EFFECTS OF NUTRIENT TIMING ON PROTEIN SYNTHESIS, MARKERS OF HEALTH AND FITNESS IN FREE LIVING OVERWEIGHT POST MENOPAUSAL WOMEN IN A RESISTANCE INTERVAL PROGRAM TRAINING (RIPT) AND WEIGHT LOSS INTERVENTION

A Dissertation

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ABSTRACT

This study examined whether immediate (I) vs. delayed (D) protein intake following exercise influenced fractional synthesis rate (FSR) and other health/fitness markers during a Resistance Interval Program (RIPT) exercise & weight management intervention. 21 sedentary women (59.8±5 yrs, 43.7±3% body fat, 31.0±3 kg/m^2) participated in a 12 week Curves Complete® program [followed an energy-reduced diet (1,500 kcal/d; 30% C, 45% P, and 25% F) while participating in circuit resistance exercise (RE) (30min; 3d/wk) and walking (10k steps, 4/d wk)]. Each ingested 15 g of protein immediately post (I) or 2 hours post (D) RE. Body composition, body mass, resting energy expenditure (REE) and FSR [determined using a deuterium oxide (^2H_2O or D_2O) ingestion and muscle biopsy protocol] were examined. Data were analyzed by repeated measures MANOVA and/or ANOVA, and are presented as changes from baseline after 4, 8, and 12 wks [body composition, body mass, resting energy expenditure (REE)]; 0 and 12 weeks (FSR) for the I and D groups, respectively. Significant time effects were observed (body mass, fat mass, and body fat) yet no significant group x time effects resulted. The D group generally experienced more favorable body mass (I -2.0±1.0, -2.7±1.6, -3.6±2.2; D -2.2±2.5, -3.6±3.6, -4.2±4.2, kg, p=0.59), fat mass (I -1.7±1.0, -2.4±1.5, -3.5±1.5; D -2.8±1.7, -3.4±2.6, -4.8±3.3, kg, p=0.32), FFM (I -0.3±2.0, -0.1±2.0, -0.0±1.7; D 0.92±1.4, 0.4±1.7, 1.1±1.3, kg, p=0.24), and % body fat (I -1.2±2.1, -1.8±2.1, -2.8±1.9; D -2.6±1.9, -2.8±2.6, -4.4±3.1 %, p=0.25) changes. No REE differences were seen among groups (I -18±146, -

101±163, -82±126; D -46±137, -17±173, -90±142 kcal/d, p=0.34). No significant nutrient timing x training interactions (mean±SEM) were observed in muscle FSR expressed as percent/day of the alanine pool (I-Pre 13.6±4.3, I-Post 21.1±4.3; D-Pre 15.6±4.0, D-Post 23.8±4.0 %/d, p=0.93). However, FSR was upregulated ($p<0.05$) in response to a pre-training bout of RE (14.6±2.9 %/d), and trended 54% higher (p=0.075) in response to post-training values (22.5±2.9 %/d). Results indicate that the program was effective in promoting weight and fat loss, while maintaining FFM. Post exercise FSR increased pre-training, and trended higher at 12-wks. However, while some trends were observed warranting additional study, no statistically significant differences were seen between the I and D nutrient timing strategies

DEDICATION

I'd like to dedicate this work to my late Mother Syble, who never lost faith, confidence or admiration for my perhaps convoluted, protracted educational pursuits. I can only imagine her humble pride were she here to share in this accomplishment. Come to think of it, her pride would most likely not be so humble. Until we meet again. I'd also like to mention my late Father Earnest (I can only pray that we meet again), and voice thanks for his quiet reserve (which could hardly hide his cynicism) with regard to his not so invisible opinion(s) regarding my purpose. Thanks Lavona.

To my children: (Erin, Michael, Jacob), and my grandchildren [Kaitlyn, Liam, Benjamin and Kenneth…. I hope the example I strive to set (although most assuredly not perfect) provides some perspective, and look forward in hopes that our relationships continue to grow stronger. Lastly to my wife Canda: thanks for your support, encouragement, and love from day one. We've come so far, yet our journey has just begun.

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CHAPTER I

INTRODUCTION AND RATIONALE

Estimates of the number of obese American adults have been steadily expanding from 19.4% in 1997, to 24.5% in 2004 [1], 26.6% in 2007[2]. Previously, 75% of adults in the United States were projected to be overweight, and 41% obese by 2015 [3]. According to recent CDC figures, we currently stand at ~36.5%. That said, the beneficial effects of appropriate exercise have the ability to offset mortality rates, as well as positively affect body composition [4], and have been well-documented [5-8]. As our population ages, a significantly related consequence is sarcopenic obesity, which has been defined as gain in weight without concomitant gain in lean tissue [9,10]. This loss of muscle mass and strength has been implicated as impacting metabolic disorders (i.e., insulin resistance) as well as significantly affecting functional capacity throughout the aging process [11-14].

Hypertrophy of skeletal muscle tissue (an increase in myofiber diameter) is characterized by increased contractile force and augmented protein synthesis [15], and is consistently reported following resistance exercise. Along with an associated increase in protein synthesis, skeletal muscle hypertrophy is mediated by synergistic interactions as a result of mechanical, hormonal, and nutritional interventions [16]. Acute post resistance exercise (RE) increases in muscle protein synthesis (MPS) from 1-48 hours in humans has been well-documented [17-21]. The accepted theory behind muscle hypertrophy resulting from resistance exercise training (RET) is that the anabolic effects are cumulative. Nevertheless, as compared to acute RE, chronic RET may result in an

attenuation of the muscle protein synthesis (MPS) response [22-25]. Additionally, acute MPS may be attenuated in older men [26,27]. Understanding the impact of protein synthesis in response to such dynamic stimuli should enhance the understanding of basic functional & applied muscle physiology, contributing to more effective post-resistance exercise nutrition strategies. Subsequent implications for these age related fitness and functional interventions may be invaluable.

Practically, this information may be enlisted to optimize the anabolic hormonal response of resistance exercise through ingestion of macro and/or micronutrients [28]. The combination of RE with nutrition appears to facilitate a potent beneficial interaction between anabolic stimulation, and a concomitant attenuation of skeletal muscle breakdown [28-34]. Although meal intake alone has been reported to increases human muscle protein synthesis, it is believed that extra-cellular amino acid concentrations supplied by the diet mediate this response [35-37]. Post-prandial hormone secretion (insulin) supports this process, primarily by inhibiting protein breakdown [38-40]. Post-absorptive RE has been reported to increase mixed muscle [17,20-23,25,41-43], myofibrillar [23,43] and sarcoplasmic [44] protein synthesis in animals and humans, while (understandably) concomitantly facilitating breakdown [20].

Recent nutrient timing studies have shown recovery and protein synthesis benefits in younger, more athletic populations [45-49], yet similar studies targeting aging, overweight female populations are relatively sparse. Two longer (12 weeks) studies [50,51] have supported the notion that protein consumption in the early period following resistance exercise is paramount for optimal muscle growth in senior men and

women. Interestingly, several acute RE investigations which directly measured MPS with macronutrient intake before [32,47,52-55], during [34,56], or after [28-31,47,54,57] exercise have reported mixed results. Recent research seems to suggest that an improved anabolic profile may remain available for around 2 hours post-exercise [49,58,59].

Strength nutrition applications [60] including liquid carbohydrate (CHO) and protein (PRO), both whole foods (milk) and specific PRO fractions (whey and casein), amino acid constituents [essential amino acids (EAA) and branch chain amino acids (BCAA)], as well as mixed nutrient ingestion (CHO/PRO) have steadily gained popularity [61]. The availability of EAA following exercise, especially BCAA, (often leucine in particular) has been reported to influence recovery by optimizing protein re-synthesis (as well as glycogen re-synthesis) rates after exercise [31,54,58,59,62,63]. However, most investigations have used a fasted subject population, which is obviously neither practical, nor optimal during normal free-living conditions.

Previous work in the ESNL [64] has shown that older women, following a higher protein diet while participating in a circuit training program showed significant fat mass (FM) loss while maintaining fat free mass (FFM). Theoretically, timed nutrient intake after exercise may preserve and/or promote increased FFM in aging women attempting to lose weight. In particular, the insidious health concerns of post-menopausal women (sarcopenia, increasing obesity, stroke, cancer, diabetes, hormonal imbalances etc.), suggests our population of interest may stand to gain valued benefit from any nutrient timing effect. Therefore, utilizing the $^{2}H_{2}O$ stable tracer methodology described in

chapters 2 & 3, we seek to explore the acute & chronic effects of post exercise timed nutrition (via a commercially available shake) using 20 overweight untrained to trained aging women (50-70 years) following an energy and macronutrient controlled diet over 12 weeks of moderately intense circuit training on: FSR, resting energy expenditure (REE), general body composition, and muscular strength. The results of this study should offer an important contribution toward determining whether timed post exercise nutrition would be an effective nutritional intervention for optimal anabolic dividends in overweight/obese aging women.

Statement of the Problem

Does timed post exercise nutritional intake significantly affect markers of training adaptation for aging overweight/obese women on a higher protein diet participating in a 12-week circuit-training program?

Purpose of the Study

The purpose of this study was to determine if timed post exercise nutritional intake significantly affects protein synthesis and/or training adaptations in aging overweight/obese women on a high protein diet participating in a circuit training program.

General Study Overview

Twenty overweight to obese (~ 27+ BMI) aging women (50-70 years) who met screening and health approval criteria were randomly assigned to one of two groups for participation in a higher protein diet & exercise program. The early timed nutrition (I) group (n=10) received immediately post exercise nutrition, while the delayed timed

nutrition (D) group (n=10) were instructed to ingest the same nutrition 2 hours post exercise. The independent variable is the nutrient timing. The dependent variables include: body composition [weight, FM, FFM, percent body fat (BF)]; REE, upper body [bench press (BP)] and lower body [leg press (LP)] strength & endurance; and skeletal muscle protein synthesis [fractional synthesis rate (FSR)] as measured via biochemical analysis of the collected blood serum & muscle biopsy tissue samples. Blood was drawn for monitoring metabolic pools for the FSR, via $^{2}H_2O$ analysis. Statistical analyses were performed for body composition parameters, FSR, upper/lower body (BP & LP) strength, and REE.

Hypotheses

Based on the volume of published research studies cited, and the variables denoted herein, the following hypotheses were evaluated:

H_A1: Timed nutrition will significantly enhance pre training FSR in the I compared to the D group.

H_A2: Timed nutrition will not significantly enhance post training FSR of the I compared to the D group.

H_A3: Timed nutrition will significantly enhance FM loss in the I compared to the D group over time.

H_A4: Timed nutrition will significantly enhance FFM gain in the I compared to the D group over time.

H_A5: Timed nutrition will significantly improve upper body strength in the I compared to the D group over time.

H_A6: Timed nutrition will significantly improve lower body strength in the I compared to the D group over time.

H_A7: Timed nutrition will significantly improve REE in the I compared to the D group over time.

Delimitations

The research study followed guidelines listed below:

1. Approximately 20 sedentary overweight, but otherwise relatively healthy post-menopausal female participants (BMI ~ 27+) participated in this study. Participants were screened, and medically cleared by a physician when necessary as determined by ACSM guidelines.
2. Participants were recruited using flyers posted on the University campus, and physician offices/clinics. Advertisements were run in the campus & local newspapers, and/or radio segments.
3. Familiarizations and testing sessions were conducted in the Exercise and Sport Nutrition Laboratory (ESNL), and the Human Countermeasures Laboratory at Texas A&M University.
4. Participants were randomly assigned to one of two treatment groups (I or D).

Limitations

1. Participants were sedentary and overweight post-menopausal (50-70 years) females (BMI ~ 27+) who met medical clearance qualifying criteria and were able to seek physician approval if necessary.

2. Participants were required to adhere to the Curves 30 minute fitness program as described during pre-study familiarization & screening throughout the 12 week investigation.
3. Participants were required to follow the Curves International Fitness &Weight Management Program within a free-living environment.
4. Participants were required to honestly complete forms and questionnaires legibly, and follow the prescribed regimens as outlined throughout the recruitment, familiarization, and participation processes.
5. Participants completed the Eating Satisfaction Survey, which has not been proven valid or reliable.
6. The sensitivity and specificity of the laboratory procedures utilized to identify changes in the criterion variables are subject to quantified limitations. Unavoidable variations in testing times, dietary intakes, metabolic/physiological status etc. due to a model system involving free-living humans is a limitation.
7. Participants were drawn from a convenience sample at the Texas A&M campus and from surrounding communities, thus somewhat limiting the ability to infer results to a larger population.

Assumptions

1. Participants followed the Curves Fitness & Weight Management Program as specified by the assigned diet regimen.
2. Participants adhered to verbal and written instructions, on more than one occasion,

to refrain from exercise for 48 hours prior to all testing sessions.

3. Participants followed instructions to refrain from dietary intake (with the exception those randomly assigned to the I group) for at least two hours pre & post exercise.

4. Participants followed instructions to fast for a minimum of 12 hours prior to all appropriate testing sessions.

5. Participants followed the intensity guidelines for all workouts per coach's instructions, and as guided via the Curves Smart technology.

6. All assay methods & equipment used in the analysis of blood and muscle samples were accurate and reliable in quantification of dependent variables.

Relevant Terminology

1. **Adiposity** - usually refers specifically to tissue made up primarily of fat cells such as the yellow layer of fat beneath the skin.

2. **Anthropometry** - the study of human body measurement for use in anthropological classification and comparison.

3. **Anthropometric measures** - measures of or relating to anthropometry.

4. **Bioelectrical Impedance Analysis (BIA)** – body composition assessment technique whereby a small current of electricity is passed between electrodes placed on the body, thereby quantifying the volume of body water and body fat.

5. **Body Composition** - Test used to determine body fat percentage.

6. **Body Mass Index (BMI)** - statistical measure of the weight of a person scaled according to height defined as kilograms per meter squared.

7. **Food Record (Log) -** Form used to record all fluid and food intake during a specified

period in order to standardize nutritional intake.

8. **Deuterium (Heavy Water; D_2O, or ^2H_2O) -** Heavy water is water that contains a higher proportion than normal of the isotope deuterium, as deuterium oxide, D_2O or ^2H_2O.

9. **Dual-Energy X-Ray Absorptiometry (DEXA) -** Procedure used for limited x-ray technology to determine body composition and bone mineral density.

10. **Eating Satisfaction Survey -** a questionnaire used to determine eating satisfaction of an assigned diet.

11. **Elderly -** of or pertaining to persons in later life.

12. **Fat Free Mass (FFM)** – Typically refers to lean muscle tissue.

13. **Fat Mass (FM) -** Term used to describe the fat weight of the human body.

14. **Fractional Synthesis Rate (FSR)** – quantification method for the rate of protein synthesis. $FSR = E_A/(E_{BW} \cdot 3.7 \cdot t)$, where E_A represents amount of protein-bound ^2H Ala (%), E_{BW} is the quantity of ^2H_2O in body water (%), 3.7 represents the exchange Of ^2H between body water and alanine, and *t* is time of label exposure.

15. **Gerontology -** the study of aging. It is to be distinguished from geriatrics, which is the study of the diseases of the aging.

16. **Isotonic Exercise** – involves movements with constant external resistance. The amount of force required to move the resistance varies depending, primarily, on the angle and length of each agonist muscle.

17. **Gas Chromatography-Mass Spectrometry (GS MS) -** in combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining

metabolic activity. GC MS is an analytical technique for the determination of the elemental composition of a sample. It is also used for elucidating the chemical structures of molecules, such as peptides and other chemical compounds.

18. **Macronutrient** – carbohydrate, lipid, and protein nutrients.

19. **Micronutrient** – small quantities of vitamins & minerals that facilitate energy transfer and tissue synthesis.

20. **Modified Bruce Protocol**- A standardized multistage treadmill test for assessing cardiovascular health. An alteration of the Bruce protocol

21. **Mole Percent Excess** - A quantitative measure of the concentration of a stable isotope, analogous to the specific radioactivity of a radioisotope; the enrichment of that isotope, as a percentage of all isotopes, over and above its usual occurrence in nature.

22. **Psychometric Assessments** - Questionnaires completed by participants to determine the eating satisfaction and quality of life throughout the length of the study.

23. Quality of Life (QOL/SF-36) - Questionnaire used to measure health-related quality of life by assessing eight different dimensions: physical functioning, role limitations caused by physical health problems, bodily pain, general health perceptions, energy/fatigue, social function, role limitation caused by emotional problems, and emotional well-being.

24. **Sarcopenic Obesity** - the combination of obesity and loss of muscle mass.

25. **Senior** - of, for, or pertaining to a senior citizen, or senior citizens as a group.

26. **Total Body Water (TBW)** - The sum of intracellular water and extracellular water (volume), about 60% of total body weight.

27. **VAT** – Visceral Adipose Tissue.

28. **Waist to Hip Ratio (WHR)** - waist circumference divided by hip circumference.

CHAPTER II

LITERATURE REVIEW

Aging in the U.S.

One of the fastest growing segments of developed countries is felt to be those aged 65 and over. Currently this group accounts for approximately 15% of the population in western European countries and the United States. The proportion is expected to grow to 19%-26% by the year 2025, and it becomes clear that it is especially important to focus on obesity in the elderly, due to the effects on morbidity and mortality compared to younger individuals [65]. Addressing health concerns during the early aging stages should make a significant impact on overall health as our population ages. It is also important to note that due to the incidence of co-morbid conditions in this population, obesity intervention plays a key role in the management of these diseases [66]. A 1993 study found that 14 percent of all deaths in the United States were felt to be attributed to inadequate nutrition and insufficient activity [67].

The Centers for Disease Control (CDC) data has reported that 28-34 percent of adults aged 65-74, and 35-44 percent of those aged 75 or older are inactive (defined as no leisure-time physical activity). Their findings also suggest that older people are more inactive than those persons who are middle aged [66].

National data tracked by the U.S. Department of Health and Human Services supports that few older persons engage in regular physical activity. Thirty one percent of Individuals aged 65 to 74 years reported 20 minutes of moderate physical activity on three or more days per week, while approximately 16% reported 30 minutes of moderate

activity on 5 or more days per week [68]. Vigorous physical activity that produces large increases in heart rate and moderately heavy sweating produces greater gains in cardiopulmonary fitness. Individuals that participate in vigorous activity are even fewer in number, and continue to decline with age [66]. The estimates for 2000 indicate only 13% of individuals aged 65 to 74 reported engaging in vigorous physical activity for 20 minutes three or more days per week. There were only six percent of those age 75 and older reporting vigorous physical activity [68].

Even midlife increases in physical activity, through change in occupation or recreational activities, have been associated with a decrease in mortality [69-72]. Despite this evidence, the vast majority of adults in the United States remain effectively sedentary. A 2003 study reported that less than one-third of Americans met the minimal recommendations for activity as outlined by the CDC, ACSM, and AHA expert panels [73].

The growing number of aging, obese adults puts increased demands on medical, social, and public health system services. The total US combined costs of overweight and obese in the year 2000 was estimated at $117 billion [68]. Of this number, eighteen percent of adults aged 65 and older were obese. This data also showed that another 40 percent were overweight. As the American population grows in number and the percentage of elderly increase, medical care costs will also continue to rise. The CDC has shown that the cost of inactive adults is substantially higher than those of their active counterparts (Figure B1, pg. 131). More recent research continues to emphasize increasing costs of healthcare in aging populations with decreased physical activity and

disability [74-76]. The excess costs are especially notable in women. Data suggests that improving physical activity in elderly women stands to reap more benefits in terms of lower health care costs as compared to any other age group [66].

Obesity in the Aging Population

The definition of obesity currently relies upon Body Mass Index as a tool for assessing weight status. Both the National Heart, Lung and Blood Institute of Health (NHLBI) of the National Institute of Health (NIH) and World Health Organization (WHO) now recommend 25 kg/m^2 as the upper limit of ideal body weight for all adults regardless of age [77]**.** Parameters for normal weight and obesity in the elderly are often disputed in the literature due to potential adverse effects of weight loss such as muscle wasting, nutritional deficits and disease states. A meta-analysis [78] concluded that the federal guidelines for ideal weight (BMI 18.7 to < 25) might be too limiting for older adults. They determined that a BMI ranging from 27-30 are optimal based on all-cause and CHD mortality and CHD incidence. Some experts support the definition of obesity in the elderly as a BMI > 30. Figure B2 (pg. 133) shows the US median BMI by age from 1990-2000.

There is some debate over ideal body weight due to the concern that senior adults may benefit more from maintenance of activity versus weight loss. The concern is due to obesity related conditions that may be worsened such as sarcopenia and bone density changes during weight loss [79]. Due to these discrepancies and decreased reliability of BMI in the elderly, researchers have suggested the use of a more precise measure of body fat in the older population. Hence, methods that measure three, four and/or five

compartments are recommended [79]. This study will utilize a multi-compartment method for assessing body composition changes (e.g., Bioelectrical Impendence (BIA), Dual Energy X-Ray Absorptiometry (DEXA), waist and hip measure etc.).

Physiology of Aging and the Effects of Exercise

The process of aging can impair the body's ability to regulate its internal environment. While this process occurs in all individuals, exercise can offset some of the effects of aging. The body systems and related effects of aging are represented in Table A1 (pg. 127) which illustrates how aging affects many physiological processes.

The onset of these changes usually begins in the third and fourth decade of life and continues to deteriorate with aging. Fortunately for humans, the body system has been shown to respond quickly despite advanced age. Obesity in the absence of intervention complicates and produces further stress on a body system and leads to cellular and system malfunction, ultimately producing a disease state. Research has shown many times over that obesity is associated with increased risk of many common & chronic disease states including heart disease, stroke, some cancers and diabetes mellitus as well as disease risk factors such as hypertension and hyperlipidemia [80]. Obesity has also been linked as a co-morbidity in a milieu of related and seemingly unrelated conditions such as osteoarthritis, gallstones, asthma, depression, and sleep disorders [81-86].

Research has repeatedly shown that exercise and the resultant weight management can improve or mediate such conditions. Resistance training impedes the rate of sarcopenia [87-94] (see below) [94], and may also substantially improve physical

function [95]. An examination of the association between physical activity (PA) and visceral adipose tissue (VAT) in older adults conducted by Riechman, et.al. (2002) reported mean visceral, subcutaneous, total adipose, and visceral percent adipose tissue were all higher in older (64.5 ± 5.2 years) men and women subjects reporting physical difficulties (PD). Reported trends for these mean differences were significant for all comparisons except for subcutaneous adipose in women, and visceral percent adipose tissue in men (as well as the total cohort). The study suggests however, that age was only "weakly" associated to increased VAT and decreased subcutaneous adipose, which resulted in a significant association of age to visceral percent adipose tissue. Hence, the accumulation of the relative VAT which accompanies aging in many, may contribute to the exacerbation of PD [96]. Hence, it stands to reason that any intervention which enhances FFM, and reduces FM should have a positive impact on overweight female populations which might experience a reduction in PD's associated with VAT.

In the human aging process, there is a significant decline in neuromuscular function and performance. One main characteristic of this decline is the inevitable reduction in skeletal muscle mass, and the associated loss of strength that occurs even in the healthy elderly [97]. This prevalent, physiological specter of aging is a condition called sarcopenia. Sarcopenia is defined as loss of muscle mass and strength, and is generally considered to be a result of disuse. However, the loss of muscle mass can be accelerated by inadequate nutrition and chronic illness. The loss of muscle mass is currently felt to be approximately 5% per decade after the 4th decade of life, and currently thought to occur more rapidly after age 65 [98]. More importantly for this

study, it is also currently reported that the impact of muscle loss and function in women may be greater due to a lower initial muscle mass compared to men [98].

Table A2 (pg. 128) depicts a simplified summary of the alterations in muscle physiology with aging and post exercise intervention. The advancement of techniques such as muscle fiber typing has allowed for increased knowledge in the area of muscle fiber changes through the lifespan and in studying training adaptations. ATPase staining techniques are utilized to assess changes in Type I and II muscle fibers and their respective phenotypes. Type I fibers are predominantly comprised of myosin heavy chain 2a (MHC 2a), and type IIB fibers are predominantly myosin heavy chain 2x (MHC 2x). One study reports that mRNA levels of MHC 2a and 2x decrease with age [99]. Slow twitch type I fibers are fatigue resistant with greater oxidative capacity, higher mitochondrial content, and greater capillary density. Type II fibers are fast-twitch fibers with a high glycolytic capacity. Type II fibers can be classified into type IIA, having intermediate oxidative and glycolytic capacity and are more fatigue resistant. Types IIB and IIC are more glycolytic.

In a comprehensive study looking at muscle fiber changes in the vastus lateralis, commonly used as a site for muscle tissue biopsy, 43 male cadavers examined between the ages of 15-83, showed an age-related loss in fiber number. Between the ages of 20 and 80 there was a reported 50% reduction in the total fiber number [100]. This loss has been noted to be more rapid over the age of 50. Measurements of a cross sectional area of muscle, show a selective loss of fast-twitch type II fibers in comparison to slow-twitch type I fibers. This shift in aging appears to be independent of vigorous endurance

exercise based on muscle biopsies taken from a 20-year longitudinal study of distance runners [101]. The metabolic consequences of muscle mass decline have been well documented in a number of cross-sectional and longitudinal studies. The reduction in muscle mass significantly affects the amount of metabolically active cell mass. Previous research supports a 15% decline in resting metabolic rate between age 30 and 80 [101-103]. This reported rate of decline may be seen as corresponding with an estimated caloric needs decrease of ~250kcal per day.

A decline in total energy expenditure is also impacted by a decrease in physical activity. The decline in physical activity is often due to age-related muscle weakness, fatigability, and loss of endurance. Concomitantly, the loss of muscle mass (FFM) in aging is accompanied by gains in fat mass (FM). This process is exacerbated by a decreased daily expenditure/physical activity, and a resultant decline in function which adds to the age-related accumulation of visceral & total body fat, as well as decreased insulin sensitivity. This constellation of changes increases the likelihood of Type II Diabetes [104]. Due to the loss of muscle mass in the elderly, which serves as the main metabolic organ responsible for glucose disposal and fatty acids post meals, post prandial hyperglycemia is also more common in this population [98].

The balance between protein synthesis and protein breakdown is the main contributor to the maintenance and repair of skeletal muscle. Muscle quality and mass are reliant upon the efficient synthesis of new structural proteins. Previous research has shown that the synthesis of mixed muscle protein is reduced by 30% with age

[26,99,105]. It is proposed that this conditionally selective decrease in muscle protein synthesis may explain the age-related decrease in muscle mass [98].

The number and function of mitochondria have been associated with the degree of muscle fatigability, reduced endurance capacity, and possibly loss of strength. The ATP generated by mitochondria play a crucial role in generating contractile force. It has been shown that aerobic exercise can significantly increase mitochondrial enzyme activity. In men and women aged 60-70 who trained for 9-12 months by walking or jogging at 80% of maximal heart rate for 45 minutes, 4 days per week had a 24% increase in the mitochondrial enzymes succinate dehydrogenase, citrate synthase, and beta-hydroxyaacyl-CoA dehydrogenase [106].

The phenomenon “anorexia of aging” is suggested to be one of the most modifiable variables in the development, and progression of sarcopenia [97]. This condition is simply defined as the decline of food intake over the lifespan. From differing perspectives between two separate papers, a steady dietary supply of protein may or may not provide sufficient (or optimal) support for mature adults pursuing maintenance or enhancement of FFM [51,107] (an area of interest that is central to the purpose of study). There are many complex mechanisms and interactions that contribute to decreased food intake, including: early satiety secondary to decreased relaxation of the stomach, increased release of cholecystokinin (stimulates digestion of fat/protein) in response to fat intake, and increased leptin (appetite suppression) levels. The elevation of leptin levels are thought to be in part due to increased fat mass with aging. Other

contributors include effects of neurotransmitters such as opioids and neuropeptides [108].

Hormone therapy has also shown promise in combating age related muscle mass loss. The hormones that have received the most attention include testosterone, growth hormone (GH), Insulin-like growth hormone (IGF-1) and dehydroepiandrosterone (DHEA) [98]. Bioavailable levels of testosterone have been shown to be decreased, especially in the years immediately following menopause. Testosterone supplementation in elderly women appears to be an area which lends itself to further study [98]. In post hysterectomy patients with oopherectomy, bioavailable testosterone levels have been noted to be even lower due to the loss of ovarian androgen production [109,110]. Women taking estrogen replacement therapy may have a further reduction in bio-available testosterone by binding more androgen.

Studies have reported that growth hormone and its peripheral mediator, IGF-1, decrease with age [111-113], and the effects of growth hormone continue to be of interest [114,115]. Deficiency in growth hormone leads to muscle mass loss and increased adipose tissue. The benefits and effects of growth hormone replacement in the elderly continue to be controversial. Supplementation with recombinant growth hormone or IGF-1 in elderly women has been noted to increase net protein synthesis [116]. These changes may lead to increased muscle mass. A 2003 study in elderly males failed to show beneficial effects from growth hormone in relation to muscle protein synthesis or strength compared to exercise alone [98]. It is also important to note the significant adverse effects due to growth hormone administration that contribute to

study withdrawal. These adverse effects may include fluid retention, carpal tunnel compression, gynecomastia, and arthralgias [117,118].

DHEA and DHEA-S (DHEA sulfated form) are produced by the adrenal cortex. Although the exact practical biological role of these hormones has not been thoroughly examined, early studies have suggested that DHEA levels slowly decline after the second decade of life. The decline is linear, and felt to be approximately 10% per decade until age 80 when the decline seems to accelerate [119-121]. Research on DHEA supplementation has produced mixed findings. It is currently thought to have no effect on insulin sensitivity, resting metabolic rate, total energy expenditure, and/or muscle protein synthesis rates throughout the aging process [122-125].

The delivery of oxygen and fuel to muscle tissue is dependent upon a vast capillary network. This intricate delivery system can be significantly altered due to partial occlusion of vessels by atherosclerotic plaque and defects in arterial compliance. Other age associated conditions such as hypertension can reduce blood flow and compromise oxygen delivery. In 1997, the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure reported that Hypertension occurs in more than two thirds of those over age 65 [126]. Subsequent reviews have shed further light onto these reported relationships [127,128]. Such noted changes are more common in the elderly, and can measurably impact protein synthesis and mitochondrial function, but the degree of these effects merits further study [98].

The progressive loss of innervations, motor neurons, muscle stimulation, and declining neurological function as we age play crucial roles in age-related muscle

dysfunction and atrophy [129-132]. Muscle denervation leads to atrophy, the result of which can result in decreases of muscle mass to less than one half its original weight within a month [98]. Tomlinson and Irving [133] studied motor neuron quantification in the entire lumbo-sacral spinal cord of 47 individuals aged 13-95. After the age of 60, there was as much as a 50% loss of neurons in the elderly compared to younger or middle aged subjects.

There is an abundance of research supporting the idea that older people have "trainable" physiology, and that they would reap many health benefits from exercise. It is currently felt that prevention of age-related muscle loss is the key to healthy aging. Exercise research has shown the greatest promise for both prevention and treatment of sarcopenia. Ades, et. al. looked at a group of community dwelling men and women over the age of 65. Participants were randomized to either a weight-training or no training program for 3 months. The intervention group showed significant improvements in both leg strength and walking endurance [134]. Resistance training has also been shown to be effective in the elderly. An earlier study looked at nursing home residents with an average age of 87 [135]. The participants underwent a 10-week resistance training and nutritional intervention, showing gains in strength, improving by 125% compared to less than a 3% change in the control group. Other benefits included improved gait velocity, stair-climbing power and spontaneous physical activity. Through training adaptation, muscle physiology is enhanced via increases in muscle fiber size and muscle protein synthesis in response to resistance training [99,105,136,137]. Increases have also been

shown to occur in specific muscle proteins such as myosin heavy chain, which some [99] considered to be the key contractile protein.

In consideration of these findings, it seems apparent that the process of sarcopenia is complex, and is likely to have a profound impact on numerous body systems as the aging process progresses. Although it has yet to be specifically proven that there is a clear cause and effect relationship between muscle mass loss and such physiological changes, it stands to reason that reduced synthesis rates of specific muscle proteins, mitochondrial dysfunction, poor nutrition, reduced anabolic hormone levels, alterations in perfusion, altered neurological function, and reduced physical activity could all be reasonably connected to losses in muscle strength, mass and function. In order to offset these losses, potentially slow the aging process, and maximize functional capacity throughout the aging process, further interventions in the areas of exercise, nutrition and preventive health are inevitable.

Exercise Recommendations

The current guidelines by the CDC and the American College of Sports Medicine (ACSM) recommend 30 minutes per day on most days of the week. Research has shown that these guidelines allow men and women to experience decreased rates of cardiovascular disease and premature mortality [138]. The NIH has done extensive research in the area of exercise intervention in promoting health and established the following guidelines. Weight loss is recommended to lower elevated blood pressure in overweight and obese persons with high blood pressure. Exercise is also strongly recommended to lower elevated levels of total cholesterol, LDL-cholesterol and

triglycerides and to raise low levels of HDL-cholesterol in overweight and obese persons with dyslipidemia [77]. If weight loss is achieved it can lower elevated blood glucose levels in overweight and obese persons with type 2 diabetes. The combination of a reduced calorie diet and increased physical activity is recommended, since it produces weight loss, decreases abdominal fat, and increases cardiorespiratory fitness [77].

Due to research supporting that older women are higher risk in regards to inactivity, obesity and fall risk, innovative programs are needed to meet the needs of this population. Curves International has had an overwhelming response by those who would not ordinarily frequent a gym or workout center as evidenced by the 10,000 centers world-wide. The 30 minute circuit and nutrition plans are offered in a women's only environment that allows the female population to feel less intimidated in pursuing weight loss and a higher level of fitness. This environment also allows for socialization and accountability by their peers and work out supervisors. Members of the Curves International clubs are able to provide support to each other in a non-intimidating setting that has the added benefit of work out attendants providing support, encouragement and assistance with machine use and technique. Extensive & ongoing research supports the effectiveness of the Curves program in positively affecting women's health and fitness [64,139-141].

Barring any medical contraindications, cardiovascular fitness may be addressed at any age. Another advantage of consistent exercise is the ability to prevent and/or reduce declines in functional ability that is often viewed as normal aging [4]. The positive effects of exercise help offset conditions that the general public views as

inevitable during the aging process. Some of these conditions include improved bone health with subsequent reduction in risk of osteoporosis, improved postural stability, reducing the risk of falling and possible fracture, as well as increased flexibility and range of motion.

It has been established that weight loss in obese older populations can improve functional fitness, overall health and independence. The cardiovascular system is one critical body system that steadily decline with age. For example, $VO_{2\ max}$ declines approximately 30% between the ages of 20-65. The greatest rate of VO_2 decline reportedly occurs after the age of 40 [142]. One area in the elderly that contributes significantly to VO_2 levels is decreased muscle mass. The loss of muscle mass and subsequent strength can profoundly affect the quality of life in the elderly. The lack of muscular support can affect stability, functional capacity for activities of daily living and metabolism. All of these factors can cause and/or exacerbate obesity [142]. In research done in younger age groups utilizing the Curves circuit and nutrition plans, it has been shown that cardiovascular benefit and maintenance of muscle mass during weight loss are obtainable [140].

Metabolic Disorders & Obesity

According to recent estimates, over 50 million and perhaps 75 million Americans meet criteria for metabolic syndrome [143]. The criteria include: abdominal obesity (waist size > 40 inch in men or > 35 inches in women), triglycerides ≥ 150 mg/dL, HDL < 40 mg/dL in men or < 50 mg/dL in women, systemic hypertension (BP > 130/85 mm Hg), and fasting blood glucose > 110 mg/dL. It is also current knowledge that weight

loss decreases diastolic blood pressure and serum LDL cholesterol, triglycerides, insulin, glucose and increases HDL cholesterol [143].

Based on the defining characteristics noted above in diagnosing Metabolic Syndrome, waist-hip ratio (WHR) measures and waist circumference (WC) play an integral part in assessing risk for metabolic disorders and have been proven to be a better indicator of cardiovascular risk. Schneider et al. (2006) looked at whether WHR or WC are more sensitive measures for visceral obesity, and which measure may be more indicative of cardiovascular risk. This group of researchers studied 5,377 unselected subjects (2,016 men and 3,361women) without arteriosclerotic disease, aged 20-79 years from the DETECT (a cross-sectional, clinical-epidemiological study) laboratory sample in a primary care setting. The intervention included measuring anthropometric parameters and assessing CHD risk by clinical exam, patient history, and a standardized laboratory program. The associations of BMI< WC, hip circumference, WHR and waist-to-height ratio (WHtR) to cardiovascular risk by calculating the area under the receiver-operating characteristics (ROC) curve in combination with adjusted odds ratio for metabolic syndrome, dyslipidemia and type 2 Diabetes. Their results showed the area under the ROC curve for WHtR was significantly higher than for all other anthropometric parameters with respect to all risk conditions in women and to dyslipidemia and Diabetes Type II in comparison to men. The odds ratio for presence of risk conditions with one standard deviation increase of each anthropometric parameter was highest for WHtR or WC. They concluded that there are some indications that

WHtR or WC may predict prevalent cardiovascular risk better than BMI or WHR despite the small differences noted in this study [144].

Some researchers have tested whether both BMI & WHR may be a better determinant of cardiovascular risk than either separately. Zhu et al. (2004) put this theory to the test by combining waist circumference (WC) and BMI in evaluating 8,712 white men and women from the Third National Health and Nutrition Examination Survey. The optimal combination of BMI and WC using current cut-off points was also examined. Specificity, sensitivity, and receiver operating characteristics curves were compared between the combined measures and BMI alone. The results showed that for white men, the optimal combination of BMI and WC for the identifying CHD risk factors was 0.68 x BMI + 0.32 x WC. This combination generated a score that better estimated the odds of having CHD risk factors than either alone. For white women, WC alone largely determined the likelihood of having CHD risk. Combined measures showed a higher sensitivity or a shorter distance in receiver operating characteristic curves in the identification of CHD risk factors. Reported conclusions support combining measures of BMI and WC so a higher overall test performance for CHD risk factors may be utilized in some ethnic groups as an improved screening tool [145].

Despite the means of measure, it is well known & widely accepted that obesity is a major risk factor for the development of chronic diseases and mortality [146,147]. The risk of CHD increases with increased BMI [147]. There have also been several prospective studies that have shown that increased abdominal fat accumulation is an independent risk factor for type 2 diabetes mellitus as well as cardiovascular risk

conditions such as CHD, stroke and hypertension [148-151]. The accumulation of visceral fat is associated with increased free fatty acid (FFA) secretion, hyperinsulinemia, insulin resistance, hypertension and dyslipidemia [152,153]. Since Gerald Reavins established the diagnosis of Syndrome X in 1988 (currently metabolic syndrome) there have been new insights shed in the manifestations of this disorder. Insulin resistance once thought of as one of four components of Metabolic Syndrome is now thought to be the single dominant factor predicting this pathology [154-156]. Insulin resistance occurs at the cell membrane and is an early indicator of disease.

Serum Markers

The physiological effects from obesity can affect the entire body system. The deposition of intra-abdominal fat and sarcopenic obesity can alter metabolic pathways and lead to excess energy (fat) redirected towards peripheral organs. The excess lipids can also enter non-oxidative pathways resulting in production of toxic, reactive lipid species [157]. This process can lead to lipotoxicity induced apoptosis. The accumulation of these reactive lipids can be found in organs such as the liver and heart. The alterations in metabolism and fat deposition play a major role in the development of comorbidities of obesity which include: coronary artery disease, stroke, type 2 diabetes, hypertension, dyslipidemia, musculoskeletal disorders, some cancers and deep vein thrombosis [157]. There are also hormonal mechanisms associated with obesity that include abnormal alterations in leptin, ghrelin, insulin and/or adiponectin.

Insulin, one of the most powerful hormones produced by the body is a major regulator of glucose metabolism. One primary regulator of basal insulin secretion between meals is the presence of fatty acids [158]. In obesity, there is an overabundance of free fatty acids secreted from a large reservoir of adipose tissue. The fatty acid regulation of basal insulin secretion between meals serves as a significant determinant of whole body "insulin sensitivity". Persistent elevation of free fatty acid levels has direct effects on metabolism in muscle and liver, dampening the effect of insulin [159-162]. The continued process of adipocyte hypertrophy (e.g., fat mass) produces an oversupply of fatty acids in the blood that contribute to metabolic disturbances via many pathways in the obese [163]. Sustained elevated levels of insulin, despite the cause, typically leads to generalized insulin resistance [163]. In transgenic mice, hyperinsulinemia caused insulin resistance, reduced insulin-receptor binding as well as increased triglycerides [163]. These findings support the idea that hypersecretion of insulin can be a cause, and the result of insulin resistance [163].

The presence of cytokines adds to the complex state of obesity [164-168]. TNF-α can produce insulin resistance at the level of target cells for insulin [163]. In research on obese mice, the insulin resistance of macrophages is associated with hypersecretion of cytokines and the enhanced density of macrophages found in fat depots bolsters the idea that cytokines play a major role in insulin resistance [163]. Despite the complexity of insulin resistance and cell signaling, one key intervention in treating the obese patient remains to be moderate caloric restriction, moderate exercise, 5%-10% weight loss and treatment of co-morbid conditions associated with obesity [163].

Adipose tissue has been referred to as one of the largest endocrine organs, having paracrine, autocrine and endocrine function [157]. Its effects on the body system have also been shown to differ by location. Adipose tissue plays a role in regulating body weight. It secretes products such as leptin, adiponectin, interluekin-6, TNFα, angiotensinogen, plasminogen activator-inhibitor 1 (PAI-1), adipsin (complement factor D), sex steroids, and glucocorticoids. Many of these products have been present in the literature since the mid-1990s but were not always connected to obesity [169]. These hormones play an intricate role in the etiology of obesity. Leptin, a cytokine-like polypeptide, can have an effect on long-term control of energy intake, whereas ghrelin and insulin appear to have short-term impact on energy intake [169]. Insulin, an anabolic hormone, has the known function of direct storage and utilization of energy in adipocytes. In obesity, insulin is often in abundance in the body. Insulin is often found in high amounts due to the lack of communication between insulin and its receptor located on the cell membrane. Insulin secretion subsequently increases by the beta cells of the pancreas [169].

Abdominal obesity and Type II Diabetes have proven to be related to insulin resistance. Leptin and adiponectin have also been shown to play a role in insulin regulation. They both have been linked to increase insulin sensitivity and leptin has been shown to decrease insulin secretion. Leptin and other anorectic (pertaining to anorexia) cytokines may also mediate levels of ghrelin. Ghrelin is found in high concentrations in the stomach and its expression and secretion increases with fasting. This area of research shows a lot of promise in better understanding the pathophysiology

of obesity on a cellular level [169].Adiponectin, a protein secreted by adipose tissue, is present at lower levels in the obese. It has been shown to affect different aspect of the immune system and subsequent inflammatory responses. Low levels of adiponectin have been associated with cardiovascular disease, diabetes and insulin resistance [169]. Hyperlipidemia, defined as abnormal lipid levels is a known risk factor for Coronary Heart Disease (CHD). In particular, concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol have been highly correlated with CHD. Low levels of High density Lipoprotein (HDL) are an individual risk factor for CHD. Elevated triglycerides are also associated with CHD and often co-exist in those with low HDL and high LDL. Triglycerides also respond very well to non-drug therapy. Pharmaceutical companies track prescription compliance of statin therapy and have noted that approximately 30% of patients initiated on statins do not continue their prescriptions [170].

Due to the financial burden of these drugs, many patients admit to taking their medicine every other day, or a few times a week to decrease cost. Outcome studies on statin therapy base results on daily compliance. Recent literature also suggests that abrupt stoppage of this medication may be more harmful than if therapy had never been initiated. In the elderly population, it is often debated whether long term statin therapy benefits out way the risks. These drugs are financially taxing and can cause unpleasant symptoms as well as negative side effects in muscle and liver tissue. Due to the multi-system benefits of exercise, this population stands to reap tremendous benefit from lifestyle modification.

Fahlman et.al (2002) examined the relationship between exercise and plasma lipoprotein levels in the elderly population. The purpose of the study was examining the effects of endurance and resistance exercise on plasma lipoprotein levels in elderly women who were active but non- exercising prior to the study. The total number of participants equaled 45 healthy, active women, aged 70-87 years, randomly assigned to either an aerobic training (AT, n=15), resistance training (RT, n=15) or control (C, n=15). The AT group walked three days a week at 70% heart rate reserve. The duration on day one was 20 minutes, and the time increased by five minutes each day until participants were walking a total of 50 minutes per session (week 3) [171]. The training session for the RT group consisted of one to three sets of an eight repetition maximum. The C group maintained their normal routine. Weight and diet were unchanged across groups. The interventions lasted a total of ten weeks, while serum blood samples were obtained at week zero and eleven. The AT training group had significant decrease in 1-mile walk times and heart rate at completion of the walk and there was a significant increase in eight repetition maximum of all RT exercises. Both the AT and RT groups experienced increased HDL cholesterol and decreased triglycerides at week 11 in comparison to week 0. There were no positive changes in lipoproteins for the control group. Both triglycerides and the total cholesterol to HDL ratio increased significantly while total cholesterol, HDL cholesterol and LDL cholesterol remained unchanged. The RT group also had significant lower LDL cholesterol and total cholesterol compared with controls at week 11. Both resistance and endurance training resulted in favorable changes to plasma lipoprotein levels for elderly women in only ten weeks. These

changes occurred without changes in weight or diet. The researchers concluded that high-intensity exercise alone can be used to modify lipoproteins in healthy elderly women [171]. Likewise, positive results have been reported comparing resistance training & multi-component exercise (ME), with ME yielding the better results in a similar population [172].

Protein Synthesis

Fractional synthesis rate (FSR) has been established as the preferred measure of protein synthesis, and measures the rate of tracer incorporation from the muscle intracellular free (MIF) pool into the protein-bound amino acid pool (PBAAP) [173,174]. The free amino acid pool (FAP) in muscle is often used as an acceptable surrogate of the precursor for muscle protein synthesis[174,175]. Control of the processes surrounding protein synthesis in tissue of free living humans is a complex process requiring continuous integration of multiple positive and negative stimuli [176].

Hypertrophy of skeletal muscle tissue following resistance exercise and the associated increase in protein synthesis is mediated by a synergistic interaction of mechanical, hormonal, and nutritional stimulation [16]. Mechanical and nutritional stimuli converge on cell signaling pathways that are under the influence of anabolic hormones and growth factors such as insulin and insulin-like growth factor 1 (IGF-1), resulting in increases in both global (e.g. cap-dependent) and specific (e.g. 5' TOP) mRNA translation [15]. Consideration of the impact of transduction at the cellular and molecular levels on protein synthesis, in response to various types of stimuli (such as

mechanical, hormonal, and nutrient signals), may enhance our understanding of basic muscle physiology.

Enhanced understanding of these principles can be pursued through their application in post-resistance exercise nutrition strategies. Such strategies seek to optimize the anabolic hormonal response of resistance exercise through ingestion of macronutrients [177]. Feeding a complex diet of macronutrients results in increased blood concentrations of insulin and amino acids, which is associated with insulin-dependent and insulin-independent stimulation of protein synthesis. Conversely, a decrease in plasma insulin and amino acid levels in the blood is observed upon reversal of the effects of the feeding, with protein synthesis machinery assembly returning to pre-prandial levels [178]. It has been reported that as muscles age they become increasingly resistant to the anabolic effects of amino acids [179-181]. Specifically, feeding (hyperaminoacidemia) has been reported to double muscle protein FSR [35,182,183]. However, potentially less favorable results have been suggested in elderly populations [184]. These data support the plausible importance of addressing this concern during the earliest stages of the aging process.

Hypertrophy of skeletal muscle is defined as an increase in myofiber diameter, and is characterized by increased contractile force and augmented protein synthesis [15]. Protein synthesis is comprised of three phases: initiation, elongation, and termination. Initiation of messenger RNA (mRNA) translation is a key site of regulation for gene expression and protein biosynthesis in skeletal muscle tissue [185,186]. Mitogens (e.g. insulin and IGF-1) and nutrients (e.g. amino acids) converge on cell signal transduction

pathways to affect translation initiation [187]. Nutritional signals control gene expression through modulation of cell transduction pathways that have traditionally been thought to be stimulated by the action of hormones [188]. For example, the distinct signals from insulin, amino acids, and resistance exercise reportedly converge on the (phosphoinositide 3-kinases) PI3K – (protein family also called protein kinases B) Akt/PKB – (mammalian target of rapamycin) mTOR signaling pathway to promote muscle hypertrophy following resistance exercise [187]. Hence, the overall molecular detection of protein synthesis may not be best marked by select protein up-regulation. Rather, a coordinated response from multiple points in the signal transduction pathways is essential for complete understanding of the process of mRNA translation associated with muscle hypertrophy [187].

Branched chain amino acid mediated increases in protein synthesis are attributed to regulation at the initiation phase of translation [189]. Immediately following resistance exercise and without supplementation, 4E-BP1 (eIF4E-binding proteins) phosphorylation is significantly reduced [20]. However, recent findings in the ESNL have suggested that it may be significantly increased at two hours post-exercise when BCAA and LEU supplementation is ingested before and after exercise [190]. While BCAA (and especially LEU) modulate specific and global mRNA translation through a mitogen (a chemical substance that encourages initiation of cell division)-independent mTOR mechanism, they appear to modulate signaling through the pathway that is shared by the action of mitogens (e.g. insulin), rather than to directly regulate translation initiation [190]. Apparently there is a synergy between BCAA and the insulin effects on

the PI3K-Akt/PKB-mTOR signaling pathway, whereby optimized translation initiation may require a combination of amino acids and insulin [185,188,191,192].

Considering that the protein synthetic pathway is sensitive to the availability of both nutrients and hormones to modulate the initiation of mRNA translation, current literature suggests that the provision of carbohydrate (CHO) with amino acids may best elicit a coordinated mitogenic (e.g. insulin) and nutrient (e.g. amino acids) effect on both global and specific protein synthesis initiation markers [190]. Miller, et. al. (2003) demonstrated that a combination of amino acids and CHO consumed immediately before and one hour after an acute resistance exercise bout resulted in a significantly greater skeletal muscle protein synthesis as compared to either separately, as determined by stable isotope methodology [193]. The enzymes Akt, mTOR, p70(S6K), rpS6, GSK3, and glycogen synthase interact in the control of protein and/or glycogen synthesis in skeletal muscle, and each has been found to respond to exercise and nutrient supplementation.

Ivy (2008) investigated whether nutrient supplementation post exercise, in the form of a carbohydrate-protein (CHO-PRO) supplement, would alter the phosphorylation state of these enzymes in a manner that should increase muscle protein and glycogen synthesis above that produced by exercise alone. After a 45 min cycling session followed by sprints, and again 15 min later, 8 subjects (well-conditioned males, who were 23.6 ± 1.2 years of age) ingested 400 ml of a CHO-PRO drink (7.8% dextrose and 1.8% protein-electrolyte) or a placebo drink. At 45 min after supplementation, CHO-PRO treatment yielded greater phosphorylation of Akt (65%), mTOR (86%), rpS6

(85-fold), and GSK3alpha/beta (57%) than pre-exercise levels. Although p70(S6k) showed an exercise response after 45 min, there were no differences between treatments. Glycogen synthase (GS) phosphorylation was significantly reduced 45 min after exercise for both treatments, but the reduction in phosphorylation was greatest during the CHO-PRO treatment (3-fold decrease). Thus, indicating greater activation of GS following supplementation. No difference between treatments was detected prior to exercise for any of the enzymes [194]. These results suggest that a post exercise CHO-PRO supplement alters the phosphorylation levels of the enzymes tested in a manner that should accelerate muscle glycogen synthesis and protein initiation during recovery from cycling exercise.

More practically, a 2006 study compared dietary protein intake along with post-exercise supplementation. Reported conclusions suggested that post-exercise supplementation may have a more direct impact on favorable FFM profiles than a simple increase in daily dietary protein support [51]. Cumulatively, these data seem to support the use of a combined PRO and CHO supplement in the post-exercise recovery period to activate enzymes associated with muscle protein synthesis via insulin-mediated PI3K-mTOR pathways. Emerging data in the literature suggest that a combined CHO - PRO [perhaps more specifically select amino acid(s)] supplement stimulates protein translation [197] through mTOR activation [195].

Direct measurement of muscle protein synthesis can provide relevant information regarding the metabolic state of the tissue, and therefore, should be included as an outcome variable when examining anabolic/catabolic conditions. As suggested

previously[196], direct incorporation methods using tracers such as [^{13}C]leucine or [^{13}C]-, [^{15}N]-, or [^{2}H]phenylalnine have traditionally been used to measure tissue specific protein synthesis and are generally accepted for providing reliable measurements. Briefly, by providing a known amount of labeled (tracer) and unlabeled amino acid (tracee) the tracer and tracee will mix with the endogenous pool and become incorporated into protein over time. The tracer and tracee are generally administered as a primed-constant infusion (a priming bolus of tracer and tracee is administered and is continuously provided at a lower concentration to maintain enrichment), or as a flooding dose (a supraphysiological bolus of tracer and tracee is provided over seconds to minutes). Protein synthesis can be determined by measuring the enrichment (tracer/tracee) of the protein against the enrichment of the precursor (i.e., precursor: product labeling ratios). However, two central concerns that have existed with the precursor-product method have been identification and measurement of the true precursor (aminoacyl-tRNA), and the inability to conduct the tracer experiments in a free-living environment over an extended period of time, an integrative/cumulative assessment. Specifics with regard to these concerns have been addressed previously in the literature [196], and investigators have proposed various methods by which they may be addressed [38,39,175,196-198].

Currently, a more convenient but less familiar approach has come into favor which allows for circumvention of these noted limitations. The use of $^{2}H_2O$ as proposed in this paper was first described [199], re-investigated [200], and has been used more recently in studies at Texas A&M[196]. In this recently published study by Gasier, et.

al., the effect of a high-intensity bout of resistance exercise in the fed state with energy and macronutrients controlled over 24 h in humans was examined. Although no stimulatory effect of exercise on FSR in the mixed vastus lateralis was observed, there was a significant effect on the myofibrillar FSR with exercise.

Deuterium (^2H) & Heavy Water (^2H_2O)

Deuterium [heavy hydrogen, (^2H)]: The adult human body naturally contains deuterium equivalent to the amount in about 5 grams of heavy water, and comparable doses of heavy water are still used as safe non-radioactive tracers for metabolic experiments in humans and other animals. Because it would take a very large amount of heavy water to replace 25% to 50% of a human being's body water (which in turn is 70% of body weight) with heavy water, accidental or intentional poisoning with heavy water is unlikely to the point of practical disregard, and cannot be considered toxic to humans [201]. It has been estimated that a 70 kg person might drink 4.8 liters of heavy water without serious consequences. For example, a 70 kg human containing 50 kg of water and drinking 3 liters of pure heavy water per day, would take almost 5 days to reach 25% deuteration and about 11 days to approach 50% deuteration. Thus, it would take a week of drinking nothing but pure heavy water for a human to begin to feel ill, and 10 days to 2 weeks (depending on water intake) for severe poisoning and death. The U.S. issued a patent for the use of heavy water to treat hypertension (high blood pressure). Reportedly, decreased blood pressure may partially explain reported incidents of dizziness upon ingestion of relatively larger amounts. Small doses of heavy water (a few grams in humans, containing an amount of deuterium comparable to that normally

present in the body) are routinely used as harmless metabolic tracers in humans and animals [202].

Typically, dosages are in the range of 40 mg per kg of $^{2}H_2O$ [203]. These values are derived from considerations of instrumental accuracy at the end of the test period, when as little as 10% of the isotope remains in the body water. Using this dosing regime, FSR can be measured with a coefficient of variation of better than 5%. Recently, it was proposed that much smaller dosages might be effective [207]. However, considerable care in experimental design would be required for this to be done without severely prejudicing the quality of the results [204,205].

Deuterium oxide ($^{2}H_2O$), has been used successfully to measure protein synthesis in mice, rats and humans[199,206-211]. Studies have shown that by administering a dose of $^{2}H_2O$ rapid equilibration (~ 20 min) of the tracer in body water will occur[209,212] and both rapid and extensive labeling of intracellular amino acids (e.g. alanine) primarily via transamination[199,213], which can then become incorporated into protein. Reportedly, since alanine is readily produced intracellularly and has four possible sites for ^{2}H-labeling[213], its labeling should reflect that of body water over a wide variety of conditions [206,209,212]. Additionally, maintaining a steady concentration of $^{2}H_2O$ in body water (thus ^{2}H-labeled alanine) over days to weeks by providing periodic maintenance dosages of $^{2}H_2O$ in drinking water is relatively practical [199,206,208,209], albeit admittedly somewhat expensive. Finally, ^{2}H-labeling of alanine does not appear to be influenced by common metabolic activities such as feeding[209,210,212] and/or exercise[211]. Therefore, it stands to reason that non-

steady-state protein metabolism may be assessed over short and long periods of time, providing a free living account of what is actually occurring within selected tissues of interest.

Previously, cumulative assessments of whole body protein synthesis have been made using oral [1-13C]lysine[214] or intravenous [1-13C]leucine [215]. However, few have examined skeletal muscle over extended periods. The human rate of protein synthesis (RPS) has been estimated at 1.5-2% per day [216], but this is an estimation based on calculations collected over only up to a six hour period. A recent study [196] comparing these more invasive tracer protocols to the use of deuterium oxide (^2H_2O) as a tracer methodology questioned such extrapolations, and suggests that the ^2H_2O methodology might produce more accurate results when measuring FSR over longer periods of time (e.g. 24 hours or longer) in free living environments.

^2H_2O has been reported to accurately, and relatively non-invasively, measure protein synthesis over short and long periods of time [207,208,210,212]. However, only recently has this methodology been investigated in reference to RE and feeding. Recently published observations[196,211] suggest that there may be differences between cumulative myofibrillar v. mixed muscle protein synthesis after an acute bout of high intensity RE. However, studies have not investigated the effects of acute or subacute moderate intensity combined exercise (aerobic/anaerobic) with timed nutrient intake on protein synthesis in overweight post-menopausal women. Studies have also not investigated the effects of a commercially available PRO/CHO supplementation (acutely or sub acutely) in such an untrained and/or trained population. Neither have studies

investigated the effects of timed nutrient intake in overweight post-menopausal women over extended training periods utilizing the $^{2}H_2O$ stable isotope methodology.

Nutrition

A key component of weight management is sound dietary strategies and a consistently well-balanced diet. The appropriate percentage of macronutrients is also critical in balanced nutrition. Recommendations should include increased fruits and vegetables, decrease fat content to < 30% with < 10% from saturated fat, lean protein sources, protein intake for older men and women is between 1.0-1.25 g of high quality protein and appropriate percentages of macronutrients [4]. This study will offer one nutrition plan (higher protein diet) with two variations: 1.) with, & 2.) without post-exercise nutrient timing.

The problems of obtaining accurate assessments of dietary intake from free-living subjects are well known [217], and doubly labeled water (DLW) has been used on many occasions to assess the performance of dietary assessment techniques [218]. The assumption is that for a subject in energy balance, total energy expenditure (TEE) and energy intake are equal; therefore, any difference between DLW results and declared energy intake must be due to inaccuracies in reporting [203]. Efforts at validation of dietary self-assessment and/or energy expenditure have been long pursued [219-223]. A recent publication has attempted to provide a framework for the validation of dietary self-assessment on this basis [222]. Small concentrations of heavy water are nontoxic. Dosages of the two isotopes of doubly labeled water DLW [^{2}H (deuterium), and ^{18}O (Oxygen-18)] equilibrate with total body water (TBW). Oxygen-18 is a natural, stable

isotope of oxygen and one of the environmental isotopes. Deuterium (^{2}H) leaves the body as water (H_2O), while ^{18}O leaves as water and carbon dioxide (CO_2), two elements common to normal human metabolism and respiration [224].

Successful aging involves the ability to maintain three key behaviors: decreased risk for disease and related disability, high mental and physical function, and active participation in life [225]. Nutrition is considered one of the major determinants in this process. Nutritional choices are not only critical to physiological well-being, but also contribute to social, cultural and psychological quality of life. The most influential behaviors influencing aging aside from genetics include consistently eating a healthy diet, being physically active, and avoiding tobacco [225].

Normal aging is accelerated by variables that are associated with the concomitant frailty of aging. Aging adults experience age-related declines in food intake as well as other complicating factors in achieving adequate nutrition such as poor dentition, financial resources and alterations in brain function that can all contribute to poor nutrition. One such factor, the decline in food intake has been termed the anorexia of aging. Many experts in the field of gerontology consider the decline in food intake to be an important factor in the development and progression of sarcopenia [97]. Normal aging in humans progressively leads to the decline in neuromuscular function, ultimately affecting the ability to perform daily activities. Sarcopenia has also been coupled with the condition of obesity (sarcopenic obesity). This combination of conditions obviously compounds the concerns for the elderly.

As we age, many variables (e.g. financial constraints and access to food options) can and do affect sound nutritional habits. Extensive research has been conducted to compare various macronutrient combinations for health, weight reduction and maintenance in aging populations. Two areas that have received considerable attention include higher protein/lower carbohydrate and "normal" carbohydrate/lower protein diets. The Curves program offers both of these options. Previous Curves research has yielded positive results with both diets during a 14 week intervention including exercise [139].

Previously cited research has suggested that inadequate protein intake most likely plays a key role in the complex equation of sarcopenic obesity. It is well accepted that adequate protein intake is crucial in maintaining cellular integrity, function, and health by contributing amino acids that serve as precursors for essential molecules which serve as building blocks for all cell components [226]. It is also important to note that adequate intake of carbohydrates, fats, vitamins, minerals; fiber and water also need to be ingested in order for these processes to occur. High protein diets have also shown improvement in weight loss, maintaining muscle mass and improving blood markers in all ages. There is also a substantial amount of research that supports certain diagnoses may actually respond more favorably to high protein nutrition plans. The majority of nutritional research in weight loss and maintenance has been done on the adult population spanning from young adult (age 20) to the young old (aged 60). There is perhaps, a relative shortage of research in the healthy elderly. Nutritional research in

this age group appears to be disease specific, and primarily focused on institutionalized or hospitalized patients.

Noakes et.al. performed a study comparing hypocaloric high protein (HP) diet to a high carbohydrate (HC) diet [142]. Approximately 100 participants with a body mass index of 32 ± 6 and age of 49 ± 9 years completed the study. They were randomly assigned to one of two isocaloric 1,337 kilocalorie (5600 kJ) dietary interventions for twelve weeks according to parallel design. Weight loss achieved was 7.3 ± 0.3 kg with both diets. The participants with high triacyglycerol lost more fat mass with the HP diet than with the HC diet (SEM= 6.4 ± .7 and 3.4 ± .7 kg, respectively) and had a greater decrease in triacyglycerol concentrations with the HP (~5.9 ± 0.19 mmol/L) that with the HC (~0.03 ± 0.04 mmol/L) diet. Fasting LDL-cholesterol, glucose, insulin, free fatty acid, and C-reactive protein concentrations decreased with weight loss. Serum vitamin B-12 increased 9% with both diets; homocysteine did not change significantly. Bone turnover markers increased 8-12% and calcium excretion decreased by 0.8 mmol/d. Creatinine clearance decreased from 82 ± 3.3 to 75 ± 3.0 mL/min. This research suggested the HP diet was effective in producing weight loss as well as improving risk factors for diabetes and heart disease.

Meckling, et.al. (2004) studied a population of adult overweight and obese men and women (age 24-61) to compare the effects of a low-fat (LF) diet versus a low-carbohydrate (LC) diet. The participants in the LF diet consumed approximately 17.8% of energy from fat, compared with their habitual intake of 36.4% and had a resulting energy restriction of 606 kcal/day (2540 kJ/d). Participants on the LC diet consumed an

average of 15.4% carbohydrate, compared with habitual intakes of about 50% carbohydrate, and had a resulting energy restriction of 763 kcal (3195 kJ/d). Significant weight loss was reported in both groups over the ten week intervention, and there were almost identical improvements in body weight and fat mass.

In the Meckling study, LF participants were reported to have lost an average of 15 pounds (6.8 kg) with a decrease in body mass index of 2.2 kg/m^2, compared with a loss of 7.0 kg and a decrease in body mass index of 2.1 kg/m^2 in the LC participants. The LF group showed a better preserved lean body mass when compared with the LC group. However, the LC group showed a significant decrease in circulating insulin concentrations. Reported group results indicated the diets were equally effective in reducing systolic blood pressure by about 10 mmHg and diastolic pressure by 5 mmHg. Blood β hydroxbutyrate (supporting adipose tissue utilization for energy) concentrations increased in the LC group only at the 2 and 4 week time points. Data reported in this study suggest that energy restriction achieved by the LC diet is equally effective as the LF diet strategy for weight loss and decreasing body fat in overweight and obese adults [227].

Other benefits noted in literature for low carb/high protein diets include: decreased central adiposity and reduction in cardiovascular disease rates [142]. Visceral adiposity is of concern because it is felt to be more threatening possibly due to its position in the body and drainage via venous portal veins. It is important to note that long term studies need to be done in this area to confirm short duration studies. It has

also been noted that low carbohydrate/high protein diets are not always well tolerated long term and safety in patients with altered renal function is unknown as well [142]. Layman, et.al (2005) tested the benefits of high dietary protein, while maintaining the recommended macronutrient composition noted above. Their study included forty-eight overweight and obese women (40-56 years) in two diet groups (with and without exercise). The higher-protein, reduced-carbohydrate (PRO) diet was designed to provide dietary protein at 1.6 g/kg per day (~30% of calories) and ~ 30% of calories from dietary fat, allowing a carbohydrate-protein ratio of < 1:5. The higher-carbohydrate, moderate-protein (CHO) diet was designed to provide 0.8 g/kg per day of dietary protein (~15% of calories) and ~30% of calories from dietary fat, yielding a carbohydrate-protein ratio of >3:5. Both of the diet groups were within the boundaries of Acceptable Macronutrient Distribution Ranges (AMDR) defined by the Institute of Medicine. Each group was randomized into either supervised exercise or control, following the NIH guidelines of walking 30 minutes per day at least five days per week. Conclusions were that diets higher in protein and moderate in carbohydrate appear to have advantages for weight reduction, body composition, and plasma triacylglycerol. The high carbohydrate diet was shown to more efficiently decrease total and LDL cholesterol levels. Hence, it was also concluded that diets should consider clinical outcomes and pre-existing lipid values [228].

Based on research to date, the high protein and normal carbohydrate diets both produce favorable weight loss in studies up to one year. Evidence from some short term studies suggest that the high protein diet may offer more in regards to specific serum

markers (e.g., lipids) and blood pressure, but it has been suggested that it is unknown how tolerable these diets are over the long term. There is also interest regarding the effects which low carbohydrate diets may have on phytochemical composition and fiber. Additionally, the long term safety of these diets (> one year) in patients with altered renal function appears yet to be determined [142]. Longitudinal studies to test compliance and tolerance of diets, as well as long term effects in specific areas such as blood markers, bone density, muscle mass and ability to maintain weight loss are definitely of interest based on the reported studies.

The purpose of the dietary intervention in this study is to compare results in a higher protein diet with & without timed nutrient intake in female participants of the Curves Fitness and Weight Management Plan.

Nutrient Timing

A developing trend in research suggests that carbohydrate ingestion within 15 minutes post-exercise helps to restore depleted glycogen reserves [49,59]. Intake of an adequate amount of carbohydrate within two hours of prolonged exercise is essential to resupplying/maintaining adequate glycogen stores for continued training. Intakes with longer than a two hour delay are reported to result in 50 percent less muscle glycogen resupply. The carbohydrate consumption stimulates insulin production, which in turn aids in the reestablishment of muscle glycogen levels. However, the effect of carbohydrate consumption on glycogen storage does plateau. Additionally, combining protein with carbohydrate in the two hour "window" after exercise nearly doubles the insulin response, which results in more stored glycogen [57]. Higher protein content,

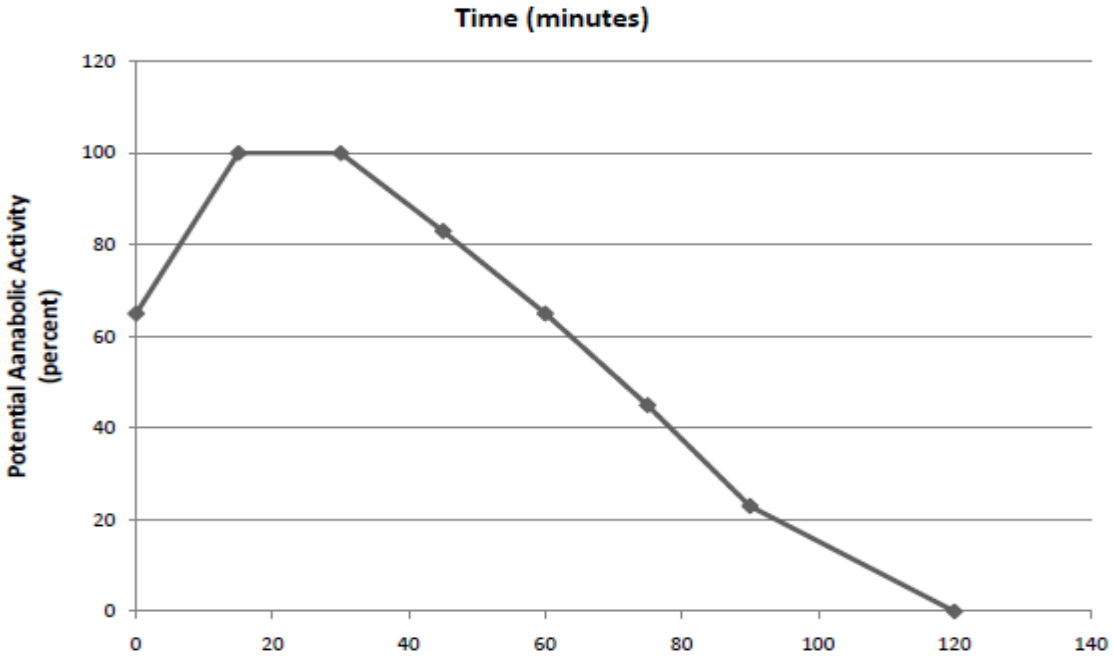
however, has been suggested to have a negative impact because of slowed rehydration and glycogen replenishment [57].

Athletes who refuel with carbohydrate and protein are reported to obtain 100 percent greater muscle glycogen stores than those who consume carbohydrate alone. Additionally, insulin is also highest in those who consume a carbohydrate and protein drink. For resistance training, a ratio of 2:1 carbohydrate to protein has been shown to be most effective. For long, strenuous endurance exercise, a 4:1 ratio of carbohydrate and protein seems to be a better choice. While solid foods may provide the required nutrients, a liquid may assimilate more rapidly, make it easier to achieve the preferred ratio, and thus more efficiently meet the 2-hour window. It has been reported that the "anabolic phase" wherein the optimal effects of nutrient ingestion begins to deteriorate, or "close" within 45 minutes post exercise, and peaks within 15-30 minutes post exercise [229] (Figure 1). The general premise is that by consuming critical nutrients [e.g. carbs, protein (specific amino acids), and vitamins] during a time sensitive window, one might spare muscle glycogen, achieve greater muscular endurance, stem the rise of the catabolic hormone cortisol (hence reducing muscle damage), and facilitate a more favorable environment for efficient recovery following exercise.

Consuming protein has other important uses after exercise. Protein provides the amino acids necessary to rebuild damaged muscle tissue during intense, prolonged exercise. It can also increase the absorption of water from the intestine, improve hydration status, and benefit the immune system. A 2004 study reported that the administration of a carbohydrate beverage with additional protein calories showed

significant improvements in cycling time to fatigue, and reductions in post-exercise muscle damage (measured indirectly using plasma CPK levels) in comparison with a carbohydrate-only beverage [230]. Obese individuals eating a slightly higher protein diet (25% of calories from protein), lost significantly more weight and body fat than those eating a lower

Figure 1. Nutrient Timing Window (Adapted from Ivy & Portman, 2004).



protein diet (12% of calories from protein) [231]. Prolonged strenuous exercise substantially depletes glycogen stores, which has important implications for post-exercise nutrition. Specifically, muscle glycogen replenishment is of the highest priority, and a carbohydrate/protein combination may play a major role in this process. Recent research has focused on the timing, amount, and type of carbohydrates, and the

ratio of protein to carbohydrates, in post-exercise meals. One study suggests that an immediate intake, within 30-45 minutes following exercise, comprised of simple (high glycemic index) carbohydrates will stimulate both glucose transport and glycogen synthase activity, promoting faster muscle glycogen resynthesis[232]. Although prolonged strenuous exercise will not be the training design for this study group, it will be of interest to determine whether or not results indicate relative positive influences.

Perhaps most interestingly, a 2006 study suggested that post exercise intake may be more influential than total daily protein intake as an effective method for maintaining FFM during periods of either increased energy expenditure (e.g. exercise) or "marginal" protein intake [51]. Subjects of said study included (in addition to 22 men of the same age range) untrained (≤ 3 hours/wk) women (n=30, 60-69 years) who participated in a 12 week moderate resistance exercise (RE) program. The post exercise supplement consisted of a Boost HP beverage (Novartis/Mead Johnson) composed of 240 kcals (15 g protein, 33 g CHO, and 6 g fat per 8 oz.) or an alternate, presumably equivalent supplement (3 subjects who were intolerant of the Boost) given immediately post exercise in an amount corresponding to 0.4 g of protein/kg of FFM. This approach seems particularly related to the type of routine proposed during our study. Finally, the 2006 study suggested that a randomized double-blind study would be necessary to confirm the reported results. Although there are a plethora of studies supporting timed nutrition within a "window" surrounding exercise, a current study suggests that the timing period may be quite liberal depending on when the pre-workout meal was consumed[272].

Conclusion

The aging population is currently one of the fastest growing segments in our nation [65] and lends itself to increasing investigational studies. Due to the high prevalence of obesity in this age group, it has been estimated that almost one-third of health care expenditures is for older adults (over age 65) [66]. There is currently a limited amount of research in elderly nutrition intervention in non-institutionalized, healthy populations. Hence, it has yet to be determined what the ideal macronutrient and caloric percentages are needed for this population. Current literature supports that there are risks associated with weight loss in this age group. There is debate surrounding ideal weight for this population due to potential harmful effects of weight loss on muscle and bone mass, as well as the ideal amount of weight loss necessary to produce optimal health. Perhaps the best defense is a good offense. If so, addressing these concerns early will offset some of the effects commonly observed in our aging population (an ounce of prevention). This study will provide additional information regarding whether the current Curves HP diet, along with the addition of a timed nutrition option, is more or less effective in retaining muscle mass in overweight females as compared to the standard Higher Protein option. A decline in muscle mass alters daily energy expenditure and insulin sensitivity as well as functionally related variables such as muscle weakness, fatigability and decline in endurance. As a counter measure to the aging co-morbid conditions discussed in this paper, it stands to reason that an early preventive approach to deterrence would be advisable.

CHAPTER III

METHODS

Participants

Approximately 20 sedentary, overweight female post-menopausal participants (50-70 years; BMI ~ 27+) participated in this 12 week prospective longitudinal clinical trial. Participants were medically cleared by their physician in case any screening procedures (via ACSM guidelines) determine it a necessity, and were not allowed to participate if they had any uncontrolled metabolic disorder, or known electrolyte abnormalities; heart disease, arrhythmias, diabetes, or thyroid disease; a history of hypertension, hepatorenal, musculoskeletal, autoimmune, or neurological disease. Nor were they accepted for participation if they were taking hypoglycemic, or androgenic medications; and/or, if they had taken ergogenic levels of nutritional supplements that may have affected muscle mass (e.g., creatine, HMB), anabolic/catabolic hormone levels (e.g., DHEA), or weight loss (e.g., thermogenics) within three months prior to beginning the study. Exceptions were only allowed if the prospective participant had a medical condition, or history that the participant's personal physician concluded was controlled, and therefore not be a limitation for participation in the study. Participants that otherwise met eligibility criteria were informed of the study requirements, and signed consent statements in compliance with the Human Participants Guidelines of Texas A&M University and the American College of Sports Medicine.

Study Site

All testing was conducted in the Exercise & Sport Nutrition Laboratory (ESNL) and the Human Countermeasures Laboratory (HCL), and sample processing conducted in the Muscle Biology Laboratory (MBL) Department Health and Kinesiology at Texas A&M University. Exercise training will be conducted at the Curves circuit within the ESNL.

Entry and Familiarization

Participants who expressed interest in participating in this study were interviewed (screened) via phone to determine whether they qualified to participate. Those deemed to meet eligibility criteria were invited to attend an entry/familiarization session held within the ESNL. Familiarization sessions were designed to provide detail as to requirements and expectations of the study. During this session, participants signed Informed Consent Statements, and completed personal and medical histories; and were familiarized as to study protocol via a verbal (audiovisual) and written explanation outlining the study design, expectations, and implications. The explanation included a description of the training and dietary program, and familiarization of the participants as to the tests which were to be performed. Participants were required to obtain medical clearance from their personal physician prior to participating in baseline assessments. Those who qualified and accepted were scheduled for day 1 of T1 testing, after which they were matched and randomized, based on age and lean mass (LM), into either the I or D group. Upon completion of day 1, they were scheduled for their second and third days of T1 testing.

Randomization

Participants were randomized into one of two groups based on age & LM. The groups include: 1.) Immediate Timed Nutrition (I), and 2.) Delayed Timed Nutrition (D). Both groups were assigned the same higher protein diet, with the same balance of macronutrients and caloric content. Both groups obtained a measured portion of their caloric and macronutrient content from the post exercise nutrition (shake) on days when they exercised & were tested (T1 & T4).

Experimental Design

Table 1 and Figure 2 (pg. 63, 64) outline the general research design and time course for the various assessments. The independent variable is the timed nutrition. The dependent variables are: FSR as measured via muscle biopsies; body composition [fat free mass (FFM), & fat mass (FM)], isotonic strength [Leg Press (LP) and Bench Press (BP)]; and resting energy expenditure (REE).

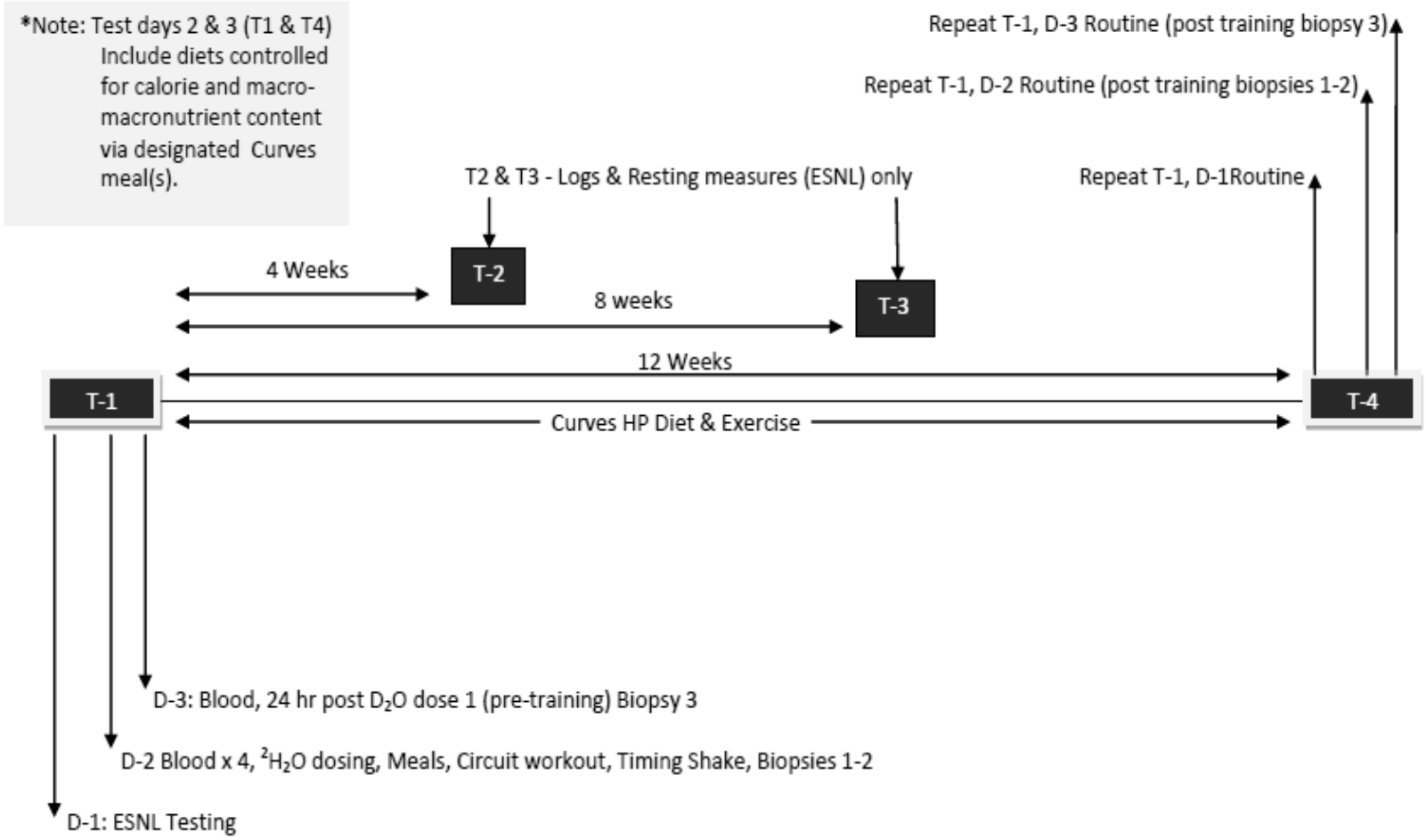
Once medical clearance was obtained, participants were given an appointment for their T1 testing sessions as summarized in Table 1. T1 was divided into 3 days of testing. T1-Day 1 included of all standard ESNL resting and exercise procedures as outlined in Table 1, and described in detail later in this chapter. T1-Day 2 was 7 days post T1-Day 1 to avoid any potential physiological implications from T1-Day 1, and to facilitate scheduling issues. T1-Days 2 & 3 included $^{2}H_{2}O$ dosing, blood measures, biopsies, assigned meals (breakfast, lunch and dinner) as applicable according to the Curves high protein diet plan. Participants completed their first exercise session on T1 Day 2.

Table 1. Overview of Testing

T-0 (Entry)	T-1	T-2/T-3	T-4
-Phone Interview -Familiarization -Assessment to determine study qualifications -General nutrition Review	Day 1 REE; DEXA; BIA; Hip:Waist; Food/Activity Logs; Fasting Blood; QOL/Eating Satisfaction Surveys; Maximal GXT/ECG; 1 RM BP/LP; & Circuit Testing Participants randomized according to BMI and age into either the **EARLY** or **LATE** group Day 2[1] **0600** Blood; $^{2}H_2O$ dose 1 **0630** Curves Breakfast[2] **0800** $^{2}H_2O$ dose 2 **0830** Blood; Biopsy 1 **1000** Circuit Workout, **1030** post exercise intake (EARLY Group only) **1045** Blood **1100** Biopsy 2 **1230** post exercise intake (LATE Group only) **1300** Curves Lunch[2] **1330** $^{2}H_2O$ dose 3 **1515** Blood **1530** $^{2}H_2O$ dose 4 **1700** Curves Dinner[2] Day 3[1] **0545** Blood **0600** Biopsy 3	REE; DEXA; BIA; Hip:Waist; Food/Activity Logs; Fasting Blood; QOL/Eating Satisfaction Surveys	Day 1 REE; DEXA; BIA; Hip:Waist; Food/Activity Logs; Fasting Blood; QOL/Eating Satisfaction Surveys; Maximal GXT/ECG; 1 RM BP/LP Day 2[1] **0600** Blood; $^{2}H_2O$ dose 1 **0630** Curves Breakfast[2] **0800** $^{2}H_2O$ dose 2 **0830** Blood; Biopsy 1 **1000** Circuit Workout, **1030** post exercise intake (EARLY Group only) **1045** Blood **1100** Biopsy 2 **1230** post exercise intake (LATE Group only) **1300** Curves Lunch[2] **1330** $^{2}H_2O$ dose 3 **1515** Blood **1530** $^{2}H_2O$ dose 4 **1700** Curves Dinner[2] Day 3[1] **0545** Blood **0600** Biopsy 3

1. Day 2 is scheduled 1 week after Day 1 testing; Day 3 follows Day 2.
2. All assigned meals matched for macronutrient and caloric content.

Figure 2. Study Timeline



Those randomized into the I group were administered their post exercise nutrition immediately post exercise. The D group was instructed to drink their nutrition at 2 hours post exercise. On testing days, the D group was given the shake at 2 hours post exercise. The T1-Day 3 biopsy was at 24 hours post T1-Day 2 $^{2}H_{2}O$ dose 1. Subsequent to the T1 series, T2, (4 wks) and T3 (8 wks) testing collected resting measures and program compliance data. The final testing series (T4) was scheduled at the 12 week point of the study. The timeline for the T4 series was identical to that of T1 series, as were the testing procedures.

Training Protocol

All participants attended, and participated in the 30 minute Curves circuit three times per week for 12 weeks while maintaining a greater than 80% compliance record. The circuit is comprised of 13 hydraulic resistance exercise stations involving bidirectional patterns working all major muscle groups, interspersed with floor-based exercises/recovery stations designed to maintain a target heart rate specific to each participant. Stations were timed from 30-60 seconds, monitored & adjusted for subsequent sessions via the Curves Smart technology to optimize intensity levels of each workout throughout the training period. On one of the three days, a Zumba® exercise dance session was implemented into the workout. The Curves circuit is located in the ESNL. Trained research assistants, Circuit Coaches, and a Zumba instructors monitored sessions. Attendance was monitored through the Curves Smart Admin application.

Dietary Intervention

Participants maintained Phase I (1200 calories/day) exercise & diet program for one week, then progressed to Phase II (1500 calories/day). They continued Phase II for the remaining 11 weeks of this 12 week prospective exercise, diet & timing clinical trial. Previous research has shown that a Curves 10-week program promotes a 10 – 15lb weight loss [140,233]. All participants followed the same HP diet plan as outlined in the Curves high protein diet program [141], which outlines a predetermined macronutrient intake of 30% carbohydrate, 45% protein, and 25% fat throughout the study.

Table 2 presents the dietary intervention protocol employed during the study. Participants in the I group (01) were provided the timed nutrition immediately post exercise. This intake consisted of a Curves Shake mixed with water, and is similar in taste, appearance, texture, and composition to other readily available commercial nutritional products. The timing of the intake was the only difference in the dietary routine between groups. The D group (02) ingested their shake at 2 hours post exercise. A detailed nutrient analysis of the timed supplement is presented in Table A3 (pg. 130). Meals were controlled for caloric and macronutrient content during the T1 and T4 testing routines of all participants as typified in Table 2. It is of interest to note: based on diet as designed with our participants, we estimate a protein consumption of approximately 3-4g/kg Lean Mass (LM)/day throughout the 12 week intervention.

Table 2. Dietary Intervention Outline

Diet Period	Study Group	Macro-Nutrient	Gms/ Day	Kcals/ Day	Percentage of Daily Diet
Phase I	Nutrition (1,200 kcals/day) (n=20)	Protein	135	540	45
		Carbohydrate	90	360	30
(One week) 3 x/week Monitored Exercise, & 1x/wk Zumba		Fat	33.3	300	25
	+				
	Timed Shake (accounted for in diet)	Protein	15	60	.05
		Carbohydrate	8	32	.03
		Fat	1.5	13.5	.01
Phase II	Nutrition (1,500 kcals/day) (n=20)	Protein	168.75	675	45
		Carbohydrate	112.5	450	30
(Eleven Weeks) 3 x/week Monitored Exercise, & 1x/wk Zumba		Fat	41.67	375	25
	+				
	Timed Shake (accounted for in diet)	Protein	15	60	.04
		Carbohydrate	8	32	.02
		Fat	1.5	13.5	.009

Caloric content: Fat (1g=9kcal); CHO (1g=4kcal); Pro (1g=4kcal); Alcohol (1g=7kcal)

Testing Procedures

Following the familiarization session and during the week prior to T-2, participants recorded all food/drink (other than water) consumption on dietary record forms (Food Logs) for four days. Food logs were turned in at each testing session, & analyzed by Food Processor SQL Nutrition Software (ESHA Nutrition Research, Salem, OR). Activity logs were turned in weekly, and utilized for exercise & activity compliance tracking.

Participants were instructed to refrain from exercise for 48 hours, and dietary intake for 12 hours prior to the initiation of each scheduled testing session. They reported to the ESNL, or the Human Countermeasures Laboratory (HCL) for testing. Upon reporting to the ESNL, participants completed the SF-36 Quality of life (QOL)

inventory [238], an appetite/eating satisfaction questionnaire, body image, activity log, and International Physical Activity Questionnaire (IPAQ). Participants were required to complete a follow-up side effect report at each testing session to facilitate monitoring for any concerns regarding exercise or diet. Validity and reliability of these tools are detailed in the previous chapter. Following the study, participants were asked to complete a post-study questionnaire to assess impressions about the Curves Fitness & Weight Management Program.

Resting Energy Expenditure

REE assessments were administered according to standard protocols using the Parvo Medics TrueMax 2400 Metabolic Measurement System (Sandy, UT). This involved participants lying down on an exam table and placing a see-through metabolic canopy over the participant's neck and head so that metabolic gas exchange could be measured. The participant remained motionless (without sleeping) for 20-minutes. Metabolic measurements were recorded to determine resting oxygen uptake and energy expenditure.

Bioelectrical Impedance Analysis

Total body water was determined by bioelectrical impedance assessment (BIA) via the Impedimed DF-50 Bioelectrical Impedance Analyzer (San Diego, CA). This unit measures bio-resistance of water and body tissues based on a minute low energy, high frequency current (500 micro-amps at a frequency of 50 kHz) transmitted through the body. The analyzer is commercially available, and BIA has been used in the health care/fitness industry as a means to assess body composition and body water for over 20

years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water, and the current to be used has been deemed safe. Measurement is obtained via four electrodes placed on the body per ACSM protocol: one electrode was placed on the posterior surface of the right wrist, between the radial and ulna styloid processes (wrist bones), another electrode was placed on the posterior surface of the right hand at the distal base of the second metacarpal; the third electrode was placed on the anterior surface of the right ankle between the lateral and medial malleoli, and the fourth electrode placed on the foot at the distal end of the first metatarsal. Participants were positioned supine on an exam table with electrodes connected to the analyzer. After connecting the participant; age, gender, weight, and height were manually entered into the unit. After measurement (approximately 30 seconds), the unit calculates total body water, intracellular and extracellular fluid, as well as a measure of body composition. Bioelectric impedance analysis has been determined to be a valid measurement for total body water [235-237].

Resting HR & BP

Resting HR and BP were determined before or after REE/BIA measurements. Resting heart rate was determined by palpitation of the radial artery using standard procedures [238], or via GE Dinamap Pro 1000 monitor (Milwaukee, WI), and prior to GXT preparation. Blood pressure was assessed in the supine position after resting for 5-min (and prior to GXT prep) using a mercurial sphygmomanometer as per standard procedures [238] or via the GE Dinamap Pro 1000.

Body Composition

Body composition/bone density assessment measures were determined using a calibrated Hologic (Bedford, MA) Discovery Dual Energy X-ray Absorptiometer (DEXA) by qualified personnel with x-ray technology training under the supervision of Richard B. Kreider, PhD, MX. Quality control (QC) calibration procedures were performed on a manufacturer supplied & calibrated spine phantom prior to each testing session. In addition, bi-weekly automated uniformity calibration procedures were performed when initiated by the instrument software.

The DEXA body composition test involved having the participant lye supine in a standardized position while wearing a pair of shorts/t-shirt or a gown. A low dose of radiation scanned the entire body for approximately seven (7) minutes. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass. Radiation exposure from DEXA for the whole body scan is approximately 1.5 mR per scan, which is similar to the amount of natural background radiation a person would receive in one month while living in the Bryan/College Station, TX area. The maximal permissible x-ray dose for non-occupational exposure is 500 mR per year. Total radiation dose was estimated to be less than 5mR for the entire study. Test-retest reliability studies performed on male athletes with this model DEXA yielded mean deviation for total Bone Mineral Content (BMC) and total fat free/soft tissue mass of 0.31% with a mean intra-class correlation of 0.985.

Anthropometry

Hip and waist measures were performed per guidelines established by the American College of Sports Medicine (2001). Hip and waist circumference were used to establish a ratio that has been shown to be a predictor of heart disease [144].

Phlebotomy & Blood Measures

Following these assessments, participants donated approximately 20 ml of fasting blood using standard venipuncture techniques. Blood samples were analyzed by Quest Diagnostics for serum clinical chemistry profiles (glucose, total protein, blood urea nitrogen, BUN/creatinine ratio, albumin, calcium, total bilirubin, alkaline phosphatase, triglycerides, cholesterol, HDL, LDL etc.). Whole blood analysis (including hemoglobin, hematocrit, red blood cell counts, white blood cell counts, neutrophils, lymphocytes, monocytes, eosinophils, basophils) were analyzed in the ESNL via the Abbot Cell-Dyn 1800 (Chicago, IL) during the 10 week intervention, or by Quest Diagnostics (Houston, TX). Samples may have been run in duplicate to verify results if the observed values were outside control values, and/or clinical norms (according to standard procedures) when run in the ESNL via the Cell-Dyn.

Participants fasted overnight for 12 hours and donated approximately 4 teaspoons of fasting venous blood (20 milliliters). Blood samples were be obtained using standard phlebotomy procedures via sterile venipuncture of an accessible vein by laboratory technicians trained in phlebotomy, and in compliance with guidelines established by the Texas Department of Health and Human Services. Lab staff wore personal protective clothing (gloves, lab coats, etc.) as appropriate when handling blood samples, which

were processed in a protective laboratory environment. Participants were seated in a phlebotomy chair, and their arm cleaned with a sterile alcohol wipe and sterile gauze. A standard rubber tourniquet was placed on the brachium, and an antecubital vein palpated. Then, a 21-22 gauge sterile (or 23 gauge butterfly) needle attached to a plastic vacutainer holder was inserted into the selected vein using standard procedures. Two or three serum separation vacutainer tubes (red or "tiger" tops) and one EDTA vacutainer tubes (purple top) were inserted in succession into the vacutainer holder for blood collection using multiple sample phlebotomy techniques. Once samples were obtained, the vacutainer holder and needle were removed, and discarded as hazardous waste in appropriate containers.

Finally, the venipuncture site was covered with clean gauze and tape (or a Band-Aid) was placed on the site. The blood collection tubes were labeled and placed in a test tube rack pending further processing.

Laboratory technicians (who had received blood borne pathogen training and were wearing personal protective clothing) centrifuged the serum samples, transferred the serum into labeled serum storage containers, and prepared samples for storage in a refrigerator when blood was analyzed by Quest, or a -80 degree freezer for subsequent analysis within the ESNL.

Graded Exercise Testing

Participants performed a graded maximal exercise test (GXT) according to the protocol outlined in Table 3. GXT were performed by lab exercise physiology graduate students in accordance with standard procedures described by the American College of

Sports Medicine's (ACSM) *Guidelines for Exercise Testing and Prescription.* This involved preparing the participant's skin for placement of 10 ECG electrodes. Electrode sites were cleansed with sterile alcohol gauze using a circular motion, then allowed to air dry (or dried with a gauze pad). Electrodes were then placed as follows: right subclavicular fossa (RA), left subclavicular fossa (LA), right abdomen (RL), left abdomen (LL), 4th intercostal space at the right sternal border (V1), 4th intercostal space at the left sternal border (V2), equidistant between V2 and V4 (V3), 5th intercostal space at the midclavicular line (V4), 5th intercostal space at the anterior axillary line (V5), and 5th intercostal space at the mid-axillary line (V6) of the chest.

The participant was then attached to a Cardio System ECG monitoring system (Nasiff Associates, Inc., Brewerton, NY). Resting blood pressure, heart rate, and a 12-lead ECG were obtained. Prior to the GXT, trained ESNL staff reviewed the supine resting 12-lead ECG to ensure that no contraindications for exercise testing were apparent based on the ACSM guidelines. Participants were then asked to stand on the treadmill, where a standing (resting) ECG was assessed for contraindications. A sanitized clean mouthpiece was attached to a head harness and secured on the participant. A nose-clip was placed on the participant's nose, and resting expired gases were collected using the Parvo Medics 2400 TrueMax Metabolic Measurement System. Once the participant was ready to begin the test protocol, they straddled the treadmill with both legs (alternately stood on the GXT belt with hands momentarily on handrails for stability), and the automated GXT protocol was initiated at a speed of 2.0 mph and at a 0% grade for a 2-minute warm-up period. Participants placed one foot onto the belt,

and repeatedly swiped the belt in order to gauge the speed of the motion. Once the participant was familiar with this speed, they stepped onto the belt (both feet) while still gripping the handrails with both hands. Once the participant was comfortable walking on the treadmill, they released the handrails and began walking freely. Subsequent to the warm-up, each participant performed a standard symptom-limited (Bruce Protocol) treadmill maximal exercise test as outlined below in Table 3 below.

Table 3. GXT (Bruce Protocol)

Stage	Speed	Grade (%)	Duration(min)
1	1.7	10	3
2	2.5	12	3
3	3.4	14	3
4	4.2	16	3
5	5.0	18	3
6	5.5	20	3
7	6.0	22	3

Each participant was encouraged to exercise to their maximum unless they exhibited clinical signs to terminate the exercise test as stated by the ACSM's *Guidelines for Exercise Testing and Prescription* [i.e., angina, dyspnea, dizziness, a decline in systolic blood pressure, dangerous dysrhythmias (increasing or multi-form premature ventricular contractions, ventricular tachycardia, supraventricular tachycardia, new atrial fibrillation, or A-V block), lightheadedness, confusion, ataxia, cyanosis, nausea, excessive rise in systolic blood pressure over 250 mmHg or diastolic over 120 mmHg, chronotropic impairment, failure of the monitoring system, or other signs or symptoms for terminating the test].

The test was terminated at the request of the participant, or when undue signs of exhaustion were observed which might have increased risk of any adverse event. Once the exercise test was complete, participants were observed for a 3 minute active recovery period, followed by a 3 minute seated recovery period. The normal exercise time to maximum for the modified Bruce treadmill protocol in untrained women is typically about 9-12 minutes (near the completion of stage III or just entering stage IV). Heart rate (HR), ECG tracings, and expired gases were monitored continuously throughout the entire exercise test process. Blood pressure (BP) and ratings of perceived exertion (RPE) were obtained toward the end of each stage. Participants were asked to report any unusual signs or symptoms to the exercise administrator during the exercise test. This GXT determines maximal aerobic capacity, and anaerobic threshold to discern the effects of exercise training on fitness and exercise capacity. The mean coefficient of variation (assessing Vo_2Max) for this protocol is 6.5% (range 2-14%).

Strength Testing (1RM)

Participants then performed 1 repetition maximum (1RM) lifts on the bench press and leg press to assess strength. Each participant performed as many repetitions as possible with 80% of their 1RM effort for upper and lower body endurance testing. Participants completed 1-RM leg press (LP) & bench press (BP) lifts to determine lower and upper body strength respectively. While participants were seated in the LP apparatus, (Nebula Fitness, Inc., Versailles, OH) foot placement was such that the participants' feet were about shoulder width apart. This placement was recorded by using numbers placed on the leg press foot plate to ensure consistency between lifting

bouts. Placement of the leg press sled height was also recorded to ensure the correct range of motion for each participant. Proper technique was described to the participant, and technique monitored throughout the testing session to ensure internal validity and consistency between participants. Proper technique included lowering the LP sled until the knees were at a ninety degree (90°) angle, placing the hands on the handle bars located at the side of the equipment to prevent the arms from pushing on the legs, keeping the knees in line with the feet and keeping the back and gluteals in constant contact with the LP seat. Participants then completed a 1-RM LP test adapted from the National Strength and Conditioning Association (NSCA) guidelines [239]:

1) Primary staff instructed the participant to warm up with a light resistance that easily allows 5-10 repetitions.

2) Provided 2 minute rest period.

3) Estimated a warm up load to allow the participant to complete ~3-5 repetitions by adding ~30-40 pounds or ~10-20% for lower body exercise and attempt 1 repetition. If successful,

4) Provided a 2 minute rest period

5) Estimated a conservative, near maximum load that would allow the participant to complete ~2-3 repetitions by adding ~30-40 pounds or ~10-20% for lower body exercise and perform 1 repetition. If successful,

6) Provided a 2 minute rest period

7) Made a load increase of ~30-40 pounds, or ~10-20% for lower body exercise

8) Instructed the participant to attempt a 1RM.

9) If the participant was successful, provided a 2 minute rest period and returned to step 7. If failed, provided a 2 minute rest period, decreased the load by ~15-20 pounds or ~5-10% for lower body exercise, and returned to step 8.

Load was continually increased until the participant could no longer complete one repetition. Ideally, the participant's 1RM was measured within 5 testing sets. Likewise, a 1RM BP was conducted to determine upper body strength using the same protocol. All strength/exercise tests were supervised by lab assistants experienced in conducting strength/anaerobic exercise tests using these standard procedures.

Circuit Baseline Testing

Finally, on T-1 day 1, participants then moved to the Curves Circuit baseline 30 minute familiarization & assessment. This session consisted of 2 sets of 3 repetitions on each of the 13 stations under direct supervision, and with specific individual instruction by a trained ESNL research assistant staff member. The first set was to determine appropriate (optimal) range-of-motion (ROM), while the second set established a reference force for maximal effort.

T1 & T4 (Day 2 & 3) Routine

One week subsequent to the above measures (to allow for adequate recovery from baseline exercise testing) and on T-1 day 2 (as outlined in Table 1), participants ingested timed doses (1-4) of the $^{2}H_2O$, had associated intermittent blood draws to determine equilibration levels for the $^{2}H_2O$, performed the first exercise session, ingested the timed nutrient intake and received muscle biopsies 1.1 (baseline) through 1.3 at timed intervals (Table 1). Additionally, predetermined HP meals were ingested at timed intervals. These meals were controlled for macronutrient and caloric content. The exercise session consisted of a 30 minute training bout, followed closely by biopsy 1.2. Deuterium doses 3 & 4 were administered at 7.5 & 9 hours, respectively on day two of

testing. Day three of T1 testing consisted of a 5th blood draw, and biopsy 1.4 at 24 hours post exercise. This process was repeated at testing session 4 (the final 3-day testing period). Testing sessions T2 & T3 included resting measures only at 4 & 8 weeks respectively, and were designed to monitor and promote participant compliance.

$^{2}H_2O$ Dosing

Timed $^{2}H_2O$ ingestion was utilized to facilitate physiological measurement of FSR through the use of periodic skeletal muscle tissue (biopsy) analyses at timed intervals throughout the available assessment period as typified in Table 1. This method allows for measurement of alanine via stable isotope pools over the 24 hours of each (T-1 & T-4) testing session. Ingestion was administered in 4 doses of approximately 1.625 ml/kg lean mass for each participant at time-points as outlined previously, for a total administration of ~6.5 ml/kg lean mass over the 10 hour period. As suggested earlier, these administrations were implemented only during the first and last testing sessions. $^{2}H_2O$ consists of a stable isotope with no documented physiological disadvantages other than nausea, dizziness or other similar side effects when administered in larger dosages than were given during this study [202].

The 6.5 ml was diluted with distilled water, to effect a 70% $^{2}H_2O$ concentration (for palatability, and to minimize side effects). Here is an outline of a typical dosing routine for T-1 & T-4 as previously presented in Table 1.

0600 - Blood, **$^{2}H_{2}O$ dose 1.1/4.1**

0630 - Curves Breakfast

0800 - **$^{2}H_{2}O$ dose 1.2/4.2**

0845 - Blood, Biopsy 1.1/2.1

0915 - Circuit Workout, -post exercise nutrient intake (I Group only)

1030 - Blood, Biopsy 1.2/2.2

1230 - Blood, Biopsy 1.3/2.3

1300 - Curves Lunch

1330- **$^{2}H_{2}O$ dose 1.3/4.3**

1530 - Blood, **$^{2}H_{2}O$ dose 1.4/4.4**

1730 - Curves Dinner

Muscle Biopsies & Follow-up Care

The six (3 each for T1 & T4) percutaneous muscle biopsies (50-125 mg each) were obtained over the duration of the study from the middle portion of the right or left vastus lateralis muscle at the midpoint between the patella and the greater trochanter of the femur and at a depth between 1 and 2 cm. For T1 & T4 biopsies, samples were extracted from sites adjacent to previous biopsies, rather than from previously accessed sites. All biopsies were extracted by trained and experienced personnel in the Human Countermeasures Laboratory at Texas A&M University. After sample extraction, any necessary adipose tissue was trimmed from the muscle specimens. Subsequently, the muscle samples were immediately frozen in liquid nitrogen, and stored at -80°C awaiting future analysis.

Muscle samples were extracted under local anesthesia of 2% Xylocaine from the middle portion of the muscle midway between the patella and the greater trochanter of the femur. First, the participant was placed supine, to assume a comfortable reclining position on an examination table. Once the extraction point was identified, the area was wiped with an alcohol pad, and further be cleansed by swabbing (with a circular pattern outward from the selected site with Betadine (fluid antiseptic) and then draped. A small area of the skin approximately 2 cm in diameter was anesthetized with a 1.0 mL subcutaneous injection of Xylocaine. Once the local anesthesia had taken effect (approximately 2-3 minutes) each biopsy procedure took approximately 15-20 seconds. A scalpel point was used to access the biopsy site by making an incision approximately 1 cm in length through the skin, subcutaneous fat, and fascia. Due to the localized effects of the anesthetic, participants were observed to feel little or no pain during this process. The biopsy needle was advanced into the incision approximately 1 cm. During this procedure, subjects were observed to feel pressure and/or discomfort to the thigh area.

Once the muscle sample was extracted, direct pressure was immediately applied. The site was subsequently closed, and the wound dressed with a pressure bandage. Due to the small incision site, only minimal bleeding was observed. The needle and scalpel blade were cleaned in an antiseptic solution and subsequently autoclaved for future use, or discarded as hazardous waste in an appropriately-labeled plastic sharps container. All waste was discarded in an appropriately labeled biohazard waste receptacle. Tissue samples were stored at $^{-}80°C$ for future analyses.

Written instructions for post-biopsy care were reviewed and issued to participants (Appendix). Each participant was instructed to leave the bandages on for 24 hours (unless unexpected bleeding or pain occurred) and asked to report back to the lab within 24 hours to have the old bandages removed, the incision inspected and new bandages applied. Participants were further advised to refrain from vigorous physical activity (with the exception of directly supervised study related exercise) during the first 48 hours post-biopsy. These suggestions were designed to minimize pain and possible bleeding of the area. If needed, the subject was instructed to take non-prescription analgesic medication such as Tylenol or Ibuprofen to relieve pain (Appendix). However, medications such as aspirin, Nuprin, Bufferin, or Advil were discouraged as these medications may lead to ecchymosis at the biopsy site. Participants were instructed that soreness of the area may occur for about 24 hours post-biopsy.

Tissue Processing & Metabolic Analysis

Skeletal muscle biopsy samples were processed and analyzed as previously described [196,206,211,212] via gas chromatography/mass spectroscopy (GC/MS), as this method has been utilized repeatedly in the Muscle Biology Laboratory at Texas A&M University. Briefly, and as described elsewhere [196] 10-20uL of plasma and ~30mg of muscle tissue was necessary for the ^{2}H-labeled/unlabeled ratios by GS-MS. For quantification, the ^{2}H enrichment of acetone was analyzed following exchange with the ^{2}H-labeling of body water[240], and the methyl-8 derivative of protein bound ^{2}H-labeled alanine after reaching the hydrolysate with N,N-dimethylformamidedimethylacetal[241]. The use of stable isotopes do not require

parallel measurements of the tracer (alanine) concentration since the GC-MS simultaneously measures the labeled and unlabeled molecules, allowing for immediate calculation of the ratio. This is dissimilar to radioisotopes, which require knowledge of the total concentration of the amino acid for determination of the specific activity[196].

The ^{2}H labeling of protein-bound alanine was measured as previously described [212]. Briefly, ~ 0.030 g of muscle is homogenized on ice in 0.3 ml of a 10% (wt/vol) TCA and centrifuged at 3,750 rpm at 4°C for 15 min. This was repeated 3 additional times prior to dissolving the protein pellet in 6 N HCl (0.1 mL/0.030 g tissue) and reacting at 100ºC for 18 h. All samples were analyzed using an Agilent 5973N-MSD equipped with an Agilent 6890 GC system, and a DB17-MS capillary column (30 m × 0.25 mm × 0.25 µm) was used in all analyses. The following temperature program was used: 90ºC initial, hold for 5 min, increase by 5°C/min to 130ºC, increase by 40ºC/min to 240ºC, and hold for 5 min. The sample was injected at a spit ratio of 5:1 with a helium flow of 1 mL/min. Alanine eluted at ~12 min. The mass spectrometer was operated in electron impact mode. Selective ion monitoring of mass-to-charge ratios (*m/z*) 99 (M) and 100 (M + 1) was conducted using a dwell time of 10 ms/ion.

FSR of mixed muscle proteins (FSR) were obtained by measuring the incorporation of ^{2}H alanine into protein (E_A) and using the precursor-product model,

$$FSR = E_A/(E_{BW} \cdot 3.7 \cdot t)$$

where E_A represents amount of protein-bound ^{2}H Ala (%), E_{BW} is the quantity of $^{2}H_2O$ in body water (%), 3.7 represents the exchange of ^{2}H between body water and alanine [206,212,242], and *t* is time of label exposure (24 h). More recently, the Muscle Biology

Lab at Texas A&M reported the measurement as the rate at which ^{2}H-labeled alanine is incorporated into muscle protein(s) relative to the total abundance of the alanine pool per unit of time (fractional synthesis rate, FSR) and can generally be calculated by the equation:

$$FSR = [(MPE_{Ala})] / (n \times MPE_{BW} \times t) \times 100,$$

where MPE (mole percent excess)$_{Ala}$ represents the total ^{2}H-labeling of protein-bound alanine, "n" represents the exchange of ^{2}H between body water and free alanine (~3.7 in mammals), MPE_{BW} represents the labeling of body water and "t" is time[196]..

Statistical Analyses

An a-priori analysis using related studies[243,244] was conducted to determine an appropriate sample size. A small effect size was defined as power $(1-\beta) = 0.20$, a medium effect size power = 0.5, and a large effect size power = 0.80 [249]. At $\alpha = 0.05$, the a-priori power analysis suggested that a sample size of 10/group would yield a power of ~0.53, which intimates detection of a medium effect as defined by Kirk.

Study data were analyzed via Multivariate Analysis of Variance (MANOVA) with repeated measures (IBM SPSS Statistics, v. 21, 2012, Armonk, NY). MANOVA effects were examined using Wilks' Lamda time and group x time p-levels as well as univariate ANOVA group effects. Greenhouse-Geisser univariate tests of within-subjects time and group x time effects, and between-subjects univariate group effects were analyzed for each variable within the MANOVA. In some instances, repeated measures ANOVA were run on variables where group, time, and group x time interaction effects were of interest. Analysis of Covariance (ANCOVA) was used to

assess baseline homogeneity. Delta values and/or percent differences were calculated and analyzed on select variables via ANOVA for repeated measures to assess % change from baseline. Delta values were calculated by subtracting the T1 from T4 values, then performing division by T1, followed by multiplication by 100

$$[(T4-T1)/T1*100].$$

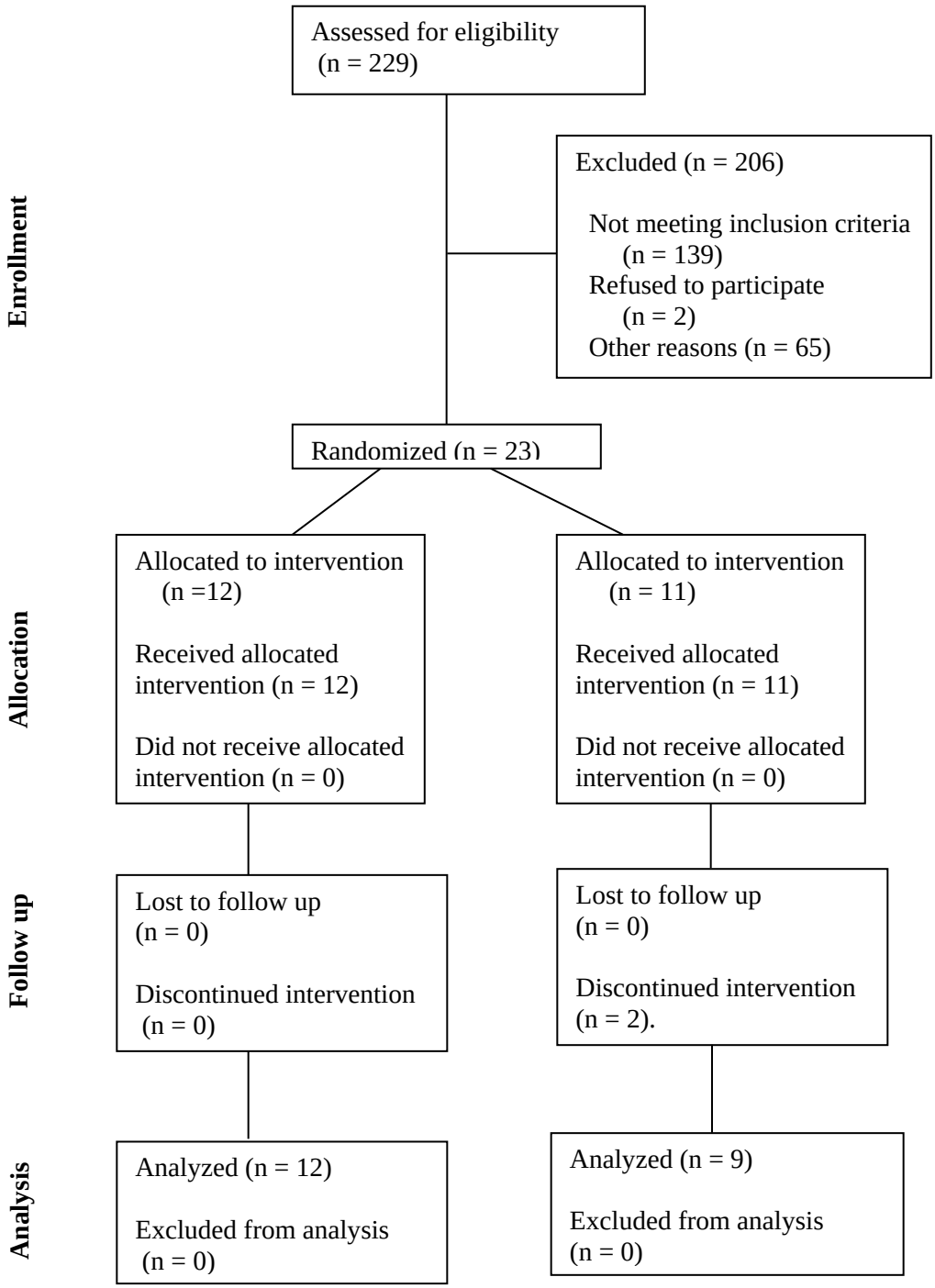
Data were considered statistically significant when the probability of type I error was 0.05 or less. Statistical trends were considered if the probability of type I error ranged between >0.05 to <0.10. If a significant group, treatment and/or interaction alpha level was observed, Tukey's least significant difference (LSD) post-hoc analyses were performed to determine where significance was obtained. Missing and outlier variable data were addressed via the group mean method. However, neither removal of cases with missing data, nor replacement of missing data was determined to significantly affect final results.

CHAPTER IV

RESULTS

This nutrient timing prospective clinical trial included 21volunteers that completed the 12 week intervention. Twenty two participants were recruited, with 1 drop after completing the first testing session due to musculoskeletal concerns under the advisement of her Chiropractor. One participant cited recurrent musculoskeletal complaints unrelated to the intervention, and did not complete lower body strength testing during T-4. A second participant experienced nausea during early stages of day two of her first testing session (she had received her first two doses of D_2O) and elected not to complete the session. All participants signed informed consent statements that were in compliance with the Institutional Review Board at Texas A&M University, and were randomized into either the I or D group. Participants were also matched, and homogeneity was compared to randomization revealing no significant differences between the two methods. All 21 participants completed a 12 week exercise and dietary weight loss intervention that included 4 testing sessions, and were included in the analyses. There were 12 participants in the Immediate (I) and 9 participants in the Delayed (D) group (n = 21).Our flowchart in Figure 3 outlines the logistical sequencing for the NT study participants.

Figure 3. NT Consort Diagram



Demographics

Baseline demographics were analyzed via ANOVA with no significant baseline differences noted between groups based on age, height, weight, BMI [(weight (kg) / [height (m)]^2 or lean mass (LM). Table 8 depicts demographic data for age, weight, height, BMI, and LM for all participants. The recruitment age was between 50-70 years old. One-way ANOVA revealed no statistically significant differences of baseline values between these variables.

Table 4. Baseline (T-1) Group Demographics

Variable	Mean ± SD	F	p
Age (years)	59.8 ± 5.2	2.3	0.15
Weight (kg)	84.85 ± 8.9	.013	0.91
Height (cm)	65.19 ± 2.5	1.4	0.25
BMI	31.00 ± 3.0	.48	0.50
LM (kg)	42.55 ± 5.3	.149	0.70

Nutritional Intervention

In lieu of the focus on timed nutrition for this study, a brief review of the dietary intake data are warranted. Both the "I" (timed nutrition immediately post-exercise) and "D" (timed nutrition 2 hours post-exercise) group were assigned a dietary calorie intake regime consisting of 45% protein, 30% carbohydrate and 25% fat; with a total intake goal of 1,200 calories/day for week 1, then 1,500 calories for the remaining 11 weeks

(maintenance phase). Nutritional goals for both groups were inclusive of the post exercise nutrition in the form of a vanilla shake containing 15g protein, 8g carbohydrate and 1.5g fat immediately following (I), or 2-hr after (D) resistance exercise as part of their nutrition program. Throughout the 12 week intervention, protein intake averaged 2.21g/kg/day Lean Mass (LM).

Table 5 outlines nutritional intake data, and provides a visual representation of caloric and macronutrient percentages for each testing session as recorded via 4-day food logs at each of the four testing sessions. There were no significant within or between group differences in caloric ($p=0.952$) or macronutrient (Pro: $p=0.543$; CHO: $p=0.685$; Fat: $p=0.182$) intake throughout the study duration.

Mixed Muscle Protein Synthesis (FSR)

Figure 4 shows FSR changes over the course of the study. Based on ANOVA analyses, no significant nutrient timing x training interactions (mean±SEM) were observed in muscle FSR, expressed as a percent/day of the alanine pool (**I**-Pre 13.6±4.3, **I**-Post 21.1±4.3; **D**-Pre 15.6±4.0, **D**-Post 23.8±4.0 %/d, $p=0.93$). However, FSR was augmented ($p<0.05$) in response to a bout of exercise for both pre-training (14.6±2.9 %/d) and post-training (22.5±2.9 %/d) interventions. FSR tended to be 54% higher ($p=0.075$) in response to the post-training bout of exercise when compared to pre-training values. Protein synthesis rates trended upward over the course of our 12-week intervention with overweight post-menopausal women.

No significant differences were observed in FSR ($\% \cdot h^{-1}$) between the Immediate and Delayed groups in mixed muscle when assessed 16 h following pre or

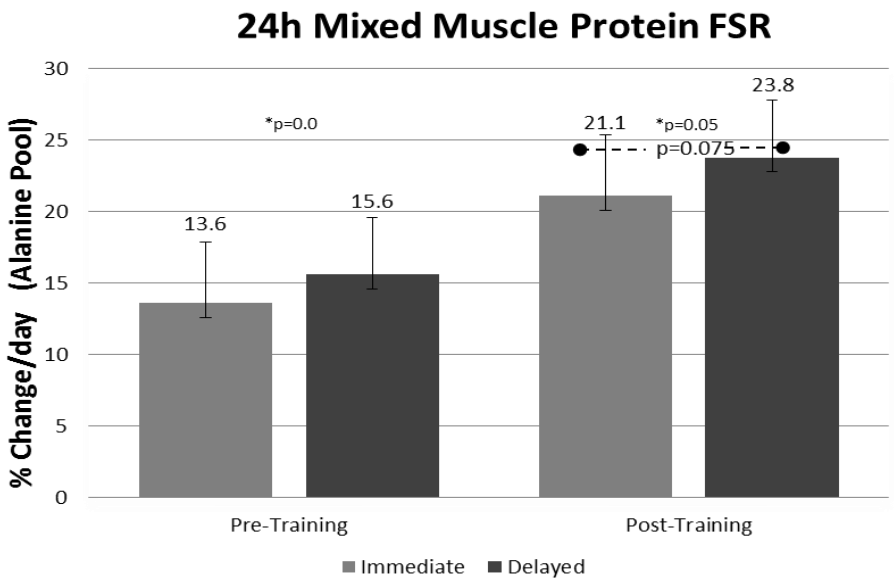
post exercise sessions (measured over 24 h). Our results are inconsistent with H_A1 and H_A2: Timed nutrition will significantly enhance pre-training FSR (H_A1), and post-training FSR (H_A2) in the Immediate compared to the Delayed group.

Table 5. Dietary Summary

Variable	Group	Baseline	1mo	2mo	3mo	p-level	
Calories (Kcal/d)	I	1,371 ±213	1,353 ±201	1,270 ±251	1,290 ±200	Group	p=0.95
						Time	p=0.07
	D	1,354 ±154	1,308 ±169	1,338 ±157	1,255 ±111	Group x Time	p=0.34
	Time Mean	1,364 ±186	1,334 ±185	1,299 ±214	1,279 ±165		
Protein (%/d)	I	21.33 ±9.04	33.22 ±8.42	30.75 ±8.31	30.23 ±7.55	Group	p=0.54
						Time	p=0.02
	D	19.29 ±4.36	30.06 ±9.28	29.30 ±7.70	33.25 ±6.73	Group x Time	p=0.25
	Time Mean	20.45 ±7.32	31.92 ±8.73	30.13 ±7.89	31.50 ± 7.20		
Carbs. (%/d)	I	41.95 ±9.6	30.80 ±6.42	34.97 ±8.37	35.69 ±7.2	Group	p=0.69
						Time	p=0.00
	D	37.22 ±5.85	34.27 ±7.46	34.04 ±5.19	32.94 ±7.2	Group x Time	p=0.46
	Time Mean	39.92 ±8.37	32.29 ±6.44	34.58 ±7.04	34.51 ±7.16		
Fat (%/d)	I	34.37 ±6.37	34.53 ±7.89	33.16 ±8.27	32.92 ±6.04	Group	p=0.18
						Time	p=0.01
	D	42.85 ±4.15	35.66 ±9.64	36.66 ±6.08	33.54 ±5.04	Group x Time	p=0.05
	Time Mean	38.01 ±6.90	35.01 ±8.47	34.66 ±7.46	33.19 ±5.51		

Note: Data represents caloric (kcal/day±sd) and macronutrient intake values (% / day) for the study. Macronutrient targets - 45%PRO; 30%CHO, and 25%FAT throughout the study. 1mo = 1 month; 2mo = 2 months; 3mo = 3 months. Carbs. = Carbohydrate; I = Immediate; D = Delayed. Wilks Lambda results- Group: $F(4,15) = 1.784$, $p = .184$, Time: $F(12,7) = 1.59$. $p = .275$, Group*Time: $F(12,7) = .720$, $p = .553$

Figure 4. Group Mixed Muscle FSR Changes



Immediate post-exercise timed nutrition did not significantly impact FSR increase when compared to 2-hour delayed post-exercise intake. If fact, the reverse seemed to be more of an encouraging possibility. Both H_A1 and H_A2 are rejected.

Figures 5 and 6 depict changes in fractional synthesis rate (FSR) for plasma percent/hour (Figure 5) and mixed muscle percent/day (Figure 6) of the alanine pool values in mole percent excess (MPE) from baseline. Data represent T1 (pre-training) and T4 (post-training) results including pre and post exercise measures. ^2H_2O dosage was administered at a dosage of 6.5mL/kg lean mass achieving an overall mean 24 hour MPE of 0.512. Body water ^2H enrichment reached ~0.5 % excess over the course of the study, and ^2H labeling of alanine did not differ between the immediate (I) and delayed (D) groups. Both plasma and muscle ^2H enrichments were adequate for FSR calculations.

Figure 5. Plasma MPE changes

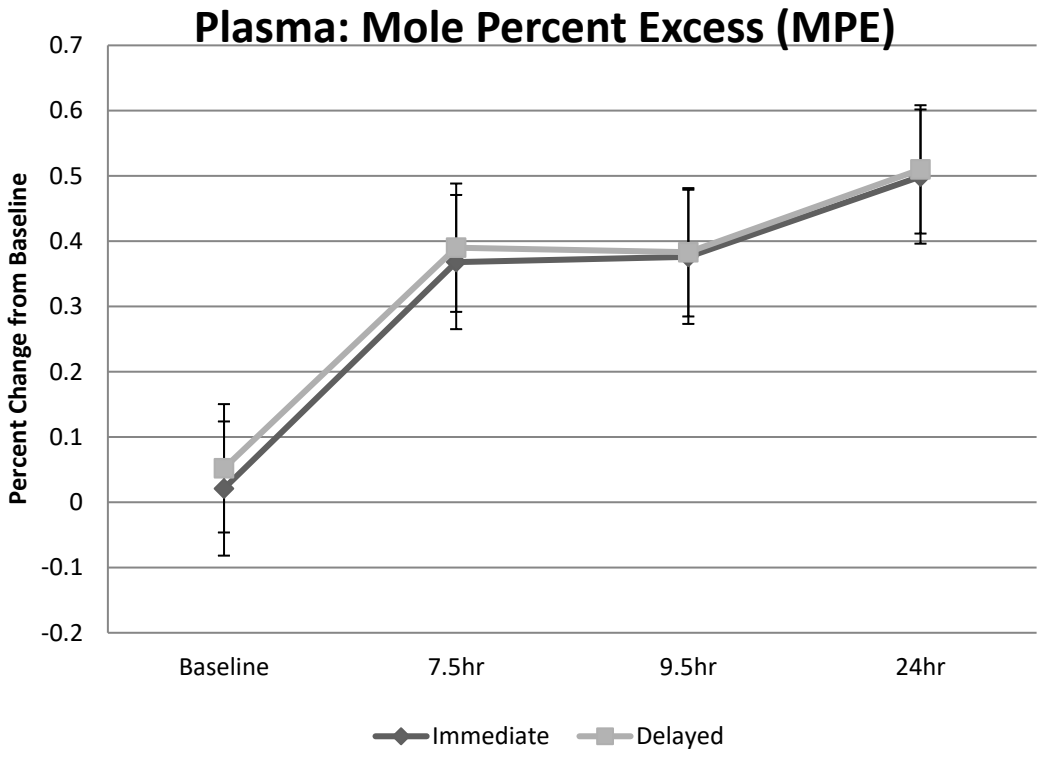
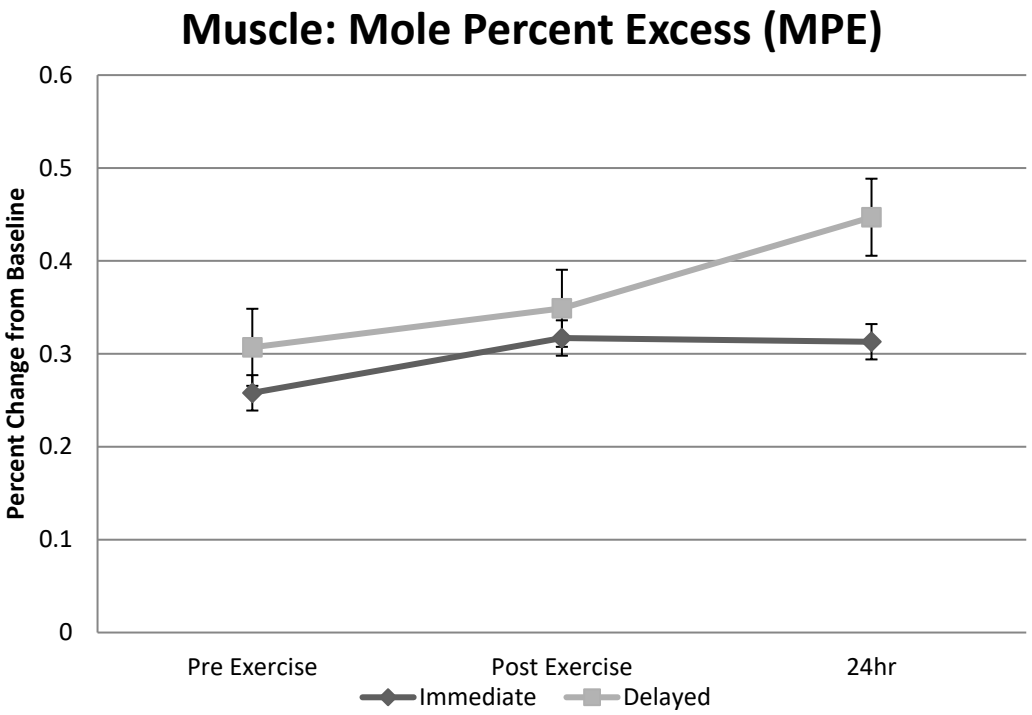


Figure 6. Mixed Muscle MPE changes



Body Composition

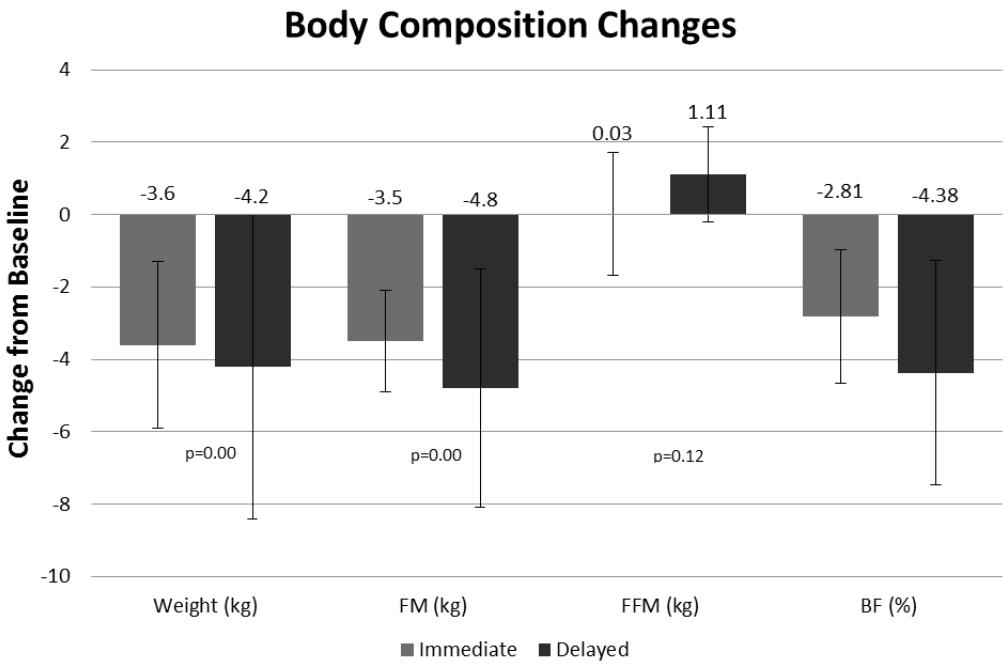
Table 6 and Figure 7 outline MANOVA body composition results over the 12 week course of diet and exercise intervention. Over the duration of the 12 week study (time effects), participants in both groups lost weight (-3.9±3.2 kg, p=000) and fat mass (-4.1±2.4 kg, p=0.000). However, no significant differences (mean±SD) were observed among groups in weight (**I** -3.6±2.3; **D** -4.2±4.2 kg, p=0.68) or fat mass (**I** -3.5±1.4; **D** -4.8±3.3, p=0.26). FFM tended to increase (0.5±1.6 kg, p=0.12) with no differences observed among groups (**I** 0.03±1.7; **D** 1.11±1.3 kg, p=0.14). While significant time effects were seen in body mass, fat mass, and encouraging time effects for REE and percent body fat; no significant group x time effects were observed.

Table 6. Body Composition Data

Variable	Group	Baseline	12 Weeks	Source	Sig. (p-value)	F	Power
Fat Free Mass (kg)	I	44.549 ±6.209	44.581 ±5.553	Group	0.878	.024	.052
				Time	0.064	3.08	.528
	D	43.584 ±4.566	44.675 ±3.880	G x T	0.538	.601	.31
	Time Mean	44.136 ±5.457	44.622 ±4.794				
Fat Mass (kg)	I	33.914 ±5.068	30.381 ±5.745	Group	0.184	1.909	.258
				Time	0.021*	3.926	.789
	D	35.115 ±3.816	30.330 ±5.192	G x T	0.040*	3.268	.641
	Time Mean	34.429 ±4.508	30.359 ±5.380				
Lean Mass (kg)	I	42.945 ±6.091	42.981 ±5.397	Group	0.788	.075	.058
				Time	0.000*	12.98	.997
	D	42.016 ±4.425	43.105 ±3.711	G x T	0.534	.661	.157
	Time Mean	42.547 ±5.335	43.034 ±4.640				
Body Fat (%)	I	43.2 ±2.7	40.3 ±3.3	Group	0.184	1.909	.258
				Time	0.021*	3.926	.727
	D	44.6 ±3.7	40.3 ±4.5	G x T	0.040*	3.268	.641
	Time Mean	43.8 ±3.2	40.3 ±3.8				
Body Weight (kg)	I	84.65 ±10.7	81.0 ±10.8	Group	0.969	.002	.050
				Time	0.000*	30.065	.999
	D	85.1 ±6.2	80.9 ±7.1	G x T	0.680	.176	.068
	Time Mean	84.88 ±5.457	80.91 ±4.794				

Note: Data represents body composition variables for the study. Data was analyzed using univariate, multivariate and repeated measures general linear models (GLM). Significant differences are Indicated by an asterisk (*). I = Immediate; D = Delayed; G = Group; T = Time. Wilks Lambda results- Group: $F(4,15) = 1.784$, $p = 0.184$, Time: $F(12,7) = 1.59$. $p = 0.275$, Group*Time: $F(12,7) = .720$, $p = 0$ .553

Figure 7. Body Composition Changes



Although significant FM reduction was observed among groups over time, these results are inconsistent with H_A3 and H_A4. Timed nutrition will significantly enhance FM loss (H_A3) and LM gain (H_A4) in the Immediate compared to the Delayed group over time. Immediate post-exercise timed nutrition did not significantly impact FM loss or LM gain when compared to 2-hour delayed post-exercise intake. Therefore, H_A3 and H_A4 are rejected.

Upper/Lower Body Strength

Table 8 (as well as Figures 5 and 6) depict changes in upper and lower body strength over the 12 week training intervention. Upper (bench press) and lower (leg press) body strength was assessed via 1RM testing during all four test sessions. ANOVA revealed there were significant changes in time main effect for one repetition maximal effort in the bench press ($p=0.02$) and leg press ($p=0.03$). The mean percent changes in bench press one repetition maximal effort among groups were: I 26.9 ± 37.5%; D 14.8 ± 20.5%. Overall mean percent 1RM change over time for bench press was 21.3 ± 27.7%. The mean percent changes for one repetition maximal effort leg press were: I 37.7 ± 47.4%, D 37.1 ± 38.8%. The overall mean percent change for one repetition leg press over time was 33.1 ± 36.9%. Figures 5 and 6 depict 1RM changes in kg for UE and LE strength, respectively.

Although significant 1RM UE and LE ($p=0.02$, 0.03 respectively) increases were observed among groups over time, these results are inconsistent with H_A5 and H_A6: Timed nutrition will significantly improve upper body strength (H_A5) and improve lower body strength (H_A6) in the I, compared to the D group over time. Immediate (I) post-exercise timed nutrition did not significantly impact UE or LE strength gain when compared to 2-hour delayed (D) post-exercise intake. Therefore, H_A5 and H_A6 are rejected.

Table 7. Upper/Lower Body Strength Data

Variable	Group	Baseline	12 Weeks	Source	Sig. (p-value)	F	Power
Bench Press 1 Repetition Maximum (Kg)	I	31.82 ±6.28	33.71 ±5.72	Group	0.39	.786	.134
				Time	0.02*	6.06	,647
	D	28.79 ±6.53	31.82 ±8.43	G x T	0.58	.329	.547
	Time Mean	30.52 ±6.41	32.9 ±6.88				
Leg Press 1 Repetition Maximum (Kg)	I	150.0 ±22.74	158.5 ±30	Group	0.40	.734	.128
				Time	0.03*	5.37	.592
	D	162.4 ±61.1	180.8 ±63.9	G x T	0.40	.736	.128
	Time Mean	155.6 ±44.9	168.5 ±48.2				

Note: Data represents upper and lower body strength testing variables for the study. LSD post hoc analysis for time main effects is indicated numerically ($p < 0.05$). * = represents $p < 0.05$ difference from baseline mean. I = Immediate; D = Delayed; G = Group; T = Time.

Figure 8. Upper Body 1RM Gains

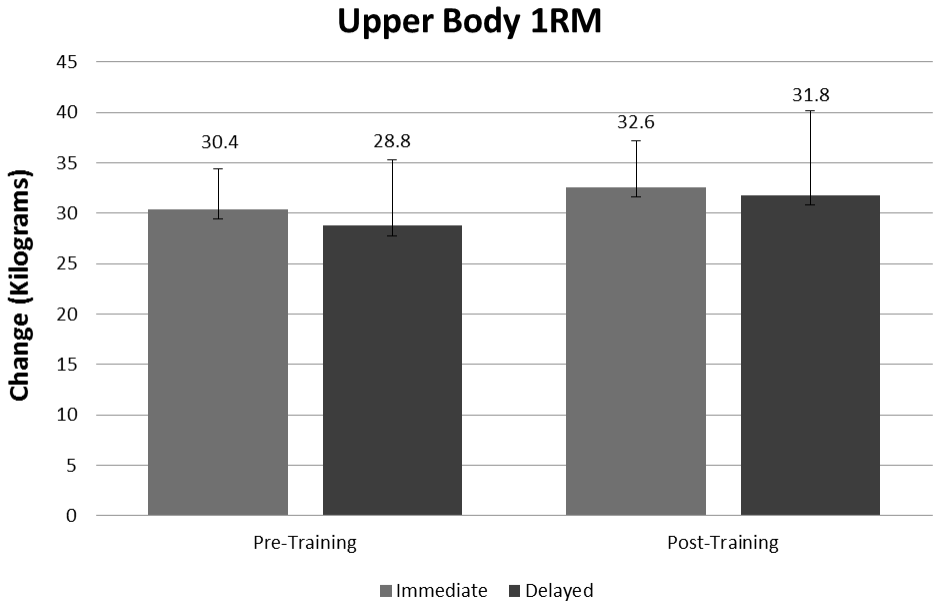
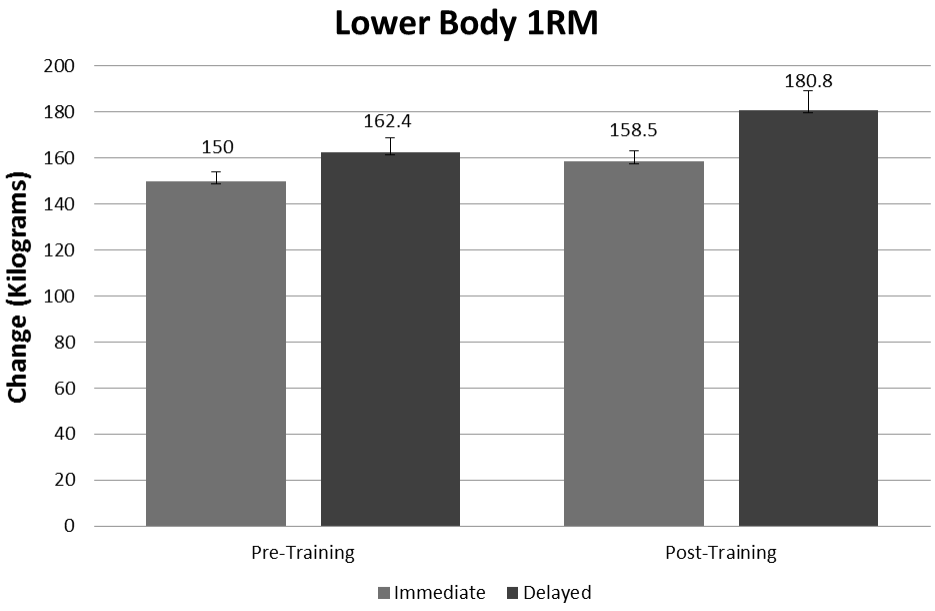


Figure 9. Lower Body 1RM Gains



Resting Energy Expenditure

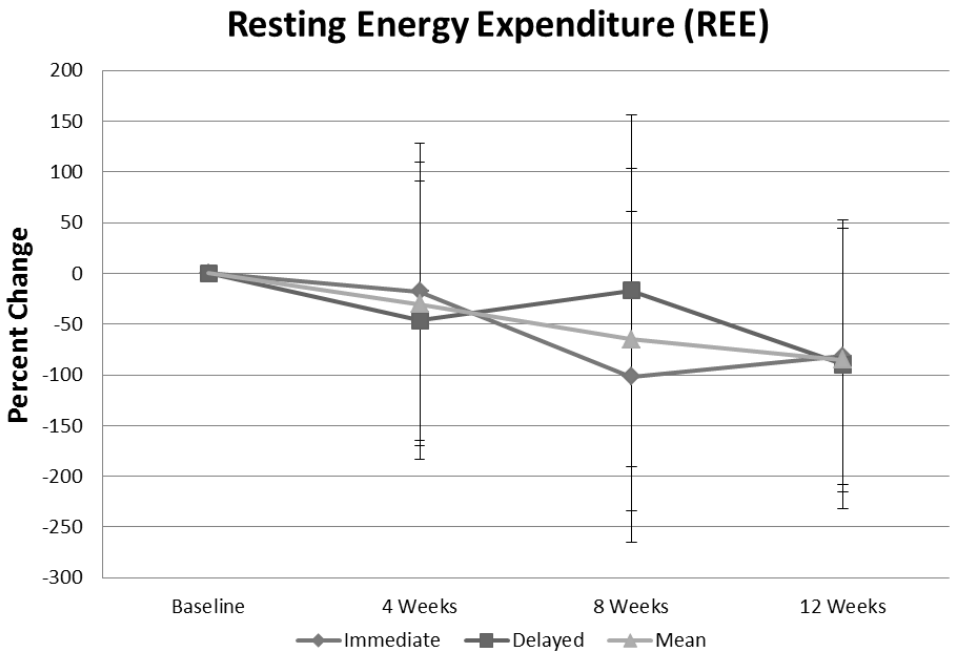
Table 20 illustrates that there are no statistically significant differences in group, time, or group x time for resting energy expenditure variables. No differences were seen among groups in REE (I -18±146, -101±163, -82±126; D -46±137, -17±173, -90±142 kcal/d, p=0.34). Interestingly, a pairwise comparison of participant combined (Groups I &D) means did suggest a trend toward decreased REE over time [(Pre) 1,363±42; (Post) 1,277±37; p= .085 (mean±SEM)]. Hence, our results are inconsistent with H_A7: Timed nutrition will significantly improve REE (Kcal/day) in the I group compared to the D group over time. Therefore, H_A7 is rejected.

Table 8. REE Data

Variable	Group	Baseline	12 Weeks	Source	Sig. (p-value)	F	Power
REE (Kcal/d)	I	1,371 ±213	1,290 ±200	Group	0.952	.004	.05
				Time	0.085	2.62	.54
	D	1,355 ±154	1,265 ±111	G x T	0.473	.875	.201
	Time Mean (±SEM)	1,363 ±42	1,277 ±37				

Note: Data represents Resting Energy Expenditure (Kcal/d) variables for the study. I = Immediate; D = Delayed; G = Group; T = Time.

Figure 10. REE Changes



CHAPTER V

DISCUSSION AND CONCLUSIONS

Discussion

Results of the current study demonstrate the effectiveness of diet with post exercise timed intake throughout a 12 week training evolution designed to elicit favorable changes in the fitness markers noted above. Throughout this 12 week study we found the following. Diet and training promoted significant improvements in that both groups lost weight (-3.9±3.2 kg, p=0.00) and fat mass (-4.1±2.4 kg, p=0.00) with no significant differences (mean±SD) observed among groups in weight (I -3.6±2.3; D -4.2±4.2 kg, p=0.68) or fat mass (I -3.5±1.4; D -4.8±3.3, p=0.26). FFM tended to increase (0.5±1.6 kg, p=0.12) with no differences observed among groups (I 0.03±1.7; D 1.11±1.3 kg, p=0.14).

Significant improvements were observed for upper (p=0.02) and lower (p=0.03) body strength (1RM). Mean percent bench press 1RM gains among groups (reported in Kg) were: I 26.9 ± 37.5%; D 14.8 ± 20.5%, while overall mean percent 1RM bench press change over time was 21.3 ± 27.7%. Mean percent changes for 1RM (reported in Kg) leg press were: I 37.7 ± 47.4%, D 37.1 ± 38.8%, and mean percent change over time for 1RM leg press was 33.1 ± 36.9%. No differences were seen among groups in REE: (I -82±126; D -90±142kcal/d, p=0.34). However, indications that REE may have slightly decreased (p=0.085) or remained unchanged over the 12 weeks of training.

Based on our analyses, no significant nutrient timing x training interactions (mean±SEM) were observed on muscle FSR expressed as a percent/day of the alanine

pool (I-Pre 13.6±4.3, I-Post 21.1±4.3; D-Pre 15.6±4.0, D-Post 23.8±4.0 %/d, p=0.93). FSR was augmented ($p<0.05$) in response to a bout of RE prior to training (14.6±2.9 %/d) and trended higher (p=0.075) in response to a bout of exercise after training when compared to pre-training values (22.5±2.9 %/d).

Both groups experienced improvements in strength, body composition and post-exercise; post-training FSR with potentially greater effects observed in the Delayed group. Total energy (Kcal) intake did not significantly differ between groups over time (p=0.073), suggesting a possible trend toward a decrease from baseline to 12 weeks. Both groups showed significant changes in macronutrient intake over time for protein (p=0.02), carbohydrate (p=0.00) and fat (p=0.01). Protein remained below target of 45%, while carbohydrate and fat approaching target (30% & 25% respectively).

The Curves exercise and weight loss program has been shown to be effective with post-menopausal overweight females in promoting weight loss and favorable body composition changes. Although there was a trend toward increased FSR with 2 hour post vs immediately post exercise nutrient ingestion, post exercise timed nutrition was generally not shown to effect significant changes.

Ingestion of carbohydrate and protein prior to, and following exercise has been reported to influence protein synthesis. For this reason, it is generally recommended to time nutrient intake around exercise to maximize results. While there is a large body of evidence suggesting that nutrient timing influences acute responses to resistance-training, it is less clear whether implementing such strategies during training may influence training adaptations. There are also reports indicating that ingesting a higher

protein while participating in a resistance-training program may be an effective way to promote fat loss while maintaining lean body mass. Theoretically, incorporating nutrient timing strategies during a weight loss program may help maintain lean mass to a greater degree.

To our knowledge, this is the first study undertaken to directly investigating the effects of post exercise nutrient timing (24hr period, pre/post) over a 12 week higher protein/reduced carbohydrate diet with concurrent mixed exercise training on mixed skeletal muscle protein synthesis and related physiological variables in overweight to obese free living post-menopausal women. Herein, we specifically considered the effects of 1) a macronutrient controlled (45% protein, 30% carbohydrate and 25% fat) diet [primary interest in post exercise timed ingestion of a commercial protein shake (15g protein, 8g carbohydrate and 1.5g fat)], and 2) a combination resistance oriented exercise/training (circuit) program designed to elicit a general fitness response.

Our dependent variables of interest included the health related fitness markers of: 1) body composition: fat free mass (FFM), fat mass (FM) and body weight (BW); 2) strength (1RM upper & lower body) and 3) resting energy expenditure (REE). Primarily, our interest was directed toward post-exercise, pre/post-training mixed skeletal muscle fractional synthesis rate (FSR) as measured via $^{2}H_2O$ ingestion and subsequent skeletal muscle biopsies before/after exercise, and at 24 hours post initial $^{2}H_2O$ loading dose as previously described herein. Along with previous studies cited using similar methods, we were interested in cumulative muscle protein synthesis to encompass perturbations (sleep, exercise, activities of daily living and rest) that occur

daily in human overweight/obese post-menopausal women. During this investigation, both the Immediate and Delayed post-exercise nutrition groups performed at equivalent training volumes, intensities, and frequencies.

Results have indicated that the exercise and diet program investigated was effective in promoting weight and fat loss without loss in lean mass. Although it is settled science that exercise (perhaps more specifically resistance training) stimulates lean mass accretion, there is limited and less convincing evidence to suggest that resistance training (without an integrated well-managed diet) is as effective when it comes to fat mass reduction. It is perhaps significant to note that a recent epidemiologic study comprised of 348 young adults over a 12 month period[246] concluded that, despite the limited effects on body mass index, exercise was associated with beneficial changes in body composition. Most interestingly as related to our study, this meta-analysis suggested that any exercise type positively affected LM in normal-fat participants, and reduced FM in overfat and obese adults. Not surprisingly, they also reported that adults with excess body fat may benefit particularly from resistance exercise. Our exercise intervention was of the mixed training variety, yet resistance training accounted for the bulk of training. Arguably, perhaps most appropriately classified as resistance interval program training (RIPT).

The exercise, diet and/or post-exercise intake approach was effective in stimulating muscle protein synthesis post exercise during pre and post training interventions. Protein synthesis stimulus persisted over the 24hr assessment period, and tended to be more pronounced following 12 weeks of training. Specifically, 24hr group

Pre/Post (Immediate +7.5%; Delayed +8.2%) and total participant Pre/Post (Immediate +2%; Delayed +2.7%) mean increases in FSR were observed over the 12 week diet and exercise intervention. The mean FSR change from Pre to Post assessment for both groups combined was +7.85 percent. Although several studies[18,21,137,247-249] have included a 24hr assessment for protein synthesis, virtually none have done so using our D_2O method in a similar population with similar diet and exercise interventions. Therefore, it seems premature to compare 24hr synthesis rates from our study with others at the 24hr time point.

Based on joint TAMU Exercise Sport Nutrition Lab (ESNL) and Muscle Biology Lab (MBL) analyses of data collected in the ESNL and Human Countermeasures (HCL) labs, the anabolic effect of exercise was not significantly affected by prolonged training (although $p = 0.075$). Nevertheless, FSR trended higher (54%) overall following 12 weeks of the exercise and diet intervention. It may be tempting to hypothesize that post-exercise timed nutrition (nutrient timing) stimulated this increase in our population. However, as neither nutritional intake, nor exercise type/volume between groups was significantly different, might we just as easily attribute such results to our well-managed diet, total dietary protein intake, simply the training effect, or (perhaps more likely) both?

Reduced metabolism and lowered FSR rates throughout the aging process[27,179,250] may, if not hypothetically account for the afore mentioned trend. Exercise notwithstanding, since FSR in women has been reported as higher and less affected than that of men over the lifespan[251,252], a study such as ours may be of

interest in a male population as well. Nevertheless, while a possible trend was observed warranting additional research, there did not appear to be any clearly distinguishable advantage of either immediate or delayed nutrient timing on 24-h FSR in our population of interest wherein a reduced carbohydrate, higher protein controlled diet was implemented throughout the study.

The ability to establish, or regain and maintain a healthy fitness level is a recommended component for optimizing quality of life. In particular, this objective is perhaps of special concern for the post-menopausal overweight/obese female population. Considering numerous previously reported nutrient timing outcomes in young athletic[47,49,253] and more relevantly older populations[50,51,254-256] it is reasonable to posit that the timed post exercise nutrition administered during this study might significantly enhance FSR, thereby supporting anabolism and preserving, perhaps increasing lean mass. Indeed, recent substantiating evidence for the positive effects of post exercise nutrient timing do exist[257-261]. However alternate studies which report mixed and/or questionable /inconclusive results also exist[28,30,54,262-264]. Nevertheless, surprisingly few (if any) have utilized our methods, and/or targeted our specific population of interest.

The exercise and diet program administered during our study has shown marked benefits during previous studies consisting of younger and senior populations[139,265-267], with more favorable results in participants randomized into higher protein diet groups. The purpose of this study was to examine potential effects of timed (I and/or D) post-exercise protein intake, combined with the higher protein diet in obese post-

menopausal women in the areas of mixed muscle FSR, measures of body composition to include fat mass, lean mass, upper and lower body strength (1RM), and resting energy expenditure (REE).

As previously noted herein, similar ESNL exercise and nutrition interventions have proven to be an effective approach for weight loss, specifically fat mass, while preserving muscle mass. The greatest gains have been seen in groups where reduced dietary carbohydrate and higher protein were consumed. Additional body composition gains were supported by time effects in waist and hip circumference. Overall, both of our groups observed weight loss (I: -3.6kg; D: -4.2kg), fat loss (I: -3.5kg; D: -4.8kg), percent body fat (I: -2.8%; D: -4.38%) and maintained fat free mass (I: +0.03kg; D: +1.11kg). Therefore, our results appear to be consistent with previous ESNL studies.

The addition of post exercise nutrient (protein) timing did not independently appear to significantly influence results. Indeed, the addition of a control group for post exercise nutrition (although not particularly necessary within the parameters of our study) may have been of interest. Pertinent trends did appear intriguing. However, post exercise timing of intake did not appear to independently influence FSR results. Significant time effects were seen in body mass, fat mass, and body fat, yet no significant group x time effects were observed. Participants in the delayed intake (D) group generally experienced more favorable changes in body mass, fat mass, lean mass and body fat. There were significant time main effect changes for 1RM bench press and leg press (with an overall mean percent change). No significant differences were seen among groups in REE, although values trended slightly downward over time throughout

the 12 weeks. No significant nutrient timing x training interactions were observed on muscle FSR expressed as a percent/day of the alanine pool. However, FSR was augmented in response to a bout of RE prior to training and tended to be 54% higher in response to a bout of exercise after training when compared to pre-training values.

Considering the bulk of previous investigations surrounding protein synthesis and resistance training, it is possible that, had we measured FSR over the immediate time following exercise (up to 3h), we may have observed more clear evidence supporting FSR elevations. However, considering our RIPT exercise design compared to the majority of other NT/FSR studies utilizing a resistance only intervention, this does not appear to be a fair comparison. It has been shown that protein synthesis begins to plateau at/or about the 24hr time point[18,268]. Based on our observations, adequately controlling dietary demands during a training cycle may offset (at least in part) any metabolic need that post-exercise nutrition might provide toward up-regulation of muscle protein synthesis. Hypothetically speaking, if all metabolic needs/energy reserves are adequate (on board) via a well-managed diet, there may be no need for a nutrient "boost" to meet the metabolic demands of a particular bout of exercise or its subsequent recovery period for our population of study.

Perhaps most interestingly as it pertains to our study, an earlier (2006) similar investigation suggested that post exercise intake may be more influential than total daily protein intake as an effective method for maintaining FFM during periods of either increased energy expenditure (e.g. exercise) or "marginal" protein intake [108]. Subjects of said study included (in addition to 22 men of the same age range) untrained

(≤ 3 hours/wk) women (n=30, 60-69 years) who participated in a 12 week moderate resistance exercise (RE) program. The post exercise supplement consisted of a HP beverage composed of 240 kcals (15 g protein, 33 g CHO, and 6 g fat per 8 oz.) or an alternate, presumably equivalent supplement (3 subjects who were intolerant of the supplement) given immediately post exercise in an amount corresponding to 0.4 g of protein/kg of FFM. This approach seems particularly related to the type of routine proposed during our study with a few notable variations. Examples being, number/type of dietary data collection (food logs, etc.), variability of post exercise protein intake, exercise programming (active vs. passive rest, aerobic component etc.), macronutrient profile of post exercise intake, dietary controls, etc. The authors suggested that a randomized double-blind study would be necessary to confirm the reported results.

While solid foods may provide the required nutrients, a liquid may assimilate more rapidly, make it easier to achieve the preferred ratio, and thus more efficiently meet the 2-hour window. It has been reported that the anabolic phase, wherein the optimal effects of nutrient ingestion begins to deteriorate, or close within 45 minutes post exercise, peaks within 15-30 minutes post exercise [229]. The general premise is that by consuming critical nutrients [e.g. carbs, protein (or specific amino acids), and perhaps vitamins] during a time sensitive window, one might spare muscle glycogen, achieve greater muscular endurance, stem the rise of the catabolic hormone cortisol (hence reducing muscle damage), and facilitate a more favorable environment for efficient recovery following exercise.

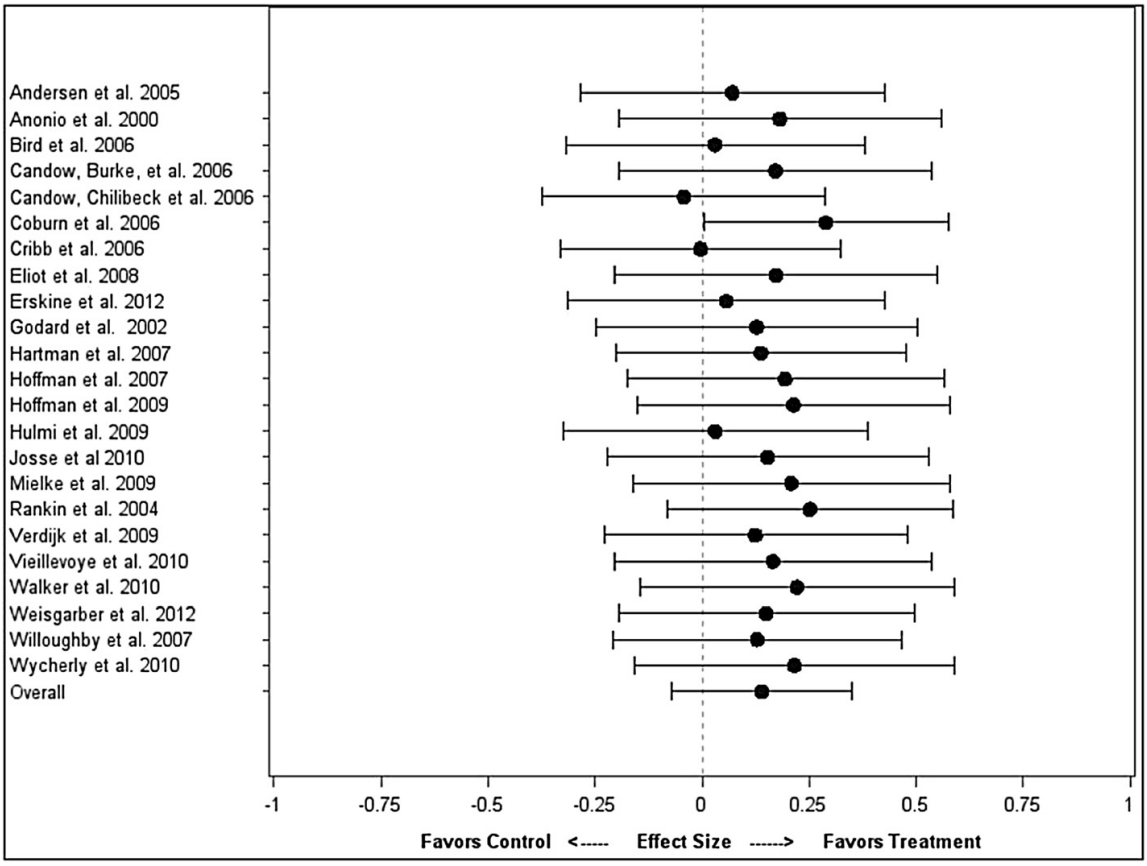
Finally (perhaps conveniently), a recent meta-analysis addressed the effects of protein timing on muscle strength and hypertrophy[264]. Therein, 23 studies with an average "Physiotherapy Evidence Database" (PEDro) scale rating of 8.7/10 (High Quality) were analyzed. Of the 23, only one[269] included a sample of older untrained women, and the total sample of 34 included men and women aged 57±7years. Study participants were randomly assigned to one of two groups for the 16 week intervention. Either an "immediately" pre RT exercise, or an "at least 2h following RT" meal group wherein a HP meal (860 kJ, 21 g protein, 0.7 g fat, 29.6 g carbohydrate) energy restricted diet was ingested. Considering that 1) the energy restricted dietary profile, 2) the focus on type 2 diabetes, and 3) no protein syntheses analyses were undertaken, it does not appear that we can draw any direct comparisons between these results and ours with regard to protein synthesis. Arguably, the Shoenfeld et al. meta-analysis, wherein most all samples were of younger males participating in a progressive resistance training program, and does not include consistently comparable timed intake or dietary profiles could be determined to depict a reasonably suitable study of reference when considering our results. However, in consideration of the body of work with regard to protein synthesis/FSR response to pre/post exercise nutrition in general as presented within our study, it is worthy to mention: the meta-analysis suggested "a majority of any gains in muscle mass would have been due to higher protein consumption by the treatment group."

Perhaps one of the most pertinent findings lay in data addressing the impact of protein timing on hypertrophy, adjusted by protein intake as depicted in Figure 9.

Although reported as non-significant, graphically these data appear supportive of dietary intake as more critical than timing. Nevertheless, a follow up "comment" on the Shoenfeld Meta-Analysis[271] suggested that: 1. the included studies afforded no reasonable assessment of whether timing of protein intake around exercise was beneficial because higher total daily protein intake is a confounding factor, and that 2. the authors' assertion that the study had "good statistical power" due to the sample size of about 500 subjects is flawed due to the use of only 3 "relevant" studies.

These findings are perhaps consistent with a slightly earlier study[270] which reported that protein supplementation alone (although timing effects were not evaluated) produced beneficial adaptations when combined with resistance training. Shoenfeld et. al. posited that their results directly lends support suggestive of the possibility that meeting target protein requirements is paramount to exercise induced muscle protein accretion, whereas timed pre and/or post-workout intake may, at best, be a minor consideration. There does appear to be a void when it comes to studies centered around the exploration of potential nutrient timing benefits on FSR when it comes to a population such as ours.

Figure 11. Timing Effects Adjusted for Protein Intake



Schoenfeld et. al. Journal of the International Society of Sports Nutrition 2013, 10:53

Conclusion

Our study investigated pre and post exercise acute and 24hr mixed skeletal muscle fractional synthesis rates in obese post-menopausal women as influenced by diet, resistance interval program training (RIPT) and post exercise timed ingestion of a commercially prepared protein shake over a 12 week training cycle. Admittedly, a limitation of this study, in addition to those enumerated earlier was our small sample size. Therefore, we can neither confirm nor deny the possibility that immediate (or

delayed) post-exercise protein consumption were responsible (or to what degree) for observed increases in protein synthesis within or between groups. Thus, more work with larger sample sizes in this area focusing on pertinent protein pools and nutrient timing should be conducted.

Nutrient timing does not appear to influence 24-hour FSR, body composition changes, and/or training adaptations in women participating in resistance interval program training (RIPT) while trying to promote weight loss. Other studies have not focused on determining if nutrient timing influences FSR or training adaptations in women using resistance exercise and a higher protein diet as a means of preserving FFM and REE during a weight loss trial. Other studies have focused on attempts to increase FFM without a hypo-energetic diet.

Participation in our exercise and weight management program with the addition of timed post-exercise nutrition may indeed promote more favorable changes in body composition. In consideration of the trend toward greater results in the 2hr post exercise timed group, a more delayed time of ingestion may be preferable to immediately post-exercise ingestion within our population of interest. With a relatively higher protein intake compared to previous studies, it also seems plausible that the post exercise intake may have boosted overall dietary protein intake, although target values were not achieved via assessment of our four 4-day food log records. Our results suggest that we are unable to conclude that post exercise protein consumption is responsible for the results achieved during this study. As discussed earlier, perhaps total dietary protein is

all it takes to get the job done as long as nutritional intake is appropriately well managed in a population such as was employed within this study.

Ultimately, our findings suggest that rather than the timing of ingestion, daily nutrient (perhaps more specifically protein) intake may be of equal interest when it comes to maintaining muscle protein anabolism with exercise. Future free living model research in this area might focus on larger sample sizes and additional controls to examine whether nutrient timing (NT) affects health related training adaptations in post-menopausal overweight women (or aged males) who participate in lower carbohydrate, fat and higher protein diet interventions wherein these types of resistance interval program training (RIPT) designs are implemented.

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APPENDIX A

Table A1 *Body System and Related Effects of Aging*

Effect	Functional Significance
Cardiovascular	
Blood, plasma, and red cell volumes ↓	Decreased venous return and stroke volume
Capillary/fiber ratio ↓	Decreased muscle blood flow
Cardiac compliance ↓	Decreased early diastolic filling and increased contribution of atrial priming
Endothelial dysfunction	Decreased nitric oxide secretion; reduced blood flow control
Sodium-potassium pump activity ↓	Decreased management of cell water and electrolytes
Cardiac muscle and heart volume ↓	Decreased maximal stroke volume and cardiac output
Elasticity of blood vessels ↓	Increased peripheral resistance, blood pressure, and cardiac afterload
Myocardial myosin-ATPase ↓	Decreased myocardial contractility
Sympathetic stimulation of SA node ↓	Decreased maximum heart rate
Respiration	
Condition of elastic lung support structures ↓	Increased work of breathing
Elasticity of support structures ↓	Decreased lung elastic recoil
Size of alveoli ↑	Decreased diffusion capacity and increased dead space
Number of pulmonary capillaries ↓	Decreased ventilation/perfusion equality
Muscles/Joints/Soft Tissues	
Accumulated mechanical stress in joints ↑	Stiffness, loss of flexibility, and osteoarthritis
Action potential threshold ↓	Loss of strength and power
Blood insulin ↑	Hypertension, coronary heart disease
Ca++, Myosin)ATPase ↓	
Insulin sensitive ↓	Diabetes, coronary heart disease. Obesity, hyperlipidemia, hypertension
Lactate dehydrogenase ↓	Slows glycolysis
Muscle mass ↓	
Number of type IIa and IIb fibers ↓	
Oxidative enzymes: SDH, cytochrome oxidase, and MDH ↓	Decreased muscle respiratory capacity
Size and number of mitochondria ↓	Decreased muscle respiratory capacity
Size of motor units ↓	
Stiffness of connective tissue in joints ↑	
Total protein and N_2 concentration ↓	
Water content in intervetebral cartilage ↓	Atrophy and increased chance of compression fractures in the spine
Bone	
Bone minerals ↓	Osteoporosis- increased risk of fracture
Body Composition and Stature	
Abdominal fat deposition ↑	Coronary Artery Disease, insulin resistance, hyperlipidemia, back pain
Body fat ↑	Impaired mobility and increased risk of disease
Fat-free weight ↓	Decreased metabolic rate
Kyphosis ↑	Loss of height

Adapted from: Brooks, et.al., Exercise Physiology; Human bioenergetics and its applications. 2005; Boston, McGraw Hill

Table A2. *Summary of Muscle Adaptation to Aging and Training in the Elderly (adaptedfrom Kirkendall et al., 1998).*

Variable	Aging	Training
Muscle mass	Decrease	Increase/No change
Type I %	Increase	No change
Type II %	Decrease	No change
Type I area	No change	Increase
Type II area	Decrease	Increase
Oxidative capacity	Decrease	No change
Glycolytic capacity	No change	Increase
Capillary density	Decrease	Increase
Relaxation time	Increase	Decrease/No change
Shortening velocity	No change	Increase

Table A3. Curves Chocolate Shake Nutrient Analysis (Daily Values)

Nutrient	Amount	DRI	Tolerable Upper Limit
Vitamin A (palmitate)	2500 IU	3000 IU	10,000 IU
Vitamin C (ascorbic acid)	30 mg	90 mg	2,000 mg
Vitamin D (Cholecalciferol)	400 IU	600 IU	2,000 IU
Vitamin E (acetate)	15 IU	22 IU	67 IU
Vitamin B1 (Thiamin Hydrochloride)	0.75 mg	1.2 IU	ND
Vitamin B2 (Riboflavin)	1 mg	1.3 mg	ND
Vitamin B6 (Pyridoxine Hydrochloride)	1 mg	1.7 mg	100 mg
Folic Acid	200 mcg	400 mcg	1000 mcg
Vitamin B12 (Cyanocobalamin)	3 mcg	2.4 mcg	ND
Biotin	150 mcg	30 mcg	ND
Pantothenic Acid (d-calcium pantothenate)	5 mg	5 mg	ND
Calcium (dicalcium & tricalcium phosphate)	1000 mg	1000 mg	2500 mg
Iron (ferrous fumarate)	4 mg	18 mg	45 mg
Phosphorus (dicalcium, tricalcium, & dipotassium phosphate)	650 mg	700 mg	4000 mg
Iodine (potassium iodide)	75 mcg	150 mcg	1100 mcg
Zinc (zinc oxide)	7.5 mg	8 mg	40 mg
Selenium (sodium selenite)	21 mcg	55 mcg	400 mcg
Copper (copper sulfate)	1 mg	0.9 mg	10 mg
Manganese (manganese sulfate)	1 mg	1.8 mg	11 mg
Chromium (chromium picolinate)	96 mcg	25 mcg	ND
Molybdenum (sodium molybdate)	50 mcg	45 mcg	2000 mcg
Sodium	100 mg	1500 mg	2300 mg
Potassium (dipotassium phosphate)	350 mg	700 mg	4000 mg
Hydroxy Citrate (garcinia cambogia extract)	250 mg	ND	ND
Soy Isoflavones (soy protein isolate)	25 mg	ND	ND

Notes: Table adapted from Food and Nutrition Board, Institute of Medicine, National Academies (2004); DRI reports (www.nap.edu). The DRI's are reported for various nutrients as either RDA's or AI's (adequate intake). The UL (Tolerable Upper Intake Levels) were defined as: "The maximum level of daily nutrient intake that is likely to pose no risk of adverse effects," and represents "the total intake from food, water, and supplements." ND is described as "not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake." 1 cup of soymilk contains about 30mg of soy isoflavones.

Table A4. ^{2}H-labeling of body water and skeletal muscle protein (T-1).

	Plasma (MPE)				Mixed SM (MPE)		
Subject	Pre	7.5 h	9.5 h	24 h	Pre	Post	24hr
1	0.019	0.379	0.366	0.481	1.086	0.464	0.417
2	0.017	0.444	0.427	0.541	0.049	0.114	0.929
3	0.026	0.318	0.369	0.497	0.092	0.261	0.486
4	0.014	0.341	0.348	0.496	0.114	0.041	0.058
5	0.029	0.385	0.382	0.520	0.078	0.040	0.089
6	0.021	0.396	0.393	0.505	0.179	0.269	0.375
7	0.026	0.384	0.383	0.524	0.232	0.108	0.114
8	0.015	0.299	0.376	0.499			
9	0.027	0.389	0.391	0.503	0.080	0.633	0.147
10	0.012	0.401	0.390	0.493	0.378	0.065	0.208
11	0.019	0.384	0.391	0.521	0.184	0.137	0.485
12	0.015	0.385	0.400	0.480	0.324	0.279	0.083
13	0.042	0.360	0.357	0.492	0.533	0.355	0.634
14	0.009	0.360	0.365	0.513	0.098	0.052	0.123
15	0.003	0.401	0.397	0.532	1.161	0.616	0.327
16	0.027	0.386	0.367	0.468	0.093	0.455	0.106
17	0.020	0.342	0.352	0.479	0.073	0.083	0.144
18	0.030	0.361	0.390	0.479	0.167	0.457	0.301
19	0.025	0.347	0.357	0.468	0.161	0.042	0.196
20	0.023	0.392	0.375	0.507	0.041	0.456	0.119
21	0.018	0.344	0.366	0.526	0.102	1.509	0.634
22	0.019	0.297	0.325	0.448	0.191	0.220	0.602
Mean	*0.021*	*0.368**	*0.376**	*0.499**	*0.258*	*0.317*	*0.313*
SEM	*0.017*	*0.075*	*0.046*	*0.050*	*0.068*	*0.073*	*0.052*

Plasma ^{2}H labeling measured prior to the start of the study (0h), 1h post-exercise (+7.5h), 3h post-exercise (9.5h) and 16h post-exercise (+24h) in the plasma, and ^{2}H labeling of protein-bound alanine measured 16h post-exercise (+24h). MPE = mole percent excess. *Plasma ^{2}H labeling was significantly higher at 7.5, 9.5 and 24 h than pre (time 0) values, $p < 0.001$.

Table A5. ^{2}H-labeling of body water and skeletal muscle protein (T-2).

	Plasma (MPE)				Mixed SM (MPE)		
Subject	Pre	7.5 h	9.5 h	24 h	Pre	Post	24 hr
1	0.021	0.404	0.377	0.497	0.191	0.251	0.265
2	0.036	0.415	0.403	0.519	0.228	0.219	0.261
3	0.025	0.404	0.389	0.518	0.762	1.930	0.522
4	0.018	0.394	0.381	0.500	0.231	0.198	0.277
5	0.186	0.388	0.390	0.563	0.213	0.296	
6	0.021	0.426	0.416	0.540	0.358	0.472	0.666
7	0.035	0.382	0.379	0.505	0.206	0.252	0.271
8	0.030	0.365	0.372	0.495	0.242	0.567	0.259
9	0.025	0.376	0.360	0.464	0.196	0.232	1.196
10	0.023	0.388	0.385	0.504	0.222	0.224	0.255
11	0.031	0.377	0.378	0.498	0.271	0.253	0.261
12							
13	0.028	0.407	0.395	0.522	0.497	0.281	0.324
14	0.192	0.370	0.382	0.488	0.231	0.216	0.305
15	0.244	0.403	0.398	0.537	0.887	0.352	1.474
16	0.028	0.388	0.387	0.503	0.179	0.153	0.201
17	0.022	0.414	0.398	0.493	0.286	0.263	0.279
18	0.022	0.382	0.368	0.546	0.249	0.287	0.261
19	0.017	0.356	0.355	0.487	0.158	0.229	0.222
20	0.033	0.399	0.398	0.528	0.283	0.197	0.250
21	0.024	0.385	0.371	0.496	0.240	0.254	0.334
22	0.027	0.372	0.367	0.479	1.229	0.281	0.994
Mean	*0.052*	*0.390**	*0.383**	*0.510**	*0.307*	*0.349*	*0.447*
SEM	*0.010*	*0.006*	*0.004*	*0.005*	*0.043*	*0.082*	*0.077*

Plasma ^{2}H labeling measured prior to the start of the study (0h), 1h post-exercise (+7.5h), 3h post-exercise (9.5h) and 16h post-exercise (+24h) in the plasma, and ^{2}H labeling of protein-bound alanine measured 16h post-exercise (+24h). MPE = mole percent excess. *Plasma ^{2}H labeling was significantly higher at 7.5, 9.5 and 24 h than pre (time 0) values, $P < 0.000$.

APPENDIX B

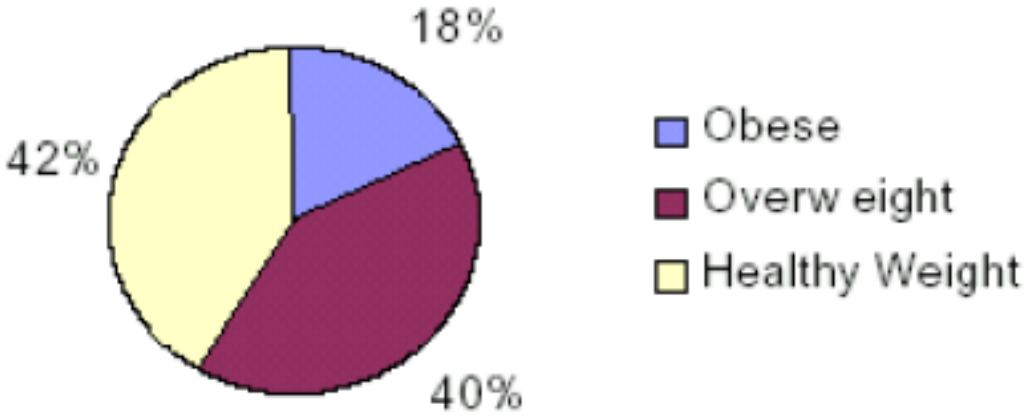

Figure B1. Centers for Disease Control and Prevention. National Center for Chronic Disease Prevention and Health Promotion. Behavioral Risk Factor Surveillance System. Trend Data, Nationwide. April 1, 2002.

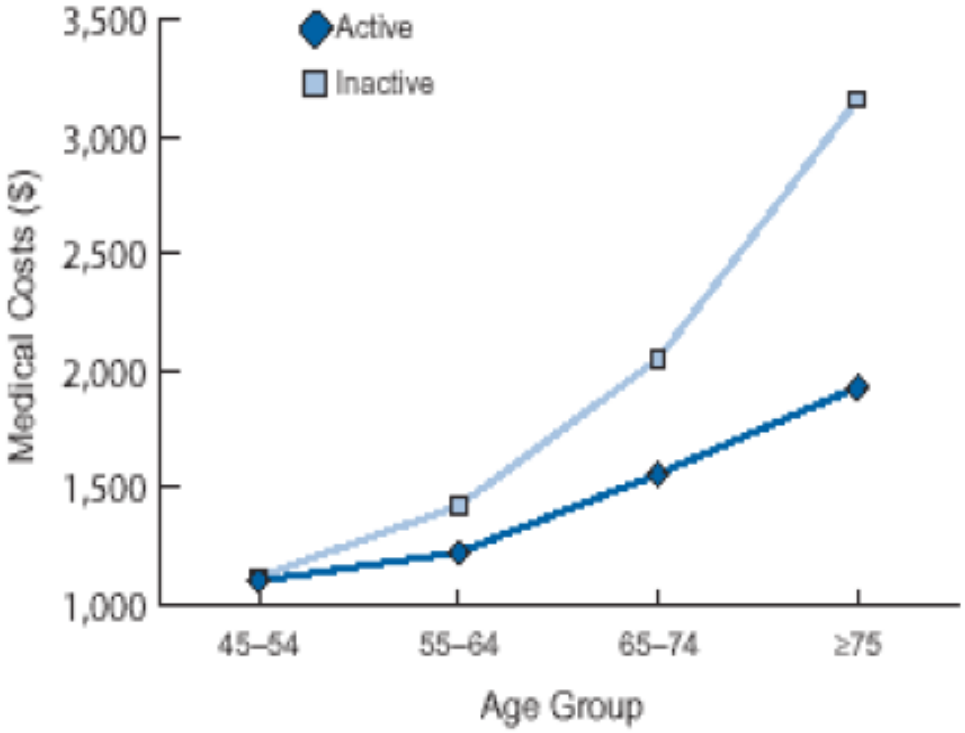

Figure B2. Annual Medical Costs of Active and Inactive Women (Aged 45 or Older) Without Physical Limitations. Figure retrieved from Centers for Disease Control and Prevention. Promoting Active Lifestyles Among Older Adults. National Center for Chronic Disease Prevention and Health Promotion. Nutrition and Physical Activity. www.cdc.gov/nccdphp/dnpa/physical/recommendations/older adults.htm

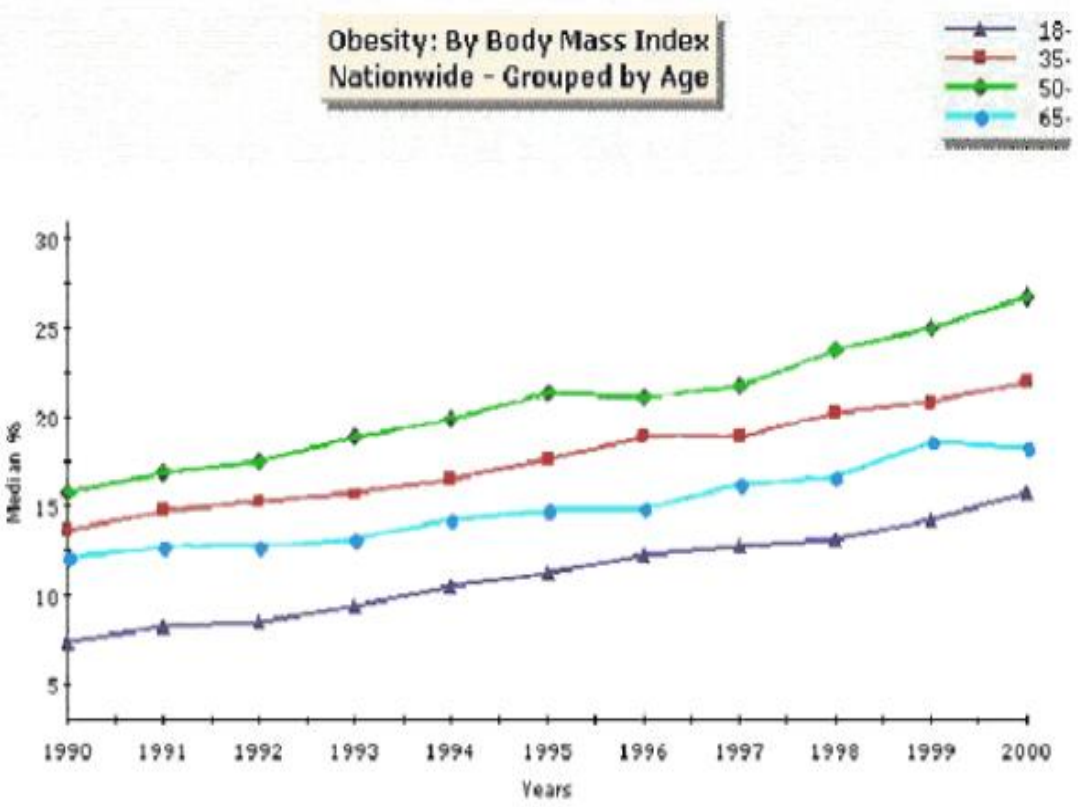

Figure B3. Centers for Disease Control and Prevention. National Center for Chronic Disease Prevention and Health Promotion. Behavioral Risk Factor Surveillance System. Trend Data, Nationwide. April 1, 2002.