THE EFFECTS OF DIETARY CHOLINE ON MUSCLE RESPONSES TO RESISTANCE EXERCISE IN OLDER ADULTS

A Dissertation

by

CHANG WOOCK LEE

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Steven E. Riechman
Committee Members,	Stephen F. Crouse
	James D. Fluckey
	Stephen B. Smith
Head of Department,	Richard B. Kreider

May 2016

Major Subject: Kinesiology

ABSTRACT

Choline is an essential nutrient for humans. It participates in many important physiological processes including membrane signaling/integrity, neurotransmission, methylation, and lipid transport. Since choline is a precursor to acetylcholine (ACh), a neurotransmitter that mediates muscle contraction, studies have been conducted to examine the relations between choline intake and endurance exercise performance. However, the results were equivocal, mostly due to lack of nutritional control, and there has been no study that examined the effects of choline associated with resistance exercise (RE).

The purpose of this research was to investigate the effects of dietary choline on muscle responses to RE in three study populations. It was hypothesized that low choline consumption would negatively influence changes in lean mass and strength in response to RE in older adults.

The first study examined the effects of habitual choline intake in the context of commonly recommended “healthy eating”, on changes in strength and lean mass following 12 weeks of full body resistance exercise training (RET). The results showed that lower intake of choline (<50% of Adequate Intake [AI]) was associated with significantly diminished gains in strength and lean mass compared with higher choline intakes (~63% or ~85% of AI).

The second study investigated the effects of choline supplementation from egg yolk for 12 weeks on muscle responses to RET in a randomized double-blind placebo-

controlled trial. The results showed that lower (~51% of AI) choline consumption significantly impaired strength but not muscle gains compared with moderate choline intake (~68% of AI) while higher (~118% of AI) choline intake did not provide additional benefits on strength gains.

The third study examined the effects of choline supplementation for 3 weeks on EMG amplitude and strength responses. No choline effect was observed on isometric force outputs, maximum strength on leg press/leg extension, or EMG amplitudes.

The results of these studies suggest that only lower choline intake (~50% of AI) for more than one month may negatively affect change in strength associated with RET. Consumption of varying amounts of choline for a short period or higher than recommended amounts of choline may not influence muscle responses to RE.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Age related loss of muscle mass (sarcopenia) and the associated decline of strength, force, functionality, and mobility is a serious issue in the U.S. where the population over the age of 65 is expected to increase steadily from 14.9% in 2015 to 23.6% by 2060 (1). Fortunately, resistance exercise has been shown to delay and/or reverse the detrimental effects of sarcopenia, and the efficacy and efficiency of resistance exercise programs can be influenced by dietary intakes of various nutrients such as protein and carbohydrate as well as micronutrients including vitamins and minerals (2, 3).

Choline is an important micronutrient playing vital roles in many physiological processes including cell membrane formation/integrity/signaling, lipid transport, and methylation/reduction of homocysteine. Choline deficiency is known to cause liver/muscle damage, steatosis (fatty liver), birth defects, and cognitive dysfunction. Choline is also a precursor to acetylcholine (ACh), the main neurotransmitter in α-motor neurons, which control muscle contraction and force generation, and ACh production and release is dependent on available choline levels (4, 5). Therefore, dietary choline may affect exercise performance and responses to exercise by mediating availability of ACh at the neuromuscular junction (NMJ) and/or through other mechanisms such as its roles as a structural component of membranes and its effects on cell signaling, lipid metabolism, methylation reactions, and oxidative stress (6-10).

Several studies have examined the relationship between dietary choline consumption, blood choline levels, and exercise performance, but the results are equivocal. Exercises with prolonged duration and high in intensity such as marathon runs and 2 hour long high speed bicycle riding decreased blood choline levels, but the effects of decreased blood choline concentration on exercise performance were unclear or not measured (11-13). Other types of exercises (treadmill or cycling at moderate intensity) did not affect blood choline concentrations (14, 15), and choline supplementation prior to exercises did not improve exercise performance even though the supplementation either prevented a drop in blood choline or increased choline concentrations (13-17).

However, many of these studies lacked nutritional control and/or only examined the effects associated with acute endurance type of exercises, leaving the effects of choline intakes on resistance exercise (RE) and training responses largely unknown. The low frequency α-motor neuron output and lower number of total motor units activated during endurance exercise may allow sufficient time to re-synthesize ACh. Conversely, the high frequency α-motor neuron output and increased number of active motor units during RE may limit ACh availability and influence neural activation, muscle contraction, and force generation. Currently, there is no study that examined the effects of dietary choline on RE and training responses. Even though some of the aforementioned exercise studies utilized a barbell squat, push-ups, or sit-ups in their exercise protocols, the exercise loads used were very light (either a 45 kg barbell or body weight only), therefore, it is difficult to regard those as RE (15, 18).

Despite the importance in human health and physiology, data suggest that choline intakes are lower than recommended values (Adequate Intakes [AI] of choline: 550 mg/d and 425 mg/d for adult men and women, respectively) (19) in general public and especially in older adults. A recent data analysis of National Health and Nutrition Examination Survey (NHANES) 2007-2010 shows that mean daily intakes of choline in men and women between the ages of 51 and 70 years were 396 mg and 274 mg, respectively, and more than 90% of these individuals consumed less than the recommended amounts of choline (20, 21). Yonemori et al. (22) also reported that the mean choline intakes of 188,147 subjects aged between 45 and 75 were 372 mg/d in men and 304 mg/d in women. The prevalence of low choline intake in older adults may have devastating effects if choline influences responses to RE, which is one of the most effective means to delay or counter the effects of aging on muscle mass and strength in older population (2).

The purpose of this dissertation was to examine the effects of dietary choline on exercise performance and physiological responses to RE in older adults. Considering the detrimental effects of sarcopenia, it is important for these individuals to regularly perform RE and maintain optimal nutrient intakes (3).

CHAPTER II

BACKGROUND

Property and Physiological Roles

Structure and Sources of Choline

Choline was first discovered in 1862 by Adolph Strecker via heating lecithin (phosphatidylcholine [PC]) obtained from bile of pig and ox (23). Choline is a water soluble quaternary ammonium cation with the chemical formula of $(CH_3)_3N^+(CH_2)_2OH$, and it usually exists as an ionic compound combined with various type of anions such as chloride, tartrate, hydroxide, or phosphate. (**Figure 1**).

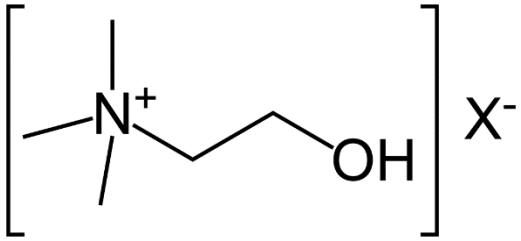

Figure 1. Chemical Structure of Choline

There are two sources of choline for humans: dietary intake and endogenous de novo synthesis (**Figure 2**). Choline from foods (exogenous choline) is absorbed in the intestine via choline transporters and promptly converted to phosphocholine by choline

kinase, or betaine by choline oxidase/choline dehydrogenase (CHDH) and betaine aldehyde dehydrogenase (BADH) reactions (24). Phosphocholine is converted to cytidine diphosphocholine (CDP-choline) by CTP:phosphocholine cytidylyltransferase enzyme, and PC is synthesized from CDP-choline by CDP-choline:1,2-diacylglycerol choline phosphotransferase. PC is the most abundant phospholipid (>50%) in all cellular membranes and the major form of choline reservoir in animals. Approximately 95% of the total choline exists in the form of PC, and the remaining 5% exists as free choline, phosphocholine, glycerophosphocholine, CDP-choline, or ACh (24). PC is then broken down to phosphatidic acid (PA) and choline by phospholipase D (PLD).

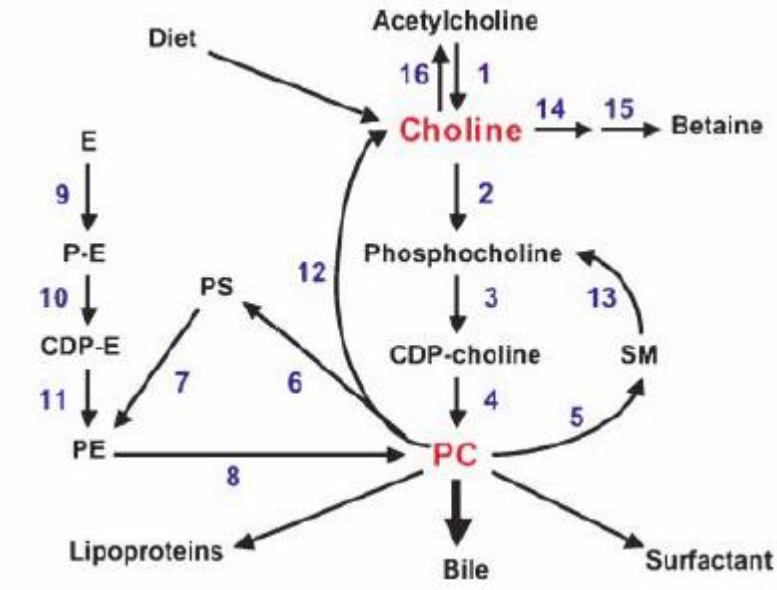

Figure 2. Pathways of PC and Choline Synthesis (24)

Choline (endogenous choline) can be also obtained from PC synthesized de novo from phosphatidylethanolamine (PE). PE is sequentially methylated by phosphatidylethanolamine *N*-methyltransferase (PEMT) enzyme and *S*-adenosyl methionine (SAM) to be converted to PC in the liver. Choline can then be generated from PC by phospholipase enzymes. Animal models showed that approximately 70% of hepatic PC is produced via CDP-choline pathway, and PEMT pathway accounts for the remaining 30% (24), but since PEMT activity is meaningfully active only in the liver, it is estimated that 85% of total required choline is obtained from diet and 15% is synthesized endogenously in rodents (25). However, when choline intake is low, and perhaps when choline use is elevated, PEMT pathway can play an important role to compensate for the insufficient amount of choline and PC (24).

Membrane Integrity/Signaling

Choline is a critical component of PC, which is the most abundant phospholipid and major constituent of all cellular membranes and pulmonary surfactant. PC is also a precursor to signaling molecules such as PA, which is required to activate mammalian target of rapamycin (mTOR) that plays a critical role in protein synthesis and cell growth/proliferation (26). PC can be converted to sphingomyelin or phosphatidylserine, and choline is needed to make lysophosphatidylcholine and choline plasmalogen, which are also essential components of all cellular membranes. Choline deficient diet results in compromised cell membranes, and intracellular enzymes can leak from liver and muscle cells. da Costa et al. (6) reported that serum creatine kinase (CK; a marker of muscle

damage) increased by 33-66 fold in three of four male subjects who were fed a choline deficient diet (50 mg/70 kg/d), and the increase in CK was reversed with higher choline intake during a recovery period. The study also observed 3.5 times more CK leaked from mouse myoblasts incubated in choline deficient medium compared with control. Also, in the cells grown in choline deficient environment, apoptosis was induced, and reduced concentrations of PC, choline, phosphocholine, and glycerophosphocholine, and higher membrane osmotic fragility were observed (6).

Bile and Lipoprotein Secretion

Choline in the form of PC is required for bile secretion from the liver. 5% of PC in bile is excreted as feces, and 95% is reabsorbed in the intestines, but only 40% of the reabsorbed PC returns to the liver for recycling (27). Choline is also used to package and export triacylglycerol (TAG) into very low density lipoprotein (VLDL) from the liver. Both the CDP-choline pathway and the PEMT pathway are required to produce PC for VLDL secretion in the liver (9, 24). Therefore, dietary choline is a necessary component for VLDL synthesis.

Methylation/One Carbon Reaction

A portion of choline can be irreversibly converted to betaine by CHDH and BADH reactions **(Figure 3)**. Betaine is then used to convert homocysteine to methionine by betaine homocysteine S-methyltransferase (BHMT). Methionine is used to generate SAM, which is a universal methyl donor in various biochemical reactions including

synthesis of DNA, RNA, carnitine, creatine, and epinephrine. Therefore, choline plays an important role in methylation/one-carbon reaction in mammals. Methylation is a key component in many physiological reactions such as lipid biosynthesis, epigenetic control of gene expression/protein synthesis, and regulation of metabolic pathways (10, 28).

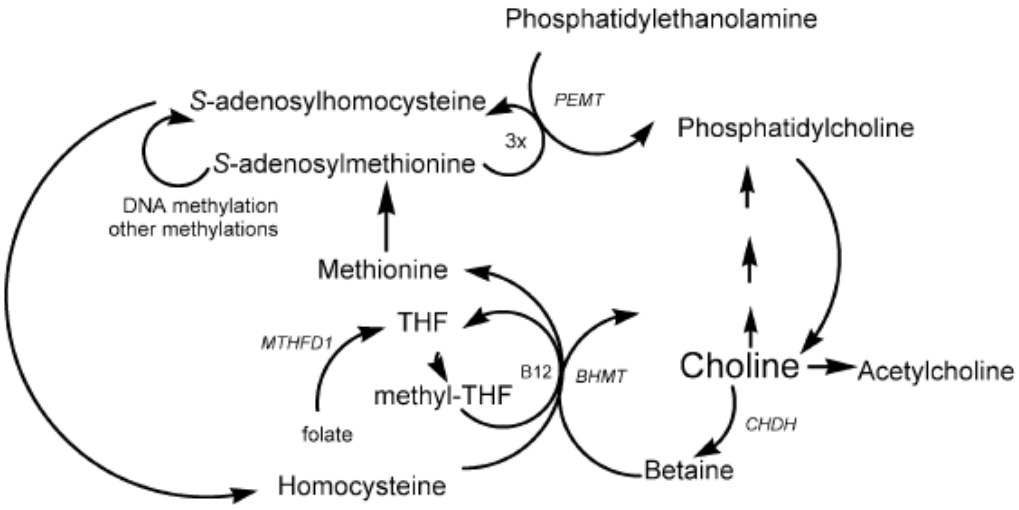

Figure 3. Metabolic Pathway of Choline (29)

Neurotransmitter Synthesis

Choline is a precursor to ACh, a neurotransmitter which allows cholinergic nerves to communicate with other cells, and relays signals from motor neurons to skeletal muscles. ACh is synthesized from acetyl-CoA and choline by choline acetyltransferase (ChAT). Once released from nerve endings, ACh is diffused through

synaptic cleft (a gap between nerve ending and other tissues such as muscle or other nerves) and binds to ACh receptors on the surface of other cells. This produces rapid flux of ions across membranes and generates depolarization/action potentials (30). ACh is then catabolized by ACh esterase to acetate and choline, which can be taken up by choline transporters on the nerve ending and recycled. Studies showed that availability of choline in surrounding environment affects ACh synthesis and release from nerve endings (5). For example, Bierkamper and Goldberg (4) reported that the amount of ACh release in nerve ending was directly related to the concentration of available choline in a vascular perfused rat phrenic nerve-hemidiaphragm. As the concentration (μmol) of choline in the perfusate increased from 0 to 30, and to 60, ACh release (pmol/min) was increased from 5.13 ± 0.6 to 6.75 ± 0.1, and to 9.78 ± 1.7, respectively, in response to electrical stimulation (7 Hz) of the phrenic nerve (4).

Reduction of Oxidative Stress and Increased Fatty Acid Oxidation

Choline may also play a role in reducing oxidative stress in humans. Sachan et al. (7) reported that one week of choline supplementation (0.94 g/d) resulted in marked decrease in serum thiobarbituric acid reactive substances (TBARS; a biomarker of oxidative stress) in 18-54 year old women, which occurred most likely by preventing membrane fatty acid peroxidation and stabilization of membranes (7). When choline and L-carnitine was supplemented together, an antioxidant sparing effect was also observed. After two weeks of choline/L-carnitine supplementation, serum concentrations of retinol

and α-tocopherol were significantly higher in the supplement group than placebo group, suggesting less oxidative damage had occurred in the supplement group (7).

Increased choline consumption may also increase fatty acid oxidation through its effect on carnitine conservation. In humans and guinea pigs, supplementation of choline decreased urinary excretion of carnitine and increased uptake of carnitine in all tissues including skeletal muscle. The functional implications of increased cellular uptake and accretion of carnitine may be increased fatty acid oxidation and possibly reduction of body fat (8, 31).

Intertwined with Other Nutrients

Choline is interconnected with other nutrients such as folate, methionine, B vitamins, and glycine at the point of conversion of homocysteine to methionine (**Figure 3**). As discussed, a portion of choline is converted to betaine, which is used to convert homocysteine to methionine (BHMT pathway), but there is another pathway (methionine synthase [MS] pathway) that performs the same task. Methyl tetrahydrofolate (methyl-THF) donates methyl group to homocysteine in a reaction catalyzed by MS and vitamin B_{12}, and this reaction produces methionine and THF. Methionine is used to make SAM, which is, after donating a methyl group, converted to *S*-adenosylhomocysteine (SAH), which is, in turn, converted back to homocysteine. THF is also converted back to methyl-THF, and folate is needed to produce THF. Homocysteine can also be converted to cysteine by cystathionine synthase and vitamin B_6. Cysteine can then be used to produce glutathione, and glycine and serine are needed in the process. Therefore,

activation of one pathway can decrease the reliance on the other pathways, and deficiency of one nutrient can increase the requirement of the other nutrients. Thus, choline, betaine, folate, vitamin B_{12}, vitamin B_6, methionine, serine, and glycine are all interconnected with each other, and higher intake of one nutrient may induce sparing effects of other nutrients.

Choline as an Essential Nutrient

AI for Choline

Since there exists a pathway for endogenous synthesis (mainly in liver), choline was not considered an essential nutrient for humans for a long time, and it was thought choline requirement can be met with de novo synthesis in humans. However, endogenous synthesis alone has since been demonstrated to be insufficient for several of choline's functions. For example, Burt et al. (32) reported that total parenteral nutrition (TPN), which does not contain choline, caused a significant drop in plasma choline levels and increase in alanine aminotransferase (ALT; a marker of liver damage) in blood. Buchman et al. (33) observed that hepatic steatosis in patients receiving TPN was completely resolved with administration of choline. Also, Zeisel et al. (34) found that 3 weeks of choline-deficient diet (with normal folate, methionine, and vitamin B_{12}) in a hospital setting lowered plasma choline and PC concentrations by 30%, and increased serum ALT levels by 48% in healthy males, and these changes were quickly reversed within one week after the subjects started to consume a normal diet.

As a result of accumulation of study data supporting the importance of choline intake, the Food and Nutrition Board of the Institute of Medicine (IOM), National Academy of Sciences, established AI values for choline (**Table 1**) in 1998 (19, 35, 36), but Recommended Dietary Allowance (RDA) has not been established yet. Both AI and RDA are parts of Dietary Reference Intakes (DRI), which are reference values for healthy people to plan and assess their diets, but RDA is a nutrient intake level sufficient to meet 98% of healthy individuals' diet requirement, whereas AI is a nutrient intake goal used when sufficient scientific data do not exist to set RDA (35). More studies are needed to establish DRI values that accurately reflect various conditions which may require elevated choline consumption such as physical activities, mental stress, and recovery from illness/injury.

Table 1. AI for Choline (37)

IOM RECOMMENDATIONS		
AI for Infants	0 to 6 months	125 mg/day, 18 mg/kg
	6 to 12 months	150 mg/day
AI for Children	1 through 3 years	200 mg/day
	4 through 8 years	250 mg/day
	9 through 13 years	375 mg/day
AI for Males	14 through 18 years	550 mg/day
	19 and older	550 mg/day
AI for Females	14 through 18 years	400 mg/day
	19 years and older	425 mg/day
AI for Pregnancy	All ages	450 mg/day
AI for Lactation	All ages	550 mg/day

Individual Variability in Dietary Choline Requirement

The current AI values of choline are set at the levels of intake which are expected to be sufficient to prevent liver dysfunction (determined by increased serum ALT levels; ~7 mg/kg/d) in sedentary people without consideration of physical activity levels (35). Therefore, it is possible that active individuals may need elevated choline intake because physical activities require increased ACh synthesis/release, lipid oxidation/metabolism, membrane integrity, and methylation reactions, and choline plays vital roles in many of these important physiological processes.

However, there is evidence that the current AI may not be sufficient to prevent choline deficiency in some individuals. For example, Fischer et al. (38) reported that 6 of 26 male subjects (23%) showed signs of liver/muscle damage (5 fold increase in serum CK; 1.5 fold increase in ALT, aspartate aminotransferase [AST], gamma-glutamyl transferase [GGT], or lactate dehydrogenase [LDH]; or >28% increase in liver fat content) when they were fed a diet containing 550 mg/70 kg/d of choline (~100% AI) for 10 days in a hospital setting.

Also, it appears that there exists a great variability in choline requirement among individuals. In the same study, the subjects were fed a choline deficient diet (50 mg/70 kg/d; <10% of AI) for up to 42 days in a hospital setting. However, 6 of 26 men, 3 of 15 postmenopausal women, and 9 out of 16 premenopausal women did not show any signs of choline deficiency. When the remaining subjects who developed the symptoms of choline deficiency were fed a repletion diet with gradually increasing the amount of

choline in their foods, 10, 3, and 5 of them needed choline intakes at 25%, 50%, and 75% of AI to reverse the conditions of choline deficiency, respectively (38). It appears that some individuals are more tolerant of low choline intake than others, possibly through variability in choline use and/or variation in the ability to synthesize choline endogenously in the liver.

Effects of Gender and Genetic Variations

As discussed earlier, choline can be synthesized de novo from PE by PEMT and SAM. *PEMT* gene, which codes for PEMT enzyme, has estrogen response elements, and estrogen induces *PEMT* gene expression and increases PEMT enzyme activity in a dose-dependent manner (39). This may explain why premenopausal women are more resistant to choline deficiency than men and postmenopausal women. Fisher et al. (38) reported that almost 80% of men and postmenopausal women showed signs of choline deficiency in response to a severely low choline diet (<10% AI) while only 44% of premenopausal women became choline deficient in their hospital setting study. Another study (40) also observed the effect of estrogen on dietary choline requirement and susceptibility to choline deficiency. It reported that only 18% of postmenopausal women with estrogen treatment developed organ dysfunction while 73% of those who received placebo showed signs of organ dysfunction, when they consumed a low choline diet (40).

Also, there is evidence suggesting that individual choline requirement is dependent on one's genetic predisposition. In their 2006 study, da Costa et al. (41) identified single nucleotide polymorphism (SNP) in *PEMT* gene and examined the effect

of the SNP on susceptibility to choline deficiency. They recruited 57 males and females aged between 18 and 70 and had them consume a low choline diet (<50 mg/70 kg/d) for up to 42 days or until they developed signs of organ dysfunction. They found that 18 of 23 women and 11 of 13 postmenopausal women with an SNP in *PEMT* gene (-744 G→C; rs12325817) developed organ dysfunction (41). Fischer et al. (40) also reported that 80%, 43%, and 13% of premenopausal women with 2, 1, and 0 variant alleles of the SNP on *PEMT* gene showed signs of organ dysfunction with a very low choline diet, respectively.

Other SNPs in different genes were also identified. An SNP (+318 A→C; rs9001) in *CHDH* gene, which codes for CHDH enzyme, had a protective effect in all the subjects, and another SNP in *CHDH* gene (+432 G→T; rs12676) increased susceptibility to organ dysfunction in premenopausal women (41). More recently, additional SNPs (in choline kinase A/B [*CHKA, CHKB*] and solute carrier 44A1 [*SLC44A1*] genes, which affect PC synthesis from choline and transport of choline across membranes) that affect requirement of dietary choline were discovered, and these SNPs appear to vary in their distribution across ethnic/racial groups, confirming the effect of genetics on choline metabolism and dietary requirement (42). While the influence of genetics makes it difficult to accurately estimate individual choline requirements, da Costa et al. (42) suggest that setting the dietary recommendations of choline at higher levels would be the most practical solution that ensures enough choline intake for all populations including those who may have greater choline requirement due to their genotypes and other factors.

Dietary Sources of Choline

The US Department of Agriculture (USDA) developed and released a database for choline in collaboration with University of North Carolina in 2004 and 2008 (43, 44). According to the most recent version that contains choline and other choline containing compounds in more than 630 food items, choline is abundant in animal foods in general (**Table 2**), especially in eggs and organ meats (44). Egg yolk is by far the most concentrated source of dietary choline, providing 680 mg/100 g, and beef liver (330 mg/100 g), chicken liver (190 mg/100 g), turkey liver (220 mg/100 g), smoked sockeye salmon (220 mg/100 g), beef chuck (100 mg/100 g), and defatted soy flour (190 mg/100 g) are some of the most choline-rich foods, according to the USDA database (44).

Table 2. Foods High in Choline (29)

Food	Choline (mg/serving)
Chicken, liver, cooked (3 oz)	247
Soy flour, defatted (1 cup)	201
Salmon, sockeye, smoked (3 oz)	187
Egg, whole, raw, fresh (1 large)	125
Quinoa, uncooked (½ cup)	60
Chicken, broilers or fryers, meat and skin, roasted (3 oz)	56
Turkey sausage, cooked (3 oz)	55
Wheat germ, toasted, plain (2 tbsp)	50
Milk, nonfat, fluid, with added vitamin A (8 oz)	38
Cauliflower, cooked, boiled (½ cup)	24
Peas, green, frozen, cooked, drained (½ cup)	22
Bacon, pork, cured, cooked (2 pieces)	20
Almonds (1 oz)	15
Broccoli, cooked, boiled, drained (½ cup)	15
Frankfurter, beef (1)	15
Oat bran, raw (½ cup)	15
Pecans (1 oz)	15
Tomato paste, canned (2 tbsp)	12
Flaxseed (2 tbsp)	11

Source: (USDA, 2008).

Prevalence of Low Choline Intake

Despite the importance of choline as an essential nutrient, data indicate that majority of general public do not consume adequate amount of choline from their diet. A recent analysis of NHANES 2011-2012 (45) shows that mean daily intakes of choline in men and women with 20 years of age and older are 402 mg and 272 mg, respectively. Xu et al. (46) reported that the mean and median choline intake values in 3,064 subjects residing in Long Island, NY were 325 mg/d and 321 mg/d, and Chiuve et al. (47) found

that 80% of their 1,477 women subjects consumed less than 361 mg/d of choline. Also, 920 men and 1,040 women (mean age: 54 years with a range of 28-82 years) in Framingham Offspring Study consumed 313 mg of choline per day on average, and only 20% of them had daily choline intakes of >401 mg (48). Another data analysis showed that older adults with >70 of age consume only 264 mg/d of choline on average (29).

The prevalence of low choline intake may be attributed to the current diet guidelines' effect. The foods rich in choline (eggs and other animal foods) are also rich in cholesterol and/or saturated fat in general, and the diet guidelines recommending limited intakes of those foods have been in place for decades, and changes are beginning to occur only recently. In their Scientific Report recently submitted to the U.S. Department of Health and Human Services (HHS) and USDA, the 2015 Dietary Guidelines Advisory Committee said cholesterol intakes do not need to be limited while they recommend reduced consumption of red meat and saturated fat (20).

Choline Deficiency

Choline deficiency, especially when coupled with insufficient consumption of methionine and/or folate, can lead to many diseases and critical unhealthy conditions.

Non-Alcoholic Fatty Liver Disease (NAFLD)

Insufficient consumption of choline can result in fatty liver even with adequate levels of folate and methionine intake (33, 37, 38). Since choline and PC is required to package and export TAG as VLDL, choline deficiency impairs VLDL secretion, and this

in turn, leads to accumulation of TAG in hepatocytes (37). Other mechanisms for development of fatty liver associated with choline metabolism have been suggested recently (49). Aberration of PEMT pathway and/or methylation function (50), impaired mitochondrial function and beta oxidation through perturbation of mitochondrial membranes (51), and endoplasmic reticulum stress (52) are all related to NAFLD. Fatty liver is an early indicator of liver dysfunction and can progress to hepatitis, fibrosis, cirrhosis, and cancer (49).

Birth Defects / Neural Tube Defects

Folate deficiency is known to be associated with neural tube defects. However, evidence indicates that dietary choline intake may also be linked to neural tube defects. Inhibition of choline uptake/metabolism resulted in growth retardation and defects in neural tube in mouse embryo models (53), and Shaw et al. (54) reported that higher choline intake (>498.46 mg/d) during pregnancy has 50% less risk of inducing neural tube defects compared with lower choline intake (<290.41 mg/d). The authors suggested that perturbation of DNA methylation and/or increased apoptosis resulted from choline deficiency may be the underlying mechanism for the neural tube defects (54).

Cancer

Choline deficiency may even be related to cancer. Xu et al. (46) reported that higher intake of choline (>455.8 mg/d) was associated with 24% lower risk of breast cancer compared with lower choline intake (<196.5 mg/d) in their 2008 population-

based study. They suggested that aberrant DNA methylation resulted from low choline intake may be the underlying mechanism. Indeed, several studies showed that choline deficiency is related to membrane integrity, DNA damage, and apoptosis (6, 55). When choline supply from diet is insufficient, PEMT pathway can be enhanced to produce required choline and PC from PE. However, this may result in shortage of SAM. The conversion of PE to PC uses three molecules of SAM, which is to be used for methylation of various other molecules including DNA and RNA. This might be linked to aberrant DNA methylation.

Homocysteine/Cardiovascular Disease

Reliance on PEMT pathway also produces three molecules of SAH, which is then broken down to adenosine and homocysteine. Homocysteine disrupts endothelial function and is an independent cardiovascular risk factor (56), therefore, limited consumption of choline may influence cardiovascular risks by relying more on PEMT pathway of choline synthesis and generating more homocysteine, or low choline intake may simply reduce the availability of betaine, which is used to convert homocysteine to methionine. Choline deficiency has been shown to increase blood concentrations of homocysteine. For example, in choline deficient mice and humans, blood homocysteine levels were twice greater in mice and 35% higher in humans in response to methionine load, compared with control (57). Chiuve et al. (47) observed blood homocysteine level was 8% lower in women who consumed more choline and betaine than in women with lower choline and betaine consumption. Also, Olthof et al. (58) examined the effect of

choline supplementation on plasma homocysteine levels in 26 men with a cross over design. When the subjects consumed 2.6 g/d of choline for 2 weeks, fasting plasma homocysteine levels were 18% lower compared with placebo consumption (58).

Tolerable Upper Intake (UL)

IOM also set UL levels for choline. UL is the highest level of daily intake for almost all individuals without risks of adverse health effects. The current UL values for choline are: 1.0 g/d for 1-8 year olds, 2.0 g/d for 9-13 year olds, 3.0 g/d for 14-18 year olds, and 3.5 g/d for 19 years and older (19, 35). Too much choline consumption exceeding liver's ability to oxidize trimethylamine (TMA), a product of bacterial degradation of choline in intestines, and/or inability to properly metabolize TMA in liver by amine oxidase enzyme, can result in fish odor syndrome (trimethylaminuria), where offensive smell of rotten fish emanates from one's body (59). Also, a recent study suggests that too much choline consumption (more than 5 to 10 times of normal intake values) may be related to development of atherosclerosis, depending on individual's intestinal microbial environment (60).

Choline and Exercise

Since choline is a precursor to ACh, which relays signals from the nerves to skeletal muscles and thus controls muscle contraction, interest among exercise scientists about the roles of choline has arisen, and as a result, several studies have been conducted to examine the effects of choline associated with exercise and performance. ACh is

synthesized in cholinergic neurons from acetyl-CoA and choline by ChAT enzyme, and ACh synthesis and release from nerve endings can be affected by the availability of choline.

As discussed earlier, Bierkamper and Goldberg (4) showed that the release of ACh in response to electrical stimulation increased by 32% and 89% when the concentrations of choline in the perfusate increased from 0 μM to 30 μM, and to 60 μM, respectively, in vascular perfused hemidiaphragm-phrenic nerve preparation of rat. The similar results were observed in different tissues suggesting ACh synthesis is dependent upon the availability of choline. Haubrich et al. (61) reported that intravenously administered choline chloride resulted in rapid increase in ACh in adrenals, kidney, lung, and liver of guinea pigs. Cohen and Wurtman (62) observed that concentrations of ACh in the brain were 28% and 45% higher in the rats that consumed 20 mg/d and 129 mg/d of choline, respectively, for 11 days compared with the animals that did not consume choline. Therefore, it is reasonable to hypothesize that increase in choline levels may increase ACh synthesis and release, and reduced choline concentrations may decrease ACh release and consequently affect exercise performance especially at high rates of muscle contraction with large muscle groups involved.

Exercise and Blood Choline Levels

Researchers hypothesized that since physical activities increase nutritional demand, exercise may decrease circulating choline levels, and this, in turn, may negatively influence exercise performance. Conlay et al. (11) measured blood

concentrations of choline prior to and immediately after a marathon run in 1986 Boston Marathon participants. Running the marathon reduced the blood choline levels by 40% on average, from 14.1 ± 1.2 μM to 8.4 ± 0.6 μM (11). The decrease in blood choline with an acute bout of exercise was also observed in a different group of marathon runners. In 1997, choline concentrations in plasma were measured before and after a marathon in 23 male and female runners aged 25 to 49 years (12). Plasma choline levels decreased from 19.2 ± 4.5 μM to 14.6 ± 4.2 μM and from 2,565.2 ± 516.4 μM to 2,403.4 ± 643 μM, respectively, following a marathon. However, the changes in plasma choline levels were not correlated with performance (finish time), which might be expected considering the large number of factors influencing a marathon performance. The same research group measured blood choline levels again before and after another marathon race in 2,000, and observed decreases in blood choline concentration (from 9.6 ± 2.5 μM to 7.0 ± 3.6 μM) following a marathon in trained athletes (17). The decrease in blood choline was also observed in elite triathletes. In a study with top level triathletes, the mean plasma choline concentration dropped by 16.9% (from 12.08 ± 0.54 μM to 10.04 ± 0.72 μM) after 2 hours of bicycle exercise at the speed of 35km/h (13).

However, there are several other studies that failed to observe decrease in blood choline levels in response to exercise. Spector et al. (14) reported that in young trained male cyclists, acute bouts of either sprinting exercise on cycle ergometer at 150% of VO_2max until exhaustion (for approximately 2 minutes) or prolonged cycling exercise at 70% of VO_2max until exhaustion (for approximately 75 minutes) did not induce decrease in blood choline levels. Similarly, von Allworden at al. (13) found no

difference in choline concentrations between pre-exercise and post-exercise plasma samples when elite level adolescent athletes (aged 14-20 years) ran 30-60 minutes of cross country races. Warber et al. (15) also examined the effect of strenuous and prolonged exercises on blood choline levels. They had highly trained soldiers walk on a treadmill at the speed of 5.6 km/h for 4 hours carrying 34.1 kg of load (at intensity of 38% of VO_2max). At the completion of the treadmill walk, after removing the load, they underwent a run-to exhaustion test on a treadmill. The blood choline level did not drop with the 4 hour-long load carriage walk and run test. A similar load carriage exercise did not reduce blood choline levels in a different group of soldiers. In their 2002 study, Deuster et al. (16) reported that 1.5 hour treadmill load carriage exercise at 70% of VO_2max did not affect blood choline concentrations in young soldiers.

These results suggest that not all exercises can lower blood choline levels, and it appears that only those exercises that are both very high in intensity and long in duration (such as marathon) can reduce blood choline levels. Either performing exercises with moderate intensity for an extended time or engaging in physical activities with high intensity for short duration does not seem to affect blood choline concentrations. However, these studies did not examine the effect of decreased blood choline concentration on exercise performance at all or in a precise way. Thus, it is unclear whether lower levels of circulating choline negatively affect exercise performance. The only reported outcome was finish time of a marathon race, which was not correlated with plasma choline levels (12), but it is difficult to draw a conclusion from this result,

because many other confounding factors such as body composition, age, training history, and nutritional intakes were not controlled.

Choline Supplementation and Exercise Performance

Another important scientific question is whether increasing choline intake has any effect on blood choline concentrations and/or exercise performance, and this has inspired several exercise studies using choline as a supplement. In a randomized, placebo-controlled, crossover study, von Allworden et al. (13) recruited 10 triathletes with 23-28 years of age (6 males and 4 females) and had them perform a 2 hour long bicycle exercise at the speed of 35km/h with and without pre-exercise lecithin supplement (0.2 g lecithin [90% PC; 12% choline]/kg body weight, consumed 1 hour prior to exercise). The athletes also consumed the same supplement without exercise as a control in a separate occasion. The authors reported that the 2 hour cycling without lecithin supplement resulted in 17% drop in blood choline concentration while the supplement prevented the decrease in blood choline levels (pre-exercise: 10.76 ± 0.23 μM vs. post-exercise: 11.10 ± 0.24 μM). When the subjects consumed the supplement without exercise, their blood choline levels increased by 27% on average. The authors also had 13 adolescent athletes (age 14-20, 3 girls and 10 boys) run a 30-60 minute cross-country race with and without the same lecithin supplement. After exercise, the mean plasma choline levels increased by 18.4% compared with the pre-exercise values (from 13.85 ± 0.60 μM to 16.25 ± 0.76 μM) when the subjects consumed the pre-exercise supplement while the subjects' plasma choline levels remained largely

unchanged with placebo consumption (pre-exercise: 14.51 ± 0.81 μM vs. post-exercise: 14.95 ± 0.46 μM). When the subjects took the supplement without exercise, the mean plasma concentrations increased from 14.15 ± 0.24 μM to 21.75 ± 0.71 μM (54% on average). The results showed that choline supplementation prior to exercise can maintain or increase blood choline concentrations in endurance trained athletes (13).

To examine the effects of choline supplementation on exercise performance, Spector et al. (14) conducted a randomized, double-blind, crossover study with 20 highly trained male cyclists with ages between 21 and 29. The subjects consumed a 200 mL fruit drink containing 6% glucose solution, 70 mg sodium, 25 mg potassium, and B vitamin complex with or without 2.43 g of choline bitartrate, twice before performing exercise tests wherein one group of subjects (n=10) cycled at 150% of their VO_2max until exhaustion, and the other group (n=10) performed a prolonged cycling at 70% of VO_2max until exhaustion. The choline supplement induced 37% and 52% increase in blood choline concentration in both groups within one hour of ingestion, and the increased blood choline levels were maintained throughout the exercise and until 45 minutes after exercise. On the other hand, blood choline levels were maintained between 8.0 and 11.5 μM and did not drop with the placebo treatment. The results showed no effect of pre-exercise choline ingestion on exercise performance at either intensity/duration of cycling exercises. There was no difference between choline and placebo treatments in performance measures such as heart rates, ventilation, oxygen uptake, carbon dioxide production, respiratory exchange ratio, time to exhaustion, and total work output (14). The results suggest that pre-exercise choline ingestion as well as

the resultant increase in blood choline concentration may not modify exercise performance in trained cyclists.

The effect of choline supplementation was also examined in marathon runners. Buchman et al. (17) randomly assigned 12 established marathon runners (21 to 50 years old, 7 males and 5 females) into either lecithin supplement group or placebo group. The lecithin group consumed approximately 2.2 g of choline over two days (one day prior to and on the marathon day). The plasma free choline levels were increased by 46% post-race compared with baseline values (from 8.0 ± 1.2 μM to 11.7 ± 2.5 μM) in the supplement group, while there was 26% decline in placebo group (from 9.6 ± 2.5 μM to 7.0 ± 3.6 μM). However, the mean finish time of lecithin group was 256.3 ± 46.3 minutes, and that of placebo group was 240.8 ± 62 minutes, suggesting there was no effect of choline ingestion and blood choline levels on exercise performance in marathon runners (17).

Warber et al. (15) also examined the effects of choline supplementation on exercise performance in 14 young male soldiers. The soldiers consumed either placebo or choline drinks before and during a 20 km load carriage walk exercise on a treadmill at the speed of 5.6 km/h in the double-blind crossover study. After the walk exercise, they also participated in a run-to exhaustion test as well as a squat-to failure test with 45.5 kg barbell at the rate of 25 repetitions per minute. Even though 6 g of choline supplementation before and during the 4 hour exercises resulted in 128% increase in blood choline concentration at the end of the tests compared with the beginning of the tests, there was no difference in oxygen uptake, heart rate, rating of perceived exertion

(RPE), time-to-exhaustion, or number of repetitions to failure between placebo and choline treatments. Deuster et al. (16) also examined the effect of choline supplementation on physical and cognitive performances following military style load carriage and no-load carriage treadmill exercises. The results showed that choline administration (50 mg/kg) increased plasma choline levels of the subjects but did not improve physical and mental performances (16).

Also, in a study conducted by Hoffman et al. (18), healthy college students performed a 4 minute reaction test, a 30-second Wingate Anaerobic Power test, maximum push-ups for one minute, and maximum sit-ups for one minute, followed by another bout of the same 4-minute reaction test, after consuming either placebo or supplement composed with α-glycerophosphocholine (150 mg), choline bitartrate (125 mg), phosphatidylserine (50 mg), vitamins B_3 (30 mg), B_6 (30 mg), and B_{12} (0.06 mg), folic acid (4 mg), L-tyrosin (500 mg), anhydrous caffeine (60 mg), acetyl-L-carnitine (500 mg), and naringin (20 mg). The acute consumption of the choline supplement combined with caffeine, carnitine, and other nutrients did not have any effect on muscular endurance or power performance, compared with placebo. The supplement only had a positive effect on maintaining reaction time following the acute bout of high intensity exercise session (the supplement alleviated exercise induced decline of reaction time), but it is unclear which ingredient(s) of the supplement mix induced the effect. The authors speculated that the ingredients in the supplement may have had neuro-protective effects through strengthened membrane integrity. Additionally, to determine if there is any effect of prolonged supplementation, the subjects repeated the same tests after

consuming either placebo or supplement for 4 weeks, but the results were very similar to the acute consumption tests (no effect on power and muscular endurance as well as reaction time) (18).

Summary of Exercise and Choline Studies: Unanswered Questions Still Remain

As discussed so far, it seems that some exercises can lower blood choline levels but most other exercises do not have any effects on blood choline concentrations. Only those exercises that are very long in duration and very strenuous in intensity at the same time seem to be able to lower blood choline levels. However, the effect of low blood choline levels (either through diet or exercise) on exercise performance has been understudied and is currently unclear. Likewise, the effects of choline consumption to prevent drop in blood choline concentration or restore normal blood levels of choline on exercise performance are also unknown.

The current body of literature shows that there is little to no effect of choline supplementation on exercise performance when the exercise does not lower blood choline levels. It appears that choline does not have meaningful effects on exercise performance in general, if one can maintain normal range of blood choline concentration (through either diet or exercise). Data also indicate that higher choline intake increases blood levels of choline, but increased choline consumption (higher than potential threshold levels) and resultant higher blood choline concentration may not provide additional benefits on exercise performance. However, since many of these studies used mostly acute aerobic/endurance type of exercise models at low to moderate intensities,

the relationship between choline intake and resistance exercise/training is largely unknown. Moreover, these studies mostly examined the effect of acute consumption of a large amount of choline supplements with lack of nutritional control in many cases, leaving the effects of chronic/habitual consumption of (lower) choline, which may limit the optimal muscle responses to RET, unexamined and unknown.

There is evidence suggesting that neuromuscular transmission failure plays a significant role in fatigue of skeletal muscles composed with predominantly fast-twitch fibers (63). Unlike aerobic/endurance exercises, high intensity RE mostly stimulates and utilizes fast-twitch fibers. Therefore, if ACh availability mediated by choline intake plays a role in neuromuscular transmission failure, it may be possible to observe different effects of choline on exercise performance and muscle responses to RE. While endurance exercise, due to its low frequency nerve firing nature, allowing nerve fibers enough opportunity to re-synthesize ACh, may not affect ACh levels at NMJ, RE may limit the ACh availability and influence neural activation, muscle contraction, and force generation, due to its high frequency nerve firing pattern, the ability to recruit greater number of motor units, and usage of larger muscle masses utilizing more ACh. Data also suggest that blood choline is regulated at relatively constant levels even with prolonged period of moderately low choline consumption, and blood concentration of choline does not reflect intracellular levels of choline effectively (38, 64), because our bodies can synthesize a certain amount of choline in the liver, and choline can be provided by degradation of PC. There might be situations where low choline intake affects muscle responses through subtly limiting ACh synthesis and release, preventing optimal muscle

action/response to RE (through impaired neuromuscular transmission) while maintaining blood choline concentration at relatively stable levels.

As discussed earlier, myocytes incubated in choline deficient medium are more prone to mechanical stress due to compromised membrane integrity (6). This may be another potential mechanism by which low choline intake can affect exercise performance and muscle responses to RE, which imposes greater mechanical stress/load on muscle cells than endurance exercise does. The lack of sufficient choline and PC may result in structurally weak cell membranes, and limit the muscle's ability to withstand mechanical stress of exercises and to produce forces, compromising exercise performance. Also, choline deficiency may affect optimal supply of PC for enlarging muscle cells and membranes in response to RET, limiting hypertrophy and associated strength gains.

CHAPTER III

LOWER INTAKE OF CHOLINE IS ASSOCIATED WITH DIMINISHED STRENGTH AND LEAN MASS GAINS FOLLOWING 12 WEEKS OF RESISTANCE TRAINING IN 60-69 YEAR OLD MEN AND WOMEN

Introduction

Skeletal muscle constitutes ~50% of total body mass and is responsible for more than half of the whole body metabolism (65). It is also the largest reservoir of glycogen and amino acids, and skeletal muscle dysfunction is related to many important health issues including cardiovascular diseases, diabetes, obesity, and osteoporosis. In addition, after the age of 40, skeletal muscle mass and strength is steadily lost by as much as five percent per decade in a condition referred to as 'sarcopenia' (66, 67). Therefore, optimizing skeletal muscle mass and function has utmost importance considering the effects of skeletal muscle on overall health and well-being.

It is well known that resistance exercise (RE) has a significant impact on increasing/maintaining muscle mass and the associated strength/function. It is also well established that the effects of RE can be, at least in part, moderated by nutritional intake. However, besides total energy intake, carbohydrate, and protein, the effects/roles of other macro/micronutrients on the muscle responses to RE are still unclear (3).

Choline plays important roles in human physiology including cell membrane integrity/signaling, lipid transport, and methylation. Because choline deficiency (<10% of Adequate Intake [AI]) is associated with muscle damage, steatosis, organ dysfunction,

and neurological disorders, choline was officially acknowledged as an essential micronutrient by the Institute of Medicine (IOM) in 1998 (19, 29), but little data on choline content of foods were available until 2004 (43). Choline is also a precursor to acetylcholine (ACh), a neurotransmitter which relays a signal between motor neurons and skeletal muscles to generate force. Therefore, it is reasonable to hypothesize that choline intake may be associated with exercise performance and skeletal muscle responses to exercise.

There have been a few studies that examined the relationship between choline and exercise performance in various circumstances. Several studies reported that marathon runs or high speed bicycle rides for long duration resulted in decreased blood choline concentrations, but the effect of lowered blood choline levels on exercise performance was equivocal (11-13). Other studies reported treadmill exercises, cycling, and cross country races had no effect on blood choline, and choline supplementation to maintain or increase blood choline concentrations did not affect exercise performance (13-17).

However, these studies were conducted in the context of acute bouts of aerobic/endurance exercises, and there has been no study that examined the effect of choline intakes on skeletal muscle mass and strength responses to resistance exercise training (RET). Considering ACh synthesis is proportional to neural activation rate, and endurance exercise has a relatively low frequency activation rate, it is not surprising that choline effects are equivocal with endurance exercise. Moreover, many of the previous studies lacked nutritional control in general and specifically for choline due to dearth of

information on choline content of foods, which may have further obscured potential effects of choline on muscle responses to exercise.

The purpose of this study was to examine the relationship between variability of choline intake in the context of a "healthy diet", as recommended by the American Dietetic Association (ADA, now known as the Academy of Nutrition and Dietetics), and muscle responses to a standard RET program in older adults. We hypothesized that variability of choline intake would be linearly associated with the magnitude of strength and lean mass gains following 12 weeks of RET.

Methods

Participants

Forty-six, 60-69 year old men and women (males: 64.3 ± 3y; females: 63.7 ± 3y, postmenopausal for more than 2 years), recruited through advertisements, flyers, and mailings to local community and senior centers, completed a 12-week RET program designed to increase lean mass and muscular strength. All the participants were non-smokers, generally healthy, and untrained: Individuals with uncontrolled high blood pressure (>160/100 mmHg), diabetes, cardiac arrhythmias, cancer, hernia, aortic aneurysm, kidney disease, and/or lung disease were excluded, and those who engaged in more than one hour of RET per week on average in the previous 12 months were not eligible for the study. This study was approved by the Kent State University and Texas A&M University Institutional Review Boards, and all the participants provided written informed consent. Other details on this cohort are provided elsewhere (68, 69).

Pre-Study Orientation

Before starting the 12-week RET program, participants attended six orientation sessions over two weeks. Each orientation session comprised one hour of exercise familiarization, wherein the participants learned correct exercise techniques by exercising at low intensity (40% of their estimated maximum strength based on the Omnibus-RE Scale [OMNI-RES] ratings of perceived exertion [RPE]) (70) to reduce the possibility of injury and standardize early gains in strength by motor learning, and 30 minutes of nutrition education by a registered dietitian on proper nutrient intake and accurate diet log documentation. Participants were instructed to maintain isocaloric food intake (50% carbohydrate, 30% fat, 20% protein, and <10% saturated fat) and consume >1.0 g/kg/d of protein, 25-30 g/d of fiber, and <300 mg/d of cholesterol, as recommended by the ADA, throughout the study.

Testing

Upon completion of the orientation and at least 72 hours before the first RET session, body composition was measured by dual energy X-ray absorptiometry (DEXA) using Hologic 4500 QDR (Bedford, MA), and maximum strength (1RM) for all the exercises included in the RET program was determined using the following protocol (70): After a three-minute warm-up on cycle ergometers (Schwinn Fitness, Inc., Denver, CO) and dynamic full body stretching, participants performed four warm-up repetitions with a resistance set at 55% of investigator/participant estimated 1RM based on OMNI-

RES RPE. The resistance was then adjusted to 75% of re-estimated 1RM, and participants performed one repetition. The resistance was increased again to 90% of re-estimated 1RM for participants to perform one repetition. The attempts for true 1RM were made by gradually increasing resistance in a manner that the total number of 1RM attempts was minimized (70). The exercise order for 1RM measurement was the same for all participants, and the rest interval between each attempt was 60 seconds. The same testing was repeated at the completion of the 12-week program to determine RET responses. 1RM and body composition tests were performed 48 hours and 72 hours after the last RET session, respectively.

Resistance Exercise Training

For 12 weeks, participants performed whole body RET three times per week (on non-consecutive days) using Cybex RET machines (Cybex International Inc., Medway, MA). The exercises comprised chest press, lat pull down, leg press, knee extension, seated leg curl, triceps extension, biceps curl, and calf raises. On each exercise day, the participants performed a 10-minute warm-up on the Schwinn cycle ergometers, five minutes of stretching, and three sets of 8-12 repetitions of the eight exercises. Resistance was set at 75% of each participant's 1RM, and the participants performed as many repetitions as possible until they reached 12 repetitions or muscle failure (inability to complete a repetition with proper form and full range of motion). When a participant achieved 12 repetitions on all three sets of an exercise, the exercise weight was increased so that the participant would only be able to achieve eight repetitions per set in the next

session. This is to ensure that relative exercise intensity (75% of 1RM) was maintained throughout the study as participants gained strength. All the exercise sessions were monitored by research assistants, and the rest time was 60 seconds between sets and two minutes between exercises. The participants were instructed to maintain their non-RET physical activities at current levels but not to perform additional RET outside of the study.

Nutrition

The participants were required to maintain and submit diet logs three times per week on non-consecutive days to investigators for the entire duration of the study. Feedback on the diet logs were provided weekly to ensure compliance to the diet recommendations. To help ensure enough protein consumption for muscle gain and to minimize potential effects of variable protein intake, participants consumed post-workout supplements (Boost High Protein; Nestle S.A., Vevey, Switzerland) immediately after each exercise session. The amount of the supplement was adjusted to each participant's lean mass so that 0.4 g of protein/kg of lean mass was consumed with each supplement.

The average intake values of all macro/micro nutrients during the 12-week intervention were calculated using the diet logs entered into Nutribase software (version 5; Cybersoft Inc., Phoenix, AZ) and the USDA database for choline (44). Then participants' mean choline intakes during the 12 weeks were categorized into three groups (Low: <5.6 mg/kg lean mass/day, Med-Low: 5.6-8.0 mg/kg lean/d, and

Adequate: >8.0 mg/kg lean/d) based on the data distribution (median and natural breakpoints).

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics software (version 21; IBM Corporation, Armonk, NY). The assumption of normal distribution was checked using Shapiro-Wilk test, and non-normal variables were log or square root transformed before tested with parametric statistical procedures. Non-parametric tests were also conducted with non-transformed data to demonstrate consistency of the test results.

Student's independent t-tests were performed to examine differences between two groups (e.g., men and women), and paired t-tests were used to compare pre- and post-training values. Pearson's correlations were used to examine associations between nutrient intakes and RET responses, and linear regression analyses were performed to examine the independent association of choline consumption with changes in composite strength and lean mass. Composite strength was defined as chest press 1RM + leg press 1RM, and percent change was calculated as 100 x (post training measurement - pre training measurement) / pre training measurement.

The differences in RET responses between choline intake groups were examined using one-way analysis of variance (ANOVA). A two-way ANOVA was performed to determine if there were differences in RET responses between choline groups or gender, and analysis of covariance (ANCOVA) tests were used to account for the effects of

potential confounders (other nutrients, age, etc.). The assumption of equal variance was checked using Levene's test, and the Bonferroni method was used to perform multiple comparisons. P values of <0.05 were considered statistically significant, and data are presented as mean ± standard deviation (SD) unless stated otherwise.

Results

Participants

The baseline characteristics of the 46 participants who completed the study are presented in **Table 3**. There was no significant difference between choline intake groups in age, body weight, body fat, or BMI, but there were more male participants in the Low choline group, of which participants had greater baseline lean mass compared with the other groups.

Table 3. Participant's Baseline Characteristics

	Low (n=20)	Med-Low (n=19)	Adequate (n=7)
Age (years)	65.0 ± 3.2	63.3 ± 3.0	63.0 ± 2.5
Male/Female	16/4	3/16†	0/7†
Height (inches)	68.2 ± 3.9	65.3 ± 3.6	63.6 ± 2.4*
Weight (kg)	84.2 ± 16.4	78.7 ± 11.9	71.9 ± 16.0
Body fat (kg)	24.4 ± 7.7	29.5 ± 8.0	28.6 ± 11.2
Lean mass (kg)	58.2 ± 10.9	47.3 ± 8.4†	42.0 ± 5.5†
BMI ($kg \cdot m^{-2}$)	27.9 ± 3.5	28.6 ± 4.0	27.7 ± 6.9

Data are presented as mean ± SD. Low: choline intakes of 2.9-5.5 mg/kg lean/d. Med-Low: choline intakes of 5.6-8.0 mg/kg lean/d. Adequate: choline intakes of 8.1-10.6 mg/kg lean/d. * denotes a significant difference from Low group ($p<0.05$). † denotes a significant difference from Low group ($p<0.01$).

Nutritional Intakes

All the participants met dietary recommendations for caloric intake, protein consumption, cholesterol intake, and proportions of macronutrients, and there was no difference between choline groups in these nutrient intakes except for protein/kg (higher in Adequate group) and percent of kcal from carbohydrate (lower in Med-Low group) (**Table 4**). No significant differences were observed in intakes of micronutrients related to choline metabolism such as vitamins B_5, B_6, B_{12}, folate, and betaine (data not shown).

The average choline consumption during the study period was 304.2 ± 70 mg/day (6.1 ± 1.6 mg/day and 3.9 ± 0.9 mg/day when adjusted for kg lean mass and kg body

weight, respectively), which is consistent with previously reported choline intake data (313 mg/d in the Framingham Offspring Study, 335 mg/d in NHANES 2011-2012; 372 mg/d in men and 304 mg/d in women in Yonemori et al.'s study) (22, 45, 48). Dietary choline (mg/kg lean/d) was correlated with kcal/kg lean/d ($r=0.516$, $p<0.001$), protein intake (g/kg lean/d, $r=0.733$, $p<0.001$), carbohydrate intake (g/kg lean/d, $r=0.295$, $p=0.047$), fat intake (g/kg lean/d, $r=0.442$, $p=0.002$), and dietary cholesterol (mg/kg lean/d, $r=0.519$, $p<0.001$), but none of these nutrients were significantly correlated with muscle responses to RET except for dietary cholesterol which was significantly correlated with percent changes in composite strength ($r=0.386$, $p=0.009$) and lean mass ($r=0.364$, $p=0.013$).

Table 4. Nutritional Intakes

	Low (n=20)	Med-Low (n=19)	Adequate (n=7)
Total Energy (kcal/kg/d)	23.0 ± 4.7	21.7 ± 6.3	26.7 ± 6.4
Protein (g/kg/d)	0.9 ± 0.1	1.0 ± 0.2	1.2 ± 0.2‡
Carbohydrate (g/kg/d)	2.7 ± 0.8	2.3 ± 0.8	2.9 ± 0.9
Fat (g/kg/d)	0.7 ± 0.2	0.8 ± 0.3	0.9 ± 0.3
% of total kcal from carbohydrate	53.8 ± 8.0	48.0 ± 6.7*	49.0 ± 6.3
% of total kcal from protein	16.0 ± 2.2	18.1 ± 3.8	18.1 ± 4.7
% of total kcal from fat	28.6 ± 6.5	32.4 ± 6.1	30.7 ± 5.8
Cholesterol (mg/kg/d)	2.5 ± 1.0	3.0 ± 1.0	3.4 ± 1.0

Data are presented as mean ± SD. Low: choline intakes of 2.9-5.5 mg/kg lean/d. Med-Low: choline intakes of 5.6-8.0 mg/kg lean/d. Adequate: choline intakes of 8.1-10.6 mg/kg lean/d. * denotes a significant difference from Low group ($p<0.05$). ‡ denotes a significant difference from the other groups ($p<0.05$).

Effects of Choline on RET Responses

Choline intake (mg/kg lean/d) was significantly correlated with percent change in composite strength with medium effect size ($r=0.36$, $p=0.015$), and a simple regression analysis indicated that there was a significant linear relationship where choline intake (mg/kg lean/d) predicts percent change in composite strength ($\beta=0.655$, $t=2.534$, $R^2=0.13$, $p=0.015$), while a trend was observed between choline consumption (mg/kg lean/d) and percent change in lean mass ($r=0.265$, $p=0.075$).

Also, there was a significant difference in composite strength gains between choline groups (**Figure 4**). While RET resulted in significant increases in lean mass and strength in all three groups (**Table 5 and 6**), the Low choline group gained significantly less composite strength compared with the other groups (Low: 30.9 ± 15.1%, Med-Low: 70.3 ± 48.5%, Adequate: 81.9 ± 68.4%; p=0.004). The Low choline group also showed significantly reduced changes in 1RM for leg press and chest press compared with the higher choline intake groups (**Table 6**), and similar trends were observed for calf raises (p=0.08) and triceps extension exercises (p=0.07). Additionally, non-parametric tests (Spearman's Rho and Kruskal-Wallis test) showed the same results (data not shown) that are consistent with Pearson's correlation and ANOVA tests, confirming the strong association between choline intake and composite strength gain.

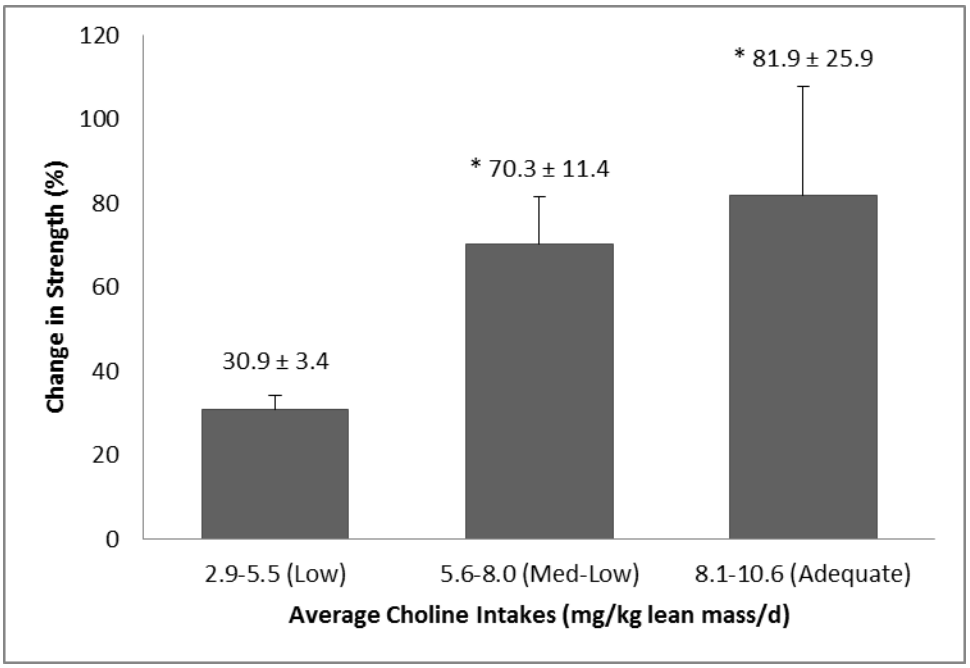

Figure 4. The Effect of Choline Intake on Changes in Composite Strength
Data are presented as mean ± standard error (SE). Composite strength is defined as chest press 1RM + leg press 1RM. * denotes a significant difference from Low group ($p<0.01$).

Table 5. The Effect of Choline Intakes on Body Composition

	Low (n=20)	Med-Low (n=19)	Adequate (n=7)	P values
Change in lean mass (kg)	0.9 ± 1.7	1.4 ± 1.7	1.5 ± 0.6	0.50
Percent change in lean mass	1.5 ± 2.8	2.9 ± 3.3	3.7 ± 1.8	0.15
Change in body fat (kg)	-1.9 ± 1.6	-1.6 ± 2.1	-1.5 ± 2.1	0.88
Percent change in body fat	-7.5 ± 6.6	-4.8 ± 5.8	-5.5 ± 5.8	0.40

Data are presented as mean ± SD. Low: choline intakes of 2.9-5.5 mg/kg lean/d. Med-Low: choline intakes of 5.6-8.0 mg/kg lean/d. Adequate: choline intakes of 8.1-10.6 mg/kg lean/d. All changes from baseline are statistically significant except for change in body fat (kg) in Adequate group. No differences were observed between choline intake groups ($p>0.1$).

Table 6. The Effect of Choline Intakes on Changes (%) in 1RM

	Low (n=20)	Med-Low (n=19)	Adequate (n=7)
Leg Press	35.7 ± 19.7	77.4 ± 50.6*	97.5 ± 87.1*
Chest Press	19.4 ± 10.5	50.9 ± 50.2*	42.7 ± 29.3
Lat Pull Down	36.5 ± 26.5	44.4 ± 33.0	49.4 ± 36.8
Knee Extension	42.6 ± 27.7	56.0 ± 47.0	61.9 ± 71.0
Leg Curls	30.8 ± 24.3	47.5 ± 35.4	18.2 ± 26.0
Calf Raises	74.1 ± 40.3	102.7 ± 77.8	156.1 ± 92.9
Triceps Extension	29.4 ± 17.3	36.7 ± 18.2	48.7 ± 24.8
Biceps Curls	31.0 ± 22.6	53.8 ± 45.7	39.6 ± 24.6

Data are presented as mean ± SD. Low: choline intakes of 2.9-5.5 mg/kg lean/d. Med-Low: choline intakes of 5.6-8.0 mg/kg lean/d. Adequate: choline intakes of 8.1-10.6 mg/kg lean/d. All changes from baseline are statistically significant. * denotes a significant difference from Low group ($p<0.05$).

Since there was a difference between choline groups in gender distribution, and women had higher intakes of protein (g/kg lean/d), cholesterol (mg/kg lean/d), and choline (mg/kg lean/d), a two-way ANOVA test was conducted to determine if there was a difference in composite strength gains (%) between choline groups or gender. There was no gender x choline group interaction, and the effect of gender was not significant while the main effect for choline intake was statistically significant ($p<0.05$). Multiple comparisons showed the Low group gained significantly less composite strength compared with the other groups ($p<0.05$).

Choline consumption was correlated with total energy, protein, carbohydrate, fat, and cholesterol intakes in the present study. Since many choline rich foods (e.g., eggs, fish, and meat) are also rich in fat, protein, and cholesterol (44), and choline metabolism is intertwined with betaine, folate, and vitamins B_5, B_6, B_{12} metabolism (29, 37), ANCOVA tests were conducted to separate the effects of these dietary factors as well as other covariates such as age and baseline lean mass from the effects of choline. Significant ($p<0.05$) differences in composite strength gains (%) between choline groups were observed in the presence of these covariates, separately or in various combinations (data not shown), indicating independent effects of choline intake on strength gains.

Multiple linear regression analyses were also conducted to further separate the effects of choline from those of other variables such as age, gender, and other nutrients, especially cholesterol, because cholesterol was previously reported to be associated with strength and lean mass in older adults (69) and was correlated with RET responses in the present study. All of the aforementioned variables were initially entered into the equation, and after a series of backward elimination procedures, only low choline intake, age, and cholesterol intake remained in the final model, which showed that choline intake independently predicts percent change in composite strength (adjusted $R^2=0.268$, $p=0.001$) (**Table 7**).

Table 7. Multiple Regression Analysis of the Independent Effect of Choline Consumption, Dietary Cholesterol, and Age on Change in Composite Strength (%) Following 12 Weeks of RET

	Unstandardized Coefficients		
Predictors	β	SE	P values
(Constant)	13.135	4.149	0.003
Low Choline Intake	-1.833	0.847	0.036
Age (years)	-0.002	0.001	0.089
Cholesterol Intake (mg/kg lean/d)	0.341	0.236	0.156

SE: standard error. Low Choline Intake: 2.9-5.5 mg/kg lean/d.

Because RET responses, nutritional intakes, and demographic characteristics were similar between Med-Low and Adequate groups (**Tables 3-5, Figure 4**), the two groups were pooled for further analyses. ANCOVA tests showed that the Low group gained significantly diminished lean mass compared with the higher choline intake (5.6-10.6 mg/kg lean/d) group (1.3 ± 0.6% vs. 3.2 ± 0.6%; folate/kg lean/d as a covariate; estimated marginal mean ± standard error [SE], $p<0.05$). Two-way ANOVA test showed a trend ($p=0.076$) indicating the effect of choline intake on percent change in lean mass, while gender and choline intake x gender interaction were not statistically significant ($p>0.5$). Non-parametric independent-samples Mann-Whitney U test also indicated that the distribution of percent change in lean mass was significantly different between the two choline intake groups ($p<0.05$).

Discussion

The purpose of this study was to examine the relationship between habitual, food-based choline intake and changes in strength and lean mass following 12 weeks of RET. To our knowledge, the present study is the first to examine the effect of habitual choline intake on muscle responses to RET in older adults. The major finding of the present study was that choline intake is strongly associated with the magnitude of strength gains following 12 weeks of RET in older adults. Our data also indicated a moderate effect of choline intake on lean mass gains.

The mechanism through which choline may affect strength and lean mass gains with RET is unknown, however, there exist a few potential mechanisms. Choline is a precursor to ACh, the main neurotransmitter in α-motor neurons, and evidence shows ACh production and release can be mediated by availability of choline (4, 5). Therefore, dietary choline may affect availability of ACh at the neuromuscular junctions (NMJ), influencing muscle contraction and force production in skeletal muscles. While previous studies utilizing endurance exercise models failed to observe meaningful effects of choline, dietary choline might have different effects on ACh in the context of RE.

Neuromuscular transmission failure is suggested as a major factor in fatigue of skeletal muscles predominantly composed of fast-twitch fibers (63), which are recruited mostly with RE. If ACh availability plays a key role in neuromuscular transmission failure (71, 72), dietary choline may differentially affect exercise performance with RE. The low frequency motor output and lower number of active motor units associated with endurance exercise might allow sufficient re-synthesis of ACh in motor neurons while

high frequency motor output and greater number of motor units recruited with RE might reduce ACh availability at the NMJ.

Choline is also a precursor to phosphatidylcholine (PC), which is the most abundant phospholipid in all cellular membranes, and choline deficiency has been shown to induce weakened cell membranes and muscle damage (6). Structurally weak cell membranes may negatively influence muscle's ability to handle mechanical load, which is greater with RE, and an increase in lean mass as a result of RET may require more PC as a substrate for enlarging muscle cells and membranes. In addition, PC is a precursor to phosphatidic acid (PA), which is required to activate mammalian target of rapamycin (mTOR), a central element in regulation of protein synthesis (26). Choline also plays an important role in methylation reactions, crucial steps for control of gene expression, protein synthesis, and other metabolic pathways (10, 28). Therefore, insufficient choline consumption might limit skeletal muscle hypertrophy and strength gains associated with RET through several physiological processes.

We also observed that the majority of the subjects in the present study consumed less than the recommended amount of choline (90th percentile of choline intake was 393 mg/d), which is similar to previously published data (only 20% of the subjects [mean age:54y] consumed >401 mg/d choline in the Framingham Offspring Study, and 80% of 1,477 women [mean age: 52y] consumed less than 361 mg of choline per day in Chiuve et al.'s study) (47, 48), providing additional evidence showing that inadequate choline consumption is prevalent, at least in older populations. The current AI of choline for adults is ~7 mg/kg/d (550 mg/d for males and 425 mg/d for females) (19).

In the present study, the Low, Med-Low, and Adequate groups consumed approximately ~49%, ~63%, and ~85% AI of choline, respectively. We observed that the Low group (< ½ of AI) gained significantly less strength and lean mass following 12 weeks of RET compared with higher choline intake groups. Reduced strength gains with inadequate choline intake reported presently adds to the established list of consequences identified with extremely low choline consumption (<10% AI) including muscle/liver damage, cardiovascular disease, fatty liver, neurological disorder, and cancer. These results suggest that even normal/average intakes, which are below AI but do not present overt clinical signs, may still have consequences that affect health and well-being of older adults. Since the results of the present study provide evidence only in the low to near adequate levels of choline consumption, the effects of higher choline intake on lean mass and strength gains still remain unknown. Even though previous studies did not observe any effect of choline supplementation (higher than AI) on endurance exercise performance, higher choline consumption might influence muscle responses to RET differently.

It should be noted that there are several limitations to our study. We were not able to determine blood choline concentrations, therefore the associations of blood choline with muscle responses to RET were not examined. However, we believe this does not change the overall conclusions of our observation because blood choline levels do not effectively reflect moderate changes in choline intake (64). Data also suggest that blood choline is regulated at relatively constant levels with moderately low choline

consumption in resting individuals, and blood concentration of choline does not represent intracellular levels of choline (38, 64, 73).

Also, the well-known limitations of dietary records may have affected the accuracy of our analyses. However, the impact of potentially inaccurate diet records was minimized by combined effects of pre-study nutrition education, collection of weekly diet report for 12 weeks (>36 records per subject), and provision of regular feedback to encourage compliance to the diet guidelines and accurate documentation of food intake.

In addition, there was a significant difference in gender distribution between choline groups in the present study (**Table 3**), which may have confounded the results of our analyses. However, extensive efforts to separate the influence of gender as well as other potential confounders from that of dietary choline using various statistical techniques including two-way ANOVA, ANCOVA, and multiple regression tests, consistently indicated that the significant effects of choline intake observed presently was independent of gender and/or other confounders. Moreover, studies suggest that physiological responses to different amounts of choline intake are similar between men and postmenopausal women (38), and skeletal muscle responses to RET are not different between genders, at least in older adults (74, 75), supporting the conclusion of the present study.

Summary

Our data suggest that dietary choline has a linear relation (in the 49^{th}-85^{th} percentile of AI) with skeletal muscle responses to 12 weeks of RET in generally healthy

60-69 year old men and women. Lower intake of choline was associated with reduced gains in strength and lean mass in this population. The potential mechanisms of these effects of dietary choline may include ACh availability at the NMJ, membrane integrity, cell signaling, and/or methylation reactions. Considering the effects of skeletal muscle to overall health and well-being, especially in older population whose choline intake is persistently low, it is important to optimize nutritional factors to maximize the benefits of RET. Future studies are warranted to confirm the results of the present study and elucidate the mechanism through which choline affects skeletal muscle responses to RET.

CHAPTER IV

THE EFFECTS OF CHOLINE SUPPLEMENTATION AND RESISTANCE TRAINING ON STRENGTH AND LEAN MASS GAINS IN OLDER ADULTS: A RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED CLINICAL TRIAL

Introduction

Choline plays crucial roles in several physiological processes such as neurotransmission and muscle contraction via synthesis of acetylcholine (ACh), lipid transport via lipoprotein synthesis, and methyl-group metabolism as a precursor to betaine. It also supports cell membrane integrity/function as a precursor to phosphatidylcholine (PC), the most abundant phospholipid in all cellular membranes.

Choline can be obtained from the diet (exogenously) or from de novo PC synthesis (endogenously) through sequential methylations of phosphatidylethanolamine (PE) (24). However, the amount of choline produced via de novo synthesis is not sufficient to support total choline requirement. Therefore, the majority of the required choline must be acquired from diet (19), and choline deficient diet (<10% of Adequate Intake [AI]) has been associated with negative health conditions including liver/muscle damage, nonalcoholic fatty liver disease, atherosclerosis, birth defects (neural tube defects), and neurological disorders (29).

We have observed that lower intake of choline was associated with reduced strength and lean mass gains following 12 weeks of resistance exercise training (RET) in 60-69 year old individuals (76), providing additional evidence that sufficient choline

intake is important for older individuals' health and well-being. In that study, subjects' habitual, food based choline consumption ranged between 49% and 85% of AI, and a linear relation was observed between choline intake and percent change in strength (between 31% and 82%) within that range, suggesting lower choline intake may negatively affect muscle responses to RET (76). However, it is still unclear whether higher choline intake (≥AI) positively affects strength and lean mass gains with RET.

Previous studies regarding choline supplementation and exercise generally reported that consumption of choline exceeding AI, sufficient to increase blood levels of choline, does not positively affect exercise performance. Spector et al. (14) reported that choline supplementation of ~970 mg (~200% of AI) prior to cycling did not affect heart rate, ventilation, oxygen consumption, time to exhaustion, and total work output in young athletes. Similarly, 6 g of choline supplementation (11 times AI) before and during four hours of treadmill walk, run-to-exhaustion, and squat-to-failure tests did not affect oxygen uptake, heart rate, or time to exhaustion in young male soldiers (15). Other studies also reported no effect of pre-exercise choline supplementation (greater than AI values) on exercise performance with military style load-carriage test, a marathon run, or Wingate/push-up/sit-up tests (16-18). However, these studies lacked nutritional control of habitual choline intake and only examined the acute effects of choline supplementation on endurance performance variables, leaving the effects of choline supplementation greater than AI on resistance exercise (RE) and training responses largely unknown.

The purpose of the present study was to determine the effects of various amounts of choline intakes (including higher than AI) on muscle responses to RET. We supplemented subjects' habitual choline intake from a standard healthy diet, as recommended by the American Dietetic Association (ADA, now known as the Academy of Nutrition and Dietetics), with choline in the form of egg yolk and examined the effects of dietary choline on changes in strength, lean mass, muscle quality, clinical markers of muscle/liver damage, and blood lipids in older men and women following 12 weeks of RET, with a randomized double-blind placebo-controlled study design.

Methods

Participants

Thirty-seven, 50 to 69 year old, generally healthy men and women were recruited via flyers and advertisements in a local newspaper. Smokers and individuals with any of the following health conditions were excluded: hypertension (> 160/100 mmHg), cardiac arrhythmias, cancer, hernia, aortic aneurysm, kidney disease, diabetes, lung disease, blood cholesterol >240 mg/dl or <160 mg/dl, or taking cholesterol lowering medications. Those who participated in one hour or more of RET in the previous year were not eligible for participation, and women needed to be postmenopausal for more than two years. The eligible subjects were randomly assigned into one of three choline groups in a double-blind manner: zero additional egg yolk (Low), one additional egg yolk (Med), or three additional egg yolks (High) per day. This study was approved by Texas A&M

University Institutional Review Board, and all the participants provided written informed consent prior to participation in the study.

Orientation

During two weeks of a pre-study orientation period, the participants attended two sessions of nutrition education by a registered dietitian (RD) and four sessions of exercise orientation/familiarization. Each nutrition education session lasted for two hours, and the participants learned about proper nutrient intake, calorie/portion control, and study specific diet guidelines. They also practiced the use of a nutrition software (Nutribase; version 7; Client Intake Module; Cybersoft Inc., Phoenix, AZ) with which they maintained diet logs throughout the study. The exercise orientation provided the participants with information on the benefits of regular exercise and principles of RE. Correct exercise techniques were explained/demonstrated, and the participants became familiarized with RE by practicing the techniques with light weight initially and gradually increasing the intensity to 40% of their estimated maximum strength (4/10 on the Omnibus-RE Scale [OMNI-RES] ratings of perceived exertion [RPE]). (77) The purpose of the exercise orientation was to allow rapid motor learning while minimizing skeletal muscle adaptations to standardize strength measures, estimate maximum strength (1RM) prior to testing, and reduce the possibility of exercise-induced injury.

Testing

Following the orientation and at least 72 hours before the first RET session, 1RM, peak power, body composition, and resting metabolic rate (RMR) were measured. 1RM's for all the exercises included in the RET program were determined by gradually increasing exercise weights until the maximum resistance, at which only one repetition can be completed with proper form in full range of motion, was reached (77) using Keiser 300 series pneumatic exercise machines (Keiser, Palo Alto, CA). Following a three minute warm-up on a cycle ergometer (Schwinn Fitness, Inc., Denver, CO) and stretching, participants performed four warm-up repetitions with an exercise weight corresponding to 55% of an estimated 1RM based on RPE on the OMNI-RES. The weight was then increased to 75% of a re-estimated 1RM (based on RPE) to perform only one repetition. After 60 seconds of rest, the weight was increased again to 90% of a re-estimated 1RM to perform one repetition. Additional attempts for 1RM were made after 60 seconds of rest until the true 1RM value was obtained, in a manner that the total number of 1RM attempts was minimized (77). The same procedure was performed for all exercises and in the same order for all participants.

The power (force x speed) output for each exercise was also measured during 1RM tests. The participants were instructed to perform the concentric phase of repetitions at their maximal speed, and the Keiser machines calculated the power output for each repetition. The highest value of the power outputs recorded during 1RM tests was used as peak power for each exercise.

Body composition was assessed by dual energy X-ray absorptiometry (DEXA) using Lunar Prodigy (General Electric, Fairfield, CT), and RMR was measured with ParvoMedics TrueMax 2400 Metabolic Measurement System (Sandy, UT) in the morning after an overnight fast. On the first day of the RET program, fasted (12 hours, overnight) blood samples were collected from antecubital veins, and blood lipid/metabolic panels were run with standard methods at St. Joseph Regional Health Center's CDC certified laboratory (Bryan, TX) to examine the effects of choline intake on clinical markers of liver/muscle damage and blood lipid profiles since choline deficiency is shown to cause liver/muscle damage and to perturb lipid metabolism (29) . All tests were repeated at the completion of 12 weeks of RET. 1RM and body composition were measured 48 and 72 hours after the last RET session, respectively, and the blood collection was performed on the last day of the RET.

Resistance Exercise Training

Participants performed a full body RET program three times per week (on non-consecutive days) for 12 weeks using Keiser 300 series exercise machines. The RET program consisted of 10 minutes of warm-up on a cycle ergometer (Schwinn Fitness, Inc., Denver, CO), five minutes of dynamic stretching, seated chest press, lat pull down, leg press, calf raises, seated leg curls, knee extension, biceps curls, and triceps extension exercises. Participants performed three sets of 8-12 repetitions with resistance set at 70% of 1RM. They were instructed to perform as many repetitions as possible until they reached 12 repetitions or muscle failure on a given set. When a participant was able to

complete 12 repetitions on all three sets of an exercise, the weight was increased so that only eight repetitions would be possible in the next exercise session. The rest between each set was one minute, and the rest between each exercise was two minutes, during which muscle specific stretching was performed. All the exercise sessions were supervised by Exercise Physiology graduate students, and the participants were instructed to maintain their non-RET physical activities at the pre-study level, but not to perform any additional RET.

Nutrition Control

Participants were instructed to consume 50% of total calories from carbohydrate, 30% from fat, 20% from protein, and <10% from saturated fat; to meet daily caloric consumption goals as determined by RMR test. They were also instructed to consume >1.0 g/kg/d of protein, 25-30 g/d of fiber, and <200 mg/d of cholesterol, as recommended by the ADA. Participants were required to maintain 24-hour diet logs at least four times per week (three week days and one weekend day) during the study period. Feedback on the diet logs were provided weekly, and adjustments were made as necessary to ensure adherence to the dietary guidelines of the study.

To minimize any potential effect that the variability of protein consumption may have, participants consumed protein supplements (0.4 g/kg lean mass/supplement; MET-Rx protein [MET-Rx USA Inc., Boca Raton, FL] + egg protein) every 12 hours throughout the study period. The supplement contained different amounts of whole egg powder so that Low, Med, and High groups were provided with 0.0, 2.2, or 6.8 mg of

choline/kg lean/d from egg yolk, respectively. Egg white powder and peanut oil was used to achieve equivalent amounts of protein and fat content for each group's supplement, and the supplement additionally provided 0.7 mg/kg lean/d of choline as well as 0.9 g/kg lean/d of carbohydrate and 0.3 g/kg lean/d of fat equally for all groups.

Thigh Muscle Quality

From the DEXA scans of each participant, thigh muscle quality-strength (TMQ-S) was assessed and defined as leg press 1RM (kg) / total thigh lean mass for both legs (kg). Total thigh lean mass was determined through the construction of a four-sided polygon encompassing the entire region of each thigh and combining lean mass of both thighs together (**Figure 5**). The first line segment of the polygon consisted of one point (point a) inferior to the pubic bone immediately below any flesh as a reference point with the other point (point b) positioned as to obliquely transverse the intertrochanter crest of the femur bone. The next line segment transected the tibio-femoral joint (c-d), and two more line segments were drawn to enclose the entire thigh tissue (b-c, a-d). Lean mass values located inside the polygon were calculated with DEXA and defined as thigh lean mass. All thigh lean mass measurements were performed by two independent raters. Inter-rater reliability was $R^2>0.99$, and the coefficient of variation was <1.5%. Deviations from these norms were reanalyzed. Means of the two raters were used for data analyses.

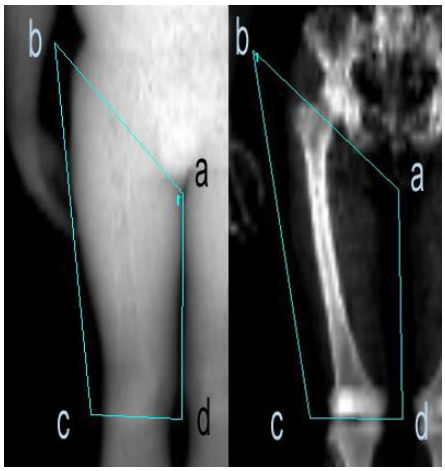

Figure 5. Example of DEXA Scan Image of Right Thigh
A four-sided polygon was constructed with one point below the pubic bone (a) that was connected to another point (b) as to obliquely cross the intertrochanter crest of the femur bone. Points (c) and (d) were positioned as to be traversing the tibio-femoral joint. Points (b)-(c) and (a)-(d) were connected to ensure the entire thigh was encompassed.

Data Analysis

All statistical analyses were conducted using SAS/STAT software (version 9.4; SAS Institute Inc., Cary, NC). The mean intakes of all nutrients were calculated from the diet logs that were entered into Nutribase software (version 7; Cybersoft Inc., Phoenix, AZ) or direct calculations from the USDA database for choline (44). The assumption of normal distribution was checked using Shapiro-Wilk test, and non-normal variables were log transformed for parametric statistical tests. Student's independent t-tests were used to compare means of two different groups (e.g., gender), and paired t-tests were performed to examine the difference between pre- and post-training values. Pearson correlations were used to examine associations between nutrient intakes and RET

responses (changes in lean mass and 1RM's), and to identify potential covariates for further analyses.

The differences between choline groups were analyzed by one way analysis of variance (ANOVA). The assumption of equal variance was checked using Levene's test, and the Tukey method was used to perform pairwise comparisons. When the equal variance assumption was not met, Welch's variance-weighted ANOVA test was performed. Analysis of covariance (ANCOVA) tests were conducted to examine the effect of dietary choline controlling for effects of potential confounders (cholesterol and other nutrients, gender, age, and/or lean mass, etc.) on RET responses.

Multiple linear regression analyses were performed to examine the independent effects of choline consumption and any other factors on RET responses. Composite strength was defined as chest press 1RM + leg press 1RM, and percent change was calculated as 100 x (post training measurement - pre training measurement) / pre training measurement. P values of <0.05 were considered statistically significant, and data are presented as mean ± standard deviation (SD) unless stated otherwise.

Results

Demographics

The baseline characteristics of the 37 participants who completed the 12 weeks of RET are presented in **Table 8**. There were no differences between choline groups in age, gender distribution, height, weight, lean mass, or adiposity. Furthermore, there were no significant differences between men and women in age and body fat, and BMI while

males were taller and had more lean and total body mass compared with females (data not shown).

Table 8. Participant's Baseline Characteristics

	Low (n=13)	Med (n=11)	High (n=13)	P values
Age (years)	58.9 ± 7.3	60.7 ± 4.6	60.0 ± 4.5	0.78
Male/Female	6/7	5/6	4/9	0.69
Height (inches)	67.2 ± 3.8	66.5 ± 3.5	65.5 ± 3.0	0.47
Weight (kg)	83.5 ± 14.9	80.8 ± 22.9	78.5 ± 15.9	0.77
Body fat (kg)	31.3 ± 12.2	27.7 ± 10.7	31.3 ± 7.9	0.65
Lean mass (kg)	48.8 ± 9.7	44.6 ± 8.1	44.0 ± 11.3	0.42
BMI ($kg \cdot m^{-2}$)	28.8 ± 5.8	28.1 ± 5.7	28.2 ± 4.7	0.94

Data are presented as mean ± SD. Low: choline intake of 6.2 ± 1.2 mg/kg lean mass/d. Med: choline intake of 8.1 ± 1.6 mg/kg lean/d. High: choline intake of 14.2 ± 3.0 mg/kg lean/d. No differences were observed between choline groups.

Nutritional Compliance

Participants successfully followed the dietary guidelines of the study and met all the requirement for nutritional intake. On average, the participants consumed 27 kcal/kg/d of calories, 3.2 g/kg/d of carbohydrate, 1.4 g/kg/d of protein, and 1.0 g/kg/d of fat throughout the study, and there was no difference in nutrient intake between choline groups except for cholesterol consumption (**Table 9**).

Table 9. Nutritional Intakes

	Low (n=13)	Med (n=11)	High (n=13)	P values
Total Energy (kcal/kg/d)	27.7 ± 5.8	25.6 ± 6.3	27.7 ± 6.9	0.58
Carbohydrate (g/kg/d)	3.4 ± 0.9	3.0 ± 0.7	3.3 ± 0.8	0.39
Protein (g/kg/d)	1.4 ± 0.2	1.4 ± 0.3	1.4 ± 0.2	0.45
Fat (g/kg/d)	1.0 ± 0.4	0.9 ± 0.2	1.0 ± 0.4	0.36
% of kcal from carbohydrate*	49.6 ± 4.3	47.9 ± 2.7	49.5 ± 4.9	0.58
% of kcal from protein*	16.9 ± 3.0	18.4 ± 1.4	17.1 ± 2.3	0.25
% of kcal from fat*	31.7 ± 4.5	31.5 ± 2.1	30.8 ± 5.8	0.86
Folate (DFE/kg/d)	5.8 ± 1.6	6.3 ± 2.8	7.3 ± 2.1	0.21
Vitamin B_5 (mg/kg/d)	0.07 ±0.02	0.08 ± 0.05	0.09 ± 0.02	0.33
Vitamin B_6 (mg/kg/d)	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.35
Vitamin B_{12} (mcg/kg/d)	0.06 ± 0.03	0.07 ± 0.04	0.08 ± 0.03	0.16
Betaine (mg/kg/d)	0.7 ± 0.5	0.9 ± 1.1	1.0 ± 0.9	0.61
Cholesterol (mg/kg/d)	1.9 ± 0.4	4.0 ± 0.7	7.7 ± 1.1	<0.001

Data are presented as mean ± SD and include values from supplement. Low: choline intake of 6.2 ± 1.2 mg/kg lean mass/d. Med: choline intake of 8.1 ± 1.6 mg/kg lean/d. High: choline intake of 14.2 ± 3.0 mg/kg lean/d. * denotes values from participants' diet only. No differences were observed between choline groups except for cholesterol intake.

The mean choline consumption was 6.2 ± 1.2 mg/kg lean mass/d for Low group (~51% of AI), 8.1 ± 1.6 mg/kg lean/d for Med group (~68% of AI), and 14.2 ± 3.0

mg/kg lean/d for High group (~118% of AI). Choline intake from participants' own diets was 5.9 ± 2.2 mg/kg lean/d (Low: 5.5 ± 1.2 mg/kg lean/d, Med: 5.3 ± 1.6 mg/kg lean/d, and High: 6.7 ± 3.0 mg/kg lean/d, p= 0.20), and the supplement provided additional 0.7, 2.8, and 7.5 mg/kg lean/d of choline for Low, Med, and High groups, respectively. Choline intake (mg/kg lean/d) was significantly correlated with folate (DFE/kg lean/d, r=0.58, p<0.001), vitamin B_5 (mg/kg lean/d, r=0.52, p=0.001), vitamin B_6 (mg/kg lean/d, r=0.53, p<0.001), vitamin B_{12} (mcg/kg lean/d, r=0.59, p<0.001), and cholesterol (mg/kg lean/d, r=0.88, p<0.001) intakes.

RET Responses

RET resulted in significant increases in lean mass and strength from baseline in all three groups while only Low and Med groups lost significant body fat (**Tables 10 and 11**). However, there was no difference between groups in changes in lean or fat mass. Since there was no difference between men and women in muscle responses to RET (men vs. women; percent change in lean mass: 3.6 ± 2.0 vs. 3.6 ± 3.6, p=0.99; percent change in composite strength: 41.0 ± 38.8 vs. 33.7 ± 23.5, p=0.52), data were pooled for further analyses. Observed correlation coefficients (r) of percent change in composite strength were 0.29 (p=0.097) with choline (mg/kg lean/d) and 0.31 (p=0.07) with betaine (mg/kg lean/d). Percent change in lean mass was correlated with vitamin B_5 intake (mg/kg lean/d, r= 0.35, p=0.04) while choline consumption was not significantly correlated with lean mass gains with RET (r=0.25, p=0.14).

Table 10. The Effect of Choline Intakes on Body Composition

	Low (n=13)	Med (n=11)	High (n=13)	P values
Change in lean mass (kg)	1.6 ± 1.5	1.6 ± 1.5	1.7 ± 0.9	0.95
Percent change in lean mass	2.9 ± 3.3	3.9 ± 3.7	4.1 ± 2.3	0.58
Change in body fat (kg)	-0.8 ± 1.4	-1.2 ± 1.1	-0.6 ± 1.8	0.75
Percent change in body fat	-3.4 ± 5.8	-4.8 ± 5.0	-2.0 ± 6.7	0.55

Data are presented as mean ± SD. Low: choline intake of 6.2 ± 1.2 mg/kg lean mass/d. Med: choline intake of 8.1 ± 1.6 mg/kg lean/d. High: choline intake of 14.2 ± 3.0 mg/kg lean/d. All changes from baseline are statistically significant except for change in body fat in High group. No significant differences were observed between groups.

Since choline consumption was significantly correlated with folate, vitamin B_5, vitamin B_6, vitamin B_{12}, and cholesterol, and RET responses were associated with betaine and vitamin B_5, ANCOVA analyses using these and other potential confounders including age, lean mass, and other major dietary factors (e.g. protein intake) as covariates were conducted. The results showed a significant difference in percent change in composite strength between choline groups (**Figure 6**, p=0.034). Low choline group showed reduced composite strength gain (%) when compared with Med (p=0.035) or High (p=0.085) choline groups (Low: 19.4 ± 8.2%, Med: 46.8 ± 8.9%, High: 47.4 ± 8.1%), after adjusting for covariates. The covariates appearing in the final model were lean mass, protein, betaine, and vitamin B_{12}. The other potential confounders were

removed during the model selection/simplification processes due to their insignificant contribution to variability of percent gains in composite strength.

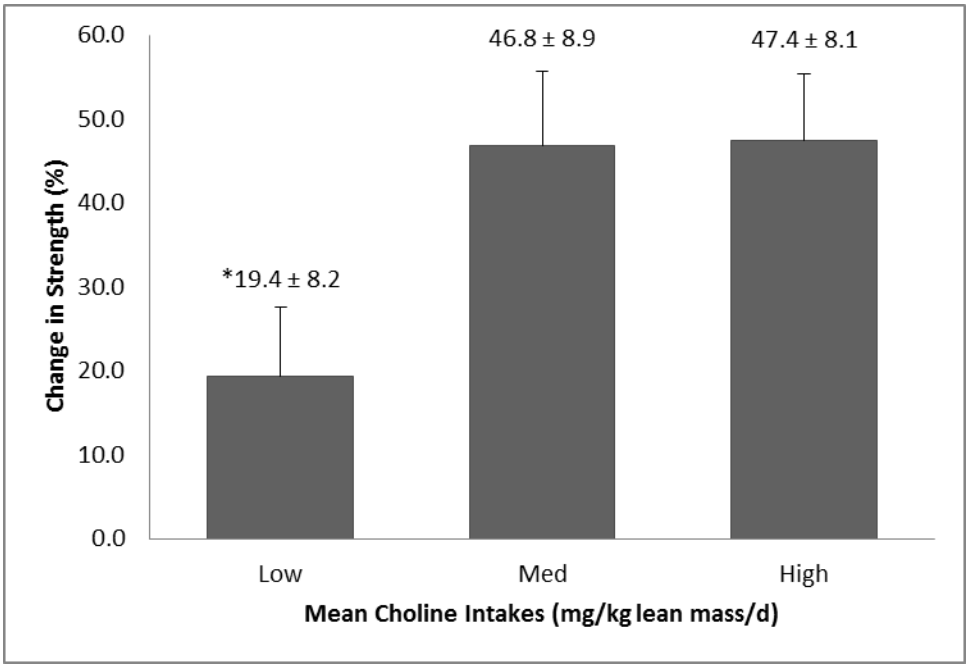

Figure 6. The Effect of Choline Intake on Change in Composite Strength
Data are presented as least squares mean ± standard error (SE). Low: choline intake of 6.2 ± 1.2 mg/kg lean mass/d. Med: choline intake of 8.1 ± 1.6 mg/kg lean/d. High: choline intake of 14.2 ± 3.0 mg/kg lean/d. Low group gained less strength when compared with Med (p=0.035) or High (p=0.085) groups. Composite strength is defined as chest press 1RM + leg press 1RM. Covariates appearing in the model: lean mass (kg), protein (g/kg lean/d), betaine (mg/kg lean/d), and vitamin B_{12} (mcg/kg lean/d). * denotes a significant difference from the other groups.

Table 11. The Effect of Choline Intakes on Changes (%) in 1RM

	Low (n=13)	Med (n=11)	High (n=13)	P values
Leg Press	25.3 ± 24.0	45.7 ± 40.2	50.3 ± 46.4	0.22
Chest Press	25.4 ± 13.4	23.0 ± 15.1	31.0 ± 24.0	0.55
Lat Pull Down	17.2 ± 13.3	23.1 ± 26.7	30.6 ± 23.7	0.30
Knee Extension	21.7 ± 23.0	14.8 ±30.3	37.5 ± 44.9	0.27
Leg Curls	20.9 ± 19.9	35.6 ± 25.0	36.0 ± 26.6	0.21
Triceps Extension	26.8 ± 17.6	31.0 ± 21.2	36.8 ± 27.5	0.53
Biceps Curls	41.4 ± 52.8	38.2 ± 26.0	36.8 ± 39.8	0.96

Data are presented as mean ± SD. Low: choline intake of 6.2 ± 1.2 mg/kg lean mass/d. Med: choline intake of 8.1 ± 1.6 mg/kg lean/d. High: choline intake of 14.2 ± 3.0 mg/kg lean/d. All changes from baseline are statistically significant, but no significant differences were observed between groups.

Since RET responses in Med and High groups were similar (**Figure 6, Table 11**), additional analyses were conducted after the Med and High groups were pooled. Independent t-test results showed significant differences between Low and Med-High groups in changes (%) in leg press 1RM (Low: 25.3 ± 24.0 vs. Med-High: 48.2 ± 42.8, $p<0.05$) and composite strength (Low: 129.9 ± 84.2 vs. Med-High: 182.1 ± 147.3, $p=0.05$). ANCOVA analysis using lean mass (kg), protein (g/kg lean/d), betaine (mg/kg lean/d), and vitamin B_{12} (mcg/kg lean/d) as covariates also showed that Low group had significantly diminished thigh muscle quality-strength (TMQ-S) improvements compared with Med-High group while differences in leg press and composite peak power were not statistically significant (**Table 12**).

Table 12. The Effect of Choline Intakes on Changes (%) in Peak Power and Thigh Muscle Quality

	Low (n=13)	Med-High (n=24)	P values
Leg Press Peak Power (W)	19.0 ± 13.5	30.0 ± 22.4	0.072
Composite Peak Power (W)	17.4 ± 12.5	25.5 ± 17.3	0.110
TMQ-S†	12.3 ± 9.6	46.4 ± 7.0	0.010

Data are presented as mean ± SD. Composite Peak Power: Leg press peak power + Chest press peak power. TMQ-S = leg press 1RM (kg) / total thigh lean mass for both legs (kg). All changes from baseline were statistically significant. † denotes ANCOVA analysis results presented as least squares mean ± standard error (SE). Covariates appearing in the model: lean mass (kg), protein (g/kg lean/d), betaine (mg/kg lean/d), and vitamin B_{12} (mcg/kg lean/d).

Multiple linear regression analyses were also conducted to evaluate independent association of dietary choline and other nutrients as well as aforementioned potential confounders. All variables were initially entered into the regression equation, and the variables were sequentially removed at each step with backward elimination method. The final model showed that Low choline intake independently predicted percent change in composite strength, with betaine intake, male gender, and lean mass remaining in the model (adjusted R^2=0.215, p=0.02, **Table 13**). Even though cholesterol was previously shown to be associated with strength and lean mass gains (69), it did not predict variability of strength gains in the present study.

Table 13. Multiple Regression Analysis of the Independent Effect of Low Choline Intake, Lean Mass, and Betaine Intake on Change (%) in Composite Strength Following 12 Weeks of RET

Predictors	Unstandardized Coefficients		P values
	β	SE	
(Intercept)	0.86	1.04	0.42
Low Choline Intake	-0.62	0.27	0.03
Male Gender	-0.89	0.50	0.09
Lean Mass (kg)	0.00006	0.00003	0.03
Betaine Intake (mg/kg/d)	0.23	0.11	0.04

SE: standard error. Low Choline Intake: choline consumption of 6.2 ± 1.2 mg/kg lean mass/d.

Blood Lipids and Liver Damage Markers

Since choline deficiency is reported to cause liver/muscle damage and altered lipoprotein/blood lipid metabolism, blood markers for liver damage (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and muscle damage (creatine kinase [CK]) and blood lipid profiles (triacylglycerol [TAG], total cholesterol, high density lipoprotein cholesterol [HDL-C], and low density lipoprotein cholesterol [LDL-C]) were also measured. The results showed no effect of choline intake on any of these clinical blood markers (**Table 14**).

Table 14. The Effect of Choline Intakes on Pre and Post RET Values of Select Blood Lipids and Enzymes

		Low (n=10)*	Med (n=10)*	High (n=10)*	P values†
ALT (U/L)	Pre	42.3 ± 8.6	32.6 ± 9.6	39.4 ± 24.9	0.36
	Post	36.5 ± 8.7	35.6 ± 14.9	37.2 ± 12.1	0.96
AST (U/L)	Pre	38.4 ± 11.1	28.7 ± 5.4	35.0 ± 16.5	0.17
	Post	33.4 ± 7.8	32.9 ± 9.2	31.2 ± 9.2	0.83
Total cholesterol	Pre	184.4 ± 25.7	193.9 ± 20.3	181.2 ± 31.3	0.53
(mg/dL)	Post	176.8 ± 15.1	206.3 ± 29.8	191.6 ± 36.4	0.08
HDL-C (mg/dL)	Pre	55.8 ± 10.7	55.2 ± 10.4	52.8 ± 12.4	0.81
	Post	53.2 ± 9.2	54.8 ± 9.5	53.9 ± 12.8	0.94
LDL-C (mg/dL)	Pre	107.8 ± 20.6	115.3 ± 20.1	104.0 ± 30.6	0.57
	Post	104.1 ± 16.2	127.0 ± 31.1	111.6 ± 33.4	0.18
TAG (mg/dL)	Pre	103.8 ± 34.6	117.1 ± 48.9	122.3 ± 78.6	0.73
	Post	97.1 ± 37.5	122.2 ± 62.0	131.4 ± 80.0	0.42
CK (U/L)*	Pre	119.0 ± 50.4	98.7 ± 47.9	128.1 ± 78.9	0.70
	Post	77.3 ± 25.4	85.8 ± 41.4	99.3 ± 29.3	0.54

Data are presented as mean ± SD. *: Sample sizes are reduced due to blood sample availability (n's for CK: Low=4, Med=6, High=8; CK was measured in blood samples taken 48 hours after the 1st and last exercise sessions of RET). Low: choline intake of 6.2 ± 1.2 mg/kg lean mass/d. Med: choline intake of 8.1 ± 1.6 mg/kg lean/d. High: choline intake of 14.2 ± 3.0 mg/kg lean/d. ALT: alanine aminotransferase, AST: aspartate aminotransferase, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TAG: triacylglycerol. CK: creatine kinase. † denotes p values for between group comparisons. There was no difference between or within groups in any of the blood marker concentrations.

Discussion

The purpose of the present study was to examine the effects of choline supplementation in the form of egg yolk (approximately zero, one, or three additional egg yolks per day) on strength gains and lean mass changes with 12 weeks of RET in older men and women. The major finding of the present study was that low choline intake (~50% of AI) negatively affects strength gains with 12 weeks of RET in 50 to 69 year old individuals, which is consistent with our other findings (76). Our data also suggest that choline intake greater than AI may not provide additional positive effect on RET responses.

There have been several studies that examined the effect of choline supplementation on exercise performance, and the results showed that choline consumption above AI did not positively affect exercise performance. However, these studies utilized acute bouts of aerobic/endurance exercise protocols, thus direct comparison to our study is somewhat difficult (14-17). One study that examined the effect of chronic choline supplementation on performance outcomes utilizing an anaerobic exercise protocol (18) had college students perform a four minute reaction test, a 30 minute Wingate Anaerobic Power test, and maximum number of push-ups and sit-ups for one minute after taking ~50mg of choline or placebo. The subjects repeated the same tests after four weeks of the supplement/placebo consumption, and the results showed no acute or chronic effect of choline supplementation on exercise performance. However, since the exercise loads used for testing were very light, there was no nutritional control, and the amount of choline in the supplement was small and mixed

with other ingredients such as caffeine, it is difficult to interpret their results as related to RET responses.

Choline may affect muscle responses to RET through one or many potential mechanisms. Since choline is a precursor to a neurotransmitter, ACh, which relays a signal from motor neurons to skeletal muscle to contract and generate force, and of which synthesis is reported to be affected by availability of choline (4, 5), insufficient choline consumption may limit the availability of ACh at neuromuscular junction (NMJ) and in turn, muscle contraction and force generation. ACh is synthesized in cholinergic nerves by choline acetyltransferase (ChAT) using acetyl-CoA and choline as substrates. After it is released into the synaptic cleft and binds to ACh receptors on the muscle membrane, it is broken down to acetate and choline by ACh esterase. Choline is then taken up by choline transporter proteins on nerve cells and recycled to re-synthesize ACh by ChAT.

Crockett et al. (78) reported that ChAT activity is inversely related to the size of the muscle fiber and positively related to muscle fiber's resistance to fatigue. Since fast twitch (FT) muscle fibers are in general larger in size and less resistant to fatigue, ChAT activity may be lower in FT fibers, and FT fibers may have less ability to recycle choline and re-synthesize ACh, making them more sensitive to and reliant on choline supplied by the circulation (eventually from diet), compared with slow twitch (ST) fibers. Since FT fibers are mostly recruited and utilized with RE, if choline affects RET responses via ACh, this may explain, at least in part, why choline intake may influence muscle responses to endurance exercise and RE differently. Moreover, Herscovich and Gershon

(79) reported that aging decreases activities of ChAT, suggesting that the importance of sufficient choline intake may be amplified in the older population.

Choline can also influence methylation reactions. A portion of choline is irreversibly converted to betaine, which is used to convert homocysteine to methionine, which is then used to generate *S*-adenosyl methionine (SAM), a universal methyl group donor. Therefore, choline and its metabolite betaine may affect methylation, which plays crucial roles in lipid synthesis, epigenetic control of gene expression/protein synthesis, and regulation of many other metabolic pathways (10, 28).

Since betaine, via SAM, may contribute to synthesis of creatine, and is an important osmolyte maintaining fluid balance, studies have been conducted to examine the effect of betaine ingestion on exercise performance and body composition. The results generally showed positive effects of betaine on performance and body composition, with some inconsistencies (80). For example, body composition was favorably affected, and training volume was increased with six weeks of betaine supplementation in Cholewa et al.'s study (81) whereas 10 days of betaine supplementation did not affect muscle creatine content, or 1RM/power of bench press/squat in another study (82). Also, Hoffman et al. (83) reported 15 days of betaine supplementation did not increase peak force on isokinetic chest press in college-aged men while Lee et al. (84) reported 14 days of betaine supplementation resulted in increased vertical jump power and isometric squat force. In the present study, together with choline, betaine was independently associated with change (%) in composite

strength suggesting multiple mechanisms are at work. Future studies are warranted to investigate the independent roles that choline and betaine each plays on RET responses.

Choline is also a precursor to PC, the predominant form of phospholipid in all cell membranes. PC is also a precursor to many other components in membranes including sphingomyelin and phosphatidylserine. Therefore, choline contributes to the stability and integrity of cell membranes, and choline deficiency results in weakened cell membranes and leakage of intracellular enzymes to the circulation. da Costa et al. (6) reported that blood levels of creatine kinase (CK), a muscle cell damage marker, increased up to 66 fold with severely low (<10% of AI) choline intake. Insufficient choline consumption and the resultant decrease in cytidine diphosphocholine (CDP-choline) pathway (which makes PC from dietary choline) can also lead to perturbation of PC homeostasis and induce cell death (85). Therefore, choline deficient diets may result in weak and compromised cell membrane/structure, which may negatively affect skeletal muscle's ability to withstand mechanical stresses imposed by exercise, especially during RET.

However, we did not observe negative effects of low choline consumption on plasma CK concentrations. This may be explained by the difference in the amount of choline intakes between da Costa et al.'s (6) study (<10% of AI) and the present study (Low group: ~50% of AI). It appears that only severely low choline intake may induce muscle cell membrane damage and leakage of CK into the circulation. The similar results were observed with liver damage markers. In the present study, there was no effect of low choline intake on blood concentrations of ALT and AST, suggesting that

only severely deficient choline consumption may result in release of those enzymes. However, hepatic steatosis was observed in a previous study (38) without leakage of ALT or AST in many subjects in response to a choline deficient diet, suggesting that liver dysfunction can occur even without increases in intracellular enzyme concentrations in the blood. It remains to be determined whether moderately low choline intake (as in the present study) can subtly compromise and weaken membrane integrity negatively affecting muscle functions while still not allowing leakage of intracellular enzymes into the circulation.

We have also examined the effect of choline intake on blood lipid profiles, since total blood cholesterol and LDL-C were previously reported to be positively associated with lean mass gains (69), and dietary choline influences synthesis of lipoproteins that transport cholesterol and fat in the circulation (24). However, there was no effect of choline consumption on any of the blood lipids and lipoproteins, indicating that moderately low choline intake does not negatively affect blood lipid profiles.

In addition, while dietary cholesterol was previously reported to be associated with lean mass and strength gains with RET in older adults (69), it did not contribute much to the variability of strength gains in the present study. The reason for this discrepancy is unclear, but it can be speculated that the results of the previous study may have been confounded by inability to separate choline's effect from that of cholesterol because information on choline content of foods was not widely available when the previous study was conducted. Since many choline rich foods are also rich in cholesterol, future studies may need separate administration of choline and cholesterol to

examine the independent roles that choline and cholesterol each plays on skeletal muscle responses to RET.

Our study has some limitations including the inability to determine mechanisms through which choline may affect RET responses. Since we have consistently observed effects of dietary choline (especially the negative effects of low choline intake [~50% of AI]) on muscle responses to RET, future studies should be focused on elucidating the mechanism(s) of choline's effect. Also, the well-known tendency to under-report food intake associated with diet logs may have obscured the accuracy of our data (86). However, we believe that, compared with the three-to-four-day food records commonly used in nutrition studies or the seven-day weighed food records which are considered as the best method currently, >48 day food records (at least four days of diet records/week for the entire 12 weeks) we required from our participants have minimized the issues related to inaccurate reporting of food intake. We also expect that error associated with this method would be consistent across choline groups.

Summary

Our data suggest that low choline intake (~50% of AI) for 12 weeks may impair strength gains with RET in 50-69 year old generally healthy individuals, that is consistent now in two studies, whereas higher choline intake (greater than AI) may not have additional benefits on strength gains. However, choline intake did not affect change in lean mass in the present study, which is inconsistent with our other findings. It

appears that having inadequate dietary choline may have a negative effect on strength gains but subtle or no effect on lean mass changes.

CHAPTER V

THE EFFECTS OF 3 WEEKS OF RESISTANCE EXERCISE AND CHOLINE SUPPLEMENTATION ON EMG AMPLITUDE AND STRENGTH RESPONSES IN 50-65 YEAR OLDS: A RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL

Introduction

Choline is an essential nutrient playing important roles in many physiological processes including membrane formation/signaling, lipid transport, methylation, reduction of homocysteine, and brain/memory development (29). Choline is also a precursor to acetylcholine (ACh), the main neurotransmitter of α-motor neurons, which mediates muscle contraction and force generation. Therefore, dietary choline may affect exercise performance through availability of ACh at the neuromuscular junction (NMJ) and/or other mechanisms listed above. Even though a limited number of studies examined the relationship between choline and exercise performance, the results were equivocal, perhaps because previous studies lacked control of dietary choline intake and only examined the effects associated with endurance exercise (14-17). Considering the difference between endurance and resistance exercises (RE) in muscle fiber recruitment and nerve firing patterns (i.e., low vs. high frequency neural activation rate, respectively), it is reasonable to hypothesize that dietary choline may influence muscle responses to the two different exercise modes differently.

ACh transmits action potentials from motor neurons to skeletal muscle through

NMJ (87). When action potentials travel to skeletal muscle via ACh, electrical waves are formed along the surface of muscle fibers (30), and these electrical activities can be measured by electrodes placed on the skin surface enclosing the activated muscle fibers (88). The surface electromyography is a non-invasive method to detect electrical activities of skeletal muscle, and surface electromyogram (EMG) represents global electrical activities of motor unit (MU). The amplitude of surface EMG reflects the net MU activity which consists of recruitment and rate coding of active MUs (88), and EMG can be used as an indirect tool to examine the activities of motor neurons, which are mediated by ACh.

Any change in EMG amplitude can be interpreted as changes in motor neuron/NMJ functions or membrane conductivity. However, Warren et al. (71) reported that membrane conductivity was not impaired even after muscle damaging, high volume eccentric contractions. They also reported that EMG amplitude was reduced by 59% when 3 mg/kg of succinylcholine (a competitive inhibitor of ACh) and 0.4 mg/kg of tubocurarine (antagonist of ACh receptor) were administered to partially block ACh at the NMJ in mice (71). In addition, Suzuki et al. (72) observed a marked decrease in EMG amplitude after administration of vesamicol hydrochloride which inhibits ACh release from the motor nerve terminals in cats. Therefore, EMG amplitude can represent ACh status at the NMJ, and if dietary choline affects muscle responses through ACh availability, it can be reflected on EMG amplitudes.

We have previously reported that 12 weeks of low choline intake (~50% of Adequate Intake [AI]) resulted in diminished strength and/or lean mass gains in older

men and women in response to resistance exercise training (RET) in two separate study populations (76, 89). However, it is unclear whether low choline consumption for a shorter period of time (<one month) still has negative effects on muscle responses to RE. Also unclear is whether ACh availability is the main mechanism through which dietary choline affects skeletal muscle function and exercise performance.

The purpose of the present study was to determine whether varying levels of choline consumption for less than one month have any effects on changes in neuromuscular activation and strength in response to RE in older adults. We also measured clinical markers of liver/muscle damage and blood lipids since choline deficiency was reported to cause liver/muscle damage and perturbation of lipid metabolism (29). We hypothesized that 3 weeks of moderately low choline consumption would result in reduced neuromuscular activation (EMG amplitudes), isometric force production, and strength gains compared with higher choline intake, without affecting liver/muscle damage markers and/or blood lipid profiles.

Methods

Participants

Thirty-one, generally healthy, previously untrained, community dwelling, 50-65 year old males and females (post-menopausal for more than a year), recruited through emails, flyers, and newspaper advertisement, underwent 3 weeks of diet/RE intervention. Individuals with following conditions were excluded: resting blood pressure of >160/100 mmHg, cardiac arrhythmias, hernia, aortic aneurysm, kidney disease, lung disease,

smoking habit, diabetes, participation in RET program of one hour or more per week in the previous 12 months. All participants provided written informed consent prior to participation, and the study protocol was approved by Texas A&M University Institutional Review Board.

Pre-Study Orientation

Prior to the start of the study, participants had one week of an orientation period during which they attended two sessions of nutrition education and exercise familiarization. Participants learned about optimal nutrient intake, serving size/portion control, study specific diet requirements, general information about RE, and proper forms/techniques of RE. They also became familiarized with the exercises to be performed during the study, by practicing the techniques with light resistance (approximately 40% of estimated maximum strength [1RM] based on the Omnibus-RE Scale [OMNI-RES] ratings of perceived exertion [RPE]) (77). The purpose of the exercise orientation was to reduce the possibility of injury and standardize strength measures by inducing rapid motor learning.

Nutritional Control

Following the orientation, participants started recording their daily food intakes using nutrition software (Nutribase; version 10; Client Intake Module; Cybersoft Inc., Phoenix, AZ). Body composition was measured with dual energy X-ray absorptiometry (DEXA; Lunar Prodigy, General Electric, Fairfield, CT), and the participants were

randomly assigned to one of three choline groups (Low: 0.7 mg/kg/d, Med: 3.2 mg/kg/d, and High: 5.7 mg/kg/d of supplemental choline, respectively) in a double-blind manner and underwent three weeks of diet intervention. During the intervention, the participants' choline consumption from their own diet was limited to approximately 3 mg/kg/day (~43% of AI) by limiting choline rich foods such as egg yolk, liver, organ meat, etc. (90), and each group's choline requirement was met with daily supplementation of choline bitartrate powder (Prescribed For Life, Fredericksburg, TX), mixed in a protein drink (Boost High Protein; Nestle S.A., Vevey, Switzerland) which additionally provided 0.2 g/kg/d of protein, 0.44 g/kg/d of carbohydrate, 0.08 g/kg/d of fat, and 0.7 g/kg/d of choline equally for all participants. Foods high in betaine such as spinach, beet, and wheat bran/germs were also limited in order to minimize potential effects of betaine intake on choline availability (24, 90).

Each participant's daily caloric goal was calculated with Mifflin-St Jeor equation (91), and the participants were instructed to consume 50% of total energy from carbohydrate, 30% from fat, 20% from protein, and <10% from saturated fat; and to consume >1.0 g/kg/d of protein, as recommended by the Academy of Nutrition and Dietetics. Participants were required to maintain and submit daily diet logs during the entire period of the study, and feedback was provided weekly to ensure participants' compliance to the study specific diet guidelines.

Resistance Exercise

The participants performed four bouts (on days 10, 12, 17, and 19; **Figure 7**) of high intensity leg press and leg extension exercises with emphasis on eccentric contractions on Keiser 300 pneumatic exercise equipment (Keiser, Palo Alto, CA). The exercises were performed after a 10-minute warm-up on a cycle ergometer (Schwinn Fitness, Inc., Denver, CO), and the participants performed three sets of each exercise with 8-12 repetitions per set, with resistance set at 75% of 1RM. When a participant achieved 12 repetitions on all three sets of an exercise, the resistance was increased so that only eight repetitions would be completed in the next exercise session. The rest between each set was one minute, and the rest between the exercises was two minutes. All the exercise sessions were supervised by Exercise Physiology graduate students, and the participants were instructed not to perform any RE outside of the study program but to maintain their usual non-resistance type activities at the current level.

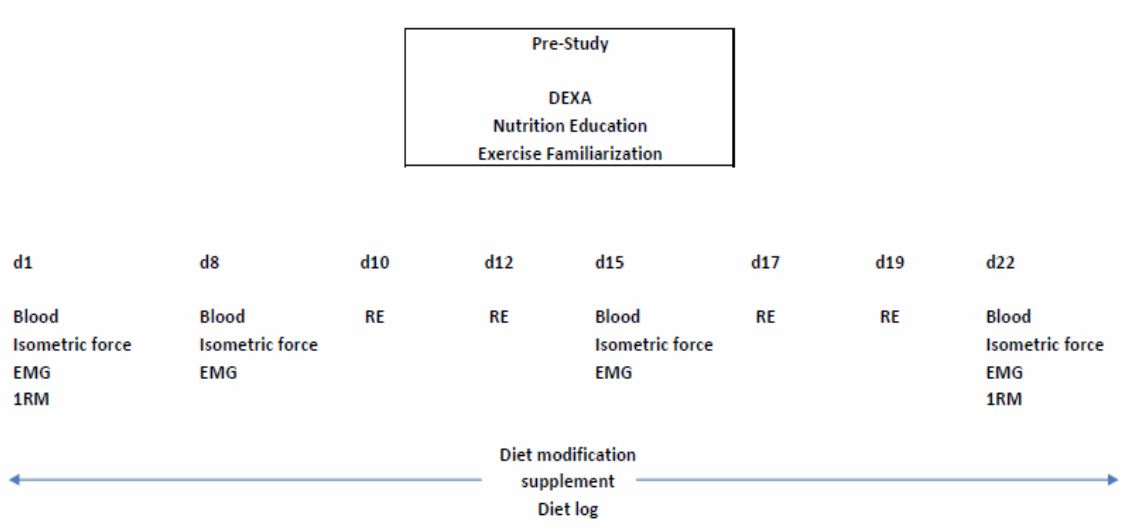

Figure 7. Study Timeline
EMG: surface electromyogram on rectus femoris muscles. 1RM: maximum strength tests of leg press and leg extension. RE: resistance exercises (leg press and leg extension; 8-12 reps, 3 sets for each exercise, 75% of 1RM).

Testing

1RM tests on leg press and leg extension exercises were conducted on the first (d1; 9 days prior to the first RE session) and the last (d22; 72 hours after the last RE session) days of the study using a standard method (77) by progressively increasing exercise weight until the maximum resistance was reached at which only one repetition was successfully completed with proper form throughout the entire range of motion. The details of 1RM procedure were described elsewhere (89), and 1RM test was performed in a manner that the total number of 1RM attempts was minimized. Blood samples were collected from antecubital veins using 10 ml Vacutainer tubes four times throughout the study (on days 1, 8, 15, and 22), and complete metabolic panels were run at St. Joseph

Regional Health Center's CDC certified laboratory (Bryan, TX) to measure muscle/liver damage markers and lipid profiles in blood.

Four times over the three weeks of the study (on days 1, 8, 15, and 22; **Figure 7**), maximal isometric force (MIF) outputs were measured using Kin-Com 125 AP dynamometer (Isokinetic International; Chattanooga, TN) at four different angles (starting at 90°, then 75°, 60°, and 45°; 0° is full extension) of knee extension with participants' dominant legs. Participants were instructed to maximally contract their quadriceps muscles exerting 100% of force and maintain the maximal contraction for five seconds at each angle. The rest between angles was 10 seconds, and all participants conducted the tests in the same order of the knee angles. Peak force and average force was assessed during the MIF knee extension tests, and feedback to participants was displayed on the monitor screen as a time-force curve (on X and Y axis respectively) during the tests.

To monitor neural/muscle activation status, a surface EMG was obtained with 3M Red Dot self-adhering sticky gel Ag/AgCl monitoring electrodes (3M; St. Paul, MN) attached to participants' rectus femoris muscles during the MIF tests. Prior to placement of the electrodes, participants' skin was shaved as needed, cleaned with alcohol wipes containing 70% isopropyl alcohol to remove oily residue, and gently abraded with 3M Red Dot Trace Prep abrasive tape (3M; St. Paul, MN) to reduce skin impedance and enhance trace quality. Two electrodes were placed at the mid-point on the line connecting the anterior superior iliac spine and the superior center of the patella, in the longitudinal direction of the rectus femoris muscle. The electrode size was 8 mm, inter-

electrode distance was 5 cm, and reference (earth) electrode was placed around the ankle. The EMG signal was amplified, band pass filtered (10-450 Hz), sampled at 1,000 Hz, and processed using FE132 bio-amp, PowerLab data acquisition system, and LabChart Pro software (version 7; ADInstruments; Colorado Springs, CO). All EMG data are expressed as root mean square (RMS) normalized to the baseline values obtained during MIF test on day 1 for comparison within and between choline groups. EMG procedures were performed following the guidelines by International Society of Electrophysiology and Kinesiology (ISEK) (92) and Surface EMG for the Non-Invasive Assessment of Muscles (SENIAM) (93).

Data Analysis

All statistical analyses were conducted using IBM SPSS Statistics software (version 23; IBM Corporation, Armonk, NY). The mean intake values of all macro/micro nutrients were calculated from the 21 daily diet logs that were entered into Nutribase computer software (version 10; Cybersoft Inc., Phoenix, AZ), or using the USDA database for choline (44). Student's independent t-tests were used to compare means of two different groups (e.g., gender), and paired t-tests were performed to examine the difference between pre- and post-study values. The Pearson correlation was used to examine associations between nutrient intakes and muscle responses to RE.

The differences between and/or within choline groups were analyzed by one-way analysis of variance (ANOVA) and two-way repeated measure ANOVA (group x time). The assumptions of equal variance and sphericity were checked using Levene's and

Mauchly's tests respectively, and the Bonferroni method was used to perform pairwise comparisons. When the equal variance assumption was not met, Welch's variance-weighted ANOVA test was performed. Analysis of covariance (ANCOVA) tests were conducted to examine the effect of dietary choline controlling for the effects of potential confounders (other nutrients, gender, age, and/or lean mass, etc.) on muscle responses. Percent change was calculated as 100 x (post-study measurement - pre-study measurement) / pre-study measurement. P values of <0.05 were considered statistically significant, and data are presented as mean ± standard deviation (SD) unless stated otherwise.

Results

Participants

The baseline characteristics of the 31 participants who completed the study are presented in **Table 15**. There were no significant differences in age, gender distribution, height, body weight, lean mass, or body fat percentage between choline groups. Also, there were no significant differences between genders in age and adiposity while men were taller and had more lean and total body mass compared with women (data not shown).

Table 15. Participant's Baseline Characteristics

	Low (n=10)	Med (n=11)	High (n=10)	P values
Age (years)	59.2 ± 4.1	59.0 ± 5.0	59.2 ± 4.3	0.993
Male/Female	4/6	2/9	4/6	0.459
Height (inches)	68.2 ± 3.3	66.4 ± 3.6	67.5 ± 5.1	0.581
Weight (kg)	80.5 ± 35.9	80.8 ± 16.2	81.6 ± 24.7	0.995
Lean mass (kg)	50.0 ± 17.8	46.0 ± 9.3	51.4 ± 16.0	0.610
Body fat (%)	33.0 ± 10.2	40.0 ± 9.0	34.0 ± 9.1	0.195

Data are presented as mean ± SD. Low: choline intake of 3.6 ± 0.6 mg/kg/d. Med: choline intake of 6.0 ± 0.6 mg/kg/d. High: choline intake of 8.8 ± 0.8 mg/kg/d. No differences were observed between choline groups.

Nutritional Intakes

Participants successfully met all the study specific requirements for nutritional intake. During the three weeks of the study, they consumed 25 kcal/kg/d of calories, 3.2 g/kg/d of carbohydrate, 1.2 g/kg/d of protein, and 0.9 g/kg/d of fat on average, and there was no difference in nutrient intake between choline groups except for cholesterol (**Table 16**). Participants' consumption of the macro/micro nutrients met or exceeded nutritional recommendations (Dietary Reference Intakes [DRI]), except for folate (consumed 82% of DRI) and betaine (DRI has not been set) (94).

Table 16. Nutritional Intakes

	Low (n=10)	Med (n=11)	High (n=10)	P values
Total Energy (kcal/kg/d)	27.1 ± 7.6	24.0 ± 3.7	24.0 ± 5.4	0.372
Carbohydrate (g/kg/d)	3.4 ± 0.9	3.0 ± 0.5	3.1 ± 0.9	0.547
Protein (g/kg/d)	1.2 ± 0.3	1.2 ± 0.2	1.1 ± 0.2	0.754
Fat (g/kg/d)	0.9 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	0.588
Folate (DFE/kg/d)	4.2 ± 2.5	4.0 ± 1.7	3.9 ± 1.9	0.925
Vitamin B_5 (mg/kg/d)	0.07 ± 0.04	0.07 ± 0.02	0.07 ± 0.02	0.932
Vitamin B_6 (mg/kg/d)	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.278
Vitamin B_{12} (mcg/kg/d)	0.09 ± 0.07	0.06 ± 0.02	0.07 ± 0.03	0.279
Betaine (mg/kg/d)	0.7 ± 0.2	0.7 ± 0.1	0.9 ± 0.8	0.601
Cholesterol (mg/kg/d)	2.3 ± 0.6	2.1 ± 0.4	1.7 ± 0.3*	0.026

Data are presented as mean ± SD and include values from diet and supplement. Low: choline intake of 3.6 ± 0.6 mg/kg/d. Med: choline intake of 6.0 ± 0.6 mg/kg/d. High: choline intake of 8.8 ± 0.8 mg/kg/d. No differences were observed between choline groups except for cholesterol intake. * denotes a statistically significant difference from Low group ($p<0.05$).

The average choline consumption was 3.6 ± 0.6 mg/kg/d for Low group (~51% of AI), 6.0 ± 0.6 mg/kg/d for Med group (~86% of AI), and 8.8 ± 0.8 mg/kg/d for High group (~126% of AI). The mean choline intake from the participants' own diets was 2.9 ± 0.6 mg/kg/d (Low: 2.9 ± 0.6 mg/kg/d, Med: 2.8 ± 0.6 mg/kg lean/d, and High: 3.1 ± 0.8 mg/kg lean/d, p= 0.20), and the supplement provided additional 0.7, 3.2, and 5.7 mg/kg/d of choline for the Low, Med, and High groups, respectively.

Maximum Strength

Four sessions of leg press/leg extension resulted in significant increases in strength from baseline in all three groups except for leg extension in the High group (**Table 17**). However, there was no difference between choline groups in changes in 1RMs. Since there was no difference between men and women in strength responses (men vs. women; percent change in leg press 1RM: 13.0 ± 14.6 vs. 16.4 ± 8.9, p=0.514; percent change in leg extension 1RM: 9.5 ± 17.3 vs. 14.0 ± 14.6, p=0.385) and nutrient intakes (data not shown), they were pooled for further analyses.

No nutrient was correlated with changes (%) in leg press or leg extension 1RM while percent change in leg extension 1RM was correlated with percent changes in isometric knee extension peak force at 90º (r=0.415, p=0.032) and 60º (r=0.524, p=0.004). ANCOVA tests using various potential confounders (e.g., age, gender, lean mass, and/or other nutrients including cholesterol and folate) as covariates also showed no difference between choline groups in changes in leg press or leg extension 1RMs (data not shown).

Table 17. The Effect of Choline Intakes on Changes in 1RM

	Low (n=10)	Med (n=10)§	High (n=10)	P values
Leg Press (kg)	62.6 ± 81.0	87.8 ± 54.0	91.0 ± 74.6	0.622
Leg Press (%Δ)	12.4 ± 12.0	17.5 ± 10.7	15.8 ± 10.7	0.588
Leg Extension (kg)	15.6 ± 13.9	9.5 ± 5.4	4.2 ± 13.5§†	0.117
Leg Extension (%Δ)	17.9 ± 13.0	15.3 ± 18.8	5.9 ± 12.2§†	0.209

Data are presented as mean ± SD. Low: choline intake of 3.6 ± 0.6 mg/kg/d. Med: choline intake of 6.0 ± 0.6 mg/kg/d. High: choline intake of 8.8 ± 0.8 mg/kg/d. §: Sample sizes are reduced due to data availability. N's for Leg Extension: High=9. All changes from baseline are statistically significant except for † (not significant). No significant differences were observed between groups.

Isometric Forces and EMG Amplitudes

There was no significant effect of time, choline group, or time x group interaction on average or peak force outputs at four different knee angles except for 45º where there were significant time effects between days on both of the force outputs ($p<0.01$) (**Figures 8 and 9**). Similarly, no differences were observed in EMG amplitudes within or between choline groups except for 75º where there was a significant linear effect of time between days ($p=0.018$) (**Figure 10**). There was no effect of group x time interaction on EMG amplitudes.

Average and peak isometric forces were highly correlated with EMG amplitudes at each respective knee angle and time point ($r=0.40$ to 0.90, $p=0.027$ to <0.001). ANCOVA analyses also did not show any effects of choline on isometric forces or EMG amplitudes after controlling for various potential confounders such as age, gender, lean

mass, and/or other nutrients that may influence choline and ACh metabolism such as vitamin B_5 (pantothenic acid, the backbone of acetyl-CoA which is used for synthesis of ACh from choline), folate, and betaine (data not shown).

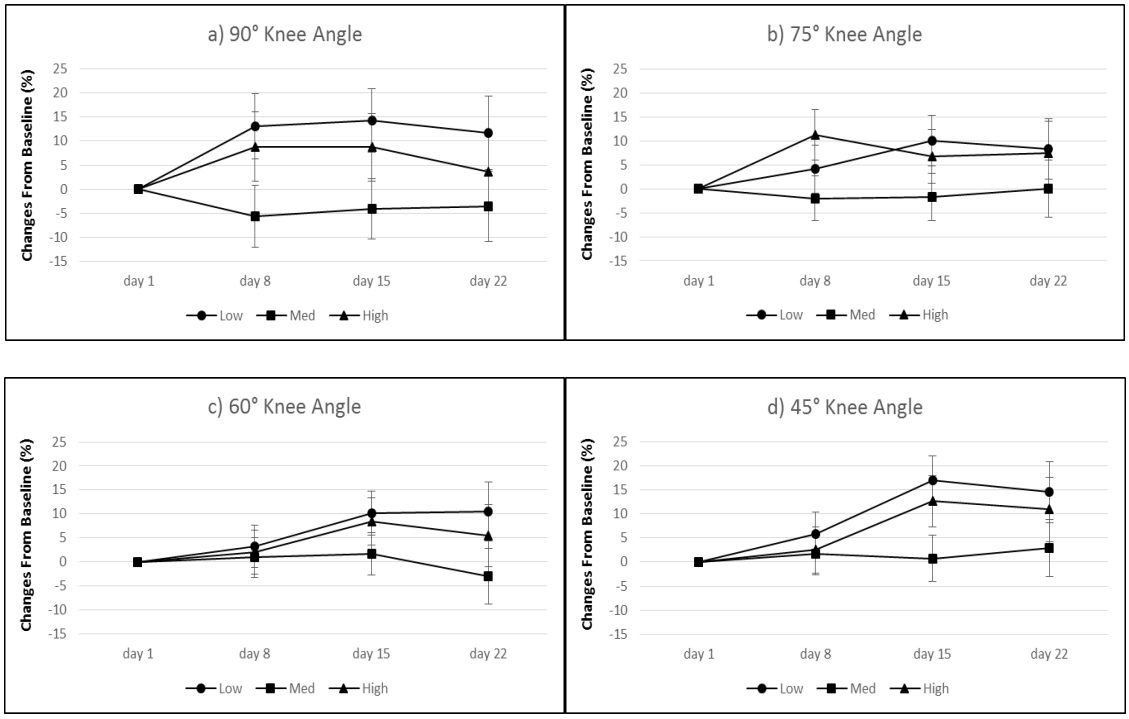

Figure 8. The Effect of Choline Intake on Changes (%) in Average Isometric Forces
Data are presented as mean ± standard error (SE). Low: choline intake of 3.6 ± 0.6 mg/kg/d. Med: choline intake of 6.0 ± 0.6 mg/kg/d. High: choline intake of 8.8 ± 0.8 mg/kg/d. No significant differences were observed within or between groups except for 45º where there was a significant time effect between days (p=0.004).

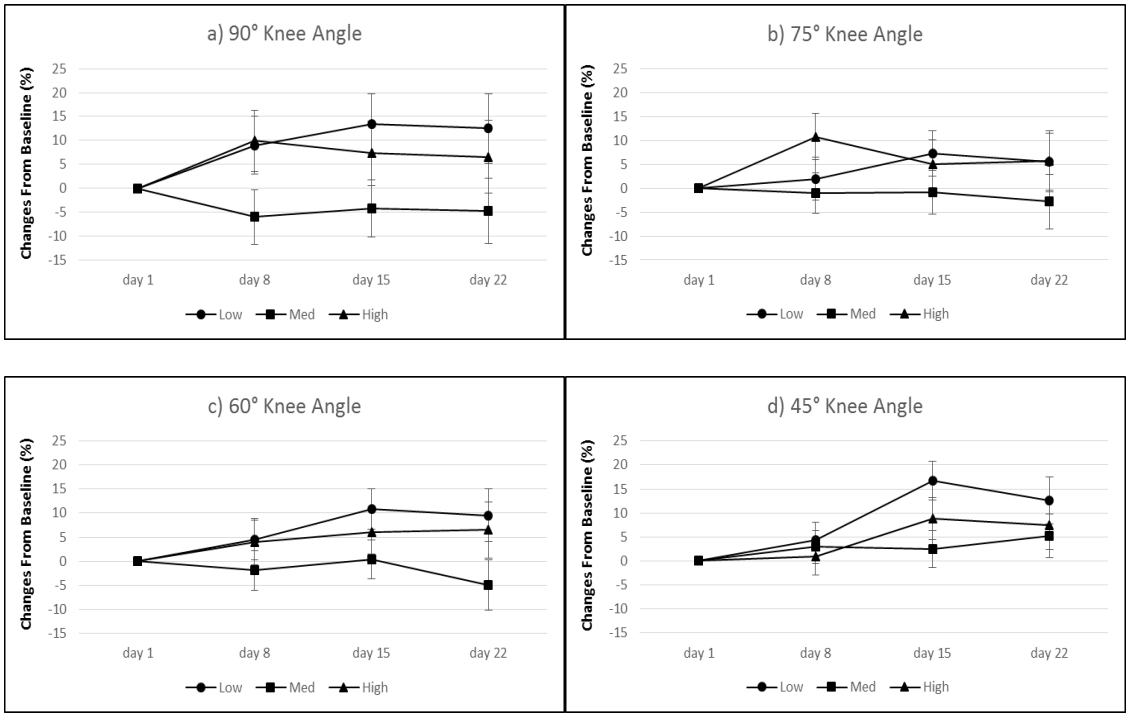

Figure 9. The Effect of Choline Intake on Changes (%) in Peak Isometric Forces
Data are presented as mean ± standard error (SE). Low: choline intake of 3.6 ± 0.6 mg/kg/d. Med: choline intake of 6.0 ± 0.6 mg/kg/d. High: choline intake of 8.8 ± 0.8 mg/kg/d. No significant differences were observed within or between groups except for 45º where there was a significant time effect between days (p=0.001).

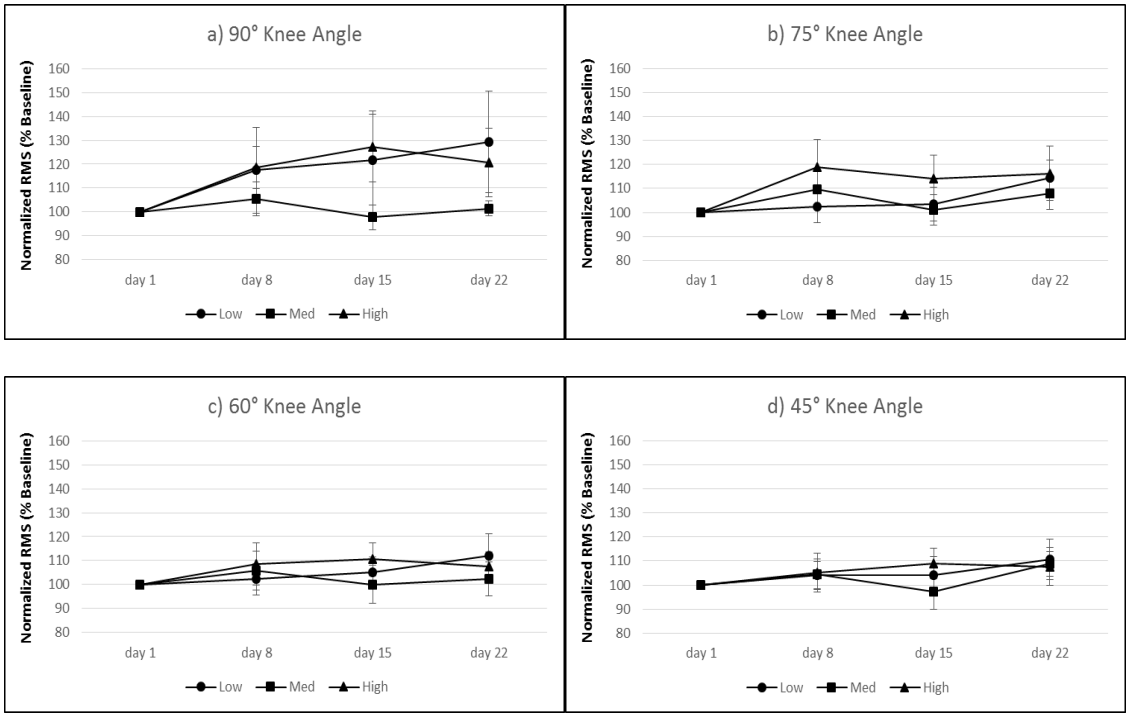

Figure 10. The Effect of Choline Intake on Changes in EMG Amplitudes
Data are presented as mean ± standard error (SE) of RMS values normalized for baseline. Low: choline intake of 3.6 ± 0.6 mg/kg/d. Med: choline intake of 6.0 ± 0.6 mg/kg/d. High: choline intake of 8.8 ± 0.8 mg/kg/d. No significant differences were observed within or between groups except for 75º where there was a significant effect of time between days (p=0.018).

Blood Lipids and Liver Damage Markers

Since choline deficiency (<10% of AI) results in liver/muscle damage and altered lipoprotein/blood lipid profiles, blood concentrations of clinical markers of liver damage (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and muscle damage (creatine kinase [CK]) and blood lipids (triacylglycerol [TAG], total cholesterol, high density lipoprotein cholesterol [HDL-C], and low density lipoprotein cholesterol

[LDL-C]) were measured. The results showed no effect of choline intake on any of these clinical blood markers (**Table 18**).

Table 18. The Effect of Choline Intakes on Pre and Post Study Values of Select Blood Lipids and Enzymes

		Low (n=10)	Med (n=11)	High (n=10)	P values†
ALT (U/L)	Pre	20.0 ± 8.3	18.9 ± 4.6	20.9 ± 5.7	0.773
	Post	20.0 ± 7.7	19.8 ± 5.1	22.9 ± 6.8	0.506
AST (U/L)	Pre	20.5 ± 4.9	21.4 ± 4.4	22.2 ± 4.5	0.712
	Post	21.2 ± 4.3	20.6 ± 4.3	22.0 ± 2.8	0.690
Total cholesterol	Pre	205.0 ± 40.0	220.7 ± 29.2	193.8 ± 41.0	0.258
(mg/dL)	Post	195.2 ± 32.5*	218.6 ± 25.2	203.0 ± 32.3	0.209
HDL-C (mg/dL)	Pre	57.5 ± 19.5	60.6 ± 13.1	53.4 ± 11.7	0.561
	Post	56.3 ± 17.2	58.9 ± 10.5	52.8 ± 12.4	0.592
LDL-C (mg/dL)§	Pre	121.0 ± 35.1	141.8 ± 26.0	118.9 ± 34.9	0.211
	Post	113.0 ± 28.0	138.5 ± 24.5	121.8 ± 33.4	0.149
TAG (mg/dL)§	Pre	119.6 ± 65.6	91.8 ± 42.2	107.4 ± 40.0	0.466
	Post	126.1 ± 94.0	106.2 ± 48.9*	142.6 ± 68.0	0.509
CK (U/L)§	Pre	77.4 ± 29.7	94.1 ± 52.5	93.3 ± 43.1	0.625
	Post	88.9 ± 24.4	79.0 ± 14.8	97.9 ± 43.9	0.352

Data are presented as mean ± SD. There was no difference between or within groups in the blood marker concentrations except for total cholesterol in Low group and TAG in Med group. † denotes p values for between group comparison.* denotes significant difference from pre-study values ($p<0.05$). §: n=9 for Low group for LDL-C and TAG, and n=10 and n=9 respectively for Med and High groups for CK due to blood sample availability.

Discussion

The purpose of this study was to evaluate the effects of choline intakes of ~51%, ~86%, and ~126% of AI for three weeks on neuromuscular responses to four sessions of lower body RE in 50-65 year old generally healthy individuals, with a randomized double-blind design. Based on our previous findings (76, 89) where lower choline intake (~50% of AI) negatively influenced strength and/or lean mass gains after 12 weeks of RET, with no additional positive effects of higher choline consumption (~118% of AI), it was hypothesized that low choline group would show diminished neuromuscular activation, isometric force production, and strength gains compared with higher choline intakes. However, there was no difference between choline groups in changes in leg press/leg extension 1RMs, average/peak isometric force outputs, or EMG amplitudes.

It is difficult to identify the reason(s) why there was no effect of varying choline intakes on muscle responses in the present study while different amounts of choline consumption influenced strength and/or muscle gains in our previous studies. However, the differing results may be attributed to the difference in duration of diet intervention/study design. In the present study, diet intervention was conducted for three weeks whereas participants in our previous studies underwent 12 weeks of diet intervention. Even though previous studies by others used mostly endurance exercise as the exercise protocols, these studies consistently showed that acute (one time) or short-term (~2 days) choline supplementation did not have any effects on exercise

performance (14-17). The only study that examined the effect of prolonged (four weeks) choline supplementation did not observe any effect on exercise performance either (18). Moreover, even severely low choline diet (<10% of AI) for 42 days did not result in signs of choline deficiency in 6 of 26 men and 3 of 15 postmenopausal women in a clinical setting under a sedentary condition (38). It appears that longer than one month of time may be needed before differing choline intakes start to show an observable difference in muscle responses, probably due to the ability to synthesize choline de novo.

In addition, difference in methods of choline administration may explain, at least in part, why a choline effect was not observed in the present study. Wurtman et al. (95) reported that oral intake of lecithin (phosphatidylcholine [PC]) affects blood choline levels more effectively than choline chloride in healthy young subjects: Taking 2.3g (>400% of AI) of choline as choline chloride after 12 hours of fast increased blood choline levels by 86% after 30 minutes of ingestion, which returned to baseline within four hours whereas blood choline concentration increased by 265% in one hour after taking the equal amount of choline as lecithin, and the increase remained for 12 hours (95). Hirsch et al. (96) also reported that after intake of lecithin, increase in blood choline lasted considerably longer than choline chloride consumption (12 hours vs. 4 hours, respectively).

The disparity in effectiveness between lecithin/PC and choline salt consumption can be explained by the roles that intestinal bacteria play. It is estimated that approximately 65% of free choline liberated from choline salts can be converted to trimethylamine (TMA) by intestinal (stomach and small intestine) bacteria and excreted

in the urine (97) while choline in the form of lecithin/PC is less susceptible to the transformation by intestinal bacteria. de La Huerga and Popper (98) reported that 2 g of choline from lecithin resulted in considerably lower (1/3) urinary excretion of TMA compared with the same amount of choline in the form of choline bicarbonate. The consequence of bacterial degradation of choline to TMA and subsequent excretion is reduced choline availability for absorption and utilization.

Therefore, it may be possible that the Med and High groups in the present study did not have sufficiently more available choline than the Low group, effectively negating the difference in choline intake between groups, because of bacterial degradation of choline to TMA, since supplemental choline was provided as choline bitartrate in the present study. Considering the fact that vast majority of choline is ingested as lecithin/PC, and <1% of the choline exists as free choline in food (95), it is likely that the participants in our previous studies that examined the effects of habitual choline intake or egg yolk supplementation, consumed choline mostly from PC, thus, more choline was absorbed and utilized in the higher choline intake groups, resulting in meaningful differences in muscle responses. This also suggests that choline intake in the form of whole foods may be more effective and preferable than consumption of choline from isolated dietary supplements. While it is generally recommended that healthy dietary patterns be achieved through whole foods instead of isolated nutrient or dietary supplements (20), the general public appears to value isolated nutrients more than whole foods that contain them, which may be problematic (99).

The present study attempted to determine indirectly whether ACh availability

mediated by dietary choline is one of the main mechanisms responsible for choline's effect on muscle responses to RE by measuring surface EMG activities. The electrical current detected on the surface of skeletal muscle can represent the activities of motor neuron that control muscle contraction via ACh, which may be affected by dietary choline, unless there is a problem in electrical conductivity on muscle membranes, which is unlikely to occur in most cases since plasmalemmal conduction is properly maintained even after high volume, muscle injury-inducing eccentric contractions (71).

However, no difference in EMG amplitudes was observed between choline groups while EMG activities were highly correlated with average and peak isometric force production, which were also not different between choline groups, making it difficult to draw any conclusion on whether ACh availability is the major mechanism responsible for muscle responses to dietary choline and exercise. If there was a dissociation between force outputs and EMG amplitudes (i.e., difference between choline groups in force outputs but no difference in EMG amplitudes), it would mean the mechanism responsible for the force disparity may be excitation-contraction coupling failure (100), rather than ACh. On the contrary, if there was a difference between choline groups both in the force outputs and EMG amplitudes, and the two outcome variables were highly correlated with each other, it would mean ACh may be the main mechanism through which dietary choline influences strength/force production. Since the present study observed high correlations between force outputs and EMG amplitudes while there was no difference between groups in force production, it is difficult to exclude ACh from the list of potential mechanisms. Future studies with longer duration of dietary

choline intervention with EMG measurements are warranted to examine the relationship between ACh activities, force outputs, and choline intake.

In addition to muscle responses to choline and exercise, since liver and muscle damage as well as perturbed lipoprotein metabolism was associated with a low choline diet (37, 38), clinical markers of liver/muscle damage and blood lipid profiles were measured in the present study. Consistent with our previous study results (89), there was no effect of dietary choline on blood levels of ALT, AST, CK, HDL-C, LDL-C, TAG, or total cholesterol (**Table 18**), confirming that moderately low choline consumption does not result in alterations of clinical markers of muscle/liver damage or lipid metabolism.

In summary, the present study observed that three weeks of choline intakes ranging from 51% to 126% of AI did not have any effects on changes in strength and neural activation measures such as 1RMs of leg press/leg extension, average/peak isometric force outputs, and EMG amplitudes in response to lower body RE in 50-65 year olds. It appears that only prolonged period of differing choline consumption may be able to influence muscle responses and exercise performance. Future studies may be needed to examine the roles that intestinal bacteria play in choline metabolism associated with exercise.

CHAPTER VI

CONCLUSIONS

The purpose of this dissertation was to investigate the effects of varying amount of choline consumption on muscle responses to resistance exercise in older men and women. It was observed in CHAPTER III that mean choline consumption in 60-69 year old generally healthy men and women was 304.2 ± 70 mg/day (3.9 ± 0.9 mg/kg/d), which is approximately 56% of AI (~7 mg/kg/day; 550 mg/d and 425 mg/d for adult men and women, respectively), when they followed a "healthy diet" recommended by the American Dietetic Association (ADA, now known as the Academy of Nutrition and Dietetics). This was consistent with previously reported choline intake data (313 mg/d in the Framingham Offspring Study, 335 mg/d in NHANES 2011-2012; 372 mg/d in men and 304 mg/d in women in Yonemori et al.'s study) (22, 45, 48), suggesting that inadequate choline intake is prevalent in this age group. The prevalence of low choline intakes in older adults may have devastating effects if dietary choline affects responses to RE, which is one of the most effective means to delay or counter the effects of aging on muscle mass and strength in older men and women (2).

In CHAPTER III, it was shown that lower choline intake (~49% of AI) was associated with significantly reduced gains in strength and lean mass following 12 weeks of RET compared with higher choline intakes (63% or 85% of AI), and there was a significant linear relationship between choline intake and change in strength in 60-69 year old men and women. CHAPTER IV also showed that lower intake of choline

(~51% of AI) induced significantly diminished strength gains in response to 12 weeks of RET compared with higher choline intakes of 68% or 118% of AI in 50-69 year old individuals while there was no effect of dietary choline on lean mass gains. Meanwhile, choline intake higher than AI did not provide additional positive effect on RET responses. It appears that having adequate dietary choline may have a strong effect on strength gains and subtle or no effect on lean mass changes.

With evidence that 12 weeks of low choline intake negatively affects muscle responses to RET, CHAPTER V examined whether low choline consumption for a shorter period of time (< a month) still impairs muscle responses to resistance exercise in older adults. There was no effect of dietary choline on changes in 1RM on leg press/leg extension or average/peak isometric force outputs from knee extension in 50-65 year olds after three weeks of dietary choline intakes ranging from ~51 to ~126% of AI, suggesting that only prolonged period of differing choline consumption may be able to influence muscle responses and exercise performance.

An attempt was made, in CHAPTER V, to determine indirectly whether ACh availability mediated by dietary choline is the main mechanism through which choline influences muscle responses to RE by measuring surface EMG, since EMG amplitude represents the activities of motor neuron and ACh (71, 72, 88). However, no difference observed in EMG amplitudes between choline groups and high correlation between EMG and maximal isometric forces, which were also not different between choline groups, made it difficult to draw any conclusion on whether ACh is the main mechanism for muscle responses to dietary choline and RE.

It was reported that severely low choline consumption (<10% of AI) causes liver/muscle damage and altered lipoprotein/blood lipid metabolism. However, in the studies of this dissertation, moderately low choline intake (~50% of AI) did not affect blood levels of clinical markers of liver/muscle damage or lipid metabolism while low choline intake negatively influenced strength and/or lean mass gains with 12 weeks of RET. These results indicate that choline intake levels which are below AI but do not present overt clinical signs may still have negative consequences that affect health and well-being of older adults.

Taken together, it was concluded from the observations of this dissertation that lower choline consumption (≤ 50% of AI) for 12 weeks may impair strength and/or lean mass gains associated with RET while less than a month of low choline intake may not have observable effects on muscle responses to RE in generally healthy 50-69 year old individuals. The potential mechanisms of these effects of dietary choline may include ACh availability at the NMJ, membrane integrity, cell signaling, and/or methylation reactions.

Considering the effects of skeletal muscle on overall health and well-being, especially in older population who are exposed to devastating effects of sarcopenia and whose choline intake is persistently low, it is important to optimize nutritional factors to maximize the benefits of RET which is one of the most effective tools to fight sarcopenia with. Future studies are needed to confirm the results of this dissertation and if so proven, to elucidate the mechanism through which dietary choline affects skeletal muscle responses to RE.

REFERENCES

1. U.S. Census Bureau. Percent distribution of the projected population by sex and selected age groups for the United States: 2015 to 2060. December 2014. available from: https://www.census.gov/population/projections/data/national/2014.html [last accessed December 2015]
2. Landi F, Marzetti E, Martone AM, Bernabei R, Onder G. Exercise as a remedy for sarcopenia. Current Opinion in Clinical Nutrition and Metabolic Care 2014;17(1):25-31.
3. Mithal A, Bonjour JP, Boonen S, Burckhardt P, Degens H, El Hajj Fuleihan G, Josse R, Lips P, Morales Torres J, Rizzoli R, et al. Impact of nutrition on muscle mass, strength, and performance in older adults. Osteoporos International 2013;24(5):1555-66.
4. Bierkamper GG, Goldberg AM. Release of acetylcholine from the vascular perfused rat phrenic nerve hemidiaphragm. Brain Research 1980;202(1):234-7.
5. Wurtman RJ, Hefti F, Melamed E. Precursor control of neurotransmitter synthesis. Pharmacological Reviews 1980;32(4):315-35.
6. da Costa KA, Badea M, Fischer LM, Zeisel SH. Elevated serum creatine phosphokinase in choline-deficient humans: mechanistic studies in C2C12 mouse myoblasts. The American Journal of Clinical Nutririon 2004;80(1):163-70.

7. Sachan DS, Hongu N, Johnsen M. Decreasing Oxidative stress with choline and carnitine in women. Journal of the American College of Nutrition 2005;24(3):172-6.

8. Daily JW, Hongu N, Mynatt RL, Sachan DS. Choline supplementation increases tissue concentrations of carnitine and lowers body fat in guinea pigs. The Journal of Nutritional Biochemistry 1998;9(8):464-70.

9. Yao ZM, Vance DE. The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. The Journal of Biological Chemistry 1988;263(6):2998-3004.

10. Niculescu MD, Craciunescu CN, Zeisel SH. Dietary choline deficiency alters global and gene-specific DNA methylation in the developing hippocampus of mouse fetal brains. The FASEB Journal 2006;20(1):43-9.

11. Conlay LA, Sabounjian LA, Wurtman RJ. Exercise and neuromodulators: choline and acetylcholine in marathon runners. International Journal of Sports Medicine 1992;13 Suppl 1:S141-2.

12. Buchman AL, Jenden D, Roch M. Plasma free, phospholipid-bound and urinary free choline all decrease during a marathon run and may be associated with impaired performance. Journal of the American College of Nutrition 1999;18(6):598-601.

13. von Allworden HN, Horn S, Kahl J, Feldheim W. The influence of lecithin on plasma choline concentrations in triathletes and adolescent runners during

exercise. European Journal of Applied Physiology and Occupational Physiology 1993;67(1):87-91.

14. Spector SA, Jackman MR, Sabounjian LA, Sakkas C, Landers DM, Willis WT. Effect of choline supplementation on fatigue in trained cyclists. Medicine and Science in Sports and Exercise 1995;27(5):668-73.

15. Warber JP, Patton JF, Tharion WJ, Zeisel SH, Mello RP, Kemnitz CP, Lieberman HR. The effects of choline supplementation on physical performance. International Journal of Sport Nutrition and Exercise Metabolism 2000;10(2):170-81.

16. Deuster P, Singh A, Coll R, Hyde D, Becker W. Choline ingestion does not modify physical or cognitive performance. Military Medicine 2002;167(12):1020-5.

17. Buchman AL, Awal M, Jenden D, Roch M, Kang SH. The effect of lecithin supplementation on plasma choline concentrations during a marathon. Journal of the American College of Nutrition 2000;19(6):768-70.

18. Hoffman JR, Ratamess NA, Gonzalez A, Beller NA, Hoffman MW, Olson M, Purpura M, Jäger R. The effects of acute and prolonged CRAM supplementation on reaction time and subjective measures of focus and alertness in healthy college students. Journal of the International Society of Sports Nutrition 2010;7:39

19. Institute of Medicine. Dietary reference intakes for folate, thiamin, riboflavin, niacin, vitamin B12, pantothenic acid, biotin, and choline. Washington DC: National Academy Press, 1998:390-422.

20. 2015 Dietary Guidelines Advisory Committee. The scientific report of the 2015 Dietary Guidelines Advisory Committee. February 2015: available from: http://health.gov/dietaryguidelines/2015-scientific-report/ [last accessed December 2015]

21. USDA Agricultural Research Service. What we eat in America, NHANES 2007-2010, Dietary Intake Data. Beltsville, MD: available from: http://www.ars.usda.gov/Services/docs.htm?docid=13793 [last accessed December 2015]

22. Yonemori KM, Lim U, Koga KR, Wilkens LR, Au D, Boushey CJ, Le Marchand L, Kolonel LN, Murphy SP. Dietary choline and betaine intakes vary in an adult multiethnic population. Journal of Nutrition 2013;143(6):894-9.

23. Strecker A. Ueber einige neue Bestandtheile der Schweinegalle. Justus Liebigs Annalen der Chemie 1862;123(3):353-60.

24. Li Z, Vance DE. Phosphatidylcholine and choline homeostasis. Journal of Lipid Research 2008;49(6):1187-94.

25. Zeisel SH. Dietary choline: biochemistry, physiology, and pharmacology. Annual Review of Nutrition 1981;1(1):95-121.

26. Fang Y, Vilella-Bach M, Bachmann R, Flanigan A, Chen J. Phosphatidic acid-mediated mitogenic activation of mTOR aignaling. Science 2001;294(5548):1942-5.

27. Robins S. Recirculation and reutilization of micellar bile lecithin. The American Journal of Physiology 1975;229(3):598-602.

28. Mehedint MG, Niculescu MD, Craciunescu CN, Zeisel SH. Choline deficiency alters global histone methylation and epigenetic marking at the Re1 site of the calbindin 1 gene. The FASEB Journal 2010;24(1):184-95.

29. Zeisel SH, da Costa KA. Choline: an essential nutrient for public health. Nutrition Reviews 2009;67(11):615-23.

30. Nicholls JG. How acetylcholine gives rise to current at the motor end-plate. Journal of Physiology 2007;578(Pt 3):621-2.

31. Daily JW, Sachan DS. Choline Supplementation Alters Carnitine Homeostasis in Humans and Guinea Pigs. The Journal of Nutrition 1995;125(7):1938-44.

32. Burt M, Hanin I, Brennan M. Choline deficiency associated with total parenteral nutrition. The Lancet 1980;316(8195):638-9.

33. Buchman AL, Dubin MD, Moukarzel AA, Jenden DJ, Roch M, Rice KM, Gornbein J, Ament ME. Choline deficiency: a cause of hepatic steatosis during parenteral nutrition that can be reversed with intravenous choline supplementation. Hepatology 1995;22(5):1399-403.

34. Zeisel SH, Da Costa KA, Franklin PD, Alexander EA, Lamont JT, Sheard NF, Beiser A. Choline, an essential nutrient for humans. The FASEB Journal 1991;5(7):2093-8.

35. Yates AA, Schlicker SA, Suitor CW. Dietary reference intakes: the new basis for recommendations for calcium and related nutrients, B vitamins, and choline. Journal of the American Dietetic Association 1998;98(6):699-706.

36. Zeisel SH. A brief history of choline. Annals of Nutrition & Metabolism 2012;61(3):254-8.

37. Zeisel SH. Choline: an essential nutrient for humans. Nutrition 2000;16(7–8):669-71.

38. Fischer LM, daCosta KA, Kwock L, Stewart PW, Lu TS, Stabler SP, Allen RH, Zeisel SH. Sex and menopausal status influence human dietary requirements for the nutrient choline. The American Journal of Clinical Nutrition 2007;85(5):1275-85.

39. Resseguie M, Song J, Niculescu MD, da Costa K-A, Randall TA, Zeisel SH. Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes. The FASEB Journal 2007;21(10):2622-32.

40. Fischer L, da Costa K-A, Kwock L, Galanko J, Zeisel S. Dietary choline requirements of women: effects of estrogen and genetic variation. The American Journal of Clinical Nutrition 2010;92(5):1113-9.

41. da Costa KA, Kozyreva OG, Song J, Galanko JA, Fischer LM, Zeisel SH. Common genetic polymorphisms affect the human requirement for the nutrient choline. The FASEB Journal 2006;20(9):1336-44.

42. da Costa KA, Corbin KD, Niculescu MD, Galanko JA, Zeisel SH. Identification of new genetic polymorphisms that alter the dietary requirement for choline and vary in their distribution across ethnic and racial groups. The FASEB Journal 2014;28(7):2970-8.

43. USDA Agricultural Research Service. USDA Database for the Choline Content of Common Foods. Beltsville, MD: 2004. available from http://nal.usda.gov/fnic/foodcomp/

44. USDA Agricultural Research Service. USDA Database for the Choline Content of Common Foods Release Two. Beltsville, MD: 2008. available from: http://catalog.data.gov/dataset/usda-database-for-the-choline-content-of-common-foods [last accessed December 2015]

45. USDA Agricultural Research Service. Nutrient intakes from food and beverages: mean amounts consumed per individual, by gender and age, What we eat in America, NHANES 2011-2012. Beltsville, MD: 2014. available from: http://www.ars.usda.gov/Services/docs.htm?docid=13793 [last accessed December 2015]

46. Xu X, Gammon MD, Zeisel SH, Lee YL, Wetmur JG, Teitelbaum SL, Bradshaw PT, Neugut AI, Santella RM, Chen J. Choline metabolism and risk of breast cancer in a population-based study. The FASEB Journal 2008;22(6):2045-52.

47. Chiuve SE, Giovannucci EL, Hankinson SE, Zeisel SH, Dougherty LW, Willett WC, Rimm EB. The association between betaine and choline intakes and the plasma concentrations of homocysteine in women. The American Journal of Clinical Nutrition 2007;86(4):1073-81.

48. Cho E, Zeisel SH, Jacques P, Selhub J, Dougherty L, Colditz GA, Willett WC. Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. The American Journal of Clinical Nutrition 2006;83(4):905-11.

49. Corbin K, Zeisel S. Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. Current Opinion in Gastroenterology 2012;28(2):159-65.

50. Song J, da Costa KA, Fischer LM, Kohlmeier M, Kwock L, Wang S, Zeisel SH. Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease (NAFLD). The FASEB Journal 2005;19(10):1266-71.

51. Hensley K, Kotake Y, Sang H, Pye QN, Wallis GL, Kolker LM, Tabatabaie T, Stewart CA, Konishi Y, Nakae D, et al. Dietary choline restriction causes complex I dysfunction and increased H2O2 generation in liver mitochondria. Carcinogenesis 2000;21(5):983-9.

52. Soon RK, Yan JS, Grenert JP, Maher JJ. Stress signaling in the methionine-choline-deficient model of murine fatty liver disease. Gastroenterology 2010;139(5):1730-9.e1.

53. Fisher MC, Zeisel SH, Mar M-H, Sadler TW. Inhibitors of choline uptake and metabolism cause developmental abnormalities in neurulating mouse embryos. Teratology 2001;64(2):114-22.

54. Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. The American Journal of Epidemiology 2004;160(2):102-9.

55. da Costa K-A, Niculescu MD, Craciunescu CN, Fischer LM, Zeisel SH. Choline deficiency increases lymphocyte apoptosis and DNA damage in humans. The American Journal of Clinical Nutrition 2006;84(1):88-94.

56. Joubert LM, Manore MM. Exercise, nutrition, and homocysteine. International Journal of Sport Nutrition and Exercise Metabolism 2006;16(4):341-61.

57. da Costa K-A, Gaffney CE, Fischer LM, Zeisel SH. Choline deficiency in mice and humans is associated with increased plasma homocysteine concentration after a methionine load. The American Journal of Clinical Nutrition 2005;81(2):440-4.

58. Olthof MR, Brink EJ, Katan MB, Verhoef P. Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men. The American Journal of Clinical Nutrition 2005;82(1):111-7.

59. Rothschild JG, Hansen RC. Fish odor syndrome: trimethylaminuria with milk as chief dietary factor. Pediatric Dermatology 1985;3(1):38-9.

60. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, Feldstein AE, Britt EB, Fu X, Chung Y-M, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472(7341):57-63.

61. Haubrich DR, Wedeking PW, Wang PFL. Increase in tissue concentration of acetylcholine in guinea pigs in vivo induced by administration of choline. Life Sciences 1974;14(5):921-7.

62. Cohen E, Wurtman R. Brain acetylcholine: control by dietary choline. Science 1976;191(4227):561-2.

63. Pagala MKD, Namba T, Grob D. Failure of neuromuscular transmission and contractility during muscle fatigue. Muscle & Nerve 1984;7(6):454-64.

64. Abratte CM, Wang W, Li R, Axume J, Moriarty DJ, Caudill MA. Choline status is not a reliable indicator of moderate changes in dietary choline consumption in premenopausal women. The Journal of Nutritional Biochemistry 2009;20(1):62-9.

65. Riechman SE, Lee CW, Chikani G, Chen VC, Lee TV. Cholesterol and skeletal muscle health. World Review of Nutrition and Dietetics 2009;100:71-9.

66. Melton LJr, Khosla S, Crowson CS, O'Connor MK, O'Fallon WM, Riggs BL. Epidemiology of sarcopenia. Journal of the American Geriatrics Society 2000;48(6):625-30.

67. Evans WJ. What is sarcopenia? The Journals of Gerontology Series A, Biological Sciences and Medical Sciences 1995;50 Spec No:5-8.

68. Andrews RD, MacLean DA, Riechman SE. Protein intake for skeletal muscle hypertrophy with resistance training in seniors. International Journal of Sport Nutrition and Exercise Metabolism 2006; 16(4): 362-72.

69. Riechman SE, Andrews RD, Maclean DA, Sheather S. Statins and dietary and serum cholesterol are associated with increased lean mass following resistance training. The Journals of Gerontology Series A, Biological Sciences and Medical Sciences 2007;62(10):1164-71.

70. Gearhart RF, Jr., Lagally KM, Riechman SE, Andrews RD, Robertson RJ. RPE at relative intensities after 12 weeks of resistance-exercise training by older adults. Perceptual and Motor Skills 2008;106(3):893-903.

71. Warren GL, Ingalls CP, Shah SJ, Armstrong RB. Uncoupling of in vivo torque production from EMG in mouse muscles injured by eccentric contractions. Journal of Physiology 1999;515(2):609-19.

72. Suzuki T, Nagai H, Katsumata N, Ogawa S, Suzuki H. Investigation of fading responses induced by non-depolarising muscle relaxants in the evoked EMG of the gastrocnemius muscle of the cat. Acta Anaesthesiologica Scandinavica 1999;43(6):658-62.

73. Mills JL, Fan R, Brody LC, Liu A, Ueland PM, Wang Y, Kirke PN, Shane B, Molloy AM. Maternal choline concentrations during pregnancy and choline-related genetic variants as risk factors for neural tube defects. The American Journal of Clinical Nutrition 2014; 100(4):1069-74.

74. Physical Activity Guidelines Advisory Committee. Physical activity guidelines advisory committe report 2008. Washington, DC: U.S. Department of Health and Human Services, 2008.

75. Churchward-Venne TA, Tieland M, Verdijk LB, Leenders M, Dirks ML, de Groot LC, van Loon LJ. There are no nonresponders to resistance-type exercise training in older men and women. Journal of the American Medical Directors Association 2015;16(5):400-11.

76. Lee CW, Galvan E, Lee TV, Chen VC, Bui S, Crouse S, Fluckey J, Smith S, Riechman SE. Lower intake of choline is associated with diminished strength and lean mass gains following 12 weeks of resistance training in older adults. The American Journal of Clinical Nutrition 2015;Under Review.

77. Gearhart RF, Jr., Lagally KM, Riechman SE, Andrews RD, Robertson RJ. Safety of using the adult OMNI resistance exercise scale to determine 1-RM in older men and women. Perceptual and Motor Skills 2011;113(2):671-6.

78. Crockett JL, Edgerton VR, Max SR, Barnard RJ. The neuromuscular junction in response to endurance training. Experimental Neurology 1976;51(1):207-15.

79. Herscovich S, Gershon D. Effects of aging and physical training on the neuromuscular junction of the mouse. Gerontology 1987;33(1):7-13.

80. Cholewa JM, Guimaraes-Ferreira L, Zanchi NE. Effects of betaine on performance and body composition: a review of recent findings and potential mechanisms. Amino Acids 2014;46(8):1785-93.

81. Cholewa JM, Wyszczelska-Rokiel M, Glowacki R, Jakubowski H, Matthews T, Wood R, Craig SA, Paolone V. Effects of betaine on body composition, performance, and homocysteine thiolactone. Journal of the International Society of Sports Nutrition 2013;10(1):39.

82. del Favero S, Roschel H, Artioli G, Ugrinowitsch C, Tricoli V, Costa A, Barroso R, Negrelli AL, Otaduy MC, da Costa Leite C, et al. Creatine but not betaine supplementation increases muscle phosphorylcreatine content and strength performance. Amino Acids 2012;42(6):2299-305.

83. Hoffman JR, Ratamess NA, Kang J, Gonzalez AM, Beller NA, Craig SA. Effect of 15 days of betaine ingestion on concentric and eccentric force outputs during isokinetic exercise. Journal of Strength and Conditioning Research / National Strength & Conditioning Association 2011;25(8):2235-41.

84. Lee EC, Maresh CM, Kraemer WJ, Yamamoto LM, Hatfield DL, Bailey BL, Armstrong LE, Volek JS, McDermott BP, Craig SAS. Ergogenic effects of betaine supplementation on strength and power performance. Journal of the International Society of Sports Nutrition 2010;7:27.

85. Cui Z, Houweling M. Phosphatidylcholine and cell death. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 2002;1585(2–3):87-96.

86. Fischer LM, Scearce JA, Mar M-H, Patel JR, Blanchard RT, Macintosh BA, Busby MG, Zeisel SH. Ad libitum choline intake in healthy individuals meets or

exceeds the proposed adequate intake level. The Journal of Nutrition 2005;135(4):826-9.

87. Gonzalez-Freire M, de Cabo R, Studenski SA, Ferrucci L. The Neuromuscular junction: aging at the crossroad between nerves and muscle. Frontiers in Aging Neuroscience 2014;6:208.

88. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. Journal of Applied Physiology 2004;96(4):1486-95.

89. Lee CW, Lee TV, Galvan E, Chen VC, Bui S, Crouse S, Fluckey J, Smith S, Riechman SE. The effect of choline supplementation and resistance training on strength and lean mass gains in older adults: a randomized double-blind placebo-controlled clinical trial. The American Journal of Clinical Nutrition 2015;Under Review.

90. Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. Journal of Nutrition 2003;133(5):1302-7.

91. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. The American Journal of Clinical Nutrition 1990;51(2):241-7.

92. Merletti R. Standards for reporting EMG data. Journal of Electromyography and Kinesiology 1999;9(1):1.

93. Freriks B, Hermens HJ, Commission des Communautés européennes B, Health Research P. SENIAM : European recommendations for surface

electromyography : results of the SENIAM project. 2nd ed. Amsterdam, The Netherlands: Roessingh Research and Development, 2000.

94. National Research Council. Dietary reference intakes: the essential guide to nutrient requirements. Washington, DC: The National Academies Press, 2006.

95. Wurtman RJ, Hirsch MJ, Growdon JH. Lecithin consumption raises serum-free-choline levels. Lancet 1977;2(8028):68-9.

96. Hirsch MJ, Growdon JH, Wurtman RJ. Relations between dietary choline or lecithin intake, serum choline levels, and various metabolic indices. Metabolism 1978;27(8):953-60.

97. de la Huerga J, Popper H. Urinary excretion of choline metabolites following choline administration in normals and patiens with hepatobiliary diseases. Journal of Clinical Investigation 1951;30(5):463-70.

98. de la Huerga J, Popper H. Factors influencing choline absorption in the intestinal tract. Journal of Clinical Investigation 1952;31(6):598-603.

99. Schuldt JP, Pearson AR. Nutrient-centrism and perceived risk of chronic disease. Journal of Health Psychology 2015;20(6):899-906.

100. Ingalls CP, Warren GL, Williams JH, Ward CW, Armstrong RB. E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. The Journal of Applied Physiology 1998;85(1):58-67.