

# Florida State University Libraries

---

Honors Theses

The Division of Undergraduate Studies

---

2012

## The Acute Effects of Late Evening Whey and Casein Ingestion on Fasting Blood Glucose, Blood Lipid Profile, Resting Metabolic Rate, and Hunger in Overweight and Obese Individuals

Charles Blay



## Abstract

**Background:** Theoretically, protein ingestion before sleep should affect obesity rates and promote cardiovascular health by increasing nocturnal metabolism and decreasing morning hunger. However, there is little research linking nighttime protein ingestion and morning cardiovascular health, metabolism, and hunger.

**Purpose:** To evaluate the acute effectiveness of evening (before sleep) consumption of whey protein (WP) and casein protein (CP) on improving blood glucose, blood lipids, resting metabolic rate, and hunger in overweight and obese individuals.

**Methods:** Forty (n=40; 5 men; 35 women) overweight or obese (age,  $28.9 \pm 6.6$  years; height,  $166.2 \pm 8.8$  cm; weight,  $99.0 \pm 20.2$  kg; body mass index (BMI),  $35.7 \pm 5.9$  kg/m<sup>2</sup>; % body fat,  $46.0 \pm 5.8$  %) participated in this double blind, placebo-controlled study. Resting baseline measures of glucose (GLU), total cholesterol (TC), triglycerides (TRG), TC/HDL, high-density lipoproteins (HDL), low-density lipoproteins (LDL), non-high density lipoproteins (Non-HDL; TC-HDL), resting metabolic rate (RMR), respiratory quotient (RQ), and a hunger-satiety visual analogue scale (VAS) were taken in a fasted state after ~8 hours of sleep. Participants were randomly assigned to WP, CP, or a carbohydrate placebo (PL) supplement to consume before bed. Participants returned to the lab in a fasted state to repeat baseline measures the next morning under identical conditions (6 to 8 am).

**Results:** No significant group by time differences were measured for any dependent variable. Group differences were measured for GLU to be higher and HDL to be lower for CP compared to both WP and PL. In addition, RMR was elevated to a greater extent for WP and CP compared to PL, although the difference was not significant. Improvements in hunger, satiety, and desire to eat were observed from baseline to acute; however there are no group differences.

**Conclusion:** No significant differences were measured among or between groups for our dependent variables. However, there was a greater magnitude of change in RMR for WP and CP compared to PL. Furthermore, the late evening ingestion of WP, CP, and PL before bed improved morning hunger and satiety after ~8 hours of sleep.

THE FLORIDA STATE UNIVERSITY  
COLLEGE OF HUMAN SCIENCES

THE ACUTE EFFECTS OF LATE EVENING WHEY AND CASEIN INGESTION ON  
FASTING BLOOD GLUCOSE, BLOOD LIPID PROFILE, RESTING METABOLIC RATE,  
AND HUNGER IN OVERWEIGHT AND OBESE INDIVIDUALS

By

CHARLES J. BLAY

A Thesis submitted to the  
Department of Nutrition, Food, and Exercise Science  
in partial fulfillment of the requirements for graduation with  
Honors in the Major

Degree Awarded:  
Spring, 2012

The members of the Defense Committee approve the thesis of Charles J. Blay defended on April 16<sup>th</sup>, 2012.

---

Assistant Professor, Dr. Michael J. Ormsbee  
Thesis Director

---

Dean of Undergraduate Studies, Dr. Karen Laughlin  
Outside Committee Member

---

Associate Professor, Dr. Arturo Figueroa  
Committee Member

## **Acknowledgements**

Without the cumulative efforts of the following people, this undergraduate thesis project would have not been possible to complete. First and foremost, I would like to thank Dr. Michael J. Ormsbee, my undergraduate thesis director, for allowing me to take part in the acute phase of his research project, as well as his guidance in concise scientific writing and data analysis. I would also like to thank my other two committee members, Dr. Arturo Figueroa and Dean Karen Laughlin of Undergraduate Studies, for their efforts in directing my project. This project certainly would not have run so efficiently if not for graduate students Amber W. Kinsey and Wyatt R. Eddy who recruited, scheduled, and conducted an immense portion of the project. Also graduate student Takudzwa A. Madzima and his insight in the field of acute protein ingestion. I would also like to thank the undergraduate student workers like Bruce Lee, Emily Mattei, Kelly Knoth, and Yasmine Kahok. A sincere thanks to our dedicated participants involved in this study and the Florida State University Council on Research and Creativity for their support of this research project.

## TABLE OF CONTENTS

<u>Section</u>	<u>Page number</u>
Review of Literature Table of Contents .....	6
Review of Literature .....	7
Introduction .....	17
Methods .....	18
Results .....	20
Discussion .....	21
Conclusion .....	25
References .....	27
Figures and Tables.....	31
Appendices	
Appendix A: Institutional Approved Informed Consent .....	35
Appendix B: Health and Fitness History Questionnaire .....	40
Appendix C: Visual Analogue Scale: Hunger/Satiety/Desire to Eat .....	44
Appendix D: Descriptives, Measurements, and Cholestech Data Sheet .....	45
Appendix E: Supplement Compliance.....	46

**Review of Literature**  
**Table of Contents**

<u>Section</u>	<u>Page Number</u>
Overweight and Obesity .....	6
Evening Food Ingestion .....	8
Whey and Casein .....	9
<i>Insulin and Blood Glucose</i> .....	9
<i>Blood Lipids</i> .....	10
<i>Blood Pressure</i> .....	12
<i>Resting Metabolic Rate</i> .....	13
<i>Appetite Suppression</i> .....	14
Conclusion .....	15

## Review of Literature

### **Overweight and Obesity**

The United States has a public health crisis due to the growth of obesity. National surveys, like that conducted by the National Health and Nutrition Examination Study (NHANES) (9), have revealed the prevalence of obesity continuously increasing over the last three decades. Furthermore, there are no indications of this crisis ceasing or slowing down (43).

Wang et al. analyzed the NHANES data from the 1970s to 2004 for rising overweight/obesity trends (44). If these trends continued until 2030, 86.3% of the adult population would be overweight or obese (Body Mass Index (BMI) > 25) with 51.1% of the population considered obese (BMI > 30). By 2048 all adult Americans would be overweight or obese. Obesity is not only a health problem in the United States, but it causes severe economic strain as well. In fact, it is estimated that U.S. health-care costs attributable to the increases in obesity/overweight prevalence would reach 860.7–956.9 billion US dollars by the year 2030, accounting for 16–18% of total US health-care costs (44).

The rising prevalence of obesity in the U.S. is a problem due to the widespread associated health concerns. In fact, it has been suggested that obesity, including excess visceral fat tissue, is linked to a greater likelihood of cardiovascular disease (CVD) and death (19). Many obese individuals are susceptible to developing dyslipidemia, type II diabetes, and hypertension. As visceral obesity increases, factors for metabolic syndrome (MetS) and CVD increase as well.

Scaglione et al. (37) reviewed the known impact of obesity on health components such as blood lipids, blood glucose, blood pressure, and fat metabolism. Obesity adversely affects several components of blood lipids. The visceral adipose tissue acts as a source of free fatty acids (FFA) formed by the hydrolysis of triglycerides. An excessive quantity of FFA in the liver

induces an increased synthesis of triglycerides and promotes the production of very low-density lipoprotein (VLDL). High triglyceride concentrations are correlated to increased high-density lipoprotein (HDL) clearance. With less HDL circulating in the blood, there is a decrease in the cholesterol removing action of HDL and, thereby, atherosclerosis may increase. Atherosclerosis is also promoted with the hydrolysis of triglycerides into FFA to produce low-density lipoprotein (LDL) (37).

Excessive FFA concentrations in the blood also induce hepatic gluconeogenesis leading to hyperglycemia. Furthermore, excessive FFA concentrations may increase insulin resistance in the muscles by interfering with the intracellular signaling (37). It is likely that a reduction in the muscles' sensitivity to insulin may raise blood glucose levels even further, and exacerbate hyperglycemia.

Hypertension is also associated with having excess adiposity (22). The mechanism responsible for obesity hypertension is multi-faceted, however, accumulation of visceral fat and elevated serum insulin and glucose concentrations are thought to be implicated. In addition, increased renal reabsorption of sodium as a result of these metabolic and hormonal perturbations may increase blood volume and ultimately, raises blood pressure (7).

Chronic decreases in overall metabolism are expressed through MetS and its underlying components including dyslipidemia, hyperglycemia, and hypertension. MetS can be a fatal consequence of obesity. With increasing age and obesity, the components of MetS worsen and the vicious cycle of obesity continues (37).

## **Evening Food Ingestion**

Late evening ingestion of food is often thought to increase the likelihood of weight gain. For this reason it is recommended to limit caloric intake in the evening hours because metabolic rate decreases during sleep (20). Food intake prior to sleep is not metabolized for energy as much as during earlier times in the day. As a result, more of the food intake is sent towards storage, rather than being used to provide energy, and leads to weight gain and body composition changes (20). Postprandial hyperglycemia is often related to late evening food ingestion because glucose tolerance decreases during sleep (41).

On the other hand, a cross sectional study with non-diabetic late night eaters reported that ad libitum trials included more kilocalories (kcal) per day than the controlled diet trials (17). They reported that weight gain is likely due to excessive kcal intake and not to the late evening meal (25).

Night eating syndrome (NES) is frequently described in obese patients (49). The prevalence of obese patients seeking weight loss treatment is 6-14%. An even greater prevalence (from 51 to 64%) has been reported among patients with severe obesity that has been resistant to any treatment. The concurrent appearance of NES and obesity is also evident because the presence of obese and overweight NES patients is 57.1% and 28.6%, respectively (49).

## **Whey and Casein Protein**

Whey originates from soluble portions that are removed during the formation of cheeses. Casein's origins are from the solid micelles of curd when skim milk is exposed to a low pH (34).

The acute differences in the levels of plasma amino acids from whey and casein ingestion are due to the differences in the rate of gastric emptying between the two protein sources.

Clotting of casein in the stomach appears to delay its digestion. Due to the quick movement of whey protein from the stomach to the duodenum (26), large amounts of amino acids are absorbed within a shorter window of time, resulting in amino acid concentrations much higher than that of casein. It is thought that this mechanism is responsible for whey's greater stimulation of muscle protein synthesis (MPS) compared to casein and other protein types (26).

Borie et al. (8) studied the postprandial differences in plasma amino acid content in sixteen average male and female young adults using intrinsically labeled  $^{13}\text{C}$  leucine within whey and casein samples. After ingesting whey, plasma amino acid concentration rose quicker and to a greater extent at 100 minutes than after ingesting casein. However, the effects from the casein ingestion were prolonged over a period of 300 minutes. Although whey ingestion created an early leucine spike, it returned to basal levels after 100 minutes, while casein ingestion created better overall leucine balance (8).

### *Insulin and blood glucose*

Hoppe et al. (18) examined the effect of milk proteins, whey and casein, on insulin secretion and blood glucose. These authors reported that whey increased fasting insulin significantly more than casein. Also, insulin resistance and pancreatic beta cell function were significantly increased in the whey group, and not in the casein group. The greater content of BCAAs, leucine, and isoleucine, in whey seem to be the main stimulus for increased insulin release compared to casein (30). The effect of whey ingestion appears to be dose-dependent because after acute consumption of various amounts of whey protein, ingestion of more than 20 grams led to increased insulin concentrations; lower blood glucose more than 5 and 10 gram doses (35). With the prevalence of Type II diabetes increasing, this alternate means of lowering

blood glucose provides a plausible and cost-effective means of protecting and improving overall health.

### *Blood Lipids*

Total cholesterol (TC) is an extremely important blood lipid measurement derived by the sum of LDL and HDL. The National Cholesterol Education Program recommends that your TC levels should not surpass 200 mg/dL (29). Once TC exceeds this number, the likelihood of coronary heart disease (CHD) greatly increases. Cholesterol promotes atherosclerosis by building up a plaque on the damaged artery wall and decreasing lumen diameter (22).

Triglycerides are broken down into free fatty acids (FFA) and monoglycerides in the lumen of the small intestine by pancreatic lipase. They are then absorbed into the enterocytes and are packaged into chylomicrons to move to the liver. Within the liver, the liposomes form lipoproteins like low-density lipoprotein (LDL), which is used to carry cholesterol throughout the body because it is insoluble in the blood. High-density lipoprotein (HDL) is used to collect cholesterol throughout the body and return it for degradation and excretion in the liver.

Pal et al. (33) studied the long-term effect of chronic ingestion of milk proteins on blood lipids in eighty-nine overweight and obese individuals between the ages of 18–65 years. Their findings suggest that fasting triglyceride (TRG) concentrations decreased in the WP group by 13% and 22% after 6 weeks and 12 weeks of whey protein supplementation. Low-density lipoprotein (LDL) plasma levels were reduced at week 12 in the whey group by 7% compared with baseline. Similar reductions were seen when compared to casein and the control groups. Total cholesterol (TC) plasma levels were decreased at week 6 in the whey group when compared to the control. After 12 weeks, WP comparatively decreased in plasma TC levels by

7% to baseline, 9% to CP and 11% to control (33). Whey protein inhibits the formation of new cholesterol in the liver (48) and inhibits the expression of genes involved in intestinal FFA and cholesterol absorption and synthesis (10).

There were no significant changes in body composition after chronic ingestion of whey protein in 70 middle-aged overweight and obese men and women during a 12-week trial (33). Beneficial changes in TC, LDL, and TRG from whey supplementation must have been unaided by changes in body fat mass (33). A meta-analysis by Baigent et al (4) reported that a reduction in just 1 mmol per L of blood of LDL cholesterol resulted in a decrease of 19% in coronary mortality. Reductions of TRG levels of 20–24% have also been shown to reduce the progression of CHD (27).

### *Blood Pressure*

Blood pressure is the most common predictor of future onset of CVD. Increased systolic blood pressure (SBP) in hypertensive subjects is likely caused by increases in arterial stiffness. This stiffness causes less cushion in the arteries and a faster pulse wave velocity (PWV) of the ejected blood. This causes higher left ventricle afterload as the heart contracts during the systole. Altogether, stiffness in the arteries, which is predicative of CVD, corresponds to the higher SBP (38).

Blood pressure regulation begins with the renin–angiotensin system and is often manipulated for hypotensive medication. Supplements that can inhibit the renin–angiotensin system can be used to treat hypertension (11). This can be accomplished by inhibiting angiotensin-converting enzyme (ACE) or by blocking angiotensin (AT1) receptors. Previous

evidence indicates that dairy milk proteins (whey and casein) inhibit ACE activity (14, 21, 39) and in vitro studies specifically indicate that whey has an anti-hypertensive effect (14, 21).

Previous research indicates that whey and casein contain peptides that inhibit ACE activity (13, 14). Casein and whey degradation produces casokinins and lactokinins, respectively, which inhibit ACE (13). Both casokinins and lactokinins have been shown to greatly reduce BP, specifically reductions in systolic blood pressure from 2 to 34 mmHg in both normotensive and hypertensive individuals (1, 31).

Pal et al. (32) demonstrated that 6-hour postprandial blood pressure (BP) and arterial stiffness did not have significant reduction with ingestion of 45 g whey protein when compared with 45 g casein and 45 g of a glucose control in overweight and obese postmenopausal women. They concluded that the expected hypotensive effects and improvement in measures of arterial stiffness from whey ingestion are likely only observed over a greater period of time. They did, however, mention that these unexpected results might be due to the test meal. Consumption of meal with the supplements likely slowed down the rate of gastric emptying, which could have delayed or inactivated bioactive components once they reached the small intestine. This likely reduced the positive effects of whey on BP and vascular function (32). In the present study, we will investigate the acute effect of consumption of whey and casein alone to avoid any confounding influences on our results.

Kawase et al. (21) studied the impact of whey protein ingestion on BP on twenty healthy male volunteers. They reported that after 8 weeks of milk ingestion enriched with whey protein, systolic blood pressure (SBP) was significantly reduced (21). A similar study showed that 12 weeks of chronic ingestion of whey (54 grams/day) improved arterial stiffness when compared with casein and a glucose control. Also, both whey and casein reduced diastolic blood pressure

(DBP) when compared with the control after 12 weeks. This implies that a higher dose of whey and, possibly, a longer duration of supplementation is required for observable effects (33). The remaining questions to answer are the acute effect of whey and casein on BP without a meal test and the effect of nighttime ingestion of whey, casein, and carbohydrate on the dependant variables.

### *Resting Metabolic Rate*

By increasing metabolic rate, our bodies become more efficient in utilizing fat stores, which then leads a decreased fat mass. Decreased fat mass has already been shown to have serious health implications, especially when applied to overweight and obese individuals. Not only does protein deter fat accumulation through satiety, but also it may increase our utilization of fat stores for fuel.

Acheson et al. (2) studied the differences between macronutrients on 23 healthy lean participants. Energy expenditure increased after test meals of whey, casein, and carbohydrate. Expectedly, the thermic effect of the milk proteins was greater than that of the carbohydrate test meal and the thermic effect of whey was greater than that of casein (34 compared to 24 kcal increase) (2). These results were conclusively attributed to a greater thermic response and fat oxidation (2). The previous concerns with BP, blood lipids, and blood glucose are all affected by visceral fat accumulation. Metabolic efficiency compounded with the acute effect of protein ingestion could have even greater positive health benefits long-term.

### *Appetite Suppression*

A high protein diet appears to play a role in body weight control because of protein's impact on decreasing hunger and increasing satiety (35,47). In fact, Weigle et al. reported that an isocaloric high-protein (30% DV) diet was able to reduce ad libitum total food intake in 19 men and women during a 2 week trial (45). This satiating effect may be due in part to greater secretion of glucagon-like peptide 1 (GLP-1) (16) and cholecystokinin (CCK) (24). GLP-1 and CCK are released upon entry of chyme into the small intestine for the purpose of increasing satiety. It has also been reported that protein is a requirement for the release of CCK into our blood (24).

A high insulin response after whey protein ingestion is due to the insulinotropic effect of whey caused by certain amino acids that have insulinogenic properties (15, 30). GLP-1 stimulates the synthesis of insulin secretion, inhibits glucagon, slows the rate of gastric motility, and inhibits hunger (12). GLP-1 is stimulated by whey's inhibition of dipeptidyl peptidase IV (DPP-IV), which is normally responsible for the breakdown of GLP-1.

There are, however, differences between whey and casein in regards to their effect on satiety. Whey at breakfast appears to suppress appetite more than casein (42). The ingestion of whey stimulated a stronger response to insulin and GLP-1. WP breakdown had an elevated production of the amino acids leucine, lysine, tryptophan, isoleucine, and threonine (42). The high-energy demands of protein breakdown may also be related to satiety (23, 47). Tryptophan has been suggested to have a direct effect on satiety because it is used as a substrate to synthesize serotonin, which is a neurotransmitter directly associated with appetite (6). Lysine has also been shown to decrease food intake in sheep (5). Threonine has been shown to reduce weight gain in rats when it was added to an already low protein diet (28). It is suggested that differences in

appetite ratings between WP and casein only appear when ingestion of each is within a certain range of protein intake (42).

## **Conclusion**

Obesity's prevalence, as well as diseases like CVD and Type II diabetes, is rising worldwide (33). It is possible that protein consumption may reduce total daily caloric intake, improve fasting blood lipids and glucose and improve metabolic rate. Therefore, protein supplementation may help in preventing obesity, reducing onset of CVD, and reducing the likelihood of Type II Diabetes. The effects of evening protein ingestion on risk factors for these diseases have been under-researched and warrant investigation. The present study will address the acute effects of protein ingestion (specifically WP and CP) in the late evening (before sleep) in an attempt to reverse diet-induced diseases like obesity, Type II diabetes, and CVD.

## **Introduction**

Overweight and obese individuals are classified as having a Body Mass Index (BMI; weight in kilograms divided by height in centimeters<sup>2</sup>) between 25-29.9 and greater than 30, respectively (9). Fat accumulation is of multi-factorial etiology, but a primary cause is caloric intake beyond our daily caloric expenditure needs. Obesity increases one's likelihood for developing life-threatening diseases like coronary artery disease and type II diabetes mellitus (T2DM), and is the leading preventable cause of death worldwide (33).

In most instances, the harmful impact of obesity is apparent when measuring blood lipids, blood glucose, blood pressure, and resting metabolic rate. Elevated blood lipids and glucose and blood pressure indicate increased risk for developing atherosclerosis, heart disease, and T2DM. In addition, variations in daily resting metabolic rate due to obesity and/or nutritional manipulation can lead to long-term changes in energy balance and, ultimately, alterations to body weight and composition. Unfortunately, these detrimental effects of obesity are quite common given that approximately 70% of the US population is considered overweight or obese (9). Therefore, appropriate research into dietary interventions to combat this growing trend in body size and disease is needed.

Obesity's devastating health effects can be offset by proper nutrient intake and exercise regimens. Interestingly, high protein diets have been shown to increase satiety and may lower total caloric intake, particularly if fat calories (9kcal/g) are replaced by protein calories (4kcal/g). Additionally, high protein diets have been demonstrated to increase energy expenditure (2). It is quite apparent from the existing evidence that including more protein in the diet will ultimately be beneficial for an overweight/obese population (3). However, which is the type of consumed protein and timing of ingestion are the most valuable questions left unanswered to date. WP and

CP have been suggested to have positive health benefits. By our inclusion of both milk proteins, WP and CP, we plan, not just to compare carbohydrates to proteins, but also to investigate differences between protein types, which are composed of specific amino acid contents.

Therefore, the purpose of this study is to investigate the acute health implications of nighttime WP ingestion in comparison to CP and PL on blood lipids and glucose, RMR, and morning hunger, which are all prognostic of health issues and disease.

## **Methods**

***Participants.*** Forty (5 men; 35 women) overweight or obese ( $\text{BMI} > 25 \text{ kg/m}^2$ ) participants (Age,  $28.9 \pm 6.6$  years; Height,  $166.2 \pm 8.8$  cm; Weight,  $99.0 \pm 20.2$  kg; Body Mass Index (BMI),  $35.7 \pm 5.9 \text{ kg/m}^2$ ; % body fat,  $46.0 \pm 5.8$ ) were recruited for this study. Each participant visited the human performance laboratory (HPL) a total of 2 times.

All participants were informed as to the experimental procedures and sign informed consent statements and medical history forms in adherence with the human subjects' guidelines of The Florida State University and with the current national and international laws and regulations governing the use of human subjects before any data collection. Exclusion criteria included uncontrolled hypertension (blood pressure (BP)  $\geq 160/100$  mmHg), current use of BP medications, diagnosed cardiovascular disease, stroke, diabetes, thyroid or kidney dysfunction, milk allergies, or pregnancy. In addition, heavy smoking ( $>20$  cigarettes per day), ingestion of cholesterol medication or nutritional supplements (except for a multivitamin), or planned exercise for more than 2 days per week for more than 40-minutes per session (within the past 6 months) were excluded.

**Procedures.** (Figure 1) The first visit to the HPL (baseline) included arriving in a fasted state (no food or drink, except water, for at least 8 hours) between 6 and 8 am in athletic clothing. Questionnaires regarding mood-state, hunger, and satiety were completed. After sitting quietly for 5-minutes, participants had their baseline blood pressure (BP) was measured twice. Resting metabolic rate (RMR) was then measured using indirect calorimetry (Parvometrics, Sandy, UT). This is a non-invasive test that involves lying down on a padded table for 30-minutes with a ventilated hood covering the head and torso. Blood was drawn for a total amount of 20 milliliters from a forearm vein (antecubital space between the upper and lower arm). The blood samples were analyzed for glucose (GLU), total cholesterol (TC), triglycerides (TRG), TC/HDL, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and non-HDL (TC-HDL) concentrations (mg/dL) utilizing the CholestechLDX blood analysis system (Hayward, CA). Hunger, satiety, and desire to eat were then assessed utilizing a Visual Analogue Scale (VAS).

After finishing the baseline visit, participants were then matched for BMI, sex, and percent body fat and randomly assigned to one of three groups in double-blind fashion: 1) 100% WP consumption in the late evening before sleep (WP), 2) 100% CP consumption in the late evening before sleep (CP), or 3) PL consumption in the late evening before sleep (Table 1). Participants in all 3 groups consumed their respective supplements as the last food or caloric beverage at night before sleep (taken at least 2 hours after dinner, but no more than 30 minutes before bed).

The WP supplement contained 30g of WP, 3g of carbohydrate, and 2g of fat for a total of 150 kcals per serving. The CP supplement contained 30g of CP, 3g of carbohydrate, and 1g of fat for a total of 140 kcals per serving. The PL supplement contained 0g of protein, 33g of

carbohydrate, and 2g of fat for a total of 150 kcals per serving. Other ingredients included small amounts sodium, potassium, and calcium for consistency and flavoring.

On the morning after nighttime consumption of the supplement (between 6 and 8 am), participants visited the laboratory in the fasted state for the second time (24 hours after visit 1). Participants were asked to eat the same foods prior to each testing day, with the exception of the evening supplement to minimize a nutritional influence on the results other than the supplement consumed. Participants were asked to bring the empty packages to ensure they complied with protocol and ingested their supplement. The identical testing procedures took place on visit 2 as were measured on visit 1 to test how acute ingestion with WP, CP, and PL supplements impacted our dependent variables.

*Statistics.* A one-way ANOVA was conducted to ensure no differences in groups for BMI and percent body fat prior to randomization of groups. Data was analyzed using a 3 x 2 (group x time) RMANOVA (JMP Pro 9, Cary, NC). A Tukey post-hoc analysis was used where appropriate to examine differences. Significance was set as  $P < 0.05$  and all data are reported as means  $\pm$  SD, unless otherwise noted.

## **Results**

### ***Blood glucose and lipids***

No significant group by time differences were measured for glucose (GLU), total cholesterol (TC), triglycerides (TRG), TC/HDL, high-density lipoproteins (HDL), low-density lipoproteins (LDL), or non-high density lipoproteins (Non-HDL; TC-HDL). Group differences were measured for GLU to be higher and HDL to be lower for CP compared to both WP and PL. (Table 2).

### ***Resting Metabolic Rate (RMR)***

No significant group by time differences were observed for RMR or respiratory quotient (RQ), although there was a magnitude change from baseline to acute for all groups. There was a main group effect ( $p=0.02$ ), which post-hoc analysis revealed there to be a significant difference between WP ( $1941.2 \pm 187.5$  kcal) and CP ( $2150.2 \pm 288.6$  kcal), but not PL ( $2035.0 \pm 211.4$  kcal) (Figure 2).

### ***Hunger, Satiety, Desire to Eat***

There was a significant time difference from baseline to acute in all three groups, but no group or group by time differences (Figure 3).

## **Discussion**

This study investigated the immediate health implications of nighttime WP ingestion in comparison to CP and PL on GLU, blood lipids, RMR, and morning hunger, which are all prognostic of health issues and disease.

Increased protein consumption has been reported to have various acute and long-term health benefits including improvements in GLU, blood lipids, RMR, and satiety (33, 2, 40, 45). In addition, protein consumed at particular times of the day has become a topic of much interest (3, 36). However, few reports have examined the impact that protein supplementation has on cardiometabolic health when consumed before sleep. Furthermore, only one study to date (36) has linked protein ingestion before bed to improvements in muscle protein synthesis, and none link nighttime protein ingestion and morning cardiovascular health, metabolism, and satiety, despite the common thought that eating before bed may be harmful to health. By our inclusion of both milk proteins, WP and CP, we planned, not just to compare carbohydrates and proteins, but also to investigate two protein types which differ in amino acid composition. When comparing

these two proteins, it is important to understand the differences in digestion of these two macronutrients. WP has been shown to more quickly raise plasma amino acid concentration when compared to CP due to the differences in the rate of gastric emptying. CP clots more in the stomach, which delays digestion while WP rapidly moves from the stomach to the duodenum (26). Due to this quick movement of WP, large amounts of amino acids are absorbed within a shorter window of time, resulting in amino acid concentrations much higher than that of CP. Acute differences in plasma amino acids content may accentuate other cardiometabolic differences between WP and CP.

Our primary findings reveal no statistically significant group by time interactions between WP, CP, and a PL in terms of fasting GLU, blood lipids, and RMR after evening ingestion. Despite the lack of significance, it is important to note that WP increased RMR by  $4.5 \pm 0.3\%$  and CP increased RMR by  $2.7 \pm 0.3\%$ , while PL decreased RMR  $2.6 \pm 0.3\%$ , which could have practical implications. In addition, significant time effects between baseline and acute were observed for all 3 groups to improve scores of hunger, with no difference between groups.

Our baseline-to-acute change in fasting GLU was not statistically significant, which agrees with a previous 7-day intervention study using WP and CP with prepubertal boys (WP: baseline  $4.52 \pm 0.38$ , post  $4.58 \pm 0.29$ ; CP: baseline  $4.47 \pm 0.26$ , post  $4.53 \pm 0.24$  mmol/L) (18). These effects on fasting GLU appear to be affected by the specific amount of macronutrient ingested and are dose-dependent (35). While we report no differences in GLU, it would be interesting to evaluate the different levels of specific branched-chain amino acids, leucine and isoleucine, within the blood, which appear to be proportional to blood insulin levels (30). This is especially appropriate considering the higher content of these specific amino acids in WP (isoleucine: 6.20%; leucine: 10.40%) compared to CP (isoleucine: 4.89%; leucine: 9.41%). Additionally,

muscle insulin sensitivity may have been affected by ingesting the supplements in the present study. Ingestion of WP for 7 days, as opposed to CP, has been shown to significantly increase insulin concentration (WP: baseline  $33.00 \pm 11.6$ , post  $39.93 \pm 14.5$  pmol/L; CP: baseline  $37.17 \pm 12.4$ , post  $40.90 \pm 23.9$  pmol/L) and increase insulin resistance using the homeostatic model of insulin resistance (HOMA) (WP: baseline  $1.12 \pm 0.42$ , post  $1.37 \pm 0.52$ ; CP: baseline  $1.25 \pm 0.46$ , post  $1.39 \pm 0.86$ ). This increase in insulin concentration and resistance occurs in proportion to one another, which is likely why hyperinsulinemia may not be recognized by GLU measurements alone (18). While we can only speculate these facts may coincide in the present study, it is possible that differences between groups may have existed in insulin concentrations. Raising insulin concentration will improve GLU only if the proportional rise in insulin resistance were combated with resistance training.

We measured no statistically significant changes in fasting blood lipids: TC, TRG, TC/HDL, HDL, LDL and non-HDL. Previous research has shown decreases in TRGs and LDLs over the course of 6 and 12 weeks with supplementation of whey in comparison to a carbohydrate placebo (33). These improvements are of significance for cardiometabolic health. TRG and LDL improvements were not associated, however, with decreases in fat mass. The divergence between our research and that of Pal et al (33) is likely due to the duration of each supplement intervention. Long-term improvements in blood lipid concentration by WP and CP ingestion, as well as the compounded effect of improvement in body fat percentage should improve cardiometabolic health over a longer period of time.

No significant changes were observed in RMR as a result of WP, CP or PL supplementation. However, WP increased RMR by  $4.5 \pm 0.3\%$  and CP increased RMR by  $2.7 \pm 0.3\%$ , while PL decreased RMR  $-2.6 \pm 0.3\%$ . These changes, albeit not significant, represent

approximately a ~140 kcal increase in daily RMR when comparing WP to PL. Extrapolating this to a full week, nighttime protein consumption could result in a ~980 kcal increase in metabolism. These positive results are consistent with Acheson et al. who also reported the greatest improvement in morning RMR in WP ( $14.4\pm 0.5\%$ ), followed by CP ( $12.0\pm 0.6\%$ ), and PL ( $6.6\pm 0.5\%$ ) during a 5-treatment (baseline measured before each morning ingestion of given supplement), diet-controlled intervention of healthy individuals (2). These increases are expected due to the complexity of proteins in comparison to simple sugars, which requires a greater amount of energy for their metabolism. The long-term practical effect of a higher protein diet may be important for decreasing fat mass and improving body composition by a consistently elevated metabolic rate.

We utilized the Visual Analogue Scale (VAS) to assess fasting hunger, satiety, and desire to eat after nocturnal sleep at baseline and following consumption of WP, CP, or PL. Other research has suggested through ad libitum trials that protein, WP in particular, decreases hunger and desire to eat by increasing satiety (40, 45). This increased feeling of satiety is due to the increased cholecystokinin (CCK) secretion in response to amino acid-rich chyme in the intestines (24). Whey is thought to increase amino acid content more so than casein and carbohydrate due to its high branched-chain amino acid composition (24). Our data reveals that whether participants ingested WP, CP, or PL they had decreased subjective feelings of hunger, increased satiety, and a decreased desire to eat the morning after ingestion of the supplement in comparison to baseline. Thus, participants felt fuller after ingesting kilocalories the night before, regardless of their composition, which has not previously been documented after an overnight fast. Serum CCK concentrations and other appetite hormones would likely reveal if any physiological measures of fullness were different between groups.

Whey and casein protein are known to have acute and long-term effects on cardiometabolic health, macronutrient metabolism, and dietary decisions after consumption in both the short-term and long-term. Our data indicate a lack of significance in any of the dependent variables; however, the benefit of WP and CP consumed before sleep may be shown to improve health when similar testing is conducted over a longer period of time or in more participants. Interestingly, the observed non-significant trend for RMR to increase for WP and CP, and a decrease for PL group may have significant influence on long-term metabolism. These results, compounded with the resulting increases in morning satiety, may provide a means of improving overall caloric balance by decreasing ad libitum intake and increasing metabolic rate. Thus, while no statistically significant findings were observed, there may be practical implications to our findings.

The study has several limitations that should be addressed. While this study was designed to test the acute impact of WP and CP on health when consumed in the evening before sleep, we might have observed different results if the study had been longitudinal in design. We also studied forty individuals, including men and women. With a larger number of participants as well as separation of data by sex, we might have found that our non-significant changes were, in fact, statistically significant. Additionally, we can only speculate as to the mechanisms behind our findings. Future investigations must include serum measurements of amino acids and insulin to reveal possible mechanisms of action. Similarly, serum concentrations of leptin and CCK might better describe the physiological response of evening WP and CP ingestion on morning hunger and satiety. In addition, we did not include measurement of regular dietary intake data for our participants, which may influence our primary outcome variables. Also, participant compliance to supplement ingestion and timing of intake (2 hours post-dinner, 30 minutes before

sleep) may not have been perfect; however, our participants reported 100% compliance with the supplement intake for this study, which was regulated by the collection of empty supplement bags at visit 2. Also, we did not control for menstrual cycle phase in the present study and this should be accounted for in future research. The addition of an exercise regimen may also alter the physiological response to these macronutrients when consumed at night before bed and round out a balanced lifestyle intervention designed to improve overall health.

## **Conclusions**

The purpose of this study was to investigate the immediate health implications of nighttime WP ingestion in comparison to CP and PL on blood GLU, blood lipids, RMR, and morning hunger, which are all prognostic of health issues and disease. No significant differences were observed among or between groups for GLU, blood lipids, RMR, and hunger. However, there was a greater magnitude of improvement for RMR for WP and CP compared to PL. Furthermore, regardless of macronutrient choice, eating before bed appeared to improve hunger, satiety, and desire to eat. Thus, protein ingestion before bed may provide a practical health benefit, although more data is warranted. It is likely that a longer duration study with more participants and exercise training would reveal significant differences between these groups.

## References

- (1) Abubakar A, Saito T, Kitazawa H, Kawai Y, Itoh T. Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. *J Dairy Sci* 1998 Dec;81(12):3131-3138.
- (2) Acheson KJ, Blondel-Lubrano A, Oguey-Araymon S, Beaumont M, Emady-Azar S, Ammon-Zufferey C, et al. Protein choices targeting thermogenesis and metabolism. *Am J Clin Nutr* 2011 Mar;93(3):525-534.
- (3) Arciero PJ, Gentile CL, Pressman R, Everett M, Ormsbee MJ, Martin J, et al. Moderate protein intake improves total and regional body composition and insulin sensitivity in overweight adults. *Metabolism* 2008 Jun;57(6):757-765.
- (4) Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005 Oct 8;366(9493):1267-1278.
- (5) Baile CA, Martin FH. Hormones and amino acids as possible factors in the control of hunger and satiety in sheep. *J Dairy Sci* 1971 Jun;54(6):897-905.
- (6) Beulens JW, Bindels JG, de Graaf C, Alles MS, Wouters-Wesseling W. Alpha-lactalbumin combined with a regular diet increases plasma Trp-LNAA ratio. *Physiol Behav* 2004 Jun;81(4):585-593.
- (7) Blaustein MP, Zhang J, Chen L, Hamilton BP. How does salt retention raise blood pressure? *Am J Physiol Regul Integr Comp Physiol* 2006 Mar;290(3):R514-23.
- (8) Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci U S A* 1997 Dec 23;94(26):14930-14935.
- (9) Center for Disease Control and Prevention (CDC). National Health and Nutrition Examination Survey. 2006.
- (10) Chen Q, Reimer RA. Dairy protein and leucine alter GLP-1 release and mRNA of genes involved in intestinal lipid metabolism in vitro. *Nutrition* 2009 Mar;25(3):340-349.
- (11) Doulton TW. ACE inhibitor-angiotensin receptor blocker combinations: a clinician's perspective. *Mini Rev Med Chem* 2006 May;6(5):491-497.
- (12) Drucker DJ. Minireview: the glucagon-like peptides. *Endocrinology* 2001 Feb;142(2):521-527.

- (13) FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. *Br J Nutr* 2000 Nov;84 Suppl 1:S33-7.
- (14) FitzGerald RJ, Meisel H. Lactokinins: whey protein-derived ACE inhibitory peptides. *Nahrung* 1999 Jun;43(3):165-167.
- (15) Frid AH, Nilsson M, Holst JJ, Bjorck IM. Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. *Am J Clin Nutr* 2005 Jul;82(1):69-75.
- (16) Gevrey JC, Malapel M, Philippe J, Mithieux G, Chayvialle JA, Abello J, et al. Protein hydrolysates stimulate proglucagon gene transcription in intestinal endocrine cells via two elements related to cyclic AMP response element. *Diabetologia* 2004 May;47(5):926-936.
- (17) Gluck ME, Venti CA, Salbe AD, Krakoff J. Nighttime eating: commonly observed and related to weight gain in an inpatient food intake study. *Am J Clin Nutr* 2008 Oct;88(4):900-905.
- (18) Hoppe C, Molgaard C, Dalum C, Vaag A, Michaelsen KF. Differential effects of casein versus whey on fasting plasma levels of insulin, IGF-1 and IGF-1/IGFBP-3: results from a randomized 7-day supplementation study in prepubertal boys. *Eur J Clin Nutr* 2009 Sep;63(9):1076-1083.
- (19) Kaplan RC, Heckbert SR, Furberg CD, Psaty BM. Predictors of subsequent coronary events, stroke, and death among survivors of first hospitalized myocardial infarction. *J Clin Epidemiol* 2002 Jul;55(7):654-664.
- (20) Katayose Y, Tasaki M, Ogata H, Nakata Y, Tokuyama K, Satoh M. Metabolic rate and fuel utilization during sleep assessed by whole-body indirect calorimetry. *Metabolism* 2009 Jul;58(7):920-926.
- (21) Kawase M, Hashimoto H, Hosoda M, Morita H, Hosono A. Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. *J Dairy Sci* 2000 Feb;83(2):255-263.
- (22) Keller KB, Lemberg L. Obesity and the metabolic syndrome. *Am J Crit Care* 2003 Mar;12(2):167-170.
- (23) Layman DK, Walker DA. Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr* 2006 Jan;136(1 Suppl):319S-23S.
- (24) Liddle RA. Regulation of cholecystokinin secretion in humans. *J Gastroenterol* 2000;35(3):181-187.
- (25) Ma Y, Bertone ER, Stanek EJ, 3rd, Reed GW, Hebert JR, Cohen NL, et al. Association between eating patterns and obesity in a free-living US adult population. *Am J Epidemiol* 2003 Jul 1;158(1):85-92.

- (26) Mahe S, Roos N, Benamouzig R, Davin L, Luengo C, Gagnon L, et al. Gastrojejunal kinetics and the digestion of [15N]beta-lactoglobulin and casein in humans: the influence of the nature and quantity of the protein. *Am J Clin Nutr* 1996 Apr;63(4):546-552.
- (27) Miller M. Current perspectives on the management of hypertriglyceridemia. *Am Heart J* 2000 Aug;140(2):232-240.
- (28) Muramatsu K, Odagiri H, Morishita S, Takeuchi H. Effect of excess levels of individual amino acids on growth of rats fed casein diets. *J Nutr* 1971 Sep;101(9):1117-1125.
- (29) National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002 Dec 17;106(25):3143-3421.
- (30) Nilsson M, Stenberg M, Frid AH, Holst JJ, Bjorck IM. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. *Am J Clin Nutr* 2004 Nov;80(5):1246-1253.
- (31) Nurminen ML, Sipola M, Kaarto H, Pihlanto-Leppala A, Piilola K, Korpela R, et al. Alpha-lactorphan lowers blood pressure measured by radiotelemetry in normotensive and spontaneously hypertensive rats. *Life Sci* 2000;66(16):1535-1543.
- (32) Pal S, Ellis V. Acute effects of whey protein isolate on blood pressure, vascular function and inflammatory markers in overweight postmenopausal women. *Br J Nutr* 2011 May;105(10):1512-1519.
- (33) Pal S, Ellis V, Dhaliwal S. Effects of whey protein isolate on body composition, lipids, insulin and glucose in overweight and obese individuals. *Br J Nutr* 2010 Sep;104(5):716-723.
- (34) Paul GL. The rationale for consuming protein blends in sports nutrition. *J Am Coll Nutr* 2009 Aug;28 Suppl:464S-472S.
- (35) Petersen BL, Ward LS, Bastian ED, Jenkins AL, Campbell J, Vuksan V. A whey protein supplement decreases post-prandial glycemia. *Nutr J* 2009 Oct 16;8:47.
- (36) Res, P. T., et al. (2012). Protein ingestion prior to sleep improves post-exercise overnight recovery. *Medicine and Science in Sports and Exercise*,
- (37) Scaglione R, Di Chiara T, Cariello T, Licata G. Visceral obesity and metabolic syndrome: two faces of the same medal? *Intern Emerg Med* 2010 Apr;5(2):111-119.
- (38) Schiffrin EL. Vascular stiffening and arterial compliance. Implications for systolic blood pressure. *Am J Hypertens* 2004 Dec;17(12 Pt 2):39S-48S.

- (39) Seppo L, Jauhiainen T, Poussa T, Korpela R. A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. *Am J Clin Nutr* 2003 Feb;77(2):326-330.
- (40) Skov AR, Toubro S, Ronn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab Disord* 1999 May;23(5):528-536.
- (41) Van Cauter E, Desir D, Decoster C, Fery F, Balasse EO. Nocturnal decrease in glucose tolerance during constant glucose infusion. *J Clin Endocrinol Metab* 1989 Sep;69(3):604-611.
- (42) Veldhorst MA, Nieuwenhuizen AG, Hochstenbach-Waelen A, van Vught AJ, Westerterp KR, Engelen MP, et al. Dose-dependent satiating effect of whey relative to casein or soy. *Physiol Behav* 2009 Mar 23;96(4-5):675-682.
- (43) Wang Y, Beydoun MA. The obesity epidemic in the United States--gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis. *Epidemiol Rev* 2007;29:6-28.
- (44) Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. *Obesity (Silver Spring)* 2008 Oct;16(10):2323-2330.
- (45) Weigle DS, Breen PA, Matthys CC, Callahan HS, Meeuws KE, Burden VR, et al. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *Am J Clin Nutr* 2005 Jul;82(1):41-48.
- (46) Westerterp-Plantenga MS. The significance of protein in food intake and body weight regulation. *Curr Opin Clin Nutr Metab Care* 2003 Nov;6(6):635-638.
- (47) Westerterp-Plantenga MS, Rolland V, Wilson SA, Westerterp KR. Satiety related to 24 h diet-induced thermogenesis during high protein/carbohydrate vs high fat diets measured in a respiration chamber. *Eur J Clin Nutr* 1999 Jun;53(6):495-502.
- (48) Zhang X, Beynen AC. Lowering effect of dietary milk-whey protein v. casein on plasma and liver cholesterol concentrations in rats. *Br J Nutr* 1993 Jul;70(1):139-146.
- (49) Calugi S, Dalle Grave R, Marchesini G. Night eating syndrome in class II-III obesity: metabolic and psychopathological features. *Int J Obes (Lond)* 2009 Aug;33(8):899-904.

### **Figure Headings.**

**Figure 1: Overall Study Design.** Study timeline. All testing was completed in the fasted state.

At baseline, participants had not consumed any evening meal the night before morning testing. At acute testing, participants had consumed whey, casein, or placebo supplementation the night before morning testing. Supplement consumption was 2-hr after the last meal and 30 min before sleep. Overweight and obese individuals participated in this study ( $n = 40$ ).

**Figure 2: Resting Metabolic Rate.** Energy expenditure at baseline and the morning after evening ingestion of whey, casein, or placebo supplements (acute) in 40 overweight and obese individuals

**Figure 3: Hunger, Satiety, and Desire to Eat.** Hunger, satiety and desire to eat as assessed using a visual analog scale in 40 overweight and obese individuals at baseline and the morning after evening ingestion of whey, casein, or placebo supplements (acute). \*,  $p < 0.05$  compared to baseline for all groups.

Figure 1

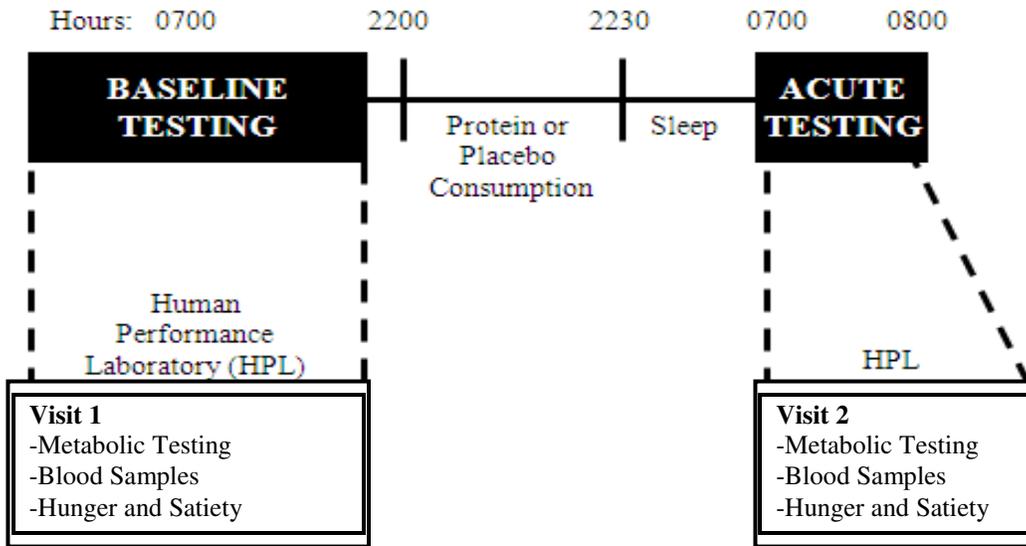


Figure 2

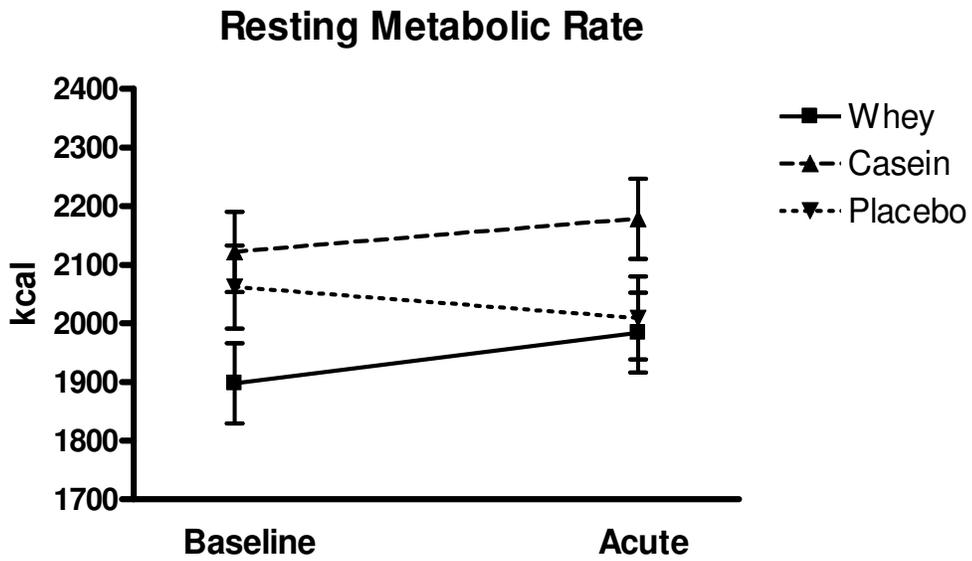
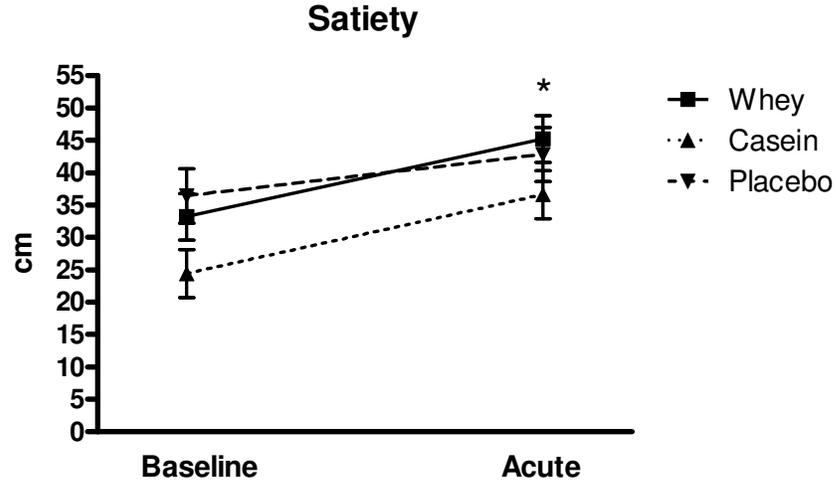
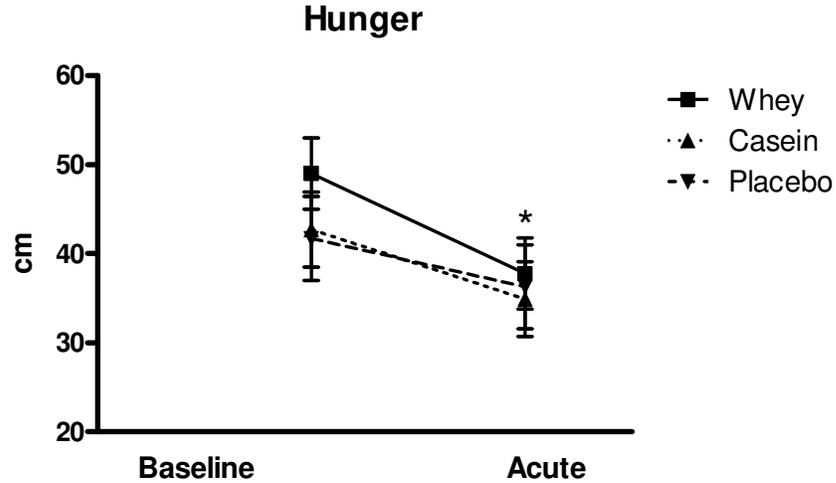


Figure 3



## Table Headings

**Table 1: Participant Characteristics.** Values are means  $\pm$  SD. BMI, body mass index; n= number of subjects.

**Table 2: Cardiovascular Measures.** Values are means  $\pm$  SD. GLU, fasting blood glucose; TC, total cholesterol; TRG, triglycerides; HDL, high-density lipoproteins; LDL, low-density lipoproteins; Non-HDL, TC-HDL; Placebo, carbohydrate;  $n = 40$ . Differences were significant at  $P < 0.05$ .

**Table 1**

	<b>Whey</b> (n=15: 2 Males)	<b>Casein</b> (n=14: 2 Male)	<b>Placebo</b> (n=11: 1 Male)
<b>Age (year)</b>	27.1 $\pm$ 5.9	32.1 $\pm$ 7.0	27.1 $\pm$ 7.2
<b>Height (cm)</b>	165.4 $\pm$ 11.2	167.0 $\pm$ 8.6	166.1 $\pm$ 5.8
<b>Weight (kg)</b>	96.5 $\pm$ 17.7	104.7 $\pm$ 24.5	95.0 $\pm$ 18.1
<b>BMI (kg/m<sup>2</sup>)</b>	35.7 $\pm$ 4.7	37.5 $\pm$ 7.8	34.2 $\pm$ 5.2
<b>% Body Fat</b>	45.6 $\pm$ 7.3	45.9 $\pm$ 6.7	46.8 $\pm$ 4.6

Values are Mean  $\pm$  SD.  $P > 0.05$  for all variables.

**Table 2**

	<b>Whey</b>		<b>Casein</b>		<b>Placebo</b>	
	<i>Baseline</i>	<i>Acute</i>	<i>Baseline</i>	<i>Acute</i>	<i>Baseline</i>	<i>Acute</i>
<b>GLU, mg/dL</b>	85.9 $\pm$ 5.4 <sup>B</sup>	84.4 $\pm$ 5.4 <sup>B</sup>	95.6 $\pm$ 5.4 <sup>A</sup>	94.8 $\pm$ 5.4 <sup>A</sup>	86.0 $\pm$ 5.4 <sup>B</sup>	86.5 $\pm$ 5.4 <sup>B</sup>
<b>TC</b>	169.0 $\pm$ 13.1	163.6 $\pm$ 13.1	161.3 $\pm$ 13.1	158.8 $\pm$ 13.1	173.6 $\pm$ 13.1	170.4 $\pm$ 13.1
<b>TRG</b>	104.6 $\pm$ 27.1	89.5 $\pm$ 27.1	99.0 $\pm$ 27.1	108.6 $\pm$ 27.1	99.3 $\pm$ 27.1	86.0 $\pm$ 27.1
<b>TC/HDL</b>	4.1 $\pm$ 5.5	6.9 $\pm$ 5.5	4.2 $\pm$ 5.2	4.0 $\pm$ 5.2	3.8 $\pm$ 5.2	3.7 $\pm$ 5.2
<b>HDL</b>	40.2 $\pm$ 8.2	41.8 $\pm$ 8.2	40.1 $\pm$ 8.3	42.1 $\pm$ 8.3	49.1 $\pm$ 8.3	48.5 $\pm$ 8.3
<b>LDL</b>	104.8 $\pm$ 16.2	105.6 $\pm$ 16.2	102.4 $\pm$ 14.5	97.3 $\pm$ 14.5	107.5 $\pm$ 14.1	108.9 $\pm$ 14.1
<b>Non-HDL</b>	120.0 $\pm$ 17.4	118.9 $\pm$ 16.3	121.8 $\pm$ 16.3	119.1 $\pm$ 16.3	124.3 $\pm$ 16.3	121.9 $\pm$ 16.3

Values not connected by the same letter are significantly different

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.

## **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, Dr. Jeong-Su Kim, Dr. Lynn Panton, 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. Sixty sedentary, overweight or obese men and women (18 to 45 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, am pregnant, or have 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 and hip circumferences, 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 noninvasive. 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 (1-RM). 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 48 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 flow 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 drawn on 3 occasions under sterile conditions (2 blood draws per each visit) and the total amount of 20 milliliters from a forearm vein (between the upper and lower arm) and finger prick and stored for later analysis. 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 three intervention groups for the duration of the four-week intervention: 1) 100% whey protein consumption in the late evening before sleep (WP), 2) 100% casein protein consumption in the late evening before sleep (CP), or 3) placebo (carbohydrate) consumption in the late evening before sleep (CON). Participants in all groups will consume their respective supplements as the last food or caloric beverage consumed prior to going to sleep.

The whey protein supplement will contain 30g of whey protein, 3g of carbohydrate, and 2g of fat for a total of 150 kcals per serving. The casein protein supplement will contain 30g of casein protein, 3g of carbohydrate, and 1g of fat for a total of 140 kcals per serving. The placebo supplement will contain 0g of protein, 33g of carbohydrate, and 2g of fat for a total of 150 kcals per serving. Other ingredients will include small amounts sodium, potassium,

and calcium for consistency and flavoring.

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 and fill out a hunger and mood-state questionnaire 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 3<sup>rd</sup> set to muscular exhaustion with a load equaling 70-85% 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. In addition, I will wear a pedometer (step-counter) daily to measure physical activity over the 4- week study.

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 1-RM and exercise training sessions. The risks associated with 1-RM 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 (1-RM), the risk will be reduced by using a submaximal strength test, the 1-RM. 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. I am aware that the facility that produces the supplements for this study may also manufacture products made from soy, wheat, and grain at the facility. It is possible that cross-contamination could occur, but is unlikely. If I have an allergy to milk, soy, wheat, or grain I must make this known to the research team.

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](mailto:mormsbee@fsu.edu)), or Amber Kinsey at [awk10d@fsu.edu](mailto: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](mailto: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

**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: \_\_\_\_\_ Age: \_\_\_\_\_  
(mm/dd/yy)

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.

Hospitalized for Disease/Operation	Duration	Age when hospitalized
---------------------------------------	----------	--------------------------

_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

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:

Date: \_\_\_\_\_

ID #: \_\_\_\_\_

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?

**Date:** \_\_\_\_\_

**ID #:** \_\_\_\_\_

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?

13. Do you exercise regularly? Y      N      What kinds of exercise?

How often? Please be detailed in a description of your average week of training.

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:

16. To what extent does snacking after dinner contribute to your weight? Circle one.

1  
Not at all

2

3

4

5  
To a very large ext

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

# Evening Protein and Exercise Training Study Data Sheet

**Subject ID:** \_\_\_\_\_ **Age:** \_\_\_\_\_ **Date:** \_\_\_\_\_ (circle one): **Baseline** **Post**

**DOB:** \_\_\_\_\_

*Women only - Start Date of Menses:* \_\_\_\_\_ *Birth Control:* \_\_\_\_\_

Heart Rate 1. \_\_\_\_\_ 2. \_\_\_\_\_

Height: \_\_\_\_\_ cm \_\_\_\_\_ in

Blood Pressure 1. \_\_\_\_\_ 2. \_\_\_\_\_

Weight: \_\_\_\_\_ kg \_\_\_\_\_ lbs

\_\_\_\_\_  
(tech initials)

Waist circumference 1. \_\_\_\_\_ 2. \_\_\_\_\_

Hip circumference 1. \_\_\_\_\_ 2. \_\_\_\_\_

Waist:Hip ratio \_\_\_\_\_

\_\_\_\_\_  
(tech initials)

## Cholestech

TC \_\_\_\_\_ LDL \_\_\_\_\_

TRG \_\_\_\_\_ HDL \_\_\_\_\_

TC/HDL \_\_\_\_\_

Non-HDL \_\_\_\_\_

GLU \_\_\_\_\_

**Notes:** (ex: clothing worn for circumferences)

---

---

---

**Supplement Compliance**

**Evening Protein and Exercise Training**

DATE: \_\_\_ - \_\_\_ - \_\_\_

Subject No: \_\_\_\_\_

Subject Initials: \_\_\_\_\_

---

	Supplement (given - initial/returned - initial)	Reminder (called - date and initial)
Initial	____ / ____	_____
Week 1	____ / ____	_____
Week 2	____ / ____	_____
Week 3	____ / ____	_____
Week 4	____ / ____	_____