MANIPULATING DIETARY PROTEIN DENSITY AND ITS EFFECT ON TRAINING-INDUCED MUSCLE PERFORMANCE AND OVERALL HEALTH AMONG MIDDLE-AGED ADULTS

BY

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THESIS

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ABSTRACT

By 2050, the aging population worldwide is expected to increase vastly. This has major implications as the progressive loss of muscle strength is a common consequence of aging and negatively impacts physical performance and ultimately, independence and quality of life. The underlying mechanistic cause of this age-related strength loss is likely related to alterations in overall muscle mass and neuromuscular function. It has been proposed that lifestyle modifications to include exercise training and higher dietary protein intakes will likely be the most effective approach to offset muscle strength loss with advancing age. Thus, the study within this thesis aimed to examine the effect of manipulating the protein density of the diet in combination with resistance training on skeletal muscle strength and performance adaptations in middle-aged men and women. Fourteen healthy middle-aged adults were randomly assigned to consume protein at the Recommended Dietary Allowance (RDA; 0.8 – 1.0 g • kg BW^{-1} • day^{-1}) or twice the Recommended Dietary Allowance (2×RDA; 1.6 – 1.8 g • kg BW^{-1} • day^{-1}) throughout a supervised 10-week progressive resistance training program. Body composition, muscle strength and performance were evaluated pre- and post-intervention. Results of this study demonstrate that resistance training induced gains in lean body mass and muscle strength are not potentiated when consuming protein in far excess of the protein RDA in middle-aged adults. Thus, consuming protein slightly above the RDA (1.2 g • kg BW^{-1} • day^{-1}) is adequate to support training-induced muscle adaptations when adhering to a healthy eating pattern consisting of equally distributed protein meals throughout the day.

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CHAPTER 1: INTRODUCTION

Maintenance of muscle mass in adulthood is important in preventing disease and promoting health. Aside from muscle being vital for movement, it serves several metabolic roles and, as such, loss of muscle mass and function are involved in the development of many age-related conditions[1].

One of the major physiological changes that accompany aging is the progressive loss of muscle mass and strength. These two decline at different rates and it has been observed that the rate of strength loss is much more rapid than muscle mass loss[2,3] but both negatively impacts physical performance and ultimately, independence and quality of life[4,5]. The mechanism behind the age-related loss of strength is most likely related to alterations in overall muscle mass and neuromuscular function[6] and the preservation of muscle mass alone does not completely prevent strength loss with advancing age. Both increasing habitual physical activity[7] and/or optimizing nutrition[8,9] have been identified as effective strategies in the prevention and management of this age-related decline in muscle mass and strength.

Strategies, however, that combine both exercise and nutrition may help delay, prevent and manage this condition. For example, ingestion of protein[10,11] or exercise alone have both been shown to be effective anabolic stimuli to increase muscle protein synthesis rates. However, when nutrition and exercise are combine the muscle protein synthetic response is potentiated[12,13]. It is generally understood that consumption of high quality protein is vital, as it provides essential amino acids that serve as building blocks for new proteins. Thus, it has been proposed that in the aging population, a greater intake of high quality protein along with exercise training will likely be the most effective approach to offset muscle strength loss with advancing age.

Aging impairs skeletal muscle anabolic signaling and protein synthesis as evidenced by the blunted response to anabolic stimuli such as protein ingestion[14,15] and resistance exercise[16,17] compared to younger individuals. In fact, nutritional needs differ and it has

been proposed that a relatively higher amount of protein is needed to maximally stimulate muscle protein synthesis rates among older individuals[18]. The current Recommended Dietary Allowance (RDA) for protein at 0.8 g • kg BW^{-1} • day^{-1} represents the minimum amount of protein required on daily bases to prevent net nitrogen (protein) loss and deficiency in physically inactive individuals[19]. As such, when the goal is to maximize muscle mass, especially with exercise training, the RDA may not the 'optimal' target. The more optimal level of protein intake for these individuals is likely greater the current RDA and is estimated to be more than 1.2 g • kg BW^{-1} • day^{-1} [20,21]. Aside from total dietary protein intake, distribution across meals is also an important consideration as it has been shown that meals with suboptimal amount of protein[22] and leucine [23,24] have less anabolic impact on skeletal muscle protein synthesis rates among older individuals.

Specific Aims and Hypothesis Tested

Lifestyle-based strategies that are most effective include an exercise component which further underlines the need to optimize protein intake to maximize adaptations. It is well-established that ingestion of protein immediately after exercise[25] and during prolonged recovery[26,27] is crucial in facilitating muscle remodeling and impacts long-term adaptations to training and improvements in physical performance. Hence, ensuring a more optimal protein intake - above the RDA, and distributing this across meals, while engaging in resistance training (RT) should be more beneficial in promoting training-induced improvements in lean mass gain and muscle performance.

Therefore, the purpose of this thesis was to examine the effect of manipulating the protein density of the diet, when adhering to an optimal protein distribution pattern, in combination with resistance training on skeletal muscle strength and performance adaptations in middle-aged men and women. Moreover, we aimed to explore other relevant health outcomes (i.e. insulin sensitivity, plasma lipid levels) and quantitative and qualitative dietary measures. To accomplish this aim, we combined dietary counseling and nutrition intervention with a 10-week progressive resistance exercise training program and measured various anthropometric, biochemical, clinical, dietary and performance outcomes.

We hypothesize that consuming protein at twice the RDA (2×RDA) level - distributed across meals and during key nutrient timing windows, would be more optimal and would enhance adaptations to RT and lead to greater training-induced gains in lean mass, muscular strength and performance. The chapters that follow will describe the current state of the literature, the methodology, results, and overall discussion and conclusions with regards to aging and muscle mass regulation.

CHAPTER 2: LITERATURE REVIEW

This literature review chapter will cover a discussion of health challenges in aging; specifically, skeletal muscle mass and strength loss, its proposed mechanisms and current strategies for prevention. Current evidence on protein and amino acid supplementation with or without RT will also be presented. A section of this review will discuss further about the macronutrient protein; specifically, its food sources, functions and significance in health promotion along with trends in protein intake and current recommendations stated in US dietary guides. These topics have been chosen to underline the need to define the optimal level of dietary protein intake among adults and its implications on outcome-focused measures such as body composition, muscular performance and overall health.

2.1 Health Challenges with Aging and the Role of Exercise and Nutrition

The age-related decline in skeletal muscle mass and function is one of the major challenges during aging as it has a significant effect on physical performance and ultimately, independence, and quality of life. Several major factors (i.e. nutritional, genetic, lifestyle, neural) seem to play a role on the onset of this condition[28]. In addition to this, the age-related decrease in muscle mass is also accompanied by a decline in strength. In fact, several studies have demonstrated that strength loss rates seem to be higher than muscle mass loss rates[2,3] and onset occurs around the fourth and fifth decade of life[3] making it a concern not only among the elderly. To date, the exact underlying mechanistic cause of this age-related strength loss has yet to be determined, but is most likely related to changes in overall muscle mass and neuromuscular function due to impairments in skeletal muscle anabolic signaling[14,15] and poor motor unit remodeling[29] respectively, that occur with aging.

Regulation of Skeletal Muscle Protein Turnover and Aging

Muscle net protein balance (NPB) is maintained by the continuous and synchronized synthesis and breakdown of protein. In older individuals, it is believed that

skeletal muscle protein synthesis is altered, causing a negative NPB; which over time, may lead to loss of skeletal muscle mass[30]. It is well-established that protein ingestion[31,32]; specifically, essential amino acids[33], and exercise[34,35] have a stimulatory effect on muscle protein synthesis (MPS) rates and are considered as principal modulators of muscle protein turnover. In fact, exercise coupled with protein ingestion has a synergistic effect and further increases the postprandial muscle protein synthetic response[13,36] leading to muscle protein accretion[37]. It is important to note that these exercise-induced increases in MPS rates can either be hypertrophic or non-hypertrophic in nature[38] and is generally referred to as muscle protein remodeling.

Compared to younger individuals, the mechanism of skeletal muscle protein turnover is impaired among older individuals as evidenced by a blunted skeletal muscle protein synthetic response to ingestion of protein[14,15] and resistance exercise[16,17]. In contrast, results from other studies[13,39] question the concept of anabolic resistance among older adults and just how much it contributes to the age-related loss of skeletal muscle mass. Overall, majority of studies conducted among middle-aged and older adults have high variability (i.e. amount and source of protein, difference in subject characteristics) making it difficult to assess the real implication of age-related impairments on muscle protein turnover and as such continues to be an active area of research.

Basal muscle protein synthesis rates and the postprandial/post-exercise muscle protein synthetic response to anabolic stimuli (i.e. muscle contraction and essential amino acids) mainly controls the maintenance of muscle mass[40]. As such, prolonged resistance training (RT) programs have been shown to benefit older adults by increasing lean mass and strength[3,41,42]. Moreover, exercise[13], particularly, RT[17,43] further augments the skeletal muscle protein synthetic response to dietary protein ingestion. This illustrates the combined potential of both nutrition and exercise in promoting muscle health. Nutritional needs, however, slightly differ among middle-aged and older adults as compared to younger individuals as a relatively higher amount of protein is required to maximally stimulate MPS among older adults[18].

Exercise Benefits Overall Health

Exercise interventions have been proven to benefit aging muscle. In particular, it has been demonstrated that resistance exercise reverses the loss of muscle strength at the phenotypic and transcriptome level[44], and recent meta-analyses have shown that it is also effective in promoting gains in lean mass and improvements in upper and lower body strength[45,46]. Also, given the different metabolic roles of muscle tissue, a decrease in skeletal muscle mass impacts other aspects of health aside from strength and physical function. It has been shown beneficial in improving other clinical and biochemical markers of health such as blood pressure(BP)[47–49] and insulin sensitivity[50,51]. A meta-analysis looking at the impact of resistance training on BP and other cardiovascular risk factors among healthy adults found a significant reduction in blood pressure, body fat and plasma triglycerides but not other blood lipid levels or fasting blood glucose (FBG)[52].

A major role muscle tissue plays is the maintenance of glucose homeostasis as majority of the glucose in the blood is taken up by the muscle[53]. Loss of muscle mass has been attributed to a changes in beta-cell function among healthy middle-aged adults without diabetes[54]. As such, the effect of resistance training on increasing muscle mass also has positive implications on preventing insulin resistance and decreased insulin secretion among adults[54,55]. The most widely used approach to gain insight into whole body glucose tolerance is conducting an oral glucose tolerance testing (OGTT). It is also possible to use the information gained from an OGTT to determine various indices of insulin sensitivity as described below. It should be noted, however, that the gold standard for assessing insulin sensitivity (hepatic and peripheral) is using hyperinsulinemic-euglycemic clamp as it directly measures the effects of insulin to promote glucose use under steady-state conditions, but this technique is labor intensive and difficult to use on a large-scale. Alternatively, an OGTT is conducted by administering 75 grams of glucose and repeatedly measuring plasma glucose and insulin concentrations over a 2-hour time course from blood samples [56]. It is a common clinical assessment tool, as its simple and cheap, for evaluating insulin sensitivity (IS) – sensitivity of tissues to insulin-mediated glucose disposal[57,58], and beta-cell function (BCF) – ability of pancreatic beta cells to secret

adequate amounts of insulin[59]. Several insulinogenic indices have been derived to assess both IS and BCF. Insulin resistance (IR) – or the impaired sensitivity of tissues to insulin is commonly assessed using the Homeostasis Model Assessment Insulin Resistance index (HOMA-IR), Matsuda – Defronzo Insulin Sensitivity Index (ISI-M), Metabolic Clearance Rate of Glucose ($MCR_{Glucose}$) and the estimated Insulin Sensitivity Index (ISI-Stumvoll). HOMA-IR assumes that hepatic and peripheral tissue sensitivity to insulin are the same and uses only basal glucose and insulin or C-peptide concentrations, with a value greater than 2.5 indicating IR[60,61]. On the other hand, ISI-M approximates whole body insulin sensitivity using the whole OGTT time course[62] which makes it a better indicator, and a value of less than 2.5 indicates IR. Stumvoll and colleagues'[63] equations to predict glucose metabolic clearance rate and insulin sensitivity - $MCR_{Glucose}$ and ISI - Stumvoll, which factor in body mass and uses the 2-hour insulin value and the 90 minute value for glucose are commonly used insulin sensitivity indices as well. On the other hand, indices used to assess BCF include Insulinogenic Index (IGI)[64] and the individual phases of insulin release – first-phase insulin release (1^{st}PH Insulin Release) and second-phase insulin release (2^{nd}PH Insulin release)[63]. IGI evaluates beta-cell function from the first 30 minutes of an OGTT with a value less than 0.4 suggesting that insulin secretion from beta cells is defective[64]. Both 1^{st} PH and 2^{nd} PH insulin release are calculated using the 30-minute glucose value and the basal and 30-minute insulin values as well as the body mass of an individual[63].

Exercise is known to positively impact glucose handling through insulin-dependent and –independent mechanisms. Acutely, exercise increases translocation of GLUT-4, a glucose transport protein, to the muscle cell membrane while chronic exercise increases relative muscle GLUT-4 protein concentration[65]. In addition, exercise training enhances insulin sensitivity by enhancing post-receptor insulin signaling[66,67]. A study conducted by Iglay in colleagues[68] demonstrated improvements in glucose tolerance, insulin signaling and lipid profile with adequate (0.9 $g \bullet kg\ BW^{-1} \bullet day^{-1}$) and moderately higher (1.2 $g \bullet kg\ BW^{-1} \bullet day^{-1}$) protein intake along with 12-weeks of resistance training. As such, lifestyle approaches aimed to promote overall health with aging should include exercise training in combination with proper nutrition as it not only benefits muscle health but also has positive implications on other metabolic health markers.

Characterizing Dietary Protein and Its Role in Skeletal Muscle Health

Protein is made up of series of amino acids linked by peptide bonds[69]. When dietary protein is ingested and hydrolyzed, it provides the structural component of various tissues, enzymes and hormones in the body that are important for daily function[69]. It also serves as an energy source yielding 4 calories (KCAL) per gram[70]. The range of amino acids available in a certain food item determines its ability to provide the building blocks needed to synthesize new proteins and is the basis of evaluating quality of a food protein source.

Various food groups such as dairy, grains, meats, legumes, nuts and seeds all contribute to dietary protein intake. In general, dietary protein sources are classified as either animal-derived or plant-derived which determines its quality. From a muscle health perspective, dietary protein quality indicates a protein food's ability to stimulate and support the increase in MPS after its ingestion[69]. Food items that contain a complete array of essential amino acids – those that cannot be synthesized by the body in adequate amounts[71]; particularly those that contain a higher proportion of leucine per total amino acid content, are deemed to be of higher quality and can induce higher MPS rates[72]. In general, protein from animal sources are superior in quality compared to plant-based protein. As a result, plant-based protein are usually fortified with free amino acids[72] and must be consumed in greater amounts or in specific combinations to optimize the amino acid profile. Moreover, with the advent of food processing technologies, derivative products (i.e. whey, casein and soy protein isolates) are also widespread in the market[70] and are commonly used as supplements. It is important to note though, that protein-rich whole food offer a unique advantage in terms of nutrient density. It not only provides calories and protein but also other micronutrients (i.e. iron, B-vitamins)[73] which help improve overall quality of the diet.

2.2 Dietary Protein Recommendations and Trends

Current US Dietary Guides and Dietary Protein Recommendations

The Dietary Guidelines for Americans (DGA), USDA Food Guidance System - *MyPlate*, USDA Food Patterns and Dietary Reference Intakes (DRI) are developed to foster good nutrition and promote health in the country[74]. The DGA serves as a set of general guidelines and overarching themes while DRIs are quantitative recommendations on nutrient intake; both of which are reflected in the USDA food patterns and translated to a visual nutrition guide; currently, *MyPlate.* Each of these dietary guides include recommendations on protein intake which highlight the important role of protein in a healthy diet and in human health.

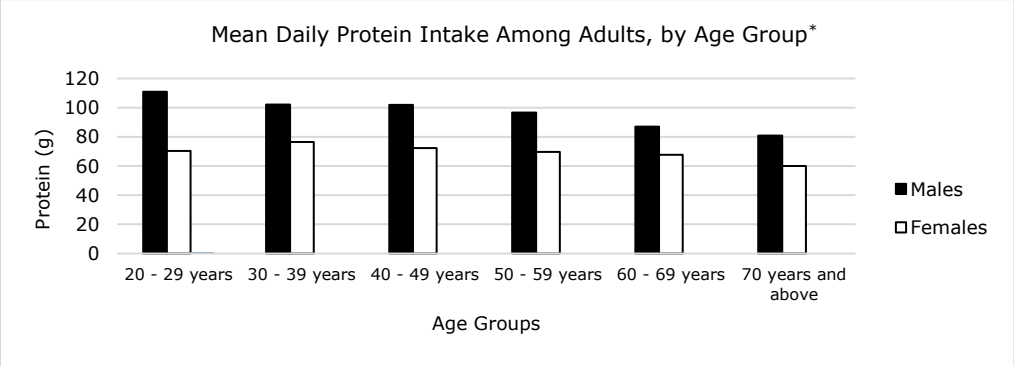
Protein as a food group is an important part of a healthy eating pattern as it contributes other important nutrients aside from protein such as B-vitamins, vitamin E, vitamin D, selenium, choline, phosphorus, zinc and copper; concentration of which, vary depending on source[74]. Protein-specific recommendations in the DGA[74] include choosing a variety of protein foods – both from animal- and plant-sources and selecting unsalted nuts and seeds and lean forms of meats and poultry to help meet quantitative recommendations on sodium, saturated fats and added sugars within a given caloric limit. In addition to this, dairy is also a significant source of protein and other nutrients (i.e. calcium, vitamin D, etc.) in the diet but also contributes to fat intake; thus, it is recommended to choose low-fat or fat-free options when consuming dairy and dairy products[74].

The USDA food patterns are quantitative food group recommendations at different calorie levels which were designed to ensure adequacy of intake based on the DRIs and adherence to DGA. As an example, the 2,000 calorie healthy US-style eating pattern recommends a daily protein intake of 5 ½ ounce-equivalents[75] which is in line with the RDA for protein at 46 grams (females) and 56 grams (males) per day[19]. An ounce-equivalent of protein is defined as 1 ounce lean meat, poultry, or seafood; 1 piece of egg , ¼ cup cooked beans or tofu, 1 tablespoon peanut butter, ½ ounce nuts or seeds[76]. It is also recommended to consume 3 cup-equivalents of dairy and dairy products per day, which

would contribute additional protein (around 26.1 grams), if met[77]. These recommendations are translated to the visual food guide, My Plate[78].

Trends in Protein Intake of the US Population

Compared to carbohydrate and fat, protein intake among adults has increased but remained relatively consistent over the years. Mean protein intake ranged between 78 – 87 grams per day with a significant small increase noted from 1971 – 2010, but not in the recent years (1999-2010)[79]. Most recent dietary data from NHANES 2013- 2014[80] show that the mean protein intake among adults ages 20 years and over is 83.6 grams per day or 16% of calories which is considered adequate based on the RDA, but clearly at lower end of the Acceptable Macronutrient Distribution Range (AMDR) for protein which is set at 10 – 35% of total calories. Looking at specific age groups among adults, protein intake seems to decrease with age (Figure 2.1).



*Data from Agricultural Research Services, United States Department of Agriculture (ARS/USDA)[81]

Figure 2.1 Mean Protein Intake Among Adults in USA

Younger adults (i.e. 20-29 years old) consume around 110.8 grams (males) to 70.2 grams (females) of protein while older adults (i.e. 70 years and above) consume an average of 80.8 grams (males) to 60 grams (females) of protein. Among adults ages 50 years and above, around 30% are not meeting the current RDA for protein with 10% of women not even meeting the estimated average requirement (EAR) for protein[82]. The decrease in protein intake may be associated with the observed overall decline in caloric intake among older individuals due to the different physiological changes that affect appetite and dietary

habits[83]. Aside from amount, the spread of protein across meals throughout the day is also an issue; this notion is completely lost when simply examining overall daily protein intakes among US adults.

Most of the daily dietary protein are concentrated on particular meals - breakfast having the least amount of protein (16% of total protein) and more carbohydrates[81], especially among older adults[82]. Protein intake is skewed towards certain meals - dinner (43% of total protein) and lunch (28% of total protein)[81]. Snacks also contribute to total protein intake (13% of total protein) with most adults consuming around 2-3 snacks in a day[81]. Overall, the average daily total protein intake of adults meet the current RDA; however, the distribution of protein intake across meals is another crucial factor that may positively impact outcome-focused measures (i.e. lean mass, muscle performance). For example, Mamerow and colleagues recently conducted a study among middle-aged adults[84] examining the effect of manipulating 24-hour dietary protein distribution on MPS rates and found that consumption of a moderate amount of protein (~30 g) at each main meal was more effective in stimulating 24-hour MPS than skewing protein intake toward the evening meal. This supports the notion that consuming larger amounts or protein in a single meal does not entail further stimulation of MPS[22], but simply results in the excess dietary amino acids being oxidized

2.3 Optimizing Protein Intake to Support Training Adaptations in Healthy Adults

Dietary Factors Contributing to Favorable Outcome-Focused Measures

Efficient and feasible lifestyle strategies aimed to positively impact outcome-focused measures (i.e. physical performance, muscle strength and power, body composition) should include both exercise and nutrition approaches. Specifically, in terms of nutrition, ensuring adequacy of total dietary protein intake is not the only factor that warrants attention; especially since NHANES data[81] show that diets of most adults are clearly not deficient in protein. The current protein RDA for adults ages 19 years and above, set at 0.8 g • kg BW^{-1} • day^{-1} was established as the minimum amount of protein required on a daily

basis to prevent a deficiency[19]. Management of age-related conditions that do not develop acutely may entail establishing preventive measures such as optimizing lifestyle practices early on in adulthood. In addition, the different physiological impairments (i.e. compromised protein digestion, amino acid absorption and muscle amino acid uptake) that occur with aging justifies the need to increase protein requirements beyond the current RDA[20,85]; however, it should be noted that promoting higher intakes of protein may bring about other issues such as nutrient displacement, cost and total energy intake. Thus, other factors such as distribution, quality and timing should also be taken into account when designing nutritional strategies. Moreover, if the goal is to maximize muscle mass, there is a need to recognize and take advantage of the synergistic effect of protein feeding and exercise[13], especially resistance exercise. It has already been shown that ingestion of protein supports adaptation to prolonged resistance exercise training[86,87] and that around 20 g of protein is necessary to induce maximal post-exercise MPS stimulation in young individuals[88]. For older adults though, it seems that a higher protein dose is more ideal. Dose-response studies have demonstrated a greater increase in MPS with the ingestion of 36 – 40 grams of protein among middle-aged and elderly individuals[89,90].

The need to ingest more protein may be a challenge among older individuals since overall energy intake decreases with age due to other physiological factors (i.e. loss of appetite, altered taste perception). Protein supplementation may aid in meeting the increased dietary requirement and has been shown to improve lean body mass[91] and measures of sarcopenia[92] among older adults. Furthermore, supplementing a suboptimal protein dose[93,94] or meals[24] with indispensable amino acids (IAA), particularly, leucine,[95] has also been shown to stimulate the muscle protein synthetic response in older adults[33,96–98]. It is proposed that around 2.1 -3.9 grams leucine[15,82] or around 10 grams of essential amino acids[15] is needed to stimulate MPS. Although protein or amino acid supplementation may be a convenient option, it is important to note that dietary protein is generally obtained from whole foods, with high-quality protein-rich whole food being the ideal option.

In general, protein from animal sources are considered of higher quality compared to plant-based protein as these contain proportional amounts of all the essential amino acids that are vital in muscle remodeling[99]. Opting for protein-rich whole food may also be a more cost-effective way of meeting overall nutrition needs. These contribute not only high-quality protein but also significant amounts of other essential nutrients (i.e. iron, folic acid)[73,100,101]. In addition, there is a potential for the various nutrients in the natural food matrix to interact and elicit a positive action on various metabolic processes[102], including synthesis of protein. Existing evidence[103–105] suggest that ingesting protein within its natural food matrix compared to the individual food components within the food matrix is more effective in stimulating postprandial MPS during the post-exercise recovery period. Moreover, aside from optimizing quantity and quality of protein intake, timing and distribution are also important variables in promoting net protein balance throughout the course of the day and supporting the skeletal muscle adaptive response to training.

Given the known interaction between protein feeding and exercise, the volume[106] and intensity[17] of exercise would then influence dietary protein factors (i.e. amount, timing and distribution). Protein ingestion during key nutrient timing windows - close to the exercise initiation or cessation (within 0 - 4 hours)[87] and before sleep (1-2 hours)[26,107], have been shown to have positive impacts on muscle adaptations from resistance-type exercise training. A recent study conducted by van Loon's group[108] demonstrated that ingestion of 40 g of protein prior to sleep led to a sufficient increase in overnight myofibrillar protein synthesis rates compared to small doses of protein - 20 g, with or without additional free leucine.

The effect of protein feeding on post-exercise MPS has been most commonly studied during the early recovery period (i.e. 4-6 hours post-exercise); however, it has been shown that the enhanced MPS response to protein, exercise or both is prolonged for up to 24-hours[27]. As such, an important area of research that has not yet been thoroughly studied is the impact of longer-term feeding strategies in training recovery and adaptations. This is highly relevant when creating nutritional strategies aimed to enhanced skeletal muscle mass with training.

Recently, the optimal pattern and distribution of protein intake to achieve and maintain of peak muscle mass has also been examined. A study conducted by Areta and colleagues[109] among young men, aimed to determine how quantity and timing of protein feeding after a single bout of resistance exercise would influence the rates MPS throughout a 24-hour period by comparing three protein ingestion protocols – pulse (10 g PRO every 1.5 hours), intermediate (20 g PRO every 3 hours)and bolus (40 g PRO every 6 hours) feeding . They found that ingestion of around 20 grams of protein every three hours led to greater myofibrillar fractional synthetic rates over the 12-hour period compared to pulse and bolus feeding. This demonstrates that not only overall quantity of protein intake can regulate MPS but also timing and distribution and as such, including around 20 – 30 g protein per meal compared to bolus intakes is more ideal for the maintenance of protein balance over the course of the day[84,109–111] and has been associated with better muscle strength among adults[112].

Lastly, ensuring adequate caloric intake is also important in supporting adaptations from training. A recent analysis of dietary intake of a large cohort of adults (19 – 72 years old), showed that adequate energy and protein intake is associated with improved markers of musculo-skeletal health regardless of the primary source of dietary protein[113].

Optimized Protein Feeding and Resistance Training on Body Composition and Performance

Main outcomes of studies assessing the effects of protein supplementation alone or in combination with resistance exercise have shown improvements in lean mass and muscular function. Body composition is commonly measured using dual-energy x-ray absorptiometry (DEXA) which provides accurate and precise measures of bone and soft tissue composition on a whole body level or specific anatomical region[114]. In addition to this, derivative values (i.e. relative fat mass, appendicular lean mass) can be compared to available body composition reference values[115] to interpret results according to the age of the individual. It is important to note that there are different adaptations to training (i.e. structural, neural, mechanical) and that the increase in strength associated with neural

adaptations is more evident than changes in mass, especially during the initial phase of the training[116]. As such, most studies employ several valid muscular strength and performance measures such as maximum load using one-repetition maximum (1RM) test[117,118] and maximal voluntary contraction (MVC) and power output via dynamometry[119,120]. Dynamometric assessment of strength, unlike 1RM measurement, eliminates the learning effect and may be able to capture changes in force-velocity characteristics of the muscle more effectively[121] and eliminates the effect of training on neural mechanisms (i.e. learning effect). The Short Physical Performance Battery (SPPB) which includes time to sit or stand, gait speed, and balance tests are also utilized to assess physical function in tasks of daily living among older adults[122–125]. In addition to this, it is also important to look at muscle power as it has been shown as a critical determinant of physical functioning and declines earlier compared to muscle strength[126].

Current evidence show that protein intake alone does not determine improvements in body composition and muscle strength and performance. A 10-week randomized controlled study among elderly men (>70 years old) that examined the effect of dietary protein intake on appendicular lean mass and muscle function among elderly men found that consuming protein at twice the RDA level had positive effects on lean body mass and leg power compared to the consuming protein at the RDA level[120]. On the contrary, another study among older adults comparing two levels of protein intake (0.9 g • kg BW^{-1} • day^{-1} versus 1.2 g • kg BW^{-1} •• day^{-1}) with 12-weeks resistance training found no difference in changes in body composition and skeletal muscle fiber size [127]. In a recent study among young healthy men[128] , protein supplementation did not enhance resistance exercise-induced gains in myofiber hypertrophy, satellite cell content, or myonuclear addition in young healthy men and that it only has a modest effect on whole-body lean mass as compared with exercise training without protein supplementation.

Protein supplementation is suggested to enhance muscle mass and performance given that training stimulus is optimized and dietary intake conforms with the recommendations for physically-active adults[129]. On the contrary, several recent meta-analyses[130], showed that protein supplementation in combination with RT may induce fat-

free mass gains but not muscle mass or strength. Another recent meta-analysis[131] concluded that protein supplementation significantly enhanced muscle strength and size with RT in healthy adults but dietary protein intake greater than 1.6 g • kg BW^{-1} • day^{-1} does not have additional benefits in training-induced gains in fat-free mass. Despite the abundance of studies examining the effect of protein supplementation with RT, it is quite difficult to generalize results. This is primarily due to the differences in supplementation protocol (i.e. quantity, source, timing) and also the number and characteristics of the population being studied (i.e. training status, stage of adulthood). It can be concluded though that protein supplementation alone does not determine enhanced responses to training and that other factors (i.e. overall dietary intake, training variables) would also play a role. Hence, aside from optimizing protein intake, a progressive resistance training (RT) protocol should also be employed in order to maximize results and achieve training goals. In general, it is recommended for untrained adults to train 2-3 times a week, utilize multiple sets with loading within the range of 8-12 RM and continuously increase the load by 2-10% when the individual can perform the workload for one to two repetitions over the set number[132].

With long-term RT, it is not yet fully established whether protein supplementation is predictive of improvements in body composition changes and physical performance. A recent review of evidence[133] showed the benefit of protein and amino acid supplementation with RT diminishes and its effect on lean mass, muscle mass and strength improvements are minor or almost negligible, partly owing to the fact that majority of training studies are underpowered. In fact, looking at studies with larger sample sizes, the effect size for protein or amino acid supplementation with RT seemed low and only applicable to a certain subset of individuals due to high individual variability[133]. In addition, it was concluded that individuals who consume adequate calories and protein will not exhibit enhanced and clinically-relevant increases in whole-body lean mass and strength outcomes with protein/amino acid supplementation during RT[133]. This highlights the need for more research in this topic with larger sample sizes, standardized protocols and comparable study designs. It is also highly important to explore the effect of optimal dietary protein intake on enhancing muscle mass and strength outcomes in response to RT

in free-living conditions. A major challenge, however, would be controlling dietary intake and achieving the target level of protein intakes especially in long-term interventions. As such, majority of studies employ strict dietary control by providing pre-packaged meals and protein supplements, which ensures adherence but has limited application in real-life.

2.4 Dietary Implications of Increased Protein Recommendations

Due to protein's ability to increase satiety[134], thermogenesis[135] and its role in muscle mass accretion, high-protein diets have been associated with weight loss and weight regulation benefits[135,136]. Evidence suggests that higher protein diets (30-35% of energy from protein) lead to a decline in energy intake[137,138] compared to low-moderate protein diets (10 – 20% of energy from protein) and may have effects as well in food choice and food preference[139]. Elevated protein recommendations may have the potential to displace other key nutrients in the diet and as such, it is also relevant to explore how this would affect overall diet – quantitatively and qualitatively.

Higher protein diets (30-35% of energy from protein) lead to a decline in overall energy intake[137,138] compared to low-moderate protein diets (10 – 20% of energy from protein) and may have effects as well on food choice and food preference[139]. It has been proposed that there is a potential for protein to displace other food in the diet that provide essential nutrients[140]; however, it is still unclear whether protein directly drives the decrease in carbohydrate and fat intake or if it is a function of the decline in overall energy intake. Concerns have also been raised on the implications of a higher protein diet on cardiovascular health as common protein food that are consumed (i.e. meat, dairy) are significant contributors of fat in the diet[73]. Despite this, protein food may improve nutrient density of diets – ability to meet recommended nutrient intakes within allotted caloric limits[141] as these food are also considered primary sources of other essential nutrients (i.e. calcium, vitamin D, iron, folate, zinc, vitamin E). Several observational studies that investigated the relationship between nutrient-dense protein food - beef[101], pork[142], nuts[143,144], beans[145] and dairy[146], and nutrient adequacy and diet quality found positive associations. Specifically, lean-beef consumers versus high-fat beef consumers[101] and nut

consumers versus non-nut consumers[143] were found to have higher diet quality scores as measured by the HEI. In addition to this, a study looking at the association between DGAI and obesity [147], showed that diets with the highest DGAI scores (healthiest) also had the highest intake of protein. Although this does not show causality, it would be interesting to observe if there would be changes in dietary intake when dietary protein is increased.

CHAPTER 3: METHODOLOGY

This chapter provides an overview of the methods used to address our specific aims and overall hypothesis of this thesis.

3.1 Study Design and Participants

In a prospective and controlled study, recruited participants were randomly assigned to consume protein at the RDA level (0.8 – 1.0 g • kg BW^{-1} • day^{-1}) or 2×RDA (1.6 – 1.8 g • kg BW^{-1} • day^{-1}) throughout a supervised 10-week progressive resistance training program (Figure 3.1). Main outcome variables – body composition and muscle strength and performance, were measured at baseline and after the intervention.

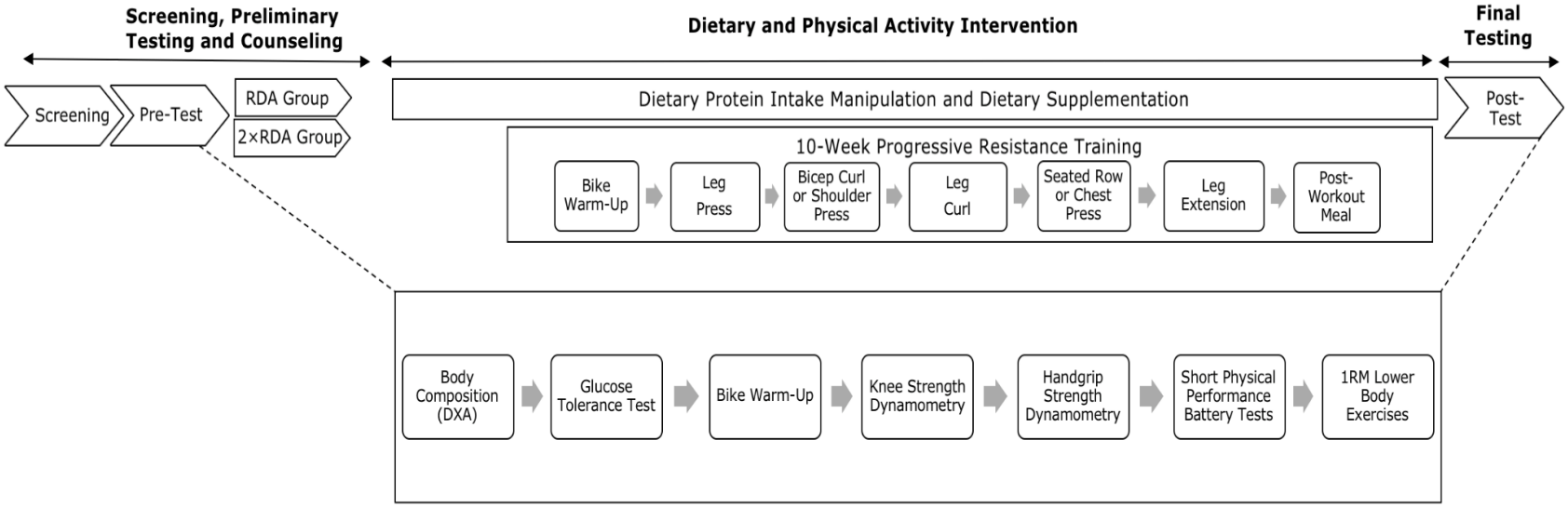


Figure 3.1 Study Design

Middle-aged and older healthy adults (40 – 64 years old) were recruited using advertisements and flyers within the University of Illinois at Urbana-Champaign and surrounding communities; as such the potential subject pool consisted of both university staff and faculty, as well as, residents of nearby communities. Individuals with body mass index (BMI) less than 30 kg/m^2, currently sedentary or not involved in any structured exercise program and weight-stable for the past 6 months were allowed to participate in the study while those with any of the following characteristics were excluded: BMI greater than 30 kg/m^2, diagnosed with phenylketonuria (PKU), uncontrolled hypertension, history of any cardiovascular disease, diabetes mellitus, GI disorders, metabolic disorders, musculoskeletal disorders, diagnosed mental illness, high alcohol consumption, history of tobacco use and those on medications/supplements known to affect protein metabolism (i.e. corticosteroids, NSAIDs, thyroid medications, androgen/estrogen containing compounds). A total of 15 participants have successfully completed the study, with 7 individuals assigned to the RDA group and 8 to the 2×RDA group.

Recruited participants were asked to come in to the lab on two separate pre-screening visits. On the initial visit, an overview of the study was discussed as well as the contents of the informed consent form approved by institutional research board (IRB) at the University of Illinois at Urbana-Champaign. Participants were asked to sign the informed consent form (Appendix 1) prior to any data collection and accomplish a medical history questionnaire and physical activity readiness questionnaire. These were reviewed by a member of the research team to confirm eligibility.

Participants were instructed to wear light clothing and remove items that may add weight prior to assessment. Weight and height were measured using a calibrated weighing scale and stadiometer while waist and hip circumference was measured using a non-elastic flexible tape and by following the minimum waist and maximum hip method[148]. Resting blood pressure (BP) was also measured twice using a digital BP monitor (Omron HEM-907XL, Omron Healthcare, Inc, Bannockburn, IL). Lastly, baseline 3-day online food recalls were administered using the Automated Self-Administered Recall System (ASA 24) version 2016 program, developed by the National Cancer Institute, Bethesda, MD

(https://epi.grants.cancer.gov/asa24) which has been shown to minimize estimation errors compared to other methods of dietary evaluation[149].

Second screening day involved body composition measurement, an oral glucose tolerance test and strength testing. Participants were asked to come in fasted (minimum 10 hours) and rested. Whole body dual-energy X-ray absorptiometry scans (Hologic QDR 4500A, Bedford, MA, USA) were conducted to determine total body weight, fat mass and lean mass. Scans were performed with participants' bladder voided and with no prior exercise on the day before and the morning of the scan. Subsequent analysis was done by identifying customized regions of interest (ROI) which were drawn using the sub-region analysis tool to capture specific segments of the body.

An oral glucose tolerance test was performed by providing the fasted participants with 75 g of glucose dissolved in water and collecting blood samples at different time points during the 2-hour window. Blood samples were collected by inserting a Teflon catheter into an antecubital vein. Prior to the ingestion of glucose load, a baseline blood sample was collected, and then subsequent blood samples were collected after the ingestion - every 15 minutes during the first hour and every 30 minutes during the second hour of the test. The blood samples were immediately analyzed for whole blood glucose concentrations (2300 Stat Plus, YSI Life Sciences, Springs, OH) and subsequently centrifuged at 3000 × g for 10 min at 4°C. Aliquots of plasma were frozen and stored at -80°C until subsequent analysis. Participants with fasting blood sugar values greater than 126 mg/dL or a post-glucose load two-hour blood sugar value greater than 200 mg/dL were excluded from the study. Plasma insulin concentrations were determined using a commercially-available immunoassay kit (ALPCO Diagnostics, Salem, NH). Other blood metabolites were measured using a point-of-care chemistry analyzer (Piccolo Xpress Chemistry Analyzer, Abaxis, Union City, CA).

3.2 Muscular Strength and Performance Testing

A light snack (100 calories (KCAL), 1 g protein (PRO), 17 g carbohydrates (CHO), 4 g fat) and water (300 ml). Participants were then asked to complete a five-minute cycling warm-up with resistance set at 1.5 kp and maintain a cadence of 70 – 80 rpm. Isometric and isokinetic maximal strength of knee extensor and flexor - defined as the highest peak torque in Newton-meters (Nm) recorded in each series of tests[120] was assessed using Biodex System 3 PRO dynamometer (Biodex Medical Systems, NY). The test was performed unilaterally using the participant's dominant leg secured by safety belts and in a seated position. Maximal voluntary isometric contractions were measured twice at three different knee-joint angles – 30°, 60° and 90°. The static peak torque in foot-pounds (ft-lbs) was recorded for each angle. Maximal isokinetic knee extensor and flexor strength was then measured twice at three angular velocities - $60° \cdot s^{-1}$, $120° \cdot s^{-1}$ and $180° \cdot s^{-1}$ and peak torque values (ft-lbs) as well as average power in watts (W) were recorded.

Handgrip strength measurement and a short physical performance battery (SPPB) tests were also conducted to measure physical function. Hand grip strength was measured as the peak-hold needle value in three trials using a hydraulic hand dynamometer with participant upright and dominant arm positioned at a 90-degree angle elbow bend. Balance and functional motilities were assessed using SPPB[124] which consists of a balance test, gait speed test and a chair stand test. In the balance test, participants were instructed to perform three different stances (side-by-side, semi-tandem and tandem) and hold for 10 seconds and was scored accordingly. In the gait speed test, the time required for a participant to walk 4 meters at a normal pace was recorded. Duration was also recorded during the chair stand test wherein participants were instructed to fold their arms across their chest and try to stand up from a chair 5 times as fast as possible. The scores from each part of the SPPB were summed to get the participant's total score out of 12 points. Time for the gait speed test and chair stand test were also noted.

Maximal weight (1RM) for lower body exercises (i.e. leg press, leg curl and leg extension) were also assessed as a measure of strength[117]. Starting with the leg press

exercise, participants were asked to perform two warm-up sets - 10 repetitions at 50% and 5 repetitions at 75% of previously estimated 1RM. This was followed by performing 1 repetition at 85% of 1RM and the next set at previously estimated 1RM with an additional 10 lbs. A series of single attempts (maximum 3 sets) was done with load increasing until participant failed to complete a full repetition and 1RM was determined. Participants were given 90-minute rest periods between sets. A similar 1RM testing protocol was done with the leg curl and leg extension exercises, except there were only 2 warm up sets (5 repetitions at 75% of 1RM and 1 repetition at 85% of 1RM). Muscle strength and physical performance testing was done at baseline and after the 10-week intervention. For the upper body exercises, 1RM was estimated using Landers Formula[150,151] from their 10RM that was assessed during the week 1 and week 10 of training.

3.3 Dietary Counseling, Intervention and Monitoring

Baseline diet recalls were reviewed, and dietary counseling was performed by a member of the research team on the third pre-test day. Participants were counseled on how to adhere to target dietary protein intake within a given caloric limit and a healthy eating pattern. Estimated energy requirement (EER) was calculated using the Institute of Medicine (IOM) factorial equations[152] and was subsequently adjusted using a standard physical activity factor to account for the increased energy expenditure from the resistance exercise training program. Target dietary intake was discussed within the context of the assigned USDA Food Pattern[75] (Appendix 2), MyPlate[78] guidelines (Appendix 3) and protein-specific recommendations in the 2015-2020 Dietary Guidelines for Americans[74]. Using food models, participants were taught how to estimate protein content of common portions of food. Participants were instructed to keep their daily protein intake at 0.8 to 1.0 g • kg BW^{-1} • day^{-1} for the RDA group and 1.6 to 1.8 g • kg BW^{-1} • day^{-1} for the 2×RDA group and to spread protein intake throughout 5 - 6 meals in a day with around 15 g protein per meal for the RDA group and 30 g protein per meal for the 2×RDA group. A sample meal plan was provided to illustrate the target meal pattern and protein

distribution across the day. In addition, protein food items were provided by the research team to supplement the participants' diets.

Dietary intake was controlled during certain time points. Participants ingested calorically-matched post-workout meals (214 KCAL) consisting of a protein-dense food and a carbohydrate beverage immediately after each training bout (RDA group – 16 g PRO, 29 g CHO, 4 g fat; 2×RDA group – 32 g PRO, 12 g CHO, 4 g fat) in the laboratory under supervision. Specifically, the RDA group's post workout meal consisted of 30 g carbohydrate recovery beverage mix (True Nutrition, Post Work-Out Carbohydrate Formula, Vista, CA: 29 g CARB, 108 KCAL) dissolved in 300 ml water and 3 ounce lean beef patty (University of Illinois Meat Science Laboratory, Urbana, IL: 16 g PRO, 0 g CHO, 2 g fat, 85 KCAL) with 2 g beef tallow (Pure Tallow, Fatworks Foods, Denver, CO: 2.2 g fat, 20 KCA). The 2×RDA group's post workout meal consisted of 12.5 g carbohydrate recovery beverage mix (True Nutrition, Post Work-Out Carbohydrate Formula, Vista, CA: 12 g CHO, 45 KCAL) dissolved in 300 ml water and a 6 ounce lean beef patty (University of Illinois Meat Science Laboratory, Urbana, IL: 32 g PRO, 4.5 g fat 169 KCAL). The beef patties were cooked until an internal temperature of 155°F was reached and maintained for 15 seconds. In addition to this, participants also ingested a pre-sleep protein beverage daily. The RDA group's protein beverage consisted of 17 g beef protein isolate powder (Isoprime 100% Beef Protein Isolate, Maximum Human Performance, West Caldwell, NJ: 15 g PRO, 0.6 g CHO, 0 g fat, 62 KCAL) mixed with 17 g of maltodextrin (Maltodextrin, True Nutrition, Vista, CA: 0 g PRO, 16 g CHO, 0 g fat, 62 KCAL). The 2×RDA group's protein beverage consisted of 30 g beef protein isolate powder (Isoprime 100% Beef Protein Isolate, Maximum Human Performance, West Caldwell, NJ: 30 g PRO, 1.2 g CHO, 0 g fat 124 KCAL). Participants were instructed to add 300 ml of water to the protein powder and to consume the supplement daily, 1 to 2 hours before sleep during the duration of the study. To monitor adherence, participants returned the supplement bottles on a weekly basis and the number of unconsumed supplements was noted by a member of the research team. In addition to this, individually packaged semi-dried lean beef jerky (Graze Bar Tasty Original, Mission Meats, Decorah, IA: 60 KCAL, 9 g PRO, 1 g CARB, 2 g FAT) was provided to the participants to

assist in meeting target protein intake in instances when dietary protein may not be readily available.

Additional three-day food recalls were collected every other week throughout the intervention to monitor adherence and serve as basis for providing feedback to participants during the continuous dietary counseling in achieving dietary goals.

3.4 Resistance Exercise Training and Physical Activity Control

Participants were instructed to maintain usual activities of daily living throughout the duration of the study and not to engage in any other strenuous structured exercise program outside the laboratory.

Participants came to the laboratory for 1-hour of supervised training three times a week for 10 weeks with each training session separated by at least one day. The training session consisted of three lower body exercises (leg press, leg curl, leg extension) and two upper body exercises (bicep curl and seated row or shoulder press and chest press) using guided weight machines. Each session began with a five-minute cycling warm up with resistance set at 1.5 kp and cadence of 70 – 80 rpm. Each exercise had two warm-up sets followed by three working sets of 10 repetitions each with 90-minute rest periods between sets. Training intensity was based on measured 1RM of participants with 70% 1RM (10 – 15 repetitions) to 80% 1RM (8 – 10 repetitions) being the target for the first two weeks of training. When 10 repetitions can be performed with proper form in all 4 working sets, workload was adjusted accordingly. Midway through the program (week 5), 1RM for lower body exercises and 10RM for upper body exercises were assessed to serve as a basis for progression.

3.5 Data Management and Statistical Analysis

Dietary Data Calculations

Dietary intake values which were below or above the 25th and 75th percentiles were considered outliers and were not included in the analysis. Macronutrient composition of caloric intake was determined and expressed a percent of calories and absolute total intake per day. In addition, if applicable, dietary variables were expressed in relative terms (i.e. g • kg BW^{-1} • day^{-1}). Eating pattern was determined by classifying eating occasions into meals and evaluating caloric intake and macronutrient composition of each meal. Baseline dietary intake was compared to their intake throughout the study by calculating the average intake from data collected using the sets of diet recalls administered from week 1 to week 10. Compliance to dietary intervention was calculated using the actual amount of food items consumed by participants compared to the amount provided throughout the intervention, expressed in percentage.

Based on the participant's assigned USDA Food Pattern (see Appendix 2), the DGAI score was determined. A maximum DGAI score of 20 consists of a food intake sub score (11 points total) and a healthy choice sub score (9 points total). The food intake sub score includes recommendations for 5 vegetable subgroups (dark green vegetables, red or orange vegetables, starchy vegetables, legumes and other vegetables), fruit, variety of fruit and vegetables, grains, meat and beans, dairy and added sugars while the healthy choice sub score consists of percentage of whole grain intake, fiber intake, total fat intake, saturated fat intake, cholesterol intake, selection of low-fat meat and dairy products, sodium intake and alcohol consumption[147]. A continuous score from 0 (complete nonadherence) to 1 (complete adherence)[153] was assigned per component. A penalty was also applied for overconsumption of certain food groups that are considered energy-dense such grains, meat, dairy and starchy vegetables. Intakes greater than 1.25-times the recommended amount are given a truncated score up to a maximum of 0.5 points [153]. The scoring guide for the updated 2015 version of the DGAI can be found in Appendix 4.

Strength Data and Training Compliance Calculations

The mean peak torque values (ft-lbs) for isometric contractions at 30°, 60° and 90° knee-joint angles were converted to Newton-meters (Nm) using the conversion factor $\frac{1.36\ Nm}{1\ ft-lb}$ and presented as isometric extension average peak torque and isometric flexion average peak torque. The mean peak torque values (ft-lbs) for isokinetic knee extensor and flexor strength at 60° • s^{-1}, 120° • s^{-1} and 180° • s^{-1} angular velocities were converted to Newton-meters (Nm) using the conversion factor $\frac{1.36\ Nm}{1\ ft-lb}$ and presented as isokinetic extension average peak torque and isokinetic flexion average peak torque. Changes in muscle strength, performance and power measures were compared by calculating percent change values.

Training compliance was calculated using the number of trainings sessions attended divided by the total number of training sessions conducted and was expressed in percentage. A training compliance threshold value was set at 80% and as such, participants with less than 80% training attendance were not included in data analysis.

Statistical Tests

Data collected were encoded and reviewed prior to any statistical analysis. Differences between group means were analyzed using independent samples t-test. Changes in main outcome variables (i.e. strength measures, body composition) and other secondary outcomes (i.e. dietary intake, clinical variables) were assessed by a 2-factor (time x condition) analysis of variance (ANOVA). For all statistically significant interaction effects identified in the ANOVA, Tukey's post hoc tests were performed to determine the differences between means for all significant main effects and interactions. All analysis were carried out using IBM SPSS Statistics (version 24, Chicago, IL) with a statistical significance level set at $P < 0.05$. All data presented are expressed as mean ± SEMs.

CHAPTER 4: RESULTS

This chapter describes the results of the study outlined in this thesis.

4.1 Study Participants

Fifteen healthy recreationally active middle-aged and older adults successfully completed the study. Baseline characteristics of participants are shown in Table 4.1 below.

Table 4.1 Participant Baseline Characteristics[1]

Variable	RDA	2×RDA
n (females)	7 (6)	8 (2)
Age (y)	54 ± 4	51 ± 3
Height (cm)	163.3 ± 3.3	175.5 ± 3.2*
Weight (kg)	73. 7 ± 3.5	87.2 ± 4.7*
BMI (kg/m^2)	27.6 ± 0.5	28.2 ± 0.8
Blood Pressure (Diastolic/Systolic, mmHg)	89 ± 8 / 125 ± 7	77 ± 3 / 124 ± 4
Fasting Blood Glucose (mg/dL)	80.8 ± 2.2	84.0 ± 3.4
Waist-to-Hip Ratio	0.95 ± 0.05	1.01 ± 0.03
Physical Activity (GLPAQ score)	16 ± 5	14 ± 5
Estimated TEE, kcal/kg BW/d[2]	29.7 ± 0.6	30.7 ± 0.8
Habitual Baseline Energy Intake, kcal/kg BW/d[3]	24.4 ± 1.2	29.8 ± 1.0*

[1]*Data are mean ± SEMs.* *significantly different between groups ($P < 0.05$). RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance.

[2]Estimated using Institute of Medicine Estimated Energy Requirements equation[152]

[3]Estimated based on 3-day food recalls

There were no significant differences in baseline characteristics between groups except for weight, height and relative energy intake at baseline ($P < 0.05$). This is due to the fact that a large number of the study participants recruited were females and were mostly randomized to the RDA group. As such, majority of the data that are presented in the subsequent sections of this paper are expressed relative to body mass (i.e. per kilogram body weight), where applicable.

Adherence to dietary intervention during the study were similar in both groups (92 ± 2% vs 96 ± 1%, P = 0.081). Training compliance was also similar (P = 0.068) between the RDA group (91 ± 2%) and the 2×RDA group (95± 2%).

4.2 Dietary Intake

Average macronutrient intakes at baseline and during the study are summarized in Table 4.2.

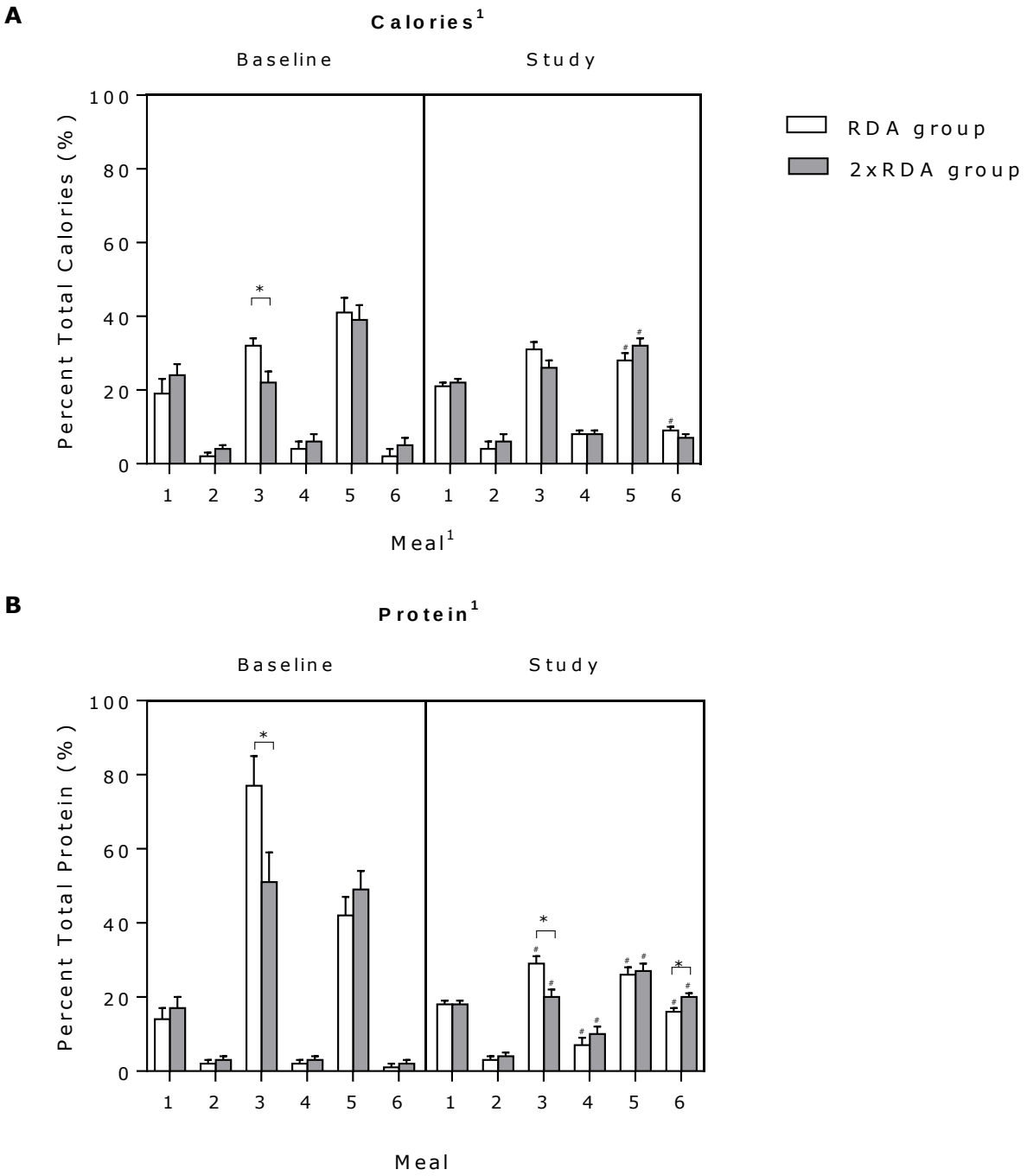
Table 4.2 Macronutrient Intake[1]

Variable	RDA (n = 7)		2×RDA (n = 8)	
	Baseline	**Study**	**Baseline**	**Study**
Energy intake, kcal • kg BW $^{-1}$ • d^{-1}	24.4 ± 1.2	22.8 ± 1.6*	29.8 ± 1.0	29.9 ± 1.4*
Protein intake, relative, g • kg $^{-1}$ • d^{-1}	1.0 ± 0.1	1.2 ± 0.1$^{\#}$	1.3± 0.1*	1.8 ± 0.1$^{\#*}$
Carbohydrate intake, relative, g • kg $^{-1}$ • d^{-1}	2.4 ± 0.3	2.5 ± 0.2	3.5 ± 0.3	3.0 ± 0.2$^{\#}$
Fiber, g	14.9 ± 3.7	13.8 ± 3.2	26.4 ± 2.9*	22.4 ± 2.5*
Fat intake				
Total, g /d	76.1 ± 5.2	62.1 ± 5.6	103.9 ± 4 .5*	105.6 ± 5.9*
Saturated Fat, g/d	29.0 ± 2.7	22.1 ± 34.8$^{\#}$	34.8 ± 2.3*	31.9 ± 2.4$^{\#*}$
Cholesterol, mg/d	194.2 ± 37.1	247.5 ± 38.2	326.9 ± 32.1*	442.8 ± 33.1$^{\#*}$
Dietary Intervention Compliance, %	-	92 ± 2	-	91 ± 1
BUN, mg/dl	13.0 ± 0.7	15.1 ± 1.0$^{\#}$	15.1 ± 0.6	19.0 ± 0.8$^{\#}$
Creatinine, mg/dL	0.7 ± 0.1	1.0 ± 0.1	0.87 ± 0.1	1.2 ± 0.9
BUN:Creatinine	15.7 ± 1.8	16.7 ± 1.0	16.6 ± 1.5	16.7 ± 0.9

[1]Data are mean ± SEMs. RDA is 0.8 – 1.0 g protein · kg $^{-1}$ · d^{-1}; 2xRDA is 1.6 g protein · kg $^{-1}$ · d^{-1}. Baseline represents measurements collected prior to start of diet lead-in week and resistance exercise training and study represents average values throughout the 10-week dietary and training intervention. $^{\#}$Different from baseline, P < 0.05. *Different between groups, P < 0.05. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance. BUN, Blood Urea Nitrogen

Caloric intake remained similar from baseline (P = 0.731) in both groups but the 2×RDA group had a higher caloric intake during the study compared to the RDA group (29.9 ± 1.4 versus 22.8 ± 1.6 g • kg $^{-1}$ • d^{-1}, P < 0.05). Relative protein intake increased from

baseline ($P < 0.05$) in the RDA group (41 ± 20%) and the 2×RDA (48 ± 9%) without difference between groups ($P = 0.714$). During the study, dietary protein intake was greater in the 2×RDA group (1.85 ± 0.09 g • kg $^{-1}$ • d^{-1}) compared to the RDA group (1.20 ± 0.05 g • kg $^{-1}$ • d^{-1}, $P < 0.001$). Fat intake during the study was similar to baseline ($P = 0.079$) in both groups but intake of saturated fat decreased significantly over time ($P < 0.05$), with no differences between the two groups ($P = 0.281$). Compared to baseline, intake of carbohydrate during the study remained the same for the RDA group (2.4 ± 0.3 versus 2.5 ± 0.2 g • kg $^{-1}$ • d^{-1}, $P = 0.5906$) but significantly decreased in the 2×RDA group (3.5 ± 0.3 versus 3.0 ± 0.2 g • kg $^{-1}$ • d^{-1}, $P < 0.05$). The average contribution of each meal to total caloric and protein intake at baseline and during the study is shown in Figure 4.1.



*[1]Average percentage total calorie (A) and percentage total protein (B) contributed by meals at baseline and during the study in the RDA (n = 7) and 2×RDA (n = 8) groups. Baseline represents measurements collected prior to start of diet lead-in week and resistance exercise training and study represents average values throughout the 10-week dietary and training intervention. Meal numbers refer to the following eating occasions: 1 – breakfast, 2 – morning snack, 3 – lunch, 4 – afternoon snack, 5 - dinner and 6 – bedtime snack. Data are expressed as means ± SEMs. *Different between groups, P < 0.05. RDA, Recommended Daily Allowance; 2×RDA, twice the Recommended Daily Allowance.*

Figure 4.1 Distribution of Calories and Protein

Compared to baseline, intake of calories was less skewed towards lunch and dinner during the study (Figure 4.1A) in the RDA group (breakfast: 19 ± 1% vs 21 ± 2 %, $P = 0.172$; lunch: 32 ± 3 % vs 31 ± 2 %, $P = 0.794$; dinner: 41± 4 % vs 28 ± 23 %, $P < 0.05$; snacks: 8 ± 3 % vs 20 ± 2 %, $P < 0.05$) and 2×RDA group (breakfast: 24 ± 4 % vs 22 ± 21 %, $P = 0.614$; lunch: 22 ± 2 % vs 26 ± 2 %, $P = 0.194$; dinner: 39± 4 % vs 32 ± 1 %, $P = 0.094$; snacks: 15 ± 6 % vs 20 ± 2 %, $P = 0.167$). Likewise, distribution of protein across meals (Figure 4.1B) was less skewed towards lunch and dinner during the study in the RDA group (breakfast: 14 ± 2 % vs 18 ± 2 %, $P = 0.190$; lunch: 77 ± 10 % vs 29 ± 2 %, $P < 0.05$; dinner: 42 ± 5 % vs 26 ± 2 %, $P < 0.05$; snacks: 5 ± 2 % vs 26 ± 2 %, $P < 0.001$) and 2×RDA group (breakfast: 16 ± 4 % vs 18 ± 1 %, $P = 0.673$; lunch: 51 ± 7 % vs 20 ± 1 %, $P < 0.05$; dinner: 49 ± 5 % vs 28 ± 1 %, $P < 0.05$; snacks: 8 ± 3 % vs 34 ± 1 %, $P < 0.001$). Table 4.3 shows that average calories and protein amounts contributed by each meal at baseline and during the study.

Table 4.3 Caloric and Protein Content of Meals[1]

Variable	RDA (n = 7)				2xRDA (n = 8)			
	Baseline		Study		Baseline		Study	
	Amount	%TI	Amount	%TI	Amount	%TI	Amount	%TI
Breakfast								
Calories, kcal	325 ± 33	19 ± 1	343 ± 28*	21 ± 1	678 ± 165	24 ± 4	564 ± 38*	22 ± 1
Protein, g	10.5 ± 2.3	14 ± 2	15.8 ± 1.4*	18 ± 1	18 ± 4	16 ± 4	29.0 ± 1.5*#	18 ± 1
Morning Snack								
Calories, kcal	45 ± 23	2 ± 1	62 ± 22	4 ± 1	100 ± 38	4 ± 1	149 ± 41	6 ± 2
Protein, g	1.2 ± 0.5	2 ± 1	2.5 ± 1.1	3 ± 1	2.8 ± 1.7	3 ± 2	5.7 ± 1.1	4 ± 1
Lunch								
Calories, kcal	575 ± 89	32 ± 3	508 ± 47*	31 ± 2	598 ± 78	22 ± 3	698 ± 68*	26 ± 2
Protein, g	27.2 ± 3.9	77 ± 10*	25.3 ± 1.9*	29 ± 2*#	29.3 ± 5.3	51 ± 7*	33.2 ± 2.8*	20 ± 1*#
Afternoon Snack								
Calories, kcal	70 ± 33	4 ± 2	122 ± 18*	8 ± 1	136 ± 42	6 ± 2	201 ± 24*	8 ± 1
Protein, g	1.8 ± 0.7	2 ± 1	6.4 ± 1.3*#	7 ± 1	3.4 ± 1.1	3 ± 1	16.9 ± 3.0*#	10 ± 2
Dinner								
Calories, kcal	720 ± 128	41 ± 4	490 ± 86*#	28 ± 3#	1063 ± 128	39 ± 4	834 ± 70*#	32 ± 1#
Protein, g	28.6 ± 4.3*	42 ± 5	23.3 ± 2.7*#	26 ± 2#	53.9 ± 6.5*	49 ± 5	44.6 ± 44.0*#	27 ± 1#
Bedtime Snack								
Calories, kcal	46 ± 21	2 ± 1	143 ± 10	9 ± 1#	140 ± 60	5 ± 2	188 ± 28	7 ± 1
Protein, g	0.6 ± 0.3	1 ± 0	13.8 ± 0.7*#	16 ± 1*#	2.7 ± 1.4	3 ± 1	31.6 ± 1.6*#	20 ± 1*#

[1]Data are mean ± SEMs. RDA is 0.8 – 1.0 g protein · kg $^{-1}$ · d^{-1}; 2xRDA is 1.6 g protein · kg $^{-1}$ · d^{-1}. Baseline represents measurements collected prior to start of diet lead-in week and resistance exercise training and study represents average values throughout the 10-week dietary and training intervention. #Different from baseline, $P < 0.05$. *Different between groups, $P < 0.05$. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance. % TI, Percent of Total Intake.

Examination of absolute intakes, there were no significant changes over time in caloric intake at any eating occasion in both groups; however, caloric intake of the 2×RDA group was higher during breakfast ($P = 0.002$), lunch ($P = 0.045$) and dinner ($P = 0.004$) compared to the RDA group during the study. At baseline, protein intake at dinner was significantly higher in the 2×RDA group compared to the RDA group (58 ± 6 g vs 30 ± 6 g, $P < 0.05$). Similarly, during the study, 2×RDA had higher protein intakes at breakfast ($P < 0.001$), lunch ($P < 0.05$), afternoon snack ($P < 0.05$), dinner ($P < 0.05$) and bedtime snack ($P < 0.001$) compared to the RDA group. Protein intake was less skewed towards dinner during the study with an increase in protein consumption during breakfast observed during the study in both RDA (10 ± 2 g vs 16 ± 1 g, $P < 0.05$) and 2×RDA group (18 ± 4 g vs 29 ± 2 g, $P < 0.05$).

Micronutrients of public health concern and those associated with intake of red meat were also assessed along with diet quality scores. Results of average intake at baseline and during the study are presented in Table 4.4.

Table 4.4 Micronutrients and Diet Quality[1]

Variable	RDA (n = 7)		2×RDA (n = 8)	
	Baseline	**Study**	**Baseline**	**Study**
Micronutrients				
B6, mg/d	1.5 ± 0.2	1.7 ± 0.1	2.8 ± 0.2	2.4 ± 0.1
Folate, µg/d	388.2 ± 68.0	290.7 ± 34.9#	645.7 ± 72.3	549.2 ± 60.5#
B12, µg/d	4.13 ± 0.8	5.6 ± 0.7	7.2 ± 0.9	9.2 ± 2.3
D, µg/d	3.0 ± 0.8	3.7 ± 0.5	8.9 ± 2.1	7.0 ± 0.9
Iron, mg/d	12.6 ± 2.1	13.7 ± 1.6	27.4 ± 5.3	21.1 ± 2.3
Zinc, mg/d	9.9 ± 1.5	11.2 ± 1.6	16.9 ± 1.2	16.6 ± 1.2
Calcium, mg/d	793.6 ± 121.4	689.8 ± 140.5	1355.8 ± 219.2	1165.0 ± 150.2
Choline, mg/d	269.3 ± 28.6	267.3 ± 26.2	394.1 ± 37.1	460.4 ± 35.6
Sodium, mg/d	3033 ± 339	3113 ± 217	4596 ± 543	4814 ± 423
Potassium, mg/d	2163 ± 242	2141 ± 263#	3702 ± 418	3599 ± 345#
DGAI[2]	5 ± 1	6 ± 0	6 ± 1	6 ± 1
Food Intake Score	3 ± 1	3 ± 0	3 ± 1	3 ± 0
Healthy Choice Score	2 ± 0	3 ± 0	3 ± 0	3 ± 0

[1]Data are means ± SEMs. Baseline represents prior to start of diet lead-in week and training and study represents average values throughout the intervention. #Different from baseline, P < 0.05. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance
[2]DGAI, Dietary Guidelines Adherence Index[154]

There were no significant changes in micronutrient intake in both groups during the study except for folate and potassium. Folate intake declined ($P < 0.05$) from baseline in the RDA group (16 ± 11%) and the 2×RDA group (12 ± 9%) without differences between groups ($P = 0.745$). In addition, DGAI scores did not significantly change over time ($P = 0.089$) in both groups as both food intake ($P = 0.874$) and healthy choice ($P = 0.090$) sub scores during the study remained similar over time.

4.3 Anthropometric, Clinical and Body Composition Outcomes

Results of anthropometric, biochemical and clinical measures collected at pre-intervention and post-intervention are presented in Table 4.5.

Table 4.5 Anthropometric and Clinical Measures[1]

Variable	RDA (n = 7)		2×RDA (n = 8)	
	Pre	**Post**	**Pre**	**Post**
Weight, kg	73.7 ± 4.4	73.9 ± 4.1	87.2 ± 4.1	88.1 ± 3.8
BMI, kg/m^2	27.6 ± 0.7	27.8 ± 0.8	28.2 ± 0.7	28.4 ± 0.8
Waist Circumference, cm	97.5 ± 3.3	97.8 ± 3.0	105.2 ± 3.6	104.1 ± 3.2
Hip Circumference. cm	102.8 ± 3.3	103.3 ± 4.2	105.8 ± 3.6	107.7 ± 4.5
Waist-Hip Ratio	0.96 ± 0.0	0.99 ± 0.0	1.0 ± 0.1	1.1 ± 0.1
Blood Pressure				
Systolic, mmHg	123 ± 8	100 ± 10$^{\#}$	123 ± 7	100 ± 9$^{\#}$
Diastolic, mmHg	93 ± 8	99 ± 14	77 ± 8	86 ± 130

[1]Data are means ± SEMs. Pre represents measurements collected prior to start of the intervention and post represents measurements after the intervention. #Different from baseline, $P < 0.05$. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance. BMI, Body Mass Index.

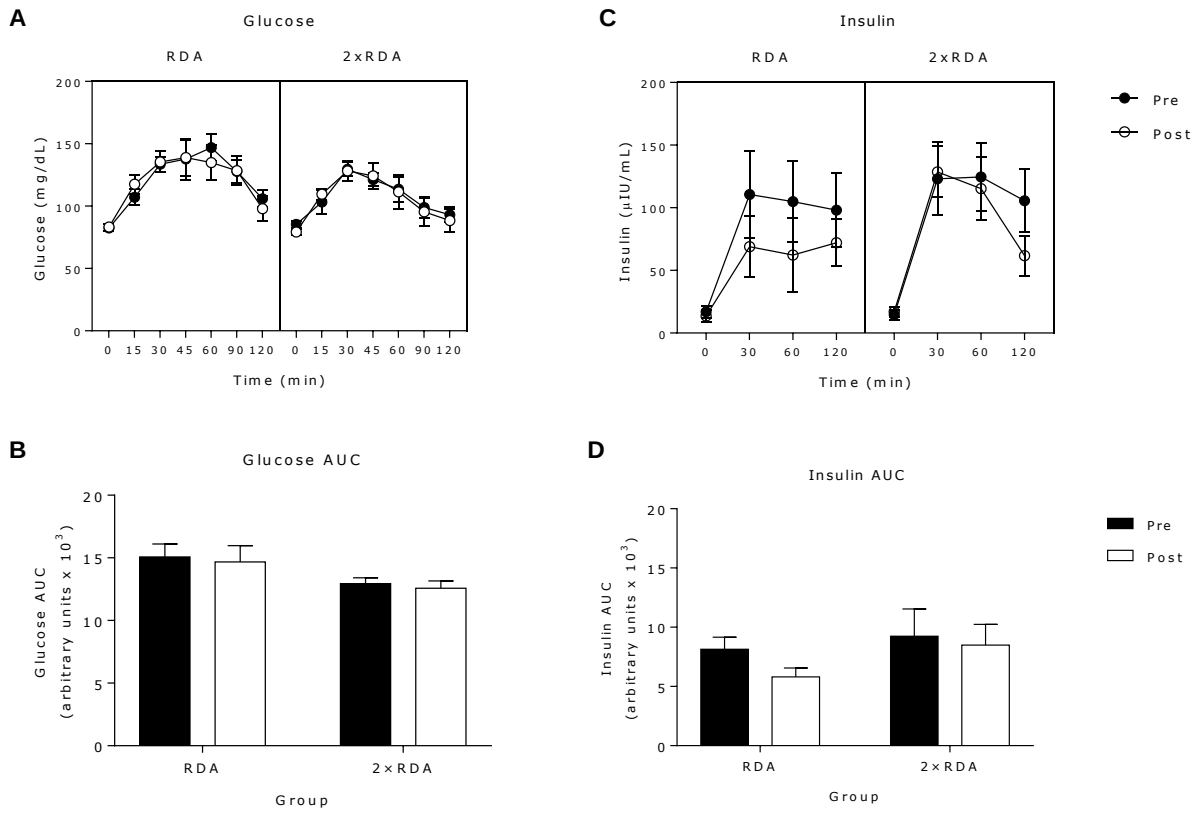
There were no significant changes from baseline in the anthropometric, clinical and biochemical outcomes except for systolic blood pressure. A significant decrease in systolic blood pressure ($P < 0.05$) over time was observed in both groups ($P = 0.967$). Table 4.6 summarizes results of biochemical measures that were assessed pre- and post-intervention.

Table 4.6 Biochemical Measures[1]

Variable	RDA (n = 7)		2×RDA (n = 8)	
	Pre	**Post**	**Pre**	**Post**
Fasting Lipid Levels				
Cholesterol, mg/dL	190.0 ± 16.3	184.0 ± 15.1	202.4 ± 11.6	199.1 ± 10.7
Triglycerides, mg/dL	120.2 ± 26.2	116.8 ± 22.2	145.9 ± 22.7	137.4 ± 19.2
HDL, mg/dL	60.5 ± 5.2	60.0 ± 4.1	49.0 ± 3.9	47.1 ± 3.1
LDL, mg/dL	121.5 ± 13.8	117.7 ± 13.1	120.1 ± 11.9	119.8 ± 11.3
VLDL, mg/dL	24.0 ± 5.3	23.5 ± 4.4	29.1 ± 4.6	27.5 ± 3.8
Fasting Blood Glucose, mg/dL	80.8 ± 2.3	82.6 ± 2.5	81.4 ± 2.3	81.3 ± 2.5
Insulin Sensitivity Indices				
HOMA-IR	3.2 ± 1.0	2.2 ± 1.0	2.4 ± 0.7	3.3 ± 0.8
ISI-M	3.3 ± 1.5	4.2 ± 1.5	4.8 ± 1.3	4.6 ± 1.3
$MCR_{Glucose}$, mg/kg/min	6.4 ± 0.94	7.2 ± 0.8	6.3 ± 0.9	7.6 ± 0.7
ISI_{est},μmol/kg/min/pM	0.07 ± 0.01	0.14 ± 0.01#	0.07 ± 0.01	0.13 ± 0.01#
Beta-cell Function Indices				
IGI	3.8 ± 1.1	1.5 ± 0.5	4.7 ± 0.9	2.2 ± 0.4
1stPH Insulin Release, pM	1282.5 ± 362.3	1130.2 ± 232.9#*	1654.0 ± 335.4	1770.1 ± 215.6#*
2ndPH Insulin Release, pM	412.2 ± 230.1	113.9 ± 14.0#	487.3 ± 91.1	138.0 ±13.0#

[1]Data are means ± SEMs. Pre represents measurements collected prior to start of the intervention and post represents measurements after the intervention. #Different from baseline, $P < 0.05$. *Different between groups, $P < 0.05$. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance. HDL, High Density Lipoprotein. LDL, Low Density Lipoprotein. VLDL, Very Low Density Lipoprotein. HOMA IR, Homeostatic Model Assessment of Insulin Resistance. ISI-M, Matsuda–DeFronzo Insulin Sensitivity Index. MCR_{est} Glucose, estimated Metabolic Clearance Rate of Glucose, ISI_{est}, estimated Insulin Sensitivity Index. IGI, Insulinogenic Index. 1stPH, First Phase Insulin Release. 2ndPH, Second Phase Insulin Release.

Fasting lipid levels also tended to decrease over time; however, these changes were not significant. Measures of insulin sensitivity and beta-cell function tended to improve in both groups from baseline. Specifically, there was a significant improvement in the ISI_{est} over time ($P < 0.001$) in both the RDA and the 2×RDA group (91 ± 67% and 348 ± 281%, $P = 0.660$). Additionally, both 1st PH insulin release ($P < 0.05$) and 2nd PH insulin release ($P < 0.001$) improved over time. The RDA group had greater improvement in 1st PH insulin release compared to the 2×RDA group (102 ± 127% and 23 ± 21%, $P < 0.05$). Figure 4.2 illustrates the glucose and insulin response curves during the OGTT pre-intervention ad post-intervention.



[1]*Average glucose (A) and insulin (C) levels during key time-points of Oral Glucose Tolerance Testing as well as average area under the curve for glucose (B) and insulin (D) in the RDA (n = 7) and 2×RDA (n = 8) groups. Pre represents measurements collected prior to start of diet lead-in week and resistance exercise training and post represents measurements collected during post-intervention testing. Data are expressed as means ± SEMs. AUC; Area Under the Curve. RDA, Recommended Daily Allowance; 2×RDA, twice the Recommended Daily Allowance.*

Figure 4.2 Glucose and Insulin Response

Improvements observed in insulin sensitivity indices and beta-cell function indices are reflected in the glucose (Figure 4.2A) and insulin (Figure 4.2C) response curves. The 2-hour glucose values tended to be lower during post-intervention in the RDA group (105.99 ± 7.96 mg/dL versus 97.85 ± 11.34 mg/dL, $P = 0.483$) and the 2×RDA group (93.01 ± 4.88 mg/dL versus 88.36 ± 7.76 mg/dL, $P = 0.832$). Consequently, 2-hour insulin values also tended to be lower during post-intervention in the RDA group (100.02 ± 12.83 µIU/L versus 71.93 ± 18.13 µIU/L, $P = 0.151$) and the 2×RDA group (105.59 ± 30.60 µIU/L versus 61.77 ± 13.83 µIU/L, $P = 0.143$). Furthermore, AUC for both glucose (Figure 4.2B) and insulin (Figure 4.2D) tended to decrease in both groups when comparing pre- and post-intervention; however, these changes are not significant.

Results of body composition analysis at baseline and after the intervention are presented in Table 4.7 below.

Table 4.7 Body Composition[1]

Variable	RDA (n = 7)		2×RDA (n = 8)	
	Pre	**Post**	**Pre**	**Post**
Body Mass, kg	73.9 ± 4.3	75. ± 3.7	87.6 ± 4.0	87.8 ± 3.4
Fat Mass, total, kg	26.9 ± 2.0	26.1 ± 2.2#	22.7 ± 1.8	23.2 ± 2.1#
Body Fat, %	36.6 ± 2.2	35.4 ± 2.6	26.0 ± 2.0	26.5 ± 2.4
Absolute Lean Mass Measures				
Whole Body Lean Mass, kg	44.8 ± 3.6	45.8 ± 3.6	62.2 ± 3.4	61.9 ± 3.4
Lean/Height^2, kg/m^2	16.7 ± 0.8	17.2 ± 1.0	20.1 ± 0.8	20.2 ± 1.0
Appen. Lean Mass/Height^2, kg/m^2	6.9 ± 0.5	7.2 ± 0.5#	8.9 ± 0.5	9.2 ± 0.5#
Relative Lean Body Mass Measures				
Whole Body Lean Mass/Total Mass, %	60.4 ± 2.1	60.9 ± 2.7	70.9 ± 2.0	69.6 ± 2.5
Appen. Lean Mass/Total Mass, %	25.0 ± 1.5	25.7 ± 1.6	31.6 ± 1.4	32.0 ± 1.5

[1]Data are means ± SEMs. Pre represents measurements collected prior to start of the intervention and post represents measurements after the intervention. #Different from baseline, within group, $p < 0.05$. *Different between groups $P < 0.05$. *P values* were determined by using Tukey's post hoc test. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance. Appen, Appendicular.
[2]Calculated by 2-factor repeated-measures ANOVA

Total fat mass changed over time ($P < 0.05$) – RDA group decreased (3 ± 2 %) while there was an increase in the 2×RDA group (2 ± 4 %) but there was no significant difference between groups ($P = 0.293$). Appendicular lean mass increased over time ($P < 0.05$) with no difference between the RDA group (5 ± 2 %) and the 2×RDA group (3 ± 2 %, $P = 0.790$). Changes in lower body lean mass and fat mass are illustrated in Figure 4.3.

[1]Changes in lower body lean mass (**A**) and lower body fat mass (**B**) after the 10 weeks of resistance training. Data are presented as Mean ± SEM. [#]Significant change over time, $P < 0.05$. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance.

Figure 4.3. Changes in Lower Body Lean Mass and Fat Mass

Lower body lean mass (Figure 4.3A) increased over time ($P < 0.05$) in the RDA group (6 ± 2 %). and 2×RDA group (3 ± 2 %) without difference between the two groups ($P = 0.816$). Lower body fat mass tended to decrease in the RDA group and increase in the 2×RDA group (Figure4.3B: -3 ± 2 % vs 2 ± 5 %, $P = 0.399$)

4.4 Muscle Strength and Performance Measures

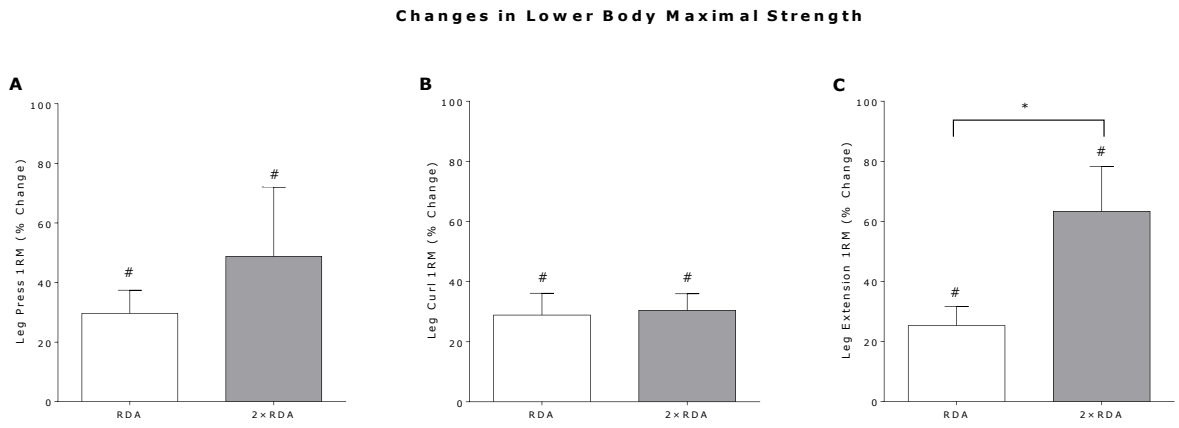
Significant improvements in muscle strength and performance were observed after the intervention. Maximum Load (1RM) for upper body exercises, handgrip strength and SPPB results are summarized in Table 4.8

Table 4.8 Muscle Strength and Physical Performance Measures[1,2]

Variable	RDA (n = 7)		2×RDA (n = 8)	
	Pre	**Post**	**Pre**	**Post**
Maximum Load (1RM)				
Chest Press, kg	32.8 ± 5.1	41.7 ± 5.3#	54.7 ± 6.8	68.80 ± 8.2#
Seated Row, kg	35.2 ± 3.4	49.5 ± 2.6#	60.0 ± 5.4	70.7 ± 5.8#
Bicep Curl, kg	15.6 ± 1.4	18.3 ± 1.3#*	24.7 ± 2.8	34.2 ± 3.2#*
Shoulder Press, kg	10.1 ± 1.5	18.8 ± 3.1#	20.5 ± 3.3	34.2 ± 4.4#
Handgrip Strength, kg	33.3 ± 2.9	31.1 ± 2.0	45.2 ± 3.6	45.5 ± 3.1
SPPB, total points	11 ± 1	11 ± 1	11 ± 0	11 ± 0
Balance test, points	4 ± 0	4 ± 0	4 ± 0	4 ± 0
Gait test, points	4 ± 0	4 ± 0	4 ± 0	4 ± 0
Gait speed time, secs	4.3 ±0.3	3.3 ± 0.3	3.5 ± 0.3	2.8 ± 0.3
Chair stand test, points	3 ± 0	3 ± 0	3 ± 0	4 ± 0
Chair stand time, secs	12.9 ± 0.8	11.2 ± 1.0	11.5 ± 0.7	9.3 ± 1.0

[1]Data are means ± SEMs. Pre represents measurements collected prior to start of the intervention and post represents measurements after the intervention. *Significant main effect or interaction, P < 0.05. #Different from baseline, within group, *P* < 0.05. *Different between groups, *P* < 0.05. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance.
[2]Calculated by 2-factor repeated-measures ANOVA

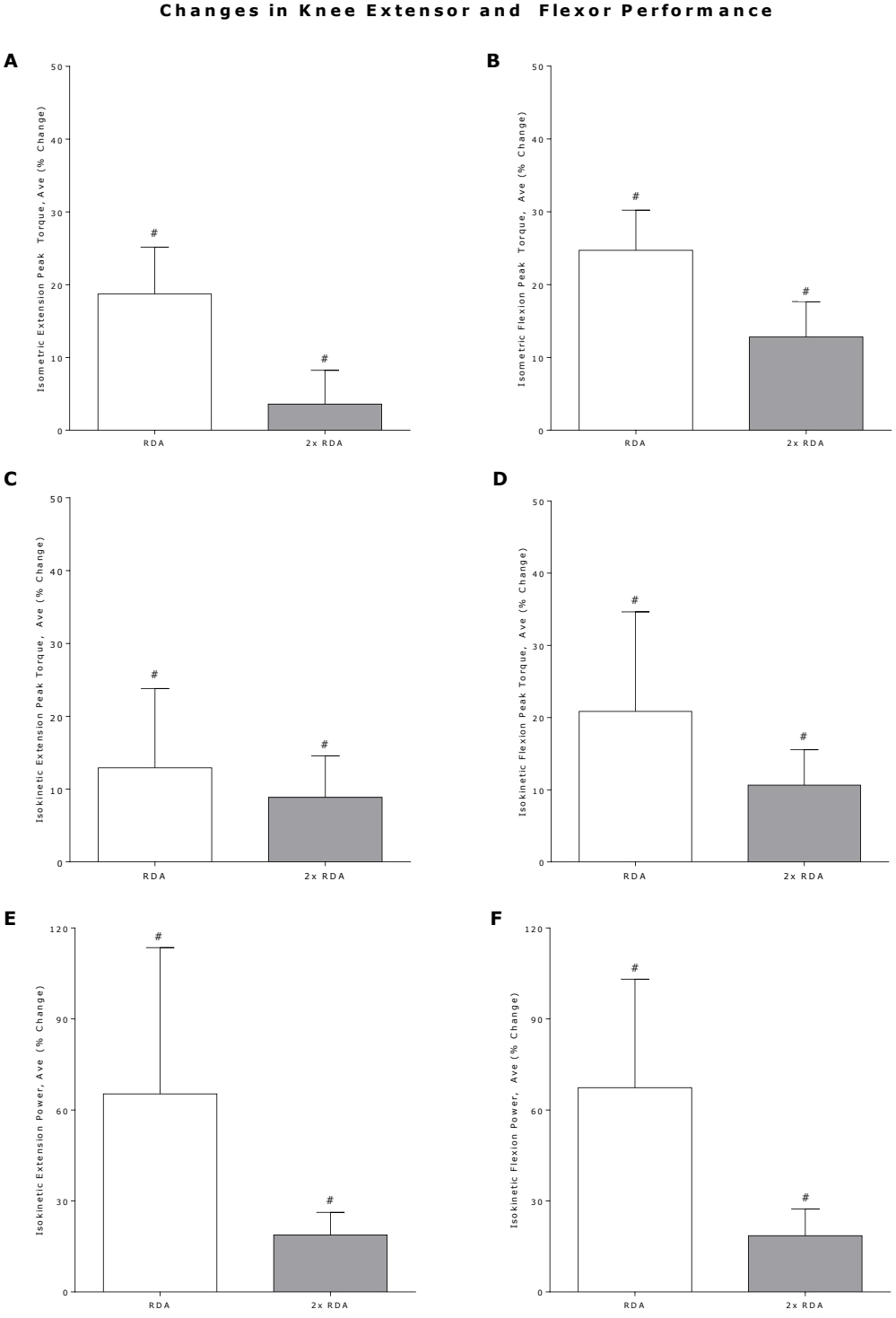
Both groups improved their maximal strength (1RM) for chest press (34 ± 21% vs. 26 ± 4%, *P* = 0.312), shoulder press (92 ± 16% vs. 79 ± 15%, *P* = 0.104), seated row (47 ± 15% vs 20 ± 5%, *P* = 0.315) and bicep curl (19 ± 4% vs. 42 ± 9%, *P* < 0.05). Handgrip strength and measures of physical function remained similar to baseline measures in both groups. Figure 4.4 below illustrate changes in lower body maximal strength.



[1]Changes in maximal strength for leg press (**A**), leg curl (**B**) and leg extension (**C**) after the 10 weeks of resistance training. Data are presented as Mean ± SEM. #Significant change over time, *P* < 0.05. *Different between groups, *P* < 0.05. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance.

Figure 4.4 Changes in Maximal Strength for Lower Body Exercises

Both groups significantly improved their dynamic strength (1RM) for leg press (Figure 1A: 30 ± 8% vs. 49 ± 23%, $P = 0.435$) and leg curl (Figure 1B: 30 ± 7% vs. 30 ± 6%, $P = 0.863$). The 2×RDA group had a greater change in 1RM for leg extension (63 ± 15% vs 25 ± 6%, $P < 0.05$) compared to the RDA group. Figure 4.5 illustrates changes in knee extensor and flexor performance measures.



[1]Changes in isometric extension (**A**) and flexion (**B**) peak torque, isokinetic extension (**C**) and flexion (**D**) peak torque and isokinetic extension (**E**) and flexion (**F**) power after the 10 weeks of resistance training. Data are presented as Mean ± SEM. #Significant change over time, $P < 0.05$. RDA, Recommended Daily Allowance, 2×RDA, twice the Recommended Daily Allowance.

Figure 4.5 Changes in Knee Extensor and Flexor Peak Torque and Power

Peak torque for isometric extension (Figure 4.5A: 19 ± 6 % vs. 4 ± 6%, $P = 0.123$) and flexion (Figure 4.5B: 25 ± 6 % vs. 13 ± 4%, $P = 0.429$) significantly improved in both groups. Similarly, peak torque for isokinetic extension (Figure 4.5C: 13 ± 11 % vs. 9 ± 6%, $P = 0.416$) and flexion (Figure 4.5D: 21 ± 14 % vs. 11 ± 5%, $P = 0.579$) improved over time without difference between groups. There were similar improvements in isokinetic extension power (Figure 4.5E: 21 ± 14 % vs. 11 ± 5%, $P = 0.094$ and isokinetic flexion power (Figure 4.5F: 21 ± 14 % vs. 11 ± 5%, $P = 0.980$) in both groups from baseline.

CHAPTER 5: DISCUSSION

Preliminary data from data within this thesis highlights that consumption of protein in far excess of the RDA does not potentiate training-induced gains in lean mass and muscle performance in middle-aged men and women. In addition to this, protein intake slightly above the RDA seems adequate to support training adaptations, given that intake is equally distributed across meals and protein is ingested during key nutrient timing windows. Thus, our work underlines the value of not only meeting total daily protein recommendations within a healthy eating pattern, but also the importance of timing and distribution.

Despite the common notion that increased physical activity or exercise leads to increase in energy intake, our results show no significant change in both overall and relative caloric intake during the study compared to baseline caloric intake. This is in agreement with the results of a recent meta-analysis which found no consistent effect of any level of increased physical activity or exercise on ad-libitum daily energy intake[155]. Moreover, caloric intake over time throughout the intervention showed no significant difference between time points in any of the groups. This implies that there was no compensatory increase in energy intake with training which may be attributed to the consistent dietary guidance received by the participants throughout the intervention. Dietary feedback was provided to the participants every other week which mainly highlighted eating healthy within caloric goals (i.e. avoid caloric deficit) as well as controlling protein intake and distribution across meals. It is important to note that caloric intake during the study was significantly lower in the RDA group compared to the 2×RDA group (22 kcal • kg $^{-1}$ • d^{-1} vs 30 kcal • kg $^{-1}$ • d^{-1}, $P < 0.05$) and can be explained by the sex difference between the groups, as well as the difference protein amounts in meals consumed outside the laboratory.

Protein intake significantly increased from baseline in both groups and can be attributed to the effect of both dietary counselling as well as the dietary intervention. However, despite consistent counseling, the RDA group demonstrated an increase in

protein intake above the actual protein RDA (0.8 to 1.0 g • kg $^{-1}$ • d^{-1}). This is not surprising given the limitations of dietary counseling in controlling actual intake. Moreover, this coincides with the average daily protein intake of the U.S. adult population - 1.2 g of protein per kilogram body weight per day[81], which also exceeds the RDA. As such, this puts a limit on the applicability of the results in identifying whether the current RDA is adequate to support training adaptation. It is still important to note that protein intake during the study between groups were significantly different wherein the 2×RDA group had a higher relative protein intake compared to the RDA group (1.85 ± 0.09 g • kg $^{-1}$ • d^{-1} vs 1.20 ± 0.05 g • kg $^{-1}$ • d^{-1}, $P < 0.001$) during the study. In addition, improvements in protein spread was also observed during the study. Intake of protein was less skewed towards dinner (RDA group: 26 ± 2 %, 2×RDA group: 32 ± 1 %) with closer proportions of protein intake at breakfast (RDA group 18 ± 2 %; 2×RDA group: 18 ± 2 %), lunch (RDA: 29 ± 2 %; 2×RDA group: 20 ± 1 %) and snacks (RDA: 26 ± 2 %; 2×RDA group: 34 ± 1 %). Similarly, percentage of calories was also more evenly distributed across meals during the study: breakfast (RDA group 21 ± 2 %; 2×RDA group: 22 ± 1 %), lunch (RDA: 31 ± 2 %; 2×RDA group: 26 ± 2 %), dinner (RDA: 28 ± 23 %; 2×RDA group: 28 ± 1%) and snacks (RDA: 20 ± 2 %; 2×RDA group: 20 ± 2 %). These results are slightly better but comparable to the average distribution of calories (17% from breakfast, 23% from lunch, 35% from dinner and 24% from snacks) and protein (16% from breakfast, 20% from lunch, 43% from dinner and 13% from snacks) in the diet of American adults[81]. Despite not employing very strict dietary control (i.e. provision of quantified meals), we show that the distribution of protein and calories is possible in an ad-libitum diet through consistent dietary counseling. Furthermore, there were no significant improvements in micronutrient intakes and DGAI scores that was observed. This may possibly be due to the small sample size in tandem with the inter- and intra-individual variability nature of dietary data collected through diet recalls which is a known limitation of all dietary assessment tools[156]. In addition, DGAI was designed to conform to the RDA and as such, an overconsumption penalty was always applied to protein component as intakes were greater than the RDA.

Despite the lack of significant changes in anthropometric measures (i.e. body mass, BMI, waist and hip circumference), other markers of metabolic health improved. This

highlights the positive impact of physical activity on health and eludes to the fact that an increasing protein intake in combination with resistance training did not seem to negatively impact overall metabolic health among the participants. A significant reduction in systolic blood pressure was observed in the RDA group (15 ± 17 %) and 2×RDA group (19 ± 7 %) after the intervention. This is in accordance with evidence that have shown the blood-pressure lowering effect of both acute and chronic resistance training[47–49]; specifically, a more pronounced effect on SBP has been observed in both normotensive and pre-hypertensive adults[52]. Preliminary results from this study also show a decreasing trend in fasting lipid levels when comparing pre-intervention and post-intervention measures; however, these changes were not significant and might be due to the small sample size. Our preliminary data also show improvements in glucose handling in both groups after the intervention. Glucose tolerance test values for glucose (RDA group: 7 ± 10 %; 2×RDA group: 4 ± 9 %) and insulin (RDA group: 31 ± 20%; 2×RDA group: 28 ± 18 %) at the 2-hour mark were significantly lower at post-testing in both groups compared to baseline. Consequently, significant improvements were also observed in certain insulin sensitivity and beta-cell function indices based on an OGTT. Based on ISI_{est}, insulin sensitivity significantly increased in both groups from baseline without difference between the groups while there were no significant changes in based on the other indices. This may be due to the fact that ISI_{est} takes into account body mass, which makes it a relative measure and may be appropriate given the unbalanced sex distribution in our groups. Other insulin sensitivity indices (Matsuda, $MCR_{Glucose}$) also indicate improvements from baseline; however, these were not significant. All other insulin sensitivity indices classify both groups as normal; except for HOMA-IR, wherein the 2×RDA group's post-intervention HOMA-IR value (3.8 ± 0.8) is indicative of insulin resistance. It is important to note that calculation of HOMA-IR is only based on the baseline values, making it a static measure. Reproducibility of this value has also been questioned, making it a more appropriate measure for epidemiological studies with larger sample sizes[60]. Examination of beta-cell function indices, a group × time interaction was observed ($P < 0.05$) with 1st PH insulin release. A significant decrease in 1st PH insulin release value was seen in the RDA group (24 ± 12%) but the 2×RDA group significantly increased 1st PH insulin release value (13 ± 9 %). In addition to this, a similar reduction in 2nd PH insulin release was seen in both groups

post-intervention (RDA group: 65 ± 8%; 2×RDA group: 65). These changes are both reflected in the insulin response curves in Figure 4.2C which illustrates an overall lower insulin response at post-intervention in both groups. It has already been established that exercise improves glucose handling either through insulin-dependent and independent mechanisms such as an increase in GLUT-4 translocation or expression[65], enhancement of post-receptor insulin-signaling[66,67]. Furthermore, this may also be related to the changes in body composition in both groups after the intervention. Our results are consistent with a similar study conducted among middle-aged and older adults wherein they saw significant improvements in lipid profile and glucose handling after 12-weeks of RT in the group with adequate (0.9 g • kg $^{-1}$ • d^{-1}) and moderate (1.2 g • kg $^{-1}$ • d^{-1}) protein intake.

Total body mass did not significantly change in both groups and there were no major changes in body composition on a whole body level, except for total fat mass wherein the RDA group demonstrated a slight decrease (3 ± 2%) while the 2×RDA group had a slight increase (2 ± 4%). This can possibly be explained by the higher relative caloric intake the 2×RDA group had during the study compared to the RDA group; although the 2×RDA group's relative caloric intake during the study was very close to the groups' estimated total energy expenditure (TEE) during the study (29.9 ± 1.4 kcal • kg $^{-1}$ • d^{-1} versus 30.7 ± 0.8 kcal • kg $^{-1}$ • d^{-1}). Alternatively, this can also be attributed to the sex imbalances between the two groups. Whole body lean mass did not increase significantly in both groups; however, appendicular lean mass relative to height similarly increased in both groups. Furthermore, examination of lower body-specific body composition changes; there was a significant increase in lean mass in the RDA group (6 ± 2%) and the 2×RDA group (3 ± 2%) after the intervention. These results are not surprising as the exercise intervention employed mostly lower-body specific exercise and that the significant increase in relative appendicular lean mass was probably mostly-driven by lean mass gains in the lower extremities. Our results differ from a study that showed consuming protein at twice the RDA level induced greater increases in lean body mass compared to the RDA level[157]; however, it is important to note that this study was conducted in elderly men (>70 years old) and not middle-aged adults and that they did not employ any exercise intervention. On the other hand, our results are in line with a study[127] comparing an intake of protein within

the RDA (0.9 g • kg $^{-1}$ • d^{-1}) and a moderate protein intake (1.2 g • kg $^{-1}$ • d^{-1}) with resistance training among older adults (average age 61 years) found that there were no differences in body composition and fiber size between the two groups. Additionally, other studies[158–160] among middle-aged and older adults wherein they provided adequate protein intake (0.8 – 1.2 g • kg $^{-1}$ • d^{-1}) with resistance training have demonstrated improvements in whole body composition. Moreover, the increase in lean mass seen in both groups may also be related to the improvements in metabolic health; in particular, glucose handling. It is well established that muscle is a major site of glucose disposal and that exercise also induces an increase in glucose uptake[50,65].

The changes in body composition observed in this study support the results of muscle performance measures. Similar gains in lean mass; especially for the lower body would explain the similar improvements in strength demonstrated by both groups. Dynamic maximal strength (1RM) for all upper body and lower body exercises similarly improved from baseline in both groups, demonstrating that all the participants got stronger and that the exercise intervention was effective. In addition to this, knee extensor and flexor dynamometry outcomes also improved in both groups without difference between the two. This further validates that there were significant improvements in muscle performance that are independent of adaptations in neural mechanisms[121]. Performance outcomes based on 1RM may be influenced by a practice effect as these are exercises they regularly perform throughout the training. It must be noted that there are instances when 1RM results conflict with dynamometry strength outcomes[161]; however, data we present agree that both groups displayed similar improvements in isokinetic and isometric peak torque, as well as, isokinetic power. Both performance indices – that reflect differential effects of training, improved over time without difference between groups. Lastly, the lack of improvements in other performance measure (i.e. handgrip strength and SPPB test outcomes) is not at all surprising. It has been shown that handgrip strength is not an appropriate measure for the evaluation of muscle strength changes in response to an exercise intervention in frail older people[162], more so younger adults and that the SPPB test may be irrelevant for our study population as was designed to assess physical functionality in older populations[124]. This basically highlights that the participants enrolled in the study

were healthy, had a good level of physical functionality which makes them fully capable of performing the exercise intervention.

We acknowledge the limitations of our current data set; specifically, the small sample size and the unequal distribution of males and females between groups. We were not able to lower the mean relative protein intake of the RDA group within a level that is within the RDA. As such, this does not directly test our original hypothesis and it is not possible to conclude that the RDA level of protein intake is not optimal to support adaptations to resistance training. Moreover, despite having significantly different protein intakes between groups, the relatively small difference may not be adequate to determine diet-specific effects on training-induced adaptations. Employing diet counseling to control dietary intake among free-living humans was also a difficult feat; especially in a population where the average protein intake is well above the RDA and may have contributed to the inability of participants to achieve a specific level of protein intake. However, this method is more applicable in a wider range of situations and reflects real-life food habits of individuals. Moreover, we cannot dismiss the fact that methods for dietary assessment has its inherent limitations. It is possible that participants were underreporting or over reporting their intakes; however, members of the research team did consistently provide participants feedback and confirmed their reported intakes throughout the intervention. In addition, multiple sets of three-day food records were collected, and it was ensured that the captured intakes would represent both weekday and weekend intakes, as well as, training and non-training days. The food records were also administered frequently enough throughout the study wherein a change in dietary intake would be reflected; however, we did not see any significant change in overall calories and macronutrient composition across the 10-week intervention. Results of the blood urea nitrogen level validate the increase in protein intake in both groups as well. The small sample size may have cause the statistical analysis to be underpowered to detect desired differences between the groups. Furthermore, the unbalanced sex distribution between the intervention groups is another major limitation. Sex-differences in dietary knowledge and habits[163,164] could have impacted dietary intake. There are also sex-related differences physical characteristics (i.e. weight, height, strength level) which can influence results of

the study; however, analysis of body composition and performance outcomes relative to body mass and in terms of relative percentages in change also yielded similar results.

In summary, results of our study support the synergistic value of nutrition and exercise in strategies that aim to offset the age-related loss of muscle mass and strength. Resistance training with moderate and higher protein intakes that were equally distributed across meals and ingested during key nutrient timing windows yielded significant increases in regional lean body mass and improvements in muscle performance and overall health. The lack of difference in terms of training adaptations between the groups with two levels of protein intake highlights the robust role of exercise in improving the sensitivity of the muscle to anabolic stimuli and the importance of providing not only adequate protein intakes but taking into account other dietary factors such timing and distribution.

CHAPTER 6: GENERAL CONCLUSIONS

Preliminary data from this study demonstrates that resistance training-induced gains in lean body mass and muscle strength and performance are not potentiated when consuming protein in far excess of the protein RDA in middle-aged adults. This is evidenced by the similar improvements in body composition and performance outcomes we observed between groups. Thus, our data suggests that consuming protein slightly above the RDA (1.2 g • kg BW^{-1} • day^{-1}) was adequate to support training-induced muscle adaptations when adhering to a healthy eating pattern consisting of adequate calories and equally distributed protein meals throughout the day. Moreover, our findings also indicate positive trends in terms improvements with glucose handling, blood pressure and lipid lipoprotein profile. This underlines the benefits of exercise not only for muscle mass and strength preservation but also metabolic health. Therefore, lifestyle-based strategies which aim to improve health and physical functionality with aging should take advantage of the synergistic effect of nutrition and exercise.

Future work should aim to address the shortcomings of the current study and further investigating the early trends observed in the study. Moving forward, it is important to increase the sample size and ensure a balanced distribution of sexes between groups in order to accurately assess the effect of the intervention. Limiting protein intake to be within the RDA in a population that consumes a moderately high protein diet highlights was a major challenge that was encountered. Reevaluating the actual dietary intervention (i.e. decrease dose of supplements) may help in meeting the target dietary protein intakes which would help test the proposed hypothesis. Moreover, given that the average intake of protein in the US adult population is well-above the RDA, a promising topic that must be further explored is the potential of optimizing distribution and timing of protein intake across meals at different levels of protein intake. This would further fine-tune recommendations for dietary protein requirements and would help shed light on whether or not there is a need to increase the total dietary intake or just address other dietary factors such as protein spread. In order to directly assess the adequacy of the current RDA for protein to support training adaptations, a very well-

controlled dietary intervention must be in place (i.e. provision of premeasured meals); however, its relevance in the real world may be limited as individual food intake is highly variable and is influenced by several non-dietary factors (i.e. culture, environment). Future studies may also delve into the primary source of protein in the diet and how that would factor in in generating recommendations for optimal protein intake in different populations. Additionally, the feasibility of achieving a high protein intake (i.e. twice the RDA for protein) without resorting to the use of supplements must also be evaluated; as it this may also be a challenge for older adults to meet given the other physiological changes that occur with aging that impact eating habits. Lastly, identifying how the optimal protein intake – assumed to be relatively higher than the current RDA, would fit into an overall healthy eating pattern given its displacement effect on other food groups must also be explored.

It is likely that the optimal protein intake to support training adaptations from resistance training among middle-aged and older adults is not far-off from the current RDA – given that it is equally distributed across meals and is likely due to the potent effect of exercise on increasing the sensitivity of the muscle to anabolic stimuli (i.e. amino acids). This has positive implications as a higher protein requirement leads to a greater demand may have other repercussions on both an individual and global level (i.e. economic, sustainability, food security). As such, utilizing the value of exercise along with eating well is a smart and effective way of achieving good health and maintaining physical functionality with aging.

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Appendix A. Informed Consent Form

INFORMED CONSENT
(Standard version)

Title of project: The influence of regular beef consumption and protein density of the diet on training induced gains in muscle strength and performance in healthy adults.

Funding: National Cattleman's Beef Association

Introduction

This form contains information about this study and it is necessary that you understand its contents prior to enrolling in this study. Please ask any questions you may have about the research; we are happy to explain anything in greater detail. You will be provided with a copy of this form to take with you. For this study, we are interested in how people adapt to exercise training when habitually consuming either the recommended amount of protein or twice this amount. You will be randomly selected consume one of these two diet patterns.

Please contact members of the research team with any questions you may have about the study: Joseph Beals
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If you wish to report a research related injury, please contact Dr. Nicholas Burd at the listed phone number or e-mail as soon as possible. This should be done within 24 hours of discovering the injury.

This study is expected to last for the next year, but your involvement will only last about 11 weeks. You will be invited to the lab on thirty-six occasions consisting of: three pre-test visits, thirty training days, and three post-test visits.

Pre-test Procedures
Prior to the intervention, you will visit the laboratory at Louise Freer Hall, UIUC for three pre-test visits (time commitment: first visit ~1 h, second and third visit will be ~3 h fasted, in the morning). During these visits, we will determine if you are eligible for this research study. If it is determined that you are not eligible for the study, your paperwork and data will be destroyed. The researchers will give you a detailed explanation of However, the following information will give you an overview of these visits:

Appendix A. Informed Consent Form (cont'd)

Pre-test 1

1. *Medical history & physical activity readiness.* You will be asked to complete a medical history and physical activity readiness questionnaire. We will also review your answers to ensure you are eligible and that it is safe for you to participate in this study.

2. *Blood pressure.* We are only enrolling participants with healthy blood pressure for this study.

3. *Food Diary.* For 3 days you will be asked to record everything you eat and the approximate amounts. You will be asked to do this 6 times over the course of the study. If you eat a large or small amount of protein we may have to exclude you from the study

4. *Fecal sampling.* You will be receiving containers for fecal/stool sample collection. You will be asked to collect three fecal samples at different time points during the study and bring them to Freer Hall within 15 minutes after the bowel movement. We will provide you with detailed instructions on how to properly use the collection containers.

Pre-test 2

1. *Body weight and height.* With these measures we will calculate your BMI; it must be less than 30 kg/m^2 for you to participate in the study.

2. *Body composition.* We will use a DEXA scan (Dual Energy X-ray absorptiometry) to determine your fat mass, bone mass, and muscle mass. For this scan, you have to remove all metal from your body such as jewelry or watches. You will be required to lie on your back on a padded table. This test lasts about 15 minutes. *For Women:* If you are pregnant we will have to exclude you from participating to avoid unnecessary radiation exposure.

3. *Blood sugar.* Your blood sugar will be checked by drawing small amounts of blood and measuring the amount of sugar it contains. You will be asked to come to the laboratory in the morning after an overnight fast (no eating for the previous 12 hours). After first measuring your blood sugar we will start an infusion of labeled glucose into one of your veins. Afterwards, we will give you a sugar drink that also contains labeled glucose. These labeled glucose molecules will help us assess how quickly you absorb and use the sugar you eat. After the sugar drink, you will lie in a bed for two hours. We will occasionally draw blood during this time. The amount of blood drawn during this visit is ~1 tablespoon. During this test you may to work on a computer, read, or watch DVDs. You will be asked to repeat this test after the study is completed. If your fasting blood sugar is too high (≥126 mg/dL) or remains high (≥200 mg/dL) 2 h after the sugar drink, we will have to exclude you from participating in the study.

4. *Maximal Strength and Muscle Performance Testing.* We will determine your maximum strength in several exercises (leg press, leg extension, leg curl, chest press, shoulder press, and seated row). For this testing, we will determine the maximum weight you can lift for this exercise. We will measure maximum strength on Pre-test 2, training weeks 3 and 5, and at Post-test 1. We will also evaluate your balance and coordination by having you perform a few simple tasks such as standing up from a seated position. Leg muscle performance will also be measured on each leg using a kicking motion once where your leg moves and once where it does not. During these tests electrodes (one on your inner and one on your outer thigh of each leg) will be placed on your leg muscle to measure the neural activation of your leg muscles. For a good signal, we will need to shave small portions of your thigh; a same-sex member of the research team will do this. Handgrip strength on both hands will also be tested using a small machine that you will squeeze as hard as you can in order for us to measure your grip strength.

Appendix A. Informed Consent Form (cont'd)

Pretest 3

1. *Cognitive Tasks* On one day at the beginning of the study and one at the end, you perform some cognitive testing. You will be asked to visit the laboratory following a 4 hour fast. At the start of your visit, your eye health will be assessed using a macular densitometer. During this test we will ask you to look into a scope for a few minutes and observe and respond to a flickering blue light. Following the eye test, you will be seated in a comfortable chair and your brain activity will be recorded using sensors placed on your scalp and face. A trained staff member will explain where the sensors will be placed before attaching them. The sensors are both painless and harmless, and serve to record electrical signals that are naturally produced by the body. You will then be asked to take part in tasks that involve watching a series of symbols or figures that appear on a computer screen in front of you. You will be asked to press button(s) in response to the symbols or figures.

2. Dietary Counseling. A member of the research team will go over what you have been eating and discuss with you how the next few weeks will proceed with how you your diet will look during the study. This will occur before and after the diet and training intervention.

Training Intervention

Each procedure performed during these visits is explained below. These procedures will also be explained during each visit.

1. *Muscle biopsy.* On two days during this study, a small piece of muscle tissue (muscle biopsy) will be collected from your thigh. For each biopsy, Dr. Burd will clean an area over your thigh muscle and inject a small amount of numbing solution (lidocaine) into and under the skin. He will then make a small incision (~1/2 in) in the skin in order to insert a sterile needle into your thigh. Dr. Burd will quickly remove a very small piece of muscle (about the size of a corn kernel). During sample collection (~30 sec), you may feel deep pressure in your thigh and on some occasions it may feel painful. However, the discomfort very quickly passes. Following the biopsies, the incisions will be closed with sterile bandages. This will occur at weeks 0 and after week 10.

   After leaving the laboratory, you are encouraged to perform light exercise (cycling, jogging) and daily activities. However, you should refrain from excessive muscle use for the remainder of the day. Namely, weightlifting sessions that involve deep squatting motions should be avoided for that day. Once the numbing agent wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". The tightness in the muscle usually improves within 2 days. You will be provided with care instructions to take with you.

2. *Resistance Exercise Training.* Three times per week for ten weeks, you will perform resistance exercise. You will be asked to refrain from eating or drinking any food anything except water for two hours prior to the exercise. During each exercise session, you will perform a 5 min warm-up on an exercise bike. Subsequently, you will perform 4 sets of lower body exercise on leg extension, leg press, and leg curl. On a rotating basis, you will perform 4 sets of upper body exercises (either seated chest press and shoulder press *or* seated row and bicep curls). As such, you will perform lower body training and upper body training for each session. The weight lifted each session will be based on your maximum strength. As you get stronger the amount of weight you train with will increase. At weeks 3 and 5 we will re-assess your maximum strength; this will occur during a regular training session. A trained member of the research team will be present during your exercise bout and will monitor proper form. Each visit will last about an hour.

3. *Beef meal and snacks.* For this study, you will be required to eat beef. After each resistance training session, you will consume a meal of minced beefsteak and a carbohydrate beverage. The minced beefsteak will be

cooked under sterile conditions to an internal temperature of 150°F; this exceeds ServSafe requirements for the preparation of beefsteak. The meal poses no health risk to you.

You will also be given a beef isolate protein powder to consume each night 1-2 hours before bed for the study duration. Additionally, snacks will be given to you for you to consume when you are in need of an extra energy source to help you meet your targeted protein goals for the day.

You will also be asked to complete five additional 3-day food records over the course of the study so that we can monitor dietary intake.

4. *Dietary Counseling.* On four separate occasions, a registered dietitian will examine your completed 3-day food diary and may ask you to provide more information (portion sizes, type of food). The dietitian will also give you some dietary counseling at this visit in order to help you consume the proper amount of protein required for participation. You will also be provided with sample food menus to help give you a better idea of what protein sources to consume and in what quantities.

5. Blood Draw. We will collect blood samples three times over the course of ten weeks. This will occur at training sessions 1, 16, and 30.

Follow-up procedures
Upon completion of your training, we will ask you to return to the lab for 3 days of post-testing. You will also be asked to collect one fecal sample during the last week of the study. See attached timeline.

Post-test 1
This visit will be identical to **Pre-test 2**. This visit will occur in the morning 2 days after your final training session.

Post-test 2
On this visit we will have you return to the laboratory for a biopsy and a blood draw. This visit will occur in the morning 3 days after your final training session.

Post-test 3
This visit will be identical to **Pre-test 3**. This visit will occur in the morning 4 days after your final training session.

Risks and Benefits
Benefits
There may be some direct benefits from participation in this study, as you will be completing a ten-week resistance exercise training program while consuming a consistent amount of protein, but no health benefits are guaranteed. Throughout this study you will be given information about your body composition (height, weight, body fat, etc.), and muscle strength. You may wish to have this information evaluated by a health professional. However, your participation in this study will help us understand how our muscles respond to the protein that we eat after resistance exercise. Understanding this process will ultimately help provide information for designing nutrition programs to prevent or reverse the loss of muscle mass that occurs as we age. The maintenance of muscle mass is important for your metabolism as well as for removing fat and sugar from the blood.

Risks
You are not expected to be in great risk from participation in this study. The potential risks involved in participating in this study are described below.

Appendix A. Informed Consent Form (cont'd)

Potential risks with the muscle biopsy procedure
The muscle biopsies are routinely used in research and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in temporary bruising (1 in 30) lasting up to 5 days. Small lump may form under the site of the incision (~1 in 500), but this normally disappears within a few weeks by massaging the lump with your thumb. As with any incision there is also a slight risk of infection (~1 in 2,200). However, this risk is virtually eliminated through proper care. In very rare occasions there can be damage to a superficial sensory nerve, which will result in temporary numbness in the area (~1 in 1,500) lasting up to 3 months. There is also an extremely remote chance that you will be allergic to the numbing agent; the chance of lidocaine allergy is currently unknown. Your muscle may feel sore for 1 or 2 days following the procedure, as if you have performed difficult exercise. However, this is normal and will pass. While there is also a theoretical risk of damage to a small motor nerve (this is used to allow your muscle to move) of your thigh muscle, this has never been seen in past experiences.

To minimize the risk of skin infection and facilitate proper healing, on biopsy days you will be provided with explicit written instructions ("Biopsy Care Kit") that detail the proper care of the wound.

Potential risks involved with blood draws
There may be some discomfort related to the blood draws and the oral glucose tolerance test, but the blood donation procedure is very common and low risk. There is a one in five chance of bruising where the blood is collected. As with all invasive procedures there is a slight risk of inflammation and infection. There is also an extremely slim chance of sudden death during the blood draws. This risk will be minimized by the use of sterile procedures and equipment at all times. There is also a possibility of dizziness and lightheadedness associated with blood draws. You will be seated or lying down during and after the blood draw to reduce risk of falling. All staff members are trained in First-Aid and certified in CPR. There is also the risk of losing a catheter placement during the infusion trial; however, this risk is minimized through use of Tegaderm and medical tape to secure the catheters during the weightlifting session. If catheter placement is lost, there is the potential for fluid accumulation under your skin. Although this may result in slight discomfort it poses no significant health risks to you. Additionally, a member of the research team will constantly monitor catheter placements during exercise to minimize this risk.

Potential risks involved with DEXA scan
The level of radiation emitted during a DEXA is very low, <0.01 mSv. This is very minimal exposure compared to the total background radiation level per year in North America, which is approximately 3.0 mSv/year).

Potential risks involved with resistance exercise and strength testing
There is a small risk of sustaining minor muscle, bone and/or tendon injury during exercise. In addition, you may experience a feeling of discomfort after the exercise bout due to intensified use of major muscle groups. There is a theoretical risk that heart irregularities or sudden death may occur during exercise. However, these events generally happen to people who already have heart conditions. If you have been diagnosed with any type of heart condition you cannot participate in this study. There is a theoretical risk of compartment syndrome of the thigh muscles resulting from exercise. This occurs when there is excess swelling of muscle tissue that greatly increases pressure in a compartment of the body (arm or leg), leading to decreased blood and oxygen supply to the affected muscle. This can cause a feeling of extreme tightness and pain in the area, and if untreated cause permanent damage. To our knowledge, this has never occurred during research of this nature. However, we minimize this risk by monitoring you during and after the exercise session as well as by following up with you frequently during the period following the experimental trials. If, despite precautions, an emergency occurs during exercise, research staff is trained in first aid and CPR. Also, advanced lifesaving equipment (e.g. AED) will be immediately on hand to respond in any manner necessary; including calling 911 if deemed appropriate.

Appendix A. Informed Consent Form (cont'd)

Potential risks involved with cognitive testing
There are no known risks of the eye test, but it is possible that your eyes may become strained or tired. To minimize this, you can take breaks as needed.

Injury and liability
If you have any questions or problems with severe soreness, bleeding, or if the biopsy site becomes red or warm to the touch, please contact any of the researchers (found at the top of this form). If you sustain an injury as a result of participating in this research project that requires medical treatment you are strongly advised to get that treatment. However, the treatment you receive is not free of charge, and we have not set aside money to pay for related injuries. The University of Illinois does not provide medical insurance coverage for participants in this research study. Also, the University of Illinois does not provide compensation for any injury sustained as a result of participation in this research study, except as required by law. Signing this form does not waive any legal rights.

Confidentiality
Will my study-related information be kept confidential?
Yes, but not always. In general, we will not tell anyone any information about you. When this research is discussed or published, no one will know that you were in the study. However, laws and university rules might require us to tell certain people about you. For example, your records from this research may be seen or copied by the following people or groups:

- Representatives of the university committee and office that reviews and approves research studies, the Institutional Review Board (IRB) and Office for Protection of Research Subjects;
- Other representatives of the state and university responsible for ethical, regulatory, or financial oversight of research;
- Federal government regulatory agencies such as the Office of Human Research Protections in the Department of Health and Human Services
- The financial sponsor of the research, the National Cattleman's Beef Association.

Some samples obtained during this study will be stored in the laboratory (maximum 15 years), and may be used for further research. These extra samples are used for determining isotopic enrichment analyses that need to be repeated, or conducting additional cellular/molecular analyses. Also, when publishing, reviewers often ask for additional measures and these samples could be used for this as well. Instead of contacting you later, you are asked to indicate whether you will permit these samples to be used in future research by selecting the appropriate option at the bottom of this form.

Voluntariness & Compensation
Your participation in this study is voluntary. You may withdraw your consent and discontinue participation in this study at any time without penalty or loss of benefits to which you are otherwise entitled. The investigators reserve the right to withdraw you from the study if they believe that circumstances have arisen that warrants doing so.

You will receive $300 upon full completion of the study. If participation in this study is ended early, you will be paid according to what was completed. Participation we be prorated at $15 per week of training completed plus $25 per biopsy. $20 will be provided for completion of the Pre-testing days.

If you have any questions about your rights as a participant in this study or any concerns or complaints, please contact the University of Illinois Institutional Review Board at 217-333-2670 or via email at irb@illinois.edu

Appendix A. Informed Consent Form (cont'd)

My signature indicates that I understand the information provided in this form and voluntarily agree to participate in this study and, on the date provided, received a copy of this informed consent. I certify that I am over 18 years of age.

As stated above, we would like to keep your data, blood samples and biopsies for possible (related) future research. Occasionally, when publishing our work, we are asked to provide more information to clarify our findings, which can require additional blood or tissue. If we must do so, your permission will not be asked for again. The samples will be kept for a maximum of 15 years. Please check the box of your choice.

☐ I **do** authorize the researchers to use my data, blood samples and biopsies for future related research

☐ I **do not** authorize the researchers to use my data, blood samples and biopsies for future related research

(signature of participant) Date

(print name of participant)

(name of investigator) Date

Appendix A. Informed Consent Form (cont'd)

Study overview

☐ **Pre-test 1 (time commitment: 1 h)**
- Medical history
- Physical activity readiness questionnaire
- Blood pressure
- 3-day food record (take-home)
- Collect 1 fecal sample

☐ **Pre-test 2 occurs in the morning after overnight fast: (time commitment 3 h)**
- Hip-to-waist measurement
- Height & weight
- DEXA
- Oral glucose tolerance
- Muscle performance (+ neural activation)
- Maximum Strength tests
- Balance and coordination tests
- Handgrip strength

☐ **Pre-test 3 occurs in the morning after overnight fast (time commitment: 3 h)**
- Cognitive testing
- Dietary counseling (go over first food record)

☐ **Diet Lead-In week** occurs 7 days prior to training day 1
- Start Intervention diet
- Collect 1 fecal sample during 48 hours prior to muscle biopsy.

☐ **Training day 1 occurs in the morning after an overnight fast (time commitment: 1 h)**

- Muscle biopsy and blood draw

☐ **Diet and training intervention**
- Exercise training 3x per week (~1hr/session) for 10 weeks
- 3-day food records to take home every other week of exercise training (week 2,4,6,8, and 10)
- Blood draws occur at days 1, 16, and 30 of exercise training

☐ **Post-test 1 occurs in the morning after overnight fast (time commitment: 3 h)**
- Hip-to-waist measurement
- Height & weight
- DEXA
- Oral glucose tolerance
- Isometric strength (+ neural activation)
- Dynamic strength (+ neural activation)
- Single repetition maximum tests
- Balance and coordination tests
- Handgrip strength

☐ **Post-test 2 occurs in the morning after an overnight fast (time commitment: 1 h)** Muscle biopsy and blood draw
-

☐ **Post-test 3 occurs in the morning after an overnight fast (time commitment: 3 h)**
- Cognitive testing
- Dietary assessment (go over 10 week food record)

Appendix B. USDA Healthy US-Style Patterns—Recommended Intake Amounts

Calorie Level of Pattern[a]	1,000	1,200	1,400	1,600	1,800	2,000	2,200	2,400	2,600	2,800	3,000	3,200
Food Group[b]	**Daily Amount[c]** of Food from Each Group (vegetables and protein foods subgroup amounts are per week)											
Fruits	**1 c-eq**	**1 c-eq**	**1½ c-eq**	**1½ c-eq**	**1½ c-eq**	**2 c-eq**	**2 c-eq**	**2 c-eq**	**2 c-eq**	**2½ c-eq**	**2½ c-eq**	**2½ c-eq**
Vegetables	**1 c-eq**	**1½ c-eq**	**1½ c-eq**	**2 c-eq**	**2½ c-eq**	**2½ c-eq**	**3 c-eq**	**3 c-eq**	**3½ c-eq**	**3½ c-eq**	**4 c-eq**	**4 c-eq**
Dark-green veg (c-eq/wk)	½	1	1	1½	1½	1½	2	2	2½	2½	2½	2½
Red/Orange veg (c-eq/wk)	2½	3	3	4	5½	5½	6	6	7	7	7½	7½
Beans and peas (c-eq/wk)	½	½	½	1	1½	1½	2	2	2½	2½	3	3
Starchy veg (c-eq/wk)	2	3½	3½	4	5	5	6	6	7	7	8	8
Other veg (c-eq/wk)	1½	2½	2½	3½	4	4	5	5	5½	5½	7	7
Grains	**3 oz-eq**	**4 oz-eq**	**5 oz-eq**	**5 oz-eq**	**6 oz-eq**	**6 oz-eq**	**7 oz-eq**	**8 oz-eq**	**9 oz-eq**	**10 oz-eq**	**10 oz-eq**	**10 oz-eq**
Whole grains[d] (oz-eq/day)	1½	2	2½	3	3	3	3½	4	4½	5	5	5
Refined grains (oz-eq/day)	1½	2	2½	2	3	3	3½	4	4½	5	5	5
Protein Foods	**2 oz-eq**	**3 oz-eq**	**4 oz-eq**	**5 oz-eq**	**5 oz-eq**	**5½ oz-eq**	**6 oz-eq**	**6½ oz-eq**	**6½ oz-eq**	**7 oz-eq**	**7 oz-eq**	**7 oz-eq**
Meats, poultry, eggs (oz-eq/wk)	10	14	19	23	23	26	28	31	31	33	33	33
Seafood (oz-eq/wk)	3	4	6	8	8	8	9	10	10	10	10	10
Nuts, seeds, soy products (oz-eq/wk)	2	2	3	4	4	5	5	5	5	6	6	6
Dairy	**2 c-eq**	**2½ c-eq**	**2½ c-eq**	**3 c-eq**	**3 c-eq**	**3 c-eq**	**3 c-eq**	**3 c-eq**	**3 c-eq**	**3 c-eq**	**3 c-eq**	**3 c-eq**
Oils	**15 g**	**17 g**	**17 g**	**22 g**	**24 g**	**27 g**	**29 g**	**31 g**	**34 g**	**36 g**	**44 g**	**51 g**
Limit on Calories for Other Uses[e,f]												
Calories	150	100	110	130	170	270	280	350	380	400	470	610
% of Calories	15%	8%	8%	8%	9%	14%	13%	15%	15%	14%	16%	19%

Source: United States Department of Agriculture Food Patterns.
Available at: https://www.cnpp.usda.gov/USDAFoodPatterns

[a]Food intake patterns at 1000, 1200, and 1400 calories are designed to meet the nutritional needs of 2- to 8-year-old children. Patterns from 1600 to 3200 calories are designed to meet the nutritional needs of children 9 years and older and adults. If a child 4 to 8 years of age needs more calories and, therefore, is following a pattern at 1600 calories or more, his/her recommended amount from the dairy group should be 2.5 cups per day. Children 9 years and older and adults should not use the 1000-, 1200-, or 1400-calorie patterns.

Appendix B. USDA Healthy US-Style Patterns—Recommended Intake Amounts (cont'd)

[b]Foods in each group and subgroup are:
Vegetables

- Dark-green vegetables: All fresh, frozen, and canned dark-green leafy vegetables and broccoli, cooked or raw: for example, broccoli; spinach; romaine; kale; collard, turnip, and mustard greens.

- Red and orange vegetables: All fresh, frozen, and canned red and orange vegetables or juice, cooked or raw: for example, tomatoes, tomato juice, red peppers, carrots, sweet potatoes, winter squash, and pumpkin.
- Legumes (beans and peas): All cooked from dry or canned beans and peas: for example, kidney beans, white beans, black beans, lentils, chickpeas, pinto beans, split peas, and edamame (green soybeans). Does not include green beans or green peas.
- Starchy vegetables: All fresh, frozen, and canned starchy vegetables: for example, white potatoes, corn, green peas, green lima beans, plantains, and cassava
- Other vegetables: All other fresh, frozen, and canned vegetables, cooked or raw: for example, iceberg lettuce, green beans, onions, cucumbers, cabbage, celery, zucchini, mushrooms, and green peppers.

Fruits - All fresh, frozen, canned, and dried fruits and fruit juices: for example, oranges and orange juice, apples and apple juice, bananas, grapes, melons, berries, and raisins.
Grains

- Whole grains: All whole-grain products and whole grains used as ingredients: for example, whole-wheat bread, whole-grain cereals and crackers, oatmeal, quinoa, popcorn, and brown rice.
- Refined grains: All refined-grain products and refined grains used as ingredients: for example, white breads, refined grain cereals and crackers, pasta, and white rice. Refined grain choices should be enriched.

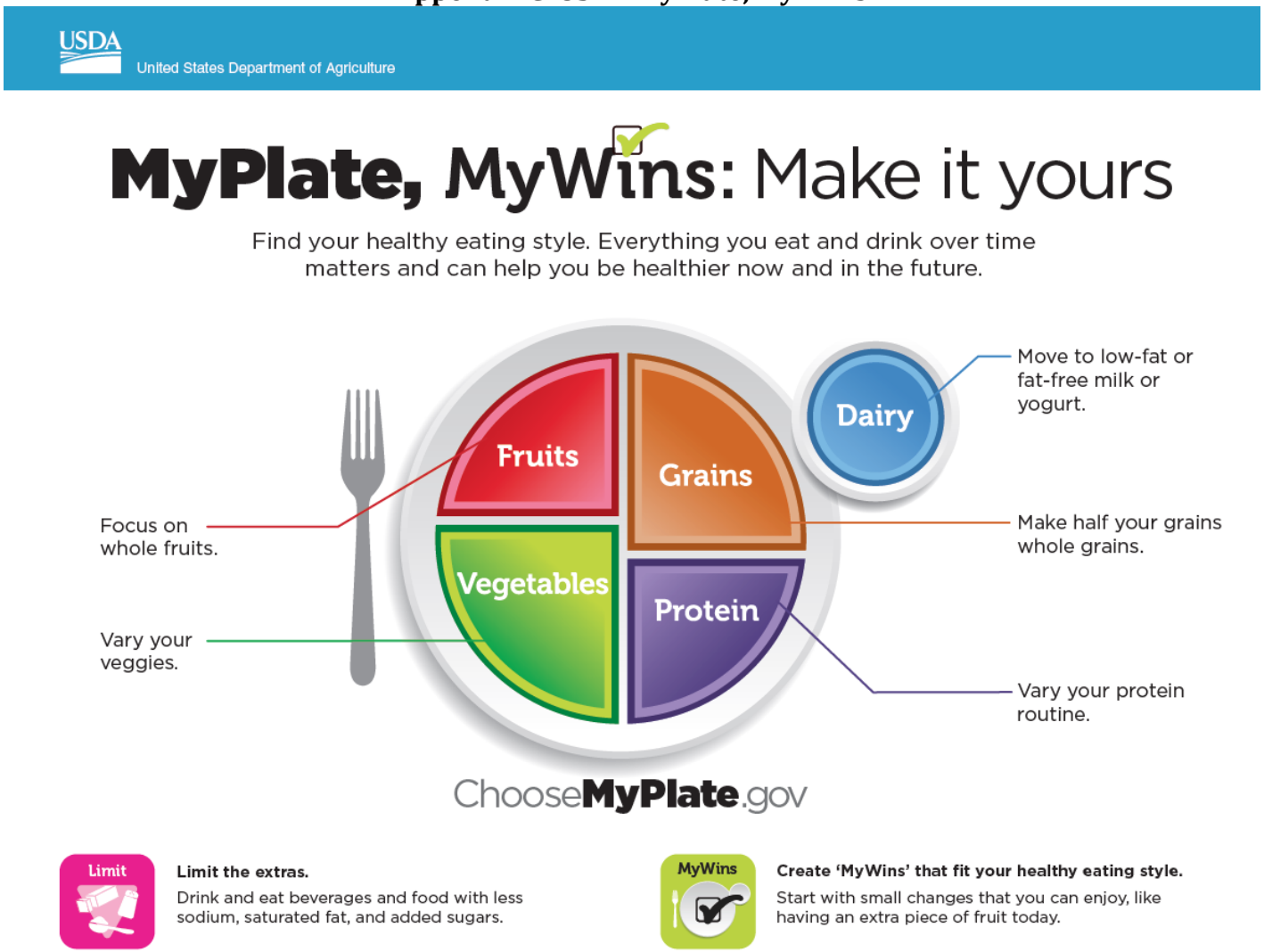
Protein Foods - All seafood, meats, poultry, eggs, soy products, nuts, and seeds. Meats and poultry should be lean or low-fat and nuts should be unsalted. Legumes (beans and peas) can be considered part of this group as well as the vegetable group, but should be counted in one group only.
Dairy - All milk, including lactose-free and lactose-reduced products and fortified soy beverages (soymilk), yogurt, frozen yogurt, dairy desserts, and cheeses. Most choices should be fat-free or low-fat. Cream, sour cream, and cream cheese are not included due to their low calcium content.

[c] Food group amounts shown in cup-(c) or ounce-equivalents (oz-eq). Oils are shown in grams (g).
Quantity equivalents for each food group are:

- Fruits and Vegetables, 1 cup-equivalent is: 1 cup raw or cooked fruit or vegetable, 1 cup fruit or vegetable juice, 2 cups leafy salad greens, ½ cup dried fruit or vegetable.
- Grains, 1 ounce-equivalent is: ½ cup cooked rice, pasta, or cereal; 1 ounce dry pasta or rice; 1 medium (1 ounce) slice bread; 1 ounce of ready-to-eat cereal (about 1 cup of flaked cereal).
- Protein Foods, 1 ounce-equivalent is: 1 ounce lean meat, poultry, or seafood; 1 egg; ¼ cup cooked beans or tofu; 1 Tbsp peanut butter; ½ ounce nuts or seeds.
- Dairy, 1 cup-equivalent is: 1 cup milk, yogurt, or fortified soymilk; 1½ ounces natural cheese such as cheddar cheese or 2 ounces of processed cheese.

[d] Amounts of whole grains in the Patterns for children are less than the minimum of 3 oz-eq in all Patterns recommended for adults.
[e] All foods are assumed to be in nutrient-dense forms, lean or low-fat and prepared without added fats, sugars, refined starches, or salt. If all food choices to meet food group recommendations are in nutrient-dense forms, a small number of calories remain within the overall calorie limit of the pattern (i.e., limit on calories for other uses). The number of these calories depends on the overall calorie limit in the pattern and the amounts of food from each food group required to meet nutritional goals. Nutritional goals are higher for the 1,200- to 1,600-calorie Patterns than for the 1,000-calorie pattern, so the limit on calories for other uses is lower in the 1,200- to 1,600-calorie patterns. Calories up to the specified limit can be used for added sugars, added refined starches, solid fats, alcohol, or to eat more than the recommended amount of food in a food group. The overall eating pattern also should not exceed the limits of less than 10 percent of calories from added sugars and less than 10 percent of calories from saturated fats. At most calorie levels, amounts that can be accommodated are less than these limits. For adults of legal drinking age who choose to drink alcohol, a limit of up to 1 drink per day for women and up to 2 drinks per day for men within limits on calories for other uses applies; and calories from protein, carbohydrate, and total fats should be within the Acceptable Macronutrient Distribution Ranges (AMDRs).
[f] Values are rounded.

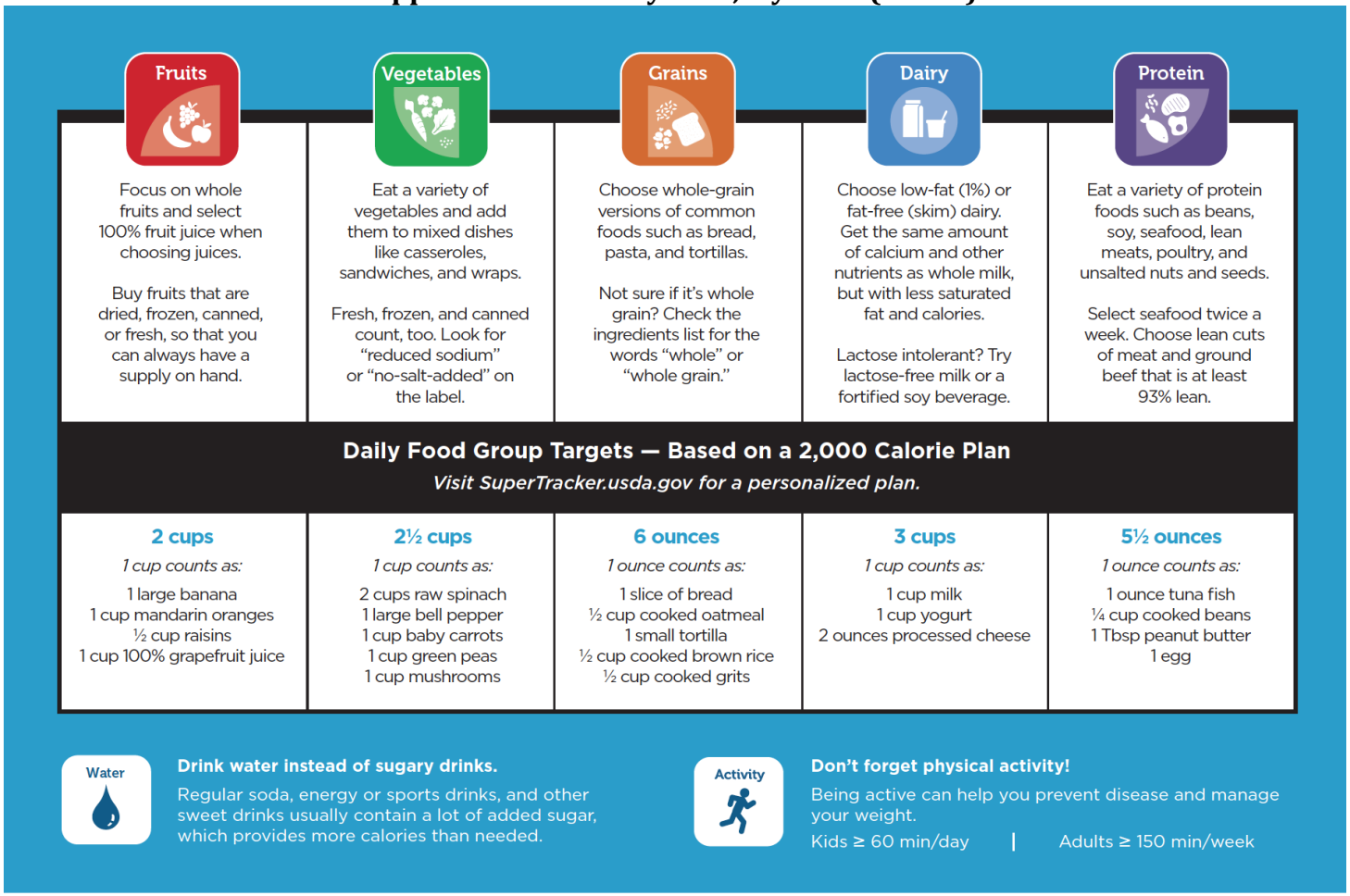
Appendix C. USDA MyPlate, My Wins



Source: U.S. Department of Agriculture, Washington, DC.

Available at: https://choosemyplate-prod.azureedge.net/sites/default/files/printablematerials/mini_poster.pdf.

Appendix C. USDA MyPlate, My Wins (cont'd)



Source: U.S. Department of Agriculture, Washington, DC.

Available at: https://choosemyplate-prod.azureedge.net/sites/default/files/printablematerials/mini_poster.pdf.

Appendix D. 2015 DGAI Scoring Criteria

Scoring criteria of the 2015 Dietary Guidelines for Americans Adherence Index (DGAI)[147,153] for individuals with 2000 kcal/day estimated energy requirement (EER)[1-4]

DGAI Components[5]					
	Scoring Criteria			Scoring Criteria	
	0 point	1 point		0 point	1 point
Food Intake Sub-score[6]			**Healthy Choice Sub-score[11]**		
Dark green vegetable *(cups/week)*	0	≥ 1.5	Whole grain *(% of grains)*	0	≥ 50%
Red/orange vegetables *(cup/week)*	0	≥ 5.5	Dietary fiber density (*gram/1000kcal)*	0	≥ 14
Legumes *(cup/week)[7]*	0	≥ 1.5	Total fat (*% Energy)*	≤ 10%, ≥ 45%	≥ 20%, ≤ 35%
Starchy vegetables *(cup/week)*[8]	0	5.0	Saturated fatty acid (*% Energy)*	≥ 15%	≤ 10%
Other vegetables *(cup/week)*	0	≥ 4.0	Cholesterol intake *(mg/day)*	≥ 450	≤ 300
Fruits *(cup/day)*	0	≥ 2	Low-fat dairy, and meat products *(%)[12]*	0%	≥ 75%
Variety of fruits and vegetables *(number of components)[9]*	0	6.0	Sodium *(mg/day)*	≥ 3450	≤ 2300
Grains *(oz-equivalent/day)*[8]	0	6.0	Alcohol *(drinks/day)[13]*	≥ 1.5	≤ 1.0
Meat and beans *(oz-equivalent/day)*[8]	0	26			
Dairy *(cup/day)*[8]	0	3			
Added sugar (*% Energy)*[10]	≥ 9%	≤ 6.0%			

[1]The 2015 Dietary Guidelines for Americans Adherence Index (DGAI) was developed based on the 2015 USDA Food Patterns (2), which has recommendations for 12 levels of energy requirement. The Canadian version of the 2015 DGAI has a total of 19 scores, since one of the Healthy Choice Sub-score components (*trans* fat) was not attainable.
[2]Estimated Energy Requirement was calculated by the IOM factorial equations using each participant's measured height, weight, physical activity level (PAL) (sedentary, low active, moderately active, highly active), age, and sex (23)
[3]One cup is defined as 237 ml (US), 0.946 cup in metric unit; 1 oz =28.35 grams
[4]Intermediate intakes between criteria for 0 and 1.0 points were scored proportionally.
[5]Possible scores for the 2015 DGAI ranged from 0-19, with higher scores indicating more healthful and varied dietary patterns.
[6]Possible maximum score of 11 points
[7]Legumes were assigned to the meat and beans group for individuals who needed to meet the 1-point criterion for meat and beans group and the extra servings were counted towards the vegetables group (legumes).
[8]An overconsumption penalty was imposed by reducing the score proportional to the amount of overconsumption up to 1.25 times higher than the recommended intake. Intakes ≥1.25 times the recommended amount were scored as 0.5 (truncation).
[9]Variety was determined by summing the 6 fruit and vegetables component scores.
[10]Added sugar available in the USDA Food Pattern for 2000-kcal/day energy requirement
[11]Possible maximum score of 8 points
[12]Adherence to recommendations of "low-fat dairy" and "low-fat meat" products was scored separately, each with a minimum score of 0 (for consuming 0% of dairy or meat products as low-fat) and maximum score of 0.5 (for consuming ≥75% of dairy or meat products as low-fat); intermediate percentages received proportional scores between 0 and 0.5. The final scores for adherence to low-fat dairy and meat were then summed for a maximum possible score of 1.0.
[13]One drink =118 ml wine; 355 ml beer; or 45 ml distilled spirits