Abstract
Low choline intake has been shown to cause elevated creatine kinase activity in blood, which indicates muscle breakdown. The link between low choline intake and muscle damage has not been previously studied, so this proposed study aims to investigate the ability for optimal choline intake to blunt muscle dysfunction. Subjects with existing low choline intake and who are at risk for choline deficiency will be recruited into the study. Participants with the predisposing genotype for choline deficiency, or the MTHFD1 1958A variant, will be targeted for recruitment into the study. Additionally, participants will also need to be Caucasian, male, and between the ages of 50 and 70 to qualify. These additional characteristics also increase the risk for choline deficiency. Focusing on this particular group will provide a sample that is more homogenous in choline needs and eliminate inter-individual variations that genetic variation, gender, ethnicity, and age group can cause. Existing muscle dysfunction will be unmasked with eccentric exercises and the associated elevated serum creatine kinase activity will be used as a measure of muscle damage.

A randomized, crossover study with 14 participants will be conducted during a six-week period. Within the six-week period, there will be a three-week period where participants will consume choline supplements and another three-week period where participants will consume placebos. There will be an exercise challenge with eccentric exercise during both periods and the absolute percent change in CK response will be compared in each period to evaluate choline's ability to blunt this response. **It is hypothesized that higher choline intake among 50-70-year-old Caucasian males with the MTHFD1 1958A variant decreases CK activity after high-intensity eccentric exercise.**

A better understanding of choline's ability to mitigate muscle dysfunction among those with a predisposing genotype can help tailor choline intake recommendations. Preventing muscle dysfunction can potentially increase exercise tolerance and the ability for individuals to engage in physical activity. Long-term health benefits of improved exercise tolerance are largely unknown, but it can potentially promote healthy aging through higher tolerated intensities of resistance training. The most effective prevention and treatment intervention strategy to reverse muscle loss associated with aging is resistance training, but muscle dysfunction can impair the ability for individuals to tolerate this type of exercise. It is therefore crucial for older adults to engage in strength training exercises and for nutrient intake to be maximized in ways to support physical exertion. The results of this study could provide evidence to increase choline intake for those exercising and striving to preserve and increase muscle mass.

A. Specific Aims

Previous studies investigating choline requirements have shown an increased need among men, post-menopausal women, and Caucasians (1). Predisposing genotypes also play an important role in choline needs since even among those who are more resistant against choline deficiency, such as pre-menopausal women, specific genetic polymorphisms have been shown to increase choline needs (2). The concept of "personalized" nutrition, or basing dietary needs on genetics, can be utilized as a basis for targeting individuals who are at risk for choline deficiency to study choline's ability to prevent muscle dysfunction, one of the consequences observed when choline intake is inadequate. A better understanding of choline's ability to mitigate muscle dysfunction among those with a predisposing genotype can help guide choline intake recommendations.

The goal of this study is to demonstrate the application of using genotype-directed nutrition guidance for individuals with a genetic variant requiring a potential increase in choline intake to prevent muscle dysfunction, a condition that can be revealed with eccentric exercises. Previous research investigating a low-choline diet indicates Caucasian men with the MTHFD1 1958A genetic variant are at an increased risk for muscle dysfunction when an inadequate amount of dietary choline is consumed (3,4). Low-choline intake is linked to muscle damage in the form of increased serum creatine kinase (CK), which can be explained by a lack of phosphatidylcholine to preserve membrane integrity. This measurable increase in serum CK level is the result of muscle cells that have undergone apoptosis (5) as a result of the eccentric exercise challenge. Carriers of the very common MTHFD1 1958A genetic variant are more likely to develop signs of choline deficiency than non-carriers because the genetic variant results in an enzyme with reduced activity that acts in the choline metabolic pathway (3). Although muscle dysfunction has been observed with a low-choline diet, the direct relationship between the choline needs of this variant to decrease muscle dysfunction has not been previously studied. Consequently, this study aims to explore the CK response after eccentric exercise is completed among Caucasian male carriers of the MTHFD1 1958A genetic variant during choline-supplemented and choline-deficient periods.

Hypothesis: Higher choline intake among 50- to 70-year-old Caucasian males with the MTHFD1 1958A variant decreases CK levels after eccentric exercise.

The following aims will be completed to test this hypothesis:

Aim 1: Unmask choline-deficiency-related muscle dysfunction with eccentric exercise, measured as increased CK response.

Aim 2: Show choline supplementation is linked to lower serum CK levels after performing eccentric exercises among individuals with MTHHFD1 1958A variant.

Targeting older adults between 50 and 70 years old will provide a group that is more likely to be malnourished and possibly choline deficient. Older adults tend to be more at risk for malnutrition because poor appetite, a decrease in the ability to taste and smell, poor dentition, and an inability to shop or prepare food are common contributing factors that lead to a diet lacking in adequate nutrition (6). Protein intake, a major source of choline, has been shown to be inadequate among older adults (7).

The results of this study will demonstrate the ability for choline to blunt muscle dysfunction, and serve as a critical nutrient needed to maintain and increase muscle mass. In the long-term, preserving muscle mass can prevent sarcopenia and promote healthy aging.

B.1 Research Strategy
B.1.1 Significance

There are great public health implications in studying the link between choline and muscle dysfunction. If choline is proven to reduce muscle dysfunction, there is a possibility that adequate choline can lead to improved exercise tolerance. Long-term health benefits of improved exercise tolerance can mean an individual can engage in more exercise without experiencing side effects associated with muscle dysfunction. Staying active through exercise is key in disease prevention and healthy aging.

This study will improve the understanding of choline's role in strength training exercise tolerance and potentially offer a dietary intervention to counter the natural progression of muscle loss. Targeting 50-70 year olds will aim at a population at risk for muscle loss and sarcopenia, as well as poor dietary intake. Targeting Caucasian male carriers of the MTHFD1 1958A variant will provide a population that has been shown to be at an increased risk for choline-deficient muscle dysfunction. Selecting this specific subgroup will allow a link between choline and muscle damage that may not be otherwise detected if the study sample is not chosen based on genetic polymorphisms to be seen. Resistance tolerance may be tolerated for longer and at higher intensities, as soreness is resolved with adequate choline, allowing for adequate exercise to be achieved to counter muscle loss that occurs as a natural part of the aging process. If this study is able to show choline's role in improved exercise tolerance, choline intake recommendations may be refined to promote healthy aging.

The link between choline and improved exercise tolerance: There is evidence to suggest choline may play an important role in strength training tolerance and subsequently serve as a practical dietary intervention to prevent muscle dysfunction, although this direct relationship has not been previously proven before. Choline deficiency has been shown to result in muscle cell damage because of its role in cell membrane biosynthesis (2). Choline-deficiency muscle dysfunction can limit exercise tolerance, as inadequate intake of this essential nutrient can lead to muscle cell apoptosis and membrane fragility (5). Strength training among those who are choline deficient can be problematic because of pain associated with muscle cell damage. Muscle damage is often experienced as stiffness, swelling, pain, reduced strength and soreness, which can impair continued efforts to exercise. In fact, the presence of pain can deter physical activity and has been a significant predictor for developing sarcopenia later in life (8). Resistance training remains the primary prevention and treatment intervention strategy to reverse muscle loss associated with aging (9,10), but implementing this type of exercise regimen can be challenging for individuals susceptible to muscle dysfunction. For those that are susceptible to choline-deficiency muscle dysfunction and experience pain when exercising, increasing choline intake may blunt this pain and allow tolerance for higher exercise intensities.

Functional status and quality of life, especially during the aging process, can be optimized with resistance training exercises. Resistance training in older adults can increase muscle strength and mass, enhance energy expenditure and body composition, reduce difficulties with completing daily tasks, and can promote physical activity. Getting adequate strength training exercise is critical in older age because age-related declines in health, such as increased body fat and decrease in muscle mass, can greatly hinder the ability for older adults to have the capacity to perform daily activities (11). Therefore, decreasing muscle dysfunction is important in promoting the ability to perform resistance training.

Possible long-term benefits of preventing muscle dysfunction: Identifying risk factors and causes of muscle dysfunction are needed to develop interventions to promote healthy living and aging. Current studies have linked specific modifiable behavioral factors in the treatment and prevention of muscle loss. In particular, there is evidence to indicate a diet adequate in protein, vitamin D, and antioxidant nutrients along with resistance exercise training can help promote muscle hypertrophy (12). A regular exercise program with an emphasis on strength training is the preferred method for reversing or slowing the progression of muscle loss and function (9, 10,13). It is therefore crucial for older adults to engage in strength training exercises and for nutrient intake to be maximized in ways to support physical exertion.

Preventing muscle dysfunction may have long-term public health benefits on the aging process. In older age, older adults are at risk for sarcopenia, the natural progressive decrease in skeletal mass with either low muscle strength or low physical performance (8). Muscle loss and fat gain naturally accelerates during the aging process. Starting as early as age 30, an average adult is predicted to gain 1 pound of fat and lose 0.5 pound of muscle annually until age 60 (14). Muscle mass loss after age 60 then accelerates with an estimated 2% loss yearly while muscle strength simultaneously decreases 3% annually (15). The prevalence of sarcopenia is estimated to be 15% among people over 65 years old and 50% of people over 80 years old (16). The consequences of sarcopenia can result in the loss of independent living and autonomy among older adults and an increased reliance on external help to accomplish day-to-day tasks (9). In addition to the natural progression of muscle loss, the risk for hospitalizations and falls is also increased in older age, resulting in greater muscle atrophy and risk for sarcopenia (17).

In the year 2000, the direct health care cost alone of sarcopenia in the U.S. was estimated to be 18.5 billion dollars (16, 18). The public health significance of sarcopenia will likely grow as the life expectancy around the world continues to increase. The world's population over the age of 60 years old is expected to increase by 300% from 600 million in 2000 to more than 2 billion by 2050 (19).

Additionally, decreasing the progression muscle loss is key in recovering and surviving through health conditions and diseases. Having adequate muscle mass is important in recovering from severe trauma or critical illness while muscle strength and function is central in the recovery process. Prolonged recovery can be required if extensive muscle loss occurs during hospitalizations and recovery of normal function may not be achieved if preexisting muscle mass deficiencies were substantial before being hospitalized (20). This is why less than 50% of women over 65 years old who break their hips never walk again (21). The ability to engage in exercise and build muscle plays a significant role in maintaining health to maintain day-to-day function and to shorten recovery time from injuries.

B.2 Background

B.2.1 Innovation

Using nutrigenetics to shape dietary recommendations: Traditional research in nutrition has generally focused on the assumption the same nutritional requirements are needed for all individuals to stay healthy (22). Current nutritional recommendations for the general public are only specific to age group and gender; however, further differences in nutritional needs that are not captured by these subgroups may be critical in maintaining health. One fundamental difference between individuals is genetics. Tailoring nutrition recommendations to incorporate nutrigenetics, a field that identifies genetic susceptibility to diseases, has the potential to eliminate a source of inter-individual variation that makes it difficult for nutrition studies to establish relationships between nutrients and health outcomes. Nutrigenetics can provide

individualized diet recommendations to meet the needs of particular genetic variants so genetic susceptibility to diseases can be prevented. As a result, nutrition recommendations can be established in a more targeted manner, allowing for diseases to be better prevented through dietary intake. Using dietary interventions in this targeted way has substantial public health application on both the population and individual levels. At the population level, studying the nutritional needs for specific genetic variants can help shape recommended dietary intake ranges to keep a greater proportion of the population healthy. At an individual level, genetic testing is already available for more than 1,700 diseases (22). Interpreting this information and creating personalized intervention strategies is greatly needed. Many other health fields, such as pharmacogenetics, have been using gene polymorphisms as a basis for tailoring medical treatments to improve effectiveness (22). Nutrition guidance in a clinical setting has the potential to be personalized as more research is conducted to integrate nutrigenetics into practical interventions.

This investigation will advance research in this area by focusing on a specific ethnic population with higher percentages of individuals carrying the MTHFD1 1958A variant and the increased choline need that is potentially required as a result of this variant to prevent muscle dysfunction. Refining a target population in this specific manner will allow for a better understanding of choline's role in muscle dysfunction.

Limitation of a previous study: Choline's effect on physical exertion has not been widely studied. A previous study evaluating choline's role in physical performance did not find choline to have an effect on physical performance after exhaustive physical activity (23). A limitation to this study, which may have led to this finding, is that key genetic variants that may require more choline intake were not targeted. By separating participants out by genetic variants, we may see a more defined role choline has in improving physical performance after exertion.

Targeting the MTHFD1 1958A variant in Caucasian males to understand choline's link to muscle dysfunction: Adequate choline intake as established by the Institute of Medicine and the National Academy of Sciences of the USA is 550mg per day for men and 425mg per day for women. Males have a higher requirement because estrogen stimulates endogenous choline production (24). Therefore, focusing this study on males will result in a more homogenous response to choline supplementation.

The MTHFD1 enzyme acts in the choline synthesis pathway and the 1958A variant results in an enzyme with reduced maximal activity, resulting in higher choline intake requirements (3). This specific allele is more common in Caucasians who have ancestry traced back to North and West Europe than those with African ancestry (4). Targeting Caucasians will yield a higher percentage of carriers with this variant. In a previous study conducted in the Raleigh-Durham-Chapel Hill area, approximately 50% of Caucasians had this variant of interest (3).

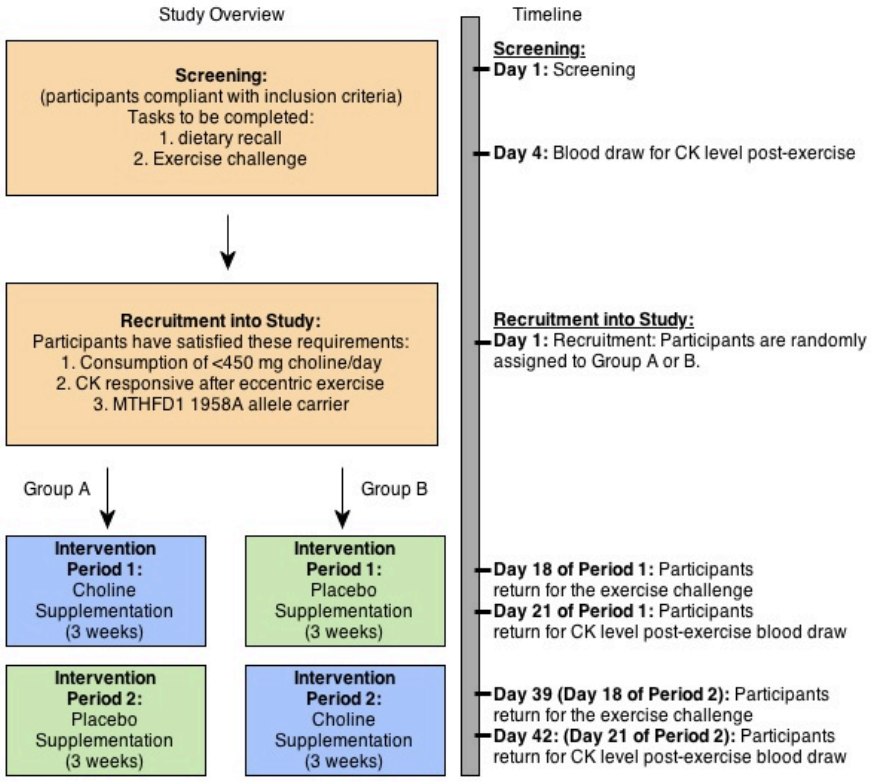
Eccentric exercise to promote measurable muscle damage: Muscle dysfunction uncovered by eccentric exercise in the form of arm extensions has been shown to correlate with changes in serum CK levels (26). In a group of CK non-responders, a 2-12% absolute percentage change in CK was observed while a 389-912% in absolute percentage change in CK was observed among CK responders after eccentric exercise was completed (25). Previous research studies have shown CK levels peak 72 hours post-exercise and returned to baseline after 2 weeks post-exercise (26). One study found individuals of Caucasian background who responded to eccentric exercise with an increase in CK level to be approximately 10% (25).

B.3 Approach

B.3.1 Study Overview

A randomized, crossover study will be conducted during a 6-week period. **Figure 1** on the right outlines the overview and timeline of the study. **Screening:** If inclusion criteria as indicated in section **B.3.2a** are met, participants will be invited to the research laboratory to complete a 24-hour dietary recall. Choline intake will be evaluated to ensure consumption is <450mg per day. If intake meets this criterion, a blood draw for genotyping and baseline CK level will be taken. Then, eccentric exercise in the form of 12 arm extensions will be conducted at 100% maximal lift capacity to challenge the muscle. Subjects will return 3 days later for a second blood draw to capture peak CK levels. **Recruitment into Study:** For those who consume <450mg choline per day, are CK- responsive 3-days post-exercise, and have the MTFHD1 1958A allele, enrollment into the study will be offered. Participants will be randomly assigned to Group A or B, with the only difference between the two group is the order of the two intervention periods where participants take either a choline or placebo supplement. Participants start with a 3-week period with choline supplementation or a 3-week period with a placebo supplementation and then switch to the other supplementation the other 3-week period. On day 18 of both intervention periods, the same exercise challenge completed for screening will be repeated to induce muscle damage. Three days later, a blood draw will be taken to capture post-exercise CK levels. Each participant will repeat the exercise regimen a total of 3 times over the course of the study.

Figure 1: Study Overview and Timeline



B.3.2 Subjects

B.3.2a Initial Inclusion criteria for screening

- Caucasian males 50-70 years old
- Access to the Internet to complete 24-hour diet recalls
- Consume on average less than 15 servings combined of eggs and meat per week for at least the past month (1 serving = 1 egg or 4 oz. meat)
- BMI 18-30

B.3.2b Exclusion criteria

- Limitations in the ability to move either arm
- Taking dietary supplements
- Smoking
- Significant health complications
- Current exercise regimen includes strength training
- History of major surgeries (i.e. hip replacements, spinal cord surgeries) or having metal in the body (i.e. screws, pins, metallic rods)
- Presence of bloodborne infectious diseases (i.e. HIV, hepatitis B or C)

B.3.2c Screening and enrollment of study subjects

Flyers and online advertisements will be used to recruit eligible participants in the Kannapolis, NC area although eligibility is not contingent upon living location. The Nutrition Research Institute is located in Kannapolis, the site where participants will come in for blood draws and eccentric exercise. The demographics found in Kannapolis are expected to provide sufficient numbers of eligible participants. Approximately 68.5% of the population is Caucasian, 47.9% are male, 60% are 18-65 years old, and 13% are above 65 years old (27). There are approximately 44,000 people living in Kannapolis (27).

Eligible subjects meeting inclusion criteria as described in sections **B.3.2a** will be invited to complete the screening process. At screening, a 24-hour recall will be completed with participants to ensure daily dietary intake of choline is <450mg. The NIH's Automated Self-Administered (ASA) 24-hour dietary recall website will be used to collect this data and analysis will be completed to ensure intake is below the desired maximum. The NIH's ASA will generate a nutrient analysis report that includes choline intake. The participants will be invited to complete an exercise challenge once the participant's 24-hour recalls satisfy the inclusion criteria. A finger stick will be done to collect a 250uL blood sample prior to the exercise challenge for genotyping and baseline CK level. The participant will then complete 12 arm extensions conducted at 100% maximal lift capacity using a dynamometer. Participants will return 3 days later for another 250uL blood draw to capture peak CK levels.

Based on these data, inclusion into the study will be granted if study participants' blood samples reveal the following: 1.) CK-responsive, as defined by a ≥ 100% percentage change in CK between pre- and post-exercise blood draws and 2.) the MTHFD1 1958A variant is present.

At least 10 participants are needed to complete the entire study. With an anticipated retention of about 75%, a target group of 14 eligible participants is needed to enroll in the study. Since at least 50% of the Caucasian population in the Raleigh-Durham-Chapel Hill area is estimated to have the MTHFD1 1958A allele and of those at least 10% will be CK-responsive, up to 280 individuals will be screened to obtain this sample size of 14. The percentage of CK-responsive individuals may be higher in our target group since this group will be consuming suboptimal levels of choline and have the genetic susceptibility to muscle dysfunction. It is estimated that screening approximately 120 eligible participants will yield the group of 14 subjects that is needed for the study.

B.3.2.d Random assignment of study subjects to dietary intervention groups

Intervention group assignments will be blinded to both the study participants and staff interacting with participants. The study assistant who will not be interacting with participants will generate subject assignments to Group A or B with a random number letter generator. Half of the participants will be assigned to Group A and the other half to Group B. In addition, the study

assistant will assign participants their supplement bottles for each period within the intervention. Supplement bottles, regardless of its contents, will be identical and the pills will also be similar in appearance. The study assistant will fill these bottles with supplements and will be the only team member who knows which pills are placebo and choline supplements. Supplement bottles will be identified by a number, which will correspond to the participant, group, and intervention period.

B.3.2.e Retention of study subjects
Participants will provide contact information at the initial screening session. This information will be used to contact participants one day before coming into the research office for appointments to improve attendance. Weekly check-ins will be conducted with participants during the intervention period to promote compliance and continued participation in the study. Monetary reimbursement will be given throughout the study for participants' time and participation. A compensation of $5 will be given to participants who are present at screening but are disqualified because choline intake exceeds 450mg/day. For participants with choline intake meeting the established criteria, $10 will be given at the follow-up appointment when a 3-day post exercise blood draw is taken. If enrolled in the study, $40 dollars will be given at the end of week 3 and $60 will be given at the end of week 6.

B.3.2.f Disqualification from the study
Participants will be disqualified from the study if inclusion criteria are no longer met at any time during the study period. Participants will be withdrawn from the study if adherence to the study protocol is not met or if appointments are missed. A waitlist of eligible participants recruited at screening will be contacted and recruited immediately into the study to replace ineligible participants.

B.4 Intervention
Participants will serve as their own control and complete a 3-week high and 3-week low choline intake period. During the high choline period, a choline supplement will be taken and a placebo will be taken during the low choline period. A total of 3,600mg of phosphatidylcholine or four 900mg phosphatidylcholine supplement tablets called PhosChol will be taken each day during the choline supplementation periods for Groups A and B. Each PhosChol tablet contains 34mg phosphorus, 134mg choline, and 621mg fatty acid. Four tablets will contain 136mg phosphorus, 536mg choline, and 2,484mg fatty acid (70% or 1738mg linoleic acid, 30% or 746mg linolenic and oleic acids). Each day of the choline-supplemented period, participants will be consuming 536mg of choline through 4 phosphatidylcholine supplements.

During the low choline period, a total of 2,900mg of polyunsaturated fatty acid or four supplement capsules with 725mg polyunsaturated fatty acid (Yes Parent Essential Oils*)* will be given as placebos for Groups A and B. Each tablet contains a proprietary blend that is estimated to be 460mg linoleic acid and 265mg linolenic acid. Four placebo pills will include 2,900mg fatty acid, or 1,840mg linoleic acid and 1,060mg linolenic acid, which is comparable in fatty acid content as the *PhosChol* pills.

Participants will take 4 tablets once a day for a total of 84 tablets over the course of each 3-week supplementation period. Each participant will consume a total of 168 tablets over the course of the entire study. Each choline and placebo pill container given to participants will contain 92 pills and participants will be asked to bring the remaining pills and pill bottle back on the last day of each supplementation period when the 3-day post exercise blood draws are done. This is one way to check for compliance, as each pill bottle should contain 8 extra pills at the

end of each supplementation period. Participants will be given a sheet of paper to record the days supplements are taken and this sheet will be collected at the last day of each supplementation period. This sheet of paper will serve as a reminder for them to take their supplements, and a weekly phone call to check-in with participants will help to remind them to take their supplements. The last three days of each supplementation period, participants will need to fill out 24-hour dietary recalls using the online ASA-24 website. Participants will be reached by a registered dietitian trained in conducting dietary recalls for assistance with using the website and entering in food items. Three dietary recalls in total will be completed for each intervention period with a total of 6 recalls combined for both intervention periods. One dietary recall will be completed on each of the last three days of each intervention period, starting with the day after participants come back to the research laboratory to complete the exercise challenge. These dietary recall will help monitor choline intake and link choline intake with CK levels.

B.4.b Intervention fidelity

The Principal Investigator (PI) will train the research team on how to conduct screening and recruitment for the study. Once trained, staff members will demonstrate competence by demonstrating the study protocols with the PI. When staff members have properly mastered the study protocols, they will be able to interact with participants. The PI or the registered dietitian will randomly evaluate 10% of visits and assess the session based on the protocols. A step-by-step script will be provided for staff members to use with patients during their visits. In addition, a phone scripts will be written for weekly phone call check-ins and dietary recall assistance to help standardize the intervention.

B.5 Assessment of Study Outcomes

Table 1 summarizes when study outcomes will be collected and evaluated.

Table 1: Schedule for Assessing Study Outcomes

Screening	Intervention Period 1	Intervention Period 2
Visit 1: • 24-hour dietary recall • Blood sample for: CK pre-exercise level, genotyping • Height and Weight for BMI • Eccentric exercise performed **Visit 2:** • Blood sample for post-exercise CK level	**Visit 1:** • Blood sample for CK pre-exercise • Eccentric exercise performed **Between visits:** • Two 24 hour dietary recall **Visit 2:** • Blood sample for CK post-exercise • 24-hour dietary recall	**Visit 1:** • Blood sample for CK pre-exercise • Eccentric exercise performed **Between visits:** • Two 24 hour dietary recall **Visit 2:** • Blood sample for CK post-exercise • 24-hour dietary recall

Genotyping: The initial blood sample at screening will be used for genomic analysis. A commercial extraction kit will be used to analyze the MTHFD1 1958A polymorphic site. The targeted DNA sequence will be amplified by multiplex PCR, purified, and analyzed with mass-spectrometry.

Dietary recall: The NIH's ASA 24-hour dietary recall program uses the USDA nutrient database to generate a dietary analysis for choline intake. Each time participants come in for their blood

draw 3-days post-exercise, a dietitian will review completed 24-hour intake recalls with them and verify items containing choline are accurately captured. Each participant will complete a total of seven 24-hour recalls during the study (1 at screening and 3 for each intervention periods). Dietary data will be invalid if average choline intake is above 450mg per day over the 3-day period.
Serum CK level: A colorimetric method will be used to analyze blood samples for serum CK. A total of six 250-uL-blood samples will be taken throughout the course of the study for each participant. Blood will be taken pre- and post-exercise on 3 occasions.
Eccentric exercise: The Biodex 4 quick set orthopedic testing and rehabilitation system will be used to have each participant complete 12 extensions of one arm at 100% maximal lift capacity on 3 occasions. Exercises will be done once at screening and once during the end of each intervention period.

B.6 Data and sample management
All files containing participant information will be kept in encrypted files and only research staff will have permission to access these electronic files. An identification number, which will be used to label all dietary recalls and blood samples, will be used to link participants to their information. Blood samples will be kept in secure laboratories.

B.7 Project Timeline

Months	Activities
1-2	• Assemble research team (1 principal investigator, 1 registered dietitian, 2 research assistants, 1 staff assistant) • Develop materials and protocols for study • Obtain IRB approval for study • Train staff members in study protocols
3	• Advertise study for recruitment • Screen eligible participants and determine participants' eligibility for intervention
4	• Form Groups A and B for intervention
5-7	• Complete intervention
8-12	• Analyze results • Complete manuscript for publication

B.3.8 Study Leadership and Organization
The research staff will include a Principal Investigator, a registered dietitian, two research assistants, and one study assistant. A biostatistician will be consulted when study results are analyzed. The responsibilities of each personnel are outlined below.

The research team includes:
Martin Kohlmeier, MD, PhD: Principal Investigator with significant experience in designing and conducting clinical and nutritional biochemistry research studies and medicine. He will lead the study, oversee study protocol development and manuscript publication, submit IRB documents, analyze samples collected from participants, and run weekly meetings with research staff team.
Olivia Dong, MPH, RD, LDN: Registered dietitian with experience in nutrition research and conducting dietary recalls. She will oversee and train the research and study assistants, analyze dietary recalls, conduct intervention fidelity checks, develop study protocols, assist participants with completing dietary recalls, and collect data from participants.
Research assistants (RA): 2 RAs with at least a bachelor's degree will be hired part-time for the study. These RAs will be responsible for flyering and advertising the study, scheduling

screening and recruitment sessions with participants, collecting data from participants, and conducting weekly check-ins with participants.
Study assistant (SA): 1 staff assistant with at least a bachelor's degree will be hired part-time for the study. This SA will create a master list linking participants with their identification number, randomly assign participants to Group A or B, handle the supplementations and package them into pill bottles, and prepare study documents for participants.

Weekly meetings will take place with the entire research team where study updates, progress, and challenges will be addressed and resolved.

B.8 Analysis
A one-sided *t* test with α = 0.05 will be used to demonstrate significance between average percent change in pre- and post- exercise serum CK levels during the choline and placebo supplementation intervention periods. The percentage change in CK level for the choline supplemented intervention period is hypothesized to be significantly lower than the placebo-supplementation period. The formula to calculate percent change for each supplementation period is shown below.

$$\%\ \Delta CK_{intervention\ period} = \frac{CK_{post-exercise} - CK_{pre-exercise}}{CK_{pre-exercise}}$$

The mean percent CK change will be calculated for each participant to obtain the overall mean percentage change for each supplementation period. An intent-to-treat analysis will be completed for participants who are either non-compliant or withdraw from the study.

B.9 Sample Size Calculation
A sample size of 10 was determined using the estimation of sample size for a 95% CI formula:

$$n = \left(\frac{Z\sigma}{d}\right)^2 = \left(\frac{1.96 * 1451}{900}\right)^2 = 10$$

The distribution for CK may vary between populations, and a specific distribution curve is currently unavailable for this target population. The variance in this sample is predicted to be lower in this study since participants have the same variant, eliminating some of the inter-individual differences caused by genetics. The variance and confidence interval width for this study is based on a serum creatine kinase 95% CI range of 600-1500 μ/L previously seen in a sample of 9 CK-responsive men 3 days post-exercise (25).

B.10 Anticipated Pitfalls
Having participants fail to adhere to the supplement regimen for six weeks is an anticipated pitfall. Measures are in place to encourage compliance and to check adherence to the regimen. Participants will be asked to keep track of the number of pills consumed each day and adherence will be monitored at weekly check-ins completed over the phone. Participants will also bring their pill bottles back to check whether they consumed the correct amount of supplements during each intervention period. Eight supplements should remain at the end of each supplementation period. If more pills than 8 pills remain, data collected for that participant will be invalid.

Dropouts are another potential pitfall for this study. We are enrolling 4 extra participants to prepare to account for dropouts. Participants will be compensated for their time with a small

monetary amount to offset the time and effort needed to participate in the study. In addition, offering appointment times outside the 8-5pm timeframe will provide participants with more flexibility as to when they can come in for their visits. Evening and weekend appointments will be offered to accommodate participants' schedules. If more than 25% of participants drop out, aggressive recruitment will occur to replace them. This means that active recruitment will begin once dropouts are seen instead of waiting until the end of the study to recruit eligible replacements. A wait list of eligible participants will be on hand to enroll new participants as needed.

A blunted CK response may occur from muscle adaptation to the repeated exercise regimen. The blunt in CK level from repeating the exercise will not be substantial enough to decrease CK level significantly. In addition, minimal changes in muscular adaptation will result from the exercise since it will be done three weeks apart from each other. The absolute percent change in CK level will be used as the outcome of interest instead of using the absolute CK value.

C. Human Subjects
C.1 Protection of Human Subjects
C.1.a Risks to Human Subjects
Human Subjects involvement, characteristics, and design: Human subjects involved in this study are ones who meet the inclusion and exclusion criteria. Up to 280 subjects will be screened and at least 14 participants will be enrolled in the study. This study will follow protocols as approved by the Institutional Review Boards at UNC Chapel Hill. The monetary compensation provided is to reimburse participants for the time and effort spent on this study. It is not a source of substantial income to encourage participation. A 3600mg daily dose of phosphatidylcholine and 2,900mg daily dose of monounsaturated fat has no known adverse side effects. No collaborating sites outside of UNC Chapel Hill will be involved in this study.
Source of Materials: Participants will provide all data and information for the study at screening. No databases will be used to recruit participants. Blood samples, information about eccentric exercise completed, and dietary recalls will be obtained from participants at visits and this information will be kept within encrypted files accessible only by research staff. Only research staff working with the study will have access to identifiable private information about the human subjects. A master document will hold participants' name and identification number. Only a number will identify all specimens and data collected from subjects, not their personal names. Specimens will be stored safely in a laboratory.
Potential Risks: Physical injuries are possible when doing eccentric exercises. Participants are eligible for the study if they are free of any medical complications, such as recent major surgeries. Subjects will sign a waiver stating they understand the risks associated with the study procedures and participants will have the choice to withdraw from the study at any time without penalty. The likelihood these exercises will cause serious damage is minimal as many gyms have similar equipment. No significant injuries are anticipated with the blood draws that will be done with a finger stick. Overall, this study will be minimally invasive and risks will be kept to a minimum.

C.1.b Adequacy of protection against risks
Recruitment and Informed Consent: Recruitment will occur in Kannapolis, NC. Flyers will be posted at local grocery stores, libraries, and anywhere else where public postings are allowed. Emails and online forums such as Craigslist will also be used to assist with recruitment. An informed consent form will be completed at the initial screening before proceeding further with study protocols. This signed form will outline the possible risks of participating in the study, that

participation is voluntary, and that they can drop out at anytime without negative consequences. Signed documents will be kept in a locked container at the Nutrition Research Institute.
Protections Against Risk: Paper files will be kept in locked containers, electronic data will be encrypted, and samples will be kept in locked laboratories. These measures will be successful in keeping people outside the study from accessing these materials. Medical attention will be initiated if injuries arise at any point during the study.

C.1.c Potential Benefits of the Proposed Research to Human Subjects and Others
Outcomes of this study can provide a reasonable nutrition intervention to help decrease elevated CK levels post-exercise. Recommending more choline intake as a result of the study findings can improve muscle dysfunction experienced when exercising. As studies have suggested the MTHFD1 1958A is linked to increased choline needs, having participants take a daily 500mg choline supplement should have minimal to no negative side effects.

C.1.d Importance of the knowledge to be gained
Since this study focuses on a subpopulation with a potential increased need for choline, outcomes of this study has the potential to refine choline recommendations to better meet the choline needs of the population. This study will provide more information on the nutritional consequences of this specific genetic variant. The little to no risks associated with having participants take a supplement of choline or monounsaturated fat outweighs the benefits of the potential increase in choline needs this subpopulation requires to avoid muscle dysfunction. The negative health outcomes associated with muscle dysfunction are greater than the outcomes associated with taking the two supplements for a total of 6 weeks.

C.1.e Data and safety monitoring
Enrollment and dropout rates will be monitored. Each participant who is enrolled in the study will be double checked by at least 1 other research staff member for accuracy. The PI will review collected data on a weekly basis for accuracy, completeness, and proper storage. Weekly staff meetings will occur and any concerns related to data and safety can be addressed and resolved at that time. Any adverse events will be reported to the PI and the IRB immediately. Research staff will also seek proper medical attention if adverse events occur.

C.1.f ClinicalTrials.gov requirements
This study will be enrolled with be registered with clinicaltrials.gov prior to beginning.

C.2 Inclusion of Women and Minorities
Women and minorities will not be enrolled in this study. The focus of this study will be Caucasian males because they are at an increased risk for being choline deficient.

C.3 Targeted/Planned Enrollment Table
Up to an estimated two hundred eighty 50-70-year old Caucasian males in the Kannapolis area of NC will be screened for this study.

C.4 Inclusion of Children
Children will not be enrolled in this study.

D. Budget

Direct Costs		
a. Personnel	**Fringe**	**Total**
Principal Investigator	N/A	$30,000
Dietitian	N/A	$22,000
2 Research Assistants	N/A	$22,000
Study Assistant	N/A	$11,000
Consultation with Biostatisticians	N/A	$2,000
Total Personnel		**$87,200**

b. Supplies	**Expense**	**Total**
Arm chair	None, already available	$0
Office supplies	$500/month x 12 months	$6,000
Supplies for sample processing, DNA extraction, storage	$2,000	$2,000
Reagents for genotyping and CK measurements	$3,000	$3,000
PhosChol supplementation	$0.47 each x 84 tablets x 14 participant	$553
Placebo pills	$0.50 each x 84 tablets x 14 participant	$588
Pill bottles	$3 per bottle x 28	$84
Stadiometer	None, already available	$0
First aid kit	None, already available	$0
2 computers	None, already available	$0
Monetary compensation for participants at screening	$670 to screen and enroll 14 participants, $1400 for 14 participants to complete study	$2,070
Reimbursement for travel	$0.50/mile x 60 miles	$30
Calling cards	$20/800 minutes	$20
Total Supplies		**$14,315**

Direct Costs		$101,515
Total Indirect Costs		$10,151
Total Costs		**$111,666**

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