The effect of weight loss on circulating biomarkers of brain health and executive function

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ABSTRACT

Obesity is associated with deficits in cognitive function, particularly within the domain of executive function (EF). EF refers to higher order cognitive processes that regulate our ability to sustain attention, inhibit subconscious tendencies, remember and manipulate information for immediate use, and remain cognitively flexible. Deficits in EF in overweight and obese individuals may impact the success of weight loss and maintenance efforts. Therefore, understanding the biological links between obesity and EF, as well as the ability to reverse EF deficits with weight loss, is imperative. The first study aimed to determine the effect of weight loss in overweight and obese, middle-aged and older adults on serum brain-derived neurotrophic fact (BDNF), S100 calcium binding protein B (S100B), and glial fibrillary acidic protein (GFAP). Serum samples (n=21; 50-75 years, BMI 25-40 kg/m²) were pooled from two prior weight loss studies. Fasting blood measurements were taken before and after 8- or 12-weeks of hypocaloric diet-induced weight loss (1200 or 1500 kcal/d). Body Mass Index (BMI), body weight, waist circumference, and percent body fat (All p<0.001) decreased with weight loss. Serum BDNF (p=0.871), S100B (p=0.898), and GFAP (p=0.506) did not change following weight loss. The second study aimed to determine the correlation between the magnitude of change in serum BDNF, S100B, and GFAP and the magnitude of improvement in EF performance on three computer-based tests. Participants (n=8; 50-75 years, BMI 25-40
kg/m²) completed 4-weeks of hypocaloric diet-induced weight loss (1200 or 1500 kcal/d), followed by 4-weeks of weight maintenance (hypocaloric diet + steps/d goal). Fasting blood and EF measurements were completed at baseline, and weeks 4 and 8. BMI (p=0.001), body weight (p=0.001), waist circumference (p=0.002), and percent body fat (p=0.001) decreased from baseline to week 8. Serum BDNF (p=0.359), S100B (p=0.277), and GFAP (p=0.585) did not change following weight loss. Go/No-Go (GNG) errors of commission (p=0.009) and AX-Continuous Performance Test (AX-CPT) correct response time (p=0.041) decreased following the weight loss. The change in serum GFAP was inversely correlated with GNG errors of omission (r=-0.716, p=0.046) and AX-CPT correct hits (r=-0.737, p=0.037), and positively correlated with AX-CPT correct response time (r=0.859, p=0.006). In conclusion, although weight loss does not influence serum BDNF, S100B, or GFAP levels, it may have a positive effect on inhibitory control in overweight and obese, middle-aged and older adults. Further research is needed to understand the relationship between serum BDNF, S100B, and GFAP and executive function.
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GENERAL AUDIENCE ABSTRACT

Obesity is associated with lower brain function, particularly in executive function (EF). EF refers to advanced thought processes that help to maintain focus, practice self-control, solve problems, and easily switch between tasks. Lower EF in individuals with overweight and obesity may impact the success of weight loss and maintenance efforts. Because of this, understanding body processes that may link obesity and lower EF, as well as the ability to improve EF with weight loss, is very important. The first study aimed to determine the effect of weight loss on blood proteins responsible for brain health: brain-derived neurotrophic fact (BDNF), S100 calcium binding protein B (S100B), and glial fibrillary acidic protein (GFAP). Twenty-one blood samples from overweight and obese, middle-aged and older adults were combined from two completed weight loss studies. In these studies, blood was measured before and after 8- or 12-weeks of a weight loss (low calorie diet; 1200 or 1500 Calories per day). Body Mass Index (BMI), body weight, waist circumference, and percent body fat all decreased with weight loss; however, levels of BDNF, S100B, and GFAP in the blood did not change. The second study aimed to determine the relationship between blood BDNF, S100B, and GFAP and performance on three computer-based tests of EF before and after weight loss. Eight overweight and obese, middle-aged and older adults completed 4-weeks of weight loss (low-calorie diet; 1200 or 1500 Calories per day), followed by 4-weeks of weight maintenance. BMI, body
weight, waist circumference, and percent body fat all decreased following the weight loss and maintenance intervention (week 8). Blood BDNF, S100B, and GFAP levels did not change, but performance on two EF measures improved: participants made less errors of commission (doing something when not supposed to) and had faster reaction time following the intervention, indicating better self-control. Additionally, greater increases in GFAP were associated with less errors of omission (not doing something when supposed to), fewer correct responses, and slower reaction time. In conclusion, although weight loss did not affect blood BDNF, S100B, or GFAP levels, it may improve self-control in overweight and obese, middle-aged and older adults. Further research is needed to understand the relationship between weight loss, blood proteins of brain health, and EF.
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ATtribution

Brenda Davy, Ph.D., RDN, was responsible for the weight loss intervention study design, developed hypocaloric diet plans, and provided dietary counseling. Kevin Davy, Ph.D., assisted in study design development with the addition of blood measures, as well as conducted fasting blood draws for data collection. Elaina Marinik, Ph.D., assisted in participant recruitment, screening, and data collection. Benjamin Katz, Ph.D., provided expertise on executive function testing and statistical analysis. Ryan McMillan, Ph.D., conducted serum protein analysis for data collection.
CHAPTER 1: INTRODUCTION

Obesity Prevalence, Classification, and Neurocognitive Implications

The prevalence of obesity continues to rise in the United States, reaching an all-time high in 2016 with 39.8% of the adult population affected.\(^1\) Obesity prevalence has risen almost 10% in the last 2 decades. In recent years, obesity has been commonly referred to as an “epidemic” as it affects all age groups. Amongst them, middle-aged (40-59 years) and older (60+ years) adults are the most affected, with prevalence rates reaching 42.8% and 41%, respectively.\(^1\) In short, obesity has become a significant problem. Body mass index (BMI), or the ratio of weight (kg) to height squared (m\(^2\)) is commonly used as a measure of obesity. BMI has four categories to classify overweight and obese individuals: overweight (25-30 kg/m\(^2\)), obesity class I (30-35 kg/m\(^2\)), obesity class II (35-40 kg/m\(^2\)), and obesity class III (>40 kg/m\(^2\)).\(^2\) The use of BMI to classify obesity status has numerous limitations, particularly in older populations where obesity is commonly underestimated.\(^3\) A waist circumference greater than 40 inches in males or 35 inches in females is used to measure abdominal obesity, which may be more relevant to risk of chronic disease development.\(^4\) Obesity-related diseases, most namely cardiovascular disease, type II diabetes mellitus, and metabolic syndrome, substantially increase ones risk for all-cause mortality.\(^4\) Additionally, obesity in mid-life has been identified as a risk factor for developing dementia in late-life.\(^5\) In 2019, about 5.8 million Americans were living with dementia; 97% of them aged 65 and older.\(^6\) The US population is aging. By 2035, older adults (65+ years old) are projected to outnumber children (<18 years old).\(^7\) Cognitive decline is part of the normal aging process; however, studies have
shown that obesity-related cognitive decline further exacerbates age-related cognitive decline.\textsuperscript{9} Altogether, the neurocognitive implications of obesity in the persisting obesity epidemic in conjunction with the high prevalence of dementia in a growing segment of the population implores the need for immediate attention for research and therapeutic intervention.

**Potential Mechanisms of Obesity and Cognitive Decline**

A number of potential mechanisms have been proposed in the pathophysiology of obesity-related cognitive decline. While comorbid conditions, such as hypertension and insulin resistance, have been suggested as mechanistic pathways, there is evidence to suggest that excess adipose tissue has an effect independent of its comorbidities.\textsuperscript{10} Adipose tissue secretes hormones and other cytokines into circulation. Dysregulated, excess adipose tissue, as in an obese state, releases inflammatory cytokines that may

(Sellbom & Gunstad, 2012)
promote systemic, as well as neural, inflammation.\textsuperscript{11,12} As depicted above, Sellbom and Gunstad describe a preliminary, cyclical model for obesity-related cognitive changes.\textsuperscript{13} Structural brain changes have been observed in obesity. In this model, obesity-related structural changes in the brain may lead to cognitive dysfunction and obesogenic behaviors that promote weight gain and obesity further.

Because the relationship between obesity and cognition is not fully understood, there are other models that attempt to explain it as well. The Scaffolding Theory of Aging and Cognition, or STAC model, depicted below, is based on compensatory neural processes (i.e., compensatory scaffolding) that work to retain cognitive function throughout the course of aging. This model was recently revised, referred to as STAC-r,
to reflect the impact of life-course, or lifestyle choices, in addition to life-span on cognition. As shown in this model, both sources of neural enrichment and neural depletion affect brain structure and function, and consequently, cognitive function and changes throughout life. Vascular disease, including obesity, is considered a source of neural depletion.\textsuperscript{14}

**Health Behaviors and Cognitive Function**

As indicated in the STAC-R model, there are many health behaviors that have the power to either deplete or enrich brain structure and function. Aside from obesity and other chronic disease states, genetics, stress, toxin exposure, and low socioeconomic status exude negative consequences on brain health, while intellectual engagement, fitness, education, and multilingualism cultivate brain health. Another review adds avoidance of fatty food, tobacco, and alcohol to the list.\textsuperscript{15}

**Obesity and Cognitive Function**

Overweight and obesity are associated with reduced cognitive function across the lifespan; however, this relationship becomes more complex with age.\textsuperscript{16,17} Studies investigating older adults (mean 73+ years) report a positive association between obesity and cognitive function, opposite of the inverse relationship observed in children, adolescents, and adults.\textsuperscript{16} This finding is in line with the “obesity paradox”; in some populations, obesity may actually be protective, such as in the elderly.\textsuperscript{18} However, there are a number of explanations for this hypothesis in regard to cognitive decline and
dementia. First, body composition changes with age, such that, fat mass increases and fat free mass decreases; therefore, the use of BMI may be a poor indicator of adiposity in the elderly. Second, unintentional weight loss has been observed to precede dementia.\textsuperscript{19,20} Therefore, lower body weight in old age may be caused by the onset of dementia. Finally, studies investigating mid-life obesity tend to be longer in nature than those investigating late-life obesity. That being said, number of obese years, rather than life stage may offer another explanation for the obesity paradox.\textsuperscript{21} While the evidence remains inconclusive in older adults, impairments in executive functioning were the most consistent cognitive deficits observed across age groups.

\textbf{Obesity and Executive Function}

The executive functions (EF) are a family of higher order cognitive processes. While many thought processes could be considered part of this family, the three most widely accepted EFs are inhibition, working memory, and cognitive flexibility.\textsuperscript{22} Inhibition, or inhibitory control, refers to the ability to suppress subconscious tendencies. Working memory is the ability to retain and manipulate information for immediate use. Cognitive flexibility is the ability to switch between tasks with ease, which requires both inhibitory control and working memory. From these core areas extend more EFs, such as, decision making, planning, problem solving, and reasoning.\textsuperscript{22} Mainly, the prefrontal regions of the brain are responsible for executive functioning. A number of reviews have described the relationship between obesity and deficits in EFs.\textsuperscript{23,24} While an older review reported deficits in specific EF domains in obese, but not overweight, individuals\textsuperscript{23}, a more recent
review and meta-analyses reported deficits in multiple EFs including inhibitory control, working memory, cognitive flexibility, decision-making, verbal fluency, and planning in obese individuals, and inhibitory control and working memory in overweight individuals. Cross-sectional analyses of middle-aged and older adults reveal an association between higher BMI and worse performance on tasks of global cognition, psychomotor speed, verbal fluency, memory, and pre-morbid intelligence, as well as intellectual and executive functioning. Further, Cohen et al. found lean middle-aged and older adults have larger orbital frontal cortex volumes, and also perform better on tests of executive function, attention, and concentration than overweight and obese counterparts. In addition to smaller brain volume, overweight and obese, middle-older aged individuals exhibited lower right parietal cortex activation while completing a working memory task. In a longitudinal study, higher BMI was not associated with worse performance on tests of executive functioning at baseline; however, overtime, individuals who started with a higher BMI had a faster decline in executive function performance. It is important to note that age has an effect on executive function. In one study, younger adults (20-50) outperformed older adults (50-80) on intellectual and executive functioning tasks. In another study, the association between mid-life obesity and late-life cognitive decline was no longer significant after controlling for age. EF deficits in overweight and obese adults could have implications in the success of weight loss and maintenance programs. Determining the direction of the relationship important to design and recommend effective therapies.
Obesity as a Cause and/or Consequence of Executive Function Deficits

The relationship between obesity and executive function is clear; however, the nature of this relationship remains ambiguous. The cross-sectional design of many studies makes it difficult to determine a causal relationship between obesity and deficits in EF. A recent review included longitudinal studies in order to elucidate the direction of the relationship, but was unable to find statistical significance; however, the authors conclude there is evidence for a bidirectional relationship. Understanding whether reduced cognitive function is a cause and/or consequence of obesity may contribute to the development of successful lifestyle interventions. Investigating the effect of weight loss on executive function is one approach to understand this relationship further.

Weight Loss and Cognitive Function

The strong relationship between obesity and lower EF introduces the question of whether weight loss has the potential to reverse EF deficits. Intentional weight loss has been shown to improve cognitive function in both overweight and obese adults, including executive function. Weight loss approaches in the literature range from behavioral to surgical interventions. Evidence for improved executive function following diet-induced weight loss is mixed. In a longitudinal study, Siervo et al. followed middle-aged and older adults on a 40% energy restricted diet until 8-12% weight loss was achieved (~3-5 months); they found that a 10% reduction in body weight improves speed of processing in both middle-aged and older adults, as well as global cognition in older adults. A few research groups have investigated the effect of different weight-reducing
diets on cognition. All three studies found significant weight loss over time, but no difference between groups. Nonetheless, Halyburton et al. reported no change in working memory or speed of processing following weight loss induced by low carb high fat (LCHF) or high carb low fat (HCLF) diets. However, once this study was extended to 1-year, working memory significantly improved in all participants. Another research group found significant weight loss after 6-months of either low or high glycemic diets, but no significant change in any cognitive measures, including reaction time, vigilance, and attention. Although one of these interventions was able to find an improvement in executive function (i.e., working memory) after 1-year of weight loss, the findings reported by all three studies are weakened by lack of a non-dieting control group. Other studies have investigated measures of cognitive function, other than executive function. 3-months of calorie restriction (30% reduced energy intake) was able to significantly reduce body weight and improve memory in middle-aged and older, normal to overweight adults; however, including 1-month of weight maintenance after weight loss caused the memory improvements to revert back to baseline. These findings may indicate that negative energy balance from calorie restriction, rather than reduction in body weight, leads to improvements in memory. Still, improvements in processing speed and executive function were observed after weight loss and maintenance in this same study, indicating body weight does have some influence on cognitive performance. Caloric restriction and consequent weight loss has been shown to have no effect on cognition as well. Diet-induced weight loss via calorie restriction for 12-weeks or 6-months did not significantly change measures of executive function. More, 15-weeks of caloric restriction
(50% reduced energy intake) followed by 3 weeks of weight maintenance has been shown to have no effect on sustained or acute attention, but impair cognitive reaction time.\textsuperscript{41} Thus, the literature on the effect of weight loss on executive function is still inconclusive. The literature can be enhanced by further investigating biological mechanisms by which obesity may interact with cognitive function. A number of circulating proteins have been associated with obesity as well as neurocognitive health. The circulating proteins of interest are discussed below.

**Circulating Biomarkers**

**Brain-Derived Neurotrophic Factor (BDNF)**

Brain-Derived Neurotrophic Factor (BDNF) is a protein encoded by the \textit{bdnf} gene. This protein belongs to a family of neurotrophins known for their support of neuronal survival by regulating synaptic plasticity and promoting neurogenesis.\textsuperscript{42} BDNF is found in almost all areas of the human brain and has various roles depending on its location. BDNF contributes to energy regulation and feeding behavior\textsuperscript{43}, memory formation\textsuperscript{44}, and executive functioning.\textsuperscript{45} Although obesity is associated with \textit{bdnf} gene mutations and reduced expression in the brain, a recent review was unable to establish a significant relationship with circulating levels.\textsuperscript{46} Nonetheless, obesity has been associated with lower levels of both plasma/serum BDNF.\textsuperscript{47,48} Further, serum BDNF has been shown to mediate the relationship between higher central adiposity and worse verbal fluency, a component of cognitive flexibility.\textsuperscript{48} Research has also demonstrated the ability to change BDNF expression and circulating levels with weight loss interventions. 3-months of calorie
restriction (25% reduced energy intake) significantly reduced body weight and increased serum BDNF in overweight and obese adults.\textsuperscript{49} Additionally, a 12-week weight-reducing ketogenic diet significantly increased serum BDNF in the first 2 weeks, followed by a return to baseline for the remainder of the intervention.\textsuperscript{50} Interestingly, the first two weeks of the ketogenic diet included caloric restriction, suggesting an effect of negative energy balance, rather than body weight on serum BDNF. Working memory and speed of processing performance improved following 12 weeks of weight loss.\textsuperscript{50} Not all studies have reported similar results. 8-weeks of weight loss via calorie restriction improves memory but had no effect on serum BDNF.\textsuperscript{37} Additionally, 8-weeks of alternate-day fasting (ADF) and caloric restriction (CR) produced similar body weight loss and no change in serum BDNF; however, after 6 months, BDNF increased in ADF and decreased in CR. Interestingly, ADF had significantly greater percent fat mass loss and lean mass gain compared to CR at follow up.\textsuperscript{51} It is well-established that exercise increases circulating BDNF.\textsuperscript{52} However, Glud et al. reported opposing findings after comparing the effect of exercise alone, diet (600 kcal/d) alone, and diet and exercise combined weight loss interventions on circulating BDNF in overweight and obese adults.\textsuperscript{53} Although body weight decreased in all groups after 12-weeks of lifestyle intervention, circulating BDNF decreased in diet alone and diet and exercise combined in women, and exercise alone in men.\textsuperscript{53} All the aforementioned interventions investigated the effect of weight loss on circulating BDNF in young to middle-age adult cohorts (<60 years). Whether weight loss influences circulating BDNF in older adults with overweight and obesity is unclear.
**S100 Calcium-Binding Protein B (S100B)**

S100 calcium-binding protein B (S100B) is a protein encoded by the *S100B* gene. S100B is highly expressed in astrocytes and oligodendrocytes in the brain. S100B binds to the receptor for advanced glycation end products (RAGE), which is well known for its role in the inflammatory response. At low concentrations, S100B can bind RAGE on neurons and induce neurotropic effects, such as neuron growth and survival; however, S100B at high concentrations binds the same receptor and induces neurotoxic effects. S100B is considered a damage-associated molecular pattern (DAMP) protein, as it is released into bodily fluids upon cell damage or death. Thus, elevated levels of S100B in serum or cerebral spinal fluid (CSF) have been considered a marker of glial cell damage. Serum S100B is significantly lower in Alzheimer’s disease patients, and is positively correlated with the clinical dementia rating scale that classifies the severity of disease. Originally, this group of proteins was believed to be nervous system specific; however, S100B was found to be expressed in adipose tissue in concentrations comparable to the nervous system. S100B has been implicated in adipose tissue dysfunction and low-grade systemic inflammation in obesity. Diet-induced obese (DIO) mice have higher levels of S100B in plasma and white adipose tissue than lean controls. Additionally, switching DIO mice from high-fat to standard chow induced weight loss and decreased plasma S100B levels. Serum S100B is correlated with both BMI and visceral adiposity in humans. However, whether circulating S100B levels are responsive to weight loss in humans is unknown. Therefore, it is unknown whether effect S100B
produced and released into circulation by adipose tissue has an effect on the CNS and neurological conditions.

**Glial-Fibrillary Acidic Protein (GFAP)**

Glial-fibrillary acidic protein (GFAP) is an intermediate filament that constitutes the cytoskeleton of astrocytes. Astrocytes make up the bulk of the central nervous system (CNS), outnumbering neurons by 5 times. As with other glial cells, astrocytes function to provide support to neurons, specifically, in the development of synapses, regulation of blood flow, maintenance of fluid homeostasis, and CNS metabolism.\(^6\) When the CNS is exposed to damage or disease, astrocytes undergo hypertrophy and hyperplasia. This is referred to as “astrogliosis” or “reactive gliosis”.\(^6\) In mild to moderate astrogliosis, GFAP is upregulated; thus, GFAP is a well-established marker of astrocyte reactivity.\(^6\) Astrogliosis is a normal physiological response to injury, with a purpose to repair and restore damaged and diseased tissues; however sustained insults can have harmful effects and lead to CNS inflammation. Obesity is associated with chronic, systemic inflammation\(^1\), including inflammation of the CNS.\(^2\) To date, it is unknown if obesity leads to CNS inflammation through similar mechanisms as obesity-related systemic inflammation, or if CNS inflammation leads to obesity through promotion of an obesogenic environment.\(^3\) In fact, rodent studies have demonstrated astrogliosis in response to a high-fat diet, even before weight gain had occurred.\(^4\) These findings suggest an effect of acute dietary intake, rather than adipose tissue, on CNS inflammation. However, both diet-induced and genetic obese mouse models display hypothalamic astrogliosis,
indicating an effect of obesity, with or without dietary change.\textsuperscript{69} Obesity-related inflammatory markers have also been shown to intensify GFAP activity\textsuperscript{70} Nonetheless, consumption of a high-fat diet significantly increased body weight, GFAP expression, and memory impairments in rats.\textsuperscript{71} Whether weight loss decreases GFAP and reverses cognitive impairments is unknown. Hypothalamic gliosis is associated with obesity humans as well.\textsuperscript{68} However, magnetic resonance imaging (MRI), rather than GFAP expression, was used to measure gliosis.\textsuperscript{68} In a case-control study, Schur et al. reported higher BMI, fasting insulin, and HOMA-IR in hypothalamic gliosis cases.\textsuperscript{72} While MRI was also used to identify cases of gliosis in this study, a separate post mortem study conducted by this research group demonstrated a positive correlation between MRI hypothalamic gliosis and GFAP upregulation in the human brain.\textsuperscript{72} Research has yet to elucidate the relationship between human obesity or weight loss and serum GFAP.
CHAPTER 3: The effect of weight loss on circulating biomarkers of brain health

ABSTRACT

Obesity is associated with a reduction in cognitive function, which may impact adherence to weight loss and maintenance efforts. Weight loss has shown promise in improving cognitive function in overweight and obese adults. The present study aimed to investigate the effect of weight loss in overweight and obese, middle-aged and older adults on circulating biomarkers of brain health: brain-derived neurotrophic factor (BDNF), S100 calcium binding protein B (S100B), and glial fibrillary acidic protein (GFAP). Serum samples (n=21; 50-75 years, BMI 25-40 kg/m²) were pooled from two prior weight loss studies. Fasting blood measurements were taken before and after 8- or 12-weeks of hypocaloric diet-induced weight loss (1200 or 1500 kcal/d). Baseline BMI was significantly correlated with the change in GFAP (r=0.560, p=0.008). BMI, body weight, waist circumference, and percent body fat (All p<0.001) decreased with weight loss. Serum BDNF (p=0.871), S100B (p=0.898), and GFAP (p=0.506) were not significantly different between baseline and following weight loss within the pooled group, or within the 8 (p=0.182; p=0.483; p=0.618) or 12 (p=0.360; p=0.536; p=0.621) week interventions. In conclusion, weight loss does not influence serum BDNF, S100B, or GFAP levels in overweight and obese, middle-aged and older adults. BMI status may predict inflammatory response to weight loss interventions.
INTRODUCTION

The prevalence of obesity in the US continues to rise, contributing to age-related cognitive decline and the development of neurodegenerative disease. Recent reports indicate that obesity and dementia affect older adults more than any other age group. The US has an aging population, which will further exacerbate the neurocognitive implications of obesity and dementia in the coming years. Because of this, understanding the biological mechanisms associated with both obesity and cognitive decline are even more pressing.

Brain-derived neurotrophic factor (BDNF), a protein known for its roles in neurogenesis, synaptic plasticity, and energy regulation, is reduced in obesity. Although bdnf gene polymorphisms and lower BDNF in specific brain regions are strongly associated with obesity, a recent systematic review and meta-analysis reported no association between circulating BDNF and obesity. Nonetheless, studies have demonstrated the ability to reverse obesity-related reductions in circulating BDNF with weight loss. However, these findings have not been replicated, as others have observed a decrease in circulating BDNF following weight loss or no change at all. Further, the effect of weight loss on circulating BDNF in older adults has not yet been studied.

S100 calcium-binding protein B (S100B), an established marker of glial cell damage, is elevated in obesity. At high concentrations, S100B binds the receptor for advance glycation end-products (RAGE) and exudes neurotoxic effects; therefore, it is considered a damage-associated molecular pattern (DAMP) protein. Although originally thought to be nervous system specific, S100B is also produced by adipose tissue.
effect of S100B produced by adipose tissue on circulating levels of S100B and consequently central nervous system damage is unknown. Rodent studies have demonstrated the ability to reverse increased plasma levels of S100B with weight loss\textsuperscript{62}; however, whether this finding extends to humans is unexplored.

Glial fibrillary acidic protein (GFAP) is another marker of glial damage, specifically of astrocytes. In the presence of central nervous system (CNS) insult, injury, or inflammation, GFAP is upregulated in the brain. This is known as “astrogliosis” or “reactive gliosis”. Obesity is associated with astrogliosis in both rodents and humans.\textsuperscript{68} Astrogliosis in human studies has been measured with magnetic resonance imaging.\textsuperscript{68,72} Schur et al. conducted two studies to demonstrate the relationship between hypothalamic gliosis, GFAP upregulation, and obesity in humans.\textsuperscript{72} Whether elevated GFAP in circulation is associated with obesity and if weight loss can change circulating GFAP levels in humans, remains to be elucidated.

The present study aims to determine the effect of weight loss on serum BDNF, S100B, and GFAP in overweight and obese, middle-aged and older adults. We hypothesize that weight loss will increase serum BDNF, and decrease serum S100B and GFAP.

METHODS AND MATERIALS

Sample characteristics

Twenty-one serum samples from two completed studies were pooled for retrospective analysis. Thirteen samples from study 1,\textsuperscript{73} were combined with 8 samples
from study 2 (data unpublished). Both studies were hypocaloric weight loss trials described in detail below. Inclusion/exclusion criteria for both studies were as follows: overweight/obese (BMI 25-40 kg/m²), 55-75 years, and no history of type II diabetes mellitus. Additional inclusion/exclusion criteria for study 1 include: weight stable (±2 kg, >1 year), no history of hypertension, cancer, heart/lung/kidney diseases, depression, or eating disorders, no allergies to foods provided, and not currently taking medication with known effects on weight. Additional inclusion/exclusion criteria for study 2 include: able to safely engage in a diet and physical activity program and no history of participating in psychology research with cognitive testing.

**Procedures**

**Baseline assessments**

Both studies included baseline assessments of body height and weight, body composition, and fasting blood draw. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer and body weight was measured to the nearest 0.1 kg using a digital scale (Scale-Tronix Model 5002, White Plains, NY). Height and weight were assessed without shoes and light clothing. Body Mass Index (BMI) was calculated according to body height and weight measurements (kg/m²). Body composition was measured using dual-energy X-ray absorptiometry (DEXA) (GE Lunar Prodigy Advance, software version 8.10e). In both studies, blood was sampled from the antecubital vein into red top serum activator tubes. Blood samples rested at room temperature for 10 minutes, or until clot formation, before being centrifuged at 3500 rpm for 13 minutes at 4°C.
Prepared serum samples were stored at -80°C prior to analyzation. Enzyme-linked immunosorbent assay (ELISA) kits (Quantikine; R&D Systems, Inc., Minneapolis, MN) were used to measure BDNF, S100B, and GFAP, according to the manufacturer directions.

**Intervention**

Participants from study 1 completed a 12-week hypocaloric diet, while participants from study 2 completed an 8-week hypocaloric diet. Following baseline assessments, all participants received instructions to follow a hypocaloric diet. The original studies randomized participants to receive either standard hypocaloric diet or hypocaloric diet with 500 ml of premeal water 3x/day, but for the purposes of this retrospective analysis, all participants were pooled into one group. Hypocaloric diets for men allowed 1500 Calories per day (kcal/d), and for women 1200 kcal/d, as developed by the United States Department of Agriculture guidelines. All participants received dietary counseling from a registered dietitian nutritionist (RDN). Consumption of fruits, vegetables, lean protein sources, low/nonfat dairy, and whole grains, as well as moderation of high-fat foods, sugar-sweetened beverage, and alcohol was emphasized. Folders containing diet plan instructions and sample menus were provided. Participants were instructed to continue with usual physical activity habits. While participants from study one focused on the low-calorie diet for the entire intervention period, participants from study 2 were given pedometers (Accusplit Eagle, AX120, San Jose, CA) and a 10,000 steps/d goal for the final 4-weeks of the 8-week intervention to promote weight maintenance.
**Post-testing**

Following 8- or 12-weeks of weight loss, all baseline assessments (body weight and height, body composition, fasting blood draw) were repeated. Participants completed an exit survey and informed of the study purpose.

**STATISTICAL ANALYSIS**

Descriptive statistics were performed on all participant characteristics for each study as well as the pooled samples. Descriptive statistics were used to assess BMI, body weight, waist circumference, percent body fat, and serum BDNF, S100B, and GFAP at baseline. A paired-samples t-test determined differences between baseline and final anthropometric and serum biomarker measurements for each study (8 and 12 weeks), as well as for the pooled samples. Change scores were calculated as the difference between final and baseline measurements. Pearson’s correlation assessed the relationship between baseline anthropometrics and the change in serum protein levels. Pearson’s correlation also assessed the relationship between the change in anthropometrics and the change in serum protein levels. Finally, percent weight loss was calculated as the difference between final and baseline body weight divided by baseline body weight. High and low weight loss categories were created from the median split. Repeated measures ANOVA determined differences between high and low percent weight loss categories for anthropometric and serum protein levels. Independent samples t-test determined differences in the change in serum proteins between male and female participants. All statistical analyses were performed using IBM SPSS version 26.
RESULTS

Sample characteristics

In this retrospective analysis, 21 samples were combined from two completed weight loss studies. Thirteen samples were from a 12-week weight loss intervention (study 1) and eight from an 8-week weight loss intervention (study 2). Baseline demographic characteristics for each study as well as for the pooled samples are shown in Table 1. The majority of the pooled samples were from female (67%), Caucasians (81%), with Hispanic (n=2), African American (n=1), and “other” (n=1) comprising the rest of the sample.

<table>
<thead>
<tr>
<th>Table 1. Baseline demographic characteristics</th>
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</thead>
<tbody>
<tr>
<td>N</td>
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<tr>
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<tr>
<td>Height, m</td>
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<tr>
<td>Body Weight, kg</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM

Serum biomarker concentrations

BMI, body weight, waist circumference, and percent body fat (All p<0.001) decreased with weight loss (Table 2). Mean percent weight loss was 6.8% (range 0.8 - 12.6%) Serum BDNF (p=0.871), S100B (p=0.898), and GFAP (p=0.506) were not different between baseline and following weight loss within the pooled samples, or within the 8 (p=0.182; p=0.483; p=0.618) or 12 (p=0.360; p=0.536; p=0.621) week interventions.
Baseline BMI was significantly correlated with the change in GFAP with weight loss ($r=0.560$, $p=0.008$; Figure 2A). Additionally, baseline body weight was correlated with the change in GFAP, although statistically insignificant ($r=0.371$, $p=0.097$; Figure 2B). There were no significant correlations between the change in anthropometrics and the change in serum biomarkers with weight loss. Change in serum S100B with weight loss was inversely correlated with the change in percent body fat, although statistically insignificant ($r=-0.391$, $p=0.080$) (Figure 3). There was no difference in the change in serum BDNF ($p=0.564$), S100B ($p=0.290$), or GFAP ($p=0.683$) between high and low percent weight loss categories (Table 3). Finally, there was no difference in the change in serum BDNF ($p=0.549$), S100B (0.488), or GFAP ($p=0.610$) between sexes (Table 4).

| Table 2. Pooled samples anthropometrics and serum biomarkers before and after weight loss |
|---------------------------------|-----------------|-----------------|
| **BMI, kg/m²**                  | Baseline        | Final           |
|                                 | $32.43 \pm 1.01$ | $30.02 \pm 0.988^{**}$ |
| **Body Weight, kg**             | $91.6 \pm 3.29$  | $85.5 \pm 3.27^{**}$  |
| **Waist circumference, cm**     | $102.73 \pm 3.11$ | $98.71 \pm 3.13^{**}$ |
| **Body Fat, %**                 | $42.49 \pm 1.69$ | $39.59 \pm 1.86^{**}$ |
| **Serum BDNF, ng/mL**           | $32.19 \pm 3.43$ | $31.31 \pm 4.00$ |
| **Serum S100B, pg/mL**          | $34.60 \pm 4.15$ | $34.97 \pm 4.35$ |
| **Serum GFAP, ng/mL**           | $1.19 \pm 0.773$ | $1.38 \pm 1.02$ |

Data are presented as mean ± SEM. Time effect $P < 0.05^{*}$. Time effect $P < 0.001^{**}$
Figure 1. Serum protein concentrations before and after weight loss

A
Serum BDNF before and after weight loss

B
Serum S100B before and after weight loss

C
Serum GFAP before and after weight loss
Figure 2. Baseline anthropometrics and change in serum GFAP with weight loss

A

**Change in Serum GFAP with Weight Loss and Baseline BMI**

$R^2$ Linear = 0.314

B

**Change in Serum GFAP with Weight Loss and Baseline Body Weight**

$R^2$ Linear = 0.138
Figure 3. Change in serum biomarkers with weight loss

Table 3. High and low percent weight loss categories

<table>
<thead>
<tr>
<th>Category</th>
<th>High</th>
<th>Low</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Percent Weight Loss, %</td>
<td>&gt;6.5</td>
<td>&lt;6.5</td>
</tr>
<tr>
<td>Serum BDNF change (ng/mL)</td>
<td>2.19 ± 9.90</td>
<td>-4.26 ± 3.64</td>
</tr>
<tr>
<td>Serum S100B change (pg/mL)</td>
<td>-2.56 ± 4.20</td>
<td>3.60 ± 3.72</td>
</tr>
<tr>
<td>Serum GFAP change (ng/mL)</td>
<td>0.29 ± 0.41</td>
<td>0.06 ± 0.36</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. Time effect P < 0.05*. Time effect P < 0.001**
### Table 4. Sex differences

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Serum BDNF change (ng/mL)</td>
<td>-7.33 ± 8.76</td>
<td>2.34 ± 6.84</td>
</tr>
<tr>
<td>Serum S100B change (pg/mL)</td>
<td>4.75 ± 5.59</td>
<td>-1.82 ± 3.21</td>
</tr>
<tr>
<td>Serum GFAP change (ng/mL)</td>
<td>0.19 ± 0.37</td>
<td>0.18 ± 0.37</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM. Time effect *P* < 0.05*. Time effect *P* < 0.001**

### DISCUSSION

In the present study, weight loss was not associated with any significant change in serum BDNF, S100B, or GFAP. These findings reject our original hypothesis that serum BDNF would increase, and serum GFAP and S100B would decrease.

Other weight loss studies have shown similar results. Catenacci et al. reported no significant correlation between changes in body weight and changes in serum BDNF after 8-weeks of caloric restriction or alternate day fasting weight loss interventions.\(^{51}\) In contrast, Araya et al. observed an increase in serum BDNF following weight loss via 25% energy intake reduction,\(^{49}\) while Glud et al. observed a decrease in serum BDNF in women following a fixed very low-calorie diet (600kcal/d).\(^{49,53}\) Although there is strong evidence that BDNF gene mutations and reductions in hypothalamic BDNF in obesity, a recent systematic review and meta-analysis was unable to establish this same relationship with circulating BDNF.\(^{46}\) Our study provides additional evidence that weight loss does not influence serum BDNF.
Circulating S100B and GFAP have been largely unstudied in humans. In mice, high fat diet-induced obesity increased plasma S100B, and switching mice from high fat to standard chow for 5 weeks induced weight loss and decreased plasma S100B.\textsuperscript{62} To our knowledge, there is no information on whether weight reduces circulating GFAP in mice or humans. However, Harrison et al. reported an increase in hypothalamic GFAP in mice following 11 days of weight loss via caloric restriction.\textsuperscript{75} Consistent with this, higher baseline BMI was associated with greater increases in serum GFAP with weight loss in our study. Future studies in a larger sample would be necessary to confirm our observation. Thaler et al observed a transient increase in hypothalamic GFAP expression in rats after only 24 hours of high fat feeding, well before any weight changes had occurred.\textsuperscript{68} The body desires to maintain energy homeostasis, which is threatened by acute dietary changes; therefore, caloric restriction may be sensed as a threat to the body. In our study, it is possible that individuals with larger bodies had greater glial responses to caloric restriction as a means of neuroprotection. However, we cannot exclude the possibility that this was a spurious finding due to our small sample size.

The present study advances the current understanding of the effects of weight loss on brain health. Importantly, our study is the first to investigate weight loss on circulating concentrations of S100B and GFAP. Prior studies were limited to those in animal models of obesity. These new findings in humans is a strength of our study. Future randomized control trials of weight loss in larger samples are needed to better understand the impact of weight loss on brain health. To date, the effects of weight loss on serum BDNF are
mixed. Our findings provide further insight into the relationship between obesity and circulating BDNF in humans.

There are a number of limitations of our study we should emphasize. First, our study was limited to small sample size. Second, our study did not include a control group. All individuals received dietary counseling for a hypocaloric diet. Third, our sample was relatively homogenous and comprised primarily by Caucasian females. Finally, the duration of our study was limited to 8 and 12 weeks, and as such, a longer duration may yield a different outcome.

CONCLUSION

In summary, we report no significant change in serum BDNF, S100B, and GFAP following 8 or 12 weeks of weight loss in overweight and obese, middle aged and older adults. Future investigations should include a randomized control trial of a larger, more heterogeneous sample and longer follow-up period. Finally, magnetic resonance imaging (MRI) is currently used to identify gliosis in humans in vivo. Adding MRI will allow for direct comparison of central nervous system inflammation and circulating BDNF, S100B, and GFAP. Future research is needed to elucidate the effect of weight loss on serum BDNF, S100B, and GFAP in overweight and obese humans.
CHAPTER 4: The effect of weight loss on circulating biomarkers of brain health and executive function

ABSTRACT

The prevalence of obesity continues to rise in the US, exacerbating age-related cognitive decline. Obesity is associated with deficits in cognitive function, particularly within the domain of executive function (EF), and may impact adherence to weight loss and maintenance efforts. The objective of the present study is to determine: 1) the effect of weight loss on brain-derived neurotrophic factor (BDNF), S100 calcium-binding protein B (S100B), and glial fibrillary acidic protein (GFAP), in overweight and obese, middle-aged and older adults and, 2) whether the magnitude of change in these circulating proteins are associated with the magnitude of improvement in executive function performance. Participants (n=8; 50-75 years, BMI 25-40 kg/m²) completed 4-weeks of weight loss via hypocaloric diet (1200 or 1500 kcal/d), followed by 4-weeks of weight maintenance. Fasting blood and EF measurements were completed at baseline, and weeks 4 and 8. BMI (p=0.001), body weight (p=0.001), waist circumference (p=0.002), and percent body fat (p=0.001) decreased from baseline to week 8. Serum BDNF (p=0.359), S100B (p=0.277), and GFAP (p=0.585) did not change following weight loss. GNG errors of commission (p=0.009) and AX-CPT correct response time (p=0.041) decreased following the intervention. The change in serum GFAP was inversely correlated with GNG errors of omission (r=-0.716, p=0.046) and AX-CPT correct hits (r=-0.737, p=0.037), and positively correlated with AX-CPT correct response time (r=0.859, p=0.006). Taken together, these observations suggest that although weight loss does not influence serum BDNF, S100B, or GFAP levels, it may enhance inhibitory control. The
relationship between these serum biomarkers and executive function require additional study.

INTRODUCTION

Obesity prevalence continues to rise in the US, now affecting 39% of adults.¹ The climbing prevalence of obesity comes with serious health implications, as obesity increases the risk of several other chronic diseases.⁴ In fact, obesity in mid-life is a risk factor for the development of dementia in late-life.⁵ Even in the absence of neurodegenerative disease, obesity has been linked with cognitive deficits,¹⁶,¹⁷ most notably in executive function (EF).²³,²⁴,³⁰ The relationship between cognitive function and obesity is more complicated in older adults (65+); in this group, obesity is actually associated with better cognitive function, rather than the opposite seen in all other age groups. This is referred to as the “obesity paradox”.¹⁸ However, the protective effect of higher body weight in this population may be explained by the inappropriate use of BMI or the unintended weight loss accompanying the onset of dementia.¹⁹,²⁰ Nonetheless, there is strong evidence to support an inverse relationship between obesity and cognitive function, specifically in EF. Still, whether deficits in EF are a cause and/or consequence of obesity is unknown.³⁰ Weight loss interventions provide a means to further elucidate the direction of this relationship.

Both behavioral and surgical weight loss interventions have been shown to improve cognitive function, including EF.³² Specifically, diet-induced weight loss interventions have been shown to improve a number of EFs, including speed of
processing, working memory, and global EF. However, the findings for diet-induced weight loss are inconsistent, as other weight loss studies have shown no effect on attention or EF.

Circulating biomarkers of brain health may offer a mechanistic link between obesity and EF. Brain-derived neurotrophic factor (BDNF), S100 calcium-binding protein B (S100B), and glial fibrillary acidic protein (GFAP) are proteins with unique functions in the brain and have all been associated with obesity; such that lower BDNF and elevated S100B and GFAP are observed in obesity. BDNF has been shown to increase following behavioral weight loss interventions (exercise, caloric restriction, etc.), as well as decrease. Only one study to date has measured the change in serum BDNF and cognitive function performance following weight loss; while memory improved, serum BDNF did not change. No studies has assessed the change in serum S100B or GFAP and executive function following weight loss in humans.

The purpose of the present study is to determine the effect of weight loss on serum BDNF, S100B, and GFAP and EF performance in overweight and obese, middle-aged and older adults. A secondary aim of the present study is to determine whether changes in these circulating biomarkers are associated with changes in EF with weight loss. Our hypothesis is that weight loss will increase serum BDNF and decrease serum S100B and GFAP in overweight and obese, middle-aged and older adults. Finally, changes in these circulating biomarkers are hypothesized to correlate with improvements in EF performance.
METHODS AND MATERIALS

Participant characteristics

Overweight and obese, middle-aged and older adults (BMI 25-40 kg/m$^2$, 50-75 years) were recruited through university-associated news advertisements and various email listservs. Interested participants were screened by phone. Eligible participants met the following inclusion/exclusion criteria: overweight or obese (BMI 25-40 kg/m$^2$), 50-75 years old, able to safety engage in a diet and physical activity program, not have type II diabetes mellitus requiring insulin, and not have previously participated in psychology research with cognitive testing. All participants were blinded to the study purpose and provided written informed consent prior to study enrollment. The study was approved by the Western Institutional Review Board.

Procedures

Baseline assessments

Anthropometrics and dietary assessment

An overview of the study is shown in Figure 1. Participants that met all inclusion/exclusion criteria completed three baseline visits over the course of one week. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer and body weight was measured to the nearest 0.1kg using a digital scale (Scale-Tronix Model 5002, White Plains, NY). Height and weight were assessed without shoes and light clothing. Body Mass Index (BMI) was calculated according to height and body weight measurements (kg/m$^2$). Body composition was measured using dual-energy X-ray absorptiometry.
(DEXA) (GE Lunar Prodigy Advance, software version 8.10e) and waist circumference was measured to the nearest 0.5 cm using Gulick measuring tape (Country Technology, Gay Mills, WI). Typical dietary intake was assessed using interviewer-administered 24-hr dietary recalls on three, non-consecutive days and analyzed using Nutrition Data System for Research (NDSR v. 2017, Minneapolis, MN).

Fasting blood draw

After an overnight fast, blood was drawn from the antecubital vein using a butterfly needle and red top serum activator tube. Blood samples rested at room temperature for 10 minutes, or until clot formation before being centrifuged at 3500 rpm for 13 minutes at 4°C. Serum samples were stored at -80°C until analyzation. Enzyme-linked immunosorbent assay (ELISA) kits were used to determine BDNF (Quantikine; R&D Systems, Inc., Minneapolis, MN), S100B, and GFAP (MilliporeSigma, Burlington, MA) according to the manufacturer directions.

Executive function testing

EF was assessed using three computer-based tests using the E-Prime 3.0 Software: Go/No-Go (GNG), Corsi-Block Tapping (CB), and AX-Continuous Performance Test (AX-CPT) (Psychology Software Tools, Pittsburg, PA). GNG was used to assess inhibitory control. Participants were instructed to respond when any letter that was not ‘X’ appeared on the screen and to refrain from responding when ‘X’ appeared on the screen. The response time for correct hits, errors of omission, errors of commission, and total
errors were used as performance measures of this task. CB was used to assess working memory. Participants were instructed to watch a sequence of blocks light up on the screen and click on the blocks in the reverse sequence. The highest level achieved was used as the performance measure for this task. Lastly, AX-CPT was used to assess attentional and inhibitory control. Participants were instructed to press the right button as soon as any letter disappeared from the screen, except, when the letter ‘X’ followed the letter ‘A’, they were instructed to press the left button. Response time for correct hits and number of correct hits were used as performance measures for this task. Testing sessions lasted about 45 minutes and included all three tasks in the order described above. Participants were provided a quiet, private space and noise canceling headphones. Testing was performed behind a closed door with a white noise machine to further minimize external noise. Before each testing session, a research assistant read a script of instructions, watched the participant complete a practice trial, and asked the participant for any questions. If there were no further questions, the research assistant instructed the participant to ring the bell to signal task completion, left the room, and closed the door.

**Intervention**

Following baseline assessments, participants received instructions to follow a hypocaloric diet. The original study design randomized participants to receive either standard hypocaloric diet or hypocaloric diet with 500 ml of premeal water 3x/day, but for the purpose of this study, all participants were pooled into one group. All participants received dietary counseling from a registered dietitian nutritionist (RDN). Hypocaloric
diets for men included 1500 Calories per day (kcal/d), and women 1200 kcal/d, as developed by the United States Department of Agriculture guidelines. Consumption of fruits, vegetables, lean protein sources, low/nonfat dairy, and whole grains, as well as moderation of high-fat foods, sugar-sweetened beverage, and alcohol was emphasized. Folders containing diet plan instructions and sample menus were provided. Participants were instructed to continue with usual physical activity habits. The first four weeks comprised the weight loss phase. During this phase, participants followed the assigned hypocaloric diet and met with a research assistant weekly for body weight checks as well as follow-up dietary counseling support. At the end of the weight loss phase, all baseline assessments (body weight, body composition, three 24-hr dietary recalls, executive function testing, fasting blood draw) were repeated. Following the weight loss phase, participants entered the weight maintenance phase. During this phase, participants were advised to continue the hypocaloric diet and given pedometers (Accusplit Eagle, AX120, San Jose, CA) to aim for a 10,000 steps/d goal.

Post-testing

Following the 8-week weight loss and maintenance intervention, all baseline assessments (body weight, body composition, three 24-hr dietary recalls, executive function testing, fasted blood draw) were repeated. Participants completed an exit survey, were informed of the study purpose, and compensated $50 for participation.
Figure 1. Schematic of study design

Individuals assessed for eligibility (n=16)

Excluded (n=6)
- Not meeting inclusion criteria (n=2)
- Declined to participate (n=2)
- Other reasons (n=2)

Baseline testing
Visit 1: informed consent, health history, height, body weight, 24-hr recall
Visit 2: cognitive function assessment, 24 hr recall
Visit 3: fasting blood draw, body composition, 24-hr recall, dietary counseling

Enrolled in 8-week intervention (n=10)

4-week weight loss (n=8)
- Weekly body weight checks, dietary counseling

4-week weight maintenance (n=8)
- Independent activity/diet goals

Discontinued intervention (n=2)
- Voluntary withdrawal

Post testing
Phone call: 24-hr recall
Visit 9: cognitive function assessment, 24-hr recall
Visit 10: fasting blood draw, body composition, 24-hr recall, exit survey

Included in analysis (n=8)
Incomplete post data (n=2)
STATISTICAL ANALYSIS

Descriptive statistics were performed on all variables at baseline, and weeks 4 and 8. A repeated measures ANOVA determined differences between baseline, week 4, and week 8 anthropometric, serum protein levels, and executive function measurements. Change scores were calculated as the difference between final and baseline measurements. Pearson’s correlation assessed the relationship between the change in serum biomarker concentrations and the change in EF performance from baseline to final (week 8).

RESULTS

Baseline Characteristics

Ten participants were enrolled in the 8-week weight loss intervention. There were two drop-outs, one due to a family emergency and the other for an unknown reason. Eight participants completed the intervention. Baseline demographic characteristics are shown in Table 1. The majority of participants were Caucasian (63%) and female (88%). Hispanic (n=2) and “other” (n=1) race/ethnic groups comprised the remainder of the sample.

<table>
<thead>
<tr>
<th>Table 1. Baseline demographic characteristics</th>
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</thead>
<tbody>
<tr>
<td>Male/ Female</td>
</tr>
<tr>
<td>Race, white/nonwhite</td>
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<td>Height, m</td>
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<td>Body Weight, kg</td>
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<tr>
<td>BMI, kg/m²</td>
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</table>

Data are presented as mean ± SEM.
Serum Biomarker Concentrations

Mean anthropometric and serum protein measurements at baseline, and weeks 4 and 8 are shown in Table 2. BMI (p=0.001), body weight (p=0.001), waist circumference (p=0.002), and percent body fat (p=0.001) decreased from baseline to final. Mean percent weight loss was 5.6% (range 0.8 – 8.0%). Serum BDNF (p=0.359), S100B (p=0.277), and GFAP (p=0.585) did not change with weight loss.

Table 2. Anthropometrics and serum proteins at baseline, and weeks 4 and 8

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 8</th>
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</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>30.3 ± 1.25</td>
<td>28.0 ± 0.948</td>
<td>27.5 ± 0.892**</td>
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<tr>
<td>Body weight, kg</td>
<td>81.6 ± 3.93</td>
<td>76.7 ± 4.35</td>
<td>75.3 ± 4.07**</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>94.4 ± 4.07</td>
<td>92.1 ± 4.21</td>
<td>89.4 ± 4.51**</td>
</tr>
<tr>
<td>Percent body fat, %</td>
<td>44.4 ± 1.23</td>
<td>42.5 ± 1.53</td>
<td>41.3 ± 1.75**</td>
</tr>
<tr>
<td>Serum BDNF, ng/mL</td>
<td>38.5 ± 6.39</td>
<td>37.6 ± 4.00</td>
<td>27.8 ± 7.34</td>
</tr>
<tr>
<td>Serum S100B, pg/mL</td>
<td>37.2 ± 4.34</td>
<td>45.3 ± 8.62</td>
<td>34.0 ± 4.40</td>
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<tr>
<td>Serum GFAP, ng/mL</td>
<td>1.36 ± 0.336</td>
<td>0.965 ± 0.250</td>
<td>1.20 ± 0.306</td>
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</table>

Data are presented as mean ± SEM. Time effect P < 0.05*. Time effect P < 0.001**

Executive Function Testing

EF performance at baseline and weeks 4 and 8 for each computer-based test is shown in Table 3. GNG errors of commission (p=0.009) and AX-CPT correct response time (p=0.041) decreased following weight loss (week 8). Executive function performance for CB highest level achieved (p=0.147), GNG errors of omission (p=0.471), GNG total errors (p=0.258), and AX-CPT correct hits (p=0.145) did not change with weight loss. The relationship between the change in serum GFAP and the change in GNG errors of omission and total errors as well as AX-CPT correct response time and correct hits are shown in Figure 2. The change in serum GFAP was inversely correlated with GNG errors.
of omission (r=-0.716, p=0.046), GNG total errors (r=-0.642, p=0.086), and AX-CPT correct hits (r=-0.737, p=0.037), and positively correlated with AX-CPT correct response time (r=0.859, p=0.006). The correlation between serum GFAP and GNG total errors was not significant (R=-0.642, p=0.086).

Table 3. Computer-based executive function tests at baseline, and weeks 4 and 8

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>Highest level achieved</td>
<td>5.60 ± 0.427</td>
<td>6.63 ± 0.420</td>
</tr>
<tr>
<td>GNG</td>
<td>Correct RT, sec</td>
<td>184.94 ± 29.2</td>
<td>174.06 ± 48.7</td>
</tr>
<tr>
<td></td>
<td>Errors of Omission, # err.</td>
<td>3.22 ± 1.24</td>
<td>3.37 ± 2.44</td>
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<tr>
<td>AX-CPT</td>
<td>Correct RT, sec.</td>
<td>451.90 ± 27.3</td>
<td>429.93 ± 22.5</td>
</tr>
<tr>
<td></td>
<td>Correct Hits, # hits</td>
<td>382.33 ± 13.7</td>
<td>403.25 ± 3.10</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. Time effect P < 0.05*. Time effect P < 0.001**.
Figure 2. The change in serum proteins and executive function performance with weight loss

A

Changes in GNG Errors of Omission and Serum GFAP with Weight Loss

B

Changes in GNG Total Errors and Serum GFAP with Weight Loss

C

Changes in AX-CPT Correct Response Time and Serum GFAP with Weight Loss

D

Changes in AX-CPT Correct Hits and Serum GFAP with Weight Loss

DISCUSSION

The primary finding of the present study was serum BDNF, S100B, and GFAP did not change following weight loss; however, weight loss was associated with an improvement in executive function. Participants made fewer errors of commission on the GNG task and had faster response times on the AX-CPT task following weight loss. Additionally, the change in serum GFAP significantly correlated with the change in four measures of executive function; a larger increase in serum GFAP was associated with greater improvements in errors of omission and total errors (fewer errors) on the GNG
task and smaller improvements in reaction time (faster time) and correct hits (more correct hits) on the AX-CPT task following weight loss.

Our finding that weight loss improved speed and accuracy on an executive function task, suggesting improved inhibitory control, is consistent with a recent systematic review and meta-analysis that reported that weight loss using a variety of approaches, including behavioral interventions and bariatric surgery, improved cognitive function across many domains, including EF. Calorie restrictive diets 3 to 5 months in duration have been shown to improve memory, speed of processing, global cognition, and EF. However, it seems evidence for improved cognitive function following diet-induced weight loss, via caloric restriction alone or in combination with controlled macronutrient composition, is mixed, as other studies found no effect. It is important to note that some of these studies did not include a non-dieting control group and others included an exercise component alongside the main dietary intervention, which may have affected the final outcomes and conclusions. It is interesting to note that our findings showed an improvement in both speed and accuracy, rather than a speed-accuracy tradeoff.

Current research is focused on the validation of circulating GFAP as a biomarker for early detection of Alzheimer’s disease (AD). In fact, a recent study demonstrated increased serum GFAP in AD, as well as a strong positive correlation with between serum GFAP and cognitive impairment in humans. Hypothalamic GFAP upregulation and gliosis are associated with obesity in rodents and humans, respectively. Further, high-fat fed rats not only gained body weight and had increased hypothalamic GFAP, but
also had impaired short- and long-term memory function. Whether weight loss reverses this increase in hypothalamic GFAP and improves cognitive function in obese animals or humans has not been studied. Our findings that the change in serum GFAP correlates with the change in four measures of executive function following weight loss in overweight and obese humans is novel. Most participants (7/8) had a faster response time on the AX-CPT following weight loss, but the participants with the largest increases in serum GFAP had the smallest improvements (Figure 1C). A greater increase in serum GFAP was also associated with less correct hits on the AX-CPT task following weight loss (Figure 1D). Elevated GFAP is a well-established marker of astrocyte reactivity, a normal neural response to damage and disease. Perhaps individuals with more robust GFAP responses were not able to improve as much due to this response; however, an outlier in the already small sample make it difficult to interpret these correlations (Figure 1C, D). When the outlier is excluded, the correlations between change in serum GFAP and AX-CPT response time (r=0.482, p=0.273) and correct hits (r=0.727, p=0.064) are no longer significant. Finally, greater increases in serum GFAP were associated with less errors of omission and total errors following weight loss. Physiologically, this finding is difficult to interpret, as we would expect higher GFAP to correlate with more errors.

The role of BDNF in eating and energy regulation as well as the association between lower BDNF and obesity suggests BDNF as a key potential mediator between adiposity, energy intake, and cognitive decline. Whether circulating BDNF levels can be manipulated with behavioral interventions remains unclear. While caloric restriction and exercise approaches have been shown to increase BDNF in overweight
and obese individuals, there is also evidence to support a decrease in circulating BDNF\textsuperscript{53} or no change at all with weight loss. Only one study has explored the relationship between diet-induced weight loss, serum BDNF, and cognitive function (specifically memory).\textsuperscript{37} Although 3-months of caloric restriction significantly reduced body weight and improved memory performance in older adults, serum BDNF remained unchanged.\textsuperscript{37} The latter observation is consistent with our findings. Furthermore, there was no correlation between changes in serum BDNF and EF performance with weight loss. Whether BDNF improvements following weight loss translate into improvements in EF remains to be elucidated.

Circulating S100B is elevated in a number of neurological conditions, including acute brain injury, neurodegenerative disease, and psychiatric disorders. Although nonspecific, circulating S100B is a well-established biomarker of neural damage.\textsuperscript{78} Elevated serum S100B has been positively correlated with BMI in a stratifying manner in humans.\textsuperscript{63} Further, increases in plasma S100B following diet-induced obesity in mice were reversed with weight loss.\textsuperscript{62} We were unable to replicate these findings in overweight and obese, middle-aged and older adults. To our knowledge, this study is the first to investigate the relationship between serum S100B and EF performance in humans. We did not observe a significant change in serum S100B with weight loss and changes in serum S100B were not associated with EF performance following weight loss. Further investigations are needed to understand the role of S100B as it relates to obesity and brain health.
Whether poor EF leads to obesity or dysregulated adipose tissue leads to poor EF remains a matter of debate; some evidence suggests a bidirectional relationship. A strength of the present study is the ability to address the direction of this complex relationship. A weight loss intervention including pre- and post- measures of EF allows for the determination of the effect of adipose tissue on EF. Another strength of this study is the inclusion of serum biomarker measurements. These findings provide preliminary evidence for the effect of weight loss on serum BDNF, S100B, and GFAP in overweight and obese, middle-aged and older adults.

This study also has limitations. First, the sample size is small and the sample was homogenous. Second, we did not include a non-dieting control group to compare the outcomes of the weight loss group. As such, we cannot exclude a practice effect on the measures of EF.

CONCLUSION

In summary, weight loss did not change serum BDNF, S100B, or GFAP. However inhibitory control was enhanced. Participants made fewer errors of commission and had a faster reaction time after weight loss. Although serum GFAP was not significantly changed over the intervention, changes in GFAP correlated with changes in four measures executive function. More research is needed to further understand the impact of obesity and weight loss on EF. Future investigations should include a larger and more diverse sample, as well as a non-dieting control group. The computer-based cognitive
testing used in this study mainly reflected working memory, and inhibitory and attentional control. Future studies should include more tests of executive function.
CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, we report no significant change in serum BDNF, S100B, and GFAP following 8 or 12 weeks of weight loss in overweight and obese, middle aged and older adults. Additionally, two measures of EF improved following 8 weeks of weight loss: participants made less errors of commission and had a faster reaction time. Without a non-dieting control group, it is difficult to decipher if improvements in EF were due to the intervention or practice effects over time. Thus, future investigation should include a non-dieting control group. Although serum GFAP did not significantly change following weight loss, changes in GFAP correlated with changes in four measures EF. More research is needed to further describe the complex relationships between and amongst obesity, weight loss, serum biomarkers of brain health, and EF.

To date, magnetic resonance imaging (MRI) is used to identify gliosis in humans; including MRI, serum biomarker, and EF measures in one study will extend our findings. Future investigations should include a larger, more diverse sample. Finally, the computer-based cognitive testing used in this study mainly reflect working memory, and inhibitory and attentional control. Future studies should also consider including more comprehensive tests of EF.
REFERENCES


CHAPTER 6: APPENDIX
Information Sheet (Version 2)
Department of Human Nutrition, Foods and Exercise
Virginia Tech

TITLE: Weight Loss in Older Adults

INVESTIGATORS: Brenda M. Davy, PhD, RD; Kevin Davy, PhD; Janet Rankin, PhD; Richard A. Winett, PhD

MEDICAL DIRECTOR: Jose Rivero, M.D.

PURPOSE
You are being asked to participate in an experimental research study. Before you agree to be a volunteer in our study, we want you to understand what your participation will involve. Please read this form thoroughly prior to your first visit and let us know if you have any questions about its contents. The following information describes the study and your role as a participant.

The incidence of obesity is higher in older Americans (over age 60) than in the overall adult population (over age 18). Increased body weight is associated with higher risk for chronic diseases such as heart disease, diabetes, and cancer, as well as functional disabilities that may limit independence with advancing age. It is therefore important to study weight loss in older adults, and how this may improve heart health.

Seventy five people will participate in this study. To participate, you must be between the ages of 55 and 75 and be overweight. If you smoke, have been told by a doctor that you have a major chronic disease, for example, diabetes, cancer, chronic lung disease, kidney disease or thyroid disease, or if you are taking drugs that could affect your weight or appetite, you may not participate in this study. If the questionnaires that you fill out for us suggest that you have an eating disorder or that you may be depressed, you will not be able to participate in the study. Finally, if you have food allergies you may not be able to participate.

Following completion of the 12-week weight loss study, you have the option of continuing your research study participation for a 12-month weight loss maintenance follow-up study. A major challenge in the treatment of obesity is maintenance of weight loss. Many dieters regain about one third of the weight lost during the next year and are typically back to baseline in three to five years. Therefore, our purpose with this component of the study is to determine effective strategies for weight loss maintenance.

PROCEDURES
If you are interested in participating in this study, you will be required to visit War Memorial Hall for initial screening tests. You would be randomly assigned (like flipping a coin) to one of three groups. Two of these groups will be prescribed a low-calorie diet to help with weight loss for 12 weeks, but the two groups will receive different dietary instructions for losing weight. The third group will be a control group; individuals assigned to this control group will undergo all study procedures but will be asked not to change their diet or exercise habits. After 12 weeks, the people in the control group will be given the option of participating in a 12-week weight loss intervention if they would like to. All participants will also be required to eat two breakfast meals in the laboratory to measure your feelings of hunger and fullness before starting weight loss, and two more breakfast meals at the end of the 12-week weight loss period. You will be asked not to change your current physical activity habits (exercise routine) during your participation in the 12-week low-calorie diet phase of the study.
There will be approximately 15-20 visits to the Human Nutrition, Foods, and Exercise Department (228 War Memorial Hall) at Virginia Tech; 2 visits will take place at Montgomery Regional Hospital. All of these visits will take place over a 4-month period. The actual number and order of visits may vary depending upon on your schedule and the availability of the study staff. All study procedures described in this document are done at no cost to participants.

**Session 1 (2 hours):** First we will explain the study to you, and have you read this information sheet. If you choose to participate, the following screening tests will be done:

- **Health History** – you will be asked to complete a medical history questionnaire. This procedure is used to screen for pre-existing disease or other reasons you should not participate in this study. Your height and weight will also be measured at this time. Your body weight will be measured on a standard balance scale and will include the weight of light indoor clothing or hospital gown without your shoes.
- **Blood Pressure** - You will be asked to sit quietly for 15 minutes. We will then measure your resting blood pressure using a stethoscope and standard blood pressure cuff and an blood pressure monitor.
- **Eating Habits and Depression Questionnaires** – you will complete two questionnaires that will be used to assess your eating habits and feelings of depression. If your scores on these questionnaires suggest that you may be depressed or have an eating disorder, you will be provided with contact information for the VT Psychological Services Center at 231-6914. You would be responsible for any costs related to follow-up care, if you decide to seek it.
- **4-Day Food Record** – you will be given instructions for how to record your food and beverage intake for four consecutive days. This may take you about 10-15 minutes total time each day. You will turn this in at the next visit.

**Session 2 (2 hours):** You will be asked to avoid eating for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process.

- **Blood Draw** – a needle will be inserted into an arm vein to draw blood (approximately 2 tablespoons) to measure the levels of cholesterol and glucose. An additional 3 teaspoons will be frozen for other blood tests which may include levels of blood hormones which influence your appetite and risk of cardiovascular disease. The tests will be restricted to those relevant to the research project described. Any blood samples remaining after 10 years will be destroyed.
- **Body Fat Analysis** - a test called a DEXA will be done to measure your percent body fat. For this test, you will have to lie very still on a table for about 20 minutes while your body is scanned, similar to having an x-ray. You will need to wear shorts and a t-shirt for this visit. Women should not wear bras with metal underwires. There is no pain associated with this test.
- **24-Hour Urine Collection** – you will be given a container for collecting your urine for a 24-hour period. We will give you instructions for how to do this test, and you will be asked to keep your urine collection refrigerated (we will provide coolers with freezer packs to help with this).
- **Food Record** - you will turn in your food record at this visit.
- **Blood Flow in Heart and Arteries** – the blood flow and diameter in the arteries in your neck, arm and leg will be measured with an ultrasound machine. An ultrasonic machine is sort-of like radar – a low frequency radio wave that bounces off the tissues and sends a picture back to a “TV-like” screen. A mobile hand unit used will be pressed gently against an artery in your neck, arm and leg. The amount of blood that your heart pumps in one minute and other measures of heart function will be determined with another ultrasound probe. For these measurements, the probe will be pressed gently against two different places on your chest. Your blood pressure will also be measured.

**Session 3 (3 hours):** You may not eat or drink anything except water for 12 hours prior to this visit. We will measure your weight when you arrive for this visit, and you will return your urine
collection from visit 2. This visit will be scheduled in the morning, typically beginning between 8a and 10:30a.

**Breakfast Meal** – you will be provided with a breakfast meal consisting of typical breakfast items (muffins, jam, fruit, etc). You may eat as much as you like.

**Visual Analog Scales (VAS)** – Visual Analog Scales (VAS) are ratings of hunger, fullness, thirst and desire to eat. They consist of questions such as “How hungry are you right now?” to address hunger, thirst, nausea, and fullness. You will be asked to complete VAS at 6 time points on each testing day; 30 minutes before the meal, immediately before and after meal, and 30, 60 and 90-minutes after the meal.

**Session 4 (4 hours):** This visit will be similar to visit 3. You may not eat or drink anything except water for 12 hours prior to this visit. We will measure your weight when you arrive for this visit. This visit will be scheduled in the morning, typically beginning between 8a and 10:30a.

**Breakfast Meal** – you will be provided with a breakfast meal consisting of typical breakfast items (muffins, jam, fruit, etc). You may eat as much as you like.

**Visual Analog Scales (VAS)** – Visual Analog Scales (VAS) are ratings of hunger, fullness, thirst and desire to eat. They consist of questions such as “How hungry are you right now?” to address hunger, thirst, nausea, and fullness. You will be asked to complete VAS at 6 time points on each testing day; 30 minutes before the meal, immediately before and after meal, and 30, 60 and 90-minutes after the meal.

**Weight Loss Diet** - at this visit, you will be instructed in a low-fat (20-30% fat), low-calorie diet which should help you lose weight. We will individualize this diet for you so that it takes into account factors like your dietary preferences and body size. We will provide you with sample menus to use during this part of the study. You will be asked to follow this diet for 12 weeks. During the 12-week period, we will check your weight each week and provide you with assistance/feedback and tips for adhering to your diet. We will also ask you for urine samples every other week to see how well hydrated you are (the amount of fluid in your body).

**Session 5 (1 hour):** This visit will take place at Montgomery Regional Hospital. You will be asked to avoid eating for at least 4 hours prior to this visit.

**Computed Tomography Scan** – the amount of total fat, fat around your internal organs, and the fat under the skin in the abdominal area will be measured by computed tomography (CT scan). The CT scan imaging will be performed at Montgomery Regional Hospital. For this procedure, you will be asked to lie still on a table. An x-ray machine (the CT scanner) will rotate around you and the table will move back and forth slightly making it possible to take X-rays from several angles. The actual x-ray time is approximately 2 minutes or less. You will be lying on the table for approximately 15 to 30 minutes. The approximate time required for the entire procedure is one hour. A longer period of time may be required due to heavy scheduling and/or emergency need of the CT scan at the Montgomery Regional Hospital.

**Sessions 6-16 (15-30 minutes each):** Once you begin your diet, you will be asked to come into the lab every 1-2 weeks to be weighed and have your blood pressure taken, and we will ask you if you are having any problems with following your diet that we can help you with. Every other week, you will be asked to keep another 24-hour urine collection and record everything that you eat and drink.

**Session 17 (2 hour):** This session will be the same as session 2.

**Sessions 18 and 19 (3 hours each):** This session will be the same as session 3.

**Session 20 (1 hour):** This session will be the same as session 5.

**The total time commitment for this study will range from approximately 24-27 hours.**
Name of Subject (please print)_________________________________

Signature of Subject_________________________________________ Date_______

Name of Person Obtaining Consent (print) ____________________________

Signature of Person Obtaining Consent ______________________________ Date_______
RESEARCH SUBJECT INFORMATION SHEET

Title:  Weight Loss and Cognitive Function in Middle-Aged and Older Adults

Protocol No.:  18-462
               WIRB® Protocol #20181536
               18-462

Sponsor:  Brenda Davy, PhD RDN

Investigator:  Brenda Davy, PhD RDN
               221 Wallace Hall
               Department of Human Nutrition, Foods and Exercise
               Virginia Tech
               Blacksburg, VA 24061
               USA

Daytime Phone Number:  540-231-6784

You are being invited to take part in a research study. A person who takes part in a research study is called a research subject, or research participant.

What should I know about this research?

- Someone will explain this research to you.
- This form sums up that explanation.
- Taking part in this research is voluntary. Whether you take part is up to you.
- You can choose not to take part. There will be no penalty or loss of benefits to which you are otherwise entitled.
- You can agree to take part and later change your mind. There will be no penalty or loss of benefits to which you are otherwise entitled.
- If you don’t understand, ask questions.
- Ask all the questions you want before you decide.

Why is this research being done?

More than two-thirds of middle-aged and older adults are overweight, which places them at greater risk of disability and higher medical expenses. Lifestyle interventions which promote weight loss for this population are needed. However, it is not known how different weight loss approaches impact brain (cognitive) functions, such as memory and attention.
About 100 subjects will take part in this research. You will **not** be eligible to participate in this study if any of the following apply:

- You are **not** between the ages of 50 and 75 years
- You have a body mass index (weight status indicator) below 25 or above 40 kg/m$^2$
- You cannot safely participate in a diet and physical activity (walking) program
- You have type 2 diabetes which requires insulin
- You have been diagnosed with Dementia, or Alzheimer’s Disease
- You have previously participated in psychology research that involves computerized cognitive (brain function) testing.

**How long will I be in this research?**

We expect that your taking part in this research will last about 10 hours total, over 10 weeks.

**What happens to me if I agree to take part in this research?**

You are being asked to be involved in a study where you will either follow a low-calorie diet for 4 weeks, followed by a weight maintenance diet and physical activity program for 4 weeks, OR you will be placed on a waiting list for 8 weeks before you receive weight loss diet instructions. You may be eligible to participate if you are between 50 and 75 years of age, and overweight. During the study, you will be asked to record your dietary intake and your physical activity level. Your weight will be checked weekly in our lab during the 4-week weight loss phase, and again at the end of the 4-week weight loss maintenance phase.

After baseline testing you will be put into a study group by chance (like a coin toss). You have a 1 out of 3 chance of being placed in each group. You cannot choose your study group. One group will be put on a waiting list for 8 weeks, before receiving weight loss diet instructions. The other two groups will be instructed in a low-calorie diet by a Registered Dietitian Nutritionist (RDN), and asked to follow a physical activity (walking) program. We will provide a meal plan for you to use, as a guide. Three times during the study, we will also have you complete cognitive testing, using a computer. We will also measure your body composition, levels of hunger and fullness, urinary specific gravity (how concentrated or diluted your urine is), and obtain a blood sample (about 3 teaspoons).

If you decide to participate, there will be a total of 10 study sessions at the Virginia Tech campus. The entire study will require about 10 hours of your time. The actual number and order of visits will depend on your and the study staffs’ schedules. We will try to schedule all of your study sessions in the morning, at a similar time. A description of the testing sessions, time required, and location, are provided below.
Baseline Screening and Testing – Sessions 1-3:

- **Session 1.** Approximate time required: 1 hour in 338 Wallace Hall

**Medical history, height and weight:** After reviewing this form and agreeing to participate, you will be asked to complete a medical history questionnaire. This questionnaire is used to screen for health problems or reasons you should not participate in this study. Your height and weight will also be measured at this time. Your body weight will be measured on a scale. Your height will be measured with a type of ruler. We will calculate your body mass index (BMI) to be sure you are eligible to participate.

**Dietary intake and health-related quality of life:** We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session. We will also ask you a series of questions about how you feel about your general health, limitations in your activities related to your health, and feelings of sadness and worrying about how your health may limit your activities.

- **Session 2.** Approximate time required: 1 hour in 338 Wallace Hall

**Dietary intake:** We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session, including the morning of testing.

**Urinary specific gravity (USG):** We will ask you to provide us with a urine sample, so that we can measure how diluted or concentrated your urine is.

**Cognitive function:** We will ask you to complete tasks on a computer, which will assess your memory, attention, and self-control. This will involve tasks such as pressing a button when you see a particular letter, but only when this letter follows another letter.

- **Session 3.** Approximate time required: 1.5 hours in 338 Wallace Hall

**Overnight Fast:** You will be asked to avoid eating or drinking anything except water and to avoid consuming any caffeine-containing beverages for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process. You may drink only water during this time.

**Blood Draw:** Blood samples will be taken to evaluate different markers (e.g., glucose, and chemical markers that influence cardiovascular health and brain function). A small needle will be placed in your arm vein to take blood samples (approximately 3 teaspoons).

**Dietary intake:** We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session.
**Pregnancy Test:** If you are female you will be required to have a pregnancy test. This will require you to collect 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study. If you are a postmenopausal female who has not menstruated for at least 1 year then you do not have to complete this test.

**Body Weight and Composition:** These tests are to measure your body weight and body fat. Your body weight and height will be measured on a scale. Then you will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 15 minutes and there is no pain associated with the procedure. Your weight and height will also be measured at this time. This test is done in War Memorial Hall (room 228) and we will walk over there with you for this procedure.

**Eating behaviors:** We will ask you to complete two questionnaires related to various aspects of eating behavior, such as how hungry you are, how full you feel, and how much you try to consciously control your food intake.

**Dietary counseling (Note that the Waiting List Group will not do this):** We will randomly assign you to one of two low-calorie diet groups for the 4-week weight loss phase. You will be instructed in the low calorie diet by an RDN, and provided with meal plans to help you follow the weight loss plan. The diet plans will be either 1200 or 1500 calories, based upon your sex and body size. They will both be based upon the Dietary Guidelines for Americans, but different in a few ways such as the types of foods or beverages included. We will also give you a pedometer and ask you to work up to a daily step count goal of 10,000 steps per day. We will ask you to keep a brief daily log of your weight, diet and step counts.

**Weight Loss Phase - Sessions 4-7:**

- **Session 4.** Approximate time required: 20 minutes in 338 Wallace Hall
  
  **Body weight:** Your body weight will be measured on a scale. We will also ask you how you are doing with your low-calorie diet and step count goal, and talk with you about how we can support you if you are having challenges.

- **Session 5.** Approximate time required: 30 minutes in 338 Wallace Hall
  
  **Dietary intake:** We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session.

  **Urinary specific gravity (USG):** We will ask you to provide us with a urine sample, so that we can measure how diluted or concentrated your urine is.
Body weight: Your body weight will be measured on a scale. We will also ask you how you are doing with your low-calorie diet and step count goal, and talk with you about how we can support you if you are having challenges.

- **Session 6.** Approximate time required: 30 minutes in 338 Wallace Hall

Dietary intake: We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session.

Body weight: Your body weight will be measured on a scale. We will also ask you how you are doing with your low-calorie diet and step count goal, and talk with you about how we can support you if you are having challenges.

- **Session 7.** Approximate time required: 2 hours in 338 Wallace Hall

Overnight Fast: You will be asked to avoid eating or drinking anything except water and to avoid consuming any caffeine-containing beverages for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process. You may drink only water during this time.

Blood Draw: Blood samples will be taken to evaluate different markers (e.g., glucose, and chemical markers that influence cardiovascular health and brain function). A small needle will be placed in your arm vein to take blood samples (approximately 3 teaspoons).

Dietary intake: We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session, including the morning of testing.

Pregnancy Test: If you are female you will be required to have a pregnancy test. This will require you to collect 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study. If you are a postmenopausal female who has not menstruated for at least 1 year then you do not have to complete this test.

Body Weight and Composition: These tests are to measure your body weight and body fat. Your body weight and height will be measured on a scale. Then you will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 15 minutes and there is no pain associated with the procedure. Your weight and height will also be measured at this time. This test is done in War Memorial Hall (room 228) and we will walk over there with you for this procedure.

Urinary specific gravity (USG): We will ask you to provide us with a urine sample, so that we can measure how diluted or concentrated your urine is.

Cognitive function: We will ask you to complete tasks on a computer, which will assess your memory, attention, and self-control. This will involve tasks such as pressing a button when you see a particular letter, but only when this letter follows another letter.
Eating behaviors: We will ask you to complete two questionnaires related to various aspects of eating behavior, such as how hungry you are, how full you feel, and how much you try to consciously control your food intake.

Dietary counseling (Note that the Waiting List Group will not do this): We will provide you with diet and physical activity counseling for weight loss maintenance, as after this session you will begin the 4-week maintenance phase. We will ask you to continue keeping a brief daily log of your weight, dietary intake, and step counts. You will do this on your own for 4 weeks, but we will occasionally call you to see how you are doing.

Weight Loss Maintenance Phase Follow-Up Testing - Sessions 8-10:

- **Session 8.** Approximate time required: 20 minutes in 338 Wallace Hall

Dietary intake: We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session.

Body weight: Your body weight will be measured on a scale.

- **Session 9.** Approximate time required: 1 hour in 338 Wallace Hall

Dietary intake: We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session, including the morning of testing.

Urinary specific gravity (USG): We will ask you to provide us with a urine sample, so that we can measure how diluted or concentrated your urine is.

Cognitive function: We will ask you to complete tasks on a computer, which will assess your memory, attention, and self-control. This will involve tasks such as pressing a button when you see a particular letter, but only when this letter follows another letter.

Eating behaviors: We will ask you to complete two questionnaires related to various aspects of eating behavior, such as how hungry you are, how full you feel, and how much you try to consciously control your food intake.

- **Session 10.** Approximate time required: 1 hour in 338 Wallace Hall

Overnight Fast: You will be asked to avoid eating or drinking anything except water and to avoid consuming any caffeine-containing beverages for 12 hours prior to this visit so that the test results will not be influenced by the food you eat or by the normal digestion process. You may drink only water during this time.
**Blood Draw:** Blood samples will be taken to evaluate different markers (e.g., glucose, and chemical markers that influence cardiovascular health and brain function). A small needle will be placed in your arm vein to take blood samples (approximately 3 teaspoons).

**Dietary intake and health-related quality of life:** We will ask you to tell us about all the foods and beverages you consumed in the 24 hours prior to this session. We will also ask you a series of questions about how you feel about your general health, limitations in your activities related to your health, and feelings of sadness and worrying about how your health may limit your activities.

**Pregnancy Test:** If you are female you will be required to have a pregnancy test. This will require you to collect 2-3 teaspoons of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study. If you are a postmenopausal female who has not menstruated for at least 1 year then you do not have to complete this test.

**Body Weight and Composition:** These tests are to measure your body weight and body fat. Your body weight and height will be measured on a scale. Then you will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to determine the amount of bone, muscle and fat in your body. This unit is called a DEXA scan. This test takes approximately 15 minutes and there is no pain associated with the procedure. Your weight and height will also be measured at this time. This test is done in War Memorial Hall (room 228) and we will walk over there with you for this procedure.

A study debriefing will also take place.

**Waiting List Group only:** You will be instructed in the low calorie diet by an RDN, and provided with meal plans to help you follow the weight loss plan. The diet plans will be either 1200 or 1500 calories, based upon your sex and body size. They will both be based upon the Dietary Guidelines for Americans, but different in a few ways such as the types of foods or beverages included. We will also give you a pedometer and ask you to work up to a daily step count goal of 10,000 steps per day. We will ask you to keep a brief daily log of your weight, diet and step counts. We will meet with you weekly for four weeks, then again after week 8. You will not have to undergo any additional testing during this time, but we will measure your body weight if you would like us to do that.

**What are my responsibilities if I take part in this research?**

If you take part in this research, you will be responsible to:

- Provide an accurate history of any health problems or use of medications before the study begins.
- Inform the investigators of any discomfort or unusual feelings before, during or after any of the study sessions.
- Be on time and attend all of the scheduled study sessions.
• Follow all participant instructions for each session.
• Follow diet and physical activity instructions provided by the investigators.

Could being in this research hurt me?

The amount of radiation that you will receive in the DEXA (body composition) test is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to 1/20 of a chest x-ray for each DEXA scan. The more radiation you receive over the course of your lifetime, the more likely your risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks; however, the exact increase in such risk is not known.

Blood Draw: Some pain or discomfort may be experienced when the needle is inserted in the vein, but this should persist for only a short time. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood is taken. In about 1 in 10 or 10% of the cases, a small amount of bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200, while the risk of infection or significant blood loss is 1 in 1000. There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the needle is removed. If you feel faint during or after a blood draw, you should notify the study doctor or study staff immediately and lie down right away to avoid falling down. Having staff who are experienced in performing blood draws will minimize these risks.

HIV/AIDS: In the event a researcher or other staff person is improperly exposed to your blood, your blood will be tested for the presence of HIV, the Hepatitis B Virus, and the Hepatitis C Virus. There will not be any cost to you for this test. The research team will follow proper procedures for testing and reporting as outlined by Virginia State Law, which includes sending the sample to a certified laboratory. Please note that, should your blood require testing, you will be informed of your test results and provided with the opportunity to receive appropriate and timely counseling. In addition, your results will be sent to the local health department.

All other tests are non-invasive and pose no potential risk.

It is not possible to identify all potential risks in an experimental study. However, the study doctors and study staff will take all possible safeguards to minimize any known and potential risks to your well-being. We believe the overall risks of participation are minimal. All of the procedures are well established and used routinely in the study investigators laboratory. Side effects are possible in any research study despite high standards of care, and could occur through no fault of your own or the study doctors or study staff.

Will it cost me money to take part in this research?

All of the study testing will be done at no cost to you. You will have to pay for the food related to the meal plans. Neither the researchers nor the University have money set aside to pay for medical treatment that would be necessary if you are injured as a result of your participation in
this study. Any expenses that you incur including emergencies and long-term expenses would be billed to you. You should consider this limitation before you consider participating in this study.

Will being in this research benefit me?

We cannot promise any benefits to you or others from your taking part in this research. However, possible benefits to you include obtaining health information related to your body composition, and your usual dietary intake. You will receive dietary counseling by a RDN. However, you should not consider this a wellness or medical exam. You should discuss any concerns about your health information with your personal physician. Your participation will contribute to improving the understanding of how weight loss impacts cognitive function.

What other choices do I have besides taking part in this research?

This research is not designed to diagnose, treat or prevent any disease. Your alternative is to not take part in the research.

What happens to the information collected for this research?

Your private information and will be shared with individuals and organizations that conduct or watch over this research, including:

- Government agencies, such as the Food and Drug Administration
- The Institutional Review Board (IRB) that reviewed this research

We may publish the results of this research. However, we will keep your name and other identifying information confidential.

We protect your information from disclosure to others to the extent required by law. We cannot promise complete secrecy.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Data or specimens collected in this research might be deidentified and used for future research or distributed to another investigator for future research without your consent.

Who can answer my questions about this research?

If you have questions, concerns, or complaints, or think this research has hurt you or made you sick, talk to the research team at the phone number listed above on the first page.
This research is being overseen by an Institutional Review Board (“IRB”). An IRB is a group of people who perform independent review of research studies. You may talk to them at (800) 562-4789, help@wirb.com if:

- You have questions, concerns, or complaints that are not being answered by the research team.
- You are not getting answers from the research team.
- You cannot reach the research team.
- You want to talk to someone else about the research.
- You have questions about your rights as a research subject.

**What if I am injured because of taking part in this research?**

If you are injured or get sick because of being in this research, call the research team using the number listed on the first page of this form. The study researchers will provide emergency medical treatment. Your insurance may be billed for this treatment.

**Can I be removed from this research without my approval?**

The person in charge of this research can remove you from this research without your approval. Possible reasons for removal include:

- It is in your best interest
- You have a side effect that requires stopping the research
- You need a treatment not allowed in this research
- You become pregnant
- You are unable to keep your scheduled appointments

We will tell you about any new information that may affect your health, welfare, or choice to stay in this research.

**What happens if I agree to be in this research, but I change my mind later?**

If you decide to leave this research, contact the research team so that the investigators know that you no longer wish to be part of the study.

**Will I be paid for taking part in this research?**

For taking part in this research, you may be paid up to a total of $50. Your compensation will be broken down as follows:

- You will receive $20 after completing session 7.
- You will receive an additional $30 after completing session 10.
- There is no compensation for completing sessions 1-6.
Statement of Consent:

Your signature documents your consent to take part in this research.

______________________________________________________________  ______________________
Signature of adult subject capable of consent                  Date

______________________________________________________________  ______________________
Signature of person obtaining consent                          Date
MEMORANDUM

DATE: June 21, 2010

TO: Brenda M. Davy, Kevin P. Davy, Janet W. Rankin

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires June 13, 2011)

PROTOCOL TITLE: Weight Loss In Older Adults

IRB NUMBER: 06-372

Effective July 17, 2010, the Virginia Tech IRB Chair, Dr. David M. Moore, approved the continuation request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at http://www.irb.vt.edu/pages/responsibilities.htm (please review before the commencement of your research).

PROTOCOL INFORMATION:
Approved as: Full Board Review
Protocol Approval Date: 7/17/2010 (protocol's initial approval date: 7/17/2006)
Protocol Expiration Date: 7/16/2011
Continuing Review Due Date*: 6/2/2011
*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:
Per federally regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals / work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.
If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.

*Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.
MEMORANDUM

DATE: May 30, 2019
TO: Brenda Davy, Benjamin D Katz, Kevin Davy, Tina Savla, Elaina Lynn Marinik
FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Premeal Water & Weight Loss: Cognitive, Behavioral, and Physiological Aspects
IRB NUMBER: 18-462

The Virginia Tech Institution Review Board (IRB), acknowledges the Amendment request for the above-mentioned research protocol.

This acknowledgement recognizes the item(s) identified in the Special Instructions section.

NOTE: Amendments that must be submitted to BRANY for review and approval include changes to funding, conflict of interest, ANY and ALL changes to study procedures and study documents. If your study qualified for Not Human Subjects or for an Exemption please review the information at the end of your approval Letter.
SPECIAL INSTRUCTIONS:
The Virginia Tech IRB acknowledges the transfer of IRB oversight from WIRB to BRANY for this protocol. Please read the information below for more details.

Dear Investigators:

This email serves as a notice that your protocol is under active transfer from WIRB to BRANY. We ask that you do not submit any further requests to WIRB or to the Virginia Tech IRB.

FAQ's:

Q. How will I know when my protocol has been accepted by BRANY?
A. BRANY IRB will send you a notification indicating your transferred study has been accepted.

Q. Has the Virginia Tech IRB drafted guidance?
A. Yes. We have created guidance and it is available on a PID protected website.
https://internal.research.vt.edu/sirc/hrpp/brany-transfer
This link will be provided on all Authorization Letters.

Q. How do I gain access to BRANY’s IRBManager?
A. This section is very important. Not everyone listed as study personnel needs to have access to IRBManager. The PI, active Co-I(s), and study coordinators are the typical research team members that will need to have access. In order to gain access, each person will need to complete the Request for User Access form and sign it with wet ink. Digital signatures and script style font are not accepted. [ http://www.brany.com/wp-content/uploads/2018/07/BRANY-User-Access-Form-complete-sign-return-20170323-V2_.pdf ]

Q. I need to submit an amendment to my protocol. What should I do?
A. Once your protocol has been accepted in the BRANY IRBManager system, you will be able to submit requests directly to BRANY for review in their IRBManager system. Refer to the guidance provided by the Virginia Tech IRB using the web link above.

Q. I need to revise my list of study personnel. What should I do?
A. You will no longer submit personnel changes to the Virginia Tech IRB. You will submit personnel changes to BRANY through their IRBManager system. You should follow the guidance provided by the Virginia Tech IRB using the web link above.

Q. I am actively working with research subjects (including recruiting, consenting, enrolling, collecting data). Do I need to alter my consent forms? Do I need to notify my participants of the change of IRB oversight?
A. This section is very important. When you receive the notification from BRANY that your study has been accepted, instructions will be included regarding consent needs.

Q. How will I know that I need to submit a Continuing Review request?
A. BRANY will send reminder emails 45, 30, and 15 days prior to the expiration date. The automated reminders will cease when either a continuing review or closure application is received and processed by BRANY IRB.
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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this protocol is to cover any other grant proposals, please contact the HRPP office (irb@vt.edu) immediately.