function in 44 overweight/obese, but otherwise healthy, adults (36 females, 8 men; ages 50-72; mean age 63.3 ± 0.8 years; BMI range BMI 25–33 kg/m) Nilsson et al. reported significantly improved performance on the Rey Auditory-Verbal Learning test as well as a test of selective attention, during the intervention period as compared to control.[11] The intervention was described by authors as a "multifunctional, active" diet (AD) including foods with a potential anti-inflammatory action (eg. low glycemic impact meals, antioxidant-rich foods, omega-3 rich oily fish and rapeseed oil, viscous dietary fibers, soybeans, whole barley kernel products, almonds, stanols, and the probiotic strain, Lactobacillus plantarum Heal19, DSM 15313). The control diet was defined as devoid of "active" components. Both diets were devised in accordance with Nordic dietary recommendations and consumed over a 4-week period, separated by a 4-week 'washout' period.[11]

Two sets of pre- post- cognitive assessments comprised the study's primary outcome measures: 1. a computerized test of Selective Attention (SA; 10 min) and 2. the Rey Auditory-Verbal Learning test (RAVLT; 60 min). The SA test assessed split and sustained attention spatial perception and aspects of working memory; the RAVLT measured learning and memory. At visits 1-4, participants arrived fasting for the following biological assessments: blood glucose, insulin, full lipid panel, free fatty acids, lipoprotein A-1 and B, hs-CRP (high-sensitivity C-reactive protein), HbA1c (glycated hemoglobin), inflammatory markers (IL-6 (interleukin-6), TNF-α (tumor necrosis factor alpha)), and PAI-1 (plasminogen activator inhibitor 1). Fasting assessments were taken first followed by, weight, blood pressure, the SA test and a standardized

15 minute breakfast consisting of white wheat bread (75.5 g) and apricot marmalade (27.7g), 100 ml water, and a plain cup of decaffeinated coffee or tea (150). Leftovers were collected, weighed and entered for dietary analysis. Cognitive tests were administered as follows up to120 min after the start of the breakfast: SA (fasting, mins), SA (not fasting, 45 mins), RAVLT (60 mins), SA (120 mins).[11]

Protocol for the AD and control diets included detailed instructions for following a 14-day 2,000-2,100 (women) or 2,500-2,600 (men) Kcal/d meal plan, including gram weights for each item. During the AD period, participants received a checklist of active food components for meal/snack inclusion with instructions to report any deviations to protocol. Usual caffeine intake was maintained throughout the study period; alcohol was limited to 30g for women and 37g for men. In addition to the AD checklist, adherence was assessed via self-report of weekly weight. If a participant's weight changed >1 kg in either direction they were advised to see the study nutritionist for dietary reassessment.[11]

In addition to improved cognitive function after AD intervention, authors reported an inverse association between cognitive performance and plasma concentration of cardiometabolic risk markers (fasting glucose, HbA1c, cholesterol, CRP and blood pressure). This relationship was also observed between cognitive function scores and Framingham cardiovascular risk scores. By contrast, positive correlation was observed between cognitive tests and apolipoprotein A1, leading authors to speculate that it may serve a protective role against cognitive decline. Taken together, authors reported their findings as evidence that a 4-week active dietary intervention significantly improves cognitive performance and cardiovascular risk profile. They attributed this effect to anti-inflammatory components in the 'active diet', suggesting that these components may reduce the presence of inflammatory cytokines associated with disrupted

insulin receptor signaling, compromised neuroplasticity and impaired neuronal function. In turn, they proposed, dampened brain inflammation may reduce mental fatigue, increase learning capacity and enhance cognitive performance.[11]

Merits of the study include its randomized, controlled and counter-balanced cross-over design, a thorough description of study timetable and dietary prescriptions, extensive report of control and AD macro and micronutrient profiles, selection of cognitive tests that are sensitive to metabolic disturbance and have been previously validated in investigations of food effects on cognitive function, selection of a cognitive testing schedule that corresponds with fasting and postprandial blood glucose profile for assessment of delayed effect and efforts to keep participants in energy balance to minimize weight as a confound.

Significant study limitations include recruitment of obese subjects (BMI 25-33) whose weight status is independently associated with cognitive impairment; lack of focus on a particular aspect of food intake that would allow identification of more specific dietary recommendations for brain health (eg. high antioxidant, low GI etc.), minimal difference in composition between control and intervention diet (both were in close alignment with Nordic Nutrition recommendations), potential for significant practice effect on cognitive measures and the absence of compliance assessment.

Although the next two studies were published after the present one was conducted, their findings merit discussion for comparison with and evaluation of the present study. Last year (2015) in the only cluster randomized cross-over trial published to date on the longer term effect of whole food dietary manipulation and pre-adolescent cognitive function, Sørensen et al. reported that their 3-month dietary intervention improved 'school performance' (P=0.015), 'reading comprehension' (P=0.043) and EPA+DHA status by 0.21 (95 % CI 0·15, 0·27) w/w %

(percent fatty acids in whole blood; P<0.001).[12,79] Diet order was randomized at grade level (third vs. fourth) and treatment allocation was unblinded. Participants included 726 well-nourished 8 to 11 year olds at nine Denmark schools. Based on prior trials correlating iron and n-3 LCPUFA intervention with cognitive functions, investigators administered an *ad libitum* dietary intervention aimed to increase n-3 LCPUFA and Fe status through dietary changes in the school meal program (mid-morning snack, lunch and mid-afternoon snack). These changes were in accordance with 'New Nordic Diet' guidelines, met 40-44% of the daily energy requirements for an 11 year-old boy and included fish twice a week. The control diet included a home prepared bag lunch (usual diet). Primary outcome measures included iron status (serum ferritin and hemoglobin (Hb)), whole blood fatty acid composition and cognitive performance on the d2-test of attention and Danish standardized reading and math tests at baseline and the end of each intervention period. Additional measures included 24-hour dietary recall assessment (> 4 days), physical activity accelerometry, anthropometric assessment (height and weight) and socioeconomic status inquiry.

Based on analysis, authors reported that the 0.21 w/w% increase in n-3 LCPUFA status was correlated with an improvement in 'school performance' but not 'reading comprehension' pattern. Authors proposed that the latter observation may be attributed to the larger % of DHA in whole blood relative to EPA. Individual analysis of EPA and DHA status revealed that a 1% increase in EPA correlated with increased 'reading comprehension'. By contrast, a 1% increase in DHA was correlated with increased 'school performance'. Investigators proposed that the 0·21% change in EPA+DHA could explain 19% of the effect of the school meal intervention on 'school performance' as well as 11–36% of intervention effect on reading speed, number correct in reading and inattention.

Although dietary intervention resulted in increased EPA + DHA status, changes in serum ferritin and Hb did not reach statistical significance. Authors did however observe that girls with lower baseline Fe stores demonstrated poorer overall 'school performance' and poorer reading performance as compared with girls with larger Fe stores (>25 μg/l). This also held true for girls with small Fe stores (ferritin 15–25 μg/l) in the absence of iron deficiency (ID).

Sørensen et al. proposed that differences in performance based on Fe stores are likely due to Fe-depletion in the brain, when intake is minimal, regardless of ID status. They suggest that the mechanism involved in low Fe status and cognitive deficit is likely related to Fe's role in neuronal growth, neurotransmitters and myelin synthesis, as well as for the formation of Hb to ensure adequate oxygen transport to the brain.[12,79] Authors did not propose a specific mechanism for the EPA and DHA results.

Several study strengths were identified including effort to increase meal consumption by involving children in cooking, tasting, presenting and serving lunch meals to their peers 2-3 times during intervention, demographic and socioeconomic assessment detailing parental education level and immigration status, pubertal staging assessment, 7 day baseline and post intervention physical activity assessment by accelerometry (Actigraph), collection of 24-hour dietary recall data using the validated Web-based Dietary Assessment Software for Children, identification and exclusion of dietary under- and over-reporters (estimated by dividing mean reported energy intake by BMR) from data analysis, supplement assessment, detailed description of anthropometric measures, and assessment of biomarkers associated with a priori hypotheses to examine dietary effect and elucidate a potential mechanism for cognitive outcomes.

Although this study shares several key features with the present study design, namely inclusion of a pre-adolescent cohort, it exhibits a number of unique and significant limitations. Despite a large population yielding greater statistical power, Sorensen et al.'s study is limited by failure to blind participants to their condition, the absence sleep/mood assessment, infrequent (4 day) assessment of what children actually consumed, differences in diet between schools (eg. fish differed between schools). Further, and most notably, the study's whole-diet approach to intervention makes it difficult to conclude that intake of n-3 LCPUFA was responsible for intervention effect. Fish contains other nutrients (e.g. amino acids, Se, I and vitamin D) and the overall dietary intervention increased intake of several other micronutrients associated with cognitive performance.

NEUROBIOLOGICAL FRAMEWORK

Animal Studies On Diet and Cognitive Function

The inability to control for several variables in human subjects makes research conducted in animal models valuable. Numerous studies aimed at exploring the correlation between dietary pattern and cognitive function have used laboratory rats and mice as subjects. Much of the nutrition and cognition literature in animal models focuses on the effect certain food types have on the hippocampus. In humans, this brain region plays a central role in forming long-term memory for facts (semantic memory) and events (episodic memory) as well as visuospatial learning and memory. One particular subregion of the hippocampus, the dentate gyrus (DG), appears to be particularly relevant to diet and cognition in rats and mice. The DG is believed to be involved in visuospatial learning, pattern separation, and face recognition (in humans) and appears to be particularly susceptible to damage in the presence of a HF/HS diet.[80,81]

Several studies suggest that diet's effect on DG function is mediated by circulating levels of brain derived neurotrophic factor (BDNF) and pro-inflammatory cytokine levels. BDNF protects and supports brain function by regulating neuronal differentiation, promoting neuron survival and synaptic plasticity, and stimulating neurogenesis.[82] In animal models, a HF/HS diet dampens BDNF levels resulting in compromised DG related functions while *increasing* the presence of pro-inflammatory molecules (cytokines), eliciting inflammation, neural cell death and cognitive impairment.[83]

In an early study by Greenwood and Winocur, one month old rats were randomized to one of three conditions for 3 months: 1. a 20% (w/w) fat (40% of calories) high in saturated fatty acids (lard-based), 2. a 20% (w/w) fat (40% of calories) high in polyunsaturated fatty acids (soybean oil-based), or 3. standard chow.[82] Rats fed the high saturated fat lard-based diet displayed impaired learning and memory when they were placed in Olton's radial arm maze and the Hebb-Williams maze series. The soybean-oil group displayed some impairment on these measures but performed better than the lard diet group. The chow fed group outperformed the two other groups.

In another early study examining the effects of Western diet on cognition in an animal model, researchers found reduced memory function after exposing laboratory rats to a high-fat, refined sugar diet for two months. Postmortem brain analysis showed that the rats exhibited lower levels of BDNF and reduced hippocampal plasticity. Behaviorally, the rats displayed reduced memory function.[29]

In a later study, middle-aged rats were fed a high-fat high-glucose (HFHS) diet supplemented with high-fructose corn syrup in water or standard NIH chow pellets and plain water (control) for 8 months.[84] The rats receiving the HFHS diet exhibited elevated fasting

glucose as well as increased cholesterol and triglyceride levels, two clinical markers of diabetes. At study end, they displayed impaired spatial learning, and the brains revealed reduced hippocampal dendritic spine density, reduced long term potentiation at synapses critical to long term memory formation and a significant decline in BDNF levels in the hippocampus..[84]

A link between diet, BDNF, brain inflammation and cognitive dysfunction was also described in a mouse model study by Pistell et al.[53] Mice were provided either a high fat Western diet (WD, 41% fat), a very high fat lard diet (HFL, 60% fat) or standard chow for 21 weeks. Spatial ability was measured using the Stone T-maze. The WD resulted in significantly increased body weight and astrocyte reactivity in the absence of cognitive impairment on the maze task or elevations in markers for brain inflammation. In mice fed the HFL diet, there was a marked increase in weight, significant cognitive impairment, increased brain inflammation and decreased levels of BDNF.[53]

In addition to changes in BDNF and inflammatory markers that appear to precipitate neural damage and cognitive dysfunction, there is some evidence that a Western style diet causes leakage at the blood brain barrier (BBB). In a recent rat model study, Hargrave et al., explored the relationship between hippocampal-dependent cognitive functioning and blood-brain barrier (BBB) permeability in the presence of a Western diet (WD).[85] Following 10, 40, and 90 days of WD exposure in rats that were 1. diet-induced obese (DIO) , 2. diet resistant (DR) and thus insensitive to the obesogenic properties of WD, or 3. chow-fed controls,. DIO rats displayed increased body weight and adiposity throughout the study as well as elevated 10-day glucose and 90-day insulin levels. Behaviorally, these rats used a hippocampal *independent* strategy in spatial maze localization. By contrast, the chow-fed and DR rats used a hippocampal *dependent*

strategy. BBB leakage was observed in multiple subregions of the DIO rat hippocampus, but not that of the DR or chow-fed rats, suggesting a compromise in DIO hippocampal function. [85]

Authors proposed that their findings indicate that WD-induced leakage is dependent on duration of WD exposure and obesity phenotype, and that both BBB leakage and impaired glucoregulation appear to elicit changes in cognitive function (eg. maze strategy). [85] It is possible that BBB leakage may contribute to cognitive dysfunction by permitting molecules that are usually restricted from passage to enter and directly damage neural structures or promote further brain inflammation.

BDNF, pro-inflammatory cytokines and BBB leakage have recently been implicated in the diet-cognition link in humans. In a short term observational study, Karczewska-Kupczewska et al. assessed serum and plasma BDNF concentration in 18 healthy male subjects (mean age 25.6 ± 3.0 years; mean BMI 26.6 ± 4.8) at baseline, 120 min and 360 min of euglycemic hyperinsulinemic clamp with or without a intralipid/heparin infusion.[55] In addition, authors also measured plasma BDNF in 20 male subjects (mean age 22.7 ± 2.3 years; mean BMI 24.9 ± 1.5 kg/m(2)) 360 min after a high-fat meal. They reported a ~40% decrease in insulin sensitivity after 6 h of intralipid/heparin infusion ($P < 0.001$). During both clamps (with and without intralipid/heparin infusion), hyperinsulinemia had no effect on circulating BDNF. Increasing free fatty acid (FFA) concentration by intralipid infusion clamp had no effect on circulating BDNF at 120 min. However, at 360 minutes, FFA increase by intralipid infusion resulted in a significant decrease 43% in serum (43%; $P = 0.005$) and plasma (35%; $P = 0.006$) BDNF. In the second cohort of male subjects, the high-fat meal also resulted in a significant decrease in plasma BDNF (27.8%; $P = 0.04$). [8] Although cognitive measures were not administered and plasma BDNF does not reveal how much BDNF is circulating in the brain, these observed changes in response to a

high fat diet and lipid infusion suggest that the diet-cognition link may be mediated, at least in part, by circulating BDNF levels and hyperinsulemia.[55]

In a similar lipid infusion model of insulin resistance in humans, Emmanuel, et al. used phosphorus magnetic resonance spectroscopy to examine high-energy phosphate metabolism in the hippocampi of eight healthy volunteers.[39] This imaging tool enabled investigators to assess phosphocreatine-to-adenosine triphosphate ratio (PCr/ATP) in the hippocampus, which compares cellular energy production to concurrent energy expenditure. Compared to baseline, PCr/ATP ratio was significantly reduced during cognitive activity after lipid infusion ($P = 0.01$). By contrast, during cognitive activity in the absence of lipid infusion, PCr/ATP ratio did not change suggesting a role for diet induced hyperinsulemia in brain energetics and metabolism.[39]

Although the animal and human literature presented here suggests that the deleterious effects of a HF/HS diet on the hippocampus are driven by inflammation, decreased BDNF and reduced BBB integrity, they do not explain the temporal progression of events or demonstrate that these effects can be observed over the short term. Most of the animal studies on diet and cognition in the literature involve interventions that are several months long, making it difficult to draw correlations with the human brain's response to dietary intervention over the span of 2 weeks. Though the literature is sparse, there are a handful of animal studies that have examined the effect of a HF/HS dietary intervention over several days. This work is described here because it more closely aligns with the duration of the present study and may help elucidate the early sequence of events that take place in the brain following dietary manipulation. Studies involving short-term HF and HS intervention are separated so that the contribution of each to the proposed biological mechanism can be delineated.

Short-term HF Intervention in the Animal Model

In a 3-day study with wild-type mice fed a HF diet, Lee et al. reported that the intervention resulted in increased body weight, overall adipose tissue mass, liver and skeletal muscle lipid content and insulin resistance. [86] Although markers of inflammation were also selectively elevated in the adipose tissue of this cohort, authors found that immune-compromised mice incapable of inflammatory response on a HF diet, *also* experienced increased insulin resistance. This finding suggests that early-onset insulin resistance in response to a HF diet may occur independent of inflammation.

In another short-term 3-day HF (40% kcal from fat) intervention study in rats,Gan et al., reported higher levels of leptin and cholesterol.[87] Unlike Lee et al., investigators found no significant change in body weight or adipose tissue mass. In the dorsal hippocampus, Gan et al. reported that transcription of the neuroprotective peptide *galanin* was upregulated. In addition, they observed a trend toward increased BDNF. These findings suggest that, at least initially, acute exposure to a HF diet precipitates a neuroprotective response. As discussed earlier, in longer-term rat and mouse model studies, chronic HF diet exposure results in decreased BDNF levels and cognitive deficit.

Although acute HF diet intervention appears to stimulate a neuroprotective response, another short-term (9-day) study in rats suggests that even before a decline in BDNF is observed, significant cognitive impairment occurs.[31] In this study, Murray et al. examined the effect of a HF (55% kcal from fat) diet on exercise capacity and performance in 8-arm radial maze test of working memory. Exercised rats were compared with sedentary rats fed either standard chow or the HF diet. Following intervention, both exercised and sedentary HF fed rats made significantly fewer initial correct arm choices than at baseline ($P<0.05$). Given that brain glucose levels

remained unchanged, authors proposed that the mechanism for the observed cognitive impairment may involve insulin resistance. [31]

Taken together, these studies suggest that insulin resistance independent of inflammation may be responsible for early-onset HF diet related cognitive deficit, even when homeostatic responses increase the presence of neuroprotective molecules such as *galanin* and BDNF. In support of this theory, Brøns et al. reported a significant increase in fasting glucose, insulin secretion and hepatic insulin resistance in men fed a HF (60% of kcal from fat) over 5 days. [88] Although peripheral insulin resistance was not observed, an increase in insulin secretion has been shown to precede and possibly precipitate it.

Short-term HS Intervention in the Animal Model

The mechanism by which acute high sugar (HS) dietary intervention influences cognitive performance has only been explored in a small handful of studies. In a short-term study, Beilharz et al. found that following HS intervention and assessment at 5d, 11d, and 20d, HS fed rats displayed increased hippocampal inflammation, marked by elevated presence of TNF-a and IL-1b mRNA, and increased markers of oxidative stress (upregulation of NRF1 mRNA). [89] Behaviorally, a HS diet alone reduced hippocampal-dependent recognition memory as opposed to perirhinal dependent memory, underscoring the increased susceptibility of this region to dietary manipulation. Decline in hippocampal-dependent recognition in response to HS feeding was observed at all three time points (5d, 11d and 20d). BDNF levels were similar across all groups, supporting Gan et al.'s finding that BDNF levels do not decrease acutely in response to diet, and with HF feeding, appear to trend toward a neuroprotective increase.[87]

In 5-week HS intervention study, Jurdak et al. reported that HS fed rats displayed longer escape latencies on a spatial version of the Morris Water Maze task 1, 3, 4 and 10 days after

training as compared to controls.[90] At 10 days, these rats also displayed deficits in long-term spatial memory. Interestingly, although the HS fed rats were significantly heavier than controls following intervention (at training day 1), rats fed a high fat (HF) diet that reached similar weight and adiposity did not display cognitive impairment on this task. Thus, it is unlikely that weight status explains the acute cognitive impairment observed in the HS group. Although acute cognitive impairment has been observed in HF fed animals (for example in Murray et al.'s study), differences in task (Morris Water Maze vs. 8-arm radial maze) may partially explain why HF fed mice performed no different that controls in this study.

Another study conducted by Kendig et al. in rats over a period of similar duration to the present 2-week study (17 days), reported impairment in hippocampal dependent place memory but not hippocampal independent object recognition. [91] Notably, this observation was seen in sucrose fed rats and rats fed an isocaloric malodextrin solution that does not contain fructose, suggesting that the observed cognitive effects of a HS diet are not limited to fructose.

Short-term, Combined HF/HS Intervention in the Animal Model

This combined effect of a high fat and high sugar (HF/HS) diet has been explored in the rat model with attempts to elucidate a potential mechanism for observed cognitive deficit. In a study by Kanoski and Davidson, HF/HS rats displayed rapid and stable performance decline on the hippocampal-dependent feature negative discrimination portion of the radial arm maze (RAM) task as early as 3 days until study end at 90 days.[33] Further, post-mortem analysis showed that the HF/HS fed rats displayed a marked decrease in mRNA expression of tight junction proteins, Claudin-5 and -12, at the choroid plexus and the BBB. Notably, an increase in blood-to-brain permeability of sodium fluorescein was observed in the hippocampus, but not in

the striatum and prefrontal cortex, supporting the notion that the hippocampus is particularly vulnerable to the effects of a HF/HS diet.

Based on the presented literature, a combined HF/HS dietary intervention may act synergistically to precipitate cognitive decline via hippocampal inflammation, oxidative stress and insulin resistance. The correlation between these three events and cognitive deficit has been explored to some extent in the literature. Notably, elevated inflammation (defined as above median serum level of interleukin 6 and C-reactive protein) is associated with greater cognitive deficit (minimum 5 point decline on the Modified Mini-Mental State Examination [3MS]) than low levels of inflammation in elders with metabolic syndrome.[92]

In line with this, experimentally induced inflammation can elicit memory deficits. Reichenberg, et al., for example, showed impaired global memory in healthy adult subjects injected with a bacterial endotoxin.[93] Their cognitive performance was positively correlated with cytokine secretion assessment. Similarly, in rodent studies, pharmacologically induced inflammation has been shown to impair memory on hippocampal-dependent tasks. For example, Gibertini et al. showed that peripheral injections of IL-1β are sufficient to impair spatial (but not non-spatial) performance on the Morris Water Maze.[94] Further, intra-cerebral administration of IL-1β has been shown to impair contextual (hippocampal-dependent) but not auditory-cued (hippocampal-independent) fear conditioning.[95–97]

The oxidative stress observed in response to a HF/HS diet is generally characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms.[98] In a rat model of cognitive dysfunction, the antioxidant activity of catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) is lower than in controls.[99]

In the brain, this type of imbalance in antioxidant activity is associated with neuronal damage and impaired spatial learning and memory. [100–102]

While the relationship between acute diet-induced insulin resistance and reduced cognitive performance has been characterized in animal and human studies as previously described here, the mechanism by which this occurs is poorly understood. One proposed mechanism is that central (brain-related) insulin resistance occurs in response to elevated peripheral glucose presence. Work in animal models suggests that increased peripheral glucose decreases glucose transport across the BBB via GLUT1 transporters. There is also evidence that cortisol decreases the amount of insulin transported across the BBB and inhibits glucose transport into hippocampal neurons and glia. PET-(18) FDG imaging in normal elderly individuals has shown that acute cortisol administration results in a hippocampal specific reduction in glucose utilization. Given that co-localization of cortisol and glucose transporter (namely, GLUT4) is exceptionally high in the hippocampus compared to other brain regions, insulin resistance mediated by both may partially explain observed hippocampal-dependent cognitive deficit.[103] Hippocampal-dependent cognitive processes appear particularly dependent on the presence of adequate glucose, with acute glucose administration the hippocampus resulting in enhanced memory performance.[104]

In summary, the combined animal and human literature presented here suggests that *chronic* exposure to a HF/HS diet may elicit cognitive dysfunction by decreasing neural BDNF, insulin sensitivity and glucose metabolism as well as by increasing BBB permeability and pro-inflammatory cytokine presence. By contrast, cognitive decline in response to *acute* exposure to a HF/HS diet may be mediated by hippocampal inflammation and oxidative stress as well as GLUT and cortisol receptor mediated hippocampal insulin resistance and diminished glucose

uptake. The latter mechanism serves as the neurobiological framework for the present research. A visual model of this framework, separated into acute response to a high fat and then high sugar diet is provided below in Figures 1 and 2.

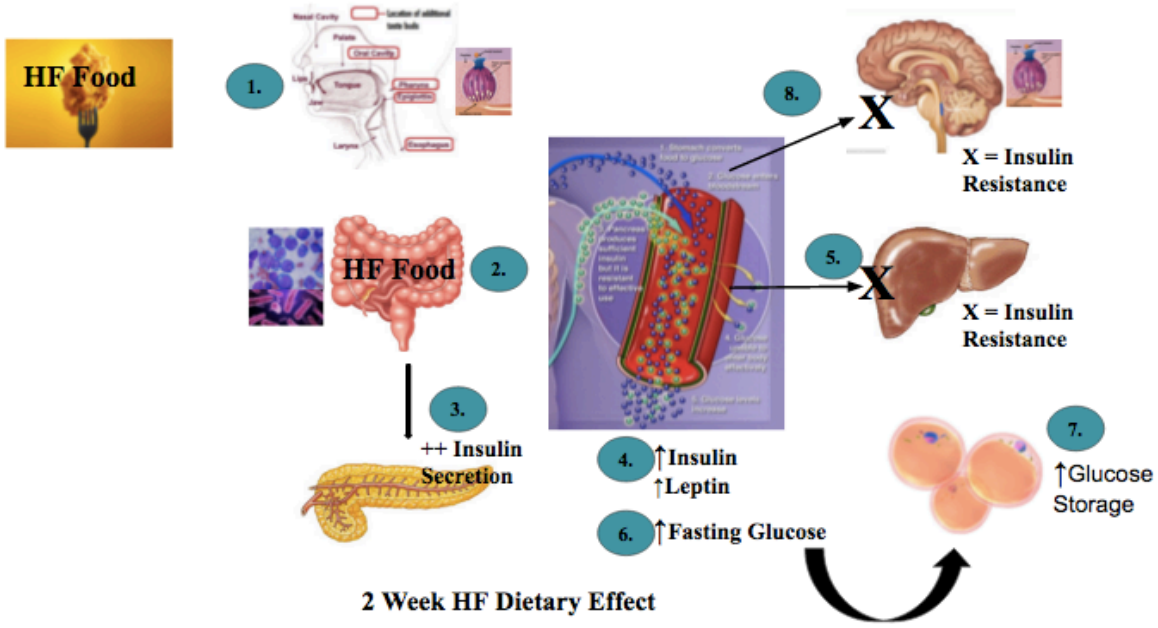


Figure 1. Proposed mechanism for the cognitive effects elicited by acute exposure to a HF diet: (1) 2 week HF diet consumption, (2) Significant increase in insulin secretion in response to hepatic insulin resistance[105], (3) Significant increase in circulating insulin and leptin[89,105], (4) Hepatic insulin resistance[105] (5) Increase in fasting glucose[105], (6) Increase in glucose storage in adipocytes[105], (7) Central insulin resistance and cognitive deficit.[106]

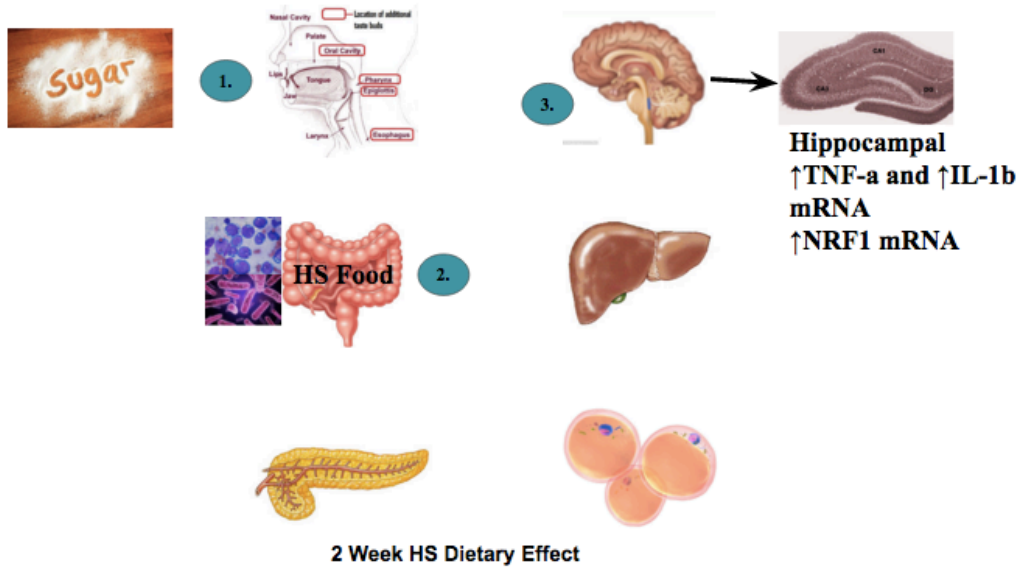


Figure 2. Proposed mechanism for the cognitive effects elicited by acute exposure to a HS diet: (1) 2 week HS diet consumption, (2) Increased hippocampal inflammation demarked by a significant elevation in TNF-a and IL- 1b mRNA[89], (3) Increased oxidative stress demarked by upregulation of NRF1 mRNA.[89]

THE INFLUENCE OF OTHER KEY VARIABLES ON COGNITIVE FUNCTION

It is important that studies examining the link between dietary intake and cognitive performance are not confounded by factors that also demonstrate significant influence on cognition. This section aims to briefly explore these variables, serving as rationale for their assessment in the present study and highlighting the need to control/adjust for these factors in future diet-cognition research.

Diet, Obesity and Cognitive Function

As mentioned earlier, obesity itself, in isolation of dietary pattern, has been implicated in cognitive dysfunction. In a study utilizing medical and cognitive examination data from a nationally representative sample of 2,519 children and adolescents aged 8-16 years included in the NHANES III (1988-1994), researchers found that obese and overweight status was correlated

with poorer performance on tests of visuospatial organization and general mental ability even after adjustment for parental and familial SES and other potential confounders such as sports participation, physical activity level, hours of television viewing, psychosocial development, BP and serum lipid profile.[107] It is important to note that only subjects without clinically diagnosed diabetes mellitus or other multiple medical co-morbidities associated with cognitive decline such as vascular and cardiac disease were included in the study.

In an effort to determine whether obesity is correlated with cognitive decline in isolation of co-morbidities that are known to affect cognitive function, Raji et al. used tensor-based morphometry (TBM) to examine differences in gray matter (GM) and white matter (WM) brain volume in 94 elderly subjects.[108] Only subjects who remained cognitively normal for at least 5 years after their scan were included in the study. Analyses with corrections for multiple comparisons strongly correlated body mass index (BMI), fasting plasma insulin (FPI) levels, and Type II Diabetes Mellitus (DM2) with atrophy (tissue death) in frontal, temporal, and subcortical brain regions. Using a multiple regression model, the researchers were able to demonstrate that BMI was negatively correlated with brain atrophy while DM2 and FPI were no longer associated with any volume differences. In an Analysis of Covariance (ANCOVA) model controlling for age, gender, and race, obese subjects with a high BMI (BMI > 30) showed atrophy in the frontal lobes, anterior cingulate gyrus, hippocampus, and thalamus compared with individuals with a normal BMI (18.5–25). Overweight subjects (BMI: 25–30) had atrophy in the basal ganglia and corona radiata of the WM. Overall brain volume did not differ between overweight and obese persons. Higher BMI was associated with lower brain volumes in overweight and obese elderly subjects.[108]

Overall, it appears that a relatively consistent finding in studies looking at the relationship between diet and human health across the lifespan is that obesity is associated with cognitive deficits, especially in executive function, in children, adolescents and adults. A study conducted by Smith et al. suggests that weight gain results, at least in part, from a neurological predisposition characterized by reduced executive function.[109] In turn, obesity itself may exert a negative impact on cognitive function via mechanisms currently attributed to low-grade systemic inflammation, elevated lipids and/or insulin resistance.

Diabetes and vascular disease have been linked to brain dysfunction. Given that many of the risk factors associated with obesity are known to contribute to cerebrovascular disease it's interesting to find that obesity is associated with reduced cognitive function among people without significant medical co-morbidity. Evidence from the Baltimore Longitudinal Study of Aging demonstrated that BMI and other measures of obesity were associated with both cross-sectional and longitudinal cognitive function in a large (n=1,703) middle-aged community sample. People with pre-existing medical conditions (AD, stroke, myocardial infarction) were excluded from the study so the results cannot be dismissed as simply a byproduct of the other co-morbidities.[110] In a separate prospective study of people who lost a substantial amount of weight through behavioral methods and bariatric surgery, study results suggest that chronic obesity alone may result in cognitive deficit. Significant memory improvements from baseline were observed 1 and 2 years following surgery ($P < 0.05$), further supporting the notion that weight alone may serve as an independent predictor of cognitive function.[111]

Allostatic load, diet and cognitive function

As briefly described in the introduction, allostatic load is the body's physiological, multi-system response to chronic stress that 'lasts several weeks to months'(McEwen, 2012).[43] AL is

typically measured as a composite score of the degree of dysregulation in multiple body systems including the cardiovascular, respiratory, endocrine and immune systems. Those who demonstrate greater dysregulation across systems in biochemical, anthropometric and physiological assessment are given a higher allostatic load score indicative of increased risk for morbidity and mortality. In addition to chronic disease outcomes, high AL is associated with cognitive decline. Given that some studies have correlated high AL with poor dietary pattern, and poor dietary pattern is associated with cognitive decline, it is entirely plausible that AL, as a measure of multiple system dysregulation, both mediates and moderates the relationship between diet and cognition. One study describing the potential correlation between AL and diet is discussed below. To date, this association has not been explored in children.

In 2011, Mattei et al. reported significant correlation between dietary pattern and composite allostatic load score as well as a number of it's 10 individual physiologic components.[47] This cross-sectional study included 1,117 45 to 75 year old participants of Puerto Rican descent living in the Boston area. Subject dietary pattern was characterized by its inclusion of 1. "meat and french fries," 2. "traditional Puerto Rican foods" (rice, beans, and oils) or 3. "sweets". After running logistic regression models adjusting for age, sex, alcohol intake, smoking, medications, energy intake, and body mass index or physical activity, authors reported significant correlation between increasing meat and french fry consumption pattern and higher allostatic load score ($P=0.002$), waist circumference ($P=0.032$), systolic blood pressure ($P=0.008$), and diastolic blood pressure ($P<0.0001$). Participants who ranked in the highest quintile of the 'meat and french fries' pattern were reported as having significantly higher allostatic load score than those in the lowest quintile ($P=0.030$). Authors also reported that this group had higher odds of having high allostatic load (odds ratio [95% confidence interval]: 1.8

[1.2 to 2.9]), low dehydroepiandrosterone sulfate (DHEA, a hormone/androgen; odds ratio 1.9 [95% confidence interval: 1.2 to 3.1]), and high glycosylated hemoglobin (HbA1c, a marker of 3 month glucose concentration, odds ratio 1.7 (95% confidence interval: 1.0 to 2.9]). The traditional Puerto Rican dietary pattern was not associated with allostatic load and a significant association between the 'sweets' pattern and allostatic load disappeared when participants with diabetes were removed from analysis.[47]

Although Mattei et al.'s study is merely correlational and thus no direct conclusions can be drawn about the direction of causality, allostatic load's relationship with both cognitive function and diet clearly merits further investigation.

SES and Cognitive function

There is substantial evidence suggesting that socioeconomic status is strongly correlated with cognitive function in childhood through adolescence. Following analysis of cognitive function and SES measures in 1292 UK children assessed at 22, 42, 60 and 120 months, Dr. Leon Feinstein reported that cognitive performance at 22 months was correlated with family background.[112] In addition, he reported, assessment at 22 months predicted educational status at 26 years of age with increased wealth serving as a moderator of this effect. Children of parents from a higher SES who initially performed poorly tended to improve dramatically enough to catch up with peers who initially did well, whereas those from families of lower SES did not.

Further substantiating Feinstein's observations, Dahl and Lochner reported that based on a panel of 4500 mother child diads from the National Longitudinal Survey of Youth datasets and changes in the Earned Income Tax Credit (EITC) over the last twenty years, a $1,000 increase in income is correlated with a combined 6% of standard deviation increase in math and reading scores.[113] The most robust results were observed for those families at greatest disadvantage.

Recently, work aimed at elucidating the neural basis for cognitive function disparity across SES groups has emerged. This critical work will likely serve as the basis for early and effective intervention. In one study driven toward this aim, Noble et al. investigated whether cortical volume differences existed in 60 children, ages 5 to 17 years (mean age:11.4) and of varying SES strata.[114] Neural regions of interest (ROIs) were areas known to support language, memory, social-emotional processing, and cognitive control. Following analysis of the MRI data, authors reported significant SES differences in hippocampal and amygdala brain volume. More specifically, income-to-needs ratio was associated with differences in hippocampal volume whereas fewer years of parental education was associated with larger amygdala volumes. These differences remained after controlling for age, total cortical volume, gender, IQ and multiple comparisons. In addition, authors reported SES × age interactions in the left superior temporal gyrus and left inferior frontal gyrus suggesting more significant disparity with increasing age. These differences remained after controlling for gender, race and IQ.[114] These and similar studies suggest a strong correlation between SES and cognitive function, independent of other important variables. Thus, it is important to either control for SES in diet-cognition studies by recruiting from a group that is relatively homogenous by SES or adjust for differences in statistical analysis. Given that even $1,000 increase in yearly salary may influence cognitive outcomes, both approaches were used in the present study.

Physical Activity and Cognitive Function

Approximately 30% of brain derived neurotrophic factor (BDNF) is produced by skeletal muscle and endothelial vasculature.[55] Given that a number of studies aimed at examining physical activity and cognitive function have observed a positive relationship in the presence of

increased BDNF, it is possible that exercise affecting skeletal muscle may increase BDNF production. In turn, an increase in BDNF may precipitate enhanced cognitive function.

Evidence supporting this hypothesis comes from a three-month study conducted by Seifert et al.[115] In a small, randomized controlled study, 12 healthy men were randomized to engage in 3 months of endurance training (n=7) or serves as controls (n=5). Following analysis of blood samples collected before and after intervention, authors reported that the intervention group's resting release of BDNF from the brain increased significantly ($P < 0.05$), while no significant change was observed in control subjects. In a mouse model study conducted in parallel by the same investigators, increased BDNF following endurance training intervention was localized to the hippocampus. [115]

Although changes in BDNF have not been examined in children, a number of studies have correlated physical activity in children with improved cognitive and academic performance.[116–121] Further, one cross-sectional study by Chaddock et al. aimed at examining the link between physical fitness, hippocampal volume (assessed by MR imaging) and memory in preadolescent children reported that children who were more fit displayed greater bilateral hippocampal volumes and superior performance on a hippocampus dependent relational memory task as compared to lower-fit children.[122] This evidence suggests that physical activity is likely a significant confounder of any study aimed at elucidating the link between a diet and cognitive function and should be accounted for either in study design or analysis.

Sleep and Cognitive Function

It is well established that lack of sleep interferes with cognitive function, particularly in children.[123–125] Thus it is important that cognition studies - even cross-over counterbalanced ones - account for this variable in study design and analysis. Within subjects, sleep patterns can

change from test to test, potentially confounding analysis of cognitive performance between groups.

Mood & Cognitive Function

The relationship between mood and cognitive function not well explored, and studies on how and even whether mood may affect cognitive functions are inconsistent. However, there is some evidence to suggest that when mood is experimentally manipulated, it can affect cognitive performance in very specific domains. In a study by Lagner et al. sixty-one male handball players were assessed on measures of memory and executive control both after winning a match and after training (control).[126] Under these two conditions mood differed significantly, while physiological arousal and motivation were comparable between cohorts. Authors reported lowered memory performance after the win as compared with training. Performance on the executive control task was unaffected by condition/mood. Once emotional states after the match were entered as covariates, differences in memory disappeared, highlighting the contribution of mood to the initial differences observed. Thus, even though evidence for a mood-cognition effect has not been fully substantiated, observations such as these demonstrate that it at least merits consideration.

SUMMARY

Despite some methodological weaknesses, as a whole, literature reporting a role for a number of physiological, biological and environmental factors on cognitive function in humans and animal models is fairly compelling. Accounting for all of these factors in study design and analysis is essential to accurate assessment of diet's contribution to cognitive performance.

To date, the present study will be the first to examine the immediate impact that highly processed, low nutrient-dense, high calorie foods have on cognitive function while

controlling/adjusting for weight,, physical activity, SES, hours of sleep and mood. In addition, it will be the first single-blind randomized controlled feeding study to explore proxy allostatic load as a measure of systemic dysregulation implicated in brain inflammation. This secondary assessment may lay foundation for gathering more evidence on the proposed mechanism by which high fat, high sugar foods disrupt cognitive function. If the hypotheses for this study are supported, further investigation would be warranted that could have dramatic implications for Federal and State child health policy, pediatric nutrition guidelines and intervention at a more local level.

CHAPTER 3: METHODS

A diet replete with an excessive amount of processed, packaged junk food may have a deleterious effect on cognitive function across the lifespan. However, evidence supporting this hypothesis is primarily limited to human adults and laboratory rats. The effect of a high fat, high sugar (HF/HS) dietary pattern on cognitive function in children and adolescents is not well understood. Relevant literature in this subpopulation is primarily based on randomized controlled single meal studies or cross-sectional time series studies.[14,15,40,54,127] Longer-term, randomized controlled studies are needed. Although a number of RCTs have been carried out in adults, the results of these studies may not be generalizable to the early adolescent population.[11,26,128] The present study proposes to fill significant gaps in the literature related to intervention duration, population, and study design by achieving the following aims:

Primary Aim

To measure the effects of a 2-week healthy moderate fat, low added sugar and nutrient dense (MF/LS) dietary intervention on cognitive functions in low-income pre-adolescents, ages 8-11, with an established high fat, high sugar (HF/HS) dietary pattern. A nutrient dense diet is defined as one that is high in food components that improve multiple body systems (eg. antioxidants, omega-3's and viscous fiber) and have been previously associated with increased cognitive function.[11] This study will use a battery of age appropriate neurocognitive tests to assess executive function, speed of processing, short-term and working memory, attention and spatial ability. The approach is a single blind, randomized controlled design that compares cognitive performance scores *between and within subjects* at baseline with their scores following either two weeks of MF/LS dietary intervention or no intervention (usual, pre-established HF/HS diet).

Primary Hypothesis

It is hypothesized that subjects consuming a MF/LS diet for 2 weeks will perform better on post-cognitive assessments than the control group (p-value < 0.05) while controlling for baseline cognitive assessment, gender, age, sleep and mood. It is also predicted that participants randomized to the control group will show no significant change in cognitive function between baseline (T1) and post-intervention measurement (T2).

Secondary Aim

To explore the role select proxy measures of allostatic load (AL) play in moderating the effect of diet quality on cognitive function. Proxy AL will be assessed using the following four secondary AL measures: BMI, waist, hip circumference and blood pressure. [129]

Secondary Hypothesis

Emerging evidence suggests that AL may moderate the effect of diet quality on cognitive dysfunction.[46,47] Thus, it is hypothesized that subjects randomized to intervention who demonstrate higher proxy measures of AL at baseline will show less improvement on cognitive assessment relative to participants in the same cohort with lower AL.

At study conclusion, we expect to 1. expand our understanding of the role diet quality plays in cognitive function independent of potential confounders (eg. sleep, mood, appetite etc.) 2. evaluate the effect a healthful MF/LS intervention diet, grounded in a neurobiological framework has on cognitive function 3. explore the role a proxy of AL plays in moderating the diet-cognition link. Thus, this study proposes to advance knowledge about the impact dietary quality may have on preadolescent cognition and provide information needed by health-care providers and policy makers to promote brain health and optimal cognitive function in early adolescents.

I. Study Procedure

A. Recruitment

Overview

For the present study we initially aimed to recruit 30 participants (15 male and 15 female). Contact with the target demographic for enrollment was made through flyers displayed throughout the Greater Harlem community as well as email and in person discussion of the study with community organization leaders, school parent coordinators and principals and direct contact with a listserve of parents interested in summer science enrichment camp scholarships at HypotheKids for their children.

Only families who 1. were eligible for the HypotheKids camp scholarship (eg. low-income) 2. had children ages 9 to 11, and 3. expressed interest in the study on the online application or via email were contacted for pre-screening. A phone screener was used to assess basic subject eligibility. Families who expressed interest and met the basic eligibility criteria described below were invited to come on site for further eligibility assessment. The recruitment period was scheduled to run from May 1st, to June 29th, 2015. However, due to exceptional difficulty with recruitment after scholarship funding was acquired in late June, the recruitment period spanned from June 22nd to July to August 14th. This period overlapped most of the active study period.

Inclusion/Exclusion Criteria

Eligible subjects for the study were male and female children between 9 to 11 years of age, provided evidence of a HF/HS dietary pattern based on three 24-hour recalls, met USDA requirements for energy intake based on age, gender, BMI and level of activity, demonstrated normal eating behaviors as assessed by select questions on the Children's Eating Behavior

Inventory (CEBI), showed no signs of coming from a food insecure home (assessed using the PEER-AID Shelf Inventory as a proxy measure), fell within a normal BMI range for age and gender, reported no adverse health conditions, neurological disorders or color blindness, reported no use of medication that may alter cognitive ability, reported no current record of a school individualized education plan (IEP) and were right hand dominant (as assessed by the Edinburgh Handedness Inventory).

Phone Screening

Pre-screening for the present study was based on information provided on the HypotheKids scholarship applications (age, gender and income) as well as a follow-up phone screener. The phone screener assessed potential participant's estimated height and weight, IEP status, eating habits (based on a subset of questions from the CEBI), food allergies, health status and diagnoses, medications/supplements and 24-hour dietary intake using the USDA 5-pass method.[130]

B. Screening

24-Hour Recalls and 3-Day Food Records

Participants who met basic eligibility criteria during the phone screening were asked to complete two additional, unannounced, 24-hour dietary recalls over the phone. These two additional recalls were conducted within one week of the initial phone screening call. An average of > 15.9% of calories from added sugar and 40% of calories from fat per day was classified as a HF/HS dietary pattern.[131] Added sugar intake was assessed using the Nutrition Data System for Research (NDSR; Nutrition Coordinating Center, Minneapolis, MN, USA) dietary analysis software (2015), contacting food item manufacturers and using nutrition labels on food packaging. High fat intake was defined in the present study as > 40% of calories from fat based

on national recommendations and average intakes from a study examining dietary intake in urban low-income adolescents.[132] High added sugar intake is defined as > 15.9% of calories from added sugars based on average intake for children and adolescents as reported by the CDC.[133] 24-hour dietary recall data was also used to assess whether each participant met 2010 USDA guidelines for energy intake based on age, gender, BMI and level of activity using the USDA Interactive DRI for Health Professionals.[134]

Informed consent

Prior to on-site screening, subjects who wished to participate in the study were asked to provide written parental consent and written child assent.

On-site Screening

Following informed consent, study eligibility was assessed using BMI, the 'PEER-AID' household food inventory, academic performance (to confirm non IEP status) and the Edinburgh Handedness questionnaire.

Recruitment Response Rate:

Of the 209 emails that were sent to parents and parent coordinators, we received 52 parent responses providing phone contact information as well as the best days and times to call. Of the 52 parents that responded, we are able to reach 40 via phone at the contact information provided. Of these 40, 31 subjects were deemed eligible to participate based on preliminary criteria included the phone screener. Over the course of the study period, 23 subjects were invited to Teachers College for additional on site eligibility screening and assessment. 4 of these subjects did not meet the eligibility criteria and 2 withdrew from the study due to schedule and other conflict. The remaining 17 subjects took part in the 2-week study between July and August 2015.

C. Randomization

Participants meeting eligibility criteria were stratified by gender and randomized into either: 1) a two-week MF/LS dietary intervention + HypotheKids camp; 2) usual HF/HS diet (*control*) + HypotheKids camp. During the recruitment period 19 eligible participants were blocked by gender and then randomized within these blocks (using randomizer.org) to intervention or control. Initially, 10 participants were randomized to the intervention and 9 were randomly assigned to control. However, 1 female participant (MF/LS) was withdrawn just prior to the study start date due to a schedule conflict. In addition, 1 male participant in the control (HF/HS) group was withdrawn midway through the study. Thus, 17 subjects were included in analysis - 8 intervention (5 boys and 3 girls) and 9 control (6 boys and 3 girls). Figure 3 below provides a visual depiction of study flow.

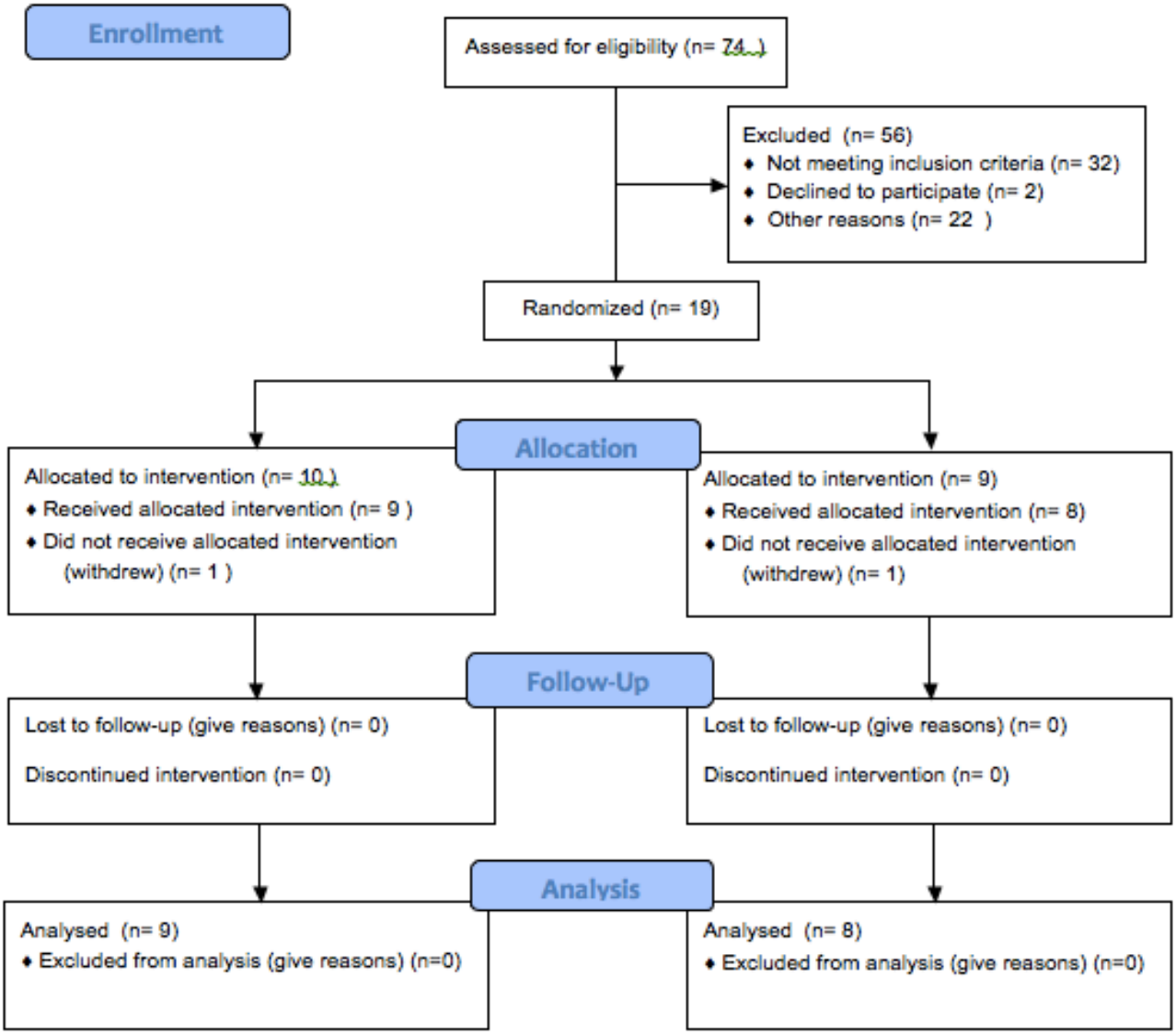


Figure 3. Study Flow Diagram.

D. Pre-Intervention Data Collection

Duplicate Assessment

All participants who had 24-hour recall data and anthropometric measurement collected more than two weeks before the study start date were asked to repeat these measures just prior to the intervention to ensure that an accurate baseline was obtained.

Food Palatability and Food Liking Assessment

Once eligible participants were randomized, they were invited to return the weekend prior to their start date for a food palatability taste testing "party". This event was used to determine palatability and preference for the both the MF/LS and HF/HS foods. During the palatability assessment, small samples of a variety of foods and beverages were provided to the subjects in conjunction with a previously validated visual analogue appetite and palatability scale.[135] This information was supplemented with data from a (*non-validated*) questionnaire on food likes. The palatability testing and food likes data were both used to inform individual diets for each participant.

E. Intervention vs. Control

Intervention

The MF/LS diet provided participants with 3-4 servings of fruits, 3-4 servings of vegetables and 4-6 servings of whole grains, as per 2010 USDA recommendations based on caloric intake.[136] Per American Heart Association recommendations, the MF/LS diet contained between 6 to 9 teaspoons of added sugar per day.[137] Percent of total fat from calories for the MF/LS diet was set at 25%, which is the lower end of the 25-35% range suggested by 2010 USDA and 2005 IOM guidelines.[136,138] Per USDA recommendations, < 10% of calories came from saturated fat.[136] The nutrient dense components of the diet included foods with high anti-oxidative capacity, foods

naturally high in omega-3s, prebiotic carbohydrates rich in viscous fiber and low-GI foods/meals. All of these components were the focus of a similar study examining diet quality and cognition in adults, which demonstrated significant effect of diet on auditory-verbal learning, selective attention and working memory.[11] Participants were expected to consume all of the food provided to them. Food palatability testing and a food-liking questionnaire were used to improve participant compliance with this study guideline. Digital food scales were used to weigh the foods to calculate calories provided as well as actual caloric intake. All participants randomized to the intervention received emergency backup MF/LS food in the event that a day of camp is missed. Prior to participant study start date**,** data from the three most recent 24-hour recalls were used to determine each participant's average caloric intake. Daily meals plans were developed for each participant based on this information and included the types and amounts of nutrient-dense food components outlined by Nilsson et al. to the greatest extent possible while still meeting dietary recommendations for each pre-adolescent participant.[11]

Control

Participants randomized to the control group also attended the HypotheKids camp during the intervention period. However the control participants were served their usual HF/HS diet. Both the intervention and control groups were served most of their daily snacks and meals during camp in the same room. Since each participant's meal plan was specifically tailored to their nutrient requirements and taste preferences, it was not necessary to match the intervention and control meals to maintain blinding. Daily 24-hour recall data was recorded in a food journal by participants in both groups throughout the 2-week intervention period.

F. Blinding Procedure

Participants randomized to intervention and control were blinded to their conditions. Provided each participant had an individualized meal plan, matching the meals for appearance and consistency was not necessary to maintain blinding to condition. Self-report questionnaires inquiring about whether each participant was provided a HF/HS or MF/LS diet each day of the week was used to confirm blinding to condition.

G. Assessments

Socioeconomic Status Assessment

SES assessment was made based on information provided in the HypotheKids scholarship application and responses to questions from the Family Affluence Scale assessment during pre-screening. [139]

Baseline (T1) AL Assessment

The following measures were used to assess AL at baseline, no more than 2 weeks prior to intervention: BMI, waist and hip circumference and blood pressure (Rogosch et al. 2011). AL assessment took place twice. Once after informed consent and again at the conclusion of the intervention period (just prior to post-cognitive testing).

Baseline (T1) Cognitive Assessment

On the scheduled intervention start date (Monday July 20th, August 3, and August 17th), participants randomized to MF/LS received a breakfast meeting this criteria on site (prior to cognitive assessment). Those randomized to HF/HS were provided a breakfast meeting this criteria. Following breakfast, all participants were asked to complete sleep and mood assessments. Cognitive assessment took place 120 minutes after eating following a procedure

used in similar studies.[11,16,140] Cognitive measures are described in detail below in Section III. (Study Measures).

Post (T2) AL and Cognitive Assessment

On the Monday morning following the 2-week intervention period, most participants were provided the same breakfast they received prior to initial cognitive assessment. (A few participants did not like their initial breakfast meal despite indicating otherwise during the tasting assessment). Study staff then assessed the same measures of AL that were collected at baseline (T1). Post cognitive assessment followed same protocol used at T1.

H. Compliance and Adherence

Weekday intervention meals and snacks (except dinner) were prepared, weighed and served at the camp site. Study staff kept meticulous record of what each child ate on site by weighing food before and after meal/snack service. Pre-weighed dinner meals with instructions for minimal food preparation were sent home with the participants each weeknight to ensure dietary compliance. Participants were asked to return their dinner bags each morning so that food consumed could be weighed. Adherence was measured through food records and pre- post- meal weight measurement.

I. Nutrition Education Follow-up

In May, once all study data are collected and analyzed, participants and their families will be invited back for an optional 2-day nutrition education workshop led by the study PI to discuss the benefits of a healthful diet and ways to overcome barriers to eating more healthfully.

III. Study Measures

Study Aim 1

BMI, SES and Food Security Assessment

BMI was assessed during screening to determine whether participants were in the normal BMI range for their age and gender. Subjects in the overweight and obese range were excluded from the study because recent literature suggests that adiposity may have a significant, independent effect on cognitive function.[56,109,141–143] SES measures included assessment of income-to-needs ratio based on records supplied in the HypotheKids scholarship application as well as questions from the previously validated Family Affluence Scale. [139] The PEER-AID Household Food Inventory was used to assess food security. Food insecure participants were excluded from the study because it places them at higher risk for malnutrition which is independently linked to cognitive dysfunction.[144,145] Further, we did not want participants to feel pressured into participating in the study to obtain food.

Academic Performance Inquiry

An academic performance questionnaire was used to assess whether participants have an IEP, indicative of developmental delays cognition. Those with an IEP were excluded from the study.

Edinburgh Handedness Assessment

This measure was used to assess handedness. Only right-handed subjects were included in the current study. Evidence suggests that left-handedness may be correlated with cognitive disadvantage.[146]

Physical Activity Questionnaire (Y-PAQ)

The Youth Physical Activity Questionnaire (Y-PAQ) was administered at initial consultation to assess physical activity (PA). Heightened PA level is associated with increased BDNF,

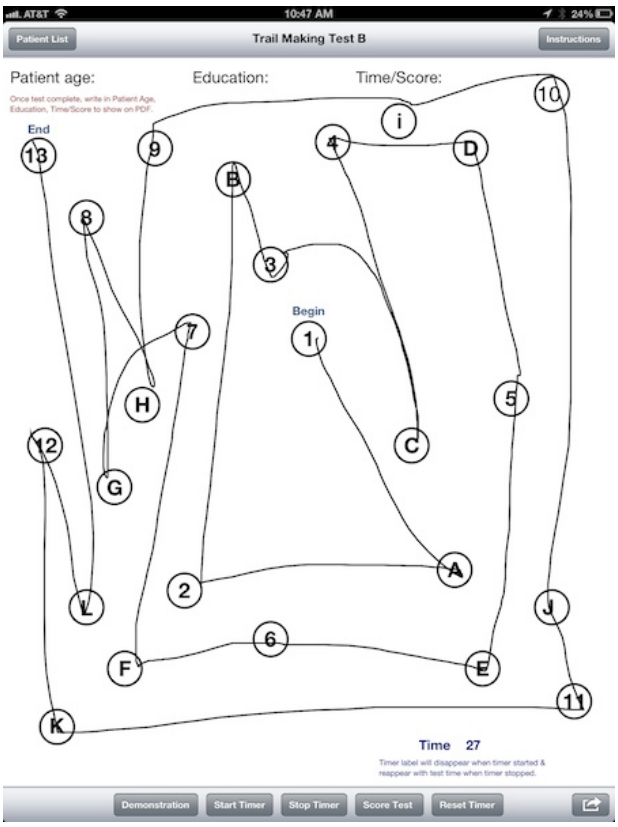
hippocampal size and enhanced cognitive function and thus may be a significant covariate in the present stud. [147,148]

Cognitive Assessment

The primary outcome measures were Trail Making B and five tasks included in the UPENN computerized neurocognitive battery (CNB). All of these tasks are considered standard and psychometrically valid**.**[149] They have been selected, in part, because earlier work has demonstrated that they're sensitive to acute changes in brain function. For example, when blood glucose levels are experimentally raised in adolescents, the Trail Making Test shows corresponding changes in performance. [17,24] Each task is described in greater detail below.

Trail-Making Test

The Trail-Making Test (TMT) is one of the most widely used instruments in neuropsychological assessment as an indicator of speed of cognitive processing, mental flexibility and executive functioning. It is made up of two components, Trail-Making A and B. For each, participants are asked to trace a line, in order from one unit to the next. Trail-Making A requires sequential tracing between alphabetically labeled points. Trail-Making B is considerably harder as it requires participants to alternate in sequential order between alphabetical and numerical points. Time of completion represents participant score. Trail-Making B was used as a primary outcome measure in prior studies and was used to power the present study.

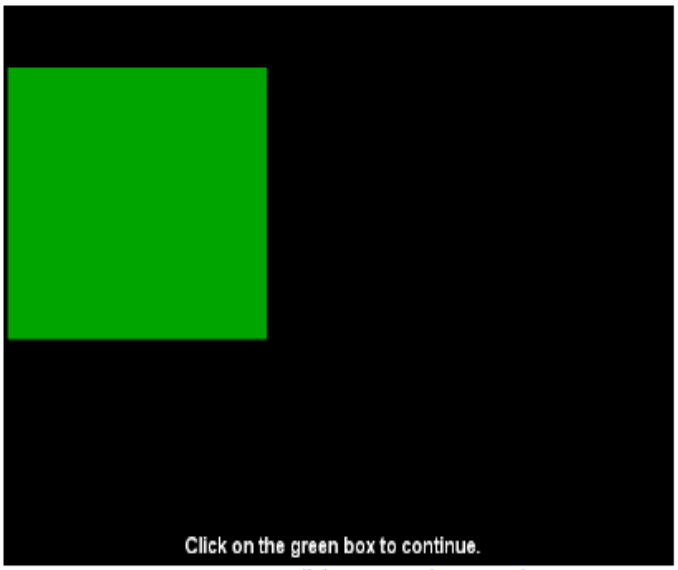


UPENN computerized neurocognitive battery (CNB)

The CNB comprise a practice test and 5 assessments presented in the following order each time the test was administered: MPRAXIS, CPW, CPF, CJLO, PCPT-NL and the sVOLT. Each of these tasks is described in greater detail below.

MPRAXIS (Mouse Practice Test)

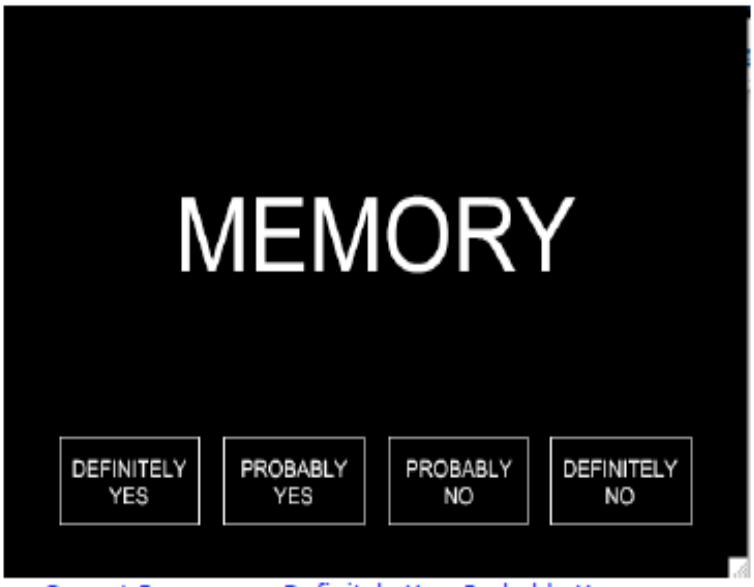
The MPRAXIS is a measure of sensory-motor ability designed to familiarize participants with the computer mouse used during all of the CNB tasks. During the MPRAXIS, the participant must move the computer mouse cursor over an ever-shrinking green box and click on it once each time it appears on a different location on the test-page. If participants can't complete the MPRAXIS, it is likely they won't be able to complete any of the other tasks.



Correct Response: Click once on the green box.

CPW (Computerized Penn Word Memory Task)

The CPW is a measure of word memory. Initially, participants are shown 20 words that they will be asked to identify later during both immediate and delayed recalls (delayed recall = CPWd). During the immediate recall (CPW), participants are shown a series, one at a time, of 40 words - the 20 stimuli they were asked to memorize mixed with 20 novel stimuli. The participants' task is to decide whether they have seen the word before by clicking with the mouse on one of four buttons, presented in a 4-point scale: "definitely yes", "probably yes", "probably no" and "definitely no".



Correct Responses: Definitely Yes, Probably Yes.

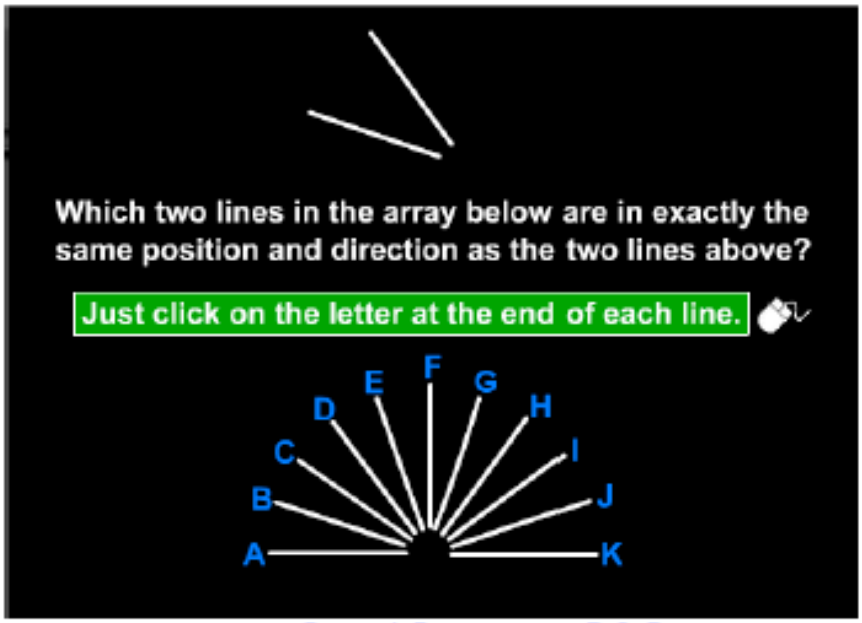
CPF (Computerized Facial Memory Task) and CPFd

The CPFd is a measure of delayed facial memory. In the first part of this test, participants are shown 20 faces and asked to identify them for immediate recall (CPF). During the delayed recall (CPFd), participants are shown a series, one at a time, of 40 faces: the 20 study stimuli/faces they were asked to memorize mixed with 20 novel faces, all different from the 20 distractors shown on the CPF. The participants' task is to decide whether they have seen each face before by clicking with the mouse on one of four buttons, presented in a 4-point scale: "definitely yes", "probably yes", "probably no" and "definitely no." Note: The CPFd takes place 15-30min after the CPF, usually with other tasks in between to control for the delay time. All facial stimuli are black and white photographs of faces rated as having neutral expressions, balanced for gender and age. Faces are pasted on a black background with hair blending into it as to remove the hair's identifying characteristic.

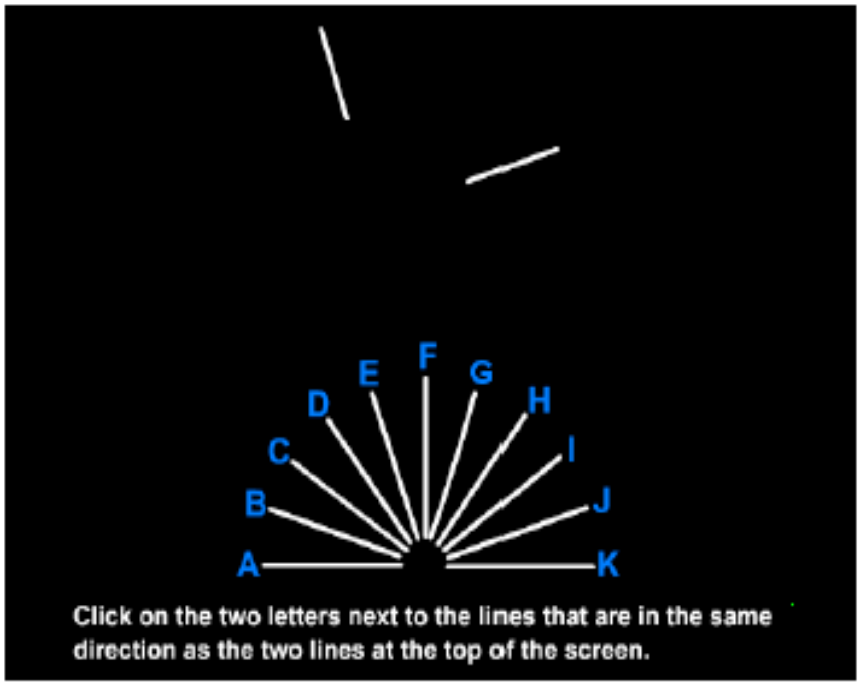
Correct Responses: Definitely Yes, Probably Yes

CJLO(Computerized Judgment of Line Orientation)

The CJLO is a measure of spatial orientation abilities. Participants are shown a pair of lines on top of the screen and asked to click with the mouse on the letter label of the matching lines arranged in a coordinate array on the mid-bottom of the screen. During the practice trials, the lines on the top of the screen are of equal length with the lines on the bottom; during the test, the lines on the top of the screen are shorter than the ones in the array, increasing the difficulty of the matching.



Correct Responses: B & D

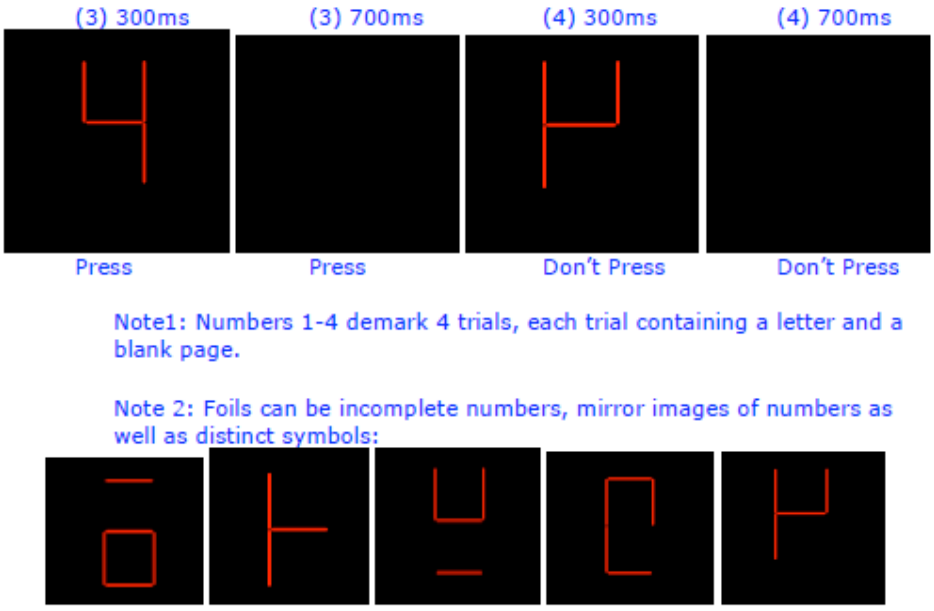


Correct Response: E & J

PCPT-NL (Penn Continuous Performance Test-Number and Letter Version)

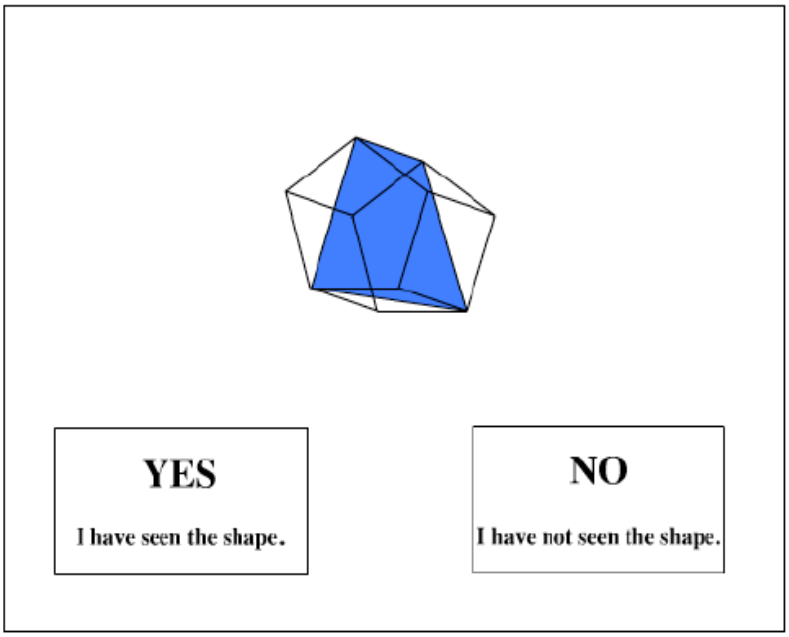
The PCPT-nl is a measure of visual attention and vigilance. In this task, a series of red vertical and horizontal lines flash in a digital numeric frame (resembling a digital clock).

Participants are instructed to press the spacebar whenever these lines form complete numbers or complete letters. The task is divided in two parts: one set of trials where the participant is looking for complete numbers lasting 3 minutes followed by another set of trials where the participant is looking for complete letters for the same duration. Each stimulus flashes for 300 milliseconds and a blank page is then displayed for 700 milliseconds, giving the participant 1 sec to respond to every trial. The participant practices both sets of trials before the task begins.



sVOLT

The sVOLT is a measure of visual object learning and memory. In the first part of this test, participants are shown 10 three-dimensional Euclidean shapes (see below) and later asked to identify for both immediate and delayed recall (delayed recall = sVOLTd). During immediate recall (sVOLT), participants are shown a series, one at a time, of 20 three-dimensional Euclidean shapes - the 10 shapes they were asked to memorize mixed with 10 novel shapes. The participant's task is to decide whether he/she has seen the shape before by clicking with the mouse on one of two buttons: "YES I have seen the shape" or "NO I have not seen the shape."



Correct Response: Yes, I have seen the shape.

Sleep and Mood Assessment

Sleep and mood were assessed using a subset of questions from the abbreviated and previously validated Children's Sleep Habits Questionnaire and POMS-A, a mood assessment tool previously validated in early adolescents and adolescents.[150,151] Both variables independently correlate with cognitive dysfunction and are thus potential confounders.[67,151–156]

Study Aim 2:

Proxy Allostatic Load (AL)Assessment

To assess proxy AL at baseline and post-intervention, the following assessments were conducted: BMI, waist and circumference and BP. These are all considered standard proxy measures of AL.[129] Actual AL is a composite measure of multi-system response to chronic stress. Additional measures were not assessed in the present study due to logistical concerns and resource limitations. Thus, instead of a composite score, each measure was analyzed for correlation with cognitive performance.

Height

Height was measured to the nearest 0.1 cm with standardized measuring equipment (Height Stadiometer)

Weight

Body weight was determined to the nearest 0.05 lb using a Tanita body composition analyzer and scale (model 2001, Tanita Corp, Tokyo, Japan).

BMI

BMI was calculated in kilogram per meter square and then converted to a sex- and age-specific BMI percentile value using a computerized formula derived from the 2000 Centers for Disease Control Growth Charts. Only children in the an at risk for overweight BMI stratum (85th to 94th percentile), or a normal BMI stratum (<85th percentile) were included in the study. Those with a BMI $\geq$ the 95th percentile were excluded.

Hip Circumference

Waist circumference was measured at the smallest circumference between the rib cage and the iliac crest, with the subject in the standing position.

Blood Pressure (BP)

Blood pressure was measured with an automated blood pressure monitor (OMRON 711 Automatic IS, Omron Healthcare, Hamburg, Germany) with a child cuff (type 40S, 15–22 cm) while the subject was sitting on a chair with the right arm held in the horizontal position. Systolic, diastolic, and mean blood pressures were recorded as the average of three readings.

IV. Data Analysis Plan

Study Aim 1

Analysis of covariance (ANCOVA) was used to examine the effectiveness of the MF/LS dietary intervention on the primary and secondary outcomes. This analysis evaluated the mean difference of the post-intervention scores between intervention and control conditions and controlled for pre-intervention scores as well as other covariates (gender, SES, mood, physical activity and sleep). Shapiro-Wilk tests was used to establish whether the data was normally distributed. Paired t tests were used to test for baseline differences between the two groups. For data that failed the Shapiro-Wilk test of normal distribution, a Wilcoxon signed-rank test was used. All data were reported as means with a difference considered significant at $P < 0.05$. Statistical testing was performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

Study Aim 2

To determine if proxy of AL acts as a moderator of the intervention effects, interaction effects between intervention and the select measures of AL in the ANCOVA models were examined. The AL data was evaluated for the main effect and the interaction effect with the MF/LS intervention. The null hypotheses for both of these aims was assessed by examining if no effect/no change is observed.

V. Expected Results and Interpretation

Study Aim 1

Expected Results

Study participants randomized to dietary intervention will have significantly higher cognitive function scores (p-value =0.05) at T2 (post-intervention) as compared to T1 (baseline). Cognitive function at T1 and T2 will not change significantly in participants randomized to control or

delayed control. There will be a significant interaction between these groups such that those receiving MF/LS will show a greater improvement than the other two groups.

Rationale

Several studies examining the relationship between diet and cognitive function suggest that a more Western typical diet, containing high amounts of fat and/or sugar has a deleterious effect on cognition as compared to one more in alignment with current national standards. Thus, participants with a HF/HS dietary pattern should show improvement in cognitive functions when placed on a more healthful MF/LS diet. Given that significant differences in cognitive function have been observed acutely, after a single meal, it is expected that such changes will be observed after a 2-week intervention.[14,15,17,24,67]

Interpretation of Expected Results

Short-term consumption of a MF/LS diet may improve cognitive function in early adolescent African-Americans exhibiting a HF/HS dietary pattern. Improved cognitive function may have important implications for academic performance.[157]

Study Aim 2

Expected Results

a) Proxy measures of AL will remain stable from baseline (T1) to post-intervention (T2) in all participants. b) Participants with high proxy measures of AL at T1 who are randomized to intervention will show less improvement in cognitive assessment from T1 to T2 than those in the same cohort who exhibit lower proxy measures of AL.

Rationale

a) If AL is defined as the body's physiological response to chronic stress, it should remain stable over a 2- to 4-week period, b) Research suggests that high AL is associated with cognitive dysfunction.[46] Thus, if the intervention diet improves cognitive function, this increase should be less pronounced in those with high proxy measures of AL at baseline as compared to those with lower proxy AL.

Interpretation of Expected Results

High AL may interfere with expected improvement in cognitive function after exposure to a 2-week MF/LS dietary intervention in early adolescent African-Americans who demonstrate a high fat, high sugar dietary pattern dietary pattern.

VI. Power Calculations

Power analyses were conducted to determine the required sample size, based on reviews of literature and a pilot study conducted in Summer 2014.[19] Using a power calculation software program G*Power 3.1.9.2, an optimal sample size was determined to be 24 children based on two groups. This calculation was based on effect size for the primary outcome, Trail-Making B test.[19] Based upon an expected attrition rate of 20%, we planned to recruit n = 30 for two groups for an expected completer n= 24 such that the primary outcome will have 90% power. While analyses of the other cognitive measures did not yield adequate power, they will still be included in the study as secondary outcomes because literature on their effect size in RCT studies in preadolescents of greater than 1-day duration is nonexistent. Thus, in a 2-week study, some of these cognitive tests may show significant effects.

CHAPTER 4: RESULTS

Characteristics of Study Participants

The 17 recruited participants for this study consisted of an ethnically diverse (10.5 African American, 2.5 Latino, 2.0 Asian and 2.0 Caucasian; including 3 children identifying as more than 1 ethnicity) low to middle income population of 8 to 11 year old (Mean: 9.44 yo) preadolescent children with an average BMI% of 57.32. No significant differences were detected between groups in mean age, female to male ratio, BMI, waist circumference or blood pressure (*See Table 1 below*). Although most participants were below the New York State percent federal poverty line (%FP) cutoff for government assistance, three participants were far above it with two at 263% and one at 496% FPL, creating a large standard deviation in this parameter across and within groups. At baseline, there appeared to be a difference between groups for vigorous physical activity and sedentary activity, with the intervention group engaging in almost twice as much vigorous activity at baseline compared to control and engaging in almost half the amount of sedentary activity as the control group. However, these differences did not prove to be statistically significant. During the intervention period, participants were enrolled in the HypotheKids Summer Science enrichment program engaging in the same vigorous and sedentary activities with the same staff.

Table 1. Baseline characteristics of the study population

	All (n =17)		Intervention (n =8)		Control (n =9)		P-value
	Mean	SD	Mean	SD	Mean	SD	
Age (yrs)	9.4	±1.1	9.5	±0.8	9.4	±1.4	ns
F:M ratio	0.4	--	0.4	--	0.4	--	ns
Ethnicity:							
% AfAm	61.7	--	68.7	--	55.0	--	ns
% Latino	14.7	--	6.2	--	22.2	--	ns
% Caucasian non-hispanic	11.7	--	6.2	--	16.6	--	ns
% Asian	11.7	--	18.7	--	5.5	--	ns
Initial Face Recognition RT (Pre)			1268.8	±438.0	1547.3	±936.5	0.2
Delayed Face Recognition RT (Pre)			1243.34	± 300.8	1336.1	± 759.0	0.11

	All (n = 17)		Intervention (n=8)		Control (n =9)		P-Value
	Mean	SD	Mean	SD	Mean	SD	
Percent Federal Poverty Line (%FPL)	176.5	± 100.6	172.7	± 134.9	180.3	± 58.7	0.457
Vigorous Physical Activity (METS)	11773.8	± 23752.8	17667.5	± 31,185.8	5880.0	± 12,531.32	0.338
Sedentary Physical Activity (METS	9690.4	± 7276.8	7174.8	± 3,192.1	12205.9	± 9,370.5	0.175
BMI	17.3	± 2.4	16.6	± 2.01	18.1	± 2.7	0.367
%BMI	57.3	± 26.9	47.19	± 29.5	67.4	± 21.4	0.765
Waist Circ. (cm)	60.9	± 5.4	58.7	± 3.06	63.7	± 6.5	0.569
Diastolic BP	89.9	± 17.3	92.8	± 24.9	87.4	± 8.2	0.633
Systolic BP	59.1	± 15.1	59.8	± 22.7	58.4	± 5.1	0.468

2-Week Intervention Period

Following randomization into the control or intervention groups, participants remained together in the HypotheKids science enrichment program for the entire duration of the intervention period. Both groups received identical camp curricula from the same instructors and engaged in identical activities throughout the day, including 45 minutes of vigorous physical activity playing "tag" or basketball.

Compliance

Based on self-report, only 4 of the participants were non-compliant with food intake instructions during the intervention period (2 intervention subjects and 2 control subjects). Each of these incidents occurred on 1 day of each participant's 2-week period. In each instance, a 24-hour recall was conducted to assess each participant's actual intake during the day they were non-compliant. This data was recorded and included in their 2-week dietary analysis with Food Processor and NDSR software. Non-compliance was reported as due to absence in 3 instances and accidental food spoilage in a 4th instance.

Caloric Intake

Following dietary analysis with Food Processor and NDSR software, mean caloric intake was calculated for each subject. As shown below in Table 2. and Fig 1, no significant differences in mean caloric intake were observed between the control and intervention groups during the 2-week intervention period. This finding was present when absolute means were considered as well as when the analysis controlled for both age and gender.

Table 2. Mean and mean adjusted caloric intake between groups during the 2-week intervention

	Intervention	Control	P-Values
Absolute Mean (Kcal)	1640.0 ± 205.1 (SD)	1645.9 ± 180.0 (SD)	0 .952
Adjusted Mean (Kcal) Controlling for Gender & Age	1633.6 (95% CI: 1450.3, 1814.8)	1649.2 (95% CI: 1508.1, 1789.7)	0.804

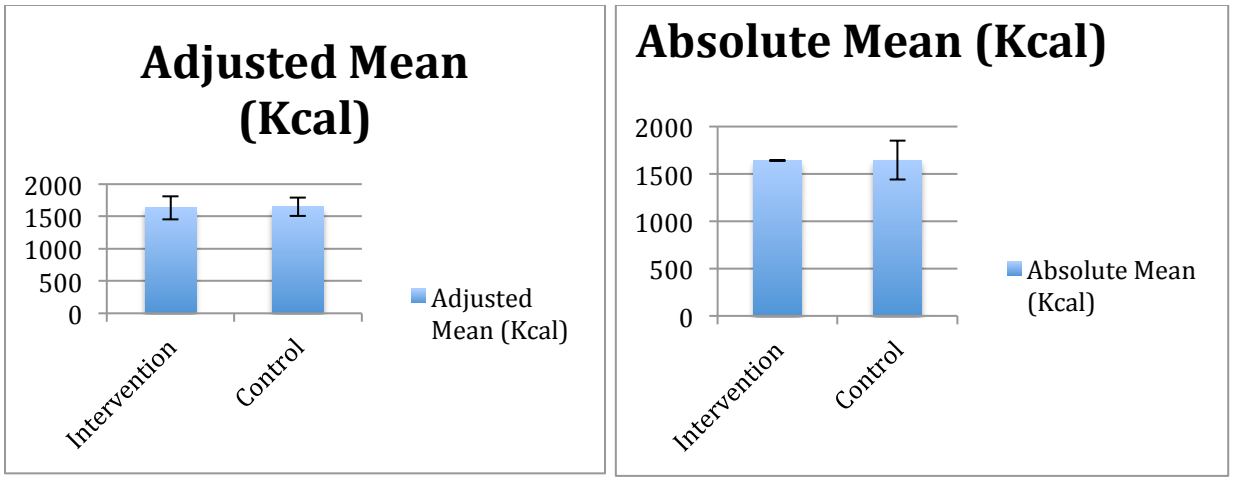


Fig. 4 Absolute mean and Adjusted mean caloric intake between groups during the 2-week intervention.

Dietary Targets

As described in the Chapter 3 as well as below in Table 2., the intervention and control groups were each assigned specific targets for % intake of calories from total fat and added sugar. In addition, targets were set for the intervention group's intake of fruits, vegetables and whole grains. Specific targets for fruit, vegetable and whole grain intake were not set for the control group at outset. However, after a number of parents requested assurance that their children would be served a similar amount of fruits and vegetables (FV) regardless of the group they were randomized to, the targets for FV were matched between groups.

Table 3. Dietary targets for both groups

	Intervention	Control
% Total Fat	25%	≥ 40%
% Added Sugar	<10%	≥ 15.9%
Fruit Servings	3-4 servings	3-4 servings
Vegetable Servings	3-4 servings	3-4 servings
Whole Grains	4-6 servings	No Target Set (Usual Intake)

Primary Dietary Targets: ***% Total Fat and % Added Sugar Intake***

As shown in Fig. 5 and 6, although % total fat and % added sugar intake targets were met in the intervention group (% total fat intake, $p = 0.122$), they were not met in the control group. In the control group, there was a significant difference between the target values for % total fat ($p = 0.00002$) and % added sugar intake ($p = 0.004$).

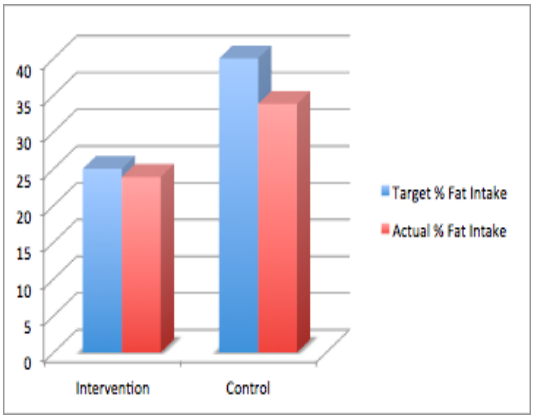


Fig. 5 Comparison of target vs. actual % fat intake in the intervention and control groups

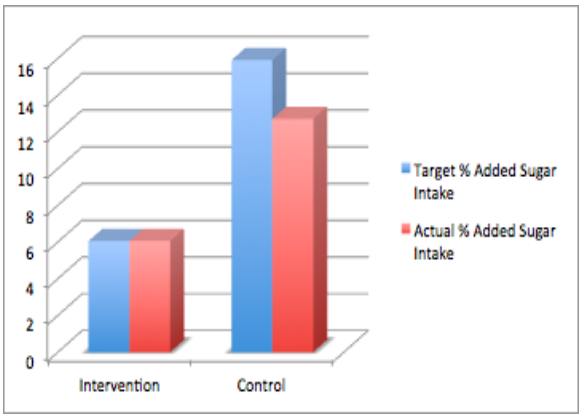


Fig. 6 Comparison of target vs. actual % sugar intake in the intervention and control groups

Table 4. Intervention Group: Actual % Total Fat and % Added Sugar Intake vs. Target Values

	Intervention Group Actual Intake	Target	P-Value
Mean %Total Fat	23.87 ± 1.81 (SD)	25	**0.122**
Mean % Added Sugar	6.05 ± 1.22 (SD)	<10	**Target Met**

Table 5. Control Group: Actual % Total Fat and % Added Sugar Intake vs. Target Values

	Control Group Actual Intake	Target	P-Value
Mean %Total Fat	33.8 ± 1.7 (SD)	40	**0.00002**
Mean % Added Sugar	12.7± 2.2 (SD)	15.9	**0.004**

Although % total fat and % added sugar intake targets were not met in the control group, independent samples t-test analysis still revealed statistically significant differences in both intake values *between* the control and intervention groups (see Fig. 7 and Table 6).

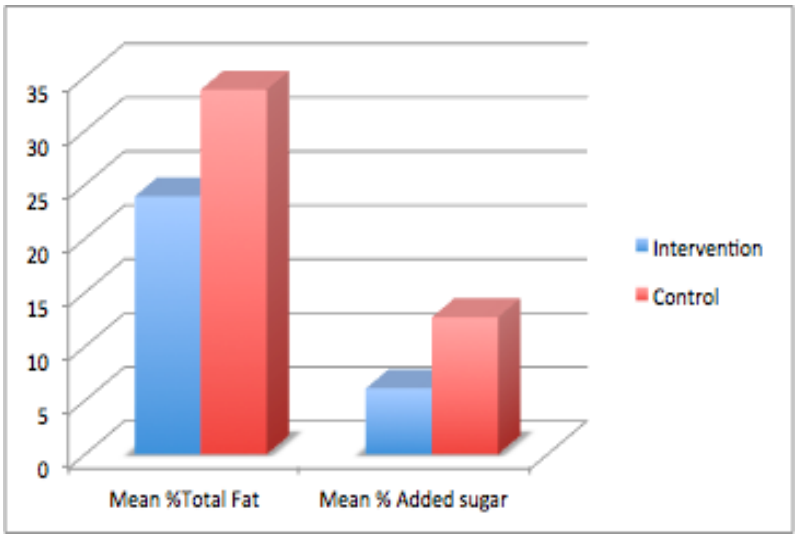


Fig. 7 Comparison of mean % total fat and % added sugar between groups.

Table 6. Comparison of mean % total fat and % added sugar between groups.

	Intervention	**Control**	**P-Value**
Mean %Total Fat	23.9 ± 1.81 (SD)	33.8 ± 1.7 (SD)	**.0007**
Mean % Added sugar	6.1 ± 1.2 (SD)	12.7± 2.2 (SD)	**.0006**

Mood & Sleep Assessment

Pre-post measures of mood and sleep were not statistically significant between groups or between administrations for each group. Thus, neither mood nor sleep were added to the model comparing cognitive performance between groups.

Primary Analyses: Dietary Intervention and Cognitive Function

Analysis of dietary intervention effect on cognitive function outcomes was conducted using two approaches, ANCOVA and Repeated Measures. The rationale for examining the data using both approaches is explored in greater detail in the discussion but in brief, each answers a different aspect of the primary research question. Presenting only one approach would not provide a full picture of the data findings requisite to understanding the data and informing future research.

ANCOVA: Analysis of Intervention Effect on Cognitive Function Outcomes

Summary

ANCOVA analysis was conducted using baseline cognitive function scores, gender and age as covariates. Overall, the intervention did not prove statistically significant for most of the cognitive tests administered and analyzed. Only 2 out of the 10 assessments analyzed were significant in favor of intervention. The full results summary is provided in the table below.

Table 7. ANCOVA: Analysis of Intervention Effect on Cognitive Function Outcomes

TEST	Intervention				Control				P-Values			
	Pre	Post	Adjust. Mean (ms.)	95% CI	Pre	Post	Adjust. Mean (ms)	95% CI	Group Effect	Group Effect Controlling for Gender	Gender Effect	Group * Sex Interaction
Trail Making B (RT)	143.7 ± 70.4 (SD)	122.3 ± 63.6 (SD)	118.8	56.2, 181.4	123.7 ± 43.5	114.8 ± 72.6 (SD)	129.8	68.6, 191.1	.762	.772	.680	.894
Initial Face Memory: Correct Responses	26.5 ± 6.0 (SD)	27.6 ± 6.3 (SD)	27.4	24.8, 29.9	25.9 ± 6.0 (SD)	27.4 ± 7.0 (SD)	27.6	25.1, 30.2	.876	.851	.820	.702

Initial Face Memory (CPF): Median Total Correct (RT)	1268.8 ±437.97 (SD)	1037.8 ±459.88 (SD)	1124.1	883.8, 1364.3	1547.3 ± 936.5 (SD)	1508.8 ± 332.0 (SD)	1539.2	1298.2, 1780.2	.054	.022*	.012*	.678
Delayed Face Memory (CPFd): Median Total Correct (RT)	1243.3 ± 300.7 (SD)	1045.1 ± 392.6 (SD)	1158.4	687.1, 1629.7	1336.1 ± 759.0 (SD)	1906.2 ±2112.8 (SD)	2110.0	1649.6, 2570.4	.789	.005*	.007*	.017*
Delayed Face Memory (CPFd): True Negative Median (RT)	1244.9 ±521.61 (SD)	1061.1 ±397.63 (SD)	1199.2	978.8, 1419.6	1443.6 ± 718.3 (SD)	1345.9 ± 572.1 (SD)	1605.10	1382.4, 1827.8	.787	.012*	.009*	.036*
Delayed Word Memory (CPWd) :Median Total Correct (RT)	1109.2 ± 489.3 (SD)	976.8 ± 549.8 (SD)	1063	67.1, 2058.9	1407.5 ± 643.7	1421.6 ± 881.5 (SD)	1398	376.4, 2419.4	.436	.689	.166	.496
Line Orientation (CJLO): Median Total Correct (RT)	5961.6 ± 29996.5 (SD)	3585.6 ±1038.8 (SD)	3754	2093.5, 5414.2	6063.4 ±1051.4 (SD)	5341.1 ±2315.6 (SD)	5529	3959.8, 7098.4	.089	.459	.204	.585
Initial Visual Object Learning (sVOLT) :Median Total Correct (RT)	1145.7 ± 301.4 (SD)	853.6 ±342.0 (SD)	979.7	656.5, 1303.0	1305.9 ±590.7 (SD)	960.9 ± 526.2 (SD)	904.8	621.7, 1187.8	.969	.922	.557	.834

***Delayed Visual Object Learning (sVOLTd) :Median Total Correct (RT)**	936.4 ± 369.2 (SD)	785.6 ± 355.9 (SD)	873.5	687.4 , 1332.4	1010.3 ± 483.0 (SD)	1073 ± 521.2 (SD)	845.2	615.4 , 1245.3	.433	.548	.723	.682

Initial Face Memory: Response Time

Participants randomized to the MF/LS intervention demonstrated a faster response time (RT) for *correct* responses (true positive 'hits' and true negatives) on the initial facial recognition task and its delayed counterpart. Compared to control, the intervention group displayed a faster total correct RT while controlling for gender ($p = .022$). Gender was controlled for in this analysis because it displayed a significant, independent effect on task performance ($p = 0.012$). A group by gender interaction was not observed and no significant effect was observed for age.

Table 8. Total median correct response time for the initial facial recognition task

Intervention				**Control**				**P-Values**			
Pre	**Post**	**Adjust. Mean (ms.)**	**95% CI**	**Pre**	**Post**	**Adjust. Mean (ms)**	**95% CI**	**Group Effect**	**Group Effect Controlling for Gender**	**Gender Effect**	**Group* Sex Interaction**
1268.8 (SD 437.97)	1037.8 (SD 459.88)	1124.1	883.82 , 1364.35	1547.3 (SD 936.53)	1508.8 (SD 331.96)	1539.2	1298.2, 1780.2	.054	.022	.012	.678

In sum, although significant differences were not found for the number of total correct responses, there was a significant difference in response time for total correct responses,

including both hits and correct rejections, favoring intervention during the facial recognition task.

Delayed Face Memory: Response Time

Participants randomized to the MF/LS intervention demonstrated faster reaction time (RT) for *correct* responses (true positive 'hits' and true negatives) on the delayed facial recognition task. Compared to control, the intervention group displayed 1) a faster delayed task median total correct RT when controlling for gender and age (p =.005) and 2) a faster delayed task true negative RT when controlling for gender and age (p = 0.012). The intervention group demonstrated a faster median total correct RT on the *delayed* facial recognition task when controlling for gender and age as compared to control (p=.005). Again, similar to the initial facial recognition task, a significant gender effect was noted (p =.007). While there was no age effect by itself or group by gender interaction effect, there was a significant group by age interaction (p = 0.017).

Table 9. Median total correct response time for the delayed facial recognition task

Intervention				Control				P-Values			
Pre	Post	Adjust.Mean	95% CI	Pre	Post	Adjust.Mean	95% CI	Group Effect	Group Effect Controlling for Age & Gender	Gender Effect	Group* Age Interaction
1243.34 ± 300.75 (SD)	1045.13 ± 392.65 (SD)	1158.41	687.09, 1629.72	1336.06 ± 758.97 (SD)	1906.25 ± 2112.85 (SD)	2109.98	1649.57, 2570.39	.789	.005	.007	.017

Total correct response time for delayed facial recognition was further analyzed by looking at its two subcomponents, true positive ('hits') and true negative response times ('correct

rejections'), respectively. Compared to control, the intervention group demonstrated a faster median true negative response time for facial recognition when controlling for both gender and age (p = .012). In this analysis a gender effect was not observed by itself. A significant group by gender interaction (p=0.03) as well as gender by age interaction was observed (p = .036) (Table 4).

Table 10. True negative median response time for *Delayed* Facial Recognition

Intervention				Control				P-Values			
Pre	Post	Adjust. Mean (ms)	95% CI	Pre	Post	Adjust. Mean (ms)	95% CI	Group Effect	Group Effect Controlling for Age & Gender	Age Effect	Group * Age Interaction
1244.87 ± 521.61 (SD)	1061.07 ± 397.63 (SD)	1199.23	978.85, 1419.60	1443.57 ± 718.27 (SD)	1345.94 ± 572.13 (SD)	1605.10	1382.37, 1827.82	.787	.012	.009*	.036

d' Analysis

d' helps to account for response bias. [158] To calculate a d′ score for each subject, the responses for each of the 40 item facial recognition memory trials must be categorized as a hit, miss, false alarm, correct rejection, or no response. A "hit" is defined as correctly identifying a previously studied stimulus as "old / studied," a "miss" is defined as incorrectly identifying a previously studied stimulus as "new / not studied," a "false alarm" is defined as incorrectly identifying an unstudied stimulus as "old / studied," a "correct rejection" is defined as correctly identifying an unstudied stimulus as "new / not studied," and a "no response" is defined as failing to make a response in the 4-second response time window.

Next, a constant of 0.05 is added to each number of hits, misses, false alarms, and correct rejections to eliminate cases of 0 and 1 hits and false alarms which would result in an undefined value for d′. Then, each participant's "hit rate" is determined by calculating "number of hits / (number of hits + number of misses)," and each subject's "false alarm rate" is determined by calculating "number of false alarms / (number of false alarms + number of correct rejections)." Finally, item and relational d′ scores are calculated for each subject by subtracting the z-score of the false alarm rate from the z-score of the hit rate (with chance performance represented by a d′ score of 0). The results of an independent t-test revealed no significant differences in d' pre ($p = .631$) or d' post ($p = .490$)

Repeated Measures: Analysis of Intervention Effect on Cognitive Function Outcomes

Using a repeated measures approach, controlling for both age and gender, none of the cognitive analyses proved statistically significant. This data is presented in the table below. This approach is a more conservative analysis and the merits of using repeated measures over ANCOVA remains somewhat controversial within the statistical literature. Some argue that when successful randomization results in no significant differences in pretest performance, treating the baseline pretest as a covariate in an analysis of covariance (ANCOVA) provides a more powerful test, because ANCOVA is more parsimonious than repeated-measures ANOVA.[159] However, regardless of approach applied, it is evident that the effect of a 2-week MFLS dietary intervention on cognitive function in a population with a pre-established HFHS diet is unclear and warrants further investigation in a larger sample.

Table 11. Repeated Measures Analysis of Intervention Effect on Cognitive Function Outcomes

TEST	Intervention		Control		P-Values			
	Pre	Post	Pre	Post	Time	Time* Gender	Time * Age	Time* Group
Initial Face Memory: Correct Responses	26.5 ± 6.0 (SD)	27.6 ± 6.4 (SD)	25.9 ± 6.0 (SD)	27.4 ± 7.0 (SD)	.191	.718	.243	**.778**
Initial Face Memory (CPF): Median Total Correct (RT)	1355.6 ± 524.4 (SD)	1074.7 ± 503.6 (SD)	1760.7 ± 444.8 (SD)	1483.0 ± 742.7 (SD)	.518	.054	.761	**.993**
Delayed Face Memory (CPFd): Median Total Correct (RT)	1243.3 ± 300.8 (SD)	1045.1 ± 392.7 (SD)	1336.0 ± 759.0 (SD)	1906.3 ± 2112.8 (SD)	.314	.256	.280	**.331**
Initial Word Memory (CPW) :Median Total Correct (RT)	31.8 ± 6.9	28.4 ± 7.3	30.4 ± 6.7	30.3 ± 8.0	.150	.369	.204	**.240**
Delayed Word Memory (CPWd) :Median Total Correct (RT)	1109.3 ± 489.3	976.8 ± 549.8	1407.5 ± 643.7	1421.6 ± 881.5	.434	.296	.352	**.685**
Line Orientation (CJLO): Median Total Correct (RT)	5961.6 ± 2996.5	3585.6 ± 1038.8	6063.4 ± 1051.4	5341.1 ± 2315.6	.514	.292	.737	**.255**
Initial Visual Object Learning (sVOLT) :Median Total Correct (RT)	1145.8 ± 590.7	853.6 ± 342.0	1305.9 ± 590.7	960.9 ± 526.2	.816	.800	.877	**.786**
Delayed Visual Object Learning (sVOLTd) :Median Total Correct (RT)	936.4 ± 369.2	785.6 ± 355.9	1010.3 ± 483.0	1073.0 ± 521.2	.054	.760	**.042**	**.227**
Trail Making B (RT)	143.7 ± 70.4	122.3 ± 63.6	143.7 ± 70.4	114.8 ± 72.6	.249	.714	.179	**.524**

Dose Response Analysis: ***Dose Response Effect of Dietary Intervention on Cognition***

Although the present study aimed to maintain the control group's usual intake at 40% of calories/day from total fat and ≥15.9% of calories from added sugar and 25% of calories/day from total fat and <10% of calories from added sugar for the intervention cohort, analysis of the actual intake did not quite meet these initial specifications (see Tables 6 and 7). The average % total fat from calories for the control group was 33.76% and the % added sugar intake was 12.70%. For intervention, the mean % calories from fat was 24.10% (*just shy of 25% target*) and the mean % calories from added sugar was 6.2% - meeting the <10% target.

Given that the % total fat and % added sugar intake varied within groups, additional analysis was conducted to assess whether there was a dose response effect on the cognitive measures with regard to percent total sugar intake, added sugar intake, total fat intake and saturated fat intake.

Dose response effects for mean total % sugar, % added sugar, % total fat and % sat fat of daily calories on the facial recognition task were examined by multiple regression analyses. For median total correct response time on the initial facial recognition task, there were significant dose response effects for all four variables, with more pronounced effects for % saturated fat over % total fat.

The dose response effect for % total sugar and % added sugar on this cognitive domain parameter was similar with a regression coefficient for each of 58.351 ($p = 0.009$) and 58.727 ($p = 0.009$), respectively. By contrast, the dose response effect for % total fat had a regression coefficient of 44.209 ($p = .006$) compared to % saturated fat which had a coefficient of 57.398 ($p = .018$). These results suggest that a 10% increase in % calories from total sugar, added sugar and saturated fat decreases processing speed for median total correct responses on the initial

facial recognition task by approximately 580 ms. Whereas a 10% increase in % total fat decreases median correct response processing speed on the same task by about 440 ms. These analyses (see Figures 8-11) controlled for gender and pre-test scores.

Fig. 8 Dose response effect of % total sugar on facial recognition correct RT

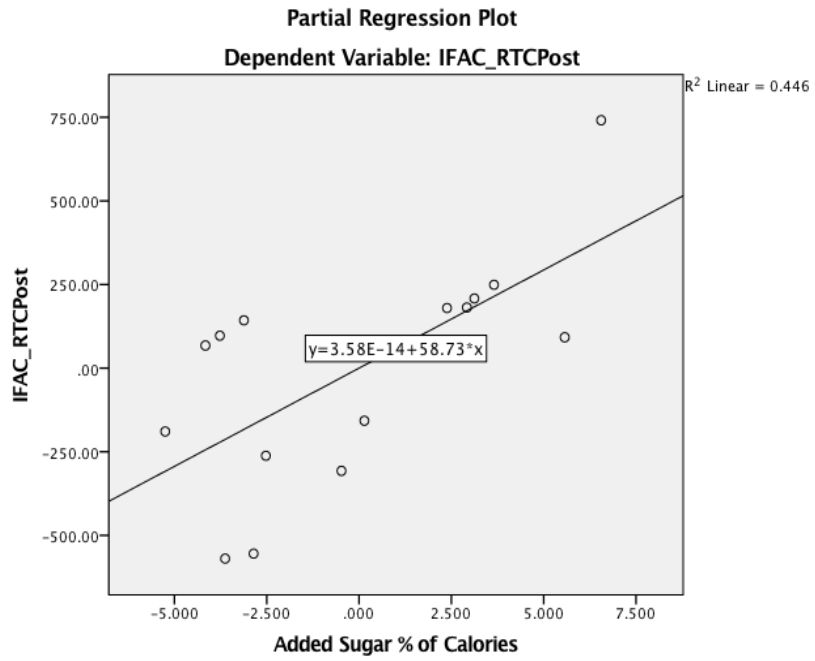


Fig. 9 Dose response effect of % added sugar on facial recognition correct RT

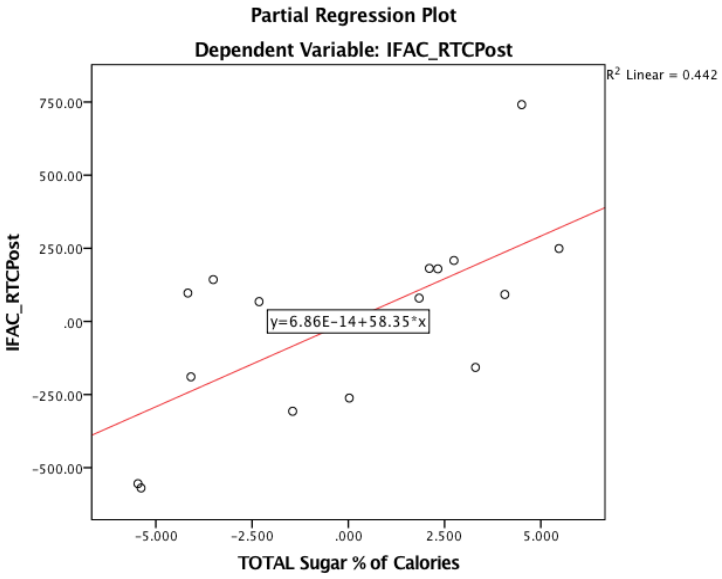


Fig. 10 Dose response effect of % total fat on facial recognition correct RT

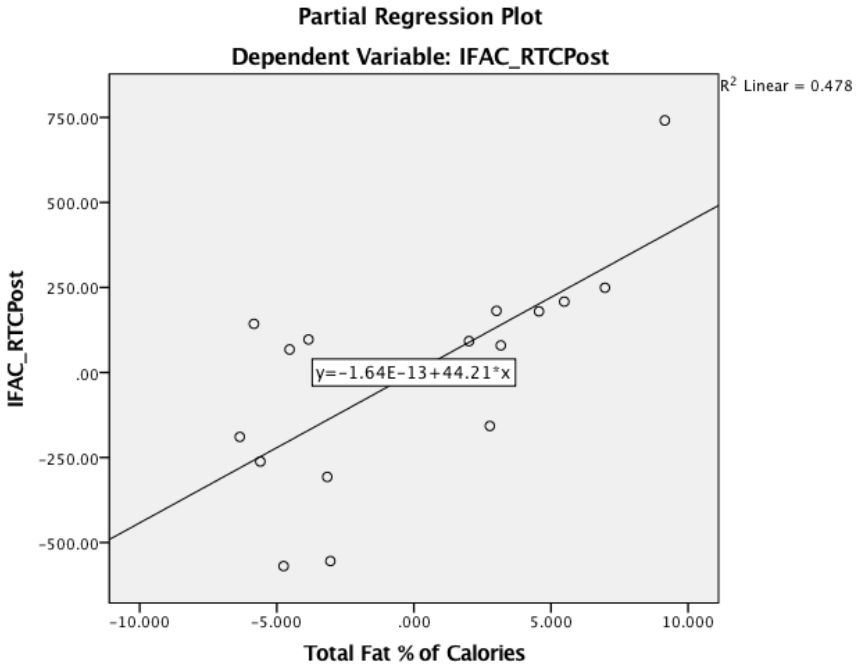
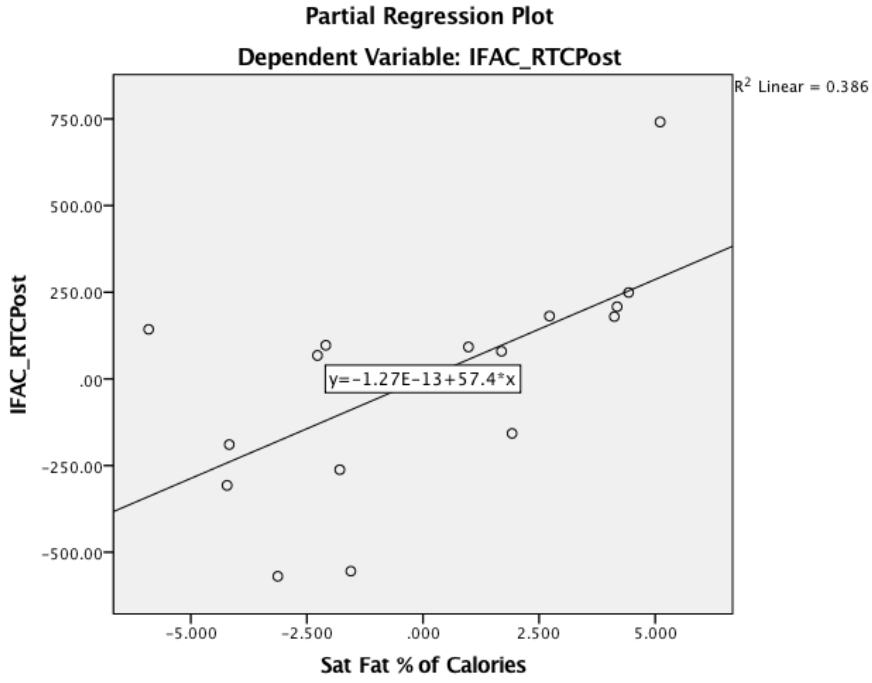


Fig. 11 Dose response effect of % added sugar on facial recognition correct RT



When the variance in median correct response time was examined for this task, 48% was explained by gender and pre-test score, 25.5% was explained by both total % fat and % total sugar and 23.7% was explained by both % saturated fat and % added sugar. A dose response effect was not observed for median correct response time on the delayed facial recognition task for % total sugar, % added sugar, % total fat or % saturated fat.

CHAPTER 5: DISCUSSION

The present study is the first 2-week single blind randomized control trial to examine the effect of diet quality on preadolescent cognitive function. To date, similar diet and cognition RCTs have only been carried out in human adults and animal models. Much of the literature in children and preadolescents is of extremely short duration (single-meal studies) or merely examines the correlation between general intake pattern and one-time cognitive assessment. The present study is unique in its RCT design, 2-week duration and analysis of actual weighed intake to the tenth of a gram while controlling for a number of key potential confounders identified in the literature. Although this study has a number of limitations, within the context of the existing literature, the findings reinforce the need for additional examination of diet's effect on pre-adolescent cognition.

Prior studies provided some evidence for cognitive change in response to acute sugar and fat dietary manipulation and suggested a proposed neurobiological framework that led to this study. The present study did not result in significant findings for initial face memory total correct responses, initial word memory median total correct response time (RT), delayed word memory median total correct (RT), line orientation median total correct (RT), initial visual object learning median total correct (RT), delayed visual object learning median total correct (RT) or Trail Making B (RT). However, results of the present study do suggest that a 2-week reduction sugar and fat intake may improve cognitive performance on working memory while increased intake of either may precipitate cognitive decline in a dose dependent manner. Allostatic load may moderate and/or mediate this relationship. The following sections will explore each of these results in greater detail within the context of similar scientific work. Next, strengths and

limitations of the current project will be discussed followed by recommendations for future research.

Research Question 1

Dietary Intervention and Cognitive Gains

The primary objective of the first research question was to assess whether a 2-week MF/MS dietary intervention diet improves certain aspects of cognitive function in low-income pre-adolescents with a pre-existing HF/HS dietary pattern as compared to control. Cognitive assessment included measures of executive function, speed of processing, working memory, attention and spatial ability. In our sample, intervention appeared to have no effect on the number of correct responses between the pre and post-test assessment for any of the measures administered, from the time series analysis. However, in an analysis of covariance (ANCOVA), on the initial and delayed face recognition tasks (2 of the 10 tests analyzed), the intervention group displayed a significantly faster response time at post-test controlling for baseline scores and relevant covariates. Analysis of the initial face recognition task showed that gender had an independent effect on task performance, warranting control for this covariate. A significant difference in median correct response time favoring intervention was observed while controlling for baseline scores and gender. Analysis of the *delayed* face recognition task revealed a significant gender effect and group by age interaction effect, thus both were controlled for in between group performance analysis. Again, the intervention group displayed a faster median correct response time while controlling for baseline scores, gender and in this analysis, age.

Although these findings appear to suggest that the study intervention elicited statistically significant differences in face recognition median correct response time, they must be interpreted with caution. Only 2 subtests out of the 10 analyzed yielded significant results in favor of

intervention. It is possible that these results are merely the product of chance, and with Bonferroni correction adjusting for multiple comparisons, the p-value would actually be 0.005 to be deemed statistically significant. Given this correction, only the delayed facial recognition task median total correct RT reached statistical significance when controlling for gender and age ($p = 0.005$). Additional research, in a larger cohort, is needed to determine if these findings are valid. Further, statistical significance does not speak to clinical or practical significance. Thus, it is important to explore the meaning behind the present study findings beyond statistical significance and ask: are the findings clinically meaningful? Further, what relevance do they hold for children's academic performance?

Clinically, delayed median correct response time on facial recognition is associated with autism spectrum disorder (ASD).[160] More specifically, a study by Behrmann et al. demonstrated that, compared to controls, individuals with ASD display a 1000 ms delay in median correct response time on facial recognition tasks. Although delayed face recognition response time is not prognostic for ASD, it may aid in developing a framework for understanding why many individuals with ASD display difficulty with emotional face processing and merits further investigation. The idiopathic neural inefficiency observed in face recognition in ASD subjects could be the result of any number of factors. Thus, it would be an extremely wide stretch to attribute similar delays in a non-ASD population to a comparable neural mechanism. Discussion of Behrmann et al.'s study is only made here to provide a rudimentary scale for the potential clinical relevance of the response delays observed in the present study.

In the context of other scientific research, the present study findings align with similar work conducted by Edwards et al. in sedentary men provided a 7-day high fat diet (74% of kcal from fat).[26] Based on analysis, Edwards et al. reported a significant increase in simple reaction

time following the high fat intervention (P<0.01). This increase in reaction time was on the scale of an 11ms difference between baseline and post-intervention testing. By contrast, the observed difference in pre- post improvement in reaction time for the present study was 192 ms. More robust findings in pre-post testing analysis was observed in Nilsson et al.'s 4-week randomized cross-over controlled study examining the effect of an anti-inflammatory "multifunctional" active diet (AD) compared to a control diet (CD) in middle aged, healthy overweight and obese adults.[11] During pre- post results for the intervention period indicated significant improvement in score on the word recognition component of the Rey Auditory-Verbal Learning test ($p<0.05$) as well as a faster reaction time on a test of selective attention ($p<0.05$). The reaction time test was administered at baseline, 45 minutes after meal consumption and 120 minutes post-meal. The time effect was significant at 120 minutes with the AD outperforming the CD by 20 ms. Thus, although the present study did not find significant differences in correct response score for the measures administered, the significant response time differences favoring intervention are well above those found in recent literature of similar study duration.

In terms of academic performance, several studies have found that better working memory is associated with superior scholastic skills, including arithmetic, reading, and writing, and general academic achievement in school-aged children.[161–163] The facial recognition task employed in this study assesses of one aspect of working memory (visual working memory) and thus it may be similarly correlated with academic achievement.[164] Given that most schools employ timed standardized tests as a measure of student achievement and overall school performance, the present results may have important implications for student test performance as well as learning more generally.

Other cognitive measures

It is plausible that the lack of robust findings for the other cognitive measures is due, in part to small sample size. The sample recruited was much smaller than the n value the study was powered for. Despite intense recruitment efforts, the offer of $1200 in camp scholarship and free meal and snack provision for 2 weeks, far fewer participants than anticipated who met eligibility criteria expressed interest or were able to accommodate the study dates.

In addition to sample size, it is possible that the intervention period was too brief. Although the animal literature cited to support the study's neurobiological framework includes acute dietary intervention spanning 3 to 17 days, it is possible that 2 weeks is too brief to elicit cognitive changes in the domains assessed in the present study population. A study of longer duration, and further, with greater adherence to the *a priori* dietary guidelines for each group may elicit more pronounced findings.

Although it is entirely possible that small sample size, intervention duration and adherence to study dietary guidelines are responsible for null findings on the other cognitive measures, examination of the raw data suggests alternative explanations for a number of the measures. On the spatial line orientation task, the absolute mean percent correct responses for all participants at baseline was 45.7% (range: 10% to 73%). Taken together, the mean and range of scores suggests that the task may have been too challenging or difficult for most participants to understand. On the Penn Continuous Performance Task, 4 participants scored near 100% at baseline, leaving very little room for improvement at post-test. Thus a 'ceiling effect' may underlie the null findings for this specific task. Similarly, one subject scored 100% at baseline on the visual object recognition task and another scored 100% on the pre-test word recognition measure. Face recognition was the only task where none of the participants scored 100% at baseline (range: 25% - 90%) and the mean for the entire group surpassed 50% (absolute mean:

65.5%). Thus, for this sample, face recognition may have most accurately assessed participant's improvement from baseline.

Research Question 2

Dose Response Dietary Intake Effect on Cognitive Performance

Although specific targets were set for dietary intake of total fat and added sugar in each group, these guidelines included a relatively wide range of values (Control: >15.9% added sugar, > 40% total fat; Intervention <10% added sugar, 25% total fat). While attempts were made to maintain participants % total fat and % added sugar at the pre-specified ranges for each group, it was not always possible to get participants to meet these guidelines. Due to ethical considerations, none of the participants were coerced into eating all of the food served to them. The only way to alter participants % total fat and % added sugar was to offer them more foods with either higher or lower percentages of fat and added sugar. On the other hand, as a result of this limitation there was enough variability in actual % total fat and % added sugar to examine dose response effect for each on face recognition median correct response time. Additionally, the dose responses for % saturated fat and % total sugar intake were examined. Based on the results, a 10% increase in any one of these was correlated with a 440 ms to 580 ms decrement in response time. Clinically, this delay is at a magnitude almost half that observed between individuals with ASD and normal controls. This finding suggests that increased consumption of fat and sugar may significantly impair children's processing speed on tasks of visual working memory, which may have important implications for both learning and testing performance.

Research Question 3

Proxy Measures of Allostatic Load as a Moderator of Dietary Effect on Cognition

Analysis of the interaction effect for proxy measures of allostatic load using WC, BP and %BMI on facial recognition median correct response time yielded no significant results. There was also no significant correlation between any of these proxy measures and baseline performance for facial recognition. This null finding is most likely attributable to the small sample size as well as limited variance in WC, BP and %BMI. The study aimed to recruit healthy non-obese preadolescent children with no evidence of adverse medical condition. Thus, with the exception of a few participants, all were within normal limits on all three parameters. Only one subject had an abnormally high blood pressure reading for age at baseline of 142/106. Five participants were overweight. Weight status is one indicator of allostatic load. However, the literature only cites obesity in children as independently associated with cognitive dysfunction. None of the participants were above the waist circumference cutoff for obesity for age and sex. Although biomarkers typically included in allostatic load composite score were not assessed, it is likely, based on the proxy measures used, that this sample does not display much variance in composite allostatic load. A larger sample including both normal and overweight subjects may yield enough variability to detect significant differences in composite allostatic load score and observe an interaction effect by group on the cognitive measures.

Strengths of the Study

Strengths of the present study include its single blind randomized controlled design, pre-study taste testing of all study food items with all recruited participants, meal observation to ensure foods were not swapped between groups or with others in the camp, maintenance of

participants daily self-report food records to assess compliance and adherence, provision of food service to participants' homes if they were unable to attend camp, effort to accurately assess and analyze participant's actual consumption during the 2-week intervention period to a tenth of a gram, similar parallel meals to ensure participants were not unblended to their condition, detailed description of actual energy and macronutrient composition provided to each group, inclusion of a previously validated computerized battery of cognitive tests sensitive to short term intervention effect, time matched pre and post cognitive testing administration in the late postprandial period, sleep and mood assessment prior to each cognitive testing administration and multiple weight assessments to ensure subjects remained in energy balance throughout the duration of the intervention. This study is also merited by a pilot feasibility study the year prior to test all of the study procedures, longer randomized controlled intervention period than previously explored in the pediatric cognition literature and inclusion of a tremendously underrepresented group in this field of research.

Limitations

This study has a number of limitations. First, the sample size is small and the study is greatly underpowered. Unexpected difficulty with recruitment led to a smaller sample than anticipated with 13 fewer participants than the study was powered for. The fact that significant results were found on two cognitive tests despite this limitation suggests that even more robust results might be found with a larger sample size. Secondly, the study was limited to an urban, non-obese low-to middle income preadolescents with a pre-established high fat high sugar diet and thus not generalizable to children outside of this demographic. Given the small sample, increased variability in the population likely would have generated too much confounding without sufficient power for subgroup analyses. Thus it is possible that different results may be

observed in other populations. Third, the study is limited by a failure to attain large differences in %fat and % added sugar intake between the intervention and control groups. At outset, the goal was to maintain the control group's usual intake at ≥ 40% of calories/day from total fat and ≥15.9% of calories from added sugar and 25% of calories/day from total fat and <10% of calories from added sugar for the intervention cohort. Parent food requests as well as nut allergy restrictions at the HypotheKids camp tremendously reduced the number of allowable foods that could be served during the study. Further, there was concern that if the foods provided were too drastically different, subjects would become unblinded to their condition.

Interestingly, of the tasks administered, facial recognition is the only one specifically correlated with dentate gyrus associated relational binding function.[165] As mentioned previously, work in animal models has implicated the dentate gyrus region specifically in cognitive deficit following prolonged intake of HF/HS food. Thus, in addition to limitations discussed below, it is entirely possible that the other tests employed in the cognitive battery do not assess the function of the specific brain region most adversely affected by sugar and fat dietary intake manipulation. In addition to including a larger sample size and increasing intervention duration, future studies should include more measures that are specifically associated with dentate gyrus function. Baym et al.'s hippocampal-dependent relational memory task employing Neurobiobehavioral Systems Presentation software, a virtual morris water maze and a virtual eight arm maze employed by Astur et al. represent potential candidates for exploring dentate gyrus specific function and dietary manipulation in preadolescent children.[40,166] Mental rotation and mental folding tasks adapted for children may also be an ideal measure to include in future work because both have been strongly correlated with spatial navigation as well as academic performance in science,

math and entrance into STEM fields.[166,167] Compared to the other tasks mentioned, mental rotation and mental folding tasks are generally relatively inexpensive and easy to administer.

Future Directions

Based on the present preliminary findings, future work should be done in a larger, more representative sample over a longer duration. Effort should also be made to employ a double blind randomized controlled cross-over design that heightens differences in dietary manipulation between groups. Ideally this work will help discern whether a high sugar or a high fat diet result in significant cognitive dysfunction independent of one another or if this effect is only observed when high sugar and fat act synergistically in altering cognitive function. Further, this work should aim to examine whether sugar type (total or added) and fat type (total or saturated) has a significant influence on cognitive performance outcomes. Most importantly, this work should be carried out in a manner that is ethically responsible and respects parental guidelines and autonomy providing adequate transparency in their child's care even though this may make it more difficult to obtain clear cut results. Children are considered a highly vulnerable population within research and every measure should be taken to ensure that future work in this field does not elicit short or long-term adverse effects on children.

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