Effects of Intermittent Fasting on Cognitive Acuity in University Students

by

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ABSTRACT

The popularity of intermittent fasting has grown in recent years and is a commonly discussed diet topic on the internet and social media. Time-restricted feeding (TRF) is one particular intermittent fasting regime that allows participants to pick windows of time per day in which they can eat or fast. While current randomized controlled trials show positive effects of TRF on weight loss, body composition, glucose, insulin, and blood pressure, there is a gap in the literature of the its effect on cognition although animal studies suggest a positive effect. The purpose of this 8-week randomized controlled trial was to investigate the effect of 18-hour time-restricted feeding on healthy, Arizona State university students. Students (n= 29) were recruited by the research team and were randomized to either an 18-hour intervention (INV) group or an 8-hour control (CON) group. INV participants were instructed to consume food within the first hour of waking and cease their eating period after 6 hours to begin their 18-hour fast. Participants were not given any other dietary restrictions and were allowed to eat ad libitum during their eating periods. Cognitive tests (Stroop Test and Trail Making Test) and blood draws were taken at baseline, week 4, and week 8. The present study demonstrated high attrition, with 7 participants dropping out of the study after their baseline visit. Interruption of the COVID-19 pandemic also impacted the data analysis, with the removal of week 8 data. Despite limitations, statistically significant differences between the INV group and CON group were seen in the Trail Making Test B at week 4 (p= 0.031). Statistically significant differences were not seen in any of the other cognitive outcomes measured (Stroop Test, Trail Making Test A, serum BDNF, serum ketones). However, a significant inverse relationship was seen between serum ketones and Trail

Making Test B. In conclusion, this study suggests that TRF may have a favorable effect on cognitive acuity among university students.

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CHAPTER 1

INTRODUCTION

Intermittent fasting has grown increasingly popular in recent years with discussion of this diet practice populating the Internet. In November 2019, over 64 million hits resulted from a Google search of "intermittent fasting", over 2 million posts on Instagram used the hashtag "intermittent fasting", and over 2,000 results came up in an Amazon search for "intermittent fasting" under books. Although there appears to be a large number of individuals interested in or practicing intermittent fasting, according to Internet and social media searches, researchers agree that there is a lack of confirmation from experiments regarding the effects of intermittent fasting in humans (Horne, Muhlestein, & Anderson, 2015; Patterson & Sears, 2017).

Cognitive function is extremely important to college students as their extensive academic workload demands a continual cognitive and intellectual output. Research has shown that metabolic syndrome has been connected to cognitive dysfunction (Yates, Sweat, Yau, Turchiano, & Convit, 2012). In a study looking at metabolic syndrome among university students, one third had a least one metabolic abnormality (Yahia et al., 2017). Erratic eating patterns, commonly associated with the lifestyle of university students, interrupt circadian rhythms and ultimately lead to metabolic disruption and chronic diseases (Manoogian & Panda, 2017). It is possible that erratic eating patterns among college students are connected to metabolic abnormalities, which ultimately can affect their cognitive abilities.

Intermittent fasting techniques are associated with cognitive improving effects. Studies conducted on animals have produced positive results, including increasing BDNF expression, preventing brain hypotrophy after exposure to distress, improving memory, enhancing neurogenesis, and strengthening of hippocampal synaptic connections (Baik et al., 2020; Dasgupta et al., 2018; Serra et al., 2019; Shojaie et al., 2017; J. Zhang et al., 2017).

Fasting has also been shown to result in a metabolic switch resulting in the production of ketones, which is connected to an improvement in neuronal networks and neuroplasticity (Mattson, Moehl, Ghena, Schmaedick, & Cheng, 2018). Additionally, intermittent fasting has been shown to increase the production brain-derived neurotrophic factor (BDNF), which can strengthen the ability of neurons to resist brain dysfunction (Mattson, 2005).

There are several different methods of intermittent fasting, including alternate-day fasting, modified eating regimens, time-restricted feeding, and religious based fasting (Patterson & Sears, 2017). In time-restricted feeding (TRF) regimes, individuals consume their calories during a specific time period and fast outside of that particular period. Rather than fasting for entire days, the time in which individuals can eat is restricted. Therefore, of all the intermittent fasting regimes, TRF may be the most feasible or desirable approach. To the best of our knowledge, 28 randomized controlled trials on humans have been published on TRF. However, of these studies, none directly address cognition or cognitive performance. Thus, a large gap exists in the literature relating to the effects of TRF, especially as it pertains to cognition, BDNF production, and ketone

production through metabolic switching. With vast Internet popularity on intermittent fasting, randomized controlled trials are needed in order to properly educate the public.

Thus, the aim of this ancillary study was to compare cognition test scores assessing cognitive acuity between Arizona State University students enrolled in a small randomized controlled study where they either fasted intermittently for 18 hours for 8 weeks (intervention group) or fasted for 8 hours for 8 weeks (control group).

Primary Hypothesis: Arizona State University students who fast intermittently for 18 hours for 8 weeks will improve their cognitive acuity compared to students in the control group that fasted for 8 hours.

Secondary Hypotheses:

(1) Arizona State University students who fast intermittently for 18 hours for 8 weeks will improve their cognitive test scores on the Trail Making Tests compared to students in the control group that fasted for 8 hours.

(2) Arizona State University students who fast intermittently for 18 hours for 8 weeks will improve their cognitive test scores on the Stroop test compared to students in the control group that fasted for 8 hours.

(3) Arizona State University students who fast intermittently for 18 hours for 8 weeks will produce greater concentrations of fasting serum Brain-Derived Neurotrophic Factor (BDNF) compared to students in the control group that fasted for 8 hours.

(4) Arizona State University students who fast intermittently for 18 hours for 8 weeks will produce greater concentrations of fasting serum ketones compared to students in the control group that fasted for 8 hours.

Definition of Terms

- **Cognition:** The psychological mechanism of knowledge, including perception, understanding, and judgment (Burdick & Goldberg, 2008)

- **Intermittent fasting:** The deliberate restriction of calories/energy during regular time periods.

- **Thermogenesis:** heat production

- **Alternate Day Fasting:** Complete fasting every other day with ad libitum eating days in between (Patterson & Sears, 2017)

- **Modified Eating Regimen:** Involves restriction of calories on particular days of the week and free consumption of calories on the remaining days (Patterson & Sears, 2017).

- **Time-Restricted Fasting:** Complete fasting occurring during specific, regular time periods throughout the day with ad libitum eating allowed outside of those time windows. For example, fasting for 16 hours a day and eating during the remaining 8 hours window (Patterson & Sears, 2017)

Delimitations:

- Convenience sample
- Participants only include Arizona State University students over 18 years old
- Participants are nonsmokers

- Participants exercise less than 420 minutes per week and are not training or competing for sports events

- Participants are free of acute and chronic illnesses

- Participants must not regularly fast

- Participants did not have significant weight loss or undergo a weight loss regime in the past 6 months

- Participants are nonpregnant and not lactating

- Participants are not on prescription contraceptives

- Participants do not work night shifts

- Participants do not have a history of weight cycling

Limitations

- Test conductor: different person could be conducting test for different people causing the participant to have a different attitude toward different researcher

- Study will be ongoing over holidays; participants might cheat more days than we ask over holiday break

- Self-reported: study participants might not actually be adhering to the fasting regime since it is self-reported

- Time of Test: the test taking time may vary among the three sessions among the participants

- At week 4 finals week is ongoing, so participants might be more stressed affecting their cognitive test scores

- Could be scheduled on an exam day for the participants they could be distracted and lose focus on the cognitive test

CHAPTER 2

REVIEW OF LITERATURE

Cognition

The intellectual mechanism of knowledge is the meaning of cognitive function (Burdick & Goldberg, 2008). However, the characterization of cognition is not restricted to the domain of knowledge alone, but rather includes several subdomains. These other aspects of cognitive function can include memory, intelligence, attention, reasoning, executive function, intellectual flexibility, language and verbal ability, motor and visual speed, and visuospatial ability (Gimeno et al., 2008; B. K. Lee et al., 2007; Simpson et al., 2016; Zamroziewicz et al., 2016). Additionally, these specific subdomains can be studied even further. For example, memory can be analyzed a variety of ways in looking at one's working memory, visual memory, verbal memory, short term memory, and long-term memory (Gaysina et al., 2014; Gimeno et al., 2008, p. 2; Krikorian et al., 2012; B. K. Lee et al., 2007).

Among younger populations (18-35 years old), the assessment of cognitive ability is approached in a different manner than for the aging population, likely due to the absence of cognitive decay. Rather than looking at the possibility for cognitive impairment among subjects, studies with younger and healthier populations tend to analyze subjects' cognitive agility. Several subdomains of overall cognition can be used to assess cognitive acuity, such as cognitive flexibility, visuomotor speed, sustained attention, processing speed, and executive function. These subdomains examine how quickly a subject can visualize and process information and execute a corresponding task

successfully. The objective among healthy, young people is to determine which factors increase or decrease mental sharpness, thus leading to more successful cognitive function among young people. For example, a study was conducted among young adults (mean age 21.05 ± 0.89 years old) on the effects of consuming grape juice on cognitive performance and analyzed participants' attention, memory accuracy, and memory reaction times in completing the Computerized Mental Performance Assessment (Haskell-Ramsay et al., 2017). The results showed that the consumption of grape juice improved cognitive performance, as demonstrated by quicker attention reaction times.

A variety of validated tests have been formulated in order to assess various cognitive subdomains. Trail Making Tests (TMT) are used to evaluate one's processing speed, cognitive flexibility, and executive function (Simpson et al., 2016). TMTs analyzes participants' ability to connect consecutive points, thus challenging their ability to process information quickly and carry out the task successfully. A participant who completes a TMT in a minimal amount of time would demonstrate a strong ability to think and process information rapidly, leading to a conclusion of proficient cognitive ability for that particular domain. TMTs are common tests among young people to assesses their cognitive flexibility and include a part A and a part B. TMT B goes even further than TMT A to assess cognitive flexibility as it incorporates two consecutive lists to be traced within the assessment. This requires the subject to switch from one rule set to another in "completing the patterned sequence" (Koven & Collins, 2014).

Another common executive function test is the Stroop test, which further analyzes one's executive function, reaction ability, or cognitive control and flexibility (Moering et

al., 2004; Uttl & Graf, 1997; N. Zhang et al., 2019). During the Stroop test, the

participant is given words of various colors to name. The challenge of the test lies when

various words of colors are displayed in opposing colors, and the participant is

challenged to name the incongruous color of the ink rather than the spelled word. For

example, the word "orange" may appear in blue text and the participant is asked to

identify the color of the text: blue. Slight variations of the Stroop Test exist across

different studies. Some studies utilize subtests, which began with a basic task of naming

colors (Stroop Test I) and ended with naming colors printed in incongruous ink (Moering

et al., 2004; Uttl & Graf, 1997; N. Zhang et al., 2019). Additionally, different outcomes

can be measured, including accuracy of responses and response time (Ludwig et al.,

2010).

In addition to assessing processing speed and cognitive flexibility, many research

studies will include intelligence tests in their assessments of cognitive ability. For

example, the Wechsler Abbreviated Scale of Intelligence (WASI) is used to formulate a

cognition score among individuals in classifying intelligence (Matsuda et al., 2017). The

two-subtest version of the WASI incorporates a vocabulary test, which analyzes the

knowledge of various words and their definitions, and a "matrix reasoning" test, which

analyzes one's nonverbal reasoning ability (Koven & Collins, 2014).

Among studies including samples of young, healthy populations, specific regions

of the brain corresponding to certain cognitive functions have been examined. For

example, one group of researchers discussed possible mechanisms associated with lower

cognitive scores resulting from physiologic dysfunction and suggested that the prefrontal

cortex could have been affected due to its known actions in executive function, memory, and spatial competencies (Kobrosly et al., 2012). Others demonstrated that the inferior parietal lobe and the fronto-parietal cortical networks play roles in visuomotor skills and visuomotor attention (Haskell-Ramsay et al., 2017). Additionally, the right superior parietal lobe has been contributed to visuomotor control, continuous attention, and increased attention and alertness (Haskell-Ramsay et al., 2017; Hosseini et al., 2017). Thus, these brain regions are important regions for observation among young populations, as they are involved in cognitive functions associated with cognitive acuity and agility.

Blood Markers of Cognition

There are a number of biomarkers that have been used in studies researching cognition, each demonstrating the specific role they play in the brain. The major cognitive biomarkers and their relationship to cognition can be found in Table 1.

A major biomarker that is very commonly analyzed when looking at cognitive function across various ages is brain-derived neurotrophic factor (BDNF). BDNF is encoded by the BDNF gene, which belongs to the neurotrophin family" (Navarro-Martínez et al., 2015). BDNF, along with other neurotrophic proteins in the neurotrophin family, is essential for brain health as it plays an important role in maintaining neurogenesis and plasticity (Koven & Collins, 2014; Poulose et al., 2017). BDNF is able to cross the blood-brain barrier in both directions, which enables it to play a variety of roles that are important for the upkeep of cognitive health (Navarro-Martínez et al., 2015). In evaluating the effect of BDNF across the various domains of cognitive

function, BDNF has one of the most diverse impacts. Because of the role it plays it neurogenesis and maintenance of neuronal plasticity, it has been shown to effect memory as well as learning abilities (Raefsky & Mattson, 2017; Wahl et al., 2016). One study found that BDNF was positively correlated with increased scores related to concentration, orientation, fixation, short term memory, and language skills in older individuals (Raefsky & Mattson, 2017; Wahl et al., 2016). Also, cognitive declines related to aging were associated with a decrease in BDNF (Navarro-Martínez et al., 2015; Wahl et al., 2016). However, BDNF is not only associated with improved cognitive abilities among aging populations. One study among middle-aged adults found that increased scores in cognitive flexibility was related to increased levels of BDNF (Koven & Collins, 2014). This work demonstrates the ability of BDNF to improve cognitive performance within aging and healthy populations due to the essential role it plays in neuron health.

Periodic ketone production may also improve during a metabolic switch may also improve cognition. Ketones can provide up to approximately 70% of the energy demands of the brain by acting as ATP substrates (Cavaleri & Bashar, 2018; Gasior et al., 2006). Ketones can be produced during the occurrence of malnutrition, starvation, periods of fasting, or in strenuous exercise (Cunnane et al., 2016). During these occurrences, the brain utilizes the ketones that are produced as "back up" energy, due to the fact that glucose has been depleted, permitting the maintenance of ATP (Cunnane et al., 2016). Increased ketone levels have been shown to be associated with improved cognitive function, especially among the cognitively impaired or those with neurological diseases, as ketones act as an alternate source of fuel specifically for cerebral neurons (Reger et al.,

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2004). Impaired glucose metabolism in the brain has been correlated senile plaque density, which may be the contributor of decreased cognition in Alzheimer's disease patients (Hoyer, 1992; Meier-Ruge et al., 1994; Møller, 2020). Thus, a switch to ketone utilization may improve cognition in this population, which was evidenced by improved paragraph recall and Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) scores with increase serum ketone levels among cognitive impaired adults and adults with Alzheimer's Disease (Reger et al., 2004).

Another study among cognitively impaired older adults found that ketone levels were positively correlated to improved memory and the researchers hypothesized that enhanced cognitive performance was linked to reduced inflammation and increased energy metabolism due to the production of ketones (Krikorian et al., 2012). Even among cognitively healthy, older adults (60 years and older), inducing serum ketone levels through a single ketogenic diet proved to be effective in improving working memory and executive function scores (Ota et al., 2016). A randomized controlled trial among young athletes showed that the consumption of a ketone ester supplement maintained executive function scores after exercise compared to a control group (Evans & Egan, 2018). A study among Type 2 diabetics (mean age 65 ± 4 years) showed that infusion of β-hydroxybutyrate increased working memory test performance significantly (Jensen et al., 2020). The mechanism associated with improved cognition related to ketosis is related to a more economical use of nicotinamide adenine dinucleotide (NAD^+) to create acetyl-coA in energy production (Xin et al., 2018). While glucose requires four NAD + to create Acetyl-CoA, β-hydroxybutyrate only requires one (Elamin et al., 2017; Newman &

Verdin, 2017). Increased NAD+ has also been a desired outcome as it is associated with signaling pathways that improved inflammation and promote longevity (Elamin et al., 2017) In addition, a decline in NAD+ and NAD+/NADH ratio has been linked to neurodegenerative disorders (Newman & Verdin, 2017). One study of healthy young adults (mean age 34.7 ± 9.4 years old) found that after inducing an increase of ketone bodies nutritionally, NAD+ increased as well as the NAD+/NADH ratio, which has the potential to provided added benefits to brain health other than providing energy (Newman & Verdin, 2017; Xin et al., 2018).

Another biomarker commonly evaluated in researching cognition among humans is cortisol. Cortisol is a stress hormone that is produced by the adrenal glands when provoked by stressful situations (Ebner et al., 2015). Internal and external stress provokes the release of cortisol through the hypothalamic-pituitary-adrenal (HPA) axis by the means of a "coordinated hormonal cascade" (Ebner et al., 2015). Stress in this case can be defined as "repeated exposure to psychosocial hazards giving rise to a bodily state that can be deleterious to multiple physiological systems" (B. K. Lee et al., 2007). An increase in cortisol due to a stressor is an indication of a properly functioning HPA-axis, therefore, increases in cortisol may be an important contributor to healthy cognitive function (Ebner et al., 2015). However, an over-activated or under-activated HPA-axis can lead to an allosteric load on the brain, which can produce detrimental effects (B. K. Lee et al., 2007). Additionally, contributing to the negative effects associated with a dysregulated HPA-axis, several studies have demonstrated that chronically elevated cortisol levels are associated with impaired cognition in the hippocampus and neocortex

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(B. K. Lee et al., 2007; Ouanes et al., 2017). One study found that high levels of cortisol were associated with lower cognitive test scores assessing language, processing speed, eye-hand coordination, executive functional, verbal learning, and verbal and visual memory in older aged adults (B. K. Lee et al., 2007). In another study with older participants, it was found that those with higher levels of cortisol in the evening time had worse scores on verbal memory, processing speed, and reaction time tests (Gaysina et al., 2014). In middle-aged adults, higher levels of cortisol resulted in worse memory and verbal fluency (Geoffroy et al., 2012). Since high levels of cortisol are commonly linked to a declining or weakening cognitive function, it is an important biomarker to assess.

Other stress biomarkers that are used to measure cognitive function are C-reactive protein (CRP) and Interleukin-6 (IL-6). IL-6 is classified as a cytokine, which is an inflammatory agent. There are anti-inflammatory and proinflammatory cytokines, and the generation of their release is similar to that of cortisol in that their release is induced by stress in order to maintain homeostasis of inflammatory cytokines that protects the function of the immune system (Sartori et al., 2012). Similarly, CRP is a protein that measures inflammation (Sartori et al., 2012). Generally, the release of IL-6 and CRP contribute to important inflammatory responses because they aid the healing process, but if the body has extended production and exposure to these proinflammatory markers, the brain tissue is susceptible to inflammation, particularly in specific cognitive domains such as memory, emotion, and attention (Sartori et al., 2012). One study found that increased levels of IL-6 and CRP were associated with poor cognitive performance with raised CRP negatively correlated with inductive reasoning and raised IL-6 negatively

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correlated with verbal fluency (Gimeno et al., 2008). Thus, brief exposure to these proinflammatory markers suggests proper function, but a chronic exposure to these markers, including IL-6 and CRP, can lead to poor cognitive health (Sartori et al., 2012).

Homocysteine is another marker that measures cognitive function and performance. Homocysteine is an amino acid that is not found naturally in dietary sources, and thus it must be derived from the essential amino acid, methionine, in all mammalian cells (FINKELSTEIN, 2000). Homocysteine levels are often measured when evaluating human vitamin B12 or folic acid status because a decrease in vitamin B levels are commonly associated with an increase in homocysteine (Teunissen et al., 2003). It can be hypothesized that increased homocysteine results in poor cognition due to the common negative relationship between homocysteine and B vitamins, however, in a study of normal aging individuals it was found that raised homocysteine levels were linked to lower cognitive scores while there was no connection between vitamin B12 and folic acid (Teunissen et al., 2003). Another study showed that homocysteine levels were inversely related to tests that challenged verbal learning and memory (Teunissen et al., 2003). In addition, the association between increased homocysteine levels and poorer cognitive performance, particularly memory and verbal abilities, has been linked to increased inflammation (Teunissen et al., 2003).

Another blood biomarker that is used to assess cognition is phosphatidylcholine (PC). PC is a phospholipid that can be acquired internally or through nutritional sources (Zamroziewicz et al., 2016). PC has been analyzed among older individuals in assessing various measures of cognitive function. For example, one study that evaluated older

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individuals found a positive relationship between PC and cognitive flexibility, which was

linked to the thickness of the left prefrontal cortex (Zamroziewicz et al., 2016). Another

study showed that lower PC was related to poorer performances on verbal learning and

memory tests and decreased neuronal activity in the frontal, occipital, and temporal lobes

of non-demented older individuals (Simpson et al., 2016). The hypothesized mechanism

behind this result was that PC may directly affect the function of docohexaenoic acid

(DHA) (Simpson et al., 2016). DHA is known to be a crucial component of the neuronal

membrane that impacts synaptic plasticity and neuroprotection, and thus, possesses

effects that lead to improved cognition (Luchtman & Song, 2013).

Table 1. Blood Biomarkers and Cognition

Marker	Relation to Cognition
Brain-Derived Neurotrophic Factor (BDNF)	BDNF is known to develop and maintain neurogenesis and neuroplasticity. Increased levels of BDNF are positively related to memory, cognitive flexibility, concentration, learning, and language. (Koven & Collins, 2014; Navarro-Martínez et al., 2015; Raefsky & Mattson, 2017)
Ketones	Ketones are known to provide energy to the brain during energy depletion, and the associated increase in ketones levels has been shown to increase memory performance (Cavaleri & Bashar, 2018; Cunnane et al., 2016; Evans & Egan, 2018; Jensen et al., 2020; Krikorian et al., 2012)
Cortisol	Elevated cortisol levels are associated with poor cognitive functions related to a variety of cognitive domains, including processing speed, verbal learning, verbal

	memory, executive function, and reaction time. (Gimeno et al., 2008)
C-Reactive Protein Interleukin-6	Prolonged elevated levels of C-Reactive protein and Interleukin-6 are associated with poor cognitive performance in domains including memory, emotion, attention, inductive reasoning, and verbal fluency (Gimeno et al., 2008; Sartori et al., 2012)
Homocysteine	Elevated homocysteine levels have shown to result in poor cognitive performance, including low scores on verbal learning and memory tests. Increased homocysteine levels and low cognition scores could also be related to a concurrent decrease in vitamin B levels (FINKELSTEIN, 2000; Teunissen et al., 2003)
Phosphatidylcholine	Phosphatidylcholine has been shown to have a positive relationship with verbal memory, verbal learning, and executive function scores (Simpson et al., 2016; Zamroziewicz et al., 2016)

Diet and Cognition

Popular Diets

Although intermittent fasting has grown in popularity, there are many other

popular diets that are used in order to improve specific health outcomes, including

cognitive function Other popular diets that will be discussed in this review include: the

Mediterranean diet (MD), the Paleolithic diet (Paleo diet), and the Ketogenic diet,

The Mediterranean diet (MD) is a common diet that incorporates a variety of beneficial eating patterns and has a relatively high adherence rate (Wade et al., 2019). The MD is characterized by lower saturated fat intake, a low glycemic index, and high antioxidant consumption (Castro-Quezada et al., 2014). The MD focuses on the consumption of plant- based foods, such as fruits, vegetables, whole grains, beans and legumes, olive oil, and nuts with a conservative amount of fatty fish, white meat, eggs, low fat dairy products, and wine (Castro-Quezada et al., 2014; McMillan et al., 2011). Additionally, compared to a traditional American diet that is high in red meat, saturated fat, and sugar consumption, the MD is high fiber and incorporates primarily monounsaturated fats (Castro-Quezada et al., 2014). Those who adhere to the MD more consistently have adequate or improved consumption of vitamins and micronutrients compared to a Western diet and are at a low risk for nutritional deficiencies (Castro-Quezada et al., 2014). Various studies have concluded diverse health effects related to the adherence to MD, including lower BMI, lower waist circumferences, reduced risk of stroke, and cardioprotective effects related to cardiovascular disease (Estruch et al., 2013; FÚart et al., 2009; McMillan et al., 2011). However, the literature is somewhat scattered when evaluating cognitive effects of the MD. One study that evaluated the MD with moderate consumption of lean pork found improvements in processing speed, but not memory, attention, or planning (Wade et al., 2019). Another study found significant improvements in reaction time, but not working memory or word recognition (McMillan et al., 2011). In addition, BDNF levels were shown to increase among those who adhered to MD with an extra consumption of nuts, although this increase only occurred in

individuals who were previously diagnosed with depression (Sánchez-Villegas et al., 2011). Thus, the researchers in this study concluded that MD plus consumption of nuts improved the impaired function of the hippocampus since those who had experienced improvements were depressed (Sánchez-Villegas et al., 2011). Additionally, when looking at the use of the MD in older adults and reducing the risk of dementia, one study concluded that the MD may have a beneficial effect in the "prodromal phase of dementia", or predementia stage, but is not effective when the disease has significantly progressed (FÚart et al., 2009). However, a supplementary intake of extra virgin olive oil while following MD also showed to have greater protective effects against cognitive decline when comparing older adults who are following the standard MD (Mazza et al., 2018). Another study among older individuals, found that the long-term implementation of the MD supplemented with extra virgin olive oil and nuts produced improved cognitive function across various domains (Martinez-Lapiscina et al., 2013). Because macronutrient or micronutrient concentrations may be somewhat variable among participants, it may be difficult to evaluate whether the diet itself or consumption of specific nutrients, such as omega-3 polyunsaturated fatty acids, which are known to maintain neuronal health and have been shown to improve cognitive performance among both young, healthy adults (Dyall, 2015).

In 2015, Morris et. al combining elements of the MD diet and the Dietary Approach to Systolic Hypertension (DASH) diet in order to develop a diet that maximizes brain health in order to prevent or delay dementia (Martha Clare Morris et al., 2015). This diet is called the Mediterranean-DASH diet intervention for

neurodegenerative delay (MIND) and emphasizes the consumption of vegetables, fruit

(nuts, olive oil, whole grains, seafood, beans, poultry, and wine while limiting animal

products and saturated fat. Unique to the MIND diet, there is a focus on the consumption

of green leafy greens and berries for brain heath (Martha Clare Morris et al., 2015). This

diet found that higher compliance to the MIND diet was associated with slower decline in

cognitive abilities among older adults. Even among young, healthy, obese women,

adherence to the MIND diet led to improvements in verbal short term memory, attention

and visual scanning, working memory, and verbal recognition (Arjmand et al., 2020).

Compounding off of the previous paragraph of the Mediterranean diet, these diets stress

high consumption of polyphenols and antioxidants, which can improve cognitive function

by protecting the brain against oxidative stress and inflammation (Arjmand et al., 2020).

High consumption of vegetables has been associated with delayed cognitive decline (M.

C. Morris et al., 2006) with more significant declines seen with greater consumption of

green leafy vegetables and cruciferous vegetables (Kang et al., 2005). Researchers

suggest that the high levels of lutein and folate found in green leafy vegetables are likely

important contributors to observed cognitive improvements (Kang et al., 2005). In

addition, the consumption of berries is associated with slower rates of cognitive decline

(Devore et al., 2012). Researchers suggest that berry consumption leads to greater

cognitive health due to the role of anthocyanidins on learning and memory. Once

anthocyanidins cross the blood-brain barrier, they are able place themselves in critical

brain regions, such as the hippocampus (Andres-Lacueva et al., 2005). Thus, anti-

inflammatory and antioxidant effects will manifest themselves in these areas, creating

improved cognitive health. In addition to these compounds, the consumption of other important compounds found richly in the MIND diet and MD, such as vitamin E and carotenoids, may protect the brain from decline and improve cognitive function (Martha Clare Morris et al., 2015).

The Paleo diet is another common diet that is based on the premise of consuming exclusively unprocessed foods that were consumed during "the Paleolithic era" by hunter-gatherers (Manheimer et al., 2015). The basis for this diet is that the increased consumption of processed foods, which are commonly high in glycemic index, salt, and omega-6 fatty acids, in the current era have contributed to metabolic abnormalities and inflammation (Manheimer et al., 2015). Thus, the removal of such foods and the inclusion of whole, unprocessed foods drives the hypothesis for improved health. While adhering to the Paleo diet, one is permitted to consume "meat, fish, poultry, eggs, fruit, vegetables, tree nuts, canola oil, mayonnaise, and honey" whereas beans and legumes, grains, cereals, dairy products, added sugars, and food industry products are prohibited (Manheimer et al., 2015; Masharani et al., 2015). In some studies, participants were allowed to consume root vegetables, such as potatoes, while others excluded potatoes. The purpose behind the exclusion of potatoes might have been the complete removal of high-glycemic carbohydrates, but overall this diet excluded most foods with a high-glycemic index. The majority of randomized, controlled trials that analyzed the effects of the Paleo diet researched adults with Type 2 diabetes. Among these experiments, positive results were associated with the adherence to a Paleo diet. Results found that those following the Paleo diet had reductions in cholesterol, HbA1c, and hepatic lipids, and

improved fat mass, insulin sensitivity, glycemic control, and leptin levels (Masharani et al., 2015; Otten et al., 2018, 2016). Interestingly, one of the studies among Type 2 diabetics compared the effects of the Paleo diet to the American Diabetes Association Diet and found that the Paleo diet was more effective in decreasing cholesterol and HbA1c, and improving weight status (Masharani et al., 2015). Among healthy individuals, the paleo diet produced improvements in glucose levels, reductions in energy and carbohydrate consumption, and decreases in fat mass (Bligh et al., 2015; Otten et al., 2019). Although studies seem to evidence the positive effect of the Paleo diet among diabetics, one recent review found no differences in effect in glucose and insulin maintenance among those with glucose metabolism disorders in comparison to other diets such as the MD or the diabetes diet (Jamka et al., 2020). Thus, Paleo diet is effective but may not be more effective in managing glucose and insulin compared to other diets. Limited studies have been published evaluating the effect of the Paleo diet on cognition. However, one study among individuals with Type 2 diabetes found that those adhering to the Paleo diet with and without the addition of aerobic exercise had increased levels of BDNF and improvements in the structure and activity of the hippocampus (Stomby et al., 2017). Another study among middle aged adults with metabolic syndrome found significant improvements in serum BDNF, Stroop Test performance time, verbal test scores, and perceived cognitive function ratings among those adhering to a carbohydrate restricted Paleo diet, with and without the implementation of exercise (Gyorkos et al., n.d.). Thus, further study will need to be done in order to determine if the Paleo diet consistently has profound effects on the brain and cognitive performance.

Another popular diet is the ketogenic diet. Unlike the other diets mentioned, which focus on the types of foods one eats, the ketogenic diet focuses on macronutrient distribution by concentrating on fat and severely restricting carbohydrates. The total percentage of calories from fat on the ketogenic diet can vary, but is typically seen between 70-90% (Cohen et al., 2019; Gasior et al., 2006; Phillips et al., 2018). Additionally, this diet practice maintains a ratio of 4:1 or 3:1 fats to carbohydrates and proteins (Lambrechts et al., 2017). Furthermore, the ketogenic diet allows for a variety of fat sources and the consumption of carbohydrates is restricted to non-starchy vegetables, such as green vegetables (Alessandro et al., 2015; Cohen et al., 2019). The premise behind the consumption of fats and under-consumption of carbohydrates is to induce a state of ketosis. When carbohydrates are constricted, the state of ketosis is activated by the increased production of blood ketones (β-hydroxybutyrate and acetoacetate) by the heightening of the breakdown of fatty acids (Colica et al., 2017). With the high consumption of fat, one would expect one's serum cholesterol to rise, but results are somewhat inconsistent. While some studies showed a corresponding increase in serum HDL, LDL, total cholesterol; others surprisingly found reduced levels of triglycerides and a lack of difference in the same blood markers in comparison to low-fat diets (Cohen et al., 2019; Phillips et al., 2018; Saslow et al., 2017; Zajac et al., 2014). Additionally, some studies have shown reductions in BMI, body weight, and fat mass resulting from adherence to the ketogenic diet (Alessandro et al., 2015; Colica et al., 2017; Saslow et al., 2017).

In addition to health outcomes related to weight and blood lipids, it is well established in the literature that the ketogenic diet has profound effects on improving seizure severity and frequency among children (Gasior et al., 2006; Lambrechts et al., 2017). More research is emerging on the effects of the ketogenic diet on cognition and other neurological disorders, but the literature is not yet as well established. However, one particular study among Parkinson's disease patients found that those who adhered to a ketogenic diet for 8 weeks experienced improved motor and nonmotor symptoms related to the disease when compared to those adhering to a low-fat diet (Phillips et al., 2018). With respect to Alzheimer's disease and memory performance, the ketogenic diet may induce promising effects as well. One study found that medium-chain triglycerides, which are often highly consumed in a ketogenic diet, enhanced memory of patients with Alzheimer's disease, leading to the conclusion that the oxidation of medium-chain triglycerides by ketones is correlated to improved memory function (Reger et al., 2004). In addition, some researchers speculate that ketosis induced by this diet is responsible for neuronal protection through antioxidant and anti-inflammatory effects (Gasior et al., 2006). Thus, the ketogenic diet may prove to be effective when analyzing cognitive outcomes and improvements, but more research needs to be done to conclude effects related on specifics neurological disease and cognitive outcomes, particularly in healthy people.

Intermittent Fasting

Mechanisms Associated with Intermittent Fasting

Various mechanisms are proposed in the literature as to how and why intermittent fasting is beneficial to human health. Mark Mattson, an expert in intermittent fasting research, frequently writes of the evolutionary adaptations that resulted from fasting, which could explain its benefits. Ancestral humans had to search and hunt for their food, which was connected with periods of food scarcity and hunger. Mattson discusses how during these energy restricted periods, human bodies adapted to specific metabolic pathways and molecular signaling that led to the improvement of the strength and usefulness of cells and organ systems (Mattson, 2014). The brain likewise experienced these metabolic adaptations while fasting in order to obtain optimal sharpness and acuity during times of searching for and obtaining food (Mattson, 2015). Interestingly, the ability to preserve enhanced cognitive function during deprivation has been connected to the fact that the brain is the one of the few organs that does not change in size, specifically in shrinkage, after deprivation (Longo & Mattson, 2014). Mattson and Longo connect this fact to observable mammal behaviors related to eating. For example, mammals tend to be idle and inactive after caloric consumption and more active and engaged mentally while experiencing hunger (Longo & Mattson, 2014).

When fasting occurs, a period of ketosis is induced. Fatty acids and glycerol are produced from triglycerides; ketones, specifically β-hydroxybutyrate and acetoacetate, are produced from fatty acids, which contribute to the brain's main source of energy during ketosis (de Cabo & Mattson, 2019). Prior to ketosis, the brain relied primarily on carbohydrates for its source of energy, so fasting periods provoke a "metabolic switch" in energy used (Mattson et al., 2018). Experiencing a periodic metabolic switch from

carbohydrates burned to ketones burned through the practice of intermittent fasting and/or exercise enhances cognitive function through improved neuroplasticity, cellular resistance, and neuronal adaptations to stress (Mattson, 2015; Mattson et al., 2018). Additionally, neurotrophic factors, including BDNF, are generated when this switch occurs or when acquired signaling pathways are activated, which enhances the production of neurogenesis, provides protection against neuronal stress periods, and stimulates the growth and plasticity of cells (Mattson, 2015; Mattson et al., 2018). Consequently, it is debated that because individuals of the present time have an abundance of food and can access desired food ad libitum, intermittent metabolic switches and adapted signaling pathways are rarely activated, putting themselves at risk for lessened cognitive ability and various chronic diseases, such as obesity, cancer, or neurodegenerative disease (Mattson, 2014).

Another mechanism that has been well discussed in the literature specific to time-restricted feeding is based on circadian biology. Modern life permits access to food and light at all hours of the day, which can be detrimental to circadian rhythms. Internal circadian oscillators located inside of the suprachiasmatic nuclei (SCN) of the anterior hypothalamus control circadian rhythms and impact daily sleep, activity, feeding, and fasting (Manoogian & Panda, 2017a; Sherman et al., 2011). These circadian oscillators in turn impact gene expression and metabolic function, leading to overall effects on the body's organ systems, including the brain (Manoogian & Panda, 2017a). With time-restricted feeding diets, circadian rhythms can be "reset" by maintaining cycles

consumption and restriction that are in alignment with the SCN, which may in turn prevent chronic disease and metabolic abnormalities (Sherman et al., 2012).

Types of Intermittent Fasting

Based on the theorized mechanisms previously discussed involving intermittent fasting, the regime appears to be promising. Three main fasting regimens are types of "intermittent" fasting, which include alternate day fasting, modified fasting, and time-restricted fasting. Religious fasting, such as the Islamic fasting practice during Ramadan, could also be considered a form of intermittent fasting. However, the main focus of this review will be the main intermittent fasting practices, as their purposes are solely health related rather than religious based. Additionally, Ramadan fasting is not meant to be a daily practice lasting longer than one month.

Alternate day fasting is the fasting regime that practices rotating days of complete fasting and ad libitum feeding. On the fasting day, there is an all-inclusive restriction of calories from food and drink. The modified fasting practice involves a significant restriction of calories on particular days of the week and free consumption of calories on the remaining days. For example, a popular modified fasting practice is the 5:2 diet. The 5:2 diet restricts daily caloric requirements by 75-80% on two nonconsecutive days of the week and allows ad libitum eating on the remaining five days (Alessandro et al., 2015; Colica et al., 2017; Saslow et al., 2017). In time-restricted fasting, individuals develop a consistent schedule of eating freely during designated time periods throughout the day and fasting outside that specific time window. Common models of this practice include the 16:8 diet and the 18:6 diet, which is the observed protocol of this particular thesis. On

the 18:6 diet, individuals fast for 18 hours of a 24-hour period and eat during a consistent 6-hour time period. For example, adherers of this particular diet may designate 8:00 am – 2:00 pm as their ad libitum feeding period and outside of this time period participants will fast.

Animals Studies of Intermittent Fasting

A number of intermittent fasting studies have been conducted on animals and provide indication of the effectiveness of its practice. Among the different types of intermittent fasting, a 2014 review article concluded that alternate-day fasting was the most widely used intermittent fasting protocol in animal studies (Longo & Mattson, 2014). More recently, Mattson wrote that the majority of animal studies which analyzed metabolic intermittent switching utilized alternate-day fasting and time-restricted feeding methods (Mattson et al., 2018). For the purposes of this study, a comprehensive review was conducted to assess experiments using commonly used intermittent fasting regimens among animals, specifically looking at cognitive effects as an outcome. A review of the literature confirmed that alternate day fasting was the most common protocol used in evaluating cognition among animals. Time-restricted feeding protocols have emerged in animal studies, with the majority of them published after 2015. Summaries of these two intermittent fasting methods and their impacts on cognition among animals can be found in Table 2 and Table 3. The majority of studies have indicated cognitive benefits associated with alternate-day fasting, with one study indicating a worsening of neuroinflammation and deficits when looking specifically at animals with Alzheimer's disease (Lazic et al., 2020). One study compared alternate day fasting with two time-

restricted feeding methods (12 hour fasting versus 16 hour fasting) and found both

alternate day fasting and the 16 hour time-restricted feeding to be most effective in

improving neuronal plasticity (Dasgupta et al., 2018). Another study comparing alternate

day fasting with 12-hour and 16-hour time-restricted feeding protocols found all fasting

regimens to be effective in promoting hippocampal neurogenesis with the 16-hour time-

restricted feeding protocol more consistently resulting in significant positive results (Baik

et al., 2020).

Table 2. Alternate Day Fasting on Cognition in Animal Studies

Alternate Day Fasting					
Reference	**Study Design/ Protocol**	**Study Objective**	**Subjects**	**Length of Study**	**Results**
(J. Lee et al., 2002)	Mice were separated into an ad libitum (AL) control group or an alternate day fasting (ADF) regimen. Mice were injected with bromodeoxyuridine (BrdU) in order to assess neurogenesis.	Assessed whether during alternate day fasting BNDF is critical in improving neurogenesis	BDNF +/- mice and wild-type control littermates	3 months	Levels of BDNF in hippocampal regions increased by ADF in wild-type and BDNF +/- mice, but AL BDNF +/- mice had reductions in BDNF The number of BrdU-labeled cells after 1 day in BDNF +/- ADF mice was larger than the amount of BDNF +/- AL mice, showing that ADF reverses the

					reduction of BDNF
					BDNF immunoreactivity was enhanced among ADF wild-type and BDNF +/- mice, while BDNF immunoreactivity decreased in AL BDNF +/- mice
					Wild-type and BDNF +/- mice on ADF diet had increased levels of BDNF
(Fontán-Lozano et al., 2007)	Mice were separated into either an ad libitum control group or ADF group	Assessed whether long-term ADF is effective in enhancing age-related learning and memory capabilities	8-week old male Swiss mice	6 – 8 months	The majority (93.3%) of ADF mice were able to complete the motor learning rotarod test during their fourth session while the majority of AL mice were not able to complete the task
					ADF mice were able to

					learn associative learning tasks faster than AL mice
					During object recognition memory tests ADF mice performed better in short term and long-term memory tests
(Arumugam et al., 2010)	Mice were randomly assigned to ad libitum (AL) or alternate day fasting (ADF) regimens. After maintaining diets for 4-5 weeks, middle cerebral artery occlusion/reperfusions (experimental strokes) were performed on the mice	Assessed the effects of ADF on mortality rate, neurotrophic factors, cytokines, and cellular stress resistance proteins in brain tissues after stroke	3, 9, and 16-month male C57BL6 mice	4-5 months	Mortality rate after stroke was higher in AL mice in all age groups compared to ADF mice Neurological damage was less among ADF young and middle-aged mice compared to young and middle-aged AL mice

BDNF levels increased in ADF mice with the greatest |

					increases in ADF mice
					ADF mice had reductions in TNF-alpha and IL-6 with the greatest reductions in young mice
(Lu et al., 2011)	Mice were divided into three groups upon being acclimated to their environment for one week: ad libitum (AL) group, alternate day fasting (ADF) group, alternate day fasting plus antioxidants (ADF +AO)	Assessed whether ADF affects bioenergetic enzymes and brain signaling pathways	Young, adult, male C57BL6 mice	6 weeks	ADF and ADF+AO groups had consistently lower blood glucose levels and enhanced brain insulin sensitivity levels
					ADF and ADF+AO groups had lowered AKT and GSK3B phosphorylation
					AMPK phosphorylation and total levels of SIRT1, PGC1-alpha, and COX4, were the same between groups
(Li et al., 2013)	Mice were assigned to one of three groups:	Assessed how early and long-term obesity	7-week-old male CD-1	11 months	ADF and HFD regimens

| | control group with ad libitum (AL) access to regular chow, alternate day fasting (ADF) with ad libitum access to regular chow every other day, or high fat diet (HFD) with ad libitum access to high fat foods | impacts learning, memory, and brain structures | wild type mice | | did not pose significant effects on the Barnes maze test times (testing spatial learning and memory), but times did improve over the sessions between all groups

ADF mice improved their context-related freezing behavior, indicating improved learning and memory

ADF mice had thicker CA1 pyramidal cell layers and increased debrin in the cerebral cortex and hippocampus

BDNF in the cerebral cortex and hippocampus did not change |

					significantly in the ADF of HFD groups ADF decreased oxidative stress markers: 4-hydroxy-2-nonenal and nitrotyrosine containing proteins
(Vasconcelos et al., 2014)	Rats were assigned to either ad libitum (AL) feeding group or alternate day fasting (ADF) for 30 days. After the 30 days, rats received the administration of 1 mg/kg of lipopolysacchari de (LPS). Thus, four groups remained: saline (control), LPS treatment, ADF + saline, ADF + LPS	Assessed whether ADF can affect neuronal ability to resist disease, injury, and inflammation, specifically in the brain	Adult 12-week old male Wistar rats	30 days	ADF and ADF+ LPS mice has significantly better performance s on the Barnes Maze test, indicating better spatial learning abilities despite inflammatio n by LPS In the inhibitory avoidance test, the control, ADF, and ADF+LPS groups had significantly greater scores showing that ADF improves long-term memory

					despite inflammation by LPS
					LPS significantly increased IL-1alpha, IL-1B, and TNF-alpha in the hippocampus and IL-1B, TNF-alpha, IL-6, RANTES, and IFN-y in the blood. However, in the ADF+LPS group these markers were significantly reduced
					LPS reduced hippocampal BDNF, but BDNF remained the same in control, ADF, and ADF+LPS groups
(Singh et al., 2015)	Middle aged rats were divided into two groups: alternate day fasting (ADF) group or ad libitum (AL) group. Young rats were fed ad libitum and used	Assessed whether alternate day fasting over a short-term period creates an impact on the motor coordination skills (Rotarod test), protein and DNA damage in peripheral organs	Wistar strain male albino rats – 15 months old and 3 months old (positive	3 months	ADF rats improved their performances on the Rotarod test in improving both their time spent on the rod

	as a positive control	and brain regions (piriform cortex, hippocampus, and hypothalamus), body weight, blood glucose, and the expression of energy regulators (NPY and Kiss), cell survival pathways (expression of pAKT, NF-kB, AP-1, and Cytc c) and markers of synaptic plasticity	control group)		and decreasing their fall rate. AL rats had worse performances and had increased fall rates Protein carbonyl decreased in brain regions among ADF rats, indicating decreased inflammation in the brain. Protein carbonyl increased in AL rats with a significant difference between the ADF and AL groups. The expression of synaptic plasticity markers, Syn, CaN, GAP43, and PSA-NCAM increased among ADF rats.
(Hu et al., 2017)	Rats were divided into three groups: ad libitum with	Assessed whether prior alternate day fasting poses an effect on cognitive	Adult male Wistar rats	12 weeks	After the 2VO procedure, recognition

| | sham operation, ad libitum with 2-vessel occlusion procedure (2VO-AL), or alternate day fasting with 2-vessel occlusion procedure (2VO-ADF) | dysfunction (tested by the Maze water maze and the novel recognition tests) after a 2-vessel occlusion (2VO) procedure, which serves as an experimental model for vascular dementia | | | memory was significantly impaired in 2VO-AL rats while 2VO-ADF rats had no recognition memory impairments In the Maze water test, 2VO-ADF rats performed significantly better than 2VO-AL rats, indicating memory was preserved with prior AD treatment

Reduced neuronal and synaptic density and BDNF expression resulted following the 2VO procedure compared to the sham operation rats, but ADF resulted in greater BDNF levels, synaptic density, and prevented |

					neuronal death.
					Oxidative stress was significantly greater in 2VO-AL rats compared to sham operation rats, but ADF prevented oxidative stress in 2VO-ADF rats
					Compared to the sham operation rats, 2VO-AL rats had significantly greater inflammatio n markers (TLR4, TNF-alpha, IL-1B, IL-6). However, 2VO-ADF rats had significantly lower inflammatio n markers than 2VO-AL rats, preventing brain inflammatio n.
(Hu et al., 2019)	Rats all underwent two-	Assessed whether alternate day	Adult male	Rats maintain	ADF decreased

vessel occlusion (2VO) surgeries, modeling vascular dementia. One week after the surgery, rats were divided into either an ad libitum (AL) control diet or alternate day fasting (ADF) diet. 2VO rats were also compared to rats who underwent a sham operation and followed an AL diet	fasting (ADF) poses postoperative effects on cognition after a two-vessel occlusion (2VO) procedure	Sprague Dawley rats	ed diet protocol 7 weeks after recovering from surgeries	negative memory effects via the Morris Water Maze and the Novel Object Recognition Test ADF maintained synaptic density and the expression of BDNF while 2VO-AL rats had significantly reduced expression The 2VO procedure significantly increased oxidative stress However, ADF significantly reduced oxidative stress across all markers. 2VO induced inflammatory markers compared to the Sham operation while ADF delayed further

					increase in inflammatory markers in the brain.
(Lazic et al., 2020)	Mice were assigned to either an ad libitum (AL) group or alternate day fasting (ADF) group	To determine the effect of alternate day fasting on cognitive decline in Alzheimer's disease mice models	Female transgenic 5XFAD mice (mouse model for Alzheimer's disease) and non-transgenic littermates	4 months	Inflammatory markers in the cortex increased to a greater degree in the ADF mice compared to the AL mice

A small, nonsignificant trend showed reduced short-term memory and increased anxiety among ADF mice compared to AL mice. |
| (Liu et al., 2020) | Four groups of animals were experimented during the study. For the first group of mice, intervention and control mice were split into either an ad libitum (AL) group or an alternate day fasting (ADF) group and were given behavioral tests and sacrificed in order to collect serum and tissue | Assessed through gut microbiota and the "microbiota-metabolites-brain axis" whether alternate day fasting impacts cognitive functional and behavior in diabetic mice | Male, diabetic BKS.Cg-Dock7^m + / + Lepr^{db}/J Homozygous Lepr$^{db/db}$ mice (heterzygous Lepr$^{db/m}$ mice used for the control) | 28 days | ADF enhanced anxiety levels and locomotor activity in intervention mice.

ADF enhanced the production of BDNF and improved postsynaptic density, as shown by |

	samples. The second group underwent the ADF or AL protocols and were sacrificed in order to collect samples to evaluate gut microbiome, metabolites, and RNA sequencing in the hippocampus, blood, and fecal matter. The third set of mice analyzed the use of antibiotics among control and diabetic mice with and without ADF. The fourth group analyzed the effects of ADF and antibiotic use.				PSD-95 levels ADF benefitted the gut microbiota by increasing the villi length, muscularis thickness, and claudin-1, and microbiome alpha diversity in the gut With the administration of gut-microbiota, cognitive scores on the Morris water maze test were slightly worse, showing the important role of microbiota on ADF pertaining to cognition. 3-Indolepropionic acid and short chain fatty acids were reduced when antioxidants

					were administered

Table 3. Time-Restricted Feeding on Cognition in Animal Studies

Time-Restricted Feeding					
Reference	Study Design	Study Objective	Subjects	Length of Study	Results
(Dasgupta et al., 2018)	After rats were acclimatized for 1 month, they were divided into four groups: Ad libitum (AL), group, alternate day fasting (ADF) group, 16-hour daily feeding (TRF16) group, or 12-hour time restricted feeding (TRF12) group. The TRF groups fasted during the dark hour cycle and all mice were fed a conventional diet. For all groups, associative interactions of synaptic inputs (weak and strong) were assessed at 120 minutes	Assessed how intermittent fasting in rats impacts associative mechanisms among pyramidal neurons that reside in the CA1 region of the hippocampus	Male C57BL6/Tac mice	6 months	Both ADF mice and TRF16 mice were found to have early long-term potentiation in the synaptic input S1 which lead to late long-term potentiation, thus signifying synaptic tagging/capture at 120 minutes. Synaptic tagging/capture was not expressed among AL and TRF12 mice. In analyzing TrkB/Fc, it was found that in ADF mice BDNF had the ability to prolong associative interaction Increases in BDNF gene expression was found among

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					ADF, IF16, and IF12 mice (in descending order of greatest increases)
(Shojaie et al., 2017)	Mice were divided into four groups: a control group, a distress group, an intermittent fasting (IF) group, and an IF plus distress group. The IF groups underwent TRF during their second week of the study as researchers restricted their access to food for two hours daily from 12:00 pm – 2:00 pm. During the second week, stress was induced in the distress and IF plus distress groups by generating emotional stress and providing them an electrical foot shock.	Assessed whether intermittent fasting poses beneficial stress effects related to cognition and cognitive performance. Inflammatory markers (corticosterone (CORT), IL-6, and TNF-alpha) and the Barnes Maze test was used to quantify this.	Male BALB/c mice	18 days	Brain hypotrophy resulted in the distress group, creating a significant difference in brain weight compared to control, IF, and IF + distress groups signifying the ability of IF to prevent brain hypotrophy with stress. CORT significantly increased in the distress group mice compared to control and IF mice groups, while IF prevented 50% of the CORT increase in IF + distress mice IL-6 and TNF-alpha significantly increased in distress mice but was not elevated in the IF + distress group, creating significant differences in levels between

					groups and signifying the ability of IF to suppress inflammation. IF and IF + distress mice performed better on the Barnes Maze test signifying enhanced cognitive ability produced by IF even with distress.
(J. Zhang et al., 2017)	At 11 weeks of age, rats were ovariectomized and assigned to either an AL feeding group or TRF group (restricted food for 3 hours per day) while fed on a high-fat diet. After two weeks on the diet protocols, rats in both groups were either infused with beta-amyloid (25-35 Alzheimer's disease) or beta- amyloid (35-25 Non-Alzheimer's disease). Thus, four groups were created: Alzheimer's AL (AD-AL), Alzheimer's	Assessed the impact of intermittent fasting on cognitive ability and metabolic effects on menopausal and Alzheimer's disease rat models	Sprague-Dawley female rats	4 weeks	Beta-amyloid deposition was significantly greater in AD-AL mice compared to AD-TRF mice. CREB phosphorylation was reduced by the increase in B-amyloid in the AD-AL mice as well, which prevented in the AD-TRF mice. Phosphorylation of hippocampal Akt and GSK-3B decreased by B-amyloid in AD-AL mice, but the same result was not seen in AD-TRF mice Tau phosphorylation increase in B-

	TRF (AD-TRF), Non-Alzheimer's AL (Non-AD-AL), Non-Alzheimer's TRF (Non-AD-TRF)				amyloid infused AD-AL mice, but this increase was diminished among AD-TRF mice AD-AL mice had worsened short-term memory and spatial memory performances on the Morris Water Maze test, while AD-TRF mice had slightly improved scores TNF-alpha increased non-significantly in AD mice while AD-TRF mice still reduced TNF-alpha levels
(Wang et al., 2018)	After acclimatization to their environment, mice were randomized to either an ad libitum group or a time-restricted feeding (TRF) group. The TRF protocol involved mice receiving food for only 6 hours per day in the middle of the active phase	Assessed whether time-restricted feeding (TRF) prevents circadian dysfunction leading to improved disease advancement in Huntington Disease mouse models.	Q175 C57BL6/J mice (heterozygous Huntington's Disease mice models)	3 months	Results showed that TRF mice had enhanced rhythms in locomotor activity, greater cage activity, stronger activity levels and bout length during the night (normal times for mice to be active), and a lower number of activity bouts compared to the control mice signifying that activity rhythms

					are enhanced by TRF.
	(according to Zeitgeber time: 15-21) while fasting the remaining 18 hours				Improvements were made to daily circadian regulation of autonomic outputs among TRF mice compared to control mice
					In testing motor performance on the rotarod test, TRF mice performed significantly better compared to age-matched controls
					In testing motor performance on the challenging beam test, decreased errors were associated with enhanced activity rhythms caused by the TRF protocol
					The expression of markers and pathways correlated to Huntington's disease in the striatum were significantly altered
(Serra et al., 2019)	Rats were divided into two groups: ad libitum (AL) group or time-	Assessed whether tissues prone to age-related neoplasm are	Fischer male rats	18 months	Senescent cell biomarkers were significantly reduced among TRF rats

	restricted feeding (TRF) group. The TRF protocol consisted of food consumed during an 8-hour period from 11:00 pm – 7:00 am (dark phase). After maintaining dietary protocols for 18 months, rats were given a pre-neoplastic hepatocyte transplant. Then for the 3 months following the dietary treatment, rats were given the AL diet. After the 3 months, the animals were sacrificed and analyzed.	impacted by long term time-restricted feeding (TRF)			compared AL rats while SIRT1 significantly increased, indicating improved liver aging in TRF rats BDNF in the hippocampus was significantly greater in the TRF rats compared to control
(Baik et al., 2020)	When mice were 3 months old, they were divided the following groups: ad libitum (AD) diet, 12-hour time-restricted feeding (TRF-12), 16-hour time-restricted feeding (TRF-16) or alternate day fasting. The 12-hour fasting group	Assessed the mechanisms associated with hippocampal neurogenesis caused by intermittent fasting	C57BL/6N male mice	3 months	Hippocampal levels of the markers, Nestin and Neu, significantly increased in TRF-16 rats while increasing non-significantly in the other intermittent fasting groups signifying growth of neurogenesis

	fasted from 7:00 pm – 7:00 am and the 16-hour fasting group fasted from 3:00 pm – 7:00 am.				Hippocampal levels of PSD95 increased significantly across all intermittent fasting groups compared to AL mice indicating enhanced strength of hippocampal synaptic connections

Significant increases in Notch 1, NICD1, and HES5 among the TRF-16 group indicates the activation of the Notch signaling pathway, which boosts neurogenesis. Non-significant increases were seen among the other intermittent fasting regimens

BDNF and p-CREB levels in the hippocampus significantly increased among TRF-16 rats and non-significantly among other intermittent fasting groups |

Adherence to and Popularity of Time-Restricted Intermittent Fasting

All three of the intermittent fasting regimens (alternate-day fasting, modified fasting, and time-restricted feeding) have been studied in humans, but some researchers have stated that time-restricted fasting (TRF) to be more appealing in comparison to the other methods as it permits individuals to consume food ad libitum during the feeding periods and does not purposefully restrict overall daily caloric intake or consumption of specific foods (Zhang et al., 2017). A cohort intervention study in which participants reduced their time-restricted eating to 10-11 hours for a 16 weeks reported that the intervention was a successful and feasible strategy to reduce eating duration and excess body weight (Gill & Panda, 2015). Significant improvements were reported in sleep, hunger at night, and energy levels, and all of the participants reported they voluntarily continued to practice after the study. However, one pilot study that asked participants to restrict their consumption in the morning and at night by 1.5 hours each reported that many participants found the protocol to be difficult to adhere to (Antoni et al., 2018). Additionally, participants reported that the regimen was not flexible enough or practical in relation to their family and/or social life and over 50% of participants reported they would not have maintained the protocol after the experiment.

In analyzing the current randomized controlled trials using time-restricted feeding protocols, 42% to 100% of participants completed the entire experiment (I. R. de O. M. Pureza et al., 2020; Ravussin et al., 2019; Stote et al., 2007; Sutton et al., 2018a; Tinsley et al., 2017, 2019). Participants noted their reasons for dropping out, which included: work or schedule changes, personal reasons, and unrelated medical issues. Low

compliance or difficulties in adhering to the fasting protocol were reasons for withdrawal, but not the most common (LeCheminant et al., 2013; Stote et al., 2007; Tinsley et al., 2017). Across all the studies, scheduling conflict was the most reported reason for dropout (Cai et al., 2019; Carlson et al., 2007; Chow et al., 2020; Cienfuegos et al., 2020; Hutchison et al., 2019; I. R. de O. M. Pureza et al., 2020; Ravussin et al., 2019; Stote et al., 2007; Tinsley et al., 2019). Dropout did occur due to difficulty to adhere to the TRF protocol, but the most studies only indicated one participant dropout for this reason (Cai et al., 2019; Carlson et al., 2007; Chow et al., 2020; LeCheminant et al., 2013; Parr et al., 2020). Of course, variance in study design would be a great contributor to perceived difficulty and compliance. Among the studies implementing both exercise and the TRF protocol, it is possible that some may have dropped due to difficulty keeping up with workouts rather than difficulty chiefly related to the diet protocol (Tinsley et al., 2017). One study with 100% compliance among participants had also the most controlled study, with participants receiving all their meals from the researchers in order to maintain an isocaloric diet (Sutton et al., 2018a). Thus, the protocol may have been easier for the participants to adhere to for this reason but may not be representative of compliance in daily living. Overall, more data is necessary in order to determine which time-restricted feeding protocols are most practical and feasible for individuals to adhere to.

Current Randomized Controlled Trials of Time Restricted Intermittent Fasting

Despite popularity of intermittent fasting practices in the public, there is a deficient in reports of controlled trials on intermittent fasting, particularly using the TRF protocol. Further, the vast majority are pilot-sized, statistically under-powered to assess

their outcomes of interest. To the best of our knowledge, 28 randomized controlled trials have been published on the effects of time-restricted feeding. Table 4 summarizes these experiments.

Among these studies, many variations of the TRF protocol were implemented. The most common protocol studied was the 16:8 method in which participants fasted for 16 hours of the day and consumed their food during an 8-hour window (Cai et al., 2019; Chow et al., 2020; Jones et al., 2020; Lundell et al., 2020; McAllister et al., 2020; Moro et al., 2016; Stratton et al., 2020; Tinsley et al., 2019). Other commons TRF protocols in the literature that have been implemented include early time-restricted feeding (eTRF), breakfast omission, and consuming limited meals per day. The eTRF protocol involves consuming calories in the morning rather than the evening, and by implementing this protocol fasting periods lasted from 15 – 18 hours per day (Hutchison et al., 2019; Jamshed et al., 2019; Jones et al., 2020; Ravussin et al., 2019; Sutton et al., 2018a).

A variety of health outcomes were observed in these studies, but the majority of these studies focused particularly on outcomes related to body weight and body composition changes. TRF protocols have shown to be successful at decreasing body weight, fat mass, and body fat percentage (Cai et al., 2019; Cienfuegos et al., 2020; Hutchison et al., 2019; Jones et al., 2020; Kahleova et al., 2014; LeCheminant et al., 2013; Moro et al., 2016; I. Pureza et al., 2020; I. R. de O. M. Pureza et al., 2020; Stote et al., 2007; Stratton et al., 2020). The majority of studies with ad libitum feeding within feeding windows found reductions in weight due to overall reduced energy intake due to lack of compensation (Cai et al., 2019; Cienfuegos et al., 2020; Hutchison et al., 2019;

Jones et al., 2020; LeCheminant et al., 2013). However, a study involving a 16:8 protocol and a corresponding resistance training program, fat mass decreased as a result of the TRF protocol despite consuming an isocaloric diet (Moro et al., 2016). Other studies found no change in weight, contributing to compensation during ad libitum feeding periods or consumption of an isocaloric diet (E. A. Chowdhury et al., 2016; Enhad A. Chowdhury et al., 2016; Tinsley et al., 2017). When comparing the effects of ADF and TRF, both protocols resulted in significant losses in weight and fat mass, however, ADF has slightly greater reductions and surpassed TRF participants with significantly greater reductions in fat mass as the study progressed (Cai et al., 2019).

Glucose and insulin response were also common outcomes measured, with improvements observed across various TRF protocols (E. A. Chowdhury et al., 2016, 2016; Cienfuegos et al., 2020; Hutchison et al., 2019; Jamshed et al., 2019; Jones et al., 2020; Kahleova et al., 2014; Moro et al., 2016; Parr et al., 2020; Sutton et al., 2018a). Other studies found increases in adiponectin among TRF participants as well (McAllister et al., 2020; Moro et al., 2016). Interestingly, in a study comparing eTRF (feeding between 8:00 am – 5:00 pm) and delayed TRF (TRFd: feeding between 12:00 – 9:00 pm), mean fasting glucose as obtained by continuous glucose monitoring was lower after eTRF rather than TRFd, however, the difference was not significant (Hutchison et al., 2019).

In addition, blood pressure has significantly decreased with the TRF protocol (McAllister et al., 2020; I. R. de O. M. Pureza et al., 2020; Sutton et al., 2018a). Triglycerides and total cholesterol has also decreased significantly as a result of TRF (Cai

et al., 2019; Hutchison et al., 2019). Muscle strength was able to increase alongside control participants during resistance straining protocols as well, showing that TRF does not attenuate growth in muscle strength (Moro et al., 2016; Stratton et al., 2020; Tinsley et al., 2017, 2019).

Currently, no studies of TRF effect on cognitive performance have been published. One study, which implemented a 18:6 eTRF protocol, found that intervention participants had increased BDNF in the evening and increased ketone levels in the morning but did not compare it with any cognitive assessments (Jamshed et al., 2019). However, another TRF study that involved a 1 meal per day intervention protocol, found no changes to BDNF (Carlson et al., 2007). In addition, two other studies utilizing a 16:8 and a 18:6 protocol that provided evidence of a metabolic switch favoring fat oxidation (Moro et al., 2016; Ravussin et al., 2019). Lastly, an experiment with a 16:8 TRF protocol found that participants had lower perceived alertness, focus, and mood ratings compared to controls with no treatment effects over time (McAllister et al., 2020). Clearly, the literature is lacking in experiments focused on cognition and no currently known studies of TRF are published utilizing cognitive tests.

Table 4. Current Human Randomized Controlled Trials of Time-Restricted Feeding

Reference	Study Design	Participants	N	Length	Dependent Variables	Result
(Carlson et al., 2007)	Randomized cross-over design: two eight-week treatments: intervention group involved consuming all calories	Healthy, adults ages 40-50 years old with BMIs in the normal range (18-25 kg/m^2) and who normally consume 3 meals per day	21	8 weeks	Glucose, insulin, glucagon, leptin, ghrelin, adiponectin, BDNF	Increased morning plasma glucose, sustained plasma glucose, and ghrelin in 1

	in 1 meal/day, control group involved consuming all calories in 3 meals/day					meal/day treatment

Decreased insulin response in 1 meal/day treatment |
| (Stote et al., 2007) | Randomized cross-over design: two eight-week treatments: intervention group involved consuming all calories in 1 meal/day, control group involved consuming all calories in 3 meals/day | Healthy, adults ages 40-50 years old with BMIs in the normal range (18-25 kg/m^2) and who normally consume 3 meals per day | 21 | 8 weeks | Blood pressure, heart rate, body temperature, body composition, lipid profile, metabolic panel, complete blood count, cortisol, physical activity | Decrease blood pressure for 3 meals/day

Increased hunger and desire to eat and decreased feelings of fullness for 1 meal/day

Decreased weight and fat mass in 1 meal/day treatment

Decreased blood urea nitrogen, increase |

						d albumin and liver enzymes in 1 meal/day treatment
						Increased cholesterol (total, HDL, LDL) and blood pressure in 1 meal/day treatment
(LeCheminant et al., 2013)	Cross-over study: 2-week intervention of night-time eating restriction between 7:00 pm and 6:00 am, 1 week wash out, 2-week control condition of normal eating	Healthy, nonsmoking, weight controlled young men ages 18-26 years old	29	2 weeks	Daily energy intake, body weight, and mood	Significant reduction in energy intake and body weight during the night-time eating restriction condition. No significant

						changes in mood.
(Betts et al., 2014)	Randomized repeated measures-controlled trial in which participants were randomized to either the breakfast group ($\geq$ 700 kcal before 11:00 am) or the fasting group (fasting until 12:00 pm).	Healthy, lean adults aged 21- 60 years old	33	6 weeks	Physical activity thermogenesis, diet-induced thermogenesis, resting metabolic rate, energy intake, thyroid hormones, appetite and energy balance hormones, body fat, body mass, blood lipid profiles, glucose, insulin.	Physical activity thermogenesis was significantly higher in the breakfast group compared to the fasting group. Diet-induced thermogenesis increased among the breakfast group compared to the fasting group. The breakfast group consumed greater daily energy intake, although the difference was

						not significant. Continuously monitored glucose had greater variability in the afternoon and evening in the fasting group compared to the breakfast group No changes resulted in resting metabolic rate, thyroid hormones, energy balance and appetite hormones, body mass, body weight, blood lipid

						profiles, fasting plasma glucose, serum insulin, insulin sensitivity
(Kahleova et al., 2014)	Randomized crossover study: participants were randomized intro either six meals per day (A6) or two meals per day (B2). Hypocaloric diets were received in both protocols.	Adults aged 30-70 years old with type 2 diabetes treated by oral hypoglycaemic agents, BMI 27-50 kg/m^2, and HbA$_{1C}$ (6-11.8%).	54	12 weeks	Body weight, hepatic fat content (HFC), insulin resistance, beta cell function.	Body weight, HFC decreased in both A6 and B2 groups, with a greater reduction in the B2 group Fasting plasma glucose decreased in the B2 group and increased in the A6 group Oral glucose insulin sensitivity increased in both

| | | | | | the A6 and B2 groups, with a greater increase in the B2 group.

Fasting plasma glucose, fasting immunoreactive insulin, HbA_{1C}, and fasting C-peptide decreased comparably in both A6 and B2 groups |
|---|---|---|---|---|---|---|
| (Enhad A. Chowdhury et al., 2016) | Randomized intervention: treatments involved breakfast consuming control group and breakfast omitting intervention group | Healthy, nonsmoking, obese adults ages 21-60 years old | 23 | 1 trial visit | Physical activity thermogenesis, resting metabolic rate, diet-induced thermogenesis, energy intake, energy balancing hormones, body mass, total cholesterol, | No significant difference among groups in daily energy intake, body weight, blood lipid, appetite regulatory |

					LDL cholesterol, fasting plasma glucose, serum insulin, insulin sensitivity, glycemic response	hormone, and CRP

Lower physical activity thermogenesis during fasting condition

Increased insulin response to oral glucose tolerance test during fasting condition and lowered insulin response during breakfast consuming condition |
| (E. A. Chowdhury et al., 2016) | Randomized, cross over study: treatments involved breakfast consuming control group and breakfast | Healthy obese adults aged 25-58 years old | 24 | 1 trial visit | Total and acylated ghrelin, peptide tyrosine-tyrosine (PYY), leptin, insulin, non-esterified | Energy intake was not significantly different between groups |

| | omitting intervention group | | | | fatty acids, glucose, urea, subjective appetite measures | Blood glucose was higher 1 hour after lunch in the fasting group, but was not higher any other time

Insulin was significantly lower in the morning, but was significantly higher after lunch in the fasting group

Non-esterified fatty acids were higher in the morning in the |

						fasting group
						Acylated ghrelin was higher 2 hours after breakfast and total ghrelin was higher all morning in the fasting group
						PYY was lower 2 hours after lunch and leptin was lower 3 hours after lunch in the fasting group
						Subjective appetite was higher in the morning

						in the fasting group, but did not differ after lunch
(Moro et al., 2016)	Randomized controlled trial with participants randomized to either 8-hr time-restricted feeding (TRF) intervention group or normal diet group.	Resistance trained males (mean age 29.21 ± 3.8 years)	34	8 weeks	Body weight, BMI, fat mass, fat-free mass, 1-repetition max for leg press and bench press, muscle areas in the thigh and arm, resting energy expenditure, respiratory ratio, and blood markers (glucose, insulin, HDL cholesterol, LDL cholesterol, triglycerides, total cholesterol, triiodothyronine (T3), thyroid stimulating hormone (TSH), interleukin-6 (IL-6), interleukin-1beta (IL-	Fat mass significantly decreased in TRF group while fat-free mass remained the same

Leg press maximal strength increased in both groups

Total testosterone and ILF-1 significantly decreased in TRF group

Blood glucose, insulin, and triglyceri |

63

					1B), tumor necrosis factor alpha (TNF-alpha), total and free testosterone, insulin-like growth factor 1(ILF-1), adiponectin, and lectin.	des significantly decreased in TRF group Adiponectin significantly increased in TRF group T3 significantly decreased in TRF group TNF-alpha and IL-1B significantly decreased in TRF group Respiratory ratio decreased significantly in TRF group
(Jakubowicz et al., 2017)	Randomized, crossover-within-	Two different groups of participants	36	1 trial visit	Clock-controlled gene expression,	There was reduced expressi

	subject clinical trial in which participants underwent two different test days of separate conditions: Consumption of breakfast and lunch (YesB) or Omission of breakfast and consumption of lunch (NoB)	were used and separated in the experiment: 1) healthy adults 2) adults with Type 2 diabetes (mean age: 66.8 ± 1.9 years old)			postprandial glucose, insulin, intact glucagon like peptide 1 (GLP-1), Dipeptidyl peptidase IV plasma activity.	on level of Per1, Cry1, Rorα, and Sirt1 and increased Clock expression in healthy individuals after breakfast. In type 2 diabetics, there was reduced expression of Per1, Per2, and Sirt1 and increased expression of Rorα. With breakfast omission, the expression of Bmal1, Cry1, Rev-erbα, Rorα, and

						Sirt1 increased in healthy individuals. Clock expression increased in type 2 diabetics. When breakfast was eaten, the expression of Bmal1, Rorα, and Sirt1 expression was increased and Bmal1, Per1, Rev-erbα, and AMPK expression increased after lunch. With breakfast omission,

						increases in Per2 and Ampk expression and decreases in Rev-erbα, Per1, and Cry1 decreased in healthy individuals. Expression of Bmal1, Per1, and Ampk increased and Rorα increased among Type 2 diabetics. $AUC_{glucose}$ increases significantly after lunch on breakfast omission days in both healthy and

| | | | | | | diabetic participants.

AUC$_{insulin}$ decreased significantly on breakfast omission days compared to breakfast days among type 2 diabetics.

AUC$_{iGPL-1}$ was significanty lower on breakfast omission days compared to breakfast days in both groups. |
|---|---|---|---|---|---|---|
| (Tinsley et al., 2017) | Randomized controlled with participants randomized to either resistance | Healthy, active men (mean age 22.0 ± 2.4 years old) | 28 | 8 weeks | Body composition and muscular strength in the lower and upper body | No changes in body weight or total body composition were |

	training (3 days/week) and time-restricted feeding (RT-TRF) or resistance training with a normal diet (RT-ND). The TRF protocol took place on the remaining 4 days in which participants did not have resistance training workouts and consumed food in any 4-hour window between 4:00 pm – midnight					found in either group Both groups significantly improved particular muscular exercises, including hip sled 1 repetition max, hip sled endurance, and bench press. RT-TRF decreased consumption of kcal, protein, fat, and carbohydrates on fasting days compared to RT-ND. On a weekly basis, RT-TRF

						decreased consumption of kcal and carbohydrate
(Antoni et al., 2018)	Pilot study in which participants were randomized into a TRF group or control group. The TRF protocol consisted of delaying breakfast for and advancing dinner by 1.5 hours each day, compared with their usual dietary pattern. TRF participants ate ad libitum during feeding hours.	Healthy adults aged 29-57 years old (BMI 20 – 39 kg/m^2)	16	10 weeks	Dietary intake, body weight, body fat, adiposity, fasting blood biomarkers (insulin, glucose, TAG, total cholesterol, LDL, HDL, LDL), and subjective feedback regarding the TRF protocol.	TRF protocol caused a decrease in daily energy intake and body fat, which was significantly different than the control group.

No differences were seen in body weight, fasting plasma glucose.

The TRF protocol produced mild reductions in LDL-cholesterol, but |

						no significant difference between groups. The average difficulty rating of the TRF protocol was a 7/10 (1: easy; 10: extremely difficult).
(Gasmi et al., 2018)	Randomized controlled trial; participants in the time restricted-feeding intervention fasted 12 hours from sunrise to sunset and ate during the night. Participants participated in this fasting regimen for 2 days per week and	Healthy men aged 20-50 years old. The intervention divided men into "young" and "aged" when evaluating results.	40	12 weeks	Exercise testing (running-based anaerobic sprint test); systolic and diastolic blood pressures; leukocyte, lymphocyte, and monocyte levels	The intervention resulted in decreases in hematocrit, total WBCs, lymphocytes, and neutrophils decreased in both younger and older men

	ate ad libitum the remaining days					No main effects resulted for body fat percentage or fat free mass between age groups Body mass decreased after the intervention for the young men, but not for the older men A significant effect resulted between age groups for physical performance, but this effect did not change after

						intervention
(Sutton et al., 2018b)	Randomized, crossover, isocaloric, feeding trial with 6-hour early time-restricted feeding (intervention) or 12-hour normal feeding (control)	Overweight men with prediabetes aged 35- 70 years old	12	5 weeks	Glucose tolerance, postprandial insulin, insulin sensitivity, CVD risk factors (diastolic and systolic blood pressure, HDL cholesterol, LDL cholesterol, total cholesterol, triglycerides), and markers of inflammation and oxidative stress	eTRF decreased insulin levels and improved insulin sensitivity and beta cell responsiveness eTRF reduced diastolic and systolic blood pressure eTRF increased morning levels of triglycerides and total cholesterol eTRF lowered 8-isoprostane eTRF reduced the desire to

						eat and capacity to eat in the morning eTRF reduced PYY
(Cai et al., 2019)	Randomized clinical trial in which individuals were randomized into either a control group, alternate-day fasting group (ADF: 3 non-consecutive fasting days in which participants consumed 25% of their	Healthy weight adults with non-alcoholic fatty liver disease (NAFLD) (18 – 65 years old)	271	12 weeks	Body weight, waist circumference, fat mass, fat-free mass, liver stiffness, fasting plasma cholesterol, LDL, HDL, triglycerides, hunger, fullness, diet satisfaction.	ADF and TRF participants experienced significant reductions in body weight at week 4 and week 12 compared to the control. No differences were seen between

	energy needs at baseline. Ad libitum eating was allowed on remaining days), or time-restricted feeding group (TRF; 16:8 diet, timing of diet was decided by the participant).					the ADF and TRF group, but the ADF group reduced body weight slightly more. ADF and TRF participants experienced significant reductions in fat mass at week 4 compared to the control, however, at week 12 ADF participants experienced a further significant reduction in fat mass compared to TRF and control

| | | | | | | participants.

ADF and TRF participants experienced significant reductions in triglycerides at week 4 and week 12 compared to the control. No differences were seen between the ADF and TRF group, but the TRF group reduced levels slightly more.

ADF participants experienced significantly |
|---|---|---|---|---|---|---|

| | | | | | | decreased total cholesterol at week 4 and week 12, compared to TRF and control groups

From week 4 to week 12, hunger and fullness levels significantly increased in ADF and TRF groups. |
| --- | --- | --- | --- | --- | --- | --- |

(Edinburgh et al., 2019)	Randomized, controlled, crossover study in which participants completed 3 separate trials, breakfast with subsequent rest (BR), breakfast before exercise (BE), and overnight fasting before exercise (FE). Each trial was followed by an ad libitum lunch. Trials were completed in random order by the participants and separated by >1 week each.	Healthy, physically active men (mean age: 23 ± 3 years)	12	1 trial visit	Main outcome assessed: 24 hour- energy expenditure Specific Measurements: Energy expenditure, substrate utilization, physical activity energy expenditure, energy intake, plasma glucose, plasma leptin, plasma fibroblast growth factor 21	There were no significant differences in energy consumed in the lab between BR and BE groups, but BE consumed significantly higher energy than FE. Carbohydrate utilization was significantly higher in the BE group compared to the FE group. The FE group has significantly higher whole-

| | | | | | | body lipid utilization compared to the BE group.

There was a positive correlation between postexercise energy balance regulation and plasma glucose utilization in the FE group.

The FE group created a greater negative daily energy balance compared to the BE. Participants did not completely |
|---|---|---|---|---|---|---|

						compensate for missed breakfast in later meals.
(Jamshed et al., 2019)	Randomized, controlled, crossover trial with 4 days each of early time-restricted feeding (feeding period between 8:00 am – 2:00 pm) or control (feeding period between 8:00 am – 8:00 pm) schedule with a 3.5-	Overweight or obese adults aged 20-45 years old	18	4 days	Glucose, cardiometabolic analytes and hormones (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, insulin, cortisol, free fatty acids, beta-hydroxybutyrate, BDNF, IGF-1, and IGF-binding proteins 1 and 3), Homeostatic	eTRF decreased morning fasting glucose and insulin resistance eTRF increased evening fasting insulin and insulin resistance without

					Model Assessment of Insulin Resistance (HOMA-IR), and morning and evening expression of genes related to glucose metabolism, the circadian system, fasting, autophagy, and oxidative stress	affecting glucose eTRF increased morning LDL, HDL, total cholesterol, and ketones eTRF decreased morning cortisol eTRF increased BDNF in the evening In the morning, eTRF increased the expression of circadian clock genes, BMAL1, CRY1, CRY2, and RORA
5-week washout period.						
(Hutchison et al., 2019)	Randomized, crossover	Healthy aged 30- 70 years old, who are	15	7 days	Body weight, waist and hip circumferenc	Body weight, glucose

	study in which participants were randomized into one of two TRF groups: TRF early (TRFe: feeding between 8:00 am – 5:00 pm) or TRF delayed (TRFd: eating between 12:00 pm – 9:00 pm). Each arm lasted 7 days and were separated by a 2-week washout period.	lightly active or sedentary with no prior diagnosis of type 2 diabetes			e, fat mass, lean mass, appetite measures, blood pressure, fasting glucose, insulin and glucose response, triglycerides, Non-esterified fatty acids (NEFA), gastric emptying	incremental under the curve, and triglycerides decreased significantly at the end of both treatments. Mean fasting glucose (measured by continuous glucose monitoring) was lower after TRFe in comparison to TRFd, but there was not significance between groups. No differences in either

						TRFe or TRFd were seen in fasting and postprandial insulin, nonesterified fatty acids, or gastrointestinal hormones
(Ravussin et al., 2019)	Randomized, controlled, isocaloric, crossover feeding trial with 4 days each of early time-restricted feeding (feeding period between 8:00 am – 2:00 pm) or control (8:00 am – 8:00 pm) schedule with a 3.5-5-week washout period.	Generally healthy, overweight or obese adults	18	4 days	24-hour energy expenditure, substrate oxidation, thermic effect of food, leptin, active ghrelin, peptide YY (PYY), glucagon-like peptide 1 (GLP-1), and subject appetite and energy levels	eTRF caused increased energy expenditure during the day and decreased energy expenditure during the night eTRF group decreased 24-hour protein oxidation, indicating increased fat

						oxidation
						eTRF lowered npRQ for 12 hours during the night
						eTRF decreased ghrelin and leptin collectively in the morning and evening
						eTRF collectively decreased the mean desire to eat and slightly increased mean fullness and stomach fullness while awake
(Tinsley et al., 2019)	Randomized, placebo-controlled, reduced	Healthy females (18 – 30 years old) with previous	40	8 weeks	Fat mass, fat free mass, body fat percentage,	Energy and protein increase

	factorial design trail in which participants were randomized into one of three groups: control diet (CD), time-restricted feeding (TRF; 16:8 diet, consuming calories between 12:00 pm – 8:00 pm), TRF with β-hydroxy β-methylbutyrate Supplementation (TRF_HMB). In addition, all groups received supervised resistance training three times per week and were provided whey protein supplements to consume on all days	experience (≥1 year) in consistent resistance training			muscle thickness of elbow flexor muscles, muscle thickness of knee extensor muscles, muscular performance, resting energy expenditure, substrate utilization, blood pressure, total cholesterol, triglycerides, HDL, LDL, glucose, insulin, and subjective ratings (mood, hunger, appetite, nausea, uncontrolled eating)	d significantly in all groups during the intervention period. All groups experienced significant increases in fat free mass, muscle thickness of elbow flexors, and muscle thickness of knee extensors with no differences between groups. All groups experienced significant decrease

						s in fat mass and body fat percentage with no differences between groups. All groups improved muscular strength and muscular endurance with no differences between groups.
(Cienfuegos et al., 2020)	Randomized controlled trial in which participants were randomized to one of three groups: 4-hour time restricted feeding (4-h TRF: eating between	Obese adults aged 18 – 65 years old.	54	8 weeks	Body weight, body composition, dietary intake, blood biomarkers, insulin resistance, blood pressure, heart rate.	Change in caloric intake, body weight, fat mass, fasting insulin, insulin resistance, and the oxidative stress marker, 8-

| | 3:00 – 7:00 pm), 6-hour time restricted feeding (eating between 1:00 – 7:00 pm), or control group. | | | | | isoprostane, was significantly reduced in the 4-h TRF group and 6-h TRF group, with significant differences between the control groups but no differences between the TRF groups. No changes were seen among inflammation markers in either group.

Lean mass reduced in the 6-h TRF group, which |

						was significantly different from the control group or 4-h TRF group.
(Chow et al., 2020)	Feasibility randomized controlled trial in which participants were randomized into a time-restricted eating (TRE: 16:8) group or control group (ad libitum eating). TRE participants were able to self-select their feeding windows but asked to keep the timing of the window consistent.	Overweight/ obese adults aged 18-65 years old. Participants must have had a prolonged eating window of ≥14 hours prior to the study.	22	12 weeks	Body weight, body composition, lipids, blood pressure, 2-hour oral glucose tolerance, 2-week continuous glucose monitoring, 2-week physical activity.	The TRE group significantly lowered eating window time, body weight, visceral fat, and lean mass compared to control group and pre-intervention measures.

The TRE group significantly lowered fasting glucose and fasting |

						triglyceride concentration from pre to post intervention. Number of eating occasions significantly decreased in the TRE and control group from pre to post intervention, but the reduction in eating occasions was significantly lower in the TRE group compared to the control group. There was a positive associati

						on eating-window restriction and fat mass loss and visceral fat loss.
(Jones et al., 2020)	Randomized controlled trial in which participants were randomized into the early time restricted feeding group (eTRF: feedings between 8:00 am and 4:00 pm) or control, caloric restricted (CON:CR) group. The intervention arm was completed 9 months prior to the control group so calories and macronutrient content	Healthy males aged 18 – 35 years old	16	2 weeks	Energy intake, physical activity energy expenditure, respiratory exchange rate, substrate CHO and fat oxidation, body weight, body composition, blood biomarkers, insulin sensitivity	eTRF resulted in statistically significant reductions in caloric intake from pre to post intervention.

Statistically significant differences from pre to post intervention occurred in body weight and android fat in both eTRF and |

90

	could be matched to the control group.					CON:CR groups. No differences were seen between groups. Statistically significant differences were found between groups of whole-body insulin sensitivity, skeletal muscle and uptake of glucose and branched chained amino acids, with improvements in the eTRF group.

| (Lundell et al., 2020) | Randomized, crossover trial in which participants were randomized into either an extended feeding group (EXF: 15-hour feeding period) or time-restricted feeding group (TRF: 8-hour feeding period). | Overweight/ Obese, sedentary men (30 – 45 years old) | 11 | 5 days | Skeletal muscle metabolic profiles and transcriptomic profiles | The TRF group demonstrated an improvement in rhythmicity of many amino acid transporter genes and metabolites.

Amino acids were the most identified skeletal muscle metabolite related to TRF.

In both TRF and EXF groups, lipids were the main periodic serum metabolite |
|---|---|---|---|---|---|---|
| (McAllister et al., 2020) | Randomized controlled | College-aged men (mean age: 22 ± 2.5 | 23 | 4 weeks | Body composition, energy | No differences or |

| | trial in which participants were randomized into either an isocaloric time-restricted eating (TRF: 16:8 hour fasting protocol) or an ad libitum control group | years) who reported regular physical activity | | | intake, blood biomarkers (fasting glucose, lipids, adiponectin, human growth hormone, insulin, cortisol, C-reactive protein (CRP) superoxide dismutase, total nitrate/nitrite, glutathione), Visual Analog scale scores | change over time occurred in caloric intake between groups

Change in adiponectin was greater significantly in the TRF group and statistically significant differences were found between the TRF and control group.

Change in plasma HDL was significantly greater in the TRF group. |
|---|---|---|---|---|---|---|

| | | | | | | Plasma LDL, total cholesterol (TC), TC: HDL ratio, total nitrite/nitrate, cortisol, insulin, and CRP was significantly higher in the control group compared to the TRF group.

Change in adiponectin significantly increased in the TRF group and was significantly greater than the ad |
|---|---|---|---|---|---|---|

						libitum group.
						Body weight decreased significantly in the TRF group
						Body fat significantly decreased in both groups, but with significantly higher body fat % and fat mass in the ad libitum group
						Change in blood pressure significantly decreased in the TRF group
						Perceived alertness, energy levels,

						focus, and mood were higher in the ad libitum group compared to the TRF group.
(Parr et al., 2020)	Randomized, crossover experiment in which participants were randomized into a time-restricted feeding (TRF: meals consumed at 10:00 am, 1:00 pm, and 5:00 pm) group or a controlled extended feeding group (EXF: meals consumed at 7:00 am, 2:00 pm, 9:00 pm). During both protocols,	Overweight/ obese, sedentary men aged 30-45 years old.	13	5 days	Perceived perception of protocols, dietary intake, total area under the curve (AUCtotal: glucose, insulin, triglycerides, and non-esterified fatty acids), glucose Nocturnal AUCtotal, blood biomarkers,	Positive feedback was provided from the TRF protocol based on subjective questionnaires. AUCtotal and for glucose had a slight reduction and Nocturnal glucose AUC was significantly lower in the TRF group compared to the

						EXF group, indicating improved blood sugar maintenance.
participants consumed an isoenergetic diet (Total energy intake: 50% fat, 30% CHO, 20% protein)						
(I. Pureza et al., 2020)	Randomized, controlled, parallel experiment in which participants were randomized into one of two groups: time restricted fasting, hypocaloric diet (HD + TRF: 12:12 fasting protocol) or hypocaloric diet alone (HD).	Obese women aged 19-44 years old.	58	21 days	Axillary temperature, blood pressure, heart rate, body composition, resting metabolic rate, hunger ratings, adherence evaluations, blood biomarkers, physical activity	Axillary temperature increased significantly in the HD+TRF group after 21 days.

Body fat percentage and decreased significantly in the HD+TRF group after 21 days.

No significant differences in weight, |

| | | | | | | thyroid hormones, insulin sensitivity, leptin, and resting metabolic rate were found for either group.

Waist circumference decreased significantly in the HD+TRF group after 81 days. |
|---|---|---|---|---|---|---|
| (I. R. de O. M. Pureza et al., 2020) | Randomized, parallel, controlled clinical trial in which participants were randomized into either a hypocaloric diet with time-restricted feeding group | Obese, low-income women (19 – 44 years old) | 58 | 12 months | Body fat, waist, circumference, blood pressure, heart rate, axillary temperature, | At the end of the experiment, neither group experienced significant differences in weight, BMI, waist circumfe |

| | (HD+TRF; feeding was restricted to 12 hours per day) or hypocaloric diet alone group (HD) | | | | | rence, body fat percentage, temperature, blood pressure, and heart rate.

The HD+TRF group experienced a significant reduction in the mean difference (final – starting values) of body fat percentage and a significant increase in the mean difference of axillary temperature compared to the HD group. |

						Significantly lower body fat percentage and waist circumferences were seen in the TRF+HD group compared to the HD when considering measurements at all 4 visits
(Stratton et al., 2020)	Randomized controlled trial in which participants were randomized to time restricted feeding intervention group (16:8 protocol) or control group. Both groups	Active males (18-35 years old)	32	4 weeks	Body composition, muscle thickness and skeletal muscle cross sectional areas, total energy expenditure, resting energy expenditure, muscular performance, blood biomarkers, psychometric parameters	Statistically significant reductions in body mass, fat mass, body fat percentage, adiponectin, and resting energy expenditure were found

	were given a 25% caloric deficit diet with 1.8 g/kg per day dietary protein. Participants followed a supervised resistance training program 3 times per day for 4 weeks in the middle of the intervention				over time in both groups with no differences between groups. Statistically significant increases were found over time in bench and leg press 1 repetition max, leg press and vertical jump peak power, vastus lateralis cross sectional area and muscle thickness, and biceps brachii cross sectional area and muscle

						thicknes s with no differenc es between groups Compare d to a normal diet with caloric restrictio n, TRF did not produce significa nt differenc es in fat mass, lean mass retention , or muscle strength

CHAPTER 3

METHODS

Participants

Study participants were healthy, non-smoking college students at least 18 years old at Arizona State University. Additionally, participants self-reported the following inclusion and exclusion criteria: absence of any acute or chronic illness, were engaged in ≤420 minutes of physical activity, were not involved in training or competing in athletic events, had a waist circumference of >80 cm (females) and >94 cm (males), did not regularly fast greater than 12 hours per day or participated in religious or dietary fasting within the past 6 months, did not attempt to lose or lose 5 or more pounds in the past 6 months, did not have a history of weight cycling, were not currently working night shifts, were not pregnant or lactating, had either regular menstrual cycles or were currently on hormonal contraceptives (i.e., birth control pills, IUDs, or patches), and were willing to follow the experiment protocol. The power was set at 80% (0.2 error level) with an alpha error level of 0.05, and the estimated sample size was set at 30.

Recruitment took place between October 2019 and December 2019. Recruitment was conducted using informational flyers, distributed either in-person or online. Additionally, recruitment included email correspondence sent to ASU faculty, who shared the flyer with their students on their class online platform or via email. These flyers and emails provided the participant with information regarding the 8-week trial and described that the purpose of the experiment was to evaluate the effects of fasting on health.

It was clearly defined that the experiment would look at the difference between an 18-hour fast and an 8 hour fast. The fliers and emails invited interested recipients to fill out the online survey to assess eligibility criteria. Responses to the online survey were evaluated by the research team and eligible participants were instructed to complete a 7-day diet record to assess additional eligibility requirements. Those completing the 7-day diet record and not fasting >12 hours per day were invited to visit the research laboratory (Arizona Biomedical Collaborative building; ASU Downtown Phoenix Campus) in order to receive an in-person screening which included obtaining written informed consent and enrollment in the experiment, if eligible. This study was approved by the Arizona State University Institutional Review Board. See Appendix A-E for IRB approval, recruitment flier, consent form, release form, and email script.

Study Design

The study was an 8-week randomized-controlled, parallel arm design. Participants were stratified by age, waist circumference, gender, and METS, and randomly assigned to either the intervention group or the control group by a coin toss. The intervention involved a daily 18-hour fast time-restricting feeding protocol, and the control condition involved a daily 8 hour fast. The study was not blinded; participants were informed that they would randomized into either the 18-hour fasting group or the 8-hour fasting group. Subjects were given no further diet instruction other than "fasting" during their designated periods and asked to maintain current physical activity habits while participating in the experiment.

Variables

The aim of this particular study was to compare effects of an 18-hour fast versus
an 8-hour fast on cognitive acuity. Cognitive acuity was assessed through the Stroop
Test, Trail Making Tests, Brain-derived neurotrophic factor (BDNF), and ketones.
Participants were instructed to initiate food and beverage consumption within one hour of
waking and to eat ad libitum during their feeding period (either 6 hours or 16 hours).
Outside of the feeding period, subjects were instructed to fast. Participants were free to
consume unsweetened, non-caloric beverages, such as tea and coffee, and sugarless gum
($\leq$ 5 sticks/day) during the fasting period. Subjects selected one "cheat" day per week that
freed them from adhering to fasting protocols, which remained the same day of the week
throughout the experiment.

Protocol

Three trial visits were scheduled throughout the study at baseline, 4 weeks, and 8
weeks, and participants appeared in a fasted state ($\geq$8 hours). Diet quality,
cardiometabolic health, anthropometrics, and mood and cognition were evaluated at each
visit among the participants. Each visit lasted 45-60 minutes. See Appendix F for the
study design flowchart. During the trial visits, a registered dietitian used a three-step
multiple-pass method to administer a 24-hour recall and utilized the REAP-S
questionnaire to assess diet quality. After receiving briefings and diet quality information,
trained study staff conducted mood and cognitive evaluations and collected
anthropometrics. At the conclusion of the visit, a certified phlebotomist took fasting

blood samples. The study reported herein is an ancillary study and focused only on the cognitive assessments (Mayra, *In Review*).

At each trial visit, calendars were given to each participant to record the start and finish of the eating period daily. Time of awakening was also recorded. Participants were instructed to complete two 3-day diet records at week 4 and 8. Non-caloric beverages and gum consumed in the fasting time period were also recorded on these food logs. The final trial visit ended with the completion of an exit survey to assess their satisfaction with the experiment participation.

Cognitive function was evaluated at all three trial visits. The cognitive tests that were implemented included two Trail Making Tests, Trail Making Test A and Trail Making Test B, and the Stroop Test. The completion of the tests took less than 15 minutes to complete. During the trial visits, the Stroop Test was conducted first followed by Trail Making Test A and Trail Making Test B.

The Stroop test assesses the participants' cognitive acuity. The Stroop test specifically analyzes one's reaction ability and processing speed (Zhang, Du, Zhang, & Ma, 2019). The Stroop Test is a computerized test that takes less than one minute to compute. A screen is placed in front of the participant and a video played where words are flashed across the screen. The word spells a particular color but reads in a different colored font. For example, the text could say "Green", but the font appears in a blue color. The participant is asked to name the actual color of the font rather than the word itself. Using the same example, the correct answer would be "blue". A member of the

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study staff evaluated how many correct answers the participant provided during the video. See Appendix G for an example of the Stroop Test.

Both Trail Making Tests are conducted on paper and evaluate one's processing speed, cognitive flexibility, and executive function (Simpson et al., 2016). Each Trail Making Test requires the participant to connect consecutive points. For Trail Making Test A, the staff member asked the participant to draw a "trail" using their pen consecutively from numbers 1 to 25 in consecutive order on a sheet of paper. The staff member evaluated the participant's trail making for correctness and collected the time (in seconds) it took the participant to draw the line correctly without picking up their pen or making any mistakes. If a mistake was made by the participant, the staff member asked the participant to pick up their pen and bring it back to the previous correct number before continuing on with the trail making. This was the only circumstance in which the participant is allowed to pick up their pen. For Trail Making Test B, the difficulty of the first test is increased as the participant is asked to connect consecutive numbers (1-12) and letters (A-L). The participant first begins on the "1" and then makes a trail to "A". The participant then continues to make a trail from number to letter consecutively until they end on "L". Once they end on "L", the time taken to complete the trail was recorded by the research professional. See Appendix H for copies of both Trail Making Test A and Trail Making Test B.

Following the completion of the cognitive tests, mood questionnaire, and quality of life questionnaires, anthropometrics measures were collected by the study staff. During the first trial visit, the participant's height was obtained using a research-grade

stadiometer (Cat. No. 2131821009, SECA 213, Birmingham, UK). The researcher instructed the participant to remove shoes, hold their feet together, and touch the backs of their feet to the measuring rod of the stadiometer. They were also instructed to stand erect with the corner of their eyes and top of their ears level (following the Frankfurt line). The researcher then obtained and documented their height to the nearest centimeter and rounded when necessary.

During all three trials, weight, BMI, waist circumference, and hip circumference measurements were obtained. Weight was measured using a calibrated total body composition analyzer (Cat. No. TBF-300, Tanita, Arlington Heights, IL) to the nearest 0.5 kg. Before stepping on the scale, the staff instructed the participants to empty their pockets, remove shoes, and remove any bulky clothing or accessories. The participant stood upright in the center of the scale with their hands at their sides. The Tanita then printed off data including the participant's weight and BMI.

Waist circumference was measured to the nearest 5 cm with a research-grade ergonomic measuring tape that was wrapped around the narrowest circumference of the participant's waist. Two waist circumferences measures were collected and averaged. A third waist measurement was taken if the first and second measurement had a difference greater than 0.5 cm. Hip circumference was measured twice as well. The same protocol was followed as the waist circumference in that if the first and second measurement is above 0.5 cm then a third measurement was taken.

Laboratory Analysis

On all three trial visits, fasting resting morning blood pressure was obtained. The phlebotomist instructed the participant to sit still with their feet planted on the floor. A non-invasive Omron auto cuff blood pressure monitor equipped with IntelliSense technology was used to obtain blood pressure in mmHg by the phlebotomist. Additionally, one 30 mL fasting blood sample was obtained from the participants' antecubital vein. The blood biomarkers measured blood glucose, insulin, LDL cholesterol, triglycerides, HDL cholesterol, and brain-derived neurotrophic factor (BDNF). Plasma was separated by a centrifugation within one hour of the blood draw and stored at -80°C immediately until analysis. Commercially available kits were used to analyze blood for all metabolites. A colorimetric essay kit was used to measure ketone and an in vitro ELISA kit was used to measure BDNF (Cayman Chemical, Ann Arbor, MI, USA; RayBiotech, Norcross, GA, USA)

Statistical Analysis

All data measures were reported as mean ± standard deviation SPSS version 23.0, and significant differences were reported with a p-value < 0.05. Descriptive statistics were used to analyze the participant's age, year in school, weight, height, BMI classification (underweight, normal, overweight, etc.), and race/ethnicity. Normality assumptions were assessed using the Kolmogorov-Smirnov test. Independent t-tests (Mann-Whitney U test) were used to assess mean differences at baseline between the control group and intervention group in order to normalize the data when the data failed to meet the Gaussian assumption. To assess the main significant effects between the

control and intervention groups on the observed measurements (diet quality, anthropometrics, blood markers, mood scores, cognitive scores, and quality of life scores) a repeated-measures ANOVA test was run. The repeated-measures ANOVA test was also run to assess interaction effects of the intervention and control groups and time based on trial visit on the observed measures. When there was an existence of a significant effect, a posthoc analysis was performed using an LSD. The personal information of the participant was de-identified by the researcher and entered into SPSS on password protected computers. Hard copies of participant's de-identified data will be stored for up to 10 years in a locked office of the PI, Dr. Carol Johnston. Document including signed informed consent forms and other participant identifying documents were separated from the data sheets and store in the locked office of the PI, Dr. Johnston.

Justification of Sample Size

Previous studies that have utilized the Trail Making Tests to assess cognitive function were analyzed to determine sample size. Using the sample sizes from these trials, the estimated sample size was set at 30. The assumption was made that the intervention group would experience a decrease of 16.8 ± 13 seconds on Trail Making Test B from baseline to the end of the study. See Appendix I for the sample size determination chart.

CHAPTER 4

RESULTS

Recruitment and allocation outcomes in our randomized controlled trial can be found in

Figure 1. Ten CON participants and seven INV participations were included in the final
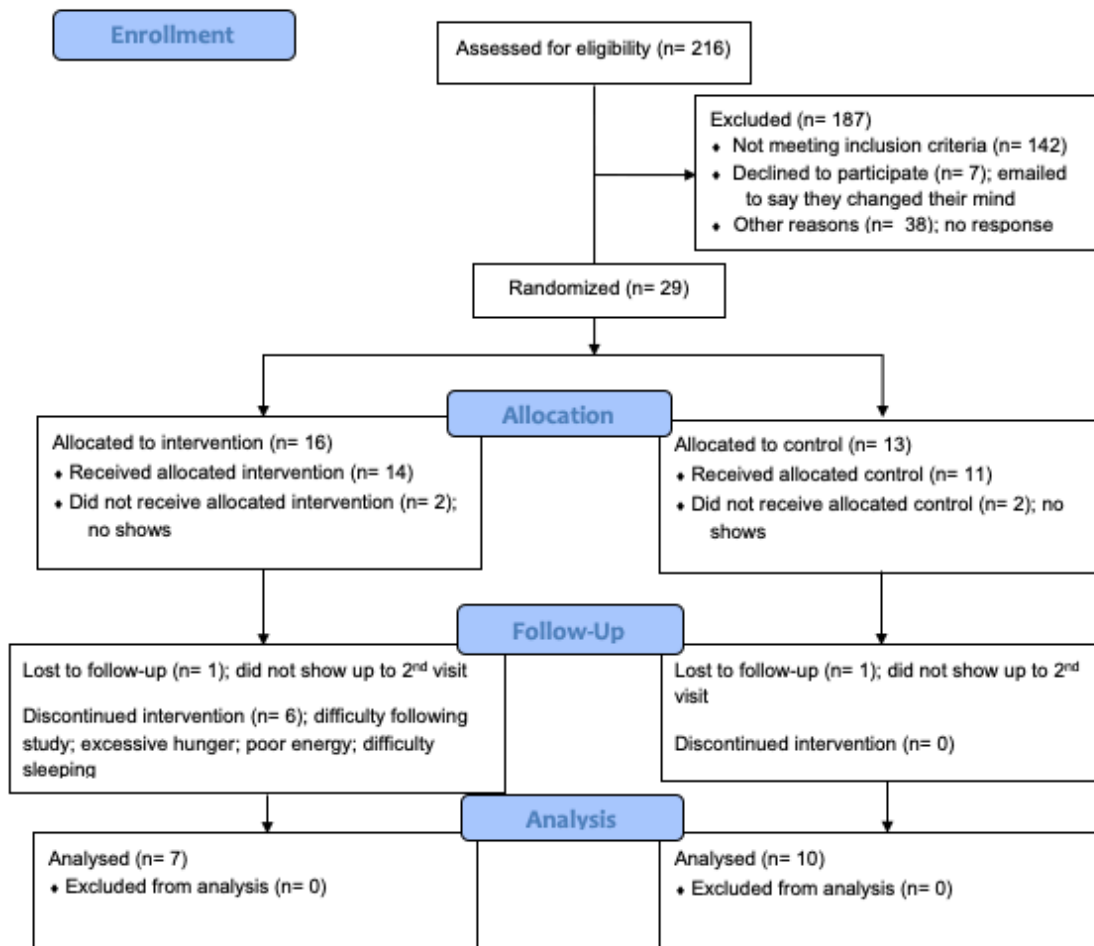
analyses.

Figure 1. Consort Flow Diagram

Descriptive statistics (mean ±SD) at baseline are reported in Table 5. Age, body

weight (kg), height (cm), waist circumference (cm), hip circumference (cm), and BMI

(kg/m2) (mean±SD) were analyzed as descriptive statistics between the intervention and

control group and between completers and non-completers of the study. There were no

descriptive variables that demonstrate statistically significant differences.

Table 5. Descriptive Statistics at Baseline

Intention to Treat				Completers and Non- Completers			
Characteristic	N	Mean	P-Value	Characteristic	N	Mean	P-Value
Age (years)				**Age (years)**			
INV	13	26.3 ± 8.4	0.095	Non-Completer	7	25.9 ± 11	0.599
CON	11	21.7 ± 3.6		Completer	17	23.5 ± 4.2	
Body Weight (kg)				**Body Weight (kg)**			
INV	13	64.4 ± 9.6	0.649	Non- Completer	7	66.8 ± 12	0.702
CON	11	66.4 ± 9.6		Completer	17	65 ± 9.4	
Height (cm)				**Height (cm)**			
INV	13	165 ± 7.7	0.875	Non-Completer	7	165 ± 9.3	0.985
CON	11	166 ± 7.2		Completer	17	165 ± 6.7	
Waist Circumference (cm)				**Waist Circumference (cm)**			
INV	13	72.5 ± 8.1	0.485	Non-Completer	7	75.3 ± 9.5	0.563
CON	11	74.8 ± 7.3		Completer	17	72.9 ± 6.9	
Hip Circumference (cm)				**Hip Circumference (cm)**			
INV	13	100 ± 9.7	0.918	Non-Completer	7	102 ± 12	0.527
CON	11	100 ± 7.1		Completer	17	99.4 ± 6.9	
BMI (kg/m²)				**BMI (kg/m²)**			
INV	13	23.7 ± 4.1	0.803	Non-Completer	7	24.5 ± 5.2	0.678
CON	11	24.1 ± 2.6		Completer	17	23.6 ± 2.5	

Descriptive statistics were performed with SPSS Statistics Analysis System (version 23.0) using an Independent Samples T-Test nonparametric analysis (Mann-Whitney U test). The data are presented as mean and standard deviation and is considered statistically significant at p-value < 0.05.

The results of the Stroop Test and TMTs are shown in Table 6. The COVID-19

pandemic interrupted the third visits of participants, which forced the researchers to

conduct their visits via Zoom. Due to the inconsistencies of the protocol with previous

procedure and unusable stressors related to the pandemic, week 8 data were removed

from the final statistical data analysis. Additionally, one outlier (#5) was removed from

the analyses of the TMTs due to standard statistical practice of obtaining a value greater

than 3 SD from the mean. The Stroop Test data at baseline and week 4 are nearly normal and the TMT data at baseline and week 4 were nearly normal. The results of the Stroop Test and the Trail Making Test A (TMT-A) showed no statistically significant change differences between the control and intervention groups (p= 0.095 and p= 0.664, respectively). The Trail Making Test B (TMT-B) and Trail Making Test B minus Trail Making Test A (TMT-B-A) results did produce statistically significant results, indicating an improved score from baseline to week four in the intervention group (p= 0.031 and p= 0.017, respectively)

Table 6. Stroop Test, Trail Making Test A (TMT-A), Trail Making Test B (TMT-B), and Trail Making Test B minus Trail Making Test A (TMT-B-A) Results and Change Between Groups Over Time

Measure		n	Baseline	Week 4	Δ	p-value
Stroop Test	CON	10	30.8 ± 0.4	31 ± 0	0.2 ± 0.4	0.095
	INV	7	30.0 ± 0.6	31 ± 0	1.0± 0.6	
TMT-A (sec.)	CON	10	25.9 ± 7.6	19.1 ± 3.5	-6.8 ± 6.4	0.664
	INV	6	23.4 ± 8.2	16.8 ± 5.8	-6.5 ± 4.2	
TMT-B (sec.)	CON	10	48.9 ± 17.1	46.1 ± 20.3	-2.8 ± 11.4	0.031*
	INV	6	45.5 ± 9.7	31.1 ± 6.7	-14.4 ± 7.8	
TMT-B-A	CON	10	23.0 ± 16.1	27.0 ± 20.2	4.00 ± 11.3	0.017*
(sec.)	INV	6	22.1 ± 11.5	14.2 ± 10.1	-7.9 ± 8.4	

Repeated Measures ANOVA tests were used to compare Stroop Test results at baseline and week 4, and to compare TMT A, TMT B, and TMT B-A at baseline and week 4 (one outlier was removed [#5 for TMT] in the INV group [>3 SD from mean]). Data represented as mean ± standard deviation. *indicates a significant change between groups.

Fasting blood ketone and fasting blood BDNF results are shown in Table 7. The data at baseline and week 4 are nearly normal. One outlier was removed from the blood BDNF analysis due to standard statistical practice of obtaining a value greater than 3 SD from the mean. There was no significant difference between the intervention and control groups with respect to blood BDNF over time (p=0.928). There was no significant

difference between the intervention and control groups with respect to blood ketones over time (Table 7), but interestingly, there was a significant inverse relationship between blood ketones and both TMT-B and TMT-B-A results at visit 2 (r= -0.633 and r = -0.492, respectively; p<0.05) (Figure 2).

Table 7. Fasting Blood Ketones and Fasting Blood BDNF Results and Change Between Groups Over Time

Measure		n	Baseline	Week 4	Δ	p-value
Ketones (mM)	CON	10	0.075 ± 0.03	0.065 ± 0.019	-0.010 ± 0.030	0.326
	INV	7	0.096 ± 0.050	0.107 ± 0.053	0.011 ± 0.032	
BDNF (ng/mL)	CON	10	30.1 ± 18.2	30.2 ± 29.5	- 0.5 ± 36.4	0.853
	INV	6	28.6 ± 16.1	25.6 ± 11.3	- 3.0± 12.1	

Repeated Measures ANOVA tests were used to compare ketones and BDNF at baseline and week 4 (one outlier was removed [#23 for BDNF] in the INV group [>3 SD from mean]). Data represented as mean ± standard deviation.
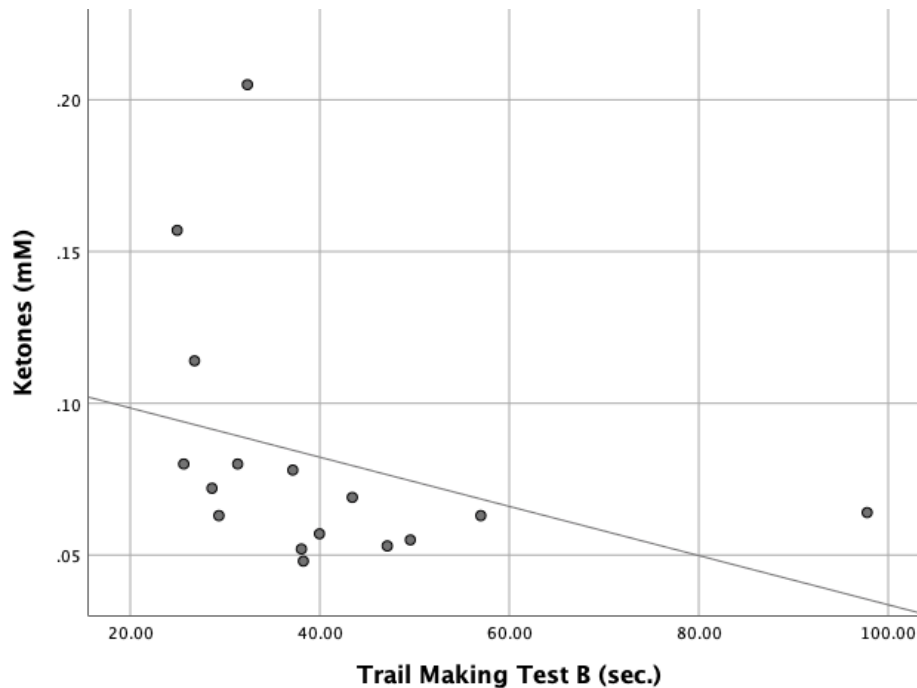

Figure 2. Association Between Ketones and Trail Making Test B Results at Week 4
Nonparametric Correlation Spearman rho was used to correlate ketones at week 4 and Trail Making Test B results at week 4.

CHAPTER 5

DISCUSSION

One primary hypothesis and four secondary hypotheses were formulated in the present ancillary study. The primary hypothesis stated that Arizona State University students who fast intermittently for 18 hours for 8 weeks will improve their cognitive acuity compared to students in the control group that fasted for 8 hours. Secondary hypotheses hypothesized improvements in the Stroop Test and Trai Making Tests and increases in BDNF and ketones would be seen among students who fast intermittently. The only secondary hypothesis supported by the study was that TRF was associated with improvements in the Trail Making Test B test. Thus, the present study suggests that 18-hour TRF may produce favorable effects on cognition.

Much more research is needed to detail changes in cognitive scores over time. As previously noted, no current randomized controlled trials of TRF exist evaluating cognition or cognitive performance. A single-armed study among overweight, older adults assessed cognitive function, but no significant results were observed (Anton et al., 2019). Animal studies point to a positive cognitive effect resulting from TRF protocols with evidence showing improved neuronal plasticity, increased growth of hippocampal neurogenesis, and increased strength of hippocampal synaptic connections (Baik et al., 2020; Dasgupta et al., 2018). Increased BDNF levels has been a consistent result among animals receiving TRF intervention (Baik et al., 2020; Dasgupta et al., 2018; Serra et al., 2019). TRF protocols have also been shown to counteract effects of distress and

neurodegenerative predispositions with observed improvements in spatial learning, spatial memory, and short term memory (Shojaie et al., 2017; J. Zhang et al., 2017).

Other diets have confirmed improved cognitive performance as well. A low-carbohydrate Paleo diet among middle-aged adults with metabolic syndrome resulted in increased serum BDNF, improved cognitive tests addressing psychomotor speed and cognitive flexibility, and improved self-perceived cognitive function ratings (Gyorkos et al., n.d.). Among healthy, obese, middle-aged adults, the MIND diet resulted in improvements in working memory, attention, and verbal recognition memory (Arjmand et al., 2020). Ingestion of ketones has produced improvements in executive function among young, healthy adults as well (Evans & Egan, 2018).

Other studies evaluating cognition in young, healthy populations confirm that a 4 to 8-week period is an appropriate time period to produce significant results related to cognition. In a randomized controlled trial among university students who participated in 3 high intensity interval training (HIIT) workouts each week for 8 weeks, significant group by time differences were observed in Trail Making Test B (Eather et al., 2019) Verbal reasoning scores significantly improved after 8 weeks of walnut consumption among healthy college students (Pribis et al., 2012). In 10 days, the Mediterranean diet significantly improved cognitive scores assessing numeric working memory, visual spatial short term memory in healthy young females with a mean age of 21 years old (McMillan et al., 2011).

Metabolic switching and regulation of circadian rhythms are two major mechanisms in favor of the cognitive effect of TRF. As previously discussed, when

glucose is depleted, the brain relies on ketones as its main sources of energy. The transition from glucose utilization to ketone utilization is known as a "metabolic switch" and experiencing this switch periodically can enhance neuroplasticity and neurogenesis, cellular resistance, and neuronal adaptations to stress (Mattson, 2015; Mattson et al., 2018). Thus, invoking periods of ketosis through the use of TRF, may result in positive cognitive outcomes.

Therapeutic use of healthful diets and aligning food intake with circadian rhythms can positively regulate metabolism, leading to improved cognition. The circadian system consists of daily circadian rhythms that regulates metabolism over a 24-hour period based on the sleep-wake cycle (Ahmad, 2020; Ravussin et al., 2019). The "circadian clock" in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus and other "clocks" located in the peripheral tissues respond to daily light-dark cycles and regulate a variety of metabolic processes (Froy, 2010; Poggiogalle et al., 2018). Metabolic processes such as energy expenditure, glucose tolerance, insulin sensitivity, and hunger hormones all follow a rhythm every 24-hours (Poggiogalle et al., 2018). Thus, metabolic disturbances in these processes can occur when circadian rhythms are interrupted, such as through erratic eating (Manoogian & Panda, 2017b; Poggiogalle et al., 2018). Cognitive dysfunction is affected among adults and adolescents in the presence of metabolic syndrome caused by multiple metabolic disturbances (Yates et al., 2012). In addition, neurogenesis functions on circadian rhythms (Tamai et al., 2008). Reductions in neurogenesis and hippocampal cell proliferation and decreases in learning and memory

performances can result from circadian disruptions and can continued to be impaired well after disruptions occur (Gibson et al., 2010).

Animal and human studies have also demonstrated the positive effect of TRF on circadian rhythms and brain function. Huntington's Disease mouse models with circadian dysfunction underwent 3 months of the 18:6 TRF diet and improvements were found in daily circadian rhythms and motor function (Wang et al., 2018). Thus, researchers concluded that TRF could be a useful strategy to align circadian rhythms and manage neurogenerative diseases. Additionally, four days of 18:6 eTRF in obese or overweight adults found increased expression of circadian clock genes while also improving evening BDNF and morning fasting glucose and insulin resistance (Jamshed et al., 2019). Our trial implemented early TRF, which may be more a more effective strategy for circadian alignment as omission of breakfast has been found to negatively affect clock-controlled gene expression (Jakubowicz et al., 2017).

BDNF is a neurotrophic protein utilized in neurogenesis maintenance and neuronal plasticity, which impacts learning and memory skills (Gyorkos et al., n.d.; Koven & Collins, 2014; Poulose et al., 2017). In order to accomplish this, BDNF binds to its high-affinity receptor tyrosine kinase B (TrkB), which leads to the initiation of proteins that activate different signaling pathways (Bathina & Das, 2015). These pathways lead to the stimulation of transcription factors that are able to influence gene expression that encode for proteins who have roles in neural plasticity, cellular stress response, and cellular longevity (Bathina & Das, 2015). BDNF not only plays an important role in brain health but is also vital in regulating metabolic processes and

energy maintenance. The expression of BDNF and TrkB have been found in the hypothalamus and dorsal vagal complex, which are critical regions for central and peripheral energy regulation (Gyorkos et al., n.d.; Pedersen et al., 2009; Yan et al., 1997). A randomized controlled trial among adults with metabolic syndrome adhering to a low-carbohydrate, Paleo diet intervention found inverse correlations between BDNF and body fat percentage, blood glucose, triglycerides, and insulin sensitivity (Gyorkos et al., n.d.). In addition, the intervention produced significant increases in serum BDNF levels and cognitive performance at four weeks compared to baseline, whether they were involved in exercise or not (Gyorkos et al., n.d.). This study did not find significant increases in BDNF production among the intervention group. Our study involved healthy individuals with no indicators of metabolic syndrome, so perhaps significant changes in BDNF would have resulted if the purpose of the study was to evaluate cognitively therapeutic interventions of TRF among those with metabolic syndrome.

It is well established in the literature that ketones act an effective source of energy to the brain in circumstances of depleted glucose, such as during fasting or extended exercise (Ota et al., 2016; Reger et al., 2004). The switch from glucose to ketone utilization is referred to as a "metabolic switch". Periodic metabolic switches are known to improve neuroplasticity, cellular resistance, neuronal adaptations to stress, and generate neurotrophic factors, such as BDNF (Mattson et al., 2018; Mattson & Wan, 2005). Especially among cognitively impaired older adults or Alzheimer's Disease patients, improved cognitive performance has resulted from increased ketone utilization (Krikorian et al., 2012; Ota et al., 2016; Reger et al., 2004). Pertaining to those with

cognitive impairment, ketone utilization may produce positive cognitive performance due to the fact that impaired glucose metabolism contributes to decreased cognition among those with Alzheimer's disease or cognitive impairment (Hoyer, 1992; Møller, 2020; Reger et al., 2004). However, even among healthy adults and young adults, increased ketone production has been shown to improve memory and executive function (Evans & Egan, 2018; Jensen et al., 2020; Ota et al., 2016). Interestingly, a study among older, cognitively healthy adults found a positive correlation between Trail Making Test B scores and plasma β-hydroxybutyrate levels, which opposes the result found in our study (Ota et al., 2016). Although no significant increases in ketones resulted from the present study, there does appears to be trend of increased ketone levels among our intervention participants, so perhaps significant results would have been resulted if the study duration was extended.

The use of ketones requires lesser utilization of NAD+ in energy production and decreased NAD+ availability has been associated with neurological disorders and neurodegenerative disorders (Verdin, 2015). In healthy, young adults, nutritional ketosis provoked by the consumption of a medium chain triglyceride supplement increased NAD+ and NAD+/NADH ratio (Xin et al., 2018). Thus, ketosis not only reduces glucose utilization in the brain but conserves the use of NAD+ and NAD metabolic pathways associated with improved inflammation (Elamin et al., 2017).

The Stroop Test is known for being an indicator of executive function and cognitive flexibility (Moering et al., 2004; Uttl & Graf, 1997). However, the present study demonstrated that the Stroop Test was a poor indicator of these cognitive domains

among students as a perfect score was obtained on average by week four. Other randomized controlled trials (RCTs) among young adults also did not produce statistically significant results using the Stroop Test while other cognitive assessments did produce significant improvements (Ahles et al., 2020; Byrn et al., 2019; Chung et al., 2012; Hidese et al., 2019). A recently published RCT looking at the effect of Long-Term *Aronia melanocarpa* Extract Supplementation on healthy, middle-aged adults (40-60 years old) after a 24-month period utilized the Stroop Test and no statistically significant effects of the treatment were found on Stroop Test performance. Similar to our results, scores steadily improved but between weeks 12 and 24 the scores had plateaued (Ahles et al., 2020). Thus, this may confirm the speculation that the Stroop Test is not ideal for testing healthy, young to middle-aged adults. In future studies, it may be beneficial to use different objectives while utilizing the Stroop Test in order to collect more accurate cognitive data. Rather than collecting data on accuracy of correct answers on the Stroop Test, response time of correct answers can be measured (Ludwig et al., 2010). The quickness of time to respond to color rather than correctness of answers alone may give us more insight into the participant's cognitive performance. A 4-week study among middle-aged adults with metabolic syndrome evaluated performance speed of the Stroop Test and found significant improvements while adhering to a carbohydrate restricted Paleo diet (Gyorkos et al., n.d.). Thus, in future studies using the Stroop Test, evaluating performance speed rather than accuracy may yield more definitive results.

The Trail Making Test (TMT) is used to test cognitive flexibility, which has been defined as the mental capability of switching between thinking about two different

concepts or to thinking about many concepts at the same time (Magnusson & Brim, 2014; Pilar, 2013). Unlike the Stroop Test, Trail Making Test B (TMT-B) did prove to be a useful marker of cognition in the current study. While the control subjects improved their scores from 48.9 ± 17.1 seconds to 46.1 ± 20.3 seconds, the intervention participants improved their scores from 45.5 ± 9.7 seconds to 31.1 ± 6.7 seconds. Our data indicated that the lowest score at 24.95 seconds on Visit 2, which was completed by an intervention participant. Mixed TMT-B results are seen in other studies among young, healthy populations. Normative data studies have found averages of 63.19 ± 28.64 seconds and 48.97 ± 12.89 seconds among young, healthy adults (P\lotek et al., 2014; Tombaugh, 2004). Tombaugh found that the minimum score among adults aged 18-24 to be 12 seconds (Tombaugh, 2004). No other TRF studies have utilized the TMT to test cognitive performance. However, other diet methods have utilized the TMT and have found mixed results. 3 months following the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet found significant improved in the TMT-A but not TMT-B among healthy, obese, middle-aged women (Arjmand et al., 2020). The implementation of a ketogenic meal saw improvements in Trail Making Test B scores among elderly adults (Ota et al., 2016). Interestingly, this study products contrasting results in that ketones were positively correlated to Trail Making Test B scores. A study evaluating outcomes of a mixed grain diet among high school students found that students both in the control and intervention groups produced significant improvements from baseline to week 9, but no differences between groups, which may suggest a learning effect (Chung et al., 2012). The average speed to complete TMT-B for the intervention among the high school

students at the end of the study was 24.07 ± 5.72, which was similar to the best score found in our study.

The study was originally powered at 80% with an estimated sample size of 30. However, despite of recruitment efforts, only 24 participants began the study and 17 students completed the study through all three visits. Recruitment limitations could have been associated with limited financial incentives, length of the study, timing of the study during a Holiday season, or appeared difficulty or low desirability of the study due to fasting. 6 individuals in the intervention group discontinued participation for reasons related to difficulty of the protocol, excessive hunger, and poor energy. Thus, feasibility of this protocol in daily living of university students despite improved cognitive scores can be put into question. There does not appear to be a trend of high attrition rates among populations of college students.

A similar study among metabolically healthy, college-aged men had high compliance with their 16:8 intervention, with only 1 dropout out of 23 participants (95% compliance) from the intervention group due to health reasons seemingly unrelated to the protocol rather than difficulty adhering to the protocol (McAllister et al., 2020). Data was not reported on participants' perceived difficulty in this particular study, but many participants remained in the study and cardiometabolic improvements were seen. Dissimilarly to our study which required participants to begin eating within one hour of waking, these participants were able to determine for themselves the specific time frame per day designated for feeding and asked to keep their feeding/fasting time period consistent. This study only lasted 4 weeks while our study involved an 8-week duration,

which may have been difficult for our participants to endure. Other TRF studies have been conducted with a stricter feeding time in the intervention group, but high compliance has been fairly consistent, and dropout was commonly related to issues not pertaining to the fasting protocol (Jones et al., 2020; Ravussin et al., 2019; Stratton et al., 2020; Sutton et al., 2018b). In a 18:6 eTRF experiment, most similar to our protocol, 7 out of 11 intervention participants completed the study (64% compliance), but reasons for drop out involved scheduling issues, personal or family reasons, or unrelated medical problems (Ravussin et al., 2019). However, this study only had a 4-day duration. In another 18:6 eTRF experiment, 100% compliance was observed and participants reported decreased hunger and desire to eat in the evening, which was during their fasting period (Sutton et al., 2018b). This study was 5-weeks in length; however, it incorporated a highly controlled feeding protocol in which participants received all their meals from the researchers. Additionally, unlike our experiment, in these two 18:6 eTRF experiments participants were required to eat isocaloric diets while participating in the intervention, which may have contributed to high compliance and lack of reported intervention difficulty. Our study allowed participants to consume food ad libitum during their feeding periods and perhaps they were not intentional about receiving enough calories during that period, leading to extreme hunger or fatigue. Interestingly, Sutton et al. noted that their participants found that it was more difficult for them to eat all their calories during the 6-hour period per day than to fast during the 18-hour period. These participants reported that a 7.8 ± 1.8 hour feeding period would be more feasible (Sutton et al., 2018b). Thus, a 16:8 protocol rather than the 18:6 may be a better TRF protocol for long-term adherence.

In looking at compliance rates and attrition among college students adhering to randomized controlled trials analyzing other health outcomes of similar duration, compliance rates varied from 68 – 96% (Antypa et al., 2009; Dhillon et al., 2018; Duan et al., 2017; H. Lee et al., 2014; McAllister et al., 2020; Pribis et al., 2012; Tan et al., 2015). In one 8-week study among college students, the intervention received 68% compliance, but when participants were asked to return for a follow up 1 month after the protocol, only 28% complied (Duan et al., 2017). Loss of interest has been reported to be a reason for drop out in randomized controlled trials among college students (Pribis et al., 2012; Zhu et al., 2013). In an 8-week study looking at walnut consumption among college students, six of seventeen dropouts occurred during the first week due to lack of interest in the study (Pribis et al., 2012). This is an interesting finding from this particular study, but when looking at multiple experiments involving college students, loss of interest does not far exceed other reasons for dropout, which include time constraints, personal reasons, scheduling conflicts, unrelated sickness, etc. Thus, more studies should be done comparing attrition rates of college students to non-collegiate adults, particularly looking at if loss of interest is a leading contributor to drop out. In addition, it would be interesting to investigate whether study completion incentives are driving force in compliance and if this is a greater motivator among college students.

Although the present study suggests that 18-hour TRF among university students may favorably affect cognition, limitations of the study must be addressed in the future. Controlling the day in which students take their "cheat day" should be addressed in future studies. As previously described, each week, students were given one day per week in

which they did not have to adhere to their fasting protocol. They were asked to keep this day consistent throughout the course of the study. Figure 3 displays which days of the week students self-selected their cheat days. 10 of the participants chose their cheat days within 1-2 days of their trial visit, which may have influenced the results. Thus, in the future, it would be best to advice students to select cheat days within 3 days from their testing days. In addition, many students changed their cheat days in between trial visits. Future researchers will need to instruct participants at their second visits to keep their cheat day consistent for their final visit.

Due to the COVID-19 pandemic, which arose in the middle of several participants' interventions, data analysis did not include week 8 data. Protocols and testing that can be implemented at-home will need to be created in order to adapt to social distancing measures and prevent inconsistencies. Thus, researchers in future studies can adapt the present study by designing tests that can be administered accurately over Zoom or by the participant at home. Due to the fact that the Stroop Test did not appear to be appropriate for the given participant group, changes to the way in which it was administered would need to be implemented. Testing Stroop performance speed rather than accuracy may give better indication of cognitive performance over time among young people. In order to improve compliance among participants, the 16:8 diet rather than the 18:6 may be implemented. In addition, asking participants to adhere to an isocaloric diet rather than consuming food ad libitum may decrease hunger and improve compliance. Finally, in future studies, allowing participants to decide their feeding

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window per day and adhering to it consistently may improve compliance and be more

feasible for participants with varying schedules.
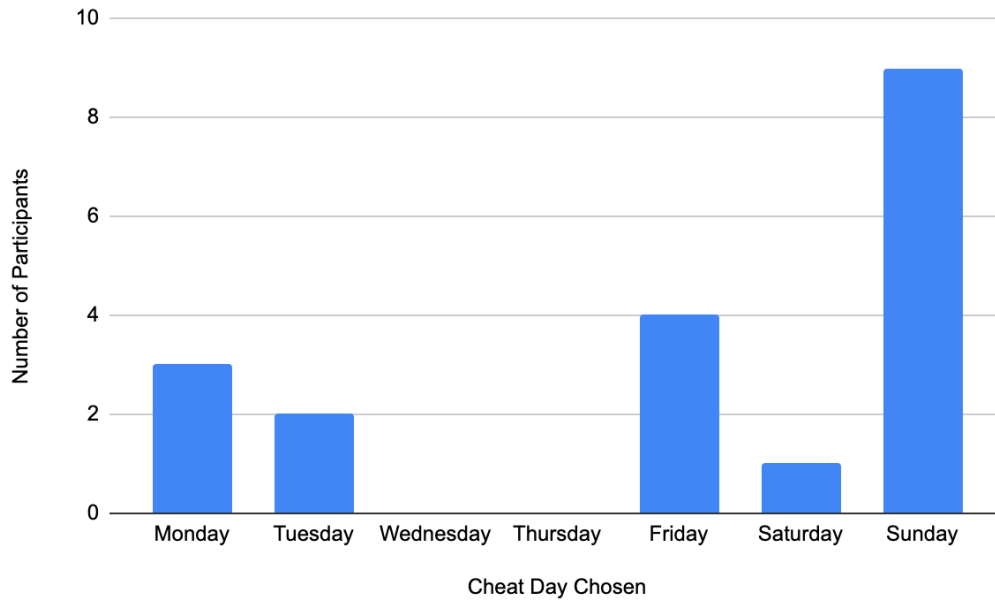
Figure 3. Self-Selected Cheat Days

CHAPTER 6

CONCLUSION

Cognitive performance will continue to be important to students and young adults as new challenges arise and they advance in their studies and careers. Late nights, often accompanied with late-night eating, is commonly associated with the lifestyles of university students, which may result in profound effects on their cognitive health. Therapeutic use of healthful diets and aligning food intake with circadian rhythms can positively regulate metabolism, leading to improved cognition.

Time-restricted feeding (TRF) is a form of intermittent fasting that appears to be the most feasible approach to sustaining a fasting regime. There is a growing amount of evidence to show that TRF has beneficial effects on a variety of factors such as weight, body composition, energy intake, glucose, insulin resistance, and blood pressure. Although TRF animal studies have produced positive results relating to brain health and cognitive performance, no TRF clinical trials exist directly address this.

The present study suggests that 18-hour TRF may produce favorable effects on cognition. The only hypothesis supported by the study was that TRF was associated with improvements in Trail Making Test B. High rates of attrition, underpowered study, and study interruptions due to the COVID-19 pandemic were a few limitations of the study that will need to be addressed with future research. Researchers can learn from the weaknesses of the current study and suggestions for improvements in order to design future experiments to rigorously test whether TRF improved cognitive acuity among

healthy, university students. Longer-term studies in larger and more heterogenous

populations are also needed.

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APPENDIX A

IRB APPROVAL

ASU Knowledge Enterprise
Development

APPROVAL: EXPEDITED REVIEW

Carol Johnston
CHS: Health Solutions, College of
602/496-2539
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 10/4/2019 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Mealtime Matters: An 8-wk Randomized-Controlled Trial to Examine the Effects of a Daily Time-Restricted Feeding Protocol on Diet Quality
Investigator:	Carol Johnston
IRB ID:	STUDY00010810
Category of review:	(2)(a) Blood samples from healthy, non-pregnant adults, (4) Noninvasive procedures, (7)(b) Social science methods, (7)(a) Behavioral research
Funding:	Name: Graduate College
Grant Title:	
Grant ID:	
Documents Reviewed:	• REAPS, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • 24-h diet recall, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Data release form, Category: Technical materials/diagrams; • survey monkey screener, Category: Screening forms; • POMS, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • trail making test, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);

	• calendar, Category: Participant materials (specific directions for them); • email for food diary, Category: Screening forms; • consent, Category: Consent Form; • generalized anxiety disorder measure, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • quality of life questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • oxford happiness questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • protocol, Category: IRB Protocol; • health questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • exit survey, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • verbal script and ad, Category: Recruitment Materials;

The IRB approved the protocol from 10/4/2019 to 10/3/2020 inclusive. Three weeks before 10/3/2020 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 10/3/2020 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:
     Kelly Chondropoulos
     Selicia Mayra
     Natalie Kopplin
     Anateresa De Leon

APPENDIX B

INFORMED CONSENT FORM

<div align="center">**Informed**
Consent</div>

Fasting and Health in College Students

INTRODUCTION The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS Dr. Carol Johnston (ASU Nutrition professor) and Selicia Mayra (registered dietitian and ASU doctoral student in nutrition), have requested your participation in a research study.

STUDY PURPOSE The purpose of this study is to investigate the effects of fasting on health including common blood biomarkers, disposition, and anthropometrics in college students.

DESCRIPTION OF RESEARCH STUDY You have indicated to us that you are a non-smoker and ≥18 years of age and healthy. You have not recently dieted for weight loss, regularly fast ≥ 12 hours per day, followed fasting regimens (i.e., alternate day fasting, Ramadan-style fasting, 5:2 fasting), and if female, you are not currently pregnant or planning a pregnancy, and you either have a regular cycle or are currently on hormonal contraceptives (i.e., birth control pills, IUDs, or patches). Also, you are not currently ill or taking prescription medications for a medical condition, and you do not work the night shift. You will be randomly assigned to a fasting arm of the study: the 18-hour fast or the 8-hour fast. You are asked to follow the fasting protocol 6 days a week for 8 weeks. You will select one day of the week (e.g., Friday or Saturday) as 'cheat day' for the entire study and on this day you do not need to 'fast'. You may eat the foods of your choice during the study, and we ask that you maintain your normal physical activities and not initiate a new exercise protocol.

This research entails three study visits to our test facilities on ASU's Downtown Phoenix campus for up to 60 minutes per session. You will be asked to complete diet recalls and mood/cognitive questionnaires and your height, body weight, blood pressure, and waist and hip circumference will be measured, and a small amount of blood (<2 tablespoons) will be collected from an arm vein during these three visits. No food or beverage aside from water is to be consumed for the 8 hours prior to this blood draw. Blood will be used to measure biomarkers related to cardiovascular health such as cholesterol and ketones.

You will receive a $25 e-gift card to Amazon once you have completed your final visit to our test facilities.

RISKS You may feel hungry and unfocused during the fasting periods; you are allowed to consume

unsweetened and non-caloric beverages such as water, coffee and tea during this period. The blood draw will be performed by trained staff (either a registered nurse or a registered radiology technician) under sterile conditions. You may feel lightheaded or nauseous when your blood is drawn and you may bruise; our staff are trained to handle such situations. You may feel discomfort when blood pressure is recorded due to the cuff constriction.

BENEFITS You may not benefit from this study, but once the study is complete you will be provided with your data, if desired. You will need to complete a study release form to receive your test results.

NEW INFORMATION If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or

identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

WITHDRAWAL PRIVILEGE You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not affect you any manner. We ask that you notify us in a timely manner if you decide to withdraw from the study, and we will ask you to complete the exit survey at that time.

COSTS AND PAYMENTS You will receive a $25 Amazon e-gift card for participation in this trial. There are no payments required for this study; however, you may need to pay for curbside parking at the test site (rate: $1.50/hour).

COMPENSATION FOR ILLNESS AND INJURY If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

VOLUNTARY CONSENT Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, 550 N. 3rd St., Phoenix, AZ 85004. [602-496- 2539]

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

_____ _____ _____ Subject's
Signature Printed Name Date

_____ _____ Contact
phone number Email

INVESTIGATOR'S STATEMENT "I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

APPENDIX C

RELEASE FORM

ASU College of Health Solutions

Nutrition Program

Research Results Acknowledgement Statement

This letter is to communicate your lab results from the ASU study: Fasting and Health in College Students

The result ranges given reflect acceptable general ranges and do not serve as definitive indicators of health or disease as the results of any test need to be evaluated in the context of other factors by a physician or clinician. Therefore, these results do not serve as a diagnosis of any kind for any medical condition and need to be interpreted by a physician or qualified health care provider. By signing below, you agree to share this information with your primary care physician to learn about how these results are relevant to your health and/or disease risk and discuss next steps.

If all your results are within the normal range please share these results with your physician or health care provider at your next routine checkup visit.

If you have results outside of the normal range we recommend you share and discuss these results with your primary care physician or qualified healthcare provider within 5 days. ASU Student Health Services is also an option for you to consider for access to care if you do not currently have a primary care provider.

ASU Health Services contact information:
• Phone 480-965-3349
• Online scheduling

APPENDIX D

RECRUITMENT FLYER

ASU STUDY SEEKS PARTICIPANTS:
FASTING AND HEALTH
THE ASU NUTRITION PROGRAM IS RECRUITING
HEALTHY COLLEGE STUDENTS
TO EXAMINE THE EFFECTS OF FASTING ON HEALTH

Participation is voluntary – **three study** visits are required, and you will be asked to fast either 18 hours/day or 8 hours/day for 8 weeks

Participation Includes:

- Three 60-minute visits to the Nutrition Research Facility (ABC1) at the downtown ASU campus (near 5th Street and Van Buren)
- At each study visit you will complete diet recalls and health questionnaires, body composition assessments, a single blood draw, and blood pressure testing
- Fasting is defined as no beverage or food with the exception of water and non-caloric beverages such as tea or coffee; however, there will be one 'cheat' day per week allowed each week of the study

You will receive a $25 Amazon card for your participation and study results
INTERESTED?? Please visit our recruitment site:
https://www.surveymonkey.com/r/FastForHealth

APPENDIX E

EMAIL SCRIPT

Hi, ...

My name is Natalie Kopplin and I'm a Master's student in the college of health solutions. I am currently recruiting participants for my thesis study that I believe some of your students might be interested in. I am writing to ask if you could post the recruitment flyer for my IRB approved research study on your Canvas platform?

Please let me know if there are any questions.

Warmest regards.

Natalie

ASU STUDY SEEKS PARTICIPANTS

FASTING AND HEALTH

THE ASU NUTRITION PROGRAM IS RECRUITING HEALTHY COLLEGE STUDENTS TO EXAMINE THE EFFECTS OF FASTING ON HEALTH

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· Fasting is defined as no beverage or food with the exception of water and non-caloric beverages such as tea or coffee; however, there will be one 'cheat' day per week allowed each week of the study

You will receive a **$25** *Amazon card for your participation*

INTERESTED?? Please visit our recruitment site:https://www.surveymonkey.com/r/FastForHealth

APPENDIX F

STUDY DESIGN FLOW CHART

Inclusion Criteria
- Healthy College Students currently enrolled at ASU
- 18 years or older
- Non-Smoking (vaping included)
- Normal to underactive (less than 420 minutes of physical activity per week)
- Not training or competition in sports
- Free of acute illnesses or diagnosed chronic illness
- ≥ 80 cm waist circumference for women
- ≥ 94 cm waist circumference for men

REAP-S Questionnaire
24-hour diet recall
Oxford Happiness Inventory
WHOQOL
Trail Making Test – A & B
Stroop Test
POMS
Blood Pressure & Blood Draw
Anthropometrics

In-person screening

Exit Survey Incentive Given

Intervention:
18 Hour Fast

Visit 1:
Baseline

Visit 2:
4 Weeks

Visit 3
8 Weeks

Trial Visits

Analysis of Data

Assessed for Eligibility

Randomization

Enrollment

Allocation

Control Group:
8 Hour Fast

Visit 1:
Baseline

Visit 2:
4 Weeks

Visit 3
8 Weeks

In-person screening

REAP-S Questionnaire
24-hour diet recall
Oxford Happiness Inventory
WHOQOL
Trail Making Test – A & B
Stroop Test
POMS
Blood Pressure & Blood Draw
Anthropometrics

Exit Survey Incentive Given

Exclusion criteria:
- Students who fast for 12 or more hours per day or adhere to a fasting regimen (at the time of the screening or within the past 6 months)
- Students who have attempted weight loss or experienced a weight change of 5 or more pounds within the past 6 months
- Students with a history of weight cycling
- Students who are currently working night shifts
- Students who are pregnant or lactating
- Students who are unwilling to adhere to the protocol
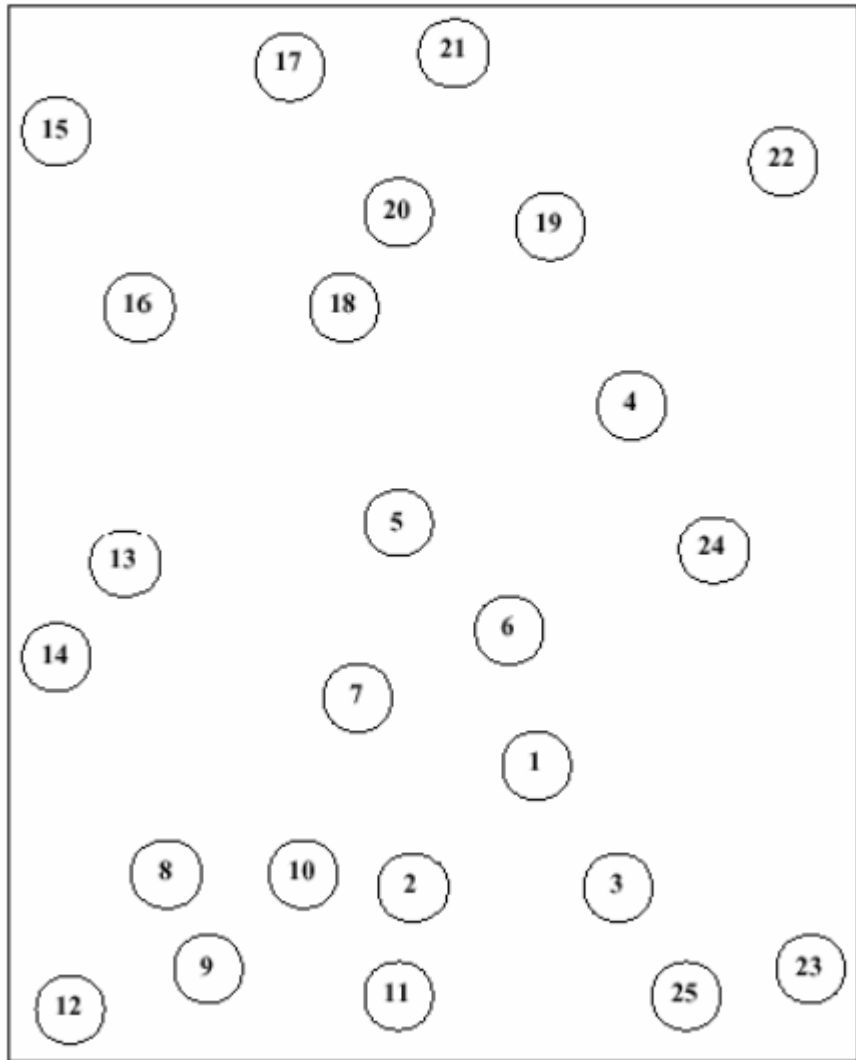
APPENDIX G

STROOP TEST

Green

APPENDIX H

TRAIL MAKING TESTS

Trail Making Test Part A
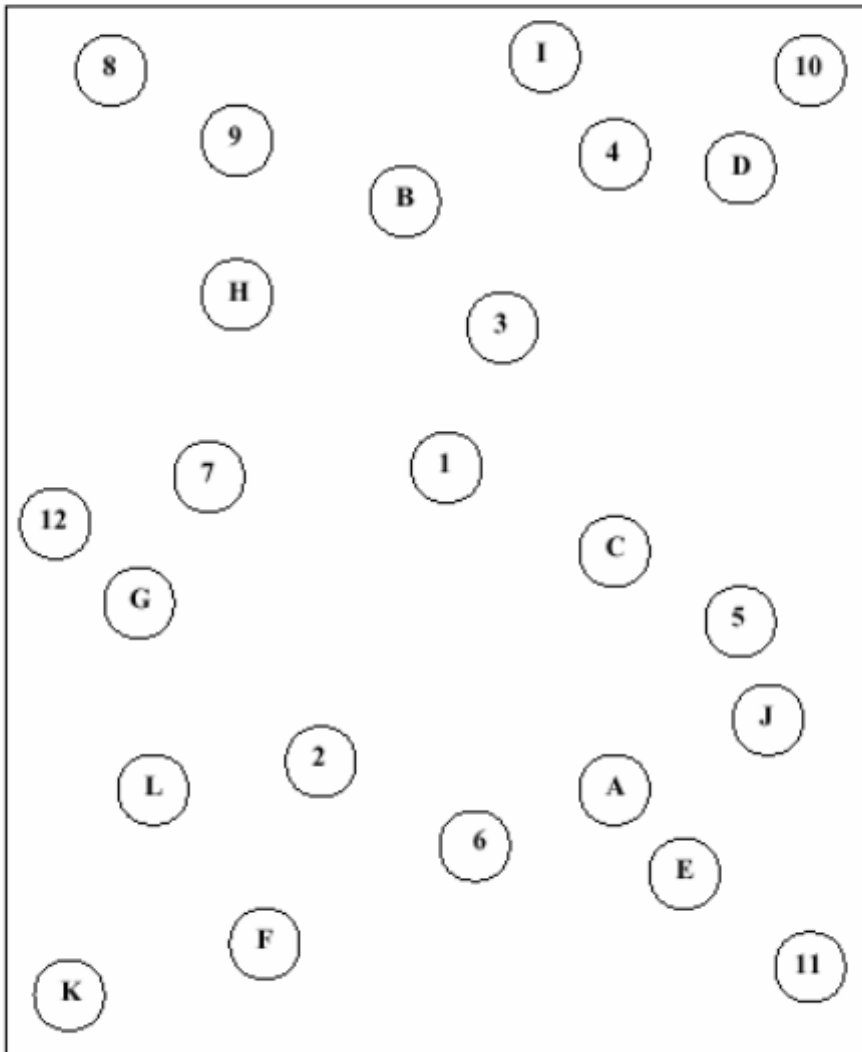
Participant ID: _____   Date: _____

Trail Making Test Part B

Participant ID: _____   Date: _____

APPENDIX I

SAMPLE SIZE DETERMINATION CHART

Author	Year	Change ± SD or correlation coefficient	n per group	Calculated n per group	Age range	Subjective health status
(Eather et al., 2019)	2019	10 ± 16	27	84	18-25	Healthy university students
(Mastroiacovo et al., 2014)	2014	16.5 ± 3	30	6	65-85	Healthy, nonsmoking, older adults with no memory concerns or impairments
(Sharma et al., 2019)	2019	24 ± 20	23	24	18-23	Healthy college students with a mean age of 21.96 ± 1.64
(Sungkarat et al., 2017)	2017	ND	33	46	62-74	Adults aged 60 and older who met Petersen's criteria for multiple domain MCI
	AVERAGE	**16.8 ± 13**	**28.25**	**40**	**40.75 - 51.75**	

*The power was set at 80% (0.2 error level) with an alpha error level of 0.05