The Effect of Nighttime Macronutrient Choice and Exercise Training on Body Composition, Strength, Cardiovascular Health, Resting Metabolism, and Appetite in Overweight and Obese Adults

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THE EFFECT OF NIGHTTIME MACRONUTRIENT CHOICE AND EXERCISE TRAINING
ON BODY COMPOSITION, STRENGTH, CARDIOVASCULAR HEALTH, RESTING
METABOLISM, AND APPETITE IN OVERWEIGHT AND OBESE ADULTS

By

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This thesis is dedicated to my wife and my late grandfather, William V. Eddy, for whom I credit my drive and ethical grounding.
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ABSTRACT

Background: Nighttime eating is often associated with metabolic syndrome and poor body composition and these conditions may be influenced by the natural decline in metabolism that occurs during sleep. However, only limited research has been conducted to determine the role of individual macronutrients at night. Previous research indicates that protein consumption increases metabolic rate more than carbohydrates or fat, and therefore may attenuate this decline when consumed at night before bed. In addition, digestion and absorption kinetics of a fast protein such as whey protein (WP) and a slow protein such as casein protein (CP) may independently influence appetite and body composition. Therefore, nighttime eating may be a window of opportunity to influence changes in body composition, strength, cardiovascular health, metabolism and appetite (hunger, desire to eat, and satiety). Purpose: To compare the effects of isocaloric maltodextrin placebo (PLA), WP and CP supplements when consumed immediately prior to nocturnal sleep when combined with four weeks of exercise training on body composition, strength, fasting glucose and lipid profiles, metabolism and appetite. Methods: Fifty-nine sedentary, overweight and obese participants were recruited and had baseline measurements of body composition (dual energy X-ray absorptiometry (DXA)), resting metabolism (ParvoMedics TrueOne 2400 metabolic cart), strength (1RM Chest- and Leg-press), blood glucose and lipid profiles (Cholestech LDX Analyzer) and appetite questionnaires (visual analogue scale) taken after an overnight fast (0600-0900 h). Forty-eight participants completed the four-week study protocol. The participants were randomly stratified by % body fat, BMI, and gender to one of three groups: PLA (n= 14, men: 4, BMI= 34.4 ± 1.5 kg/m², age= 28.1 ± 1.8 years), WP (n= 17, men: 3, BMI= 34.3 ± 1.3 kg/m², age= 30.1 ± 1.6 years), CP (n=17, men: 3, BMI= 35.4 ± 1.3 kg/m², age= 30.1 ± 1.6 years) in a double blind design. Participants were then instructed to consume their supplement at least two hours after dinner and no more than 30 minutes before bed each night for four weeks. All participants attended supervised exercise sessions (3x/week; 2
days of resistance exercise and 1 day of high-intensity cardiovascular exercise). Post-testing occurred 36-60 hours after the last supplementation and 96-144 hours after the final training session. A one-way ANOVA was performed to examine possible group differences at baseline and differences in change among groups. A two-way ANOVA with repeated measures was used to evaluate changes in dependent variables over time ([pre x post] x [PLA x WP x CP]). A Tukey test was used for post hoc comparisons. Values are reported as means ± SEM. Significance was accepted at $P<0.05$. **Results:** Eleven participants who completed baseline measurements failed to complete the four-week protocol and maintain satisfactory compliance with exercise and supplement intake (< 80% compliance). With the exception of fasting glucose, no significant group differences existed at baseline. There were no group x time interactions for resting metabolic rate (RMR), hunger, satiety, desire to eat, fat mass, lean body mass, BF%, or weight, although RMR displayed a trend ($P=0.0559$) towards significance with the PLA group decreasing by $74.3 \pm 94.5$ kcal/day and WP and CP increasing by $235.7 \pm 84.5$ kcal/day and $51.7 \pm 79.4$ kcal/day, respectively. Additionally, there was a group x time effect for $VO_2$ with WP increasing by $0.3 \pm 0.1$ ml/kg/min compared with a decrease of $0.1 \pm 0.1$ ml/kg/min and an increase of $0.1 \pm 0.1$ ml/kg/min for PLA and CP, respectively. Significant time effects were measured for satiety (pre: $31.5 \pm 2.3$ mm, post: $40.6 \pm 2.3$ mm, $P<0.008$) and lean body mass (LBM) (pre: $51.8 \pm 0.1$ kg, post: $52.3 \pm 0.1$ kg, $P<0.0001$). **Conclusion:** In conclusion, our data indicate exercise three times per week for four weeks combined with nighttime eating can be a successful method for improving LBM, BF%, strength, and satiety in previously sedentary, overweight and obese individuals. Additionally, four weeks of nighttime WP supplementation may elevate $VO_2$ 36-60 hours after the last supplementation.
CHAPTER ONE

INTRODUCTION

Obesity is an epidemic in the United States with over half of all adults now reported as overweight or obese (45). Nighttime eating is often associated with poor body composition including obesity (47, 82, 119). However, only limited research has been conducted to determine the role of individual macronutrients at night. Therefore, nighttime eating may be a possible window of opportunity to improve health, body composition, and performance.

Previous research indicates that metabolic rate decreases during sleep (83) and that protein supplementation may attenuate this decrease more than other macronutrients. Indeed, others have even demonstrated an increase in muscle protein synthesis overnight after nighttime protein ingestion (54, 114). It has been reported that the addition of protein supplementation to a normal diet can reduce body weight and improve body composition (13). Protein consumption is also known to increase resting metabolic rate (RMR) and decrease respiratory quotient (RQ) in the immediate post-prandial period (2). Similarly, protein consumption is known to have satiating properties compared to carbohydrate consumption (2, 75, 109). Due to this relationship, altering the type of protein or macronutrient consumed late at night when starting an exercise training program may influence changes in resting metabolic rate (RMR), appetite, and body composition. It is suggested that the differing absorption and digestion kinetics of whey protein (WP) and casein protein (CP) are responsible for the different biological effects of these proteins (18). Based on this, it is of interest to determine the comparative efficacy of WP, CP, and a carbohydrate placebo (PLA) when consumed immediately prior to nocturnal sleep on changes in RMR, appetite, and body composition associated with exercise training in previously sedentary individuals.

Interestingly, CP and WP have different physiological effects but similar amino acid composition (18, 113, 132). CP and WP are digested and absorbed at different rates and
absorption rates are known to be independent regulating factors for the physiological effects of protein (18, 94). Plasma amino acid availability is one of the main factors regulating muscle protein oxidation (53). CP has been reported to display prolonged amino acid absorption and thus prolonged hyperaminoacidemia compared to WP, commonly attributed to its clotting nature in the stomach (18, 90, 94) and opioid content (beta-casomorphins) (37). Due to this, CP has been reported to inhibit muscle protein oxidation for a longer period compared to WP (18). Adding importance to this is the fact that protein catabolism has been reported to be accelerated at night due to the extended length of time between meals (77). Supporting this notion is the observation that CP can result in a greater post-prandial leucine balance when compared to WP when matched for leucine content (18, 35). An additional effect attributed to CP’s clotting nature is increased satiation and reduced appetite compared to WP (5, 75), however, not all agree (138). In addition, Res et al. recently reported that protein consumption before sleep results in elevated muscle protein synthesis (MPS) compared to a placebo in healthy young men (115).

Therefore, it is plausible that consumption of protein at night before sleep may positively impact body composition, metabolism, performance, and appetite. Moreover, it is likely that differences will exist between WP and CP consumption at night before sleep in overweight and obese individuals and when combined with exercise training.

**Purpose**

The present study seeks to elucidate the differential effects of four weeks of micellar CP, WP, or PLA consumed at nighttime immediately prior to sleep and combined with exercise training on changes in body composition, strength gains, blood lipids, glucose, metabolism and appetite measured at least 36 hours after cessation of the intervention.

**Research Aims**

The present study was conducted using 48 previously sedentary, overweight and obese individuals who began a four week exercise training program in combination with the consumption of either a slow protein (CP), fast protein (WP), or carbohydrate (PLA) snack immediately prior to sleep. Our research aims were as follows:
1. To determine changes in lean body mass (LBM), fat mass (FM), body fat percent (BF%), and total body mass as measured by dual energy x-ray absorptiometry (DXA).

2. To assess the increase in maximal strength as measured by the maximal weight successfully lifted one time (1RM) during the chest- and leg-press. An increase in strength is a direct measurement and a tangible benefit of supplementation.

3. To identify any changes in fasting lipids or glucose that occurs as a result of the supplements or training protocol.

4. To measure resting metabolic rate, oxygen consumption (VO$_2$), and respiratory quotient (RQ).

5. To assess the impact of nighttime supplementation and training on fasting appetite.

**Research Hypotheses**

The hypotheses of the present study included:

1. All groups will report an improvement in LBM, FM, and BF% after four weeks of exercise training and supplementation compared with baseline. The CP group will show a greater increase in LBM compared with WP or PLA. CP supplementation has been shown to result in a more positive nitrogen balance than WP and this will be reflected with an increased LBM. The PLA group will have the least positive change in FM and BF%.

2. Strength will increase longitudinally in all groups with the CP group having the greatest increase, followed by the WP group, and lastly the PLA group.

3. There will be improvements in the fasting serum blood lipid profile and fasting serum glucose with the greatest improvement occurring in the protein groups.

4. All groups will report an increase in RMR and resting VO$_2$ and the protein groups (CP>WP) will show a greater increase. RQ will decrease in the protein groups (CP>WP) and have no change in the PLA group.

5. Appetite will decrease in the CP and to a lesser extent the WP group. No change will occur in the PLA group.
Assumptions

Assumptions for the present study include the following:

1. All participants will accurately report their age, activity level, medical history, dietary history, intake of the supplement, and will adhere to the conditions set forward in the Informed Consent Form.

2. All laboratory equipment will yield accurate and reliable results

Delimitations

The delimitations of the present study include the following:

1. Participants will be between 18 and 45 years old and will be overweight or obese (BMI ≥25), but otherwise healthy with no other pre-existing conditions.

2. Forty-eight sedentary, overweight and obese men and women aged 18-45 years from the greater Tallahassee, Florida area completed the study.

3. Participants were excluded for any of the following reasons: uncontrolled hypertension (Blood Pressure (BP)>160/100 mmHg), taking BP medications, diagnosed cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, milk allergies or any musculoskeletal complications (i.e., osteoarthritis or injury) that would impede exercising. In addition, exclusion criteria included being a heavy smoker (>20 cigarettes per day), taking cholesterol medication or nutritional supplements (except for a multivitamin), or partaking in planned exercise for more than 2 days per week for more than 40 minutes per session (within the past 6 months).

4. Participants were instructed to not alter their level physical activity or diet in any way, except for what was prescribed for the study.

5. Group assignment was randomized by BF%, BMI and gender.

Limitations

The limitations of the present study include the following:

1. Diets are self-selected.

2. Physical activity outside of exercise training is self-controlled.

3. Since the participants were previously untrained, increases in strength may be greater than what one would normally expect from a randomly chosen population.
4. The participants will be sedentary, overweight or obese volunteers recruited from one geographical area and thus may not accurately reflect the general population. Due to this they may have greater improvements in health than what occurs from an average person.

**Definitions of Terms and Abbreviations**

- **BCAA** – Branched chain amino acids. BCAA’s, leucine, isoleucine, and valine, are important in stimulating muscle protein synthesis (55).
- **BF%** -- Body Fat Percent.
- **CP** – Casein protein, the major protein component of bovine milk. Casein is a slow digesting and absorbing protein (18, 78).
- **DXA** – Whole body dual energy x-ray absorptiometry. The DXA scan entails two distinct X-ray energies passing through the participant (GE Medical Systems, Madison, WI). The manufacturer’s software then allows body composition to be determined (Encore 2006, version 9.1) (127, 151).
- **EAA** – Essential amino acids. The amino acids that the body cannot produce and must be provided by the diet. Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, histidine, and tyrosine (55).
- **Fasting** – A period of at least 8 hours without consuming any caloric food or drink.
- **FM** – Fat Mass.
- **LBM** - Lean body mass, an indirect measurement of skeletal muscle mass.
- **MPB** – Muscle protein breakdown.
- **MPS** – Muscle protein synthesis.
- **mTORC1** – A pathway that stimulates MPS by activating downstream S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) (67). Both S6K1 and 4E-BP1 drive eukaryotic translation and result in MPS.
- **PLA** - Placebo group.
- **REE** – Resting energy expenditure. The amount of calories theoretically utilized by the body in a 24-hour period at rest (149).
- **RMR** – Resting metabolic rate. The basal rate of energy expenditure required by the body when at rest which is typically expressed as calories per day (66, 149).
- **RQ** – Respiratory Quotient. The volume of CO₂ exhaled divided by the volume of O₂ consumed. An estimate of substrate oxidation, a value of 0.7 would signify beta oxidation supplying 100% of the body's energy whereas a value of 1.00 would mean carbohydrate is supplying 100% of the body's energy needs.

- **T/C Ratio** – Testosterone to cortisol ratio, an accurate predictor of the anabolic or catabolic state of the body.

- **VO₂** – The volume of oxygen utilized by the body for metabolism per minute. Typically expressed as a relative measurement ml/kg/min.

- **WP** – Whey protein. Whey is the liquid protein portion of bovine milk. Whey contains a greater amount of leucine than casein and is digested and absorbed more rapidly (18, 78).

- **1RM** – The maximal weight that can be successfully lifted during one repetition. The 1RM is the gold standard measure of muscular strength (6).
CHAPTER TWO

REVIEW OF LITERATURE

Dietary Supplements

In the United States, the sports nutrition and meal replacement industries are multi-billion dollar businesses ($2.9 and $2.7 billion) and are experiencing continued growth even in an economic downturn (5.4% and 3%) (1). One reason for the increasing popularity of dietary supplements is an attempt by the consumer to utilize nutritional interventions to improve body composition and health. Protein supplementation in particular is a large part of the dietary supplement industry and protein supplements are used as an ergogenic aid. Unfortunately, little scientific attention has been paid to distinguish the differential effects of varying protein types when consumed at night before sleep. Two of the most common types of protein supplements are the milk protein derivatives whey and casein.

Protein Supplementation

Muscle mass, measured as lean body mass (LBM), is a main determinant in resting energy expenditure (REE) and daily energy expenditure. Increased protein consumption in a normocaloric diet can be beneficial by reducing protein utilization while increasing fat oxidation (144). Additionally, increasing the relative amount of protein in the diet is associated with increased weight loss and an improvement in total and abdominal body composition (11, 43, 91, 105). One significant advantage with hypocaloric high protein diets is the sparing of LBM with concomitant fat loss (58). Maintenance of LBM will attenuate the reduction in REE that typically occurs with weight loss and subsequently will facilitate the maintenance of the reduced body weight. Additionally, protein consumption post-exercise results in an increased REE for 24 to 48 hours post-exercise compared to a carbohydrate supplement (58).

Despite the metabolic relationship between protein intake and REE, not all research has supported this relationship. Luscombe et al. compared diabetics on a hypocaloric high
or low protein diet. They reported no significant effects from the additional protein on weight loss, REE or the thermic effect of feeding (92).

**Micellar Casein vs. Whey**

Consumption of milk proteins has been shown to be superior to soy protein in stimulating muscle protein synthesis (MPS) (148). Bovine milk contains two complete proteins, micellar CP and WP. Micellar CP makes up ~80% and WP accounts for the other ~20% of the protein total of bovine milk (78, 94).

Protein forms can have different physiological effects based on their amino acid composition as well as digestion and absorption kinetics (18, 36, 118, 126). CP and WP contain slightly different amino acid profiles with WP having a higher leucine content than CP (11.77% vs. 8.77%, respectively) (18, 113, 132). Furthermore, the rate of digestion and absorption for a protein meal is dependent on many factors including gastric and intestinal motility, luminal digestion, and mucosal absorption (93).

Micellar CP is an acid insoluble protein that clots in the stomach and displays delayed gastric emptying compared to WP (18, 93, 110). CP results in a unique digestion pattern due to its lack of any appreciable secondary or tertiary structure making it rather hydrophobic and its isoelectric pH is 4.6 (90). Due to the hydrophobicity of CP and the need to solubilize the calcium phosphate found in milk, it exists as a colloidal particle known as a casein micelle (90, 110). This property causes CP to aggregate in the acidic environment of the stomach whereas WP remains in suspension (112). The compact curd formation of the CP results in a significant delay in gastric emptying compared to WP (18, 90, 94, 112). It is believed this occurs because the coagulation impedes digestion in the stomach, slowing micelle disintegration. Another contributory factor to CP’s delayed gastric emptying rate and increased transit time are beta-casomorphins which bind gastrointestinal opioid receptors (37). It is believed these naturally occurring opioid compounds must be absorbed from the intestines to have an effect, explaining the biphasic gastric emptying pattern associated with CP (37, 112).

Mahe et al. reported that consumption of 386.8 mmol of micellar CP resulted in a peak jejunal flow rate during the 20-40 minute post-prandial period and did not return to baseline until 140 minutes post-prandial (93). Additionally, it was reported that exogenous nitrogen, a marker of nitrogen absorption, first appeared within 20 minutes of
ingestion and remained stable during the 40-100 minute period, then slowly decreased during the 100-240 minute period (93). Delayed gastric emptying, as occurs with casein consumption, can result in a prolonged but moderate hyperaminoacidemia (18). Micellar casein should be differentiated from caseinate supplements such as calcium and sodium caseinate which have been previously solubilized. Due to this chemical modification, caseinates do not clot in the stomach, and are readily absorbed. Caseinate digestion and absorption markedly differs from casein and displays absorption kinetics more similar to WP (113).

WP is termed a fast protein due to its rapid digestion and absorption (18). WP is more water soluble than CP and thus its flocculent structure allows for more rapid gastric emptying which occurs in an exponential fashion (112) (90). Mahe et al. reported that after consumption of 368.2mmol of beta lactoglobulin, the primary protein found in whey, the jejunal flow rate peaked during the 20-40 minute post-prandial period and returned to basal levels after 60 minutes (93). Additionally, Mahe et al. reported exogenous nitrogen peaked in the 20-40 minute post-prandial period then decreased to basal levels during the 40-160 minute period (93). Consequently, WP results in a higher amplitude and shorter duration of plasma hyperaminoacidemia (18, 113, 132). WP contains a higher proportion of essential amino acids (EAA) than casein (~50.1% vs. ~46.5%) and as expected results in greater post-prandial plasma levels of EAA and leucine compared with casein (113, 132).

**Muscle Protein Synthesis (MPS)**

Transcription of many parts of the mammalian genome follows a 24-hour rhythmic pattern. Undulating gene expression with 24-hour periodicity has been noted in all human tissues but is most frequently studied in the suprachiasmatic nucleus (SCN), liver, and skeletal muscle (24, 96). Collectively, the area of the genome under circadian influence is known as the circadian transcriptome. This transcriptome is controlled by transcription of several key stimulatory and inhibitory gene products including the genes Clock, Cry, and Per and BMAL (152).

Exercise and feeding are the two major external influences on skeletal MPS and MPB, collectively referred to as turnover (49). Muscle metabolism, including turnover, glycogen synthesis and substrate utilization, displays a diurnal variation and often show increased anabolism during the evening (49, 136, 152). It is unknown if this is solely a
result of the increased rates of activity and feeding throughout the day or if photic input and the circadian transcriptome plays a role. If the latter is proved true, nutrient timing will become a vital component of nutrition counseling and recommendations.

The mammalian target of rapamycin complex 1 (mTORC1) cascade is a key regulator and activator of MPS. mTORC1 is a Serine/Threonine kinase that is responsive to nutrient levels and neuronal stimulation (67). The mTORC1 complex stimulates MPS by activating downstream S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) (25, 67). Both S6K1 and 4E-BP1 drive eukaryotic translation and result in MPS.

**Light Entrainment and Skeletal Muscle**

The SCN is the central circadian clock that influences other tissues and is reset daily by light exposure (25). The clock entrainment process is regulated by photic input which results in direct phosphorylation from the retinohypothalamic tract to the SCN (25). The signal is mediated by glutamatergic axons projecting from the retina onto the SCN (25, 65). Diffuse activation of the MAPK and mTOR pathways occurs throughout the SCN during the subjective day, or with light exposure at night (24, 25). MAPK and mTOR phosphorylation also lead to MPS in skeletal muscle and therefore one could speculate this would provide a direct link for light and muscle turnover. From the SCN, the light signal is passed to the pineal gland where it entrains the gland to the circadian cycle and stimulates melatonin biosynthesis. Melatonin release from the pineal gland is another postulated mechanism for central light exposure modulating skeletal muscle turnover as it is known that melatonin can potentiate testosterones inhibitory action on LH secretion (8).

Until recently, it was unknown how sunlight was able to directly stimulate melanocytes or if sunlight had any acute (immediate) peripheral effects. It was believed that sunlight played a direct role but the mechanism was unknown. To confound this, it was thought that it took many hours to days for the skin (melanocytes) to begin production of melanin in response to photic stimulation. Recently it was discovered that melanocytes located throughout the dermis contain the photopigment rhodopsin (146). It is now known that within minutes of exposure to ultraviolet A (UVA) rays melanocytes begin calcium- and retinal- dependent production of melanin (146). The significance of this in
relation to muscle turnover is unknown but this could provide a direct mechanism for peripheral light exposure directly affecting muscle turnover.

**The Molecular Clock in Skeletal Muscle**

The rate of muscle turnover is heavily influenced by circadian behaviors such as exercise and feeding (49), however, it may also be regulated by direct circadian transcriptional regulation (152). The circadian transcriptome is made up of a highly conserved gene regulatory network composed of transcriptional-translational feedback loops (152). The circadian transcriptome in skeletal muscle is essential for proper muscle structure and function (9, 99, 152). It has been shown that the circadian transcriptome in skeletal muscle accounts for ~10% of the entire genetic transcriptome (99). The positive arm of the core clock genes includes *CLOCK* (circadian locomotor output control kaput) and *BMAL1* (brain muscle arnt-like 1) (152). The core clock genes responsible for the negative feedback portion include *PERIOD* (*PER1, PER2, PER3*) and *CRYPTOCHROME* (*CRY1* and *CRY2*) (152). Several other muscle specific genes display circadian expression patterns including *MYOD1* (Myogenin), *ATROGIN1*, and *MYH1* (myosin heavy chain IIX) (152). In addition to regulation by transcription factors, acetylation and deacetylation displays a rhythmic timing and thus plays a role in the molecular control of the circadian rhythm (69).

The genes in the circadian transcriptome are responsible for a multitude of tasks including transcription, protein degradation, lipid metabolism, ion transport, and vesicular trafficking (96). To more accurately determine the group of genes under circadian control Miller et al. looked at *CLOCK* mutant mice and reported that the *CLOCK* mutation significantly inhibited cell growth and proliferation (99). Andrews et al. reported that disruption of the circadian transcription genes *CLOCK* or *BMAL1* resulted in mice with a ~30% reduction in normalized and single fiber maximal force (9). It was observed that *CLOCK* and *BMAL1* are stimulatory to transcription of myogenin, or *MyoD*, a master regulator of myogenesis, and thus a circadian rhythm for both mRNA and protein levels of myogenin occur (9). Additionally, the *CLOCK* and *BMAL1* mutant mice showed decreased expression of actin, myosins, titin, and a 40% reduction in mitochondrial volume along with abnormal mitochondrial morphology (9). In another study, *CLOCK* mutant mice had decreased expression of skeletal actin, troponin, titin, dystrophin, and myosin heavy chain mRNAs (96). In addition to the known effects of clock genes on the circadian
transcriptome, the clock genes influence the non-circadian genome. McCarthy et al. reported that 35% of the non-circadian genome in CLOCK knockout mice was disrupted as a result of a CLOCK mutation (96).

**Contraction Induced MPS**

Resistance exercise (RE) increases the rate of MPS 40-100% above resting levels (42, 133). Despite a concurrent elevation in MPB post-exercise, resistance exercise results in a positive shift in net protein balance for 48 hours post-exercise (15, 107). RE induced increases in MPS are dependent on activation of the mTORC1 pathway (42). In the presence of a potent mTORC1 inhibitor, rapamycin, contraction induced MPS is attenuated by 40% (42). mTORC1 phosphorylation occurs within minutes of an anabolic stimulus such as RE (19) and lasts at least four hours post-stimulation (42).

The size of involved muscle mass, intensity, volume, and rest intervals when performing RE determines the magnitude of the ensuing anabolic response. Higher intensities, volumes, and shorter rest intervals result in a greater anabolic response (88).

**Feeding Induced MPS**

MPS is influenced by extracellular amino acid concentrations and more specifically by plasma concentrations of EAA (17). In the fasted state, as seen during nocturnal rest, MPS is severely attenuated and MPB is accelerated (85). The mTORC1 pathway is essential for feeding induced MPS (40, 128). After protein ingestion 4E-BP1 is activated via mTOR mediated stimulation and the fractional synthetic rate (FSR) increases (85). Elevated plasma levels of the branched chain amino acids (BCAAs), specifically leucine, are essential for feeding induced MPS (21, 84, 106, 117). Plasma leucine levels can even determine the rate of MPS independently of hyperaminoacidemia (35, 116). Feeding induced MPS displays a dose dependent relationship with maximal stimulation occurring with a protein bolus of 20 grams (17, 100). Additionally, post-prandial protein synthesis is influenced by digestion and absorption kinetics of the protein source along with the absolute quantity and quality of protein consumed (106, 133).

WP is superior to CP with respect to increasing the post-prandial muscle protein FSR and total protein accretion both after exercise and at rest (106, 113, 132). Seemingly contradictory to this, CP ingestion results in a greater post-prandial leucine balance (18,
This might occur because WP induces a larger but transient increase in extracellular amino acid concentration while CP has a smaller but sustained effect.

**Time-of-Day Effects for Strength and Metabolism**

Muscle strength and exercise performance show a significant correlation with time of day (152). Gauthier et al. compared the maximal voluntary torque and electromyographic (EMG) activity across seven time points from 0600 to 2400 and reported peak torque was observed in the late afternoon at 1800 (50). An EMG was utilized to determine whether the torque variations were due to central neurological or peripheral muscular changes. The authors reported no changes in the EMG activity which suggests alterations in maximal torque are likely a result of peripheral or muscular alterations. Multiple other authors have reported maximal strength values that are ~5-9% higher in the evening compared to other times of day (14, 26, 111). Despite the paucity of evidence for this, some have observed decreased maximal torque in the evening when measuring humans (56, 57). This has been explained by suggesting daily activity may cause the muscle groups tested to be in a higher fatigue state (56). Regardless of the change, it is accepted that time of day imparts a significant effect on strength and should have been controlled for.

Muscle metabolism displays a diurnal variation with increased insulin sensitivity in the morning despite no change in maximal insulin stimulated glucose uptake (152). This suggests the change may be insulin receptor mediated without a measurable concomitant change in the muscle’s intrinsic metabolism. Additionally, lipoprotein lipase activity and RQ are affected by the day/night cycle and show increased fat oxidation during the light periods in both rats and humans (136, 152). This points to a change in muscle metabolism based on the lighting and time of day and not simply feeding or exercise. Additionally glucose metabolism has been shown to display a diurnal rhythm that is dependent on the SCN with increased plasma glucose levels and glucose uptake prior to physical activity (89).

**Protein, Time of Day, and the Endocrine Response**

**Testosterone**

Testosterone is known as both an anabolic and anti-catabolic hormone (52). Testosterone is thought to stimulate hypertrophy by activating satellite cells and increasing the number of myonuclei (79). This leads to an increased ability of the muscle to
synthesize proteins (125). Testosterone’s anti-catabolic properties are mediated by inhibiting transcription of the stress induced mTOR inhibitor, regulated in development and DNA damage response 1 (REDD1) (38, 143).

Testosterone secretion is controlled by the hypothalamic-pituitary-testicular (HPT) axis and is secreted from the Leydig cells of the testes. Once released into the circulation, 95% of testosterone is inactivated by binding to sex hormone-binding globulin (SHBG). The remaining biologically active testosterone either non-specifically binds albumin with a low affinity or remains free (140). Testosterone is a steroid hormone that binds cytoplasmic androgen receptors (AR). The bound receptor complexes then bind to a specific region of the DNA called the hormone response element (HRE) and thereby increase rates of transcription. The AR content of skeletal muscle is also an independent factor with respect to MPS. Skeletal muscle AR content is increased in glycolytic fibers and with exercise training (88).

Testosterone secretion is controlled by the hypothalamic-pituitary-testicular (HPT) axis and is secreted from the Leydig cells of the testes. Melatonin, which is released at night, indirectly inhibits the release of testosterone at the level of the hypothalamus (59). Testosterone displays diurnal variation with the highest levels occurring around 0700 and the lowest levels in the late night around 2300 (68, 81, 145). This suggests skeletal muscle would be primed for synthesis early in the day and resistant to MPS late in the night. Testosterone levels are highly correlated with the improvements typically seen with both resistance and endurance training (3). RE results in an acute increase in total and free testosterone in men (60) but the response is equivocal in women (60, 142). This response is likely affected by a reduction in plasma volume, adrenergic stimulation, lactate-stimulated secretion and Leydig cell adaptations (88). Testosterone concentrations at rest reflect the current state of the skeletal muscle with recent training resulting in elevated levels (88). For this reason chronic exercise does not affect resting testosterone levels in men or women (4, 62, 88). Additionally, protein supplementation does not reliably result in an increase in post-exercise or fasting testosterone concentrations (27, 80).

**Cortisol**

Cortisol is a metabolically active catabolic glucocorticoid released from the zona fasciculata of the adrenal cortex (88). The primary role of cortisol is to increase blood
glucose levels by stimulating gluconeogenesis and glycogenolysis and altering lipid and protein metabolism (33). Cortisol stimulates hormone sensitive lipase (HSL), mobilizes free fatty acids, and releases amino acids by proteolysis with degradation preferentially occurring in glycolytic muscle fibers (41, 68, 88). In addition to stimulating the mobilization of fuels, cortisol has been shown to inhibit protein synthesis by upregulating the mTOR inhibitor REDD1 (68, 143). Cortisol release occurs through activation of the hypothalamic-pituitary-adrenal axis (88). Cortisol displays a rhythmic pattern of release with the highest levels occurring around 0800 and the lowest levels at 2400 (68, 123). Additionally, cortisol is released during periods of fasting, stress, and intense exercise in both men and women (87)(86). This could account for the increased rate of MPB seen in the early morning.

Cortisol binds the HRE in a manner similar to other steroid hormones. In circulation, 10% of cortisol is free, 15% is bound to albumin and 75% is bound to corticosteroid-binding protein (CBP) (88). Cortisol release occurs through activation of the hypothalamic-pituitary-adrenal (HPA) axis (88). Cortisol is released during periods of fasting, stress, and intense exercise in both men and women (86, 87).

Training regimens that elicit the greatest metabolic response induce the largest cortisol release (61, 88). The cortisol response associated with exercise can be attenuated by consuming carbohydrates and EAA, either alone or in combination before and during an exercise bout (16, 134). It appears that chronic exercise does not affect resting cortisol levels although it does result in an increase in adrenocorticotropic hormone with a concomitant decrease in the number of glucocorticoid receptors (33, 88). Overtraining is an exception to this as it increases cortisol (88).

Testosterone/Cortisol (T/C) Ratio
The T/C ratio represents the anabolic to catabolic ratio of skeletal muscle and is an accurate predictor of the anabolic response to exercise (68). The T/C ratio is useful because skeletal muscle mass is determined by the net balance between synthesis and breakdown. The T/C ratio reflects this net balance. In addition to this, the T/C ratio is a marker of training status which allows it to also function as a useful indicator of overtraining (88). During periods of overtraining, or multiple consecutive weeks of
overreaching, testosterone is suppressed and cortisol is elevated (88). This shift results in a decrease in the T/C ratio.

**Insulin**

Insulin is an anabolic hormone that promotes positive nitrogen balance, inhibits proteolysis and stimulates muscle protein accretion in addition to having vasodilatory properties. Insulin is required for maximal stimulation of MPS induced either by amino acid ingestion or muscle contractions (85). Without an increase in insulin levels, feeding induced MPS is almost completely absent (85).

Insulin’s anabolic actions are primarily mediated by phosphorylation of the tyrosine kinase insulin receptor (IR) itself and the downstream insulin receptor substrate (IRS) signaling proteins (98, 120). This activation results in stimulation of the mTORC1 pathway and the downstream mitogenic enzymes (85, 98, 120, 124).

Insulin is the only hormone that directly lowers blood glucose levels and is secreted by pancreatic beta cells in response to elevated levels of glucose or BCAA’s. Insulin has been shown to suppress the release of phenylalanine by 42% and leucine by 50% and simultaneously stimulates protein synthesis independent of extracellular amino acids (51). WP ingestion has been reported to result in mild post-prandial and fasting hyperinsulinemia while micellar casein has no effect (35, 72, 97). Consumption of either a 6% carbohydrate solution or EAA during RE results in increased post-exercise insulin concentrations in men (16).

**Growth Hormone**

Somatotropin, or growth hormone (GH), is an anabolic protein hormone that is released from the anterior pituitary in a pulsatile fashion (147). In addition to its direct effects, GH mediates its anabolic effects by utilizing insulin like growth factors (IGF’s) as second messengers. Together, GH and IGFs are responsible for many of the growth promoting effects associated with exercise on skeletal muscle (48). Once released, GH binds to hepatic GH receptors and stimulates the release of IGF-1. Portal insulin levels directly result in increased IGF secretion (48). It is thought that insulin acts to sensitize the liver and results in increased IGF production from the GH stimulus (48). IGFs stimulate MPS by activating multiple downstream mechanisms including mTORC1 (139).
The majority of circulating GH is bound to growth hormone binding proteins (GHBP) to delay degradation and increase the half-life. GH binds GHBP by binding to hepatic GH receptors. The extracellular domain of the receptor is then separated by proteolytic cleavage to allow the bound GH-GHBP complex to freely circulate (153). Similarly, IGF binds IGF binding proteins (IGF-BP) when in circulation (48).

RE results in a transient increase in GH within 10 to 20 minutes of the onset of exercise, and lasts approximately 30 minutes after exercise cessation in both men and women (48, 86, 88, 101, 147). Following this release, GH release is reduced for the remainder of the 24-hour period following the exercise bout (147). This results in an unchanged 24 hour average GH concentration in response to a single exercise bout (147). The amplitude of the GH response to exercise increases with greater muscle mass involved, intensity, volume and shorter rest intervals (61, 88). Together these factors influence the metabolic properties of the RE and thus influence GH secretion in a manner similar to testosterone. One marker of the metabolic demand of an exercise bout, hydrogen ion accumulation, is suggested to be a determining factor for GH release (86). Takarada et al. reported that very low intensity exercise combined with vascular occlusion resulted in a 290-fold increase over resting values (131). Acute RE does not affect resting GH or resting IGF-1 concentrations although chronic RE has been shown to increase resting IGF-1 in men and women (48, 88). It has been reported that there is no acute change in IGF-1 concentrations during or after exercise (113), although others have reported a slight increase in IGF that is possibly due to the plasma volume reduction associated with intense exercise (48). The lack of an acute response is likely a result of the 3-9 hour delay in IGF-1 secretion that occurs after GH stimulation (86). Consumption of CP has no acute affect on IGF-1 but does increase fasting IGF-1 concentrations whereas whey has not been reported to have any effect (31, 72, 113).

**Protein Supplementation and Exercise**

Increasing protein intake and exercise training have both been reported to result in an increase or maintenance of LBM and an increase in strength in normal and overweight and obese individuals (7, 11, 80).

The timing of protein consumption with respect to exercise can have an enormous impact on physical adaptations (34). During exercise, skeletal muscle proteins are
catabolized to allow the release of amino acids, particularly leucine, to maintain or increase the free amino acid pool in the blood (20). For this reason the body is in a state of increased protein turnover post-exercise. Consuming protein during this sensitive period can have a much greater effect on muscle protein accretion compared to consumption at other times (44).

It has been reported that protein consumption immediately prior to and after RT results in increased mTOR activation (73). Another study found protein supplementation immediately following aerobic exercise significantly increased muscle fiber diameter of type I and II fibers when compared to isocaloric carbohydrate beverage (7). Esmark et al. compared the intake of protein immediately post-exercise with intake 2 hours post-exercise in elderly males (44). The authors report significant ergogenic benefits when protein is consumed immediately post-exercise. These benefits include an increase in cross sectional area and mean fiber area of the quadriceps femoris and an improvement in dynamic strength (44). Cribb et al. investigated the consumption of a protein-carbohydrate supplement immediately pre- and post- exercise compared to the same supplement at other times of day. The authors suggest that consumption of the supplement near the exercise time resulted in greater strength gains, type II muscle fiber hypertrophy as well as increases in intramuscular storage of creatine and glycogen in previously resistance trained males (34). Recently, Cockburn et al. reported that consumption of a milk based carbohydrate-protein supplement immediately post-exercise attenuated indices of exercise induced muscle damage to a greater extent than consumption of the supplement immediately pre-exercise (30).

Contradicting this precedent, Wycherley et al. concluded that the timing of protein ingestion relative to RE does not influence body composition, energy expenditure, and glycemic control or cardiometabolic risk factors in men and women with type II diabetes on a hypocaloric high protein diet (150). This study compared the effects of a protein meal consumed either immediately post-exercise or two hours post-exercise. These surprising results are likely due to the fact that the participants were sedentary, obese and type II diabetics so regardless of the timing of protein, dieting and exercise improved health indices substantially. Additionally, the participants were on a hypocaloric diet and may
have been in negative nitrogen balance. The results may have been different with healthy or trained participants or if total protein intake was increased.

**Conclusion**

In summary, CP, WP, and carbohydrate have been shown to influence body composition, strength, health, metabolism, and appetite differently. The influence of these when consumed in the late evening is largely unknown and warrants investigation. To elucidate these effects, we will look at changes in body composition, strength, blood glucose, lipid profile, metabolism, and appetite.
CHAPTER THREE

RESEARCH DESIGN AND METHODS

Participants

The study utilized sedentary, overweight or obese (BMI≥25kg/m²) but otherwise healthy men and women aged 18-45 years recruited from the greater Tallahassee, Florida area. Participants were recruited using flyers, Craig’s List, and a mass email to the university community. Participants were excluded from participation for the following reasons: uncontrolled hypertension, use of blood pressure or cholesterol medications, having diagnosed cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, milk allergies, pregnancy, or any musculoskeletal complications that would impede exercising, heavy smoking (>20 cigarettes per day) or taking nutritional supplements (except a multivitamin), or partaking in planned exercise more than two days per week for more than 40 minutes per session within the last six months. This study was approved by The Florida State University Institutional Review Board (Appendix A) and an informed consent document (Appendix B) was completed prior to participation in the study.

Experimental Design

The design was a randomized, stratified by BF%, BMI, and gender, placebo-controlled, double-blind protocol with participants assigned to one of three intervention groups for the duration of the four-week intervention (Figure 1).
All groups exercised three days per week (two resistance training (RT) days and one high-intensity interval training (HIT) day) for four weeks. Group one (whey protein, WP) consumed a WP beverage that contained 30g of WP, 3g of carbohydrate, and 2g of fat for a total of 150kcal per serving. Group two (casein protein, CP) consumed a CP beverage containing 30g of CP, 3g of carbohydrate, and 1g of fat for a total of 140 kcals per serving. Group three (placebo, PLA) consumed a placebo supplement with 0g of protein, 33g of carbohydrate, and 2g of fat for a total of 150kcal per serving. Other ingredients included small amounts of sodium, potassium, and calcium for consistency and flavoring. All groups were instructed to consume their assigned beverage every night as the last caloric food or beverage ingested before going to sleep and at least two hours after dinner and within one hour of sleep. While we cannot confirm the supplement was consumed two hours after dinner and no more than 30 minutes prior to sleep, all participants reported verbal compliance.

**Baseline Testing – Visit 1**

Participants arrived at the Human Performance Laboratory on two occasions. The first visit was for baseline data collection. The initial visit began with an informed consent and questionnaires regarding medical history (Appendix C), physical activity (Appendix D) and appetite (visual analogue scale) (Appendix G). During this visit the following measurements were made: height and weight (SECA scale, Tallahassee, FL), hip and waist
circumferences (Creative Health Products, INC; Ann Arbor, MI) brachial blood pressure (sphygmomanometer, Litmann, St. Paul, MN), heart rate, resting metabolic rate (RMR), oxygen consumption (VO₂), respiratory quotient (RQ) (ParvoMedics TrueOne 2400 metabolic cart, Sandy, UT), lipid and glucose profiles, and body composition (DXA). All results were blinded from the participant until the conclusion of the study.

Appetite was determined using a 100-mm visual analog scale (VAS) which required the participants to place a mark along a straight line 100 mm in length corresponding to how much they agreed to the question. The reproducibility and validity of the VAS method has been confirmed by Flint et al. (46). The VAS appetite questionnaire included questions on hunger, satiety, and desire to eat.

Blood pressure and heart rate were measured after the participants had been resting in a chair for at least five minutes. Blood pressure was determined by the auscultatory technique using an aneroid sphygmomanometer. Heart rate was determined by palpating the pulse at the wrist for 60 seconds. Measurements were duplicated on alternate arms with at least one minute between measurements. The value recorded was the average of two consecutive measurements no more than 12 mmHg apart for systolic blood pressure, 6 mmHg apart for diastolic blood pressure, and 5 beats per minute for heart rate.

Hip and waist circumferences were measured in centimeters using a Gulick fiberglass measuring tape with a tension handle. For the waist measurement, participants stood erect with the abdomen relaxed with the researcher positioned in front of the participant at eye-level to the waist. The tape measure was placed horizontally (parallel to the floor) around the smallest circumference of the torso, usually 1-2 cm below the last rib. Hip circumference was measured at the largest circumference around the hips or buttocks, above the gluteal fold, while participants were standing straight with their feet together and the researcher kneeling at the participant’s side, eye-level to the tape measure. All measurements were repeated until two measurements were no more than five mm apart at which point the average was recorded.

Metabolic measurements were performed in a dark and quiet climate controlled room with the participants in the supine position for 30 minutes with data collection occurring continuously for the last 20 minutes using the breath-by-breath method. The
participants breathed through a two-way non-rebreather mouthpiece which directed exhaled air into the gas chamber analyzer. A nose clip was used to prevent air from escaping. Prior to each metabolic test, the metabolic cart was calibrated to within 2% for flow (volume) and gas analysis using the recommended procedure. Participants were instructed to lie still and to not attempt to speak for the duration of the test.

A fasted venous blood sample (10mL) was collected from the antecubital region to determine baseline fasting glucose, total cholesterol (TC), triglycerides (TRG), high-density lipoproteins (HDL) TC/HDL ratio, low-density lipoproteins (LDL) and non-HDL cholesterol. Determinations of blood lipids and glucose were made using whole blood with a Cholestech LDX analyzer (Cholestech Corporation, Hayward, CA). Blood was collected under sterile conditions into a 6 ml serum vacutainer using a 23-gauge butterfly type needle. Immediately after collection, 40 μl of blood was pipetted into the Cholestech cartridge for analysis.

Body composition was determined using a DXA. Prior to commencing, a certified DXA operator briefed all participants on the risks of a DXA scan. Participants were instructed to remove all metal from their body and to lie in the supine position on the DXA scanner. A certified DXA operator properly positioned the participants and performed a total body scan. The results were analyzed using enCORE 2006 software, version 12.10. Scan results were immediately available and were used for determining stratified randomized group assignments.

After the completion of visit one, the participants were randomly assigned to either the CP, WP, or PLA group. The participants were given one week’s worth of supplement divided into daily servings in individual plastic baggies. At the end of each week the participants were instructed to return all used supplement baggies to measure compliance and seven new baggies were given out. For the remainder of the study the participants were instructed to consume the assigned supplement mixed with 12oz of water every night as the last caloric food or beverage prior to sleep, within 30 minutes of sleep, and at least 1-2 hours after their last meal.

**Exercise Training**

After baseline testing the participants completed two RT days and one HIT day per week for four weeks under the supervision of qualified instructors. One-repetition
maximum (1RM) for chest and leg press (Gold’s Gym, Tallahassee) were measured during the first training visit. Prior to completing the 1RM the participants were instructed to perform a self-selected dynamic warm-up using the corresponding equipment. For the bench press the participants were positioned on the bench so that both feet were flat on the floor and the bar was two inches superior to the xiphoid process. For the leg press the participants utilized an appropriate method using previous published criteria (135). The 1RM recorded was the heaviest weight successfully lifted during a maximum of three to five attempts with five-minute rest periods between each lift.

The RT sessions lasted approximately 45 minutes and consisted of the chest press, seated row, leg press, shoulder press, leg extension, and leg curl. Each exercise was performed with a load equaling an estimate of 70-85% of the individual’s 1RM for three total sets: two sets of 10 repetitions and a third set to muscular exhaustion. The initial starting weight for each exercise was determined by estimating 70-85% of the participants 1RM based on the previously completed chest press and leg press 1RMs. Rest periods were 90-120 seconds between all sets and exercises (101, 102). If the participant was able to perform more than 12 repetitions during the third set the weight would be increased during the following exercise session. Conversely, if the participant was unable to complete eight repetitions then the weight would be decreased. The HIT program was based on the participants rating their perceived exertion on a scale from 1 to 10 (1=resting quietly, 5=warm-up, 10=all-out exertion) (10). Participants began with a two minute warm-up at RPE level five and increased their exertion each minute for three minutes until level nine was perceived and then they recovered at level six for one minute. This pattern was repeated four times, however, on the fourth cycle participants increased their last minute of exertion to level 10, followed by one minute recovery at their initial warm-up level five. The exercise duration in total was 20 minutes.

Post-Testing - Visit 2

Post-testing occurred 36-60 hours after the last supplement was consumed and 96-144 hours (4-6 days) after the last exercise session. The participants completed training on a Friday and finished taking their assigned supplements (30) on a Sunday, Monday, or Tuesday. Post testing was completed 36-60 hours later depending on participant schedules. This delay acted as a washout period for any thermic effects of feeding or the
acute effects of exercise on metabolism, respectively. We wanted to ensure any changes we measured resulted from the chronic late night consumption of our supplements and combined exercise. During the post-test all measurements taken during the baseline visit was repeated.

**Anticipated Risks and Solutions**

Resistance exercise exposes participants to the risk of musculoskeletal injuries. Qualified exercise instructors supervised participants to ensure proper technique and form to minimize risk. Additionally, there is a small risk of local infection associated with venous blood draws. Aseptic technique was utilized to minimize this risk.

**Statistical Analysis**

A power analysis was performed which revealed a need for 16 participants per group with a power of 0.80, $\alpha=0.05$, standard deviation=2.0, difference=2.2 (23). A one-way analysis of variance (ANOVA) was performed to examine possible group differences at baseline and differences in change among groups. A two-way ANOVA with repeated measures was used to evaluate changes in dependent variables over time ([pre x post] x [PLA x WP x CP]). A Tukey test was used for post hoc comparisons. A Shapiro test was used to ensure normality for baseline BF% and BMI. Values are reported as means ± SEM unless otherwise noted. JMP Pro 10 was used to perform statistical analysis. Significance was set at $P<0.05$. 
CHAPTER FOUR

RESULTS

A total of 238 individuals were recruited from the greater Tallahassee, FL area of whom 82 participants were eligible and 59 elected to enter the study. Of the 59 who completed baseline testing, 48 completed the entire study and conformed to all study requirements including supplementation and exercise training (>80% compliance for supplement intake and did not miss more than one RT or HIT session) (PLA: n=14, WP: n=17, CP: n=17). See Figure 2 for the progression of participants throughout the study. Out of the 48 participants, 22 were African American (PLA: n=6, WP: n=10, CP: n=6), 19 were Caucasian (PLA: n=6, WP: n=6, CP: n=7), five were Hispanic (PLA: n=1, WP: n=1, CP: n=3) and two were Chinese (PLA: n=1, WP: n=0, CP: n=1).
Figure 2. Participant Recruitment

- Craigslist
- Mass email to university community
- Flyers

238 phone screenings

82 eligible

21 declined to Participate

61 elected to continue

156 ineligible:
- Diagnosed cardiovascular, thyroid, or kidney disease, stoke or diabetes
- Currently exercising more than twice a week for more than 30 minutes
- Uncontrolled hypertension or taking blood pressure medication
- Allergic to milk products

2 initially agreed to participate but failed to attend baseline testing

59 signed informed consent forms, completed baseline testing and were assigned to a supplement group

55 started training

4 withdrew from study prior to beginning training

53 completed training

2 withdrew from study prior to completion of training

48 completed final testing and followed all protocols

1 failed to show up for final testing 4 were dropped due to non-compliance
There were no significant differences among groups at baseline for any anthropometric variables (Table 1). A significant main effect of time ($P < 0.05$) was observed for BF% (pre: 45.0 ± 0.1, post: 44.6 ± 0.1 kg) and lean body mass (pre: 51.8 ± 0.1, post: 52.4 ± 0.1 kg). FM showed a non-significant change ($P = 0.1608$) with a decrease in both protein groups and an increase in PLA (Figure 3). No group x time effects were observed for any anthropometric values other than waist circumference (Table 1).
<table>
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<tr>
<th></th>
<th>PLA (n=14, 4 males)</th>
<th>WP (n=17, 3 males)</th>
<th>CP (n=17, 3 males)</th>
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<tr>
<td></td>
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<tr>
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<td>Height (cm)</td>
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Participant characteristics before and after 4 weeks of supplementation and exercise training. PLA, Placebo; WP, Whey Protein; CP, Casein Protein; W/H, waist to hip ratio. *P<0.05 main time effect. #P<0.05 different from pre in the same group. Data are mean ± SEM.
Figure 3. Δ in body composition from pre to post testing for (a) fat mass, (b) lean body mass, (c) total body weight and (d) body fat % before and after 4 weeks of supplementation and exercise training. PLA, Placebo; WP, Whey Protein; CP, Casein Protein. *P < 0.05 main effect of time. Data are mean ± SEM.
A main effect of time was observed for maximal upper (pre, 49.8 ± 0.9 kg vs. post, 57.7 ± 1.0 kg) and lower body (pre, 172.4 ± 4.7 kg vs. post, 229.5 ± 5.1 kg) strength ($P < 0.05$) in all groups with no differences observed between treatments as shown in Figure 4. No significant group differences existed among average total volume of weight lifted (sets x repetitions x weight lifted) per participant over the four week intervention (PLA=71527.0 ± 5772.1 kg; WP=66081.5 ± 5238.1 kg; CP=63006.6 ± 5238.1 kg; $P>0.05$)

**Figure 4.** Values are 1-repetition maximums for (a) chest press and (b) leg press before and after 4 weeks of supplementation and exercise training. PLA, Placebo; WP, Whey Protein; CP, Casein Protein. *$P < 0.05$ different from pre testing. Data are mean ± SEM.
A group difference was observed for serum glucose concentrations with PLA (88.9 ± 1.2 mg/dl) and WP (85.7 ± 1.1 mg/dl) being lower \( (P=0.008) \) than CP (94.6 ± 1.1 mg/dl). There were no other group, time, or group x time interactions observed for blood lipids (Table 2). In some instances the Cholestech LDX analyzer was unable to provide complete results therefore the sample size was reduced for some variables: total cholesterol n=48 (PLA=14; WP=17; CP=17), triglycerides n=48 (PLA=14; WP=17; CP=17), TC/HDL n=47 (PLA=14; WP=17; CP=16), HDL n=48 (PLA=14; WP=17; CP=17), LDL n=40 (PLA=13; WP=13; CP=14), non-HDL n=47 (PLA=14; WP=17; CP=16), glucose n=48 (PLA=14; WP=17; CP=17).
Table 2. Lipid Profile Before and After the Four Week Intervention (N=48)

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<tr>
<td>TC</td>
<td>176.3 ± 3.1</td>
<td>173.5 ± 3.1</td>
<td>-2.8 ± 4.5</td>
<td>160.3 ± 3.0</td>
<td>153.4 ± 3.0</td>
<td>-6.8 ± 4.1</td>
<td>160.4 ± 2.9</td>
<td>160.3 ± 2.9</td>
</tr>
<tr>
<td>TRG</td>
<td>112.1 ± 6.8</td>
<td>112.1 ± 6.8</td>
<td>-0.0 ± 9.5</td>
<td>91.6 ± 6.5</td>
<td>90.3 ± 6.5</td>
<td>-1.2 ± 9.2</td>
<td>93.5 ± 6.3</td>
<td>94.3 ± 6.3</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>4.3 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>0.0 ± 0.2</td>
<td>4.3 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>-0.1 ± 0.2</td>
<td>4.2 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>HDL</td>
<td>46.0 ± 2.4</td>
<td>48.9 ± 2.4</td>
<td>2.9 ± 3.3</td>
<td>39.5 ± 2.3</td>
<td>38.5 ± 2.3</td>
<td>-1.0 ± 3.2</td>
<td>38.8 ± 2.2</td>
<td>41.9 ± 2.2</td>
</tr>
<tr>
<td>LDL</td>
<td>110.4 ± 4.6</td>
<td>98.9 ± 5.4</td>
<td>-11.5 ± 7.1</td>
<td>103.9 ± 5.4</td>
<td>100.1 ± 5.0</td>
<td>-3.8 ± 7.4</td>
<td>106.0 ± 4.8</td>
<td>101.6 ± 4.8</td>
</tr>
<tr>
<td>Non-HDL</td>
<td>130.4 ± 3.2</td>
<td>128.9 ± 3.2</td>
<td>-1.5 ± 4.5</td>
<td>120.7 ± 3.1</td>
<td>114.8 ± 3.1</td>
<td>-5.9 ± 4.3</td>
<td>121.1 ± 3.2</td>
<td>118.4 ± 3.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>88.9 ± 1.2(^A)</td>
<td>86.3 ± 1.2</td>
<td>-2.5 ± 1.7</td>
<td>85.7 ± 1.1(^A)</td>
<td>87.9 ± 1.1</td>
<td>2.3 ± 1.6</td>
<td>94.6 ± 1.1</td>
<td>95.82 ± 1.1</td>
</tr>
</tbody>
</table>

Fasting lipid profile and glucose before and after 4 weeks of supplementation and exercise training. All units are mg/dl. PLA, Placebo; WP, Whey Protein; CP, Casein Protein; TC, Total Cholesterol; TRG, Triglycerides; TC/HDL, Total Cholesterol to High Density Lipoprotein Cholesterol Ratio; HDL, High Density Lipoprotein Cholesterol; LDL, Low Density Lipoprotein Cholesterol; Non-HDL, Non-High Density Lipoprotein Cholesterol. \(^A\) denotes \(P<0.05\) group difference from CP. Data are mean ± SEM.
There were no group differences for RMR, relative VO\textsubscript{2}, or RQ at baseline and there were no time or group x time interactions for RMR or RQ. However, RMR displayed a trend ($P= 0.056$) towards a group x time effect with PLA decreasing by 74.3 ± 94.5 kcal/day and WP and CP increasing by 235.7 ± 84.5 kcal/day and 51.7 ± 79.4 kcal/day, respectively, over the course of the 4-week study. Additionally, there was a group x time effect for VO\textsubscript{2} with WP increasing by 0.3 ± 0.07 ml/kg/min compared with a decrease of 0.1 ± 0.08 ml/kg/min and an increase of 0.1 ± 0.10 ml/kg/min for PLA and CP, respectively (Table 3, Figure 5). Four participant’s (PLA=3, WP=1) metabolic data were excluded from analysis due to data points falling outside two standard deviations of the mean.
Table 3. Metabolic Data Before and After the Four Week Intervention (N=44)

<table>
<thead>
<tr>
<th></th>
<th>PLA (n=11)</th>
<th></th>
<th></th>
<th>WP (n=16)</th>
<th></th>
<th></th>
<th>CP (n=17)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Change</td>
<td>Pre</td>
<td>Post</td>
<td>Change</td>
<td>Pre</td>
<td>Post</td>
<td>Change</td>
</tr>
<tr>
<td>RMR (kcal/day)</td>
<td>2209.3 ± 66.8</td>
<td>2135.0 ± 66.8</td>
<td>-74.33 ± 94.5</td>
<td>1925.0 ± 3.0</td>
<td>2160.7 ± 59.7</td>
<td>235.7 ± 84.5</td>
<td>2059.3 ± 56.1</td>
<td>2111 ± 56.1</td>
<td>51.7 ± 79.4</td>
</tr>
<tr>
<td>VO2 (ml/kg/min)</td>
<td>3.2 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>-0.1 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>3.3 ± 0.1*</td>
<td>0.3 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td>RQ</td>
<td>0.85 ± 0.01</td>
<td>0.85 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.85 ± 0.01</td>
<td>0.85 ± 0.01</td>
<td>0.00 ± 0.01</td>
<td>0.82 ± 0.01</td>
<td>0.83 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
</tbody>
</table>

Metabolic data before and after 4 weeks of supplementation and exercise training. PLA, Placebo; WP, Whey Protein; CP, Casein Protein; RMR, resting metabolic rate; VO2, volume of oxygen consumed; RQ, respiratory quotient. *P<0.05 different from pre. Data are mean ± SEM.
Figure 5. Values are for (a) resting metabolic rate, (b) $\text{VO}_2$, and (c) respiratory quotient before and after 4 weeks of supplementation and exercise training. PLA, Placebo; WP, Whey Protein; CP, Casein Protein. * $P < 0.05$ different from baseline. Data are mean ± SEM.
Neither hunger, satiety, nor desire to eat displayed any group differences at baseline. Additionally, no group x time interactions were observed in any of these appetite variables. A main effect of time ($P < 0.05$) was observed only for satiety (pre, $31.5 \pm 2.3$ vs. post, $40.6 \pm 2.3$) (Table 4, Figure 6).
<table>
<thead>
<tr>
<th></th>
<th>PLA (n=14)</th>
<th></th>
<th></th>
<th>WP (n=17)</th>
<th></th>
<th></th>
<th>CP (n=17)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Change</td>
<td>Pre</td>
<td>Post</td>
<td>Change</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Hunger</strong></td>
<td>45.5 ± 5.6</td>
<td>47.3 ± 5.6</td>
<td>1.7 ± 7.9</td>
<td>42.2 ± 5.4</td>
<td>41.4 ± 5.4</td>
<td>-0.8 ± 7.7</td>
<td>47.4 ± 5.3</td>
<td>36.8 ± 5.3</td>
</tr>
<tr>
<td><strong>Satiety</strong></td>
<td>35.1 ± 4.2</td>
<td>46.5 ± 4.2*</td>
<td>11.5 ± 5.9</td>
<td>33.1 ± 4.0</td>
<td>35.8 ± 4.0*</td>
<td>2.7 ± 5.7</td>
<td>26.2 ± 3.9</td>
<td>39.6 ± 3.9*</td>
</tr>
<tr>
<td><strong>Desire to Eat</strong></td>
<td>53.6 ± 5.8</td>
<td>48.5 ± 5.8</td>
<td>-5.1 ± 8.2</td>
<td>50.4 ± 5.6</td>
<td>46.2 ± 5.6</td>
<td>-4.2 ± 7.9</td>
<td>46.2 ± 5.4</td>
<td>37.8 ± 5.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; PLA, Placebo; WP, Whey Protein; CP, Casein Protein; a denotes significant group difference from PLA; b denotes significant group difference from WP; c denotes significant group difference from CP; * denotes significant time effect.
Figure 6. Δ in appetite scores for (a) hunger, (b) satiety, and (c) desire to eat before and after 4 weeks of supplementation and exercise training. PLA, Placebo; WP, Whey Protein; CP, Casein Protein. *P < 0.05 significant time effect of time. Data are mean ± SEM.
CHAPTER FIVE

DISCUSSION

The present study was the first to investigate the effects of four weeks of WP, CP, or PLA in the late evening combined with exercise training on changes in body composition, 1RM strength, cardiovascular health, RMR, and appetite in overweight and obese individuals. The major findings were that exercise three times per week for four weeks combined with nighttime eating can be a successful method for improving body composition and strength in this population, fasting VO$_2$ (ml/kg/min) in the morning is elevated after a 36-60 hour supplement washout following four weeks of nighttime consumption of WP and exercise training, and morning satiety is increased during the same period. In addition, there was a trend for RMR to be increased >36 hours after the cessation of four weeks of nighttime consumption of protein and exercise training. No other significant changes were measured in appetite or blood lipid profile as a result of the intervention.

There were five major hypotheses proposed in this study. The first hypothesis states all groups would report an improvement in body composition (LBM, FM, BF%) with the greatest improvement occurring in CP, followed by WP then PLA. Similarly, the second hypothesis states that strength will increase in all three groups with CP showing the greatest increase, then WP and lastly PLA. The third hypothesis states there will be an improvement the fasting serum blood lipid and glucose profiles with a greater improvement seen in the protein groups compared to PLA. The fourth hypothesis states all groups will increase RMR, and VO$_2$ with the greatest increase occurring in CP followed by WP and PLA and RQ will decrease in the protein groups only. The fifth hypothesis states appetite will decrease the most with CP, followed by WP and no change in PLA.

**Body Composition**

Previous studies report a positive association with increased dietary protein intake and improved body composition (10, 11, 13, 28, 29). We observed a non-significant
increase in FM for PLA whereas both protein groups decreased in FM over the course of the study. While not significant, this observation may be relevant for future dietary recommendations emphasizing fat loss and warrants further investigation over a longer duration.

In addition, all three of our groups displayed a significant decrease in BF% (Table 1, Figure 1). These results agree with Vikoren et al. who showed in overweight and obese adults that compared to carbohydrate, three grams per day of supplemental fish protein resulted in a significant decrease in BF% after four weeks (141). Unfortunately, the lack of exercise and the use of a different form of protein compromises the ability to directly compare results. Also in agreement with our findings, Baer et al. reported that 23 weeks of WP supplementation in free-living overweight and obese adults resulted in a decrease in FM without any adjustments to energy intake or expenditure (13).

Total body nitrogen balance follows a circadian rhythm with increased protein catabolism at night and feeding is the most prominent external regulatory factor for protein turnover (77). It is suggested that the accelerated muscle protein oxidation seen at night is partially a gluconeogenic compensation to the decrease in blood glucose that accompanies fasting (77). In addition, this state of protein loss may be magnified by the accelerated catabolism that accompanies resistance training in untrained individuals (15, 107). Consumption of a slow protein such as CP immediately prior to this nighttime catabolic period may result in an exaggerated anabolic response compared to a fast protein or carbohydrates. Compared with WP, CP results in a greater duration of hyperaminoacidemia and attenuates catabolism for a longer duration (18, 93, 113, 132). This delay is typically attributed to the CP clot that forms in the stomach in combination with the actions of beta-casomorphins (18, 37, 93). Since we were unable to directly measure muscle fractional synthetic rates or total body nitrogen balance, we used changes in LBM as a measure of accretion of muscle mass. Previous studies have reported that increased protein in a hypocaloric or isocaloric diet with exercise training has favorable effects on LBM (29, 71, 108). Additionally, the absorption kinetics of protein has been shown to be an independent regulator of the anabolic and anti-catabolic effects of protein (35). The fact that CP has been shown to result in greater post-prandial leucine balance (18, 35) and greater amino acid incorporation into skeletal muscle compared to WP (126)
makes our results even more interesting. We measured no significant differences in LBM among CP, WP, or PLA, however the magnitude of change was greater for the protein groups (Figure 1). It is possible that our participants may have already been consuming adequate protein, which may reduce the potential benefits of protein supplementation at night. This would suggest absorption rates and macronutrient type in the late evening might have a more pronounced effect in people who do not consume sufficient dietary protein, however, this has not been documented in the literature. Res et al. (115) have shown improvements in MPS concomitant with adequate daytime protein intake. Groen et al. have suggested the digestion kinetics of CP may change at night to be more similar to WP (54). This hypothesis may account for the lack of a difference we observed between CP and WP as our participants consumed all supplements at night.

No change in total body mass was measured for any group in the present study. This is most likely a result of a net equilibration between the decrease in FM and increase in LBM that was observed. The effects of protein supplementation on total body mass are controversial with some reporting no effect (92), a weight increase (141), or weight loss (13). One of the reasons it is difficult to draw a conclusion is due to the different types of proteins that are used in each study, making direct comparisons difficult.

The data only partially support the first hypothesis of improved body composition. All three groups did observe a decrease in BF% and increase in LBM but no group differences were seen.

**Strength**

Our results showed increases in all 1RM strength measures for all groups at four weeks and are in accordance with typical results utilizing untrained participants (Figure 2) (39, 129, 130). Denysschen et al. reported 12 weeks of resistance training in untrained, overweight men resulted in a 47% average increase in strength (39). Likewise, Arciero et al. reported a 37% increase in leg strength and 21% increase in upper body strength after overweight and obese participants consumed protein and completed resistance training for 12 weeks (10).

The outcome of the present study only partially supports the second hypothesis of increase strength. All three groups recorded improvements in strength but no group differences were observed.
Blood Lipids and Glucose

There were no group, time, or group x time effects for any lipid measures or glucose (Table 2). Previous research has reported an association between prolonged (>6 weeks) exercise training and protein supplementation with improvements in blood lipids and glucose (32, 39, 103, 122) but a more limited association was seen in a short-term study (<4 weeks) (104). We may have observed a different result if we were able to measure morning glucose tolerance rather than fasting glucose since glucose tolerance decreases throughout the day towards the late night thus consuming carbohydrates at night may negatively effect blood glucose uptake (137). A recent study by Sato et al. supported this assertion when they reported that postponing dinner until immediately prior to sleep resulted in impaired glucose tolerance in response to breakfast (722 Kcals, carbohydrate 63.3%, protein 16.8%, fat 19.9%) the following morning but no change in fasting blood glucose (121).

The data do not support the third hypothesis of improved fasting serum and lipid profiles as no changes were reported in any groups.

Resting Metabolism

In the present study, there was no main time effect for absolute RMR, however, there was a group x time trend ($P=0.0559$) towards an increase in RMR for both protein groups and a decrease in RMR for PLA (Table 3, Figure 5). This change agrees with previous reports that CP and WP may not differentially affect thermogenesis but protein supplementation has a greater thermic effect than carbohydrate (2, 76). In addition to the previous studies utilizing healthy, college aged individuals (2, 76) rather than overweight and obese individuals, an important distinction between our study and previous work on the thermic effect of protein is our delayed measurement of metabolism. By utilizing a 36-60 hour time period between the last nighttime supplement consumption and arriving to the laboratory for morning post-testing, we were able to effectively washout any acute post prandial affects of feeding and determine the long term alterations in metabolism that may have occurred as a result of four weeks of consecutive nighttime CP, WP, or PLA consumption. Despite removing any acute thermic effect of the protein, we observed this trend towards increased RMR throughout the study in the protein groups compared to PLA. Supporting this finding was our measurement of significantly increased relative
oxygen consumption for WP compared to CP and PLA. As TBM is both correlated with RMR (22, 74) and is a component of relative VO$_2$, our findings are bolstered by our measurement of a static TBM in all three groups throughout the study. This is a novel finding and suggests protein supplementation late at night may result in a long-term beneficial alteration of resting metabolism.

The data do not support the fourth hypothesis of an improvement in metabolism. No significant changes were observed in RMR or RQ, and only WP improved RMR.

**Appetite**

Protein has been reported to decrease appetite more than carbohydrate (12, 54, 63, 64, 109) but the affects of CP compared with WP on appetite are inconclusive with some studies reporting WP as more satiating (138) but the majority showing CP as more satiating (5, 63, 70). Our results agreed with the latter thought and suggest the CP group showed the greatest decrease in appetite, although the group difference was not significant (Table 4, Figure 4). Groen et al. measured lower hunger ratings in the morning following CP ingestion via nasogastric tube during sleep but this did not affect energy or protein intake during the subsequent breakfast (54). Of further interest is our measured increase in satiety in all groups despite concurrent exercise training. A recent study by Martins et al. reported 12 weeks of exercise, in a participant population similar to the present study, resulted in an increased appetite in the fasting state (95). Unfortunately, in the present study we were unable to measure food intake following the appetite questionnaire therefore the actual intake was unable to be determined.

The results of this study do not support the hypothesis that CP would show the greatest decrease in appetite, and that PLA would not change. All three groups reported increased satiety and thus a decreased appetite.

**Limitations**

It should be noted that the present study was limited in duration and had relatively low participant numbers. However, our power analysis revealed a need for 16 participants per group to measure significant changes and we were able to meet this number in both protein groups. After accounting for dropouts and noncompliance our PLA group finished with n= 14. In addition, to our knowledge, no previous studies have utilized a design that extends beyond one night of acute macronutrient supplementation. Thus, the present
study is the first to identify the impact of four weeks of supplementation and exercise training in overweight and obese individuals. Nevertheless, repeating the study using a larger number of participants and a time period greater than four weeks is warranted. A limitation resulting from our delayed post-testing measurement is the inability to standardize the exact duration of time between ceasing exercise/supplementation and laboratory testing due to participant’s schedule conflicts. However, each group had equal representation on all post-testing days (PLA=2.4 ± 0.3 days; WP=2.6 ± 0.2 days; CP=2.4 ± 0.2 days; \( p>0.05 \)) to avoid group differences as a result of the delay before post-testing. Another limitation was the assumption that the CP and WP displayed absorption and digestion kinetics as described in other studies (18, 37, 93). Indeed, Groen et al. suggested that these kinetics might be different in the overnight hours (54). Future research should employ radiolabeled CP and WP in utilizing techniques previously described to measure gastric emptying rate and gastrointestinal transit time during the nighttime hours (37, 112). We were also unable to measure activity or diet outside of the study. Future investigations should attempt to standardize dietary intake and control activity. Lastly, we were unable to measure hormones that could give us insight into possible mechanisms of action for our results. Future studies must incorporate hormone changes in the analysis.

**Conclusion**

In conclusion, exercise three times per week for four weeks is a successful method for improving LBM, BF%, strength, and satiety in previously sedentary, overweight and obese individuals, despite if CP, WP, or PLA is consumed before bed. However, although not statistically significant, protein appears to be more effective at improving body composition and metabolism. This is the first study to report that nighttime protein consumption causes a sustained elevation of metabolism for >36 hours and may have a beneficial effect on body composition. However, more research is needed to confirm these findings.
Informed Consent Form

1. I voluntarily and without element of force or coercion, consent to be a participant in the research project entitled “The effect of protein timing and combined resistance and high-intensity interval training on body composition, blood lipids, growth hormone, and strength in overweight and obese individuals.” This study is being conducted by Dr. Mike Ormsbee, Dr. Arturo Figueroa, Dr. Robert Moffatt, Amber Kinsey, David Thomas, and Wyatt Eddy of the Department of Nutrition, Food & Exercise Sciences at Florida State University.

2. The purpose of the proposed study is to examine how protein supplementation in the late evening before sleep and exercise training affect body composition, anabolic and appetite hormones, fat metabolism, stress, and strength. Forty sedentary, overweight or obese men and women (18 to 40 years of age) will be recruited for this study.

3. My participation in this study will require coming to the Human Performance Laboratory at Florida State University for testing on four different occasions over 4 weeks to complete the measurements and assessments as described below.

On my first visit, I will be given an informed consent document to sign and a medical history form to complete before I can participate in the study. I cannot participate in this study if I have uncontrolled hypertension (Blood Pressure (BP)>160/100 mmHg), take BP medications, have been diagnosed cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, milk allergies or any musculoskeletal complications (i.e., osteoarthritis or injury) that would impede me from exercising. In addition, I will be excluded if I am currently a heavy smoker (>20 cigarettes per day), take cholesterol medication or nutritional supplements (except for a multivitamin), or partake in planned exercise for more than 2 days per week for more than 40-minutes per session (within the past 6 months).

During this visit, I will then answer questionnaires regarding physical activity, nutritional habits, and mood-state. I will have my blood pressure, height, weight, waist circumference, body composition, and strength measured. Height and weight will be assessed using a standardized scale. Waist circumference measures will be taken a minimum of two times. My body composition and bone mineral density will be measured using dual energy X-ray absorptiometry (DXA). Very low doses of radiation are used; however, this test is non-invasive. I will lie on a padded table for
approximately 10 minutes while the scan is being completed. Testing will be completed according to the manufacturer's instructions and specifications by a certified X-ray technician. Both upper and lower body strength will be assessed using the chest press and leg press exercises, respectively. After a warm-up period, I will be progressed towards the maximum weight that I can lift 1-time through a full range of motion, also called a 1-repetition maximum (1RM). All measurements will be recorded within three and five attempts and will be supervised by trained personnel.

I will be given food record forms (to list all foods and beverages consumed over 3 days) to bring filled out on the next visit and will receive instructions on how to complete these forms. I will get familiarized with the metabolic and cardiovascular testing equipment on this day. This visit will take approximately 2 hours.

On the second visit (occurring at least 72 hours following the first visit), I will come to the laboratory in a fasted state (no food or drink, except water for at least 8 hours) between 6 and 11 am. I will turn in the 3-day food record and then have my resting metabolic rate (RMR) measured using indirect calorimetry. This is a non-invasive test that involves lying down on a padded table for 30-minutes with a ventilated hood covering my head and torso. I will have my cardiovascular function evaluated after 20-minutes of rest in the supine (lying down) position. A total of 4 cuffs, one in each extremity (around arm and ankles) and 2 tonometers (sensors applied to the skin to obtain pulse waves), one on the neck and the second on the inner thigh, will be used to measure pulse wave velocity (arterial stiffness). My blood pressure will be also monitored by placing a small cuff around the middle finger and a tonometer on the wrist and neck. Six electrodes will be positioned on the skin of my chest to measure heart rate (electrocardiogram). Arm blood flow will be measured using vascular ultrasound positioned on my skin at rest and during increased blood blow after deflation of an arm cuff (5 minutes inflation). The diameter and thickness of my neck artery (common carotid) will be measured non-invasively by ultrasound. I will also have my blood will be drawn on 3 occasions under sterile conditions and the total amount of 30 milliliters from a forearm vein and finger prick and stored for later analysis. Specifically, forearm vein blood will be drawn by inserting a catheter into the antecubital vein to avoid unnecessary needle pricks and to minimize my discomfort. The blood samples will not be used for any other research or testing purposes other than those specified in the research proposal. I will have my saliva collected by placing a salivary oral swab underneath my tongue for 2-minutes. The second visit should take approximately 90 minutes.

After finishing visit two, I will be randomly assigned to one of two intervention groups for the duration of the four-week intervention: 1) protein consumption in the late evening before sleep (PRO) or 2) placebo consumption in the late evening before sleep (CON). Participants in both groups will consume their respective supplements as the last food or caloric beverage consumed prior to going to sleep.
On the next morning (between 6 and 11 am), for my third visit (24 hours after the second visit), I will arrive to the human performance laboratory in a fasted state (for at least 8 hours). I will then have my body weight, resting metabolic rate, and cardiovascular function measured. I will also have blood and saliva collected as described above. The third visit will last approximately 90 minutes.

After the third visit, I will continue with my late evening drink consumption as previously assigned every night of the week (7 nights) and I will complete three workouts (2 resistance training days, 1 high-intensity interval training day) under the supervision of qualified instructors each week for four weeks. Each exercise session will last for approximately 45 minutes. Resistance exercises will consist of the following exercises: chest press, seated row, leg press, shoulder press, leg extension, and leg curl. Each exercise will be performed for 3 total sets: 2 sets of 10 repetitions and a 3rd set to muscular exhaustion with a load equaling 75-85% of the individual’s previously established 1-RM. Rest periods will be set to 90-120 seconds between all sets and exercises and the RE session will last for a total of 40 to 45-minutes.

The one cardiovascular training day per week will use a high-intensity interval program in which participants will rate their perceived exertion on a scale from 1 to 10 (1 = resting quietly, 5 = a warm-up level, 10 = an all-out exertion). Participants will begin with a 2-minute warm-up at level 5 and increase their exertion each minute for 3 minutes until level 9 is perceived and then recover at level 6 for 1 minute. This pattern is repeated four times, however, on the fourth cycle participants will increase their last minute of exertion to level 10, followed by 1-minute recovery at their initial warm-up level 5. The exercise duration in total will be 20-minutes.

I will repeat my 3-day food diary again during the final week of the 4-week training period and turn it into the research staff. All measurements taken during visits one and two will be replicated for visit 4 following the 4-week intervention.

4. I understand there is a minimal level of risk involved if I agree to participate in this study. I may experience some muscle soreness from the 1RM and exercise training sessions. The risks associated with 1RM and exercise training are minimal and the selected protocols have been previously used in other studies in sedentary men and women. There is the possibility of muscle fatigue or soreness related with exercise training or testing. Although there is a potential risk of muscle injury with maximal strength testing (1RM), the risk will be reduced by using a submaximal strength test, the 1RM. The risk will be minimized by using qualified exercise instructors to supervise testing and training and ensure proper exercise techniques and intensity. The risk of a cardiovascular event during testing and training will be minimized by careful review of my medical history and monitoring of my exercise sessions. In addition, my cardiovascular exercise is based off of my perceived exertion and is therefore individually tailored to my level of fitness. I understand that to reduce muscle fatigue and soreness my trainer may make adjustments to my training program.
The risk of blood drawing is small and there may be some local discomfort at the site of needle placement with possible bruising or swelling. The risk of local infection is also small. These risks will be minimized by the use of skilled technicians using sterile techniques and equipment.

Body composition will be evaluated by Dual-Energy X-ray Absorptiometry (DXA). This involves some radiation of approximately 0.5 mREM per total body scan or 1 mREM for both scans. This is much less than a traditional chest X-ray (20-50 mREM) or full dental X-ray (300 mREM). The measurement of body composition using DXA is non-invasive.

5. The possible benefits of my participation in this research project include about my body composition, bone mineral density, resting vital measures, waist and hip circumferences, resting metabolic rate, upper and lower body muscular strength, heart rate control and arterial function. Participants in both groups will have the potential to improve metabolic, cardiovascular and muscular health and may improve body composition, physical functioning, and quality of life. I will also be given 12 training sessions at no charge.

6. The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential, to the extent allowed by law. My name will not appear on any of the results. No individual responses will be reported. Only group responses will be reported in the publications. Confidentiality will be maintained by assigning each subject a code number and recording all data by code number. The only record with the participant's name and code number will be kept by the principal investigator, Dr. Michael Ormsbee, in a locked drawer in his office. Data will be kept for 10 years and then destroyed.

7. In case of an injury, first aid (free of charge) will be provided to me by the laboratory personnel working on the research project. However, any other treatment or care will be provided at my expense.

8. Any questions I have concerning the research study or my participation in it, before or after my consent, will be answered by the investigators or they will refer me to a knowledgeable source. I understand that I may contact Dr. Michael Ormsbee at (850) 644-4793 (mormsbee@fsu.edu), or Amber Kinsey at awk10d@fsu.edu for answers to questions about this research study or my rights. Group results will be sent to me upon my request.

9. In case of an injury, or if I have questions about my rights as a subject/participant in this research, or I feel I have been placed at risk, I can contact the chair of the Human Subjects Committee, Institutional Review Board, through the office of the Vice President of Research at (850) 644-8633 (humansubjects@magnet.fsu.edu).
10. The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any risk involved.

11. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise be entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies. A copy of this consent form will be given to me.

________________________________
Print name

________________________________  ______________________________
Signature        Date
APPENDIX B

IRB APPROVAL LETTER

Office of the Vice President For Research Human Subjects Committee Tallahassee, Florida 32306-2742
(850) 644-8673 * FAX (850) 644-4392

APPROVAL MEMORANDUM

Date: 3/23/2011

To: Michael Ormsbee

Address: 1493
Dept.: NUTRITION FOOD AND MOVEMENT SCIENCES

From: Thomas L. Jacobson, Chair

Re: Use of Human Subjects in Research
The effect of protein timing and combined resistance and high-intensity interval training on body composition, blood lipids, growth hormone, and strength in overweight and obese individuals

The application that you submitted to this office in regard to the use of human subjects in the research proposal referenced above has been reviewed by the Human Subjects Committee at its meeting on 01/12/2011. Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals, which may be required.

If you submitted a proposed consent form with your application, the approved stamped consent form is attached to this approval notice. Only the stamped version of the consent form may be used in recruiting research subjects.

If the project has not been completed by 1/11/2012 you must request a renewal of
approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the Committee.

You are advised that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report, in writing any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the Chair of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protection. The Assurance Number is IRB00000446.
APPENDIX C

IRB EXTENSION APPROVAL LETTER

Office of the Vice President For Research Human Subjects Committee Tallahassee, Florida 32306-2742
(850) 644-8673 * FAX (850) 644-4392

RE-APPROVAL MEMORANDUM

Date: 11/14/2011

To: Michael Ormsbee

Address: 1493
Dept.: NUTRITION FOOD AND MOVEMENT SCIENCES

From: Thomas L. Jacobson, Chair

Re: Re-approval of Use of Human subjects in Research
The effect of protein timing and combined resistance and high-intensity interval training on body composition, blood lipids, growth hormone, and strength in overweight and obese individuals

Your request to continue the research project listed above involving human subjects has been approved by the Human Subjects Committee. If your project has not been completed by 11/7/2012, you must request a renewal of approval for continuation of the project. As a courtesy, a renewal notice will be sent to you prior to your expiration date; however, it is your responsibility as the Principal Investigator to timely request renewal of your approval from the committee.

If you submitted a proposed consent form with your renewal request, the approved stamped consent form is attached to this re-approval notice. Only the stamped version of the consent form may be used in recruiting of research subjects. You are reminded that any change in protocol for this project must be reviewed and approved by the Committee prior to implementation of the proposed change in the protocol. A protocol change/amendment form is required to be submitted for approval by the Committee. In addition, federal regulations require that the Principal Investigator promptly report in
writing, any unanticipated problems or adverse events involving risks to research subjects or others.

By copy of this memorandum, the Chair of your department and/or your major professor are reminded of their responsibility for being informed concerning research projects involving human subjects in their department. They are advised to review the protocols as often as necessary to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

Cc: Bahram Arjmandi, Chair

    HSC No. 2011.7210
APPENDIX D

VISUAL ANALOG SCALE

ID# ___________________________ Date _____/____/____

TEST#: Pre______ Post______

Please indicate the level to which you are feeling ALL three of the following with a mark on the line:

1) Do you feel HUNGRY:

________________________________________________________________________________
Not at all                          Extremely

2) Satiety (feeling of fullness):

________________________________________________________________________________
Not at all                          Extremely

3) Desire to eat:

________________________________________________________________________________
Not at all                          Extremely
APPENDIX E

MEDICAL HISTORY QUESTIONNAIRE

Human Performance Laboratory
Florida State University
Nutrition, Food, and Exercise Sciences

HEALTH AND FITNESS HISTORY QUESTIONNAIRE

The following questions are designed to obtain a thorough preliminary medical history. The information you provide will help us to make the best determination about your eligibility for a particular study or other studies. Please answer all questions and provide as much information as you possibly can. This questionnaire, as well as any other medical information you provide will be kept confidential and will not be shared with any unauthorized person or organization unless you specifically request us to do so.

Name: ________________________________________________
Street Address: _________________________________________________________
City, State, Zip code: _______________________________________________________
Telephone Number: H ( ) W ( ) _______________
Email address: ____________________________________________________________
Date of Birth: (mm/dd/yy) Age: ____________________________
Sex: M ___ F
Personal Physician’s Name: ______________________ Phone: ( ) _____________
Address: ________________________________________________________________
Height _______ in. _______ cm
Weight _______ lb. _______ kg

Social Security Number: _________________________________

Signature: ________________________________________________
Date: _______ ID #: _______

**Occupation**
Current occupation: 

**Race** ________

**Personal Health History**
Have you ever been hospitalized or had surgery? Yes___ No___
Please list all hospitalizations and surgeries to the best of your recollection.

<table>
<thead>
<tr>
<th>Disease/Operation</th>
<th>Duration</th>
<th>Age when hospitalized</th>
</tr>
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<tbody>
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</tbody>
</table>

List any disease or illness you have had not listed above (e.g., mumps, measles, broken bones, etc.)

Are you allergic, sensitive or intolerant of any foods or medications? Yes___ No___
If yes, please describe:
- Food --------------------------------------------------------------
- Medication ---------------------------------------------------------
- Other -------------------------------------------------------------

Are you currently seeing a doctor or other health care provider for any reason? 
Yes____ No____
If yes, please explain:
1. Have you ever been diagnosed as having any of the following and if yes, how are you currently treating the condition?

   Y   N   High Blood Pressure  
       Please indicate last known reading:  
       Blood pressure: ___/___

   Y   N   High Cholesterol or High Triglycerides  
       Please indicate last known reading:  
       Cholesterol: ___  
       Triglycerides: ___

   Y   N   Diabetes (Circle: Type 1 or Type 2)  
       Note: Type 1 diabetes is insulin-dependent diabetes mellitus. It is typically diagnosed at an early age and requires insulin shots or an insulin pump immediately upon diagnosis. Type 2 diabetes is often diagnosed at an older age (past age 20) and is usually initially treated with changes in diet and/or medication (pills).

   Y   N   Hypoglycemia (low blood sugar)

   Y   N   Asthma

2. Have you ever had a glucose tolerance test? Y   N  
   If yes, what were the results?

3. Have you ever had a fasting blood sugar test?   Y   N  
   If yes, what were the results?

4. Does anyone in your family (immediate family including your grandparents) have a history of cardiovascular disease (heart attacks, stroke, etc.)? Please explain:

5. Do you have any neurological problems including fainting, dizziness, headaches or seizures?

6. Do you have any orthopedic or other health problems that may affect your ability to perform exercise? If yes, please explain:

7. Do you smoke or use smokeless tobacco? Y   N  
   If yes, how many cigarettes per day? ___

8. Do you drink coffee or other caffeinated beverages? Y   N  
   What kind, how much and how often?
9. Please list all vitamins, minerals and herbs and other nutritional (performance) supplements as well as medications you are currently taking. How long have you been taking them and how frequently?

Are you willing to stop taking all nutritional supplements you are currently on for the duration of this research study? (Y/N) ______________

10. Do you have any food allergies or intolerances (e.g., allergic to dairy or lactose intolerance)? Please describe:

11. How would you describe the type of diet you currently eat? Have you recently been on any special diets? What kinds of diets have you used to lose weight or lower cholesterol? Please list and describe:

12. What changes have you made in your diet in the last 6 months?


Please list the 3 most current athletic events/competitions that you have participated in:

14. How does your current exercise and physical activity compare to 6 months ago? 1 year ago?

15. Have you had a physical exam in the past 2 years? Y N Please describe your assessment of your overall health
Phone screenings will be completed in order for the research team to understand if interested participants are eligible and to avoid any unnecessary time commitment for potential subjects. Because this study requires participants to be healthy overweight or obese men and women to be free from the use of nutritional supplements for at least 4 weeks, the researchers directly involved with this study (Dr. Michael Ormsbee, Amber Kinsey, and David Thomas) will conduct a phone or email screening with the following questions.

**Nutritional Supplements and Medications**
Please list all vitamins, minerals and herbs and other nutritional (performance) supplements as well as medications you are currently taking. (examples: Medications for controlling cholesterol or blood pressure)

- How long have you been taking them?
- How frequently?

If you are currently taking any of these supplements are you willing to stop taking them for a period of one month and through the duration of the six-week study and through pre and post testing?

**EXERCISE**
Do you exercise regularly?  Y  N  What kinds of exercise?

- How often? Please be detailed in a description of your average week of training. What types of training exercises do you typically perform (be specific).
Do you have any current conditions that might prevent you from completing maximal strength testing and a whole body moderate to high intensity four week resistance and high-intensity interval training protocol (examples: tendonitis, pulled muscle, torn ligament, knee or back problem)?

**MEDICAL**
Do you have uncontrolled hypertension (BP>160/100 mmHg)?
Do you have diagnosed cardiovascular disease, stroke, or diabetes?
Do you have thyroid or kidney dysfunction?
Do you currently smoke or chew tobacco? How many/much? Frequency?
Do you take cholesterol medication or blood pressure medication? If so, what do you take?
Do you have any allergies to milk products?
# APPENDIX G

## SUPPLEMENT COMPLIANCE FORM

Supplement Compliance Form

**Protein Timing and Resistance and High Intensity-Interval Training Study**

DATE: ________

<table>
<thead>
<tr>
<th>Subject No:</th>
<th>Subject Initials:</th>
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<table>
<thead>
<tr>
<th></th>
<th>Supplement</th>
<th>Reminder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>given - initial/returned - initial</td>
<td>called - date and initial</td>
</tr>
</tbody>
</table>

|       | | |
|-------|-------------------|
| Initial | ______  ____/______  ____  _____________________ | ______________ |
| Week 1 | ______  ____/______  ____  _____________________ | ______________ |
| Week 2 | ______  ____/______  ____  _____________________ | ______________ |
| Week 3 | ______  ____/______  ____  _____________________ | ______________ |
| Week 4 | ______  ____/______  ____  _____________________ | ______________ |
REFERENCES


10. Arciero PJ, Gentile CL, Martin-Pressman R, Ormsbee MJ, Everett M, Zwicky L and Steele CA. Increased dietary protein and combined high intensity aerobic and


74. Hunter GR, Byrne NM, Sirikul B, Fernandez JR, Zuckerman PA, Darnell BE and Gower BA. Resistance training conserves fat-free mass and resting energy expenditure following weight loss Obesity (Silver Spring) 16: 5: 1045-1051, 2008.


92. **Luscombe ND, Clifton PM, Noakes M, Parker B and Wittert G.** Effects of energy-restricted diets containing increased protein on weight loss, resting energy


BIOGRAPHICAL SKETCH

Wyatt R Eddy

EDUCATION

Florida State University

Master of Science, Exercise Physiology
Cumulative GPA: 3.74
August 2010 - August 2012(Planned)

Florida State University

Bachelor of Science, Exercise Science
Upper Division GPA: 3.38
August 2006 - August 2010

PROFESSIONAL EXPERIENCE

Florida State University
Laboratory Director PET3323C
Tallahassee, FL
May 2012 – Present

- Developed revised curriculum and schedule for Functional Anatomy & Physiology II laboratory
- Worked with the publisher to implement new course tools
- Purchased all laboratory supplies

Florida State University
Teaching Assistant PET3323C
Tallahassee, FL
Functional Anatomy and Physiology II Laboratory
August 2011 - Present
Florida State University  Tallahassee, FL

Teaching Assistant PET3322L  August 2010 – May 2011

Functional Anatomy and Physiology I Laboratory

Florida State University  Tallahassee, FL

Research Assistant  April 2009 - Present

- Phlebotomy
- Treadmill VO₂ and cardiac stress testing
- Metabolic Testing

Articles in Preparation (to be submitted to referred journals)

- The Effects of Four Weeks of Nighttime Protein Intake and Exercise Training on Body Composition, Strength, Cardiovascular Health, Resting Metabolism, and Appetite in Overweight and Obese Adults
- The Acute Effects of Nighttime Protein Intake on Cardiovascular Health, Resting Metabolism, and Appetite in Overweight and Obese Adults
- Influence of Environmental Tobacco Smoke on Respiratory Carbon Monoxide

Research Presentations (requiring peer-reviewed acceptance)

- Thomas DD, Rawal S, Kinsey AW, Eddy WR, Fisher N, Spicer MM, Ormsbee MJ. The Combination of Green Tea, Caffeine, Conjugated Linoleic Acid And Branched Chain Amino Acids Have No Effect on Body Composition and Abdominal Fat Changes in


**ADDITIONAL EXPERIENCE**

**Jackson County Hospital Emergency Department**  
*Patient Care Technician*  
Marianna, FL  
July 2011 - Present

- Provided care for acute injuries
- Phlebotomy including intravenous access
- Assisted in critical care including as a member of the code blue resuscitation team

**Florida State University**  
*Tallahassee, FL*  
*Emergency Medical Responder*  
January 2009 – August 2011

- Responded to emergency 911 calls originating on FSU's campus
- Served as a preceptor for new student responders

**CERTIFICATIONS / MEMBERSHIPS / AWARDS**

Certified by the American Heart Association for the 2010 guidelines in Basic Life Support (BLS) and Advanced Cardiovascular Life Support (ACLS)
Member of the International Society of Sports Nutrition; ISSN
Certified Sports Nutritionists; CISSN
Member of the American College of Sports Medicine; ACSM
Member of the South East Chapter of the American College of Sports Medicine; SEACSM
Member of Omicron Pi Chapter of Kappa Omicron Nu National Honor Society
Member of the Golden Key International Honor Society
Member of the National Collegiate Emergency Medical Services Foundation
Member of the American Academy of Physician Assistants
Recorded over 350 hours of community service throughout college career
Received letter of commendation from the State of Florida Bureau of Emergency Medical Services for lifesaving efforts