Official Title:

EFFICACY OF NIGHTTIME PROTEIN FEEDING DURING 12 WEEKS OF RESISTANCE TRAINING ON FUNCTIONAL AND COGNITIVE ADAPTATIONS IN OLDER ADULTS

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CHAPTER THREE

METHODOLOGY

3.1 Overview of Experimental Design

The overarching aim of this project was to compare the traditional recommendation of immediate post-exercise protein consumption with nighttime pre-sleep protein intake during 12 weeks of resistance training on functional and cognitive adaptations in older adults. To achieve this, our design contained three groups who performed the same 12-week RET program two times per week and: 1) Consumed protein immediately post-exercise (Post-ex), 2) Consumed protein 30 minutes prior to sleep (Pre-sleep), or 3) Did not consume additional protein – exercise only (Ex only). Baseline total kilocalories (kcal) and protein intake were monitored to ensure no significant differences among groups occurred. The dependent variables consisted of pre- to post-intervention changes in body composition (i.e. lean mass, fat mass, and percent body fat), functional capacity (i.e. SPPB), nitrogen balance, free plasma insulin-like growth factor 1 (IGF-1), profile of mood states, and sleep evaluation, and digit symbol substitution and pattern comparison. Pre-, mid-, and post-intervention 1-RM, muscular power, and muscle thickness were assessed to determine the time course of muscular adaptations and appetite changes.

The pre-intervention measures took place over 2-3 days. All participants were instructed to refrain from vigorous physical activity at least 48 hours prior to all pre-testing measurements. On day 1, participants reported to the laboratory in the morning following an overnight fast (participants were encouraged to consume normal water intake) to complete the informed consent process, health history, and physical activity questionnaires. Thereafter, participants completed pre-training assessments including height and weight, baseline blood pressure, psychological and cognitive assessments, blood draw (IGF-1), body composition (DXA or BIA),

muscle thickness, muscular power, and the SPPB. Following the completion of day 1 assessments, participants were provided a 3-day food record, actigraphy watch, and 24-hour urine collection jug (nitrogen balance) to return prior to beginning the RET portion of the study.

Day 2 took place approximately 72-96 hours after the first visit and served as a familiarization session for maximum strength testing. Day 3 took place 5-7 days after day 2 and included retesting maximum strength. Some participants elected to complete the first maximum strength test at the end of day 1, in which case, they were instructed to return 5-7 days later to retesting maximum strength, resulting in only 2 pre-testing visits. Thereafter, participants completed 12 weeks of RET, with or without protein supplementation, in the Strength Training Laboratory of the Sandels building. Participants completed post-intervention measures during week 12 in a similar manner as pre-intervention. Maximum strength, muscle power, and muscle thickness were also assessed after week 6 of RET.

3.2 Methods for Specific Aims

3.2.1 Specific Aim 1: Body Composition and Functional Capacity

Specific aim 1 was to examine the degree to which timing of protein intake influenced body composition, muscle thickness, and functional capacity during 12 weeks of RET in older adults.

<u>Dependent Variables (Detailed protocols for each assessment are provided in the subsequent sections)</u>

- Body composition
    - Lean mass
    - Fat mass
    - Percent body fat

- Muscle thickness
- Functional capacity
    - SPPB
        - Balance
        - Gait speed
        - 5-chair stand
    - Maximum muscular strength
    - Muscular power

3.2.2 Specific Aim 2: Cognitive and Psychological Effects

Specific aim 2 was to investigate the extent by which timing of protein intake impacted psychological and cognitive processing speed during 12 weeks of RET in older adults.

Dependent Variables (Detailed protocols for each assessment are provided in the subsequent sections)

- Digit symbol substitution task
- Pattern comparison
- Appetite
- Profile of mood states
- Sleep evaluation

3.2.3 Specific Aim 3: Nitrogen Balance and Free IGF-1

Specific aim 3 was to determine the influence of timing of protein intake on changes in nitrogen balance and free plasma IGF-1 responses during 12 weeks of RET in older adults.

Dependent Variables (Detailed protocols for each assessment are provided in the subsequent sections)

- 24-hour urinary nitrogen balance
- Free plasma insulin-like growth factor 1

3.3 Participants

Healthy, sedentary (not exercising regularly for the past 6 months), older adult males (N=30; Post-ex n=9, Pre-sleep n=11, Ex only n=10) between the ages of 60-75 years old from the Tallahassee area participated in the study. Health-related exclusion criteria included diagnosis of cardiac or peripheral vascular disease, stroke, kidney dysfunction, metabolic diseases, uncontrolled hypertension, orthopedic limitations, skeletal muscle complications, milk allergy, psychiatric conditions, use of psychotropic drugs or cognitive impairment. Additional exclusion criteria included participation in a structured regular exercise program within the past 6 months, actively attempting to increase or decrease body mass, scoring 26 points or less on the Montreal Cognitive Assessment, and/or body mass index (BMI) of ≥ 35 kg/m^2. To determine exclusion criteria, all participants completed an informed consent (appendix C), health history questionnaire (appendix E), physical activity questionnaire (appendix F), Montreal Cognitive Assessment (appendix G), and height and weight measurements, prior to any participation in the intervention. Lastly, all participants submitted a physician consent (appendix D) form prior to beginning the exercise training protocol.

3.4 Resistance Exercise Training Protocol

All participants completed 12 weeks of RET, 2 times per week on non-consecutive days (separated by 72–96 hours). Participants generally completed their exercise at a consistent time (±1 hour) throughout the study (e.g., every Monday at 1 pm). Research personnel and certified

personal trainers supervised all RET sessions, which took place in Strength Training Laboratory of the Sandels building. Each resistance exercise session began with 5 minutes of cycling at 50 revolutions per minute at 0.5 kg of resistance on a stationary cycle (Monark 828E, Langley, WA) followed by a standardized dynamic warm-up consisting of body weight movements to prepare the muscles for the specific exercises being performed. Thereafter, participants completed 3 lower body (leg press, leg extension, and leg curl) exercises and 5 upper body (chest press, shoulder press, latissimus row, triceps dip, and biceps curls) and for 3-4 sets and 4-12 repetitions with a 2-3-minute rest period between each set and exercise (see Table 1 for specific details). The chest press and leg press exercises were considered as the primary exercises, while all others were considered as accessory exercises. For the primary exercises, intensity was based upon 1-RM testing results. However, estimated 1-RM values were used for accessory exercises, which were established during week 1 of the RET protocol. Thereafter, a repetitions in reserve-based rating of perceived exertion (RIR-based RPE) scale was used to adjust load accordingly (149, 379). Exercise intensity was adjusted after the mid-testing 1-RM assessment to account for changes in maximum strength. However, for secondary exercises, intensity was adjusted each session based on participants RPE rating.

The 12-week RET program was designed as a periodized mesocycle, comprised of several specific microcycles (Table 1). Week 1 was an introductory microcycle of lower volume and intensity exercise (182). The aim of this week was to acclimate participants to exercise and elicit the repeated bout effect to attenuate muscle damage in the subsequent weeks of the program. On day 1 of the introductory week participants were assigned 2 sets of 12 repetitions at 50% 1-RM and 1 set of 10 repetitions at 55% 1-RM. Thereafter, weeks 2-6 consisted of two subsequent volume-focused microcycles. On day 1 of weeks 2-4, on day 1, participants were

assigned 3 sets of 12 repetitions at 60% 1-RM and 3 sets of 10 repetitions at 65% 1-RM on day 2. On Day 1 of weeks 5-6, on day 1, participants were assigned 3 sets of 10 repetitions at 65% 1-RM and 3 sets of 8 repetitions at 70% 1-RM on day 2. Week 7 consisted of a taper on day 1, which was comprised of 2 sets of 2 repetitions at 80% 1-RM, followed by 1 set of 1 repetition at 85% 1-RM. On day 2 mid-intervention 1-RM assessments were completed for the chest press and leg press exercises followed by the normal exercise protocol for the accessory exercises. Then weeks 8-11 consisted of two subsequent intensity-focused microcycles, where intensity was based upon the mid-testing 1-RM value. For weeks 8-9, participants were assigned 3 sets of 8 repetitions at 70% 1-RM on day 1 and 3 sets of 6 repetitions at 75% 1-RM on day 2. For weeks 10-11, participants were assigned 3 sets of 6 repetitions at 75% 1-RM on day 1 and 4 sets of 4 repetitions at 80% 1-RM on day 2. Week 12 also consisted of a taper on day 1 and post-intervention 1-RM assessment on day 2. Only primary exercises were performed on taper days. Accessory exercises were completed with the same sets and repetitions as the main exercise; however, the load was selected to produce an RPE value between 6-8 and 7-9 for weeks 1-6 and 7-12, respectively. The intensity, sets, repetitions, number of exercises, and rest periods of the exercise program were in agreement with previous literature examining the effectiveness of RET on strength and LBM adaptations in older adults (270, 271).

Table 1. Overview of Exercise Training Program

Week	Focus	Day 1	Day 2
1	Introductory	2x12 @ 50%	2x10 @ 55%
2-3	Volume	3x12 @ 60%	3x10 @ 65%
4	Volume	3x11 @ 62.5%	3x9@67.5%
5-6	Volume	3x10 @ 65%	3x8 @ 70%
7	Taper/Mid 1-RM test	2x2 @ 80%, 1x1 @ 85%	Mid 1-RM test
8-9	Intensity	3x8 @ 70%	3x6 @ 75%
10-11	Intensity	3x6 @ 75%	4x4 @ 80%
12	Taper/Post 1-RM test	2x2 @ 80%, 1x1 @ 85%	Post 1-RM test

RM = Repetition Maximum

If participants were not able complete the prescribed number of repetitions within a set, the training load for the subsequent sets of that specific exercise was reduced by approximately 2.5% for each missed repetition. For example, if a participant was only able to complete 4 of 6 assigned repetitions, then the load for the subsequent sets was reduced by 5%.

3.5 Protein Supplementation

Protein and placebo supplementation were provided in a double-blind, placebo-controlled manner throughout the 12-week intervention. Participants were randomly assigned (https://www.randomizer.org) to 1 of 3 groups: 1) Post-ex consumed a protein supplement immediately post-exercise and a non-caloric placebo 30 minutes before sleep, 2) Pre-sleep consumed a non-caloric placebo immediately post-exercise and the protein supplement 30 minutes prior to sleep, or 3) Ex only did not consume protein or placebo supplementation. Participants were instructed to fast for at least 2 hours prior to each exercise session and for 2 hours post exercise (9), except for the consumption of the protein or placebo supplement, along with a 190-kcal snack bar (Nature Valley™, Oats 'N Honey, General Mills, MN), to reduce the timing effects of normal meals on exercise adaptations. Additional instructions were given to consume the nighttime pre-sleep protein or placebo supplement at least 2 hours after their last feeding (e.g. meal or snack) and approximately 30 minutes before sleep with no other food. On non-exercise days, participants were instructed to consume the protein or placebo supplement in between their normal breakfast and lunch.

To ensure a double-blind, placebo-controlled design, an assistant not associated with outcome measures created containers of protein and placebo supplements in advance and did not reveal the identity until the conclusion of the study. Participants were provided two containers with approximately 25 servings (3.5 weeks of supplements) of protein and placebo

supplementations. Each serving was weighed on a digital scale (Etekcity Co., Anaheim, CA) and placed within individual sealable bags (Ziploc; SC Johnson Co., Racine, WI) to ensure accurate servings. Furthermore, both supplements were masked for flavor, and texture. Lastly, participants were instructed to mix their supplement only with water.

The protein supplement (Dymatize Enterprises, Elite XT) consisted of a whey and casein blend (4:1 ratio) containing 40 total g of protein, 15 g of carbohydrates, and 5 g of fat for a total of 265 kcals. This amount has been recommended to maximize muscle protein synthesis in older adults (372) and is the minimum amount to optimally stimulate overnight MPS (340). To assess compliance, participants recorded the date and time of each supplement consumed on a daily compliance sheet. A compliance rate of 80% or greater was required to be included in the analysis.

3.6 Food Records and Analysis

Participants were instructed to maintain their normal dietary habits throughout the intervention, except for the consumption of either protein or placebo supplement. At pre- and post-intervention assessments, participants were asked to complete a written 3-day food record consisting of two weekdays and one weekend day of habitual dietary patterns, which was collected and used for dietary analysis. Participants were encouraged to provide photos of meals and diet logs were reviewed with research personnel after submission to enhance accuracy of the recorded food intake. Additionally, throughout the study participants were queried about changes to their dietary habits. Lastly, dietary analysis was performed by the same person for all diet logs using the MyFitnessPal® website (10).

3.7 Measurements of Dependent Variables

3.7.1 Anthropometrics, Blood Pressure, and Body Composition

Anthropometric and body composition measures took place at pre- and post-intervention points. Anthropometric measures of body mass (BM) (kg) and height (cm) were measured by a digital scale and stadiometer (SECA, Hamburg, Germany), respectively. Blood pressure was measured at baseline and the taper session of week 12 using an automated blood pressure machine (Omron, HEM-907XL, Vernon Hills, IL) after participants were seated, with their feet flat and arms rested at heart level for 5 minutes. Body composition was assessed via DXA (iDXA Lunar, GE, Madison, WI) by a trained technician. DXA is a commonly utilized, non-invasive assessment that uses x-ray to scan the body while in the supine position, which takes approximately 5-7 minutes. After completion of the body scan, the DXA system software determines the differences in attenuation of x-rays of two different energies among bone mineral content and soft tissue (lean and fat mass), from which body composition components fat mass, lean mass, and percent body fat can be calculated using machine specific algorithms (360). Unfortunately, due to a change in IRB regulations, after completing 8 DXA scans, we were no longer able to use that machine, and therefore, we measured body composition with a BIA machine (BIA 310e, Biodynamics Corp., Shoreline, WA). BIA is a commonly used, non-invasive method that uses a small test current (800 μA at 50 kHZ) to assess impedance, which is used to estimate body fat percentage. For the assessment, participants were asked to lie in a supine position, while two pairs of electrocardiography electrode tabs were placed on the right ankle and wrist. Thereafter, the test current was applied, and body composition was computed within the BIA machine. Test-rest reliability measured by intraclass correlation coefficients (model 3,1) for the body composition variables were: percent body fat, 0.969 (95% confidence

intervals: 0.753, 0.997); lean mass 0.994 (95% confidence intervals: 0.949, 0.999); fat mass 0.978 (95% confidence intervals: 0.826, 0.998).

3.7.2 Muscle Thickness

Muscle thickness was assessed via ultrasonography (HD11 XE, Philips, Netherlands) using a linear-array transducer with 3-12 MHz (L 12-3, Philips, Netherlands) and was used as an index of muscle hypertrophy for the quadriceps musculature. This technique has been previously employed to measure the hypertrophic response to RET (182) and has compared favorably to magnetic resonance imaging for muscle size (288). Ultrasound scans took place during pre-, mid-, and post-testing time points in the supine position after participants had been resting for 10 minutes to allow for any fluid shift to occur. During this time, specific anatomical locations were identified and marked with a water-soluble pen at 50% of distance between the greater trochanter and the lateral condyle of the femur and 50% of the distance between the anterior superior iliac spine and the superior border of the patella. These anatomical locations allowed for imaging of the vastus lateralis, vastus intermedius, and rectus femoris muscles (1, 146).

All ultrasound scans were completed by the same person and conducted on the right side of the body. Prior to each scan, a water-soluble transmission gel was applied to the transducer for optimal acoustic contact. Furthermore, minimal pressure was applied to the transducer to limit compression of tissues. Muscle thickness was measured as the distance (cm) from the adipose-muscle interface to the muscle-fascicle or muscle-bone interface. The values from the three sites were summed together for a total muscle thickness measure. Muscle thickness test-rest reliability measured by intraclass correlation coefficients (model 3,1) for the muscle groups were: vastus lateralis, 0.977 (95% confidence intervals: 0.835, 0.997); rectus femoris 0.995 (95% confidence intervals: 0.957, 0.999); vastus intermedius 0.977 (95% confidence intervals: 0.834, 0.998).

3.7.3 Functional Capacity Assessments

3.7.3.1 Maximum Strength

Participants underwent 1-RM testing at pre-, mid-, and post-intervention for the leg press and chest press exercises. At the pre-intervention assessment, there was a 1-RM familiarization session approximately 5-7 days before retesting 1-RM to account for any potential learning effect. The highest pre-intervention 1-RM value obtained was considered baseline maximum strength. Prior to 1-RM testing, participants completed 5 minutes of light cycling on a stationary cycle (Monark 828E, Langley, WA) followed by a full body dynamic warm up that consisted of various body weight movements lasting approximately 5 minutes. Thereafter, leg press 1-RM procedures began with a specific warm-up consisting of a loaded leg press for 10 repetitions at 30%, 5 repetitions at 50%, 2 repetitions at 75%, and 1 repetition at 85% of estimated 1-RM. All warm-up sets were completed with 1-minute rest periods. Then 1-RM attempts began, which generally consisted of a 1st attempt at 95%, 2nd attempt at 97.5%, and 3rd attempt at 100% of estimated 1-RM with 3-minute rest periods between each attempt. The 1-RM attempt procedure continued until the participants could no longer complete a full repetition with the correct form. After a leg press 1-RM was established, the same procedures were followed for the chest press exercise.

To ensure consistency among participants, strict adherence to procedural instructions for the leg press and chest press exercises was required for each 1-RM attempt. For the leg press, participants began the exercise in a seated position with their feet approximately shoulder width apart on the platform and their legs and hips flexed to position the inguinal crease just below the top of the knee. At the start of each repetition, a verbal command (e.g. start, go) was given from the researcher to begin the attempt. Then the participants moved the platform by extending their

hips, knees, and ankles until their legs reached full extension. Thereafter, they returned to the starting position in a controlled manner by flexing their hips, knees and ankle. Additionally, participants were required to keep their back and buttocks in contact with the seat throughout the movement.

For the chest press, participants began in a seated position, feet flat on the ground, with their arms abducted to approximately 45-75° and flexed until their hand is aligned with their chest, while grabbing the machine handles. At the start of each repetition, a verbal command (e.g. start, go) was given from the researcher to begin the attempt. Then participants extended their arms completely and return the handles to the starting position in a controlled manner by flexing their arms. Throughout the exercise, participants were required to keep their head, back, buttocks, and thighs in contact with the seat, along with feet flat on the ground.

3.7.3.2 Muscular Power

Power was assessed during several sit to stand tasks by affixing a Tendo unit (TENDO Sports Machines, Trencin, Slovak Republic) cord to the participants; waist (133). For this assessment, participants began seated in a standard height chair (0.47 m) with their feet on the floor, arms crossed over their chest, and hands on the contralateral shoulder. Upon a verbal command, participants were instructed to stand from a seated position as fast as possible for 3 trials separated by 60 seconds of rest. The highest peak and average power of the 3 trials were recorded and used for analysis of muscle power.

The Tendo unit consists of 2 components, a velocity sensor and display unit. The velocity sensory has a cord with a velcro sleeve that was positioned perpendicular to the floor and attached to the right side of the participants' waist during each trial. As the participants stood, the

Tendo unit cord was pulled and the display unit calculated the average and peak power output from velocity (m/s) and body mass (kg) through the sit-to-stand motion.

3.7.3.3 Short Physical Performance Battery

The SPPB is a simple, common protocol for measuring physical performance in older adults, and therefore, was used to evaluate functional ability. The SPPB consists of 3 components performed in the following order: 1) balance, 2) gait speed, and 3) 5-chair stands. The balance test required participants to maintain their balance for up to 10 seconds for one trial with their feet in 3 different positions in the order of: side-by-side, semi tandem, and full tandem position. Participants were allowed to move their arms and bend knees during the trials but were not permitted to move their feet. Each position was verbally explained and visually demonstrated prior to the test. Balance time for each trial was recorded and used for scoring. If the participants were not able to complete any balance position for 10 seconds, the balance test was ended and the SPPB was continued with the next test. For the gait speed test, participants were instructed to walk a 4-meter course at a normal pace for 2 trials, with the best time used for scoring. The 4-meter course was marked off by two pieces of tape on the floor. Participants started with both feet behind the tape and walked at a normal pace for 4-meters until they completely crossed the 2nd piece of tape. The last SPPB test was the 5-chair stands, which involved completing 5 consecutive sit-to-stand tasks as quickly as possible. Participants began in a seated position in a standard height chair (0.47 m) with their feet on the floor, arms crossed over their chest, and hands on the contralateral shoulder. Following a verbal command, they stood as quickly as possible and returned to a seatede position. The test was finished upon the 5th standing motion, or if the participants could not complete 5 chair stands.

Interpretation of the SPPB outcomes was in accordance with previously established values (140). Briefly, each SPPB test was scored on a 0-4 scale, based upon time or completion of the task, with lower values indicating worse performance and vice versa. Scoring for the balance portion of the SPPB will consisted of: 1 point for maintaining the side-by-side position for 10 seconds, 1 point for maintaining the semi-tandem for 10 seconds, and 1 point for maintaining the full-tandem position between 3–9.99 seconds or 2 points for maintaining the full-tandem position for 10 seconds. If the participant could not maintain the side-by-side position for 10 seconds, the balance portion was scored 0. The gait speed test scoring was based upon the amount of time to walk 4 meters at a normal pace using the following cut off times: 1 point for times greater than 8.70 seconds, 2 points for times between 6.21–8.69 seconds, 3 points for times between 4.82–6.20 seconds, and 4 points for times less than 4.82 seconds. If the participants could not complete the 4-meter walk, then the gait speed test was scored as 0. Lastly, the 5-chair stand scoring was also based upon cut off times including: 1 point for times greater than 16.70 seconds, 2 points for times between 13.70–16.69 seconds, 3 points for times between 11.20–13.69 seconds, and 4 points for times less than 11.19 seconds. If the participant could not complete a sit-to-stand task, then the 5-chair stand test was scored as 0. Once all portions of the SPPB were completed the scores from all 3 tests were summed together for a total score, with 12 being the highest possible score.

3.7.4 Cognitive Processing Speed

3.7.4.1 Digit Symbol Substitution Task

The digit symbol substitution (DSST) task is a well-validated psychomotor test used to assess processing speed (304). Participants were provided a series of digits (1-9) with a corresponding symbol beneath each digit, which together represents a “key” for the task. Below

this “key” was a series of digits in random order with an empty box underneath each digit. The task of the participants was to copy the associated symbol in the empty box beneath each digit, in order, as quickly as possible. This task was then scored based upon the number of symbols copied correctly within 90 seconds. A pencil and paper version of this test was used.

3.7.4.2 Pattern Comparison

The pattern comparison task is a simple 2-page, 60-second test (30-seconds per page) that assesses processing speed by requiring participants to visually examine several pairs of side-by-side images to determine if they are the same or different (58). The pencil and paper version of this test was used, which requires participants to write “S” or “D” (for “same” or “different”) depending on whether they believed the images were identical or not. The score was the correct number of judgments within 60 seconds.

3.7.5 Psychological Assessments

3.7.5.1 Appetite

A visual analog scale (VAS) was used to assess changes in appetite. The VAS is 100-mm horizontal scale with opposite extremes that represent three appetite sensations (hunger, satiety, and desire to eat) positioned at each end of the 100-mm line (0 = “not at all” and 100 = “extremely”). Following an overnight fast during the pre- and post-testing points, participants were asked to rate their subjective feelings, at that moment, by placing a vertical line along the 100-mm scale. These ratings were converted to a score in millimeters using a standard millimeter ruler. Higher scores indicated greater feelings of each sensation.

3.7.5.2. Profile of Mood States

The profile of mood states (POMS) was administrated at pre- and post-time points and assessed by requiring participants to rate their feelings on a 5-point Likert scale over the course

of 65 adjectives. The values of certain adjectives in the POMS are summed together to create values for 6 different domains: tension, depression, anger, fatigue, confusion, and vigor. A total mood disturbance score was also calculated by adding the values of all negative domains (tension, depression, anger, fatigue, confusion) and subtracting the vigor score (215). A constant of 100 was added to the total mood disturbance score to prevent a negative value.

3.7.5.3 Sleep Evaluation

Following day 1 of pre-testing, and approximately 3 days before post-testing measurements, participants were given an actigraphy watch (Fatigue Science Readiband™, Blaine, WA) to wear on their non-dominant wrist for 72 hours continuously. These data were used to assess sleep quantity and quality in their own environment (not in the laboratory). The outcome variables of this measure included total time sleeping, sleep latency (time in required to fall asleep in a resting state), and sleep efficiency (a measure of sleep quality, or the ratio of time spent asleep to the time spent in bed, including resting). The sleep efficiency measure is based on sleep latency and average number of wake episodes, with higher scores indicating better sleep quality (295). The actigraphy watch was chosen to measure sleep in favor of the more common method of administering the Pittsburgh Sleep Quality Index (PSQI), as previous studies have found that the PSQI does not correlate well with polysomnography (gold standard measure of sleep), while actigraphy watches have been validated against polysomnography (187, 223).

3.7.6 Nitrogen Balance

Nitrogen balance was analyzed at pre- and post-intervention testing points. After the day 1 visit, and after the taper session of week 12, participants were provided with a 3,000 mL 24-hour urine collection jug (VWR international, Radnor PA). Participants were instructed to store the urine collection jug in the refrigerator when not in use. After submission of the urine jug,

total volume (mL) was recorded and mixed via hand-inversion and aliquoted into two, 15-mL Falcon® tubes (Corning Inc., Corning, New York). Thereafter, samples were frozen in an -80° C freezer for later analysis using commercially available colorimetric detection assays (Thermo Fisher Scientific Inc., Carlsbad, CA). All assays were performed in duplicate. Intra-assays coefficients of variation (CV) were 1.24%, 2.19% and 1.60%. Nitrogen balance (NBAL) was calculated using the equation: NBAL = [(protein intake (g)/6.25) - ((Urine urea nitrogen (g) + 5 mg/kg body mass (fecal loss of nitrogen) + 2 (miscellaneous loss of nitrogen)] (165).

3.7.7 Insulin-like Growth Factor 1

To assess changes in free IGF-1, a venous blood sample (9 mL total) was collected from an antecubital vein into vacutainer tubes (Greiner Bio One International, NC) for plasma (EDTA) in the morning following an overnight fast on pre- and post-testing time points. Plasma was subsequently centrifuged at 1,000 g for 15 minutes at 4°C and stored in -80°C freezer for later analysis using commercially available ELISA kits (R&D Systems Inc., Minneapolis, MN). All assays were performed in duplicate. Intra-assays coefficients of variation (CV) were 6.71% and 5.52%, and the inter-assay CV was 13.07%. Intra-assay CVs were required to be less than 15% to be included in analysis.

3.8 Sample Size Justification and Statistical Analysis

An a priori analysis was conducted with an effect size F of 0.25 calculated using mean and standard deviations from previously reported changes in LBM in older men (350) with power set to 0.8 and α set to 0.05 for 3 groups and 3 measurements. With an expected dropout rate of 15% (110), a sample size of 36 was adequate and provided an even distribution between groups.

A one-way analysis of variance (ANOVA) was used to analyze baseline differences and total training volume among groups. A 3x2 mixed model ANOVA with group as the between subjects' factor (Post-ex, Pre-sleep, Ex only) and time as the within-subjects factor (pre and post) was performed on all dependent variables except for muscle thickness, 1-RM strength, muscle power, and appetite, where a 3x3 mixed model ANOVA (Post-ex, Pre-sleep, Ex only x pre, mid, post) was used. Pearson product-moment correlation, or Spearman's correlation in the event of normality violation, was used to determine the relationship between changes in free IGF-1 and changes in body composition, muscle thickness, 1-RM strength, muscular power, and cognitive assessments.

Outliers were removed and defined as values outside of the mean ± 3 standard deviations. Data were tested for normality (Shapiro-Wilk), homogeneity of variances (Levene's test), and sphericity (Mauchly's test) where appropriate. When normality or homogeneity of variances was violated, non-parametric testing or data transformation was performed. For a mixed model ANOVA, if transformation could not correct the violation, original data were analyzed, as there are no non-parametric alternatives. When sphericity was violated, the main time and group x time interactions were interpreted with the Greenhouse-Geisser estimation.

Significance was set at $p \leq 0.05$. Trends were noted for p-values between 0.051-0.10. For significant interactions and main effects, pairwise comparisons with Bonferroni adjustments were conducted (296, 332). Pearson correlation coefficients (r) or Spearman's correlation coefficients (r_s) were interpreted as "negligible" = < 0.30, "low" = 0.30–0.50, "moderate" = 0.50–0.70, "high" = 0.70–0.90, and "very high" = > 0.90 (244). Percentage change was calculated as ((post value – pre value) * 100). All analyses were performed with SPSS version 25

(IBM Corp., Armonk, NY) and GraphPad Prism version 8.1.1 (GraphPad software, San Diego, CA). Data are presented as mean values ± standard deviation, unless otherwise stated.

APPENDIX C

INFORMED CONSENT

FLORIDA STATE UNIVERSITY

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Study Title: Efficacy of Nighttime Protein Feeding during 12-week Resistance Training on Functional and Cognitive Adaptations in Older Adults.

Principal Investigator: Alex Klemp, M.S.

Introduction

We invite you to take part in a research study at Florida State University.

First, we want you to know that:

Taking part in research is entirely voluntary**.**

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of research procedures they would want to receive. If you have such beliefs, please discuss them with the research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone on the FSU research team, or with family, friends or your personal physician or other professional.

FSU Human Subjects Committee approved on 10/30/2018, void after 9/11/2019. HSC #2018.25996

FLORIDA STATE UNIVERSITY

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Study Title: Efficacy of Nighttime Protein Feeding during 12-week Resistance Training on Functional and Cognitive Adaptations in Older Adults.

Principal Investigator: Alex Klemp, M.S.

Why is this study being done?

This study is being conducted by Alex Klemp, MS (Principle Investigator) and Dr. Jeong-Su Kim (Co-Principle Investigator), who are researchers at Florida State University in the Department of Nutrition, Food and Exercise Science. It is funded by International Society of Sports Nutrition and Dymatize®, who is the manufacture of protein and placebo supplement.

The purpose of the study is The purpose of the study is to examine the effects of consuming protein either immediately after exercise or before sleep during resistance exercise training on functional and cognitive adaptations in older adults. If you agree to participate, you will be randomly assigned to one of three groups for this 12-week intervention, all of which will complete resistance exercise training 2 times per week. The three groups are: 1) Post- exercise protein group (consume protein immediately after each exercise session and placebo before sleep); 2) Nighttime protein group (consume placebo immediately after each exercise session and consume protein before sleep); or 3) Exercise only group (No protein or placebo).

Why are you being asked to take part in this study?

You are invited to participate in a research study examining the effect of protein timing during resistance exercise training on functional and cognitive adaptations. You were selected as a possible participant because you are between the ages of 60-75 years old, without chronic diseases, and not currently participating in a regular structured exercise program. We ask that you read this form and ask any questions you have before agreeing to be in the study.

FSU Human Subjects Committee approved on 10/30/2018, void after 9/11/2019. HSC #2018.25996

FLORIDA STATE UNIVERSITY

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Study Title: Efficacy of Nighttime Protein Feeding during 12-week Resistance Training on Functional and Cognitive Adaptations in Older Adults.

Principal Investigator: Alex Klemp, M.S.

How many people are expected to take part in this study?

Thirty-six healthy males between the ages of 60-75 will be recruited for this study.

Before you begin the study

We will complete a health history questionnaire and a Montreal cognitive assessment test to ensure you are eligible to participate.

Study Procedures

If you agree to participate in this study, there will be three testing time points; pre-intervention, mid-intervention, and post-intervention that will take place over a 2 to 3 day period. Day one of pre-intervention testing will take place in the Body Composition Lab at Florida State University in the Sandels Building. We will review and sign the informed consent document, complete health and physical activity questionnaires, and measure resting blood pressure and heart rate, along with your height and weight. Thereafter, we will collect a blood sample (about 9 mL or 2 teaspoons) from your arm to measure blood markers that may influence muscle growth (i.e., insulin-like growth factor-1, cortisol, and interleukin-6). Then we will complete physiological questionnaires (Montreal Cognitive Assessment, Appetite and Profile of Mood States) and cognitive assessments (Digit Symbol Substitution Task, Pattern Comparison). Lastly, we will complete several physical function tests including the short physical performance battery (balance, walking speed, and strength), along with muscular power (repeated sit-to-stand tasks). At the conclusion of day one, you will be given several items to return on day 3 including: a 3-day food record, an actigraphy watch to wear during sleep, and urine collection jug. The

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first day should take approximately one and half hours. Day two will take place between 3-4 days after day one and we will measure your body composition (bioelectrical impedance analysis (BIA), muscle thickness (Ultrasound) and complete maximum strength assessments in the leg press and chest press exercise. Finally, day 3 will take place 5-7 days after day 2 and we will reassess maximum strength in the same exercises and you will return the food record, watch, and urine jug. Mid- (week 6) and post-intervention (week 12) testing will be completed in a similar manner, except for retesting maximum strength, and therefore, and will only require 2 days.

After the pre-intervention testing days you will be assigned a group, and we will begin a 12-week resistance exercise training program (two days per week, on non-consecutive days, separated by at least 72 hours). This exercise program is designed to increase your whole body muscle strength and mass. We anticipate that each session will last approximately one hour. Then, depending on which group you are assigned, you may consume a 'shake' immediately after exercise and about 30 minutes before sleep for the entire 12 weeks. On days you do not exercise, you will take the shakes around the same time of day. Additionally, we will measure your resting blood pressure and heart rate before the first exercise session of each week.

Risks of Study Participation

The study has a minimal level of risk associated with testing procedures and resistance exercise training. For the testing procedures, the blood draw may cause fainting, some mild bruising of the arm, and infection (if puncture site is not kept clean). Additionally, you may experience some mild muscular soreness, discomfort, irritation or muscle strain from the exercise; however, this should subside during the intervention. To minimize the

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potential risk, all research personal assisting with this project will be trained and experienced with all testing and interventional procedures and will be utilized with caution.

Benefits of Study Participation

Participation in this study will provide you with several benefits including the information of: your body composition (lean & fat mass), cognitive and physical function, and muscular strength. Additionally, you will also receive 12 weeks of free personalized, supervised resistance exercise training. All of the benefits will be included free of cost.

Study Costs/Compensation

Following the completion of the post-intervention assessments, you will be compensated $100 for your time and inconveniences. If you for any reason (e.g., injury, illness, and drop out) you cannot complete the study, you will not receive any compensation.

Research Related Injury

In case of an injury, the laboratory personnel working on the research project will provide you first aid. Any other treatment or care will come at your own expense.

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Confidentiality

The records of this study will be kept private and confidential, to the extent allowed by law. In any publications or presentations, we will not include any information that will make it possible to identify you as a subject. Your record for the study may, however, by reviewed by departments at the University with appropriate regulatory oversight. Confidentiality will be maintained by assigning each subject a code number, with all data being recorded by that specific code number. The only record with the participants' name and code number will be kept in a locked drawer in the PI's office. Data will be kept for 10 years and then destroyed.

Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with the University. [Indicate any other cooperating institutions]. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Contacts and Questions

The researchers conducting this study are Alex Klemp, MS (Principle Investigator) and Dr. Jeong-Su Kim (Co-Principle Investigator). You may ask any questions you have now, or if you have questions later, you are encouraged to contact them. Alex Klemp can be reached by phone at [redacted](cell phone) or [redacted](office) or email [redacted]. Dr. Jeong-Su Kim may be contacted via telephone [redacted](office) or by email [redacted]

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If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the FSU IRB at telephone number 850-644-7900. You may also contact this office by email at humansubjects@fsu.edu, or by writing or in person at 2010 Levy Street, Research Building B, Suite 276, FSU Human Subjects Committee, Tallahassee, FL 32306-2742.

You will be given a copy of this form for your records.

Statement of Consent

I have read the above information I have asked questions and have received answers. I consent to participate in this study.

_____ _____
Signature of Subject Date

_____ _____
Signature of Investigator Date

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