A Study to Investigate the Cognitive Changes that Occur Following Keto-Adaptation and Resistance Training in Healthy Adults

THESIS

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Abstract

Purpose: The purpose of this study was to investigate the effects of keto-adaptation via a KD and a strength-power resistance training program on cognitive performance in healthy adults in both a rested and physically stressed state.

Methods: Twenty-nine subjects (25 males, 4 females) were placed in either a ketogenic diet (KD) group (N=15) or a high carbohydrate (CON) group (N=14). Resting cognition was measured using the Automated Neuropsychological Assessment Metrics (ANAM) computer battery and physically stressed cognition was measured using a symbol digit modality test (SDMT) before and after high-intensity sprints on a self-propelled treadmill (HiTrainer). These measures were assessed at baseline, and following a 9-12 week dietary and strength/power intervention.

Results: No significant difference between groups for diet was observed for resting or physically stressed cognition. For resting cognition within the KD group there existed a significant correlation between acute ketone levels prior to testing and percent change from pre to post for the tests Code-substitution delay and Go/No-go, which test aspects of delayed memory and inhibition. The strength/power intervention produced significant increases for some of the variables in both resting and physically stressed cognition. For resting cognition the tests code substitution - delayed and procedural reaction time, which are associated with learning, delayed memory, and processing speed, improved significantly from pre to post. For physically stressed cognition, the amount of correct answers on the 3rd set of SDMT following the final sets of sprints improved significantly from pre to post.

Conclusion: Exercise focused on strength and power seems to beneficially effect cognition at rest and when physically stressed. KD did not improve cognition, but some aspects seemed to be directly affected by acute ketone levels. Further research into both areas and their effects on cognition are needed.

Dedication

This document is dedicated to my loving family and friends for all the support they have given me over the years.

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I would like to offer a sincere thank you to my advisor Dr. William Kraemer. While I may not be a conventional Master's student that will go on to a PhD or use this degree directly in my career, this has been an exceptional experience for me and the skills and knowledge gained will serve me very well moving forward, in both my career and my life. It has already opened doors for me that may not have otherwise been there, and I believe that will continue to be the case throughout my career.

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Publications

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Fields of Study

Major Field: Kinesiology

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Chapter 1: Introduction

The capability of the human body to adapt in times of extreme stress in order to survive and sustain function is quite remarkable. Possibly one of the most impressive examples of these capabilities is shown in times of heavily restricted or complete caloric restriction. In these times of famine where no calories are being supplied from food to create energy, the body is forced to rely on other sources in order to continue operating. Fortunately the body has stores of glycogen, protein, and fat that can be used for energy.

Glycogen is broken down into glucose which supplies four kcal/g of energy (1). Glucose is the preferred source of 'quick' energy as it can be utilized by all cells in aerobic and anaerobic conditions. It is also the preferred source of energy for the brain due to it having the ability to cross the blood-brain barrier. However, glycogen stores in the body are limited with only ~2,000 kcal stored in the muscle and liver (83). The brain by itself consumes approximately 100-145 grams of glucose per day (14), meaning that there is only enough glycogen to support the brain for roughly five days. This does not even take into account the energy needed from all other cells in the human body.

Protein stores are greater in the body than glycogen, with energy reserves of approximately 25,000-35,000 kcal (83). Protein can be catabolized into amino acids that, if glucogenic, can then be converted to glucose via gluconeogenesis in the liver and kidneys. The amino acids alanine and glutamine are the most common amino acids precursors for gluconeogenesis (12, 29). Once converted to glucose it can be released into the blood to be utilized by all cells. If glycogen stores are depleted, and gluconeogenesis from protein stores

was required to supply the brain its energy, 500 grams of muscle would be catabolized each day (13). This catabolism can cause a negative impact. First, the stores of protein would quickly run out; also catabolizing that much protein would severely limit muscles capability to generate force (14).

Fat, the last source of fuel stored within the body, is the most plentiful with ~30,000 kcal in a thin individual to ~200,000 kcal or more in an obese individual (83). It also supplies the most energy per gram of the three fuel sources at nine kcal/g (1). In a normal fed human, fat has two ways of supplying energy to cells. Fat is stored as triglycerides, which have a glycerol backbone with three attached fatty acid chains. The fatty acid chains supply free fatty acids (FFA) which are broken down to acetyl-Coenzyme A (CoA) via beta-oxidation in the mitochondria of cells, which may enter the citric acid cycle to create energy. Most cells in aerobic environments can utilize FFA as a fuel source. The important exception is the brain, as FFA in the blood are not able to cross the blood-brain barrier. The glycerol backbone is similar to glucogenic amino acids in that it is a precursor that can undergo gluconeogenesis, and thus, supply its energy as glucose. However, glycerol only makes up approximately ten percent of the fat stores in the body (13).

Following the depletion of glycogen stores, the body's main remaining sources of glucose are via gluconeogenesis of amino acids and glycerol. With the importance of sparing protein, and the limited stores of glycerol, glucose-dependent organs would quickly run out of fuel if these were the only endogenous supplies of energy. Fortunately, the body compensates for this with the ketone bodies acetoacetate (AcAc) and beta-hydroxybutyrate (BHB), which are produced from FFA in the liver. Ketone bodies are unique in that they have the ability to cross

the blood-brain barrier and supply fuel to the brain. In a state of starvation, the body goes into a state of 'ketosis' where ketone production increases, peripheral use of ketone bodies decreases, and the kidneys adapt to help conserve ketones (13). The timeline for keto-adaptation is varied, but typically takes about two weeks (14). After adaptation occurs, ketone bodies can replace glucose as the primary fuel of the brain (11, 63), supplying nearly two-thirds of its energy needs (49). Given water is available and the environment is a temperate climate, the production of ketone bodies allows for long-term survival despite starvation. In this situation, non-obese individuals can survive for two to three months, and obese individuals even longer (14).

While starvation is not a desirable state, intermittent fasting and caloric restriction can produce similar effects and have been linked to health benefits including increased longevity and resilience against age-related diseases. It is believed that ketone bodies could play a role in these benefits, specifically BHB (61). One of the more intriguing methods of increasing circulating ketones is by eating a ketogenic diet (KD). This consists of a diet that is very low in carbohydrates (~50 g/day), which severely limits insulin production (49). Low insulin and low glucose availability allow for the production of ketones. An individual following this diet would enter a state of 'nutritional ketosis' and have circulating blood ketone levels in the range of 0.5-4.0 mmol/L, compared to the normal amount of the less than 0.2 mmol/L (64). The KD has been investigated and used as a therapeutic tool for multiple conditions including insulin-resistant conditions (55), neurological disorders (60), and cancer (53) to name a few. In addition to its benefits in clinical populations, a KD that is not calorie restricted while also meeting the daily protein needs has also been shown to be effective in healthy adults (66) and athletes (83). The production of ketones in any sense is a means of supplying an alternate fuel source to glucose-

dependent organs, most notably the brain. When in a state of nutritional ketosis, ketones have the ability to become the predominant fuel source for the brain. With such a shift in brain metabolism, it is worth inquiring how cognitive performance might be affected.

Exercise is another domain that in ways mimics the effects of starvation on the body, in that it increases the mobilization and use of stored fuels in order to meet increased metabolic demands. Glucose, fats, and ketone bodies can all be utilized during exercise in both the muscles and the brain (21). It is interesting to note that chronic exercise seems to positively affect the brain, with it being associated with increases in cognition in various studies for both aerobic (4) and anaerobic (48), however much of the focus has been on aerobic thus far. Additionally, for both aerobic and anaerobic the literature on exercise and cognition is predominately in older populations. The utilization of resistance training programs is becoming much more common in all populations, and with that so is research into its health benefits. How resistance training might impact cognitive performance in healthy adults is an area that is worth exploring further.

The purpose of this study was to investigate the effects of keto-adaptation via a KD and a strength-power resistance training program on cognitive performance in healthy adults in both a rested and physically stressed state. We hypothesized that both keto-adaption and resistance training would improve performance for both states.

Chapter 2: Literature Review

Ketogenic Diet

Ketogenic diets, as well as other low carb diets, have been the subject of much controversy and research recently. While the diet has been utilized for as far back as the 1920s, large-scale research did not flourish until roughly forty years ago when the 'fad' of low-carb diets, such as the Atkin's diet, began. Since that time, much research has been conducted, and many promising applications have ensued. Many of these applications have shown promise in improving brain function. However, most of these results have been in populations with clinical pathologies, and research on the impact of a KD in terms of cognition in a general population as well as athletes is lacking. Further research into these populations may result in broader applications, and may also help to identify the potential mechanisms behind the positive effects shown in other studies.

Safety

One of the main barriers to studying a KD, and convincing people of its benefits, has been safety. This wariness of a high fat, low carb diet seems to be attributed to two factors: the stigma placed on fats; and the negative association to the word 'ketosis'.

Within the population, foods high in fat are typically seen as unhealthy and the thought of a diet consisting of high-fat and low-carb is not seen as beneficial. In a study investigating the social stereotypes that exist within consumers about low-fat versus high-fat diets, it was found that individuals consuming a high-fat diet were significantly viewed as more unhealthy, overweight, unfit and inactive versus individuals consuming a low-fat diet (3). This stigma is

not aided by the latest release of the *Dietary Guidelines for Americans 2015-2020,* which recommends that at least 45% of calories come from carbohydrates (79). Likewise, these dietary guidelines have no mention of ketogenic or low-carbohydrate alternative options. This is despite multiple studies showing that consuming a KD is safe and does not negatively impact health (8, 17, 18, 66).

The other safety concern that tends to accompany a KD is that the word 'ketosis' is commonly associated to the medical emergency known as diabetic ketoacidosis. Diabetic ketoacidosis is a pathological state that occurs in type 1 diabetics in the complete absence of insulin. In this state, glucose in the blood cannot be taken up by cells to be metabolized, leading to it building up in the blood. The body increases lipolysis, production of ketone bodies, and gluconeogenesis to supply cells with fuel substrates. Without insulin, the amount of ketone bodies produced is not regulated and are overproduced. This subsequently leads to a drop in blood pH, dehydration, decreased blood volume, demineralization, and loss of muscle and bone mass (57). Without treatment, ketoacidosis can result in coma or death. Nutritional ketosis, which is the result of consuming a KD, produces ketone levels in the range of ~0.5-4 mmol/L (64). Ketone levels in diabetic ketoacidosis are ~10+ mmol/L and can be as high as ~25 mmol/L (81). Being in a state of nutritional ketosis would never result in ketone levels of such magnitude allowing ketoacidosis to occur. There exists feedback mechanisms that regulate ketone production, typically via insulin. Therefore nutritional ketosis is a metabolic physiological state that is both regulated and healthy (57).

Brain Metabolism

The two main ketone bodies that are produced when in a state of nutritional ketosis are AcAc and BHB. Both have the ability to be taken up by most cells in the body to be used as a fuel substrate. However, the true importance of ketone bodies as a substrate lies in the fact that they can cross the blood-brain-barrier and be used an energy substrate for the brain. The two most prominent fuel sources in circulation for the body are glucose and FFA, stored in the body as glycogen and triglycerides, respectively. While the glycerol component of the triglycerides can undergo gluconeogenesis to become glucose and cross the blood brain barrier to be used by the brain, the FFA cannot. Only after being converted to ketone bodies in the liver are FFA then able to cross.

Metabolism in the brain is significant in the human body. The brain only represents approximately 2% of the body's total weight, it consumes nearly 20% of its total calories (13), which is typically equal to about 400 to 570 calories per day. In an individual consuming a standard high carbohydrate diet, nearly all the energy for the brain comes from glucose, which equals about ~100 to 145 grams per day (14). As ketone levels rise in the blood, they may begin to replace glucose as the main fuel source for the brain. In a healthy adult, the liver can make up to ~185 grams of ketone bodies per day (49). Most of the energy demands of the brain are utilized to maintain the ion gradients of the pre- and post-synaptic space needed for glutamate neurotransmission as well as to maintain the neurons resting potentials for both excitatory and inhibitory pathways (21).

Transport of glucose in the brain is carrier-mediated by different glucose transporters (GLUTs). The GLUT1 brings glucose across the endothelium of the blood brain barrier, a different isoform of GLUT1 transports glucose into the astrocytes, and GLUT3 transports glucose into the neurons (34). With the amount of glucose transported into the brain being dependent on the intracellular and extracellular concentrations. Both GLUT1 and GLUT3 have high affinities for glucose transport, with a km (Michaelis-Menten constant) of ~1-2 mM for both (34). Activation of the brain results in a decrease in intracellular glucose and ATP concentrations, leading to an increase in the amount of glucose that is transported across the blood brain barrier from the blood stream (21). In a rested state, glucose goes through glycolysis and then through the tricarboxylic acid cycle and is typically fully oxidized to carbon dioxide and water. However, through glycolysis, pyruvate can also be converted to lactate and either oxidized elsewhere in the brain, or exit across the blood brain barrier into the bloodstream (21).

The transport of ketone bodies across the blood brain barrier is slightly different compared to glucose. Ketone bodies cross via monocarboxylic acid transporters (MCTs), of which there are multiple types. The capillaries allowing entry across the blood brain barrier express MCT1, astrocytes express MCT4, and neurons express MCT2 (65). Transport of ketone bodies across the MCTs requires the co-binding of a proton and the ketone body, translocation across the membrane of both, and a subsequent release of the ketone body. The rate-limiting step of this process is the return of the proton carrier. Transport can occur in either direction and is controlled by pH and concentration of ketones. Ketones will travel from high to low pH and from high to low concentration (67). The affinity of transport for ketones vary for different ketone bodies. BHB has Km's of 12.5 mM and 1.2 mM for MCT1 and MCT2, whereas AcAc's

are 5.5 mM and 0.8 mM, respectively. MCT4, which is located on the astrocytes, has a low affinity for ketone transport (65). As astrocytes are more glycolytic than neurons, it seems MCT4 operates more for the export of lactate, another monocarboxylate, rather than the import of ketones. Once transported into brain cells, ketones are either already in the form of AcAc. If they are in the form of BHB, they are converted into AcAc. From there, AcAc is then broken down to acetoacetyl CoA and then to acetyl CoA, which can enter into the tricarboxylic acid cycle to make energy in the form of adenosine triphosphate (ATP) (21, 49). The amount of energy contributed from ketones to brain metabolism has a direct relationship to the concentration circulating in the blood. As ketone levels rise, they will contribute a greater proportion of the brain's energy demands. At a level of ~0.3-0.5 mM, ketones supply ~3-5% of the brains energy, at ~1.5 mM they supply about ~18%, and at levels of ~4 mM or higher they supply ~60% (21). The MCT's also may play a role in the increased use of ketones for brain metabolism over time. Pierre and Pellerin (67) found that utilizing a KD in adult rats induced an increase in MCT1 expression in the brain.

Ketones have the ability to become an important contributor to brain metabolism, but are not able to replace glucose entirely. Some brain cells, such as astrocytes, are more glycolytic. In fact, glucose is essential to supply the carbon for oxaloacetate, which is needed for ketones to undergo the tricarboxylic acid cycle (21). There are also small amounts of glycogen stores in the brain which may serve as being more than just a supply of glucose. In fact it has been shown that blocking degradation of brain glycogen caused decrements in glutamatergic neurotransmission, effecting the balance of the excitatory and inhibitory neurotransmitters, even in the presence of glucose (72). Fortunately, the glucose needed for glycolytic cells, as well as

brain glycogen stores, are maintained on a KD. This is done via gluconeogenesis and the small amount of glucose still retained in the diet itself (64).

Neuroprotective Properties of Ketones

It has been well established that ketones are able to act as a fuel substrate to satisfy the metabolic needs of the brain. However, as research continues it is becoming apparent that ketones may provide additional neurological benefits. One of the first modern uses of a KD was in the 1920s by Dr. Russel Wilder as a treatment for epilepsy in children (85). The KDs success as a treatment led to it becoming a popular treatment option by the 1930s. The antiepileptic effects of the KD are profound. A recent study shows that over half of the subjects consuming the diet saw a 50% reduction in seizures by six months, with 20% having more than a 90% reduction (46). Lately, there has been a wealth of research investigating the use of a KD as a potential treatment option for other neurological disorders. The KD has been shown to improve symptoms and possibly delay progression for Parkinson's Disease (80), improve cognitive function for individuals with Alzheimer's Disease (37), improve mild cognitive impairment in the elderly (47, 69), and even enhance recovery following traumatic brain injury (54). The exact mechanisms for how a KD effects these various neurological disorders are still not all known, despite being studied as a treatment for epilepsy for almost one-hundred years. It is thought that ketones may be providing an alternate fuel source to glucose, which could be impaired in many of these neurological disorders. However, the diet has also been shown to have multiple neuroprotective effects, of which may play a role as well.

Energy Efficiency

Compared to glucose, the metabolism of ketone bodies in the brain seems to be favorable. Ketones must enter the mitochondria and undergo oxidation in the tricarboxylic acid cycle in order to create ATP for the cell. Glucose and ketones can both be oxidized to ATP through the tricarboxylic acid cycle, but ketones are more efficient, generating more ATP per unit of oxygen (81). Not only are ketones more efficient at creating ATP in the mitochondria, but increasing them in circulation while on KD leads to increased mitochondrial biogenesis in the brain and upregulation of mitochondrial proteins (6). A study investigating energy stores in cerebral slices of rats on a KD found increases in brain glycogen stores, ATP/ADP ratio, and no change in PCr concentrations (26). This was related to a significant increase in the total cerebral energy reserve, which is suggested to make the brain more metabolically stable and able to withstand fluctuations in blood glucose levels and body temperature. This increased metabolic stability may lead to an increase in the overall resilience of the neurons (26, 33).

Oxidative Stress

A normal byproduct of oxidative metabolism in mitochondria are reactive oxygen species (ROS), a product that when in excess can cause oxidative stress and damage to neurons (44). Ketones seem to have strong anti-oxidative properties, that both decrease the amount of ROS released as well as upregulate products that are involved in destroying the produced free radicals. ROS released from the mitochondria are decreased in a KD by various means. These include increased oxidation of co-enzyme Q and NADH, increased expression of certain uncoupling proteins that lower mitochondrial membrane potential, and increasing levels of glutathione peroxidase, which reduces the free radicals to alcohols and water (33, 44, 51).

Glutamate – Glutamine Cycle and GABA

Glutamate is a major excitatory neurotransmitters in the brain. Glutamate does not cross the blood brain barrier, thus it must be synthesized and controlled within the brain through a highly-regulated process (88). Glutamate is released into the synaptic space as the neurons depolarize. Following this, glutamate is removed by the astrocytes to be converted to glutamine. Glutamine is then sent back down to the neurons which convert it back to glutamate via the mitochondrial enzyme glutaminase. This process allows glutamate to be stored and ready for the next depolarization. The exchange of glutamate and glutamine between the neuron and astrocytes is known as the glutamate – glutamine cycle. A portion of the stored glutamate within the neurons is synthesized into another excitatory neurotransmitter, aspartate, while some is synthesized into GABA, a major inhibitory neurotransmitter in the brain. Having an adequate supply of GABA is vital, as excess excitatory neurotransmitters in the synaptic space can lead to excitotoxicity and neuronal damage (88). The process of converting glutamate into aspartate involves oxaloacetate, a product that is also used in the tricarboxylic acid cycle. Utilizing a KD has been shown to increase metabolism through the tricarboxylic acid cycle, which limits oxaloacetates ability to convert glutamate into aspartate (50). This process leaves more glutamate available to be converted to GABA, which offers more protection against neuronal damage as a result of excitotoxicity (33). GABA has also been shown to be connected to cognitive processes in the brain. Studies have shown that increases in GABA are linked to increases in working memory, impulsivity, and learning (70).

The neuroprotective properties that result from utilizing a KD suggest that this diet strategy may have potential means of treating and preventing many of the pathological

neurological disorders mentioned above. However, these properties occur in non-clinical populations as well and may offer benefits to cognitive performance.

Resting Cognition - KD

Much of the body of research pertaining to the KD and cognition focuses on a diseased population. Cognitive decline from dementia is one of the main symptoms that occurs with Alzheimer's disease (AD) (15). One of the potential mechanisms theorized to cause this decline are malfunctions in glucose metabolism pathways in the brain (21). Studies in both animals and humans have shown that Alzheimer's Disease can cause the metabolic rate of glucose for the entire brain to decrease by 24% percent. Subsequently, lower rates of glucose metabolism are correlated with decreases in cognition (37). This has led to interest in whether a KD can help to supplement the decrease in metabolism of glucose by increasing the levels of ketones. A study by Henderson et al.(38) investigated this in a clinical trial of 152 patients diagnosed with AD. They used a double-blind crossover design utilizing a ketone and a placebo supplement. The ketone supplement resulted in significant increases in cognitive measures in the patients utilizing the supplement compared to the placebo. These results show promise that a portion of the decreases in cognition may be diminished by supplying an alternate fuel source to glucose. It should be noted that the ketone levels in this study were raised via an oral ketogenic compound rather than a KD. In a similar study, elderly individuals who were identified to have probable Alzheimer's Disease and were currently experiencing mild to moderate cognitive decline were investigated (69). Similar to the study by Henderson et al. (38), this study utilized a double-blinded, crossover design using a meal designed to increase ketones, and a standard meal as a control. Following the consumption of the ketogenic meal, cognitive improvements were

observed versus the control, but only in subjects who were negative for the APOE ε4 allele. Being positive for this allele is associated with an increased risk for developing Alzheimer's Disease. This study only involved the consumption of one meal and only raised ketones to 0.68 mmol/L which is on the lower threshold of ketosis. Further research is needed for both acute and chronic ketosis, from both supplements and diet, to further understand potential mechanisms and treatments in this population.

Cognitive decline is not only seen in clinical populations, but also commonly seen as a normal negative association to aging (73). As we get older, it is expected for many domains of performance to decline, including cognition. It is interesting to note that a similar negative association exists between age and cerebral glucose metabolism (87), offering a potential contributor to the decreased in cognitive performance. A study conducted in elderly subjects observed the effect of raising ketones via a ketogenic meal on cognitive function (62). Following this one meal, there were significant improvements within the cognitive domains for working memory, visual attention, task switching, and integrative cognitive function compared to a control meal. While this investigation involved acute ketosis, a similar result was found in a study conducted on aged rats that were fed a KD over a span of three weeks (86), suggesting that the benefits could also be obtained over longer lengths of time.

Currently, a common use of a KD is for weight loss in individuals who are overweight and obese. Concurrent studies looking at weight loss and other health parameters have occasionally observed the effects of the KD on cognitive performance as well but with mixed results. In rats that were fed a KD versus a high-carb western diet to induce obesity it was found

that the western diet caused deficits in hippocampal-dependent cognitive functioning, deficits that seemed to be deterred by the presence of ketones in the rats consuming the KD (25). However, this particular study is contrary to the body of literature, which normally promote weight-loss and its effects on performance. Following 24 weeks of a low-carb versus a high carb diet in humans, both groups saw improvements in attention and information processing while attaining similar weight loss (52). However, there was no difference observed between the groups and no difference for time or diet in tests for reaction time, problem solving, memory, or cognitive flexibility. It should be noted that the diet was closer to a typical Atkins diet, and very likely did not restrict carbohydrates enough to induce ketosis. In a similar study, which utilized an appropriate KD for 52 weeks, no significant differences between groups were observed following the intervention for the cognitive variables of working memory and speed of processing (9). However, a separate study in obese subjects following an eight-week dietary intervention assessed working memory and processing speed. The authors found a significant difference between high and low carbohydrate groups processing speed, with the high carbohydrate group performing better. However, it is worth questioning the chosen dietary intervention and its deviation from a typical KD. The percentage of protein prescribed for the low carbohydrate group was significantly higher compared to high carbohydrate group (35% versus 24%), exceeding the typical ketogenic recommendation of 15-20%. It is very difficult to make any conclusions based on these studies as the methodology within them are very inconsistent, specifically in the basis of whether the diets can be considered ketogenic. More research within this population in regards to cognition is important, however even then it would

be difficult to make conclusions with obesity's negative association to cognition (42) and the fact that most of these studies aims involve calorie restriction and weight loss.

Despite numerous investigations involving clinical, elderly, and obese populations, a dearth of studies have been conducted exploring the potential cognitive effect of a KD in a young and healthy population. Of these few studies, results show limited promise. In two studies utilizing healthy young sedentary men, both were shown to have significant decreases in the cognitive measures of reaction time and attention in the groups utilizing a KD compared to a standard high carbohydrate control diet (27, 40). Both authors concluded that high fat diets had significant negative effects on cognition. While both studies were technically correct in their claims, they shared massive defects in their methodologies. First, the interventions were only for seven and five days, respectively. Previous research shows that it takes at least two weeks to become keto-adapted (14, 49). Second, the content and makeup of the KD interventions were not consistent with traditional KD macronutrient profiles. Due to these limitation, the results must be interpreted with caution.

Similar detriments have been shown in cognition following a KD in two studies utilizing rats (58, 89). The results of Murry ate al. (58) are questionable, as the two diet groups were fed the same weight in food (18-20 g/day), which resulted in the high-fat group consuming a significantly greater amount of calories (98 vs 65 kcal/day). The authors later conducted an additional study in rats comparing a KD versus a high carbohydrate diet and a moderate carbohydrate diet (59). This time, however, the diets were controlled for calories and it was found that the KD had significantly improved cognition compared to the other two diets. The

second study by Zhao et al. (89) claimed that the KD caused impaired memory and learning in young developing rats as well as stunted brain growth. Their results were called into question by Drs. Cunnane and Likhodii (22), as they claimed the KD supplied had a fat to protein + carbohydrate ratio that was more than double any version of the KD that had been previously prescribed to children. The authors contend that the imbalance in macronutrient ratios leading to malnutrition may be the cause of the negative results.

Based on the dearth of investigations that utilize a properly formulated KD in healthy populations, it is clear further investigation into the KD effect on cognition is warranted. Based on the extensive research that shows the benefits of ketone metabolism in the brain, it is our hypothesis that a KD will show greater increases in multiple domains of resting cognition compared to a high carbohydrate control diet in healthy adults.

Physically Stressed Cognition - KD

Athletes are an interesting population in which to study the effects of a KD on cognition. Intense exercise causes an increased metabolic demand throughout the body, including within the brain. While competing, athletes activate multiple cerebral domains for both the sensorimotor aspects, as well as cognitive aspects of the sport itself (23). As the intensity of the exercise increases, athletes experience fatigue, which can negatively impact multiple indices of cognition (39). Depletion of the glycogen in muscle, as well as hypoglycemia, may lead to a phenomenon known as central fatigue (10). Central fatigue may be involved in the cognitive decline that is often seen with intense exercise (39). Metabolic ratio is frequently used when investigating cerebral metabolism as a potential means of decreasing cognition with intense

exercise. This is due to global cerebral blood flow and cerebral metabolic rate of oxygen remaining unaffected during exercise (24). The metabolic ratio thus looks at the amount of oxygen taken up in the brain versus that of carbohydrate to see how much is oxidized. Normal MR at rest is 6, with decreases seen for brain activation. With exercise, MR may drop to 3 or below (24). This decrease in oxidized carbohydrate during exercise may offer an explanation for the decrease in cognition.

Studies utilizing MR as a means of investigating brain metabolism typically do not explore other fuel sources. In fact, Dalsgaard et al. state that "cerebral metabolism depends on oxidation of carbohydrate; the brain uptake of other energy sources, e.g. ketone bodies, amino acids, and FFA, is of little quantitative importance" (24). This may be true for an individual on a standard high-carbohydrate diet. However, for an individual consuming a KD, ketone bodies would no longer be negligible when investigating brain metabolism as blood ketone levels have been shown to increase during exercise (28, 30, 31).

Multiple investigations show that athletic performance is not hampered by consuming a KD (83). For high level athletes, including tactical athletes such as military and police members, multiple indices of cognition prove to be important to performance. Specifically, being able to quickly process and remember information and make quick and accurate decisions while performing at these high intensities are essential for performance. Due to exercise creating an increased metabolic demand that has been seen to decrease cognition, a study to investigate whether consuming a KD can deter this detrimental effect is warranted. As ketones are an additional fuel source that can spare glycogen, our secondary hypothesis for this investigation

would be that individuals who are in a state of nutritional ketosis will show less cognitive decline versus those on a standard high carbohydrate diet, following high intensity exercise.

Resistance Training

Resistance training (RT) is a subset of exercise that focuses on applying a resistance to induce muscular contraction. This type of training can lead to increases in strength, muscular endurance, and muscular size depending on the manipulation of program variables. In order to focus on strength, typically high-weight, low loads are utilized. Actions of the muscle are driven by the brain, with the motor cortex creating the neural impulse that travels down the neuronal network to the muscle to make it contract. Research seems to suggest that the relationship between the muscle and the brain for resistance training extend beyond just motor control.

Resting Cognition – RT

To this point, most of the studies investigating cognitive changes as a result of RT are in older adults. Within this population, there exists some conflict as some intervention studies have shown improvements in memory and attention (48, 84), whereas others have shown no cognitive changes (78). A study comparing differing intensities of resistance training saw increases in memory, but only for individuals using a higher resistance (48). In longitudinal studies, increased anaerobic exercise at baseline was associated with less cognitive decline and a decreased risk of cognitive impairment, dementia, and Alzheimer's Disease (7, 76). It is possible that the parity in the results of the intervention studies for anaerobic exercise and cognition is likely is due to the variance in both the length and intensity of the interventions used. Higher-intensities may be needed to see cognitive changes.

The mechanisms behind possible improvements in cognition are possibly related to IGF-1, which is shown to be increased by RT (5). IGF-1 has been shown to prevent tissue loss within the brain as well as increase brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) concentrations (19). These three molecules seem to all have the ability to effect cognitive performance. IGF-1enhances synaptic plasticity (16), BDNF is associated with neurogenesis (82), and VEGF allows for vessel growth (16).

Due to the beneficial effects that RT seem to have on the brain, it is our hypothesis that both groups will see improvements in their cognition following a strength/power intervention.

Physically Stressed Cognition – RT

As stated previously, high-intensity exercise has the ability to negatively affect cognition acutely. However, studies have shown that the extent to which to occur can be affected by current fitness status, with more physically fit individuals experiencing less decline (77). Due to this we hypothesize that both groups will experience less cognitive decline following high intensity exercise as a result of the strength/power intervention.

Chapter 3: Methods

Experimental Approach

The current investigation was a nine to twelve week intervention study designed to compare the effects of a low carbohydrate KD versus a standard high carbohydrate control diet on cognitive performance in healthy adults within a military population. The intervention and control groups were as follows:

1. Nutritional Ketosis (KD) - This group was provided a personalized low-carbohydrate diet that produced blood ketones in the 0.5+ mmol/L range with a goal of 1.0+ mmol/L average for each subject.
2. Standard High Carbohydrate (HC) - This group consumed their typical high-carbohydrate diet, supervised by registered dieticians and research key personnel but otherwise performed the same training and testing.

Both the KD and HC groups concurrently underwent a training protocol with an emphasis on strength and power. Cognitive testing required subjects from both groups to visit the lab on five separate occasions to complete consent/familiarization, pre/post resting cognition, and pre/post cognition following an acute anaerobic stressor. Each visit lasted approximately two hours with 2-3 days of rest between the two separate cognitive measures (resting/stressed) to allow recovery and flexibility for subject's availabilities. Overall the study took nine to fourteen weeks for each subject.

Recruitment

Subjects were recruited from the Ohio State University ROTC programs and local Ohio National Guard units to ensure recruitment of a military population. Additionally, to reach other military individuals, emails, word of mouth, and flyers on campus were used for recruitment. Upon expression of interest, the study was briefly explained and preliminary qualifications were determined to be met before a consent/familiarization meeting was made.

Inclusion Criteria

The inclusion criteria for this study required subjects to be: between the ages of 18-50 years old; have a body mass index (BMI) of less than 40 kg/m2; be a current or former member of the United States military; currently involved in physical training at least three times per week; be no more than moderate risk as set forth by the American College of Sports Medicine (56) for the cardiovascular risk stratification category, symptom free of cardiovascular, pulmonary or metabolic disease, and have no more than one risk factor for cardiovascular disease; not have any endocrine dysfunction, hormonal imbalances, or signs or symptoms of cardiovascular disease; not currently using drugs or supplements that may affect exercise, physical, or mental performance; physically healthy and injury free and have no limitations for any sub-maximal or maximal exercise; and mentally healthy with no history of cognitive impairment, epilepsy, traumatic brain injury, or pathological disease of the brain.

Subjects

This study was approved by the Ohio State University's Institutional Review Board for use of human subjects. Each subject had the study explained to them as to the risks and benefits of the investigation and was allowed to ask any questions before they signed the institutionally approved informed consent document. This study of cognition was part of a larger study on the effects of KD and RT. Twenty-nine recreationally trained military subjects were included in this study. Fifteen individuals were in the KD group and fourteen individuals were in the HC group. Subject characteristics can be seen in Table 2.1

Table 2.1. Subject Characteristics

	CON	KD	Combined
Sex	F=2, M=12	F=2, M=13	F=4, M=25
Age (yrs)	24.58 ± 8.98	27.4 ± 6.78	26.03 ± 7.9
Height (cm)	70.64 ± 2.06	69.08 ± 2.23	69.83 ± 2.26
Weight (kg)	79.83 ± 5.48	85.73 ± 7.78	82.88 ± 7.29
BMI	24.87 ± 2.39	27.89 ± 2.86	26.43 ± 3.02
BF (%)	22.04 ± 8.62	25.61 ± 4.96	23.89 ± 7.08

Subject Characteristics presented as means ± SD

Diet Intervention

KD Group

Following other studies KD guidelines for athletes (83), the KD consisted of no more than 50 grams of carbohydrate (personalized based on level of ketones checked daily by finger stick) and ~15-20% and ~70-75% of daily caloric intake in the form of protein and fat, respectively. Total energy intake was ad libitum to permit subjects to restrict caloric intake to

induce weight and fat loss for those who were overweight. Subjects in the keto group were provided with a handheld glucometer (Precision Xtra, Abbott Nutrition) and ketone test strips in order to check the concentration of BOHB daily from a finger stick. This finger stick was conducted upon waking prior to any activity or eating. The values obtained were then sent daily to a member of the research team and recorded. The carbohydrate level required to induce nutritional ketosis varies from person to person and thus the objective feedback provided by testing the blood ketones was a novel tool that allowed us to personalize each subject's diet by titrating the carbohydrate and protein intake to the subject's individual ketosis threshold. This aided in the attempt to achieve an average level of ketosis of 1.0+ mmol/L for each subject in the KD group throughout the intervention. A majority of the meals with known amounts of carbohydrate, fat, and protein were cooked and supplied to the KD group from the researchers. Bulk ingredients and snacks that were ketogenic friendly as well as many ketogenic recipes and resources were also supplied.

HC Group

The control group continued to eat their normal daily diet, which consisted of a typical high carbohydrate American diet of at least 45% of daily caloric intake in the form of carbohydrates. Food records were collected during the dietary intervention to ensure consistency of the diet. Similar to the KD group, the control group were not restricted in their caloric intake and were permitted to eat more or less than needed in order to meet any weight change goals.

Resistance Training Intervention

Participants in both the KD and CON groups were instructed to complete a resistance and power training program at The Ohio State University's Exercise Science Lab. Training was completed in groups or in individual sessions under the supervision of a Certified Strength and Conditioning Specialist (CSCS) to ensure participant safety and precision in complex movements. Participants completed two days per week of exercise training at the OSU lab, and one day per week of OSU Army ROTC PT. Participants not involved in the OSU Army ROTC program were instructed to complete a third day of physical training involving endurance or body-weight circuit training. Participants were further encouraged to continue their level of training volume prior to beginning the exercise intervention by also incorporating these training protocols. Thus, a participant that exercised four days per week prior to the study was instructed to complete two days of supervised strength training, one day of bodyweight endurance-based training, and one day of their regular training while completing the study.

The training program was divided into two mesocycles: strength, then power. The first day of training for both involved technique and form corrections, and low volume, to allow an easy transition into the workouts. The following 4 weeks included 2 sessions per week that included barbell back squat, bench press, a variety of other strength-based and injury preventative exercises and concluded with ~15 minutes of circuit-style metabolic training.

For the first session of week 6, the participant completed a training session instructing proper Olympic lift technique in the Olympic clean and jerk. The following 3 to 6 weeks of

training included a rotation of strength, power training, and metabolic conditioning, with a large emphasis on power training through the Clean and Jerk and plyometric exercises.

In addition to these two training sessions per week, participants also completed a third unsupervised session. Those enrolled in The Ohio State University's ROTC program completed PRT one day per week and replaced PRT with this training program two days per week. Those not enrolled in ROTC were instructed to complete an aerobic-based or body-weight circuit-style training day in addition to the training intervention.

The specific test days for cognition were as follows:

1. Consent/familiarization – Subjects were screened to ensure requirements for the study were met, all procedures were explained and questions answered, baseline visits scheduled, and consent to participate in study was obtained.
2. Baseline Cognitive Test #1 – First baseline test required subjects to take a cognitive battery on a computer fasted and rested
3. Baseline Cognitive Test #2 – Second baseline test was a measure of cognition following an acute physical stressor
4. Post-Intervention Cognitive Test #1 – A repeat of visit #2 following the feeding and training intervention
5. Post-Intervention Cognitive Test #2 – A repeat of visit #3 following the feeding and training intervention

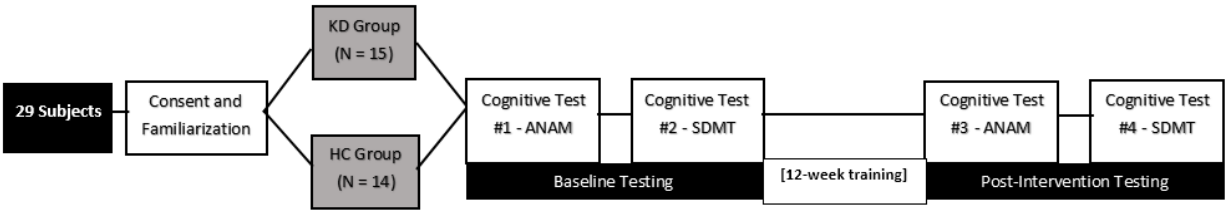


Figure 3.1. Experimental flow is shown for the study

Pre-Testing Controls

- Hydration Status - Subjects were encouraged to drink at least two cups of water the night before a test visit, and two cups of water the morning of testing. Urine specific gravity was tested (Reichert TS 400 clinical refractometer) to ensure acceptable hydration was met (all subjects had a USG <1.025) prior to continuing with data collection. If USG was >1.025 subjects were given water until USG became <1.025
- Fasted – Subjects arrived in the morning for visits fasted for at least ten hours
- Fatigue – Subjects came into testing rested and having not exercised for 72 hours prior to the day of testing to ensure there was no physical soreness or fatigue
- Mood State Survey - Subjects completed a Profile Of Mood States (POMS) survey prior to each testing visit. The POMS survey has 37 questions and is validated as a measure of psychological distress including a broad range of mental states from anger, confusion, fatigue, to vigor, friendliness (71).
- Hardiness & Resiliency Surveys - Subjects were given questionnaires designed to evaluate perceived resilience (6 questions) and a hardiness scale (12 questions). Each

scale has been previously published for use in assessment of mental resilience and hardiness (45, 74)

- Abbreviated Life Stress Survey and Recent Life Changes Questionnaire – Subjects completed this survey in order to measure life events and life stress prior to collecting physiological data (68)

Testing Visits

Testing Visit #2 (Pre) and #4 (Post) – Resting Cognition

Subject's arrived to the study laboratory in the PAES building on the Ohio State University campus. Subjects were confirmed to be fasted, rested, and hydrated and were then given the questionnaires and surveys as stated in the study controls above.

Subjects then completed a software-based cognitive assessment (Automated Neuropsychological Assessment Metrics (ANAM), Vista LifeSciences, Parker, CO, Washington D.C.) that has been used as a means of assessing different domains of cognition. ANAM was selected to assess resting cognition as its battery of tests is required prior to, and following, military deployment by the department of defense as a tool for catching potential traumatic brain injury and Post-Traumatic Stress Disorder. Its battery includes assessment of basic neural processing, associative learning, processing speed, working memory, visual spatial memory, delayed memory, and response inhibition. Within the ANAM battery subjects were asked to observe words, shapes, and arrow stimuli displayed on a screen or monitor and then select and react to stimuli according to the test instructions as quickly and accurately as possible. The test

battery includes the following tests with the accompanied descriptions from the ANAM Core Battery Administration Manual (20):

1. Simple Reaction Time – "This task measures visuomotor processing speed, simple motor speed, and attention. A series of "*" symbols is presented on the display. The user is instructed to respond as quickly as possible by pressing a button each time the stimulus appears"
2. Code Substitution (Learning) – "This test measures visual scanning, visual perception, attention, associative learning, and information processing speed. In this test the user must compare a displayed digit-symbol pair with a set of defined digit-symbol pairs (e.g., the key) presented at the top of the screen. The user presses designated buttons to indicate whether the pair in question is correct or incorrect relative to the key"
3. Procedural Reaction Time – "This test measures information processing speed, visuomotor reaction time, simple decision making, and attention. The user is presented with a number (either a 2, 3, 4, or 5). The user is instructed to press one designated button for a "low" number (2 or 3) and another designated button for a "high" number (4 or 5)"
4. Mathematical Processing – "This test measures visual-spatial processing, working memory, and visual short-term recognition memory. During this test the user views a pattern produced by eight shaded cells in a 4x4 sample grid. The sample is then removed and two comparison patterns are displayed side by side. One grid is

identical to the sample grid and the other grid differs by one shaded cell. The user is instructed to press a designated button to select the grid that matches the sample"

5. Matching to Sample – "This test assesses basic computational skills, concentration, and working memory. During this task, an arithmetic problem involving three single-digit numbers and two operators is displayed (e.g., "5 - 2 + 3 ="). The user presses buttons to indicate whether the answer to the problem is less than five or greater than five"
6. Code Substitution (Delayed) – "This test provides a measure of learning and delayed visual recognition memory. In this test the user is presented with a digit-symbol pair and must decide from memory if this pairing is correct based on the key presented during the Code Substitution – Learning test taken earlier in the test battery. The user presses designated buttons to indicate whether the pair in question represents a correct or incorrect match based on the earlier presented key"
7. Simple Reaction Time – "This is a repeat of the Simple Reaction Time test presented earlier in the battery. Results of this test are used to measure the effect of fatigue on performance as well as an index of visuomotor processing speed and attention"

   Go/No-Go – "This test assesses response inhibition. The user is presented with two characters, "x" and "o". The user is instructed to respond as quickly as possible to the "x" by pressing a button each time the stimulus appears. When the "o" appears, the user is to do nothing (inhibit response)"

The variables analyzed for each test are reaction time for correct answers (RT) and Throughput which is the amount of correct answers per minute. Exceptions are for simple

reaction time which only produced RT and for go/no-go which has DPrime instead of Throughput, but is a measure of the same thing.

Testing Visit #3 (Pre) and #5 (Post) – Cognition Following High-Intensity Exercise

Subject's arrived to the study laboratory in the PAES building on the Ohio State University campus. Subjects were confirmed to be fasted, rested, and hydrated and were then given the questionnaires and surveys as stated in the study controls above.

In order to see cognitive changes when an acute stressor is applied, subjects took a cognitive test three times (set 1, set 2, set 3) between two bouts of maximal sprints. The cognitive test utilized was the symbol digit modality test (SDMT) which is very similar to the code substitution test within ANAM. A sheet with nine different symbols matched with the numbers one through nine was printed at the top. The rest of the sheet had the symbols in rows with empty boxes below them. The objective was to fill in the correct number in the empty boxes that associated with the symbol. The subject had 90 seconds to fill out as many of the boxes as possible. Subjects were graded on amount answered and accuracy, with the amount correct being analyzed. Three different SDMT sheets were used for the three tests. Following the first SDMT test (set 1) subjects completed five sets of ten second maximal sprints on a HiTrainer (Power Systems (PS), LLC Knoxville, TN), a self-propelled treadmill. Immediately following the fifth set the subjects were administered the SDMT test a second time (set 2) (70). Following the SDMT test subjects were given two minutes of rest before doing a second set of five sprints on the HiTrainor. Immediately following the second set of five sprints, a final SDMT test (set 3) was administered.

Statistical Analysis

Data were analyzed using SPSS version 25.0(IBM, Inc., Armonk, NY, USA). Means and standard deviations (SD) were calculated for each variable. Missing data points were interpolated using an average percent change. An independent t-test was used to confirm matched groups at baseline. A mixed-method ANOVA (Diet x Time) was used to determine significant differences amongst means. A 2(79) x 2(Time) ANOVA was used to assess simple reaction time (two trials), code substitution – learning, procedural reaction time, mathematical processing, matching to sample, code substitution – delayed, and go/no-go. Significant F tests were further investigated using pairwise post-hoc comparisons with a Least-Squared difference adjustment for multiple comparisons ($p \leq .05$). Pearson's correlation was used to assess significance within the Keto group among percent change pre to post, acute ketone levels, and average ketone level for the duration of the study.

Chapter 4: Results

Subjects

A total of 29 subjects (25 men, 4 women) completed this study, (KD n=14, CON n=15). Female subjects were equally distributed between groups (KD n=2, CON n=2). Race was equally distributed between groups with 72.4% Caucasian (n=21), 13.8% Asian (n=4), 10.3% Hispanic (n=3), and 6.9% Black (n=2). There were no significant differences between groups for age (KD 27.4 yrs ± 6.8, CON 24.6 yrs ± 6.8), height (KD 175.5 cm ± 5.7, CON 179.4 ± 5.2), BMI (KD 27.9 ± 2.9, CON 24.9 ± 2.4), or body fat percentage (KD 25.6 ± 5.0, CON 22.0 ± 8.6). From the first day of training to the post-test, the KD group completed an average of 83 days in the study, 11.8 weeks, and 19 training sessions for 87.6% program completion based on weeks in the study. The CON group completed an average of 82 days in the study, 11.5 weeks, and 19 training sessions for 84.2% program completion based on individual's weeks in the study.

Automated Neuropsychological Assessment Metrics (ANAM)

Based on the results of the t-tests, there were no significant differences between groups at baseline for any of the tests. Following the intervention there were no significant effects of diet between subjects for any of the tests. When the groups were collapsed, there was a significant effect of time for code substitution - delayed (reaction time), $F(1, 27) = 4.761$, $p = 0.038$ and procedural reaction time (reaction time), $F(1, 27) = 9.446$, $p = 0.0048$. There was a significant diet/time interaction for procedural reaction time (throughput), $F(1, 27) = 6.691$, $p = 0.0154$ with the control group having a significant improvement from pre (100.13 ± 8.63) to post (108.64 ± 8.33). No other significant effects for time, diet, or time/diet interaction were observed. One

individual was not able to report his ketone level the day of testing for ANAM. The subjects data was excluded from the acute ketone correlation analysis (KD: n = 14). Within the keto group there was a significant correlation in percent change from pre to post for acute ketone level for code substitution - delayed (reaction time), r (14) = -0.6, p = 0.023; code substitution – delayed (throughput), r (14) = 0.756, p = 0.002; and go/no-go (reaction time), r (14) = -0.673, p = 0.008 with each improving from pre to post. No significant correlation between acute ketone level and percent change was seen for any other tests. No significant correlation of average ketone level was seen for any test.

SDMT

One subject was unable to complete the second set of sprints on the HiTrainer and the subject's data was excluded from analysis (KD: n = 14; CON: n = 14). There were no significant differences between groups at baseline for any of the tests. Following the intervention there were no significant effects of diet between subjects for any of the tests. When the groups were collapsed, there was a significant effect of time for correct responses on trial 3, F(1, 26) = 13.001, p = 0.001, with an improvement from pre (52.86 ± 10.987) to post (60.96 ± 9.276). No other significant effects were observed for time, diet, or time/diet interaction.

Strength/Power

There was a significant time effect for absolute max barbell back squat, F(1.75, 47.29) = 30.70, p<.01. Absolute back squat PRE (mean 110.33, SE 5.41) was both significantly less than POST (mean 125.90, SE 4.50). There was also a significant effect over time for relative barbell back squat, F(1.95, 52.66) = 51.70, p <.01. Relative back squat PRE (mean 1.34, SE .06) was

significantly less than POST (mean 1.59, SE .06). There were no significant differences between the diet groups at any time point for either absolute or relative back squat. One participant had a pre-existing shoulder injury and did not complete a max bench press for POST. The participant's data was excluded from analysis (KD: n = 14; CON: n = 14). Analysis of means revealed no significant differences between groups or over time for absolute max bench press. There was a significant time effect for relative bench press, $F(1.652, 42.963) = 11.410$, $p<.01$). Relative bench press PRE (mean 1.10, SE 0.07) was significantly less than POST (mean 1.18, SE 0.07). There were no significant differences between groups for relative bench press. There were no significant effects of time or diet in average power or maximum when PRE was used as a covariate for observed percent changes between time points.

Table 4.1. ANAM Results

Resting Cognition - ANAM						
		KD (N = 15)	CON (N = 14)	Combined (N = 29)	Diet	Time
Coded Substitution - Learning (RT)	Pre	917.29 ± 100.96	948.89 ± 163.49	932.55 ± 133.28	p = 0.518	p = 0.7647
	Post	925.77 ± 124.32	955 ± 172.2	940.07 ± 147.39		
Coded Substitution - Learning (Throughput)	Pre	64.06 ± 6.57	62.7 ± 9.96	63.4 ±8.25	p = 0.538	p = 0.5943
	Post	63.55 ± 8.98	61.39 ± 9.63	62.51 ± 9.2		
Coded Substitution - Delayed (RT)	Pre	1001.71 ± 166.54	1051.56 ± 274.98	1025.77 ± 222.75	p = 0.667	p = 0.0380*
	Post	934.37 ± 182.16	936.44 ± 135.23	935.37 ± 158.37		
Coded Substitution - Delayed (Throughput)	Pre	59.64 ± 11.37	57.27 ± 15.19	58.5 ± 13.16	p = 0.49	p = 0.3103
	Post	62.9 ± 11.69	59.45 ± 14.5	61.23 ± 13		
Matching to Sample (RT)	Pre	1435.1 ± 412.1	1498.8 ± 460.46	1465.85 ± 429.42	p = 0.581	p = 0.8791
	Post	1411.96 ± 399.85	1505.14 ± 338.7	1456.95 ± 368.03		
Matching to Sample (Throughput)	Pre	43.3 ± 11.07	40.89 ± 16.45	42.13 ± 13.72	p = 0.743	p = 0.3402
	Post	41.31 ± 10.8	40.3 ± 18.12	40.82 ± 14.53		
Mathematical Processing (RT)	Pre	2463.75 ± 611.63	2590.64 ± 854.65	2525.01 ± 728.24	p = 0.728	p = 0.7433
	Post	2459.23 ± 891.98	2521.95 ± 750.51	2489.51 ± 812.62		
Mathematical Processing (Throughput)	Pre	24.27 ± 6.2	21.99 ± 7.05	23.17 ± 6.61	p = 0.812	p = 0.9385
	Post	22.7 ± 8.34	23.72 ± 8.44	23.19 ± 8.25		
Simple Reaction Time (RT)	Pre	266.94 ± 26.21	283.48 ± 24.9	274.93 ± 26.5	p = 0.235	p = 0.6911
	Post	271.82 ± 28.3	275.26 ± 18.26	273.48 ± 23.63		
2nd Simple Reaction Time (RT)	Pre	268.34 ± 18.86	272.33 ± 16.71	270.26 ± 17.65	p = 0.528	p = 0.9118
	Post	268.35 ± 18.56	271.54 ± 16.46	269.89 ± 17.34		
Procedural Reaction Time (RT)	Pre	565.03 ± 61.76	576.02 ± 50	570.33 ± 55.67	p = 0.85	p = 0.0048*
	Post	551.5 ± 36.25	534.54 ± 41.23	543.32 ± 38.99		
Procedural Reaction Time (Throughput)	Pre	104.35 ± 10.24	100.13 ± 8.63	102.31 ± 9.57	p = 0.717	p = 0.1155
	Post	102.41 ± 9.25	108.64 ± 8.33*	105.42 ± 9.22		
Go/No-Go (RT)	Pre	325.65 ± 36.97	341.29 ± 19.64	333.2 ± 30.42	p = 0.282	p = 0.8958
	Post	328.72 ± 36.49	337.36 ± 26.65	332.89 ± 31.86		
Go/No-Go (DPrime)	Pre	4.17 ± 1.36	3.99 ± 1.17	4.08 ± 1.25	p = 0.903	p = 0.5551
	Post	3.87 ± 1.18	3.96 ± 1.13	3.91 ± 1.13		

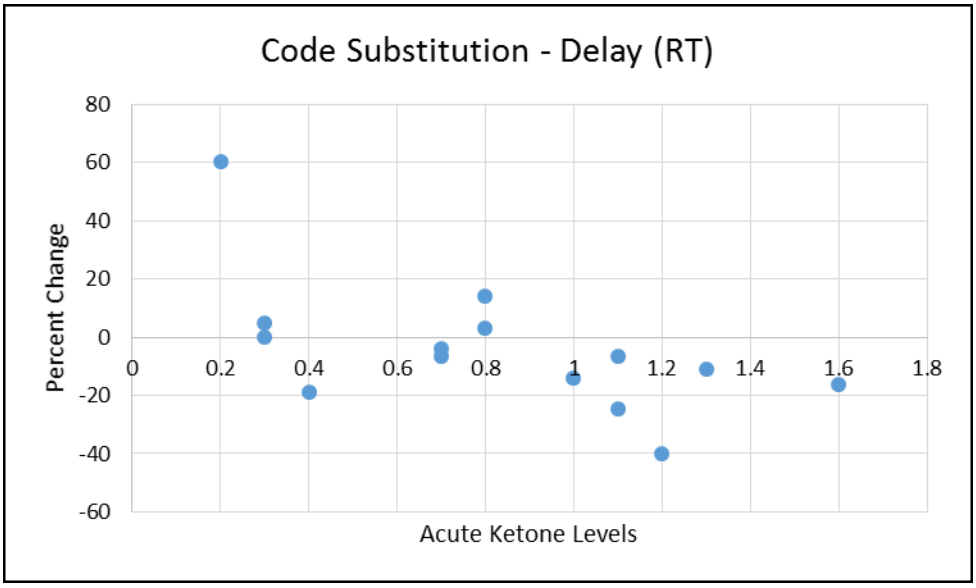
Results are presented as Means ± SD. *****represents P<0.05

Table 4.2. ANAM Ketone Correlations

	Average Ketone Level		Acute Ketone Level	
	Correlation	Significance	Correlation	Significance
Coded Substitution - Learning (RT)	r = 0.101	p = 0.719	r = -0.465	p = 0.093
Coded Substitution - Learning (Throughput)	r = -0.103	p = 0.714	r = 0.393	p = 0.164
Coded Substitution - Delayed (RT)	r = -0.075	p = 0.789	r = -0.6	p = 0.023*
Coded Substitution - Delayed (Throughput)	r = 0.173	p = 0.538	r = 0.756	p = 0.002*
Matching to Sample (RT)	r = -0.001	p = 0.997	r = -0.073	p = 0.803
Matching to Sample (Throughput)	r = -0.026	p = 0.927	r = -0.133	p = 0.649
Mathematical Processing (RT)	r = -0.224	p = 0.422	r = -0.221	p = 0.447
Mathematical Processing (Throughput)	r = 0.289	p = 0.296	r = 0.394	p = 0.163
Simple Reaction Time (RT)	r = -0.078	p = 0.783	r = 0.247	p = 0.394
2nd Simple Reaction Time (RT)	r = -0.217	p = 0.437	r = 0.028	p = 0.924
Procedural Reaction Time (RT)	r = 0.254	p = 0.362	r = 0.101	p = 0.731
Procedural Reaction Time (Throughput)	r = -0.196	p = 0.484	r = 0.025	p = 0.932
Go/No-Go (RT)	r = 0.026	p = 0.926	r = -0.673	p = 0.008*
Go/No-Go (DPrime)	r = 0.214	p = 0.444	r = -0.211	p = 0.469

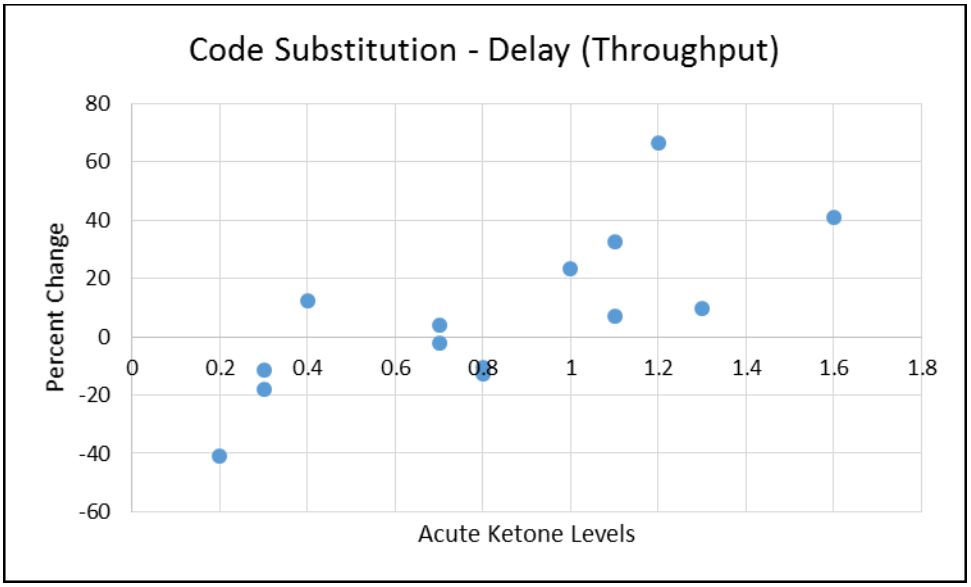
Results are presented as Means ± SD. ***** represents P<0.05

Figure 4.1 Acute Ketone Correlations: Code Substitution – Delay (RT)



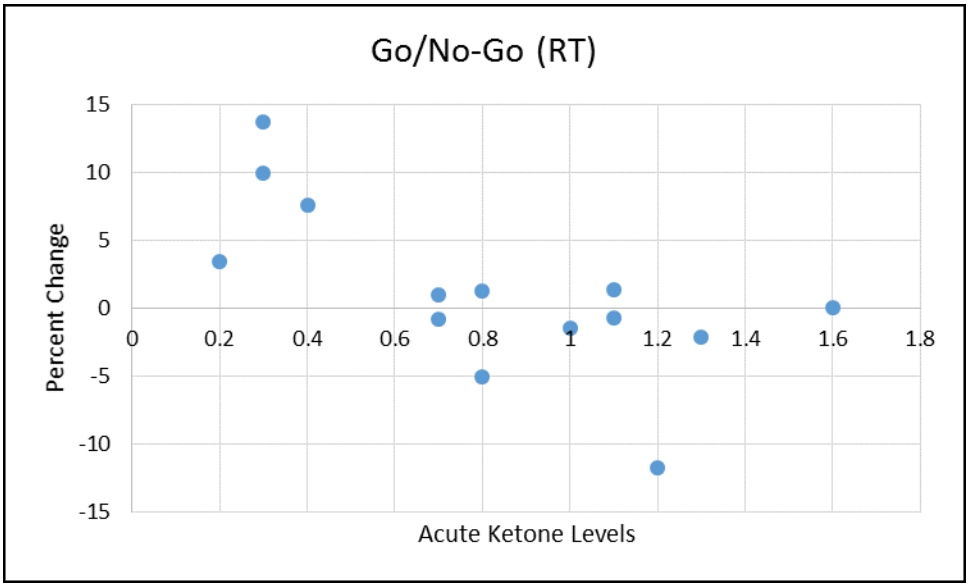
Represents the correlation (r = -0.6) that exists between the percent change Pre to Post and acute ketone levels

Figure 4.2 Acute Ketone Correlations: Code Substitution – Delay (Throughput)



Represents the correlation (r = 0.756) that exists between the percent change Pre to Post and acute ketone levels

Figure 4.3 Acute Ketone Correlations: Go/No-Go (RT)



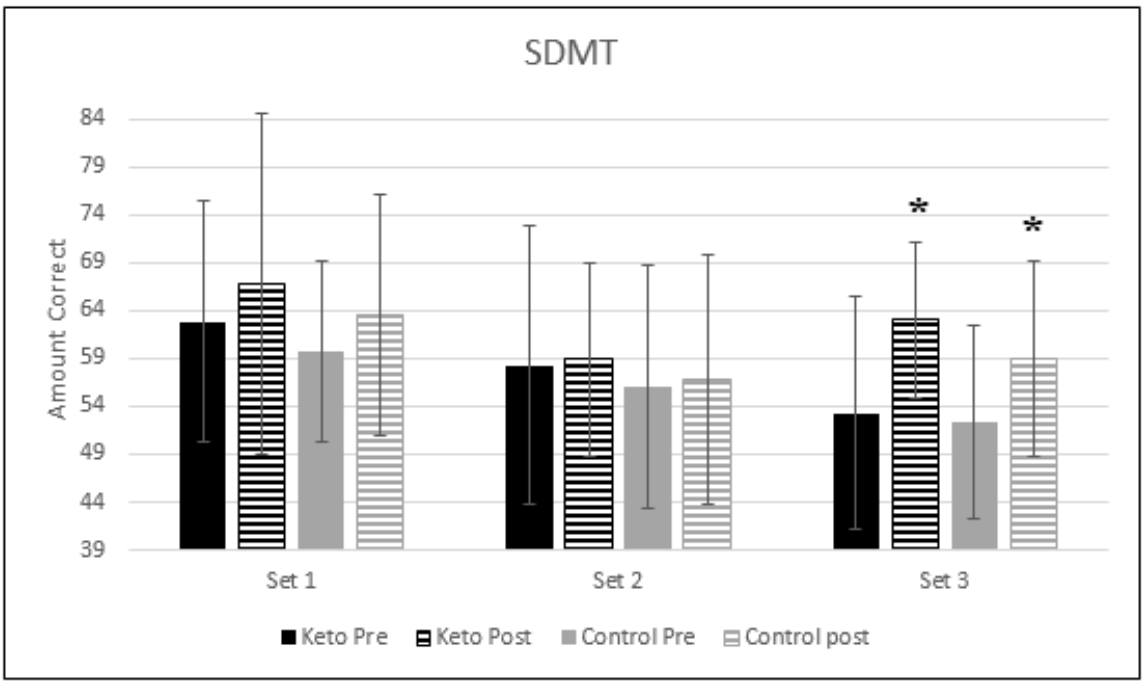
Represents the correlation (r = -0.673) that exists between the percent change Pre to Post and acute ketone levels

Table 4.3. SDMT Results

Exercise Cognition - SDMT						
		KD (N = 14)	CON (N = 14)	Combined (N = 28)	Diet	Time
Set 1	Pre	62.79 ± 12.595	59.71 ± 9.351	61.25 ± 10.997	p = 0.459	p = 0.193
	Post	66.71 ± 17.809	63.5 ± 12.593	65.11 ± 15.223		
Set 2	Pre	58.29 ± 14.51	56.07 ± 12.676	57.18 ± 13.417	p = 0.574	p = 0.816
	Post	58.93 ± 10.065	56.79 ± 13.028	57.86 ± 11.475		
Set 3	Pre	53.29 ± 12.187	52.43 ± 10.09	52.86 ± 10.987	p = 0.442	p = 0.001*
	Post	63 ± 8.162	58.93 ± 10.156	60.96 ± 9.276		

Results are presented as Means ± SD. ***** represents P<0.05

Figure 4.4. SDMT



Bars represent means and errors bars represent SD. ***** represent P<0.05

Chapter 5: Discussion

The major findings from this study were that there were no significant differences observed between groups for an effect of the KD on either resting or physically stressed cognition. However, significant effects as a result of the strength/power intervention were seen in both groups for domains of resting and physically stressed cognition.

Resting Cognition

Ketogenic Diet

The major finding of this study for resting cognition is that a ketogenic diet has no significant effect on any of the domains tested when compared to a standard control diet in healthy recreationally trained adults. This does not support our hypothesis that the neuroprotective benefits associated with a ketogenic diet would lead to concurrent benefits in cognitive performance. However, these results do contradict some of the negative effects observed in the literature that were conducted within a similar population who found that consuming a ketogenic diet for a period of five to seven days caused decreases in reaction time and attention (27, 40). Our results suggest that any decreases seen would revert back to baseline after a period of keto-adaption, a process that takes at least two-weeks in most individual's (14). This also seems to dispute the study by Halyburton et. al (36) who reported a significant increase in processing speed for a high carbohydrate diet versus a ketogenic low carbohydrate diet. The differences seen between groups was likely a result of the poorly formulated diet of the ketogenic group whose prescribed protein levels were too high to allow for appropriate levels of ketosis.

One result from this study that is intriguing is the significant correlation some tests had with acute ketone levels. As ketones are operating as the 'extra' fuel source we were curious about how the acute ketone level the morning of testing might affect scores. We found a significant correlation for the tests code substitution – delayed and go/no-go (reaction time for correct answers), suggesting that the areas in the brain associated with delayed memory and inhibition may have a higher usage rate of ketones. Ketone levels were not controlled for the day of testing resulting in some individuals within the keto group to have ketone readings of less than 0.5 mmol/L. Had levels been higher for the keto group during testing we may have seen a significant difference between groups for these tests.

Studies that have seen increases in measures of cognition while consuming a ketogenic diet in humans seem to be currently limited to populations that are not young and healthy, namely those with Alzheimer's disease (37, 38), the elderly (62, 69), and the obese (9, 52). Quite interesting is that each of these populations have been associated with decreases in the metabolism of glucose (21, 41, 87), whereas a similar decrease in ketone metabolism in these populations has not yet been observed. Thus, the cognitive improvements seen in these populations from being on a ketogenic diet may be due to having ketones as another fuel to supplement the decrease in glucose, rather than any direct beneficial effect of the ketone bodies themselves. If this were the case, it would explain why no there were no significant differences in cognition between the ketogenic and control groups in our study. The cognitive battery was completed in a rested state where the brain would not be faced with any issues of metabolic stress. This suggests that the type of fuel, glucose or ketones, may not impact cognition as long as both substrates are available and their ability to be utilized is not impaired.

While no significant differences were noticed between the ketogenic and control groups for resting cognition, much more research is needed to further investigate this claim. Some limitations of this study were that calories were not matched between the groups, ketone level the day of testing was not controlled for, learning effect was not controlled for, and the level of ketosis for the study (average of 1.23 mmol/L) may not have been high enough to induce the desired results. A future study controlling for the first three aspects and one comparing different levels of ketosis is warranted, especially given the significant correlation found in some domains for acute ketone level.

Strength/Power Intervention

While a significant effect was not observed between the keto and control groups, there was a significant effect of time for the tests coded substitution – delayed (reaction time for correct answers) and procedural reaction time (reaction time for correct answers) and a significant diet and time interaction effect for procedural reaction time (Throughput) with the control group significantly improving from pre to post. These tests are associated with the domains of delayed memory and processing speed. The improvement seen with time is likely a result of the training program both groups participated in while in the study, supporting our hypothesis. This lines up with previous studies conducted that have seen similar increases in memory (48, 84). It also seems to support the notion that higher intensities may be needed to see increases in cognition (48) as our program used intensities as high as 90% of individual's one rep maxes.

The interaction effect of time and diet on procedural reaction time (throughput) is interesting. While there was no significant difference between the diet groups, the control group

significantly improved pre to post and the keto group did not. Since there was no difference between the groups, interpretation of this result is difficult. It could be the consequence of sample distribution or possibly signify that ketone metabolism decreases the potential effect from a strength/power training program.

These results are promising for RT and its effects on cognition, however, there is potential that the increases seen were a result of a learning effect that can occur with repeated testing using the ANAM (20). As the main objective of the study was to investigate the differences in cognition as a result of a ketogenic versus control diet, learning effect was not controlled for. Due to this, it is difficult to make any outright claims on the improvements seen as a result of the exercise intervention, but it offers an exciting avenue for further research in this domain. A follow-up study with higher n-size that controls for the learning effect and focuses entirely on exercise is needed.

Physically Stressed Cognition

Similar to our findings with resting cognition, a ketogenic diet had no significant effect on cognition following high-intensity exercise, when compared to a high carbohydrate control diet in healthy recreationally trained adults. However, there was a significant effect of time for both groups for the third set following the final sprints, improving from pre (52.86 ± 10.987) to post (60.96 ± 9.276). Which supported our hypothesis for the effects of a strength/power intervention. This is supported by previous studies that cognition during and following exercise can be modulated by current physical fitness status (23, 31, 32). This is likely a result of physically trained individuals having elevated lactate metabolism during exercise compared to individuals who are untrained (43).

Increased ability to utilize lactate as a substrate for muscle and brain metabolism with increased physical fitness likely explains why post-exercise cognition improved following training for both groups. Part of the training involved high intensity, short interval training which would increase the body's ability to metabolize lactate (75). We believe that this response also offers a reason as to why no difference was noticed between groups. We hypothesized that circulating ketones would provide an additional fuel that could potentially replace the decreased use of glucose in the brain, preventing the decline in cognitive performance. However, based on our results, elevated levels of lactate may have inhibited ketones as a metabolic fuel to the brain. Like ketones, lactate is a monocarboxylic acid, and thus uses the same transporters into the brain and brain cells. Additionally, lactate has a higher affinity for these transporters compared to the main circulating ketone-body of beta-hydroxybutyrate (65, 67). This is for both MCT1 across the BBB (3.5-10 km vs 12.5) and MCT2 into the neurons (0.5-0.75 km vs 1.2). When both ketone and lactate levels are elevated in the blood, the transporters may prefer transport of lactate and act as selective inhibitors to ketones. Furthermore, lactate levels have been shown to exceed ten mmol/L following high-intensity sprinting (2), which is much higher than the ketone levels achieved when in ketosis. The ratio of lactate and ketones in the blood may have an effect on the amount of each that is transported. Lactate being the preferred substrate over ketones to the transporters could explain the lack of significance of our results between the two groups. However, lactate and ketone levels were not measured during or immediately following exercise testing, so the amounts of both available to be a substrate to the brain are unknown for this study.

As more research is warranted for resting cognition in a healthy adult population, the same holds true for athletes. Future studies should consider measuring lactate and ketones during

and immediately following exercise. A limitation of this study is that we wanted to conduct a cognitive test while the subjects were fatigued and cognition was presumed to be declined. This offered a fairly tight window and so only one cognitive test was utilized. For investigating a KD effects, discovering which cognitive domains are affected most by ketones at rest, as potentially delayed memory and inhibition were for this study, would potentially offer a more appropriate test to utilize during and following exercise. Other limitations for KD that should be addressed in future studies are similar to those stated for resting cognition.

Conclusion

In conclusion, a ketogenic dietary intervention showed no significant differences in either resting cognition or cognition following high-intensity exercise when compared to a high carbohydrate control diet in healthy recreationally trained adults. However, at the very least it seems that we have shown that a properly formulated ketogenic diet that allows for a period of keto-adaptation does not result in decreases in cognitive performance, as claimed in prior research within this population. Much more research is warranted in this population to allow for further understanding of the mechanisms of ketone metabolism in the brain and how that may affect performance. Additionally, as there WERE significant beneficial effects in both groups following the RT program, more research is needed in this population for exercise as well.

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Appendix A: Informed Consent Form

The Ohio State University Consent to Participate in Research
Study Title: Tactical Athletes in Nutritional Ketosis (TANK)
Principal Investigator: Jeff Volek, Ph.D., R.D.

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before making your decision whether or not to participate.
Your participation is voluntary. You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your future relationship with The Ohio State University. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.
You may or may not benefit as a result of participating in this study. Also, as explained below, your participation may result in unintended or harmful effects for you that may be minor or may be serious depending on the nature of the research.
You will be provided with any new information that develops during the study that may affect your decision whether or not to continue to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

1. Why is this study being done?

When dietary carbohydrate is limited to ~40 grams/day, the body increases production and utilization of ketone bodies and becomes more efficient at utilizing body fat as an energy source. These diets are often called ketogenic diets. Several new studies indicate that ketogenic diets may have therapeutic effects in managing obesity, diabetes and other chronic disease states. Ketogenic diets may also enhance certain aspects of physical and mental performance, but more work is needed to better understand how healthy active individuals respond to ketosis. The main purpose of this study is to determine the effect of a ketogenic diet on various health parameters, metabolic function and mental and physical performance, including military-specific assessments. A secondary purpose of this study is to determine if different blood levels of ketones lead to a difference in benefits. This will be assessed by adding ketone supplements to a ketogenic diet in one of the groups.

2. How many people will take part in this study?

We are planning to enroll a total of 80 people in this study.

3. What will happen if I take part in this study?

Overview

Eligibility for participation in this study is dependent on the results of the tests conducted during the screening visit. Participants who qualify will participate in either a control group or one of two dietary intervention groups. If you are in the control group, you will be asked to continue to follow your typical diet for a period of 12 weeks under the supervision of a registered dietician and/or research key personnel. If you are in one of the intervention groups, you will be provided with low-carbohydrate (ketogenic) meals for 12 weeks. All groups will participate in an exercise training program. Depending on the intervention group you are assigned to, you may also be asked to take a daily ketone supplement. Regardless of group assignment, you will undergo a comprehensive series of tests at the beginning and end of the 12 week study period. A shorter set of cognitive and physical performance monitoring will be completed at weeks 1, 4, and 8 in order to evaluate the time course of adaptations. Throughout the study, you will also be asked to maintain dietary records if you are in the control group and perform daily finger-stick blood testing of glucose and ketones if you are in the ketogenic diet groups with a meter

Screening

The purpose of the screening meeting is to determine if you meet our qualifying criteria, inform you of your rights as a participant, and to confirm your willingness to participate. We will provide you with a few questionnaires including medical history, physical activity history, dietary history, and an MRI screening form. You cannot participate in this study unless you are deemed safe to do so based upon the full screening process and you read, fully understand, and sign this informed consent document providing us with written consent.

Eligibility Criteria

We are looking for healthy men and women between the ages of 18 and 44 years. We will exclude you for any of the following reasons:

You are <18 or >50 years of age
You have a body mass index (BMI) <18.5 or ≥40 kg/m2
Exceed moderate risk according to the American College of Sports Medicine's (ACSM) risk criteria
You have gastrointestinal disorders or suffer from food allergies that would interfere with consuming the prepared meals
Regularly use tobacco products
Drink alcohol in excess of 3 drinks/day or 18 drinks/week
Have used cholesterol, diabetic, or blood pressure medications in the past 3 months
Currently use medications containing benzodiazepines or related substances (sedatives and other medications that alter sleep/consciousness)

Use of anti-inflammatory medications (aspirin, non-steroidal anti-inflammatory drugs) on a regular basis
Have indications of endocrine dysfunction, hormonal imbalance, or cardiovascular disease
Have musculoskeletal injuries or physical limitations affecting ability to exercise, or increasing risk of injury or discomfort during exercise
Have any conditions or contraindications to magnetic resonance imaging
Are pregnant or plan to become pregnant during participation in study

Taking certain dietary supplements or training outside of ROTC physical training sessions may interfere with any of the testing that will be conducted in this study. You will be asked to discontinue any additional training or usage of these supplement(s) for the full duration of the study.

Testing Sessions

The following three test sessions will be completed at the beginning of the study and will be repeated at the end of the study. Each test session will be separated by a minimum of 48 hours. The first and third sessions will occur in the PAES building on The Ohio State University campus. The address for this building is:

305 West 17th Ave
Columbus, OH 43210

The second session will occur at the Ross Heart Hospital and/or the Martha Morehouse Medical Plaza. Prior to your session, you will be informed of which location to report to. The addresses for these buildings are:

Martha Morehouse Medical Plaza
2050 Kenny Rd
Columbus, OH 43221

Ross Heart Hospital
452 West 10th Ave
Columbus, OH 43210

Test Session 1

Prior to this test session you will also need to be fasted for this session, which means you cannot consume any food or drink other than water for 10-12 hours prior to the session. To ensure proper hydration, please consume 2 cups of water the night before the session and another 2 cups the morning of the session. The following will occur during this session:

Your height and body mass will be measured.

You will provide a spot urine sample, which we will use to ensure that you are adequately hydrated.

Surveys to Evaluate Psychological Components of Mood, Resiliency, and Stress:

You will be given a brief survey prior to physiological evaluation. Profile Of Mood States (POMS) survey to be completed following a normal night's sleep. The POMS survey has 37 questions and is validated as a measure of psychological distress including a broad range of mental states from anger, confusion, fatigue, to vigor, friendliness.

You will be given questionnaires designed to evaluate perceived resilience (6 questions) and a hardiness scale (12 questions). Each scale has been previously published for use in assessment of mental resilience and hardiness.

You will be given an Abbreviated Life Stress Survey and Recent Life Changes Questionnaire. Each survey will be administered in order to measure life events and life stress on participants prior to collecting physiological data.

Your resting energy metabolism will be measured. This test requires that you first lie still and relax for 30 minutes. The room will be dark, quiet and at a comfortable temperature to allow you to rest. After the 30-minute rest period you will be instructed to remain awake but still. A plastic see-through hood will be placed over your head. This hood will allow you to breathe normally while we collect samples of the air you are breathing in and out. A measuring device, known as a metabolic cart, is attached to this hood via a plastic hose and measures the gases in the air collected by the hood to determine your resting energy expenditure. This test will last about 45 minutes.

Your body composition, which is the distribution of fat, bone, and other lean tissue, will be determined using dual-energy X-ray absorptiometry (DXA). This involves a scanner that exposes you to a small amount of X-ray radiation. You will lie quietly on the DXA bed while a scanning arm passes over your body from head to toe. You must remain still for about 7 minutes during this test. A certified technician will perform the scan. In addition to the DEXA scan for body composition, the circumference of your waist will be measured using a standard tape measurer.

You will perform a mental test battery including the Symbol Digit Modality Test (SDMT), Automated Neuropsychological Assessment Metrics (ANAM) cognitive test battery, and the Virtra Firearms Training Simulator.

During the SDMT, you will be given visual presentation of a sequence of single digit numbers that correspond to specific symbols shown in a key and requires the subject toselect and note the correct number that matches each symbol through a randomized series of approximately 120 matches Correct responses and time will be tracked throughout the SDMT testing process.

Virtra Firearms Training Simulator (Virtra, Tempe, AZ) will be utilized to assess cognitive function in a simulated combat-like scenario with interactive laser weapons. Virtra is designed to test reaction time, and higher level brain function capacity in a military relevant scenario that is depicted on a projector screen.

For the ANAM battery, you will sit in a chair and be given a touch screen computer/tablet to perform a series of tests as instructed. These tests are designed to tests your attention, concentration, mental reaction time, memory, mental processing speed, and decision-making.

Each test is explained in greater detail below:

Reaction Time Test (35)
The task is divided into five stages, which require increasingly complex chains of responses. In each case, you must react as soon as a yellow dot appears. In some stages the dot may appear in one of five locations, and you must sometimes respond by using the press-pad, sometimes by touching the screen, and sometimes both.

Stockings of Cambridge (SOC)
You will be shown two displays containing three colored balls. The displays are presented in such a way that they can easily be perceived as stacks of colored balls held in stockings or socks suspended from a beam. This arrangement makes the 3-D concepts involved apparent to you, and fits with the verbal instructions. You must use the balls in the lower display to copy the pattern shown in the upper display. The balls may be moved one at a time by touching the required ball, then touching the position to which it should be moved. The time taken to complete the pattern and the number of moves required are taken as measures of your planning ability.

Spatial Working Memory (SWM)
The test begins with a number of colored squares (boxes) being shown on the screen. The aim of this test is that, by touching the boxes and using a process of elimination, you should find one blue ‘token’ in each of a number of boxes and use them to fill up an empty column on the right hand side of the screen. The number of boxes is gradually increased, until it is necessary to search a total of eight boxes. The color and position of the boxes used are changed from trial to trial to discourage the use of stereotyped search strategies.

Pattern Recognition Memory Test (PRM)
You will be presented with a series of visual patterns, one at a time, in the center of the screen. These patterns are designed so that they cannot easily be given verbal labels. In the recognition phase, you will be required to choose between a pattern you have already seen and a novel pattern. In this phase, the test patterns are presented in the reverse order to the original order of presentation. This is then repeated, with new patterns. The second recognition phase can be given either immediately or after a delay.

Spatial Recognition Memory Test (SRM)

You will be presented with a white square, which appears in sequence at five different locations on the screen. In the recognition phase, you will see a series of five pairs of squares, one of which is in a place previously seen in the presentation phase. The other square is in a location not seen in the presentation phase. As with the PRM test, locations are tested in the reverse of the presentation order. This sub-test is repeated three more times, each time with five new locations.

Intra-Extra Dimensional Set Shift Test (39)
Two artificial dimensions are used in the test:

- color-filled shapes
- white lines

Simple stimuli are made up of just one of these dimensions, whereas compound stimuli are made up of both, namely white lines overlying color-filled shapes. You start by seeing two simple color-filled shapes, and must learn which one is correct by touching it.

Feedback teaches you which stimulus is correct, and after six correct responses, the stimuli and/or rules are changed. These shifts are initially intra-dimensional (e.g. color filled shapes remain the only relevant dimension), then later extra-dimensional (white lines become the only relevant dimension).

You progress through the test by satisfying a set criterion of learning at each stage (six consecutive correct responses). If at any stage you fail to reach this criterion after 50 trials, the test terminates.

Rapid Visual Information Processing Test (RVP)
A white box appears in the center of the computer screen, inside which digits, from 2 to 9, appear in a pseudo-random order, at the rate of 100 digits per minute. You will need to detect target sequences of digits (for example, 2-4-6, 3-5-7, 4-6-8) and to register responses using the press pad.

Electrical Impedance Myography (EIM)
These measurements will help determine the function and health of the muscle. The skin will be briefly moistened with saline (salt water) and the sensor will be placed on the skin over the muscle and held by the examiner. You will need to remain as still as possible for no more than about 1 minute as the measurements are taken. Electrical current is generated by this machine, but you will not be able to feel it. The sensor on your skin records electrical activity which is returned to the machine and analyzed by a computer. The measurement will then be repeated if necessary several times. We will continue to apply the salt water often to the skin during this time to keep it wet. Two muscles in both arms and two muscles in both legs will be measured in the same way.

The Convergence Medical Devices EIM1103 device used in this study is investigational, which means that it is not approved by the Food and Drug Administration (FDA) for routine use in

patients. However, it has undergone safety testing to ensure that any risk associated with its use is minimal and it has been used in many studies involving patients.

Skeletal Muscle Biopsy

A sample of your muscle tissue will be collected from your upper leg. For this procedure you will lie down on a comfortable surface. We will use a local anesthetic to numb an area of your skin and thigh muscle before obtaining a very small amount of muscle (about the size of an unpopped popcorn kernel) via a muscle needle biopsy. The muscle biopsy involves taking a small piece of muscle tissue from a single incision site in your thigh muscle. Prior to the incision, the skin is cleaned and made sterile. Then the skin and tissue below are injected with local anesthetic to eliminate most of the associated pain. A small incision about the size of this dash "_____" will be made through which a needle about the size of this letter "O" is advanced into the muscle. A piece of the thigh muscle is then removed with the needle. The incision site will be closed with a steri strip or a suture, if necessary and a light dressing will be applied.

We will provide you with an informational take-home sheet that addresses care of the biopsy incision sites and will provide you with extra band-aids and topical antibiotic.

The total anticipated duration of this testing session is approximately 3-4 hours.

Test Session 2

You will meet the research team at the Ross Heart Hospital or Martha Morehouse Medical Plaza for resting MRI and a maximum effort treadmill stress test followed by subsequent MRI to evaluate cardiac function. Prior to MRI the MRI screening form will be reviewed and you will be required to give consent for MRI. You will then be prepped and electrocardiogram patches will be applied to your torso to enable accurate HR measurement and MRI scan timing. Resting MRI imaging will involve laying on the MRI exam table while being scanned to evaluate visceral body fat, liver fat, and resting heart function. During scanning breath hold commands may be given to you. Total breath hold duration will not exceed 30 seconds.

We will use the MRI machine to scan your leg muscles for about 10-20 minutes. During this time, you will be asked to keep your legs as still as possible, and you will hear some noises from the machine. For the next part, we will ask you to kick your legs against several straps for 30 seconds. After the 30 seconds is over, you will lie still for another 10-20 minutes while we continue to scan the thigh muscles.

After rest imaging your blood pressure will be measured. You will be asked to stand and safely transition yourself from the MRI table to the treadmill. The exercise stress test will involve graded increases in workload. Electrocardiogram, blood pressure, and exercise performance will be monitored by a certified exercise physiologist and or physician. The treadmill exercise will be discontinued according to the American College of Sports Medicine guidelines, volitional fatigue, or desire to end exercise testing. During testing oxygen and carbon dioxide gas exchange

will be monitored with a metabolic cart. Just after exercise you will be instructed to move back to the MRI exam table immediately for post exercise imaging.

The total anticipated duration of this testing session is 1.5-2 hours.

One of the researchers of this study, Dr. Orlando Simonetti have a financial interest in the company (EXCMR, Ltd.) This company is responsible for the design and construction use of the MRI-compatible treadmill utilized during exercise testing for this study; however, they are not involved in subject selection or subject care and management in this study, and they are not participating in obtaining consent from the subjects

Test Session 3

You will need to be fasted for this session, which means you cannot consume any food or drink other than water for 10-12 hours prior to the session. To ensure proper hydration, please consume 2 cups of water the night before the session and another 2 cups the morning of the session. The following will occur during this session:

You will provide a urine sample, which we will use to ensure that you are adequately hydrated.

Mood state surveys will be administered prior to any physical activity taking place.

A catheter may be inserted into a vein in your arm to allow for repeated blood draws throughout the session. Resting blood will be drawn after you have sat quietly for 15 minutes. The blood will be taken via venipucture from a vein in your arm using a small needle. A total of up to 50 mL (~ 3 tablespoons) will be drawn at rest.

You will collect a sample of cheek cells with two small bristle brushes that you will rub on the inside of your cheeks.

You will be asked to step onto a force plate. Your center of mass will be measured via force plate. perform a series of jumps on a force plate.

* Max Vertical Squat jumps
Starting from a squat position on the force plate, you will jump in the air as high as you can and land on the force plate.

* Countermovement jumps
Starting from an upright position on the force plate, you will squat down and then jump in the air as high as you can and land on the force plate

You will complete a minimum of 2 guided warm-up sets of the back squat exercise and then perform a 1 repetition maximum test sequence to determine your maximal lower body strength.

You will do the same for the bench press exercise. If necessary, instruction for proper completion of these exercises will be provided.

You will perform a Symbol Digit Modality Test (SDMT) to measure effects of physical exertion on cognitive performance.

You will perform a series of maximal sprints on the HiTrainer self-propelled treadmill. To make this assessment military specific, you will wear military-issued combat boots and a military-style pack containing 42 kg of load. Blood will be drawn from the catheter following each sprint.

You will repeat the SDMT prior to the repeated sprint assessment after each of two sprint circuit.

Following a brief rest, and a battlespace map memory test, you will complete a military-specific obstacle course. The course will consist of a 30 m sprint, followed by a 27 m zig-zag run, and then a 10 m casualty drag with 79.5 kg of load. Similar to the repeated sprint test, the obstacle course will be completed in combat boots and a pack containing 42 kg of load. Each segment of the course will be timed to assess performance. Immediately following the obstacle course, you will be asked to locate specific points of interst that correspond to the battlespace map that you were shown just before the obstacle course to assess memory.

Up to 50 ml of blood will be drawn from the catheter immediately after completion of the obstacle course and again at 30, 60, and 120 minutes after completion. All blood draws during this session will total to no more than 150ml of blood (approximately 10 tablespoons).

The total anticipated duration of this testing session is about 4 hours.

Abbreviated Cognitive and Physical Performance Monitoring Session:

You will complete an abbreviated testing session to evaluate cognitive and physical performance at specified intervals during the intervention. This abbreviated test session will be completed at weeks 1, 4, and 8. The abbreviated is described below.

You will arrive at the PAES building (lower level) euhydrated and at least 24hrs post exercise. You will be asked to complete a urine specific gravity test prior to being asked to complete a warm up and subsequent anaerobic strength and power measurements.

Warm Up:
The warm up will require that you walk for 5 minutes on a treadmill prior to performing both static and dynamic stretches for the hip, knee, and ankle joints guided by the research personnel.

Counter Movement Vertical Jump:
The purpose of this test is to measure absolute and relative power output. Starting from an upright standing position on the force place, You will squat down and then jump in the air as high as possible and land on the force plate.

Isometric Squat Force
The purpose of this assessment is to measure absolute and relative lower body force production (strength). Standing on a force place and starting in a specific knee flexion angle squat position under a static barbell, You will push upward against the barbell with as much force as possible for thirty seconds.

After you complete the physical performance measures cognitive assessment in the form of the SDMT and Virtra firearms/combat simulator will be administered as described previously.

Controlled Feeding

If you are in one of the dietary intervention groups, we will prepare all your meals for you in the Instructional Kitchen in the Ohio Union. Daily macronutrient intake for the low-carbohydrate, ketogenic diet will consist of < 50 g carbohydrate, ~15-20% protein, and ~70-75% fat. A wide range of whole foods will be incorporated into your meals, including non-starchy vegetables, fruits, meats, nuts and seeds, oils, cheese, butter, cream, and eggs. If there is a specific food or ingredient you would prefer to avoid, we can work with you to exclude it from your meals. You will be asked to pick-up your food at the kitchen up to 3 times per week. All your food will be prepared and packaged in reusable and microwaveable containers labeled by meal (breakfast, lunch, dinner, morning or afternoon snack). A study team member will meet with you to ask and record if the amount of food was too little, too much or adequate. Finally, when you pick-up your food you will also return the plastic containers from the previous food pick-up. Please return these containers empty and rinsed, but not washed. We will wash and sanitize all the containers after you return them.

Throughout the duration of the dietary intervention, you will be asked to finger-stick testing for glucose and ketones each day. This will provide us with the information we need to adjust your diet so that you maintain a specific level of nutritional ketosis. You will be provided with the meter and we will teach you how to use it.

Depending on which group you are in, you may be asked to consume a dietary ketone supplement each day, the dosage for which will be dictated by your daily ketone measurements.

Training

You are expected to train on your normal schedule as regulated by Ohio State University ROTC. Research key personnel will assist in developing, supervising, and monitoring training. You will be asked to share detailed training logs with the research team that allow for assessment of training progress and completion. Training modifications will be submitted for approval by ROTC leadership prior to enactment.

Participant Completion

After you complete the study, an exit meeting will be scheduled with a member of the study team. You will receive your payment for participating in the study and any personal data that has been analyzed. This information will include body composition and resting energy expenditure, and any other blood analysis that is complete at this time. Analysis of most of your data will be completed after you have finished the study, and we will make that available to you as well. This will include personal data from the blood, cheek cells, and muscle tissue we collected during the study as well as the MRI. You will only receive your own data.

Analysis

All the blood, urine, cheek cells, and muscle tissue we collect from you (we refer to them as biological specimens) will be kept in cold storage at -80oC in our biochemistry lab. Your biological specimens will be labeled with your subject identifier and not your name to maintain confidentiality. During sample analysis some of your biological specimens will be sent to collaborators who will perform some of the analysis, but your name will not be shared with them. Only your subject identifier will be provided to our collaborators. We will be measuring several markers in your biological specimens related to cardiovascular health, inflammation, and antioxidant status. However, since we will be storing your samples for up to five years, we may think of other markers to measure that we did not think of prior to the start of this study. You have the right to decline the use of your samples for any potential future analysis. Below are two check boxes indicating that you either will allow us or will not allow us to use your biological specimens to measure future markers. If you select not to allow us to use your biological specimens for future analysis, then any left over biological specimens will be destroyed. Please select an option below and sign your name with today's date. The extra signature indicates that you have thought about, read and understand this option. Please keep in mind that the selection of either option will have no impact or penalty during your participation in the study, and you will not lose any benefits to which you are otherwise entitled.

Yes, I give permission to use my biological specimens for any future analysis. □

No, I do not give permission to use my biological specimens for any future analysis. □

Participant Signature: _____Date: _____

4. How long will I be in the study?

The duration of this study is expected to be 14 weeks, which includes a week for baseline testing, the 12 week dietary intervention period, and another week for final testing. We cannot perform testing when people become ill. Therefore if you become sick during the study, the feeding phase may be extended. The feeding phase could also be extended if we cannot find a good time for you to come in for testing due to scheduling issues. Based on these factors, the total duration of the study may exceed 14 weeks.

Can I stop being in the study?

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

6. What risks, side effects or discomforts can I expect from being in the study?

Finger-stick Testing
The finger stick may cause a slight immediate discomfort at the specific stick site. Under normal conditions, there are minimal risks to you when performing finger-sticks that include: bruising; light-headedness or dizziness due to fear of needles; and infection.

Blood Draws
Blood draws may cause discomfort at the skin puncture site and a small bruise may develop that may persist for several weeks. There is also a small possibility of an infection. Every precaution to avoid infection will be taken including the use of sterile disposable needles and gauze and the practice of aseptic (sterile) techniques during the blood draw.

Electrical Impedance Myography
EIM involves only minimal risk. The estimated testing duration for an individual subject is 15 to 30 minutes depending on the number of muscles tested. Testing positions and procedures will be modified as needed to increase subject comfort and measurement accuracy. Subjects may be discontinued from the study at any time and for any reason, either at the subject's request or per the decision of the principal investigator. The electrical impedance myography device has not been used in infants to date. However, it has undergone independent safety testing by Intertek and it has been used on more than 30 pediatric subjects ages 3-12 to date (including healthy controls and DMD patients).

Muscle Biopsy
The area of your leg in which the muscle biopsy will be performed contains no major neural or vascular structures. Excessive bleeding is a potential risk associated with the muscle biopsy procedure. However, you need to understand that muscle biopsies are painful. While the pain is typically quite tolerable, it is impossible to make the biopsy a completely pain-free procedure. We will make every reasonable attempt to make you as comfortable as possible.

All biopsies will be performed by a licensed physician, who has been trained in the performance of superficial minor surgical procedures and instructed in the specifics of the muscle biopsy procedure. The muscle biopsy technique has been employed thousands of times with human volunteers. When the biopsy is performed you will be lying on your back. First, a small portion of your leg will be cleaned with alcohol and the skin and muscle will be numbed with Lidocaine. Lidocaine injection can contribute to risks of signs of an allergic reaction, which include hives, difficulty breathing, swelling of your face, lips, tongue, or throat; Serious side effects, which include feeling anxious, shaky, dizzy, restless, or depressed, drowsiness, vomiting, ringing in

your ears, blurred vision, confusion, twitching, seizure (convulsions), fast heart rate, rapid breathing, feeling hot or cold, weak or shallow breathing, slow heart rate, weak pulse, or feeling like you might pass out; Less serious side effects, which include mild bruising, redness, itching, or swelling where the medication was injected, mild dizziness, and nausea. To do this, the Lidocaine will be injected using a thin needle. You will feel the poke of the needle as well as a burning sensation when the Lidocaine is injected. When the needle is placed into the muscle to numb it, the muscle will frequently respond with a rapid contraction that will feel like a brief cramp. This is normal and expected.

After the skin and muscle are numbed, your skin will be sterilized with an iodine solution called Betadine. This is done to diminish the possibility of an infection being caused by the biopsy. We will then cover the skin around this area with a sterile drape. Once you are numb, an incision will be made in your skin. Through this incision, a deeper incision will be made in the fascia around the muscle. These incisions are made so that the biopsy needle can be gently introduced into the muscle, and making the incisions will be completely painless because of the Lidocaine.

While Lidocaine does a good job of eliminating the sharp pain of an incision, it cannot fully eliminate the dull pain associated with doing the biopsy. The biopsy needle is about the same diameter as a Bic pen. After the incisions in the skin and fascia are made, the needle will be inserted through them into the muscle. Similar to when the numbing needle entered the muscle, the biopsy needle will also likely cause the muscle to cramp for a second. Once in place, the needle will be used to make three or four "snips" in the muscle, which will be removed from you. The total amount of muscle removed will be between 50 and 200 mg, or a total amount about the size of an unpopped popcorn kernel. After this, the needle will be withdrawn, some pressure will be put on the site to control bleeding, and the incision will be closed with a steri strip or one suture, if necessary. You will not notice this muscle missing, either cosmetically or functionally. There are studies where more than 400 mg have been removed without problems.

After the procedure, you will have a pressure dressing applied to the site and your thigh will be wrapped with a compressive wrap. It will be tight, but should not be painful. The night of the procedure, you will be instructed to keep your knee bent as much as possible, apply ice to your thigh, avoid heat or massage, and avoid anti-inflammatory medication.

It is vital that you understand that your thigh will hurt after a muscle biopsy. The pain you will feel will be like a deep "Charley Horse" and will typically improve over 48-72 hours. It is impossible to quantify the pain for you. Everyone's experience with pain is unique, and one's sensation of pain is influenced by multiple other factors than just the procedure itself. The exact same procedure, done the exact same way, will be felt differently by different people. It will even vary in the same person if they have multiple biopsies over time. There have been situations where people have hurt for more than a week. The more accurate expectation is 48-72 hours of tolerable aching in your thigh. After the first night, you will be allowed to exercise in any way you tolerate.

Other risks include infection, which is very rare, less than 1 in 1000 biopsies. Sterile technique is used to limit this risk, and our laboratory has never had a wound infection from a muscle biopsy. If one were to occur, it is typically easily treated with oral antibiotics. There is also a risk of nerve injury associated with incisions and the biopsy, and even the use of the numbing medication has been associated with prolonged numbness. While large incisions almost always generate permanent numbness around the incisions, because it is impossible to make an incision in the skin without severing small skin nerves, the incisions in this procedure are small enough, though, that this rarely happens with a muscle biopsy. Another nerve injury that can occur with a procedure in the lateral aspect of the thigh is an injury to the lateral femoral cutaneous nerve, which lies between the skin and the muscle. This nerve provides sensation to the lower, outer part of the thigh, and if it is injured there is resultant numbness of decreased sensation in this injury. Permanent injury to this nerve has never been reported in association with a muscle biopsy, but the nerve can be temporarily injured, causing a decreased sensation in this area. There is rarely any pain associated with this injury and the nerve does not go to any muscles, so there is no effect on strength or function. Most people do not find it troublesome. The duration of the temporary injuries to nerves is difficult to predict. They can last from a few days to several months and there is no predictable way to determine the duration of the injury at the onset.

Strenuous Exercise

The involvement of strenuous exercise in this study means that there is risk for adverse effects. However, based on eligibility criteria for this study, which is based on ACSM guidelines, your potential for risk is minimal. The risk associated with these eligibility criteria includes a 1 in 1,666 chance of abnormal heartbeats or heart attack as well as a 1 in 18,000 chance of sudden cardiac death

Body Composition-DEXA

You will be exposed to a very small amount of radiation by the scanner used to measure your body composition. Exposure to any amount of X-ray radiation, no matter how low, may cause abnormal changes in cells. However, the body continuously repairs these changes and the amount of radiation is very low in this study. DEXA risks include exposure to radiation similar to a flight from New York to Los Angeles which may elevate cancer risk. DEXA risks are minimized as the measures are only performed twice on each participant. The extra lifetime risk of dying of a fatal cancer due to the radiation exposure from this research may range from about one in 500,000 to about one in 200,000. At such low radiation exposures, scientists disagree about the amount of risk. These estimates are very uncertain, and there may be no extra risk at all. The total exposure for the whole body scan is approximately 125 times less than the average radiation from a standard chest X-ray.

Magnetic Resonance Imaging (MRI)

MRI poses little risk as there have been no harmful effects recorded for magnet and radio waves when properly screened. No contrast agents will be used during MRI. Known risk during MRI is that magnet could attract certain kinds of metal that may cause injury to you. We will ask you about metal within your body (this includes certain dyes used in tattoos and body piercings). If there is any question about potentially hazardous metal within the body, you will not be able to have an MRI study. We will also keep the MR room locked so that no one carrying metal objects

enters the room while you are having this scan performed. In addition, the MR scanner makes a loud buzzing noise that could affect hearing ability. You will be provided with earplugs and assistance in their use in order to protect your hearing. You will be able to communicate with the scanner technologist using an intercom and/or signaling device. The technologist will try to help you feel as comfortable as possible in the scanner. You can ask to stop the scan and be removed from the scanner at any time by using the intercom or signaling device.

Metabolic Rate
During this test you will be asked to breathe into a ventilated hood. There is a possibility for you to feel claustrophobic, but the hood provides enough space for your head, neck and shoulders. Also, the ventilated hood is clear, so it allows normal visibility and the ability to rotate the neck, which should minimize the chances of feeling claustrophobic.

Cheek Cell Collection
There are no risks associated with using a swab inside of your cheek to collect cheek cell samples.

Feeding Period
Since this is a controlled feeding study, it is important that you only eat the food we provide to you. This may be inconvenient. However, we will work with you to make sure that it is not too burdensome on your normal life and activities. Since we will be providing all your food during the feeding phase, your ability to travel during this time will be limited, but we can work with you to accommodate your travels. The diets are formulated with normal foods and thus we do not expect any significant side effects or discomforts. However, if you experience discomfort, we will work to adjust the diets to minimize symptoms.

Surveys
Surveys administered in this study are designed to evaluate psychological aspects of mood, resiliency, and stress, which may make participants uncomfortable. The surveys have potential to cause you to feel anxiety, stress, depressive feelings, etc. Questions are free to skip any question(s) that make them uncomfortable as needed. Research key personnel will help to provide contact information for resources such as the student health center, medical center, or emergency department if necessary to aid with intense psychological distress induced by surveys.

7. What benefits can I expect from being in the study?

You may gain insights into appropriate meal portion sizing and foods that can be made from the diets you will be exposed to during the study. This experience may help inform you about ways you could modify your own diet. Our staff and registered dietitians will also be available to answer questions you may have about the diets you will be eating to aid in your nutrition education. At the end of the study and after we have completed the blood analysis, you will also receive your own results back and you will be able to see if the diet led to any improvements in your health or performance.

8. What other choices do I have if I do not take part in the study?

You may choose not to participate without penalty or loss of benefits to which you are otherwise entitled.

9. Will my study-related information be kept confidential?

For all the data collected over the course of the study (i.e. records, biological samples and questionnaires) a unique subject identifier (i.e. a code) will be assigned and used instead of your name. This identifier, which links your name to your data, will only be available to research personnel. Any records that contain your name and identifier together will either be stored in the Kinesiology file storage room in a locked file cabinet or protected on a computer via password protection on the individual digital file and password protection on the computer the file(s) are stored on. All other records that only contain the subject identifier will be kept in either a file cabinet in our locked file storage room or on a password protected computer. Your name will never be used in any presentation or publication resulting from this study. The records will be maintained until the data are published and up to a maximum of five years after the completion of the study.

There may be circumstances where your information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law.

Also, your records may be reviewed by the following groups (as applicable to the research):
Office for Human Research Protections or other federal, state, or international regulatory agencies;
U.S. Food and Drug Administration;
The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
The sponsor supporting the study, their agents or study monitors; and
Your insurance company (if charges are billed to insurance).

If this study is related to your medical care, your study-related information may be placed in your permanent hospital, clinic, or physician's office records. Any potential incidental findings from MRI, blood biomarkers, etc. will be shared with the participant. This information is non-diagnostic and will be provided so the participant may choose to share with their physician if they would like to address potential findings and receive diagnostic medical screening or testing. Authorized Ohio State University staff not involved in the study may be aware that you are participating in a research study and have access to your information.

You may also be asked to sign a separate Health Insurance Portability and Accountability Act (HIPAA) research authorization form if the study involves the use of your protected health

information. Unpublished research information/findings from this research study will be kept confidential. Study related information will not be shared with ROTC leadership, cadre, etc.

10. What are the costs of taking part in this study?

Other than your time, there are no costs to participate in the study. You may need to pay for parking if you do not have an Ohio State University parking pass, but we have temporary passes that we can provide you with.

11. Will I be paid for taking part in this study?

Yes, if you complete the study you will receive a total of $200. No compensation will be provided for completing the screening visit or the baseline testing alone. $200.00 compensation will be distributed throughout the study and paid in 2 installments of $75.00 and $125.00. The first $75.00 payment will be received at the conclusion of week 4 of the dietary or control intervention. The second installment of $125.00 will be received at the conclusion of the post testing battery. Payments will be given in cash and/or check form

By law, payments to subjects are considered taxable income.

12. What happens if I am injured because I took part in this study?

If you suffer an injury from participating in this study, you should notify the researcher or study doctor immediately, who will determine if you should obtain medical treatment at The Ohio State University Wexner Medical Center.

The cost for this treatment will be billed to you or your medical or hospital insurance. The Ohio State University has no funds set aside for the payment of health care expenses for this study.

13. What are my rights if I take part in this study?

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study.

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled.

An Institutional Review Board responsible for human subjects research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable

state and federal regulations and University policies designed to protect the rights and welfare of participants in research.

14. Who can answer my questions about the study?

For questions, concerns, or complaints about the study you may contact Dr. Jeff Volek. His office number is 614-688-1701 and his email address is volek.1@osu.edu.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact Dr. Jeff Volek. His office number is 614-688-1701 and his email address is volek.1@osu.edu.

Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

Printed name of subject	Signature of subject AM/PM Date and time
Printed name of person authorized to consent for subject (when applicable)	Signature of person authorized to consent for subject (when applicable) AM/PM
Relationship to the subject	Date and time

Investigator/Research Staff

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

Printed name of person obtaining consent	Signature of person obtaining consent AM/PM Date and time

Witness(es) - May be left blank if not required by the IRB

Printed name of witness	Signature of witness AM/PM

Date and time

Printed name of witness

Signature of witness

AM/PM

Date and time