ACUTE METABOLIC EFFECTS OF CONSUMING EITHER WHEY PROTEIN CONCENTRATE OR HYDROLYSATE VERSUS CARBOHYDRATE IN HEALTHY YOUNG MEN

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

COLLEGE OF HEALTH SCIENCES

BY

ROSANNE M. VOGEL, B.A., B.S.

DENTON, TX

MAY 2017

DEDICATION

For my family, thank you for your unwavering support in all my endeavors, scholastic or otherwise, without which I would not be where I am today. All my love to you.

ACKNOWLEDGMENTS

With the utmost respect and appreciation, I thank my entire committee – Dr. Ben-Ezra, Dr. Nichols, and Dr. DiMarco, for their assistance, feedback, and encouragement during the preparation of my thesis. I would particularly like to thank Dr. Nancy DiMarco for her tireless efforts on my behalf throughout my residence at Texas Woman's University as a Graduate Research Assistant. None of this would have been possible without her guidance. Additionally, I would like to acknowledge Dr. Robert Wildman, who was instrumental in the conception, planning, and execution of this study, and who secured the raw materials for the test beverages.

To our Research Technician, Nathaniel Kerr, and my fellow Graduate Research Assistants, Thomas Hoover and Sarah Tucker, I owe many thanks for their hard work and support. I also owe a huge debt of gratitude to the Co-Principal Investigator of this project, Jordan Joy, who introduced me to the world of sports nutrition research and has been a mentor, colleague, and friend for many years.

Finally, I acknowledge and appreciate every one of the participants in this study for their time, commitment, and willingness to repeatedly endure spending three hours tucked under a canopy hood in the name of science. Thank you for coming back for all of your visits and making this study a possibility.

ABSTRACT

ROSANNE M. VOGEL

ACUTE METABOLIC EFFECTS OF CONSUMING EITHER WHEY PROTEIN CONCENTRATE
OR HYDROLYSATE VERSUS CARBOHYDRATE
IN HEALTHY YOUNG MEN

MAY 2017

The acute metabolic responses to consuming isocaloric drinks containing either whey protein concentrate (WPC), whey protein hydrolysate (WPH), or carbohydrate placebo (CHO) were examined. In a single-blind, randomized, crossover design, 14 healthy young men consumed each of the treatments and had their resting energy expenditure (REE) and respiratory exchange ratio (RER) assessed by indirect calorimetry over a 3-hr postprandial period. Values were compared to a pretreatment, fasting baseline measurement using repeated-measures ANOVA. WPH and WPC significantly ($p < .05$) elevated REE versus CHO, and WPC elevated REE greater than WPH at 120 min. WPH significantly ($p < .05$) lowered RER compared to CHO and WPC at 90 min. Both proteins increased REE versus isocaloric CHO, indicating a higher thermic effect of food. WPH, but not WPC, influenced substrate utilization with a shift favoring lipid metabolism versus CHO, which may help explain previous findings of fat loss with WPH, but not WPC.

TABLE OF CONTENTS

Page

DEDICATION iii

ACKNOWLEDGMENTS iv

ABSTRACT v

LIST OF TABLES viii

LIST OF FIGURES ix

Chapter

I. INTRODUCTION 1

Statement of the Problem 4
Purpose of Study 4
Hypotheses 5
Definition of Terms 5
Assumptions 6
Limitations and Delimitations 6
Significance of Study 6

II. LITERATURE REVIEW 8

Effect of Protein Ingestion on Physiological Responses 8
Effect of Protein Source on Physiological Responses 9
Effect of Whey Protein and its Hydrolyzed Form on Physiological Responses 11

III. METHODS 13

Participants 13
Inclusion Criteria 13
Exclusion Criteria 14
Instruments and Equipment 14
Procedures 15
Preliminary Screening Visit 15
Treatment Visits 17

Test Beverages 18
Calculations and Statistical Analyses 18

IV. RESULTS 20

Participants 20
Resting Energy Expenditure 21
Respiratory Exchange Ratio 23
Thermic Effect of Food 25

V. DISCUSSION 27

Possible Limitations 32
Future Research 33
Study Implications 34
Conclusion 34

REFERENCES 35

APPENDICES

A. IRB Approval Letter/IRB Modification Approval Letter 43
B. Informed Consent 46
C. Health History Questionnaire 53
D. Demographic Questionnaire 56

LIST OF TABLES

Table Page

1. Participant Characteristics 20

2. Resting Energy Expenditure Summary Data 22

3. Respiratory Exchange Ratio Summary Data 24

4. Thermic Effect of Food Summary Data 25

LIST OF FIGURES

Figure Page

1. Resting Energy Expenditure Response During Each Test Visit 22

2. Respiratory Exchange Ratio Response During Each Test Visit. 24

3. Thermic Effect of Food Cumulative Kcal Presented in 30 Min Intervals 26

4. Thermic Effect of Food Cumulative Kcal Over 180 Min 26

CHAPTER I

INTRODUCTION

Proteins, and their constituent amino acids, are indispensable nutrients. The word protein itself is derived from the Greek word “proteios” meaning “primary” or “most important” (Vickery, 1950). Ingested proteins provide the building blocks required for both the structure and function necessary to maintain life, including nearly all biological processes (Morell & Fiszman, 2016). High dietary intake of protein, while once considered potentially harmful, is now becoming more commonplace. Indeed, as the obesity epidemic continues to escalate worldwide, and along with it the economic burden of disease, great interest has been placed on the putative metabolic benefits of high protein diets. Weight loss and subsequent weight maintenance is promoted when protein intakes substantially exceed the current recommended dietary intake of 0.8 g per kilogram body weight daily (Institute of Medicine [IOM], 2006; Pencharz, Elango, & Wolfe, 2016; Phillips, Chevalier, & Leidy, 2016).

Increased dietary protein intake positively influences metabolism, satiety, and body composition when compared with isoenergetic diets containing greater proportions of carbohydrate or fat (Arciero et al., 2013; Due, Toubro, Skov, & Astrup, 2004; Halton & Hu, 2004; Mikkelsen, Toubro, & Astrup, 2000). For instance, the thermic effect of food (i.e., the increase in energy expenditure following nutrient ingestion) for protein is much greater than that of carbohydrate or fat, typically requiring 20-35% of the ingested energy for digestion, absorption and disposal, compared to approximately 5% for carbohydrate and fat (Acheson et al., 2011; Halton & Hu, 2004). The difference in the thermic effect of food may be a consequence of the

body's lack of storage capacity for protein, and hence the need for proteins to be immediately processed and metabolized into usable substrates for other processes (e.g., protein synthesis, urea production, gluconeogenesis, etc.).

Moreover, high protein diets, or those with at least 25% of energy intake as protein, are more satiating than isoenergetic diets with a lower protein content. Increased satiety, in turn, reduces subsequent energy intake, which may lead to a decrease in body weight over time (Morell & Fiszman, 2016). In fact, when looking at long term studies, high protein diets appear to be advantageous for both overall weight loss as well as fat loss, perhaps due to a combination of increased energy expenditure along with reduced energy intake (Halton & Hu, 2004). Additionally, it appears that high protein intakes are beneficial for athletic individuals seeking improvements in body composition, performance, and recovery (Arciero et al., 2013; Phillips & Van Loon, 2011; Wilson et al., 2013). As such, supplemental proteins, in the form of powders, ready-to-drink shakes, bars, and fortified foods, have become a convenient method of incorporating more protein into the diet. However, supplemental proteins derived from different sources (e.g., dairy, soy, egg) can have diverse physiological effects, owing to their unique amino acid profiles and digestion and absorption kinetics (Acheson et al., 2011; Alfenas, Bressan, & Paiva, 2010; Hulmi, Lockwood, & Stout, 2010; Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009).

Dairy proteins are widely consumed both in their natural state, as milk or milk-derived food products, or as supplemental proteins that have been processed and concentrated into various forms. Bovine milk-derived proteins, comprised of an approximate 1 to 4 ratio of whey to casein protein fractions, provide a rich source of the nine essential amino acids required by

humans for protein synthesis in the body (McGregor & Poppitt, 2013). Due to its rapid gastric emptying when compared to casein, whey protein in particular is often consumed in supplemental form by active individuals looking to increase muscle mass, improve body composition, or otherwise positively influence health (Ha & Zemel, 2003; Hulmi et al., 2010). Supplemental whey protein is produced from milk via coagulation with rennet or acid to precipitate out the casein component, followed by filtration and drying to produce a whey protein concentrate (WPC) which can be upwards of 80% protein by weight (McGregor & Poppitt, 2013). Whey protein isolate (WPI) is produced in a similar manner, with the resulting protein content accounting for more than 90% of total weight. WPC or WPI may be further broken down or partially "pre-digested" into shorter amino acid chains (peptides) by enzymatic or acid hydrolysis to produce whey protein hydrolysate (WPH). Cleaving intact whey proteins into shorter (i.e., di-, tri-, and oligo-) peptides speeds intestinal absorption and thereby augments the rate of amino acid availability following ingestion (Mannien, 2009; Wilson et al., 2013). Additionally, bioactive peptides are released during the process of hydrolysis which may confer unique health benefits when compared to the intact protein (Clemente, 2000; Nongonierma & FitzGerald, 2015; Yalçin, 2006). In acute rodent studies, lipolysis is upregulated in response to WPH feeding, whereas other intact protein forms, including WPC or egg, do not elicit the same effects (Mobley et al., 2015; Roberts et al., 2013). WPH has also been more effective at stimulating fat loss compared to WPC in a chronic resistance training model with healthy young men (Lockwood et al., 2016). Based on these findings, energy metabolism is an area of research that warrants further investigation when examining different forms of whey protein.

Statement of the Problem

Differences between the effects of protein and carbohydrate consumption on metabolism and energy expenditure are well documented; however, less is known regarding differences between various protein forms, particularly hydrolyzed versus intact proteins. Ingestion of hydrolyzed proteins may provide unique physiological advantages, such as improved glucose control (Morato et al., 2013a; Morato et al., 2013b), enhanced insulin and incretin response (Luscombe-Marsh et al., 2016; Power, Hallihan, & Jakeman, 2009) antihypertensive effects (Lacroix, Meng, Cheung, & Li-Chan, 2016), antioxidant effects (Lollo et al., 2014; Vavrusova et al., 2015), and improved body composition and weight control (Nobile et al., 2016). Recent research indicates enhanced postprandial lipolysis (Roberts et al., 2013) and decreased fat mass over time (Lockwood et al., 2016) result from ingestion of hydrolyzed whey protein when compared to its intact form. However, the mechanisms by which these effects occur have yet to be fully elucidated. As such, the purpose of the present study was to examine whether an increase in metabolic rate (as indicated by REE) or a shift in substrate utilization (as indicated by RER) favoring increased lipid oxidation may account for some of the observed acute and chronic lipolytic effects of WPH supplementation.

Purpose of Study

The purpose of this investigation was to examine the acute metabolic effects of consuming isocaloric WPH, WPC, and carbohydrate placebo (CHO) treatments in healthy young men. Specifically, the dependent variables under investigation included resting energy expenditure (REE), and respiratory exchange ratio (RER).

Hypotheses

1. Both WPH and WPC will induce a greater increase in REE than CHO over the 3-hr postprandial period.
2. WPH will induce a greater increase in REE than WPC over the 3-hr postprandial period.
3. Both WPH and WPC will induce a lower RER than CHO over the 3-hr postprandial period.
4. WPH will induce a lower RER than WPC over the 3-hr postprandial period.

Definition of Terms

Indirect calorimetry: Non-invasive method of quantifying REE based on the collection and measurement of inspired/expired gas concentrations and volumes. Relies on the use of a metabolic cart and open circuit spirometry with a mixing chamber. Gases are collected from a subject breathing into a mouthpiece or canopy hood, then analyzed and calculated by manufacturer software (da Rocha, Alves, & da Fonseca, 2006).

Resting energy expenditure (REE): The amount of energy expended while an individual is resting quietly in a supine position, typically expressed as kcal per unit of time (Plowman & Smith, 2014).

Respiratory exchange ratio (RER): The ratio of the volume of carbon dioxide produced (VCO_2) to the volume of oxygen (VO_2) consumed. Often used as an indicator of substrate utilization, or, the relative amounts of carbohydrate and lipid oxidized for energy production, assuming minimal contribution from protein oxidation (Livesey & Elia, 1988).

Thermic effect of food (TEF): The increase in energy expenditure above REE following the ingestion of a meal (Psota & Chen, 2014). It reflects energy required for digestion, absorption, and metabolism of nutrients consumed (Halton & Hu, 2004).

Assumptions

1. Participants were free of any acute or chronic metabolic or physiological condition which may have affected data collection via the use of indirect calorimetry.
2. Participants followed the same exercise, sleep, and nutrition patterns 24 hr prior to each testing visit.
3. The three treatments (WPC, WPH, CHO) remained blinded to the participants.
4. Indirect calorimetry provides a valid and reliable measure of REE.
5. RER serves as a valid indication of substrate utilization.
6. Alterations in REE following treatment consumption are indicative of TEF.

Limitations and Delimitations

1. This study was limited to the population sampled: healthy, lean, recreationally active young men recruited primarily from a college campus.
2. The study was limited by a single-blind design.
3. This study was delimited to a 3-hr postprandial period of observation.
4. This study was delimited to 0.3 g/kg lean soft tissue of protein or carbohydrate per treatment based on literature suggesting this amount is sufficient to maximally stimulate an anabolic effect in healthy young males (Moore et al., 2015).

Significance of Study

Previous research has investigated the acute metabolic effects on REE and RER of different protein sources (Acheson et al., 2011; Alfenas et al., 2010; Bendtsen et al., 2014; Lorenzen, Frederiksen, Hvid, & Astrup, 2012) yet none have directly compared WPH and WPC. Furthermore, because the underlying mechanisms by which increased fat loss observed with

WPH compared to WPC are not clearly understood, the present study sought to determine whether acute alterations in energy expenditure or substrate utilization could account for chronic changes observed by previous researchers. This study adds to the existing literature, particularly that by Lockwood and colleagues (2016), which suggests that WPH supplementation may enhance fat loss compared to WPC over an 8-week period in resistance trained men. In addition, the present study may provide rationale for consumers, athletes, and dietitians as to why WPH supplementation would be beneficial over WPC in both healthy, active individuals as well as those attempting to improve body composition and thereby overall health status.

CHAPTER II

LITERATURE REVIEW

Effect of Protein Ingestion on Physiological Responses

Increased dietary protein intake is known to positively influence metabolism, satiety, and body composition (Alfenas et al., 2010; Halton & Hu, 2004; Morell & Fiszman, 2016). Additionally, many active individuals and competitive athletes consume supplemental protein to improve performance and recovery (Wildman & Miller, 2010; Wilson et al., 2013), build and maintain lean mass (Morton, McGlory, & Phillips, 2015), as well as promote overall health (Ha & Zemel, 2003). High protein diets also result in greater maintenance of fat free mass during caloric restriction (Longland, Oikawa, Mitchell, Devries, & Phillips, 2016; Pencharz et al., 2016; Phillips et al., 2016; Phillips & Van Loon, 2011).

In a recent study by Longland, Oikawa, Mitchell, Devries, and Phillips (2016), a hypocaloric diet creating a 40% deficit in daily caloric needs combined with 6 days/week resistance and high intensity interval training resulted in substantial weight loss for both a lower protein intake (1.2 g/kg/day) group and a high protein intake (2.4 g/kg/day) group of overweight young men. Interestingly, the high protein group lost predominantly fat (nearly 5 kg) and significantly ($p < .05$) increased lean body mass (1.2 kg) over the 4-week intervention, while the low protein group did not gain lean mass (0.1 kg) and lost significantly less fat mass (3.5 kg), with no difference in total weight loss between groups ($p > .05$).

Arciero and colleagues (2013), examined the effects on body composition and thermogenesis after 8 weeks following either a high protein diet or a traditional (moderate)

protein diet, providing 35% or 15% of energy from protein, respectively, in conjunction with a regular exercise program. They found that the high protein group had more favorable body composition outcomes, including reduced fat mass, visceral adipose tissue, and total body weight, both during 4 weeks of energy balance and after 4 weeks of a prescribed 25% energy deficit.

Effect of Protein Source on Physiological Responses

Previous research has examined the different physiological effects of various sources of supplemental proteins, including whey, casein, egg, and soy, on both acute and chronic measures of metabolism, body composition, athletic performance, and a multitude of biochemical parameters. Acheson and colleagues (2011) compared the acute metabolic effects of consuming isonitrogenous amounts of either whey, soy, or casein-supplemented meals compared to an isocaloric high-carbohydrate (CHO) meal, using a crossover design in 23 healthy adults. While all three protein-rich meals caused a greater thermic effect than CHO over the 330-min postprandial period, whey meal induced the greatest thermic effect of the three proteins, resulting in a 14.4% increase in energy expenditure above fasting values, versus 12% and 11.6% for casein and soy, respectively (all, $p < .01$). Lipid oxidation was also significantly ($p < .05$) greater for whey and soy supplemented meals than for the CHO meal, and tended ($p = .097$) to be greater after whey than soy meal (16.2 g versus 13.7 g fat). Taken together, it would appear that different sources of supplemental protein have diverse effects on energy expenditure, despite being isonitrogenous.

In another acute crossover study, Tang, Moore, Kujbida, Tarnopolsky, and Phillips (2009) examined the effects of ingesting either WPH, micellar casein, or soy protein, all matched to

provide 10 g essential amino acids, on muscle protein synthesis (MPS), both at rest and following an acute bout of resistance exercise. MPS response to WPH was 93% and 18% greater compared to casein and soy, respectively, at rest, and following exercise MPS response to WPH was 122% and 31% greater than casein and soy, respectively. The greater leucine content of WPH could account for its advantage over casein and soy in stimulating MPS, as leucine is a potent stimulator of MPS, triggering the process of translation by activating the mammalian target of rapamycin complex (Anthony, Anthony, Kimball, & Jefferson, 2001). It is plausible that the diverse acute physiological effects of different dietary proteins could in turn influence long-term adaptations to exercise training.

Kanda and colleagues (2016) recently examined the acute effects on MPS of supplemental milk protein (comprising both whey and casein fractions), compared with whey, casein, or soy protein in exercised rats. While all milk-derived protein sources caused greater MPS (as indicated by fractional synthetic rate of the triceps muscle; FSR) than soy ($p < .05$), the peak FSR for each dairy protein occurred at a different time point, with whey being the fastest onset (60 min post), followed by milk protein (90 min post), and casein (120 min post). Of note, whey protein caused a significantly greater rise in plasma and intramuscular BCAA and leucine concentrations, and did so at a faster rate (30 min post administration) compared with any of the other protein sources. Even within proteins of similar source (i.e., milk-derived), it appears that differences in absorption characteristics can result in different effects on physiological responses, such as peak FSR.

Effect of Whey Protein and its Hydrolyzed Form on Physiological Responses

More recently, researchers have begun to examine the differential effects of intact whey protein versus its hydrolyzed forms. In the rat model, Roberts and colleagues (2013) investigated the acute effects of isonitrogenous feedings of either WPC or WPH on metabolic biomarkers, including the rate of appearance of individual amino acids and various di- and oligopeptides, circulating free fatty acids, and catecholamines. WPH elevated serum free fatty acid concentrations, indicating increased lipolysis, compared with WPC treatment. In addition, increased concentrations of epinephrine, ketone bodies, and carnitine esters following the WPH feeding compared to WPC were observed, indicating a shift in substrate utilization towards lipid oxidation in the WPH group. Bioactive peptides produced during hydrolysis in WPH acted as adrenal secretagogues, similar to the action of pituitary adenylyl cyclase activating peptide (PACAP), causing catecholamine release which in turn upregulated lipolysis. Such findings begin to shed light on possible mechanisms whereby WPH has advantages over WPC from a metabolic standpoint.

In the human model, Lollo and colleagues (2014) compared the effects of ingesting 1 g/kg body weight/day intact whey protein (WPC) compared to WPH or a maltodextrin placebo on body composition, performance, and biochemical markers in elite Brazilian soccer players during 12 weeks of intense training. While maltodextrin supplementation resulted in a decrease in lean body mass over the 12-week period, the WPH group maintained lean mass, and the WPC group increased lean mass over this same period. Moreover, the WPH group exhibited a significant decrease in biochemical markers associated with muscle damage and catabolism,

namely creatine kinase and lactate dehydrogenase, leading researchers to speculate that WPH may possess unique antioxidant or anti-catabolic properties not present in WPC.

Recently, Lockwood et al. (2016) reported that twice daily supplementation with 30 g of WPH during 8 weeks of resistance training in college-aged males resulted in a 6% decrease in fat mass compared to an isocaloric carbohydrate placebo, while a WPC treatment group did not exhibit these same effects. It is worth noting that all groups in the trial gained lean mass over the 8-week period, with no differences between groups. Therefore, it seems plausible that WPH supplementation may specifically enhance fat oxidation.

Results such as those observed by Lollo et al. (2014) and Lockwood et al. (2016) suggest a unique ability of WPH compared to its intact form to improve body composition over time, through the preservation of lean mass and decrease in fat mass and percent body fat in response to an intense physical training stimulus. Yet, relatively little is known regarding the acute metabolic effects of WPH compared with WPC in humans, specifically how hydrolysis influences energy expenditure and substrate utilization.

CHAPTER III

METHODS

Participants

Twenty recreationally active males between 18 and 30 years of age and willing to give informed consent were recruited for participation in this investigation. Recruitment took place via word of mouth, flyer distribution, and email outreach at Texas Woman's University in Denton, TX. Interested parties were directed to contact the primary investigator and, following an informal phone or email screening questionnaire, were scheduled to visit the Pioneer Performance Clinic for an initial screening visit. At the initial visit, they provided written informed consent and underwent a preliminary screening assessment to determine eligibility. Researchers were present to answer questions, discuss potential risks and benefits of participation, and obtain informed consent before proceeding with any data collection. The study design and procedures were reviewed and approved by the Texas Woman's University Institutional Review Board, Denton, Texas (Appendix A).

Inclusion Criteria

Participants in this study were required to be male, between the ages of 18 and 30, and healthy as determined by responses to a health history questionnaire (Appendix C). Furthermore, participants needed to be recreationally active (i.e., non-sedentary), defined as participating in some form of intentional physical activity more than twice weekly for at least 30 min/day for the past 6 months (American College of Sports Medicine [ACSM], 2013). Participants were required to have a normal blood glucose response to a 75 g oral glucose

tolerance test (OGTT), defined as a blood glucose concentration of less than 100 mg/dl before, less than 200 mg/dl after 1 hr, and less than 140 mg/dl after 2 hr.

Exclusion Criteria

Participants were excluded from the study if they presented with a history of medical conditions that may have significantly affected the study outcome, including cardiovascular disease, or any metabolic, renal, hepatic, or endocrine disorders. Participants were also excluded if they were unwilling to refrain from consuming any drug or nutritional supplement that may have significantly affected the study outcome (e.g., proteins, amino acids, thermogenic supplements, ergogenic aids, etc.). Tobacco users were excluded, as well as anyone habitually consuming 12 alcoholic beverages or more per week. Those with a body mass index (BMI) of 30 kg/m^2 or greater coincident with a body fat percentage of 25% or greater were also excluded. Finally, anyone unwilling to refrain from partaking in exercise 24 hr prior to testing, and any strenuous exercise 48 hr prior to testing were excluded, along with anyone not willing to consume the same "repeat" diet 24 hr prior to each testing visit.

Instruments and Equipment

REE and RER were measured via indirect calorimetry using a metabolic cart with a ventilated canopy hood system for assessment of metabolic rate (TrueOne 2400, Parvo Medics, Sandy, UT). The Parvo Medics TrueOne 2400 system has been previously validated against criterion methods, including the Douglas bag technique, and found to provide a reliable and accurate measurements of RMR and RER (Cooper et al., 2009; Crouter, Antczak, Hudak, DellaValle, & Haas, 2006). Before measurements, calibration of the metabolic cart was performed following the manufacturer's specifications, allowing at least 30 min of warm up time

after switching on the equipment. All assessments were made in a thermoneutral environment (18-23 °C) in accordance with currently established best practices for performing indirect calorimetry in healthy individuals (Fullmer et al., 2015). Briefly, participants were required to lie supine on an exam table with arms at their sides while the ventilated canopy hood was placed over their head and tucked in around them to minimize air leakage. Participants were instructed to lie quietly for the duration of the test, without fidgeting or falling asleep, while inspired oxygen and expired carbon dioxide gases were collected and analyzed by the metabolic cart. Gases were sampled continuously and values reported as the average of each 30 s interval by the metabolic cart software.

Procedures

In a single-blind, crossover design, participants ingested 0.3 g/kg LST of WPH, WPC, or carbohydrate placebo (as maltodextrin) in a randomized order and had their resting energy expenditure (REE) and respiratory exchange ratio (RER) measured over a 3-hr postprandial period by indirect calorimetry. Randomization occurred via a random sequence generator tool. Measured values were compared to a pretreatment, fasting baseline value obtained at each treatment visit. In total, four visits to the Pioneer Performance Clinic occurred, including one preliminary screening visit and three treatment visits, with approximately one week (but no less than 72 hr) between visits.

Preliminary Screening Visit

Following an overnight fast of 8 to 12 hr, participants reported to the Pioneer Performance Clinic between the hours of 6:00 and 8:00 a.m. to provide informed consent to participate, complete paperwork, and undergo preliminary testing to determine study

eligibility. Documents completed by participants included the IRB-approved informed consent document (Appendix B), a health history questionnaire (Appendix C), and a demographic information questionnaire (Appendix D). The purpose of each questionnaire is described below. *Health History Questionnaire:* The purpose of the health history questionnaire was to identify individuals who may have had underlying or diagnosed medical conditions which could potentially confound the results of the study (e.g., hypothyroidism, acute infection, etc.). *Demographic Questionnaire:* The purpose of the demographic questionnaire was to characterize who participated in the study. All visitors to the Pioneer Performance Clinic are required to fill out this form.

Upon completion of the required documents, participants were asked to remove their shoes for height and weight measurements using a wall mounted stadiometer and digital scale (Tanita BWB-800, Arlington Heights, Illinois, USA), with values recorded to the nearest 0.1 cm and 0.1 kg, respectively. Body composition was assessed by dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, enCORE, software version 10, GE, Madison, WI) to determine body fat percentage and lean soft tissue (LST) mass. LST is determined by the manufacturer software and consists of lean body mass minus bone mineral content. Participants subsequently underwent an oral glucose tolerance test (OGTT) by consuming a 75-g glucose solution (Trutol 75, Fisher Scientific, Middletown, VA) and having their blood glucose levels assessed via finger prick using a handheld blood glucose meter (Freestyle Lite, Abbott Diabetes Care, Alameda, CA). Blood samples of approximately 10 µl were taken immediately prior to ingestion of the glucose solution, and after 1 and 2 hr post-ingestion.

Once eligibility was confirmed, participants were distributed a blank 24-hr dietary recall journal and asked to record everything they ate or drank, including quantities and time of day, before their next scheduled appointment. They were instructed to refrain from consuming caffeine, alcohol, protein supplements, ergogenic aids, or amino acids during the 24-hr pretreatment period. Participants were given a copy of the completed journal during the first treatment visit so the diet could be replicated prior to each remaining visit. Furthermore, during the 48 hr prior to treatment visits, subjects were instructed to perform no strenuous physical activity (e.g., heavy resistance training or running), and no exercise of any type 24 hr prior to any treatment visit.

Treatment Visits

Following an overnight fast of at least 10 hr, participants reported to the Pioneer Performance Clinic between the hours of 6:00 and 8:00 a.m. Control procedures at each visit included verification that the repeat diet had been consumed as evidenced by participant food logs, along with verbal confirmation that all pre visit stipulations had been followed, including 24-hr abstention from caffeine, alcohol, protein/amino acid supplements, and exercise. If all control procedures were satisfied, participants were weighed barefoot at each visit to provide accurate data for the metabolic cart software program. A baseline, fasting REE/RER assessment was determined before (-20 min) ingestion of the test beverage. Following the baseline assessment, participants ingested the test beverage before continuous REE/RER measurements commenced. Continuous measurements took place over the subsequent 180 min, with 10-min rest breaks after each hour of testing to allow participants to use the restroom or drink water, as needed. Participants were advised to limit extraneous physical activity during the breaks.

Test Beverages

Each treatment consisted of one of the following mixed in 300 ml room temperature, filtered water: 0.3 g/kg LST whey protein hydrolysate (Lacprodan® HYDRO.365, Arla Foods, Viby J, Denmark), 0.3 g/kg LST whey protein concentrate (80% protein content; Lacprodan® SP-8011, Arla Foods, Viby J, Denmark), or 0.3 g/kg LST carbohydrate (as maltodextrin). All test beverages were sweetened with 50 mg of nonnutritive sweetener (sucralose), and flavored with 636 mg natural vanilla flavor to enhance palatability and ensure participants remained blinded to the treatment. Additionally, all beverages were provided to participants premixed in an opaque, lidded container to conceal any visual differences between treatments which may have compromised the blinding. Participants were asked to consume the entire beverage within 3 min.

Calculations and Statistical Analyses

Differences in baseline values were evaluated using simple analysis of variance (ANOVA). Thermic effect of food (TEF) was calculated from REE incremental area under the curve (AUC), determined using the trapezoidal method for the 3-hr period, as described previously by Shechter, Rising, Wolfe, Albu, & St-Onge (2014). Time points included for analyses were the pre ingestion baseline (BL; -20 min), and 15 (15P), 30 (30P), 45 (45P), 60 (60P), 90 (90P), 105 (105P), 120 (120P), 150 (150P), 165 (165P), and 180 (180P) min post ingestion, using the mean of the values recorded over the previous 10 min. Values collected for the aforementioned time points for REE and RER were analyzed using 3x11 (Treatment x Time) repeated-measures ANOVA. Differences in TEF values, calculated in 30-min intervals from REE incremental AUC data, were analyzed using repeated-measures ANOVA. If significant interaction

effects were observed, Bonferroni *post hoc* analyses were used to determine differences between treatments. $P \leq .05$ was considered significant. Results were analyzed with Statistica software (version 10, StatSoft, Tulsa, OK, USA). All data are reported as means ± standard deviation.

CHAPTER IV

RESULTS

Participants

Twenty recreationally active, healthy young males were screened for inclusion in the present study. Of these, two participants were excluded due to abnormal blood glucose responses (e.g., measured values exceeded 200 mg/dl after 1 hr) to the OGTT, one participant withdrew due to discomfort experienced during the experimental protocol (lightheadedness), one participant withdrew due to an unresolvable scheduling conflict, and two participants were excluded due to lack of compliance with the study requirements. Therefore, a total of 14 participants were included for analyses. Compliance to dietary and exercise restrictions was 100%, as participants were disallowed to participate in testing and asked to reschedule in the event they did not follow their pre visit instructions. Participant characteristics are presented in Table 1.

Table 1

Participant Characteristics

Characteristic	Mean	*SD*	Range
Age (y)	23.0	2.8	19.0 - 28.0
Height (cm)	175.1	8.8	162.5 - 190.5
Weight (kg)	76.3	12.5	57.1 - 105.1
BMI (kg/m^2)	24.8	2.8	21.1 - 30.6
Fat Mass (kg)	13.5	3.6	9.0 - 19.5
BF %	18.3	3.6	15.0 - 26.2
LST (kg)	60.1	10.1	46.5 - 84.8
REE (kcal/min)	1.37	0.03	1.06 - 1.97

Note. n = 14; BMI = body mass index; BF % = body fat percentage; LST = lean soft tissue; REE = resting energy expenditure.

Resting Energy Expenditure

REE summary data and response graph are presented in Table 2 and Figure 1, respectively. One-way ANOVA revealed no significant differences at BL between each of the three treatment visits ($p = 0.979$), with a mean fasting REE of 1.37 ± 0.03 kcal/min (1982.2 ± 301.5 kcal/day) for all three treatment visits. Two-way, repeated measures (Treatment x Time) ANOVA indicated a significant main effect for time ($F = 18.422$, $p < .001$). Additionally, a significant interaction of treatment and time was also detected ($F = 4.764$, $p < .01$). Bonferroni *post hoc* analysis indicated significant ($p < .05$) differences between WPC and CHO, wherein WPC induced a greater increase in REE for all time points after 45P. Similar differences were also observed between WPH and CHO, with WPH causing a greater increase in REE at 30P, and all time points 60P-165P (all, $p < .05$). Finally, pairwise comparison between WPC and WPH revealed a significant difference between the two treatments at 120P, wherein WPC exhibited a greater increase in REE from BL than WPH ($p < .05$).

Table 2

Resting Energy Expenditure Summary Data

	BL	15P	30P	45P	60P	90P	105P	120P	150P	165P	180P
WPC	1.37 ± 0.19	1.42 ± 0.22	1.47 ± 0.22	1.54 ± 0.23*	1.56 ± 0.27*	1.51 ± 0.21*	1.47 ± 0.19*	1.48 ± 0.20*‡	1.43 ± 0.20*	1.42 ± 0.22*	1.44 ± 0.23*
WPH	1.38 ± 0.25	1.48 ± 0.29	1.53 ± 0.31*	1.51 ± 0.28	1.54 ± 0.31*	1.49 ± 0.26*	1.45 ±0 .24*	1.44 ± 0.25*	1.43 ± 0.25*	1.45 ± 0.27*	1.42 ± 0.21
CHO	1.37 ± 0.20	1.44 ± 0.20	1.43 ± 0.22	1.44 ± 0.24	1.39 ± 0.26	1.35 ± 0.26	1.34 ± 0.19	1.34 ± 0.20	1.36 ± 0.22	1.36 ± 0.22	1.37 ± 0.21

Note. Resting energy expenditure summary data presented as kcal/min; n = 14; WPC = whey protein concentrate, WPH = hydrolyzed whey protein, CHO = carbohydrate placebo; *Significantly different from CHO ($p < .05$) ‡ WPC significantly different from WPH ($p < .05$); all data reported as mean ± *SD*.

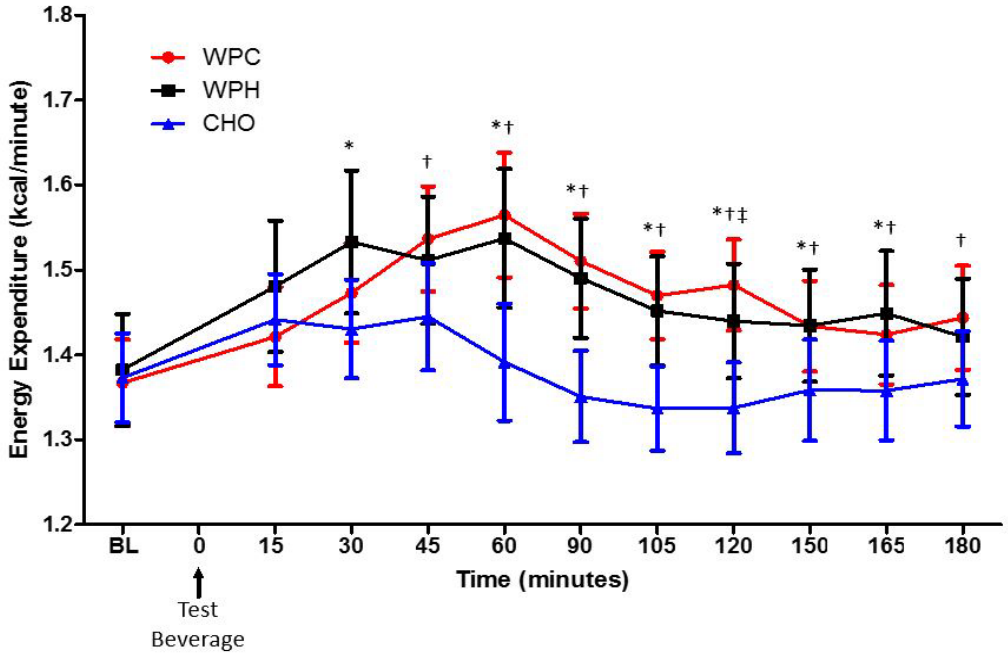

Figure 1. Resting energy expenditure response during each test visit. n = 14; WPC = whey protein concentrate; WPH = hydrolyzed whey protein; CHO = carbohydrate placebo; * WPH significantly different from CHO ($p < .05$); † WPC significantly different from CHO ($p < .05$); ‡ WPC significantly different from WPH ($p < .05$).

Respiratory Exchange Ratio

RER summary data and response graphs are presented in Table 3 and Figure 2, respectively. One-way ANOVA revealed no differences between treatments at BL for RER (p = 0.36), with a mean fasting RER of 0.82 ± 0.04 for all three treatment visits. Two-way, repeated measures ANOVA revealed a significant main effect for time (F = 8.90, p < .001) as well as a significant interaction of treatment and time (F = 2.15, p < .01). *Post hoc* analyses revealed a significant difference (p < .05) between WPH and CHO at 90P, with the two treatments producing divergent results. Compared to BL values, WPH decreased RER at 90P, whereas CHO increased RER. Similar divergent responses were also observed between WPC and WPH at 90P (p < .05), with WPC exhibiting an increase in RER over BL, while WPH decreased RER values. No other significant differences were observed at any other time points for RER.

Table 3

Respiratory Exchange Ratio Summary Data

	BL	15P	30P	45P	60P	90P	105P	120P	150P	165P	180P
WPC	0.803 ± 0.042	0.779 ± 0.052	0.792 ± 0.037	0.803 ± 0.037	0.814 ± 0.038	0.819 ± 0.031†	0.806 ± 0.037	0.803 ± 0.031	0.802 ± 0.034	0.803 ± 0.023	0.806 ± 0.031
WPH	0.821 ± 0.045	0.790 ± 0.040	0.806 ± 0.050	0.810 ± 0.032	0.825 ± 0.049	0.807 ± 0.035	0.811 ± 0.038	0.818 ± 0.036	0.810 ± 0.040	0.814 ± 0.050	0.800 ± 0.047
CHO	0.825 ± 0.043	0.775 ± 0.032	0.804 ± 0.028	0.846 ± 0.047	0.828 ± 0.045	0.849 ± 0.028†	0.840 ± 0.043	0.826 ± 0.030	0.804 ± 0.034	0.804 ± 0.038	0.806 ± 0.034

Note. Respiratory exchange ratio summary data presented as VCO_2/VO_2; n = 14; WPC = whey protein concentrate; WPH = hydrolyzed whey protein; CHO = carbohydrate placebo); † significantly different from WPH ($p < .05$); all data reported as mean ± *SD*.

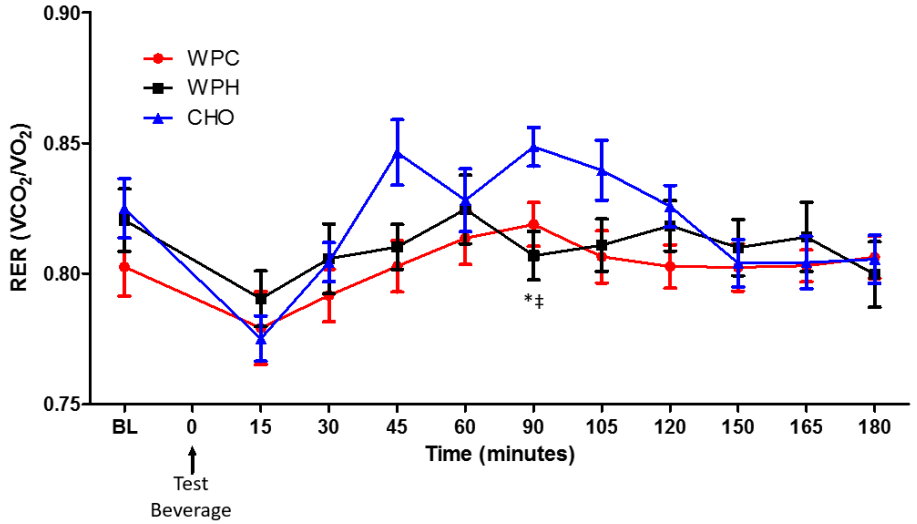

Figure 2. Respiratory exchange ratio response during each test visit. n = 14; WPC = whey protein concentrate; WPH = hydrolyzed whey protein; CHO = carbohydrate placebo; * WPH significantly different from CHO ($p < .05$); ‡ WPH significantly different from WPC ($p < .05$).

Thermic Effect of Food

TEF summary data are presented in 30 min intervals in Table 4 and Figure 3. TEF for the entire 180-min postprandial period is depicted in in Figure 4. Two-way, repeated measures ANOVA revealed a significant interaction for TEF ($F = 4.36$, $p < .05$). *Post hoc* analyses indicated no differences between WPH and WPC for TEF, however, both WPC and WPH treatments resulted in a significantly greater TEF than CHO at all time points 60P and beyond. One-way ANOVA for the total 3-hr postprandial TEF revealed significant differences between treatments ($F = 11.68$, $p < .001$), with WPH and WPC resulting in 10-fold and 13-fold higher TEF than CHO, respectively.

Table 4

Thermic Effect of Food Summary Data

	30	60	90	120	150	180
WPC	2.4 ± 1.9	7.9 ± 4.3*	12.2 ± 5.9*	15.5 ± 7.0*	17.5 ± 8.2*	20.6 ± 10.2*
WPH	3.7 ± 2.8	8.0 ± 5.5*	11.2 ± 5.9*	13.1 ± 7.0*	14.7 ± 9.5*	16.9 ± 11.3*
CHO	2.0 ± 1.5	3.5 ± 4.0	3.0 ± 5.3	2.0 ± 7.0	1.7 ± 9.1	1.6 ± 11.5

Note. Thermic effect of food summary data presented as cumulative kcal in 30-min intervals; n = 14; WPC = whey protein concentrate; WPH = hydrolyzed whey protein; CHO = carbohydrate placebo; *Significantly different from CHO ($p < .05$); all data reported as mean ± *SD*.

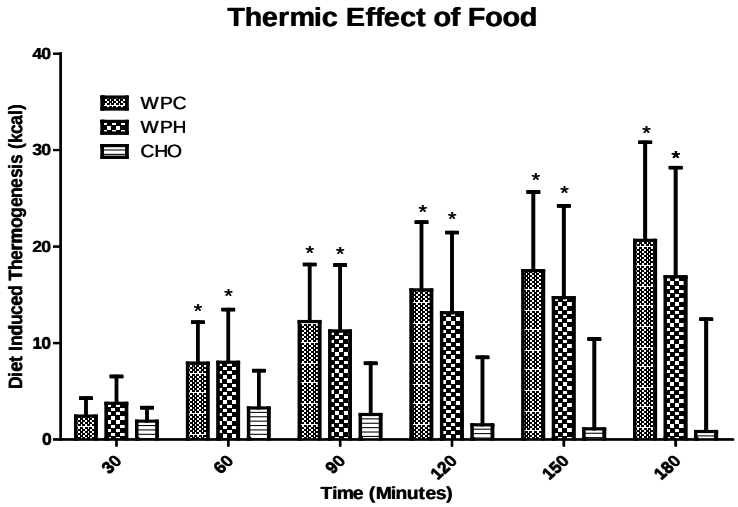

Figure 3. Thermic effect of food cumulative kcal presented in 30 min intervals. n = 14; WPC = whey protein concentrate; WPH = hydrolyzed whey protein; CHO = carbohydrate placebo; *Significantly different from CHO ($p < .05$); all data reported as mean ± *SD*.

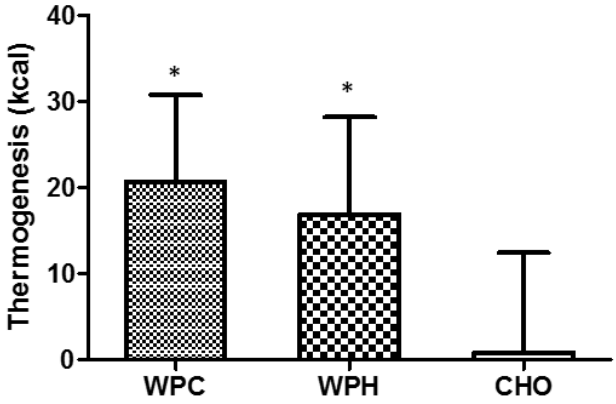

Figure 4. Thermic effect of food cumulative kcal over 180 min. n = 14; WPC = whey protein concentrate; WPH = hydrolyzed whey protein; CHO = carbohydrate placebo; *Significantly different from CHO ($p < .05$); all data reported as mean ± *SD*.

CHAPTER V

DISCUSSION

This is the first study to directly compare the acute effects of hydrolyzed whey protein (WPH) to its intact form (WPC) on REE and RER. It was hypothesized that both protein treatments would increase postprandial REE and decrease RER compared to an isocaloric carbohydrate placebo treatment (CHO). Furthermore, it was postulated that WPH would induce a greater increase in REE and decrease RER to a greater extent than WPC. These hypotheses were based on data showing enhanced lipolytic effects when comparing hydrolyzed whey to its native form (Lockwood et al., 2016; Roberts et al., 2013).

While the results of the current study support the hypothesis that both WPC and WPH treatments would induce a greater increase in REE than CHO, the results do not suggest that WPH induces a greater increase in REE than WPC. These results are similar to those observed by Acheson et al. (2011) who found REE increased following the ingestion of three different protein-rich meals when compared to an isoenergetic high carbohydrate meal. However, in the Acheson study, REE was observed for 5.5 hr post ingestion, whereas the present study examined REE effects for 3 hr. Furthermore, in the Acheson study, a test meal composed of 50% kcal from whey protein was found to elicit significantly higher REE, calculated as total AUC for the 5.5 hr period, than either an isonitrogenous casein or soy protein meal. In the present study, a significant ($p < .05$) difference in REE between the two isonitrogenous protein sources occurred at 120P, with WPC being ~3% greater than WPH (1.48 versus 1.44 kcal/min). Different digestion and absorption kinetics have been shown to exist between hydrolyzed versus intact proteins

from the same source. For instance, the rate of gastric emptying and subsequent aminoacidemia is more rapid for casein hydrolysate than for intact casein (Calbet & Holst, 2004; Koopman et al., 2009), and WPC absorption has been observed to be slower than WPH (Kobayashi et al., 2016). WPC may therefore exert its peak metabolic influence later than WPH, as is reflected by the more gradual postprandial rise in REE observed in the present study (Figure 1).

Perhaps the overall similarity in REE response between the WPC and WPH treatments can be attributed to the similar amino acid profile of the two proteins, with WPH being derived from WPC. In the present study, REE was similar following ingestion of either protein, with both treatments resulting in peak values at 60P. The major difference between the two proteins was that REE response to WPH increased more rapidly, reaching a significant difference from CHO by 30P, whereas no difference was observed between WPC and CHO at 30P. WPH also began a more rapid decline from peak REE values than WPC, and was not different from CHO by 180P.

Kobayashi and colleagues (2016) reported similar findings in their study on the rate of aminoacidemia following ingestion of WPH, WPC, or a free amino acid mixture. In their study, after 15 min, WPH resulted in increased circulating plasma levels of essential amino acids (EAA), branched-chain amino acids (BCAA), and leucine, which was maintained after 30 min, but not at 60 min. However, by 60 min post administration, WPC exhibited the highest levels of plasma EAA, BCAA, and leucine compared to the WPH or the free amino acid mixture. An important difference between the current study and that of Kobayashi et al. is that they examined effects in rats after 7 days of a protein free diet intended to simulate malnutrition and induce muscle wasting. Moreover, the rate of postprandial amino acid appearance does not necessarily

influence energy expenditure or substrate utilization, but is merely a reflection of the digestion and absorption kinetics of each treatment. Therefore, the only inferences that can be made is that a more rapid rise and decline in absorption rates with WPH may explain why WPC values are relatively greater at later time points.

Despite a significant treatment by time interaction, the results of the current study do not support the hypothesis that both protein treatments would decrease RER values relative to CHO, indicative of a shift in substrate utilization. However, there is some evidence to suggest that WPH may influence substrate utilization in favor of fat oxidation. In fact, the only significant ($p < .05$) between-treatment effect for RER was observed at 90P between WPH and both WPC and CHO, with WPH decreasing RER values relative to the other treatments. These results reflect greater fat oxidation for WPH at this time point, as an RER value approaching 0.70 theoretically indicates 100% lipid oxidation and values of 1.00 indicate 100% carbohydrate utilization (Livesey & Elia, 1988). While no previous studies have examined differential effects on RER in response to WPH and WPC feedings, Bendtsen et al. (2014) investigated the effects on 24-hr energy expenditure and substrate utilization of three isoenergetic test meals containing different protein sources, including whey, casein, and hydrolyzed casein in moderately overweight and obese young men and women. In this study, the whey protein meals induced a lower respiratory quotient (RQ; often used synonymously with RER) compared to the hydrolyzed casein meals, indicative of higher lipid oxidation, yet no difference was observed between the intact and hydrolyzed casein treatments. These results are similar to the findings in the current study, however the Bendtsen study measured REE and RQ over the course of 24 hr with participants confined to a respiratory chamber, and the test protein was consumed on 4 occasions as a

mixed meal with the protein component accounting for 26% or less of the energy provided. In the present study, 97.9% of the test beverage was protein, although the amount of energy ingested was considerably less (~80 kcal).

Furthermore, a study by Alfenas and colleagues (2010) examined the metabolic effects of three different protein shake preparations (whey, casein, or soy) versus a non-protein control shake consumed with daily breakfast in healthy young adults over a 7-day period. They found that the whey protein shake induced a lower RQ than the soy shake and control meal, but was not significantly different from the casein shake trial. This would suggest that the two milk-derived proteins did not differentially affect substrate utilization, but that whey protein increased fat oxidation compared to soy or no protein ingestion. Interestingly, the casein shake led to lower daily energy intake over the 7-day period than the whey protein shake, which the authors attributed to the slower gastric emptying rate of casein compared to whey. It is difficult to draw similarities between the present study and that of Alfenas et al., however, because the test shakes were consumed as part of a mixed meal containing 30 g carbohydrate and 6 g fat in addition to the test protein (0.5 g/kg body weight) and thus contained more total calories (~270 versus ~80 kcal) and total protein (~25 versus ~18 g) than the present study. Moreover, the Alfenas study used a 7-day trial with repeated feedings for each protein or control, whereas the present study only examined the acute metabolic effects over a 3-hr postprandial period.

Other researchers have observed an elevation in biomarkers of lipolysis in response to WPH, but not WPC feeding. Roberts and colleagues (2013) found significantly elevated free fatty acids in rats fed WPH compared to those fed WPC after 30 min, which also corresponded with a 4-fold increase in circulating epinephrine with WPH versus WPC. It was postulated that bioactive

peptides in the WPH acted as adrenal catecholamine secretagogues, mimicking the action of pituitary adenylyl cyclase activating peptide (PACAP), initiating catecholamine release which in turn upregulated lipolysis. Furthermore, lipolysis was upregulated by WPH compared to WPC as indicated by increased circulating ketone bodies (a by-product of lipid metabolism), carnitine esters (involved in fatty acid transport into the mitochondria for beta oxidation), and increased metabolites of oxidative phosphorylation. The researchers concluded that hydrolyzed whey protein appears to affect fuel partitioning in favor of fat metabolism. It has been suggested that the high BCAA content of whey proteins may enhance lipid oxidation by inhibition of glycolysis, and thus reduce reliance on carbohydrate as a fuel source (Kainulainen, Hulmi, & Kujala, 2013). Since both protein treatments were derived from whey, which is known to contain high amounts of BCAA, it is not surprising that they would induce similar changes in substrate utilization, if any.

The thermic effect of food (TEF) for the two protein treatments was significantly ($p <$ .05) greater than CHO at all time points 60P and beyond, and for the overall 180-min postprandial period. Since TEF is calculated from REE as a product of area under the curve (AUC) above the fasting baseline value, as reported by Shechter et al. (2014), this finding is not altogether surprising. Protein induces a higher TEF than carbohydrate, with 20-35% of calories consumed from protein sources being expended during the process of digestion, absorption, and utilization, compared with only around 5% for carbohydrates (Acheson et al., 2011; Halton & Hu, 2004). It is worth noting that nearly 25% of the calories ingested from each of the protein sources were expended over the 3-hr postprandial observation (~20.5 kcal or 25% intake for WPC and ~17 kcal or 21% intake for WPH) whereas CHO resulted in only about 2% of consumed

kcal being expended over the same period. Taken together, these findings lend support for the role of increased protein intake in stimulating thermogenesis and perhaps elevating total energy expenditure over time, which may in turn support weight loss or maintenance efforts.

Possible Limitations

Limitations to the present study include a relatively small protein dose (~18 g) and overall meal size (~80 kcal), which may have been suboptimal for producing more robust metabolic effects. Perhaps a larger protein dose, at least 20-30 g protein, or ~0.4 g/kg total body weight would have been more suitable for the current population (Pencharz et al., 2016). Another limitation is the use of a single-blind design, which was a consequence of the researchers having to mix and flavor the test beverages themselves. A double-blind design would have been preferable to rule out possibility of experimenter bias; however, at the time of test beverage administration a single member of the research team mixed test beverages and the remaining researchers (including the principal investigator) remained blinded during data collection. The length of observation period (3 hr) may have been too brief to detect all changes in REE/RER, and indeed previous studies have employed a longer period of observation (Acheson et. al., 2011; Alfenas, et al., 2010; Bendtsen et al., 2014), albeit the greater caloric content of the test meals provided in those studies warranted a longer observation period to accommodate a longer digestion, absorption, and metabolism period. However, it is challenging to confine participants to a supine position and ask them to refrain from moving or falling asleep for 3 hr or more. It may also be said that the study lacked a genuine random sample since most of the participants were recruited from Texas Woman's University, a campus with a relatively small proportion (~12%) of male students. Additionally, the researchers had limited control over

participant compliance to all requirements (e.g., repeat diet adherence, exercise limitations), and were dependent upon participant accuracy for providing reliable dietary recall prior to each visit. Such challenges are inherent in study designs involving free living participants, however, and are difficult to overcome unless utilizing a metabolic ward or inpatient setting where greater control is afforded.

Future Research

Future research is needed to determine whether WPH is beneficial over WPC for enhancing fat oxidation over a longer period (i.e., > 24 hr). Additionally, alterations in 24-hr energy expenditure could be observed using the doubly-labeled water method to see if any differences arise between the two protein sources under free living conditions. Also, larger doses of test protein should be administered, perhaps also within the context of a mixed meal, to simulate typical consumer use patterns in which it is common for 20-30 g of supplemental protein to be ingested in a single feeding.

It is possible that the differences in fat loss observed by Lockwood et al., (2016) are entirely unrelated to changes in REE or RER, as has been suggested by the present study's results. However, the fact that differences did occur between WPH and WPC for both REE and RER, although only at select time points, is reason enough to warrant further investigation. Finally, future studies should also examine biochemical indicators of lipolysis in humans, such as plasma free fatty acids or glycerol, so as to allow comparisons to be drawn between the acute findings observed in previous rodent studies (Mobley et al., 2015; Roberts et al., 2013).

Study Implications

The results of the current study suggest that whey protein consumption, whether intact or in hydrolyzed form, increases resting energy expenditure compared to the consumption of carbohydrate providing the same number of calories. These findings can be of benefit to individuals looking to lose weight or improve body composition, as increased energy expenditure could potentially lead to an overall energy deficit over time. Moreover, seeing as WPH supplementation may increase fat oxidation compared to both WPC and carbohydrate, it may be of value for individuals who are overweight or attempting to decrease fat mass. Finally, seeing as the majority of protein supplements on the market are whey based, the current study may have potential implications for sports nutrition applications. For example, including hydrolyzed whey protein in supplemental formulations specifically targeted at bodybuilders or physique competitors may have some advantages over whey concentrate alone from a substrate utilization standpoint.

Conclusion

In conclusion, the present study suggests that ingestion of a hydrolyzed whey protein supplement under fasting conditions increases resting energy expenditure and the thermic effect of food compared to carbohydrate, and causes a temporary shift in substrate utilization in favor of lipid oxidation compared to both carbohydrate and whey protein concentrate.

REFERENCES

Acheson, K. J., Blondel-Lubrano, A., Oguey-Araymon, S., Beaumont, M., Emady-Azar, S., Ammon-Zufferey, C., ... & Bovetto, L. (2011). Protein choices targeting thermogenesis and metabolism. *The American Journal of Clinical Nutrition*, *93*, 525-534.

Alfenas, R.C.G., Bressan, J., & Paiva, A.C. (2010). Effects of protein quality on appetite and energy metabolism in normal weight subjects. *Arquivos Brasileiros de Endocrinologia & Metabologia*, *54*(1), 45-51.

American College of Sports Medicine. (2013). *ACSM's guidelines for exercise testing and prescription* (9th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

Anthony, J. C., Anthony, T. G., Kimball, S. R., & Jefferson, L. S. (2001). Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *The Journal of Nutrition*, *131*, 856S-860S.

Arciero, P. J., Ormsbee, M. J., Gentile, C. L., Nindl, B. C., Brestoff, J. R., & Ruby, M. (2013). Increased protein intake and meal frequency reduces abdominal fat during energy balance and energy deficit. *Obesity*, *21*, 1357-1366.

Bendtsen, L.Q., Lorenzen, J.K., Gomes, S., Liaset, B., Holst, J.J., Ritz, C., ... & Astrup, A. (2014). Effects of hydrolysed casein, intact casein and intact whey protein on energy expenditure and appetite regulation: A randomised, controlled, cross-over study. *British Journal of Nutrition*, *112*, 1412-1422.

Calbet, J.A., & Holst, J.J. (2004). Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. *European Journal of Nutrition*, *43*(3), 127-139.

Clemente, A. (2000). Enzymatic protein hydrolysates in human nutrition. *Trends in Food Science & Technology*, *11*(7), 254-262.

Cooper, J.A., Watras, A.C., O'Brien, M.J., Luke, A., Dobratz, J.R., Earthman, C.P., & Schoeller, D. A. (2009). Assessing validity and reliability of resting metabolic rate in six gas analysis systems. *Journal of the American Dietetic Association*, *109*(1), 128-132.

Crouter, S.E., Antczak, A., Hudak, J.R., DellaValle, D.M., & Haas, J.D. (2006). Accuracy and reliability of the ParvoMedics TrueOne 2400 and MedGraphics VO2000 metabolic systems. *European Journal of Applied Physiology*, *98*(2), 139-151.

da Rocha, E.E.M., Alves, V.G.F., & da Fonseca, R.B.V. (2006). Indirect calorimetry: Methodology, instruments and clinical application. *Current Opinion in Clinical Nutrition & Metabolic Care*, *9*(3), 247-256.

Due, A., Toubro, S., Skov, A.R., & Astrup, A. (2004). Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: A randomised 1-year trial. *International Journal of Obesity*, *28*, 1283-1290.

Fullmer, S., Benson-Davies, S., Earthman, C.P., Frankenfield, D.C., Gradwell, E., Lee, P.S., ... & Trabulsi, J. (2015). Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non–critically ill individuals. *Journal of the Academy of Nutrition and Dietetics*, *115*, 1417-1446.

Ha, E., & Zemel, M. B. (2003). Functional properties of whey, whey components, and essential amino acids: Mechanisms underlying health benefits for active people. *Journal of Nutritional Biochemistry*, *14*(5), 251-258.

Halton, T. L., & Hu, F. B. (2004). The effects of high protein diets on thermogenesis,

satiety and weight loss: a critical review. *Journal of the American College of Nutrition*, *23*(5), 373-385.

Hulmi, J. J., Lockwood, C. M., & Stout, J. R. (2010). Effect of protein/essential amino acids and resistance training on skeletal muscle hypertrophy: A case for whey protein. *Nutrition & Metabolism*, *7*(1), 51.

Institute of Medicine. (2006). *Dietary reference intakes: The essential guide to nutrient requirements*. Washington, D.C.: National Academies Press.

Kainulainen, H., Hulmi, J.J., & Kujala, U.M. (2013). Potential role of branched-chain amino acid catabolism in regulating fat oxidation. *Exercise and Sport Sciences Reviews*, *41*(4), 194-200.

Kanda, A., Nakayama, K., Sanbongi, C., Nagata, M., Ikegami, S., & Itoh, H. (2016). Effects of whey, caseinate, or milk protein ingestion on muscle protein synthesis after exercise. *Nutrients*, *8*(6), 339.

Kobayashi, Y., Somoto, Y., Mitsuyama, E., Tanaka, A., Yuda, N., Nakada, H., ... & Nagasawa, T. (2016). Supplementation of protein-free diet with whey protein hydrolysates prevents skeletal muscle mass loss in rats. *Journal of Nutrition & Intermediary Metabolism*, *4*, 1-5.

Koopman, R., Crombach, N., Gijsen, A.P., Walrand, S., Fauquant, J., Kies, A.K., ... & van Loon, L. J. (2009). Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. *American Journal of Clinical Nutrition*, *90*(1), 106-115.

Lacroix, I. M., Meng, G., Cheung, I. W., & Li-Chan, E. C. (2016). Do whey protein-derived

peptides have dual dipeptidyl-peptidase IV and angiotensin I-converting enzyme inhibitory activities? *Journal of Functional Foods*, *21*, 87-96.

Livesey, G., & Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. *American Journal of Clinical Nutrition*, *47*, 608-628.

Lockwood, C. M., Roberts, M. D., Dalbo, V. J., Smith-Ryan, A. E., Kendall, K. L., Moon, J. R., & Stout, J. R. (2016). Effects of hydrolyzed whey versus other whey protein supplements on the physiological response to 8 weeks of resistance exercise in college-aged males. *Journal of the American College of Nutrition*, 1-12.

Lollo, P. C. B., Amaya-Farfan, J., Faria, I. C., Salgado, J.V.V., Chacon-Mikahil, M. P. T., Cruz, A. G., ... & Arruda, M. (2014). Hydrolysed whey protein reduces muscle damage markers in Brazilian elite soccer players compared with whey protein and maltodextrin: A twelve-week in-championship intervention. *International Dairy Journal*, *34*(1), 19-24.

Longland, T. M., Oikawa, S. Y., Mitchell, C. J., Devries, M. C., & Phillips, S. M. (2016). Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: A randomized trial. *American Journal of Clinical Nutrition*, *103*, 738-746.

Lorenzen, J., Frederiksen, R., Hoppe, C., Hvid, R., & Astrup, A. (2012). The effect of milk proteins on appetite regulation and diet-induced thermogenesis. *European Journal of Nutrition*, *66*, 622-627.

Luscombe-Marsh, N. D., Hutchison, A. T., Soenen, S., Steinert, R. E., Clifton, P. M., Horowitz, M., & Feinle-Bisset, C. (2016). Plasma free amino acid responses to intraduodenal whey protein, and relationships with insulin, glucagon-like peptide-1 and energy intake in lean healthy men. *Nutrients*, *8*(1), 4.

Manninen, A. H. (2009). Protein hydrolysates in sports nutrition. *Nutrition & Metabolism*, *6*(1), 1.

McGregor, R. A., & Poppitt, S. D. (2013). Milk protein for improved metabolic health: A review of the evidence. *Nutrition & Metabolism*, *10*(1), 1.

Mikkelsen, P. B., Toubro, S., & Astrup, A. (2000). Effect of fat-reduced diets on 24-h energy expenditure: Comparisons between animal protein, vegetable protein, and carbohydrate. *American Journal of Clinical Nutrition*, *72*, 1135-1141.

Mobley, C. B., Fox, C. D., Ferguson, B. S., Pascoe, C. A., Healy, J. C., McAdam, J. S., ... & Roberts, M. D. (2015). Effects of protein type and composition on postprandial markers of skeletal muscle anabolism, adipose tissue lipolysis, and hypothalamic gene expression. *Journal of the International Society of Sports Nutrition*, *12*(1), 1.

Moore, D. R., Churchward-Venne, T. A., Witard, O., Breen, L., Burd, N. A., Tipton, K. D., & Phillips, S. M. (2015). Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *70*(1), 57-62.

Morato, P. N., Lollo, P. C. B., Moura, C. S., Batista, T. M., Camargo, R. L., Carneiro, E. M., & Amaya-Farfan, J. (2013a). Whey protein hydrolysate increases translocation of GLUT-4 to

the plasma membrane independent of insulin in Wistar rats. *PloS One, 8*(8), e71134. doi:10.1371/journal.pone.0071134

Morato, P. N., Lollo, P., Moura, C. S., Batista, T. M., Carneiro, E. M., & Amaya-Farfan, J. (2013b). A dipeptide and an amino acid present in whey protein hydrolysate increase translocation of GLUT-4 to the plasma membrane in wistar rats. *Food Chemistry, 139*, 853-859.

Morell, P., & Fiszman, S. (2016). Revisiting the role of protein-induced satiation and satiety. *Food Hydrocolloids*. Retrieved from http://ezproxy.twu.edu:3361/science/article/pii/S0268005X1630340X

Morton, R. W., McGlory, C., & Phillips, S. M. (2015). Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. *Frontiers in Physiology*, *6*.

Nobile, V., Duclos, E., Michelotti, A., Bizzaro, G., Negro, M., & Soisson, F. (2016). Supplementation with a fish protein hydrolysate (Micromesistius poutassou): Effects on body weight, body composition, and CCK/GLP-1 secretion. *Food & Nutrition Research*, *60*.

Nongonierma, A. B., & FitzGerald, R. J. (2015). The scientific evidence for the role of milk protein-derived bioactive peptides in humans: A Review. *Journal of Functional Foods*, *17*, 640-656.

Pencharz, P. B., Elango, R., & Wolfe, R. R. (2016). Recent developments in understanding protein needs–How much and what kind should we eat? *Applied Physiology, Nutrition, and Metabolism*, *41*, 577-580.

Phillips, S. M., Chevalier, S., & Leidy, H. J. (2016). Protein “requirements” beyond the

RDA: Implications for optimizing health. *Applied Physiology, Nutrition, and Metabolism*, *41*, 565-572.

Phillips, S. M., & Van Loon, L. J. (2011). Dietary protein for athletes: from requirements to optimum adaptation. *Journal of sports sciences*, *29*(sup1), S29-S38.

Plowman, S. A., & Smith, D. L. (2013). *Exercise physiology for health fitness and performance* (4th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

Power, O., Hallihan, A., & Jakeman, P. (2009). Human insulinotropic response to oral ingestion of native and hydrolysed whey protein. *Amino acids*, *37*(2), 333-339.

Psota, T., & Chen, K. Y. (2013). Measuring energy expenditure in clinical populations: rewards and challenges. *European Journal of Clinical Nutrition*, *67*(5), 436-442.

Roberts, M. D., Cruthirds, C. L., Lockwood, C. M., Pappan, K., Childs, T. E., Company, J. M., ... & Booth, F. W. (2013). Comparing serum responses to acute feedings of an extensively hydrolyzed whey protein concentrate versus a native whey protein concentrate in rats: A metabolomics approach. *Applied Physiology, Nutrition, and Metabolism*, *39*(2), 158-167.

Shechter, A., Rising, R., Wolfe, S., Albu, J. B., & St-Onge, M. P. (2014). Postprandial thermogenesis and substrate oxidation are unaffected by sleep restriction. *International Journal of Obesity*, *38*, 1153-1158.

Tang, J. E., Moore, D. R., Kujbida, G. W., Tarnopolsky, M. A., & Phillips, S. M. (2009). Ingestion of whey hydrolysate, casein, or soy protein isolate: Effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *Journal of Applied Physiology*, *107*, 987-992.

Vavrusova, M., Pindstrup, H., Johansen, L. B., Andersen, M. L., Andersen, H. J., & Skibsted, L. H. (2015). Characterisation of a whey protein hydrolysate as antioxidant. *International Dairy Journal, 47*, 86-93.

Vickery, H. B. (1950). The origin of the word protein. *The Yale Journal of Biology and Medicine*, *22*(5), 387.

Wildman, R. E., & Miller, B. S. (2004). *Sports and fitness nutrition*. Belmont, CA: Wadsworth.

Wilson, J.M., Lowery, R.P., Loenneke, J.P., Duncan, N., Zanchi, N., & Wilson, S.M.C. (2013). Optimizing protein consumption for body composition and sports performance. In A. Smith-Ryan & J. Antonio (Eds.), *Sports nutrition & performance enhancing supplements* (pp. 31-53). Ronkonkoma, NY: Linus Learning.

Yalçin, A. S. (2006). Emerging therapeutic potential of whey proteins and peptides. *Current Pharmaceutical Design*, *12*, 1637-1643.

APPENDIX A

IRB Approval Letter

IRB Modification Approval Letter

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378
email: IRB@twu.edu
http://www.twu.edu/irb.html

DATE: January 29, 2016

TO: Dr. Nancy DiMarco
Nutrition & Food Sciences

FROM: Institutional Review Board (IRB) - Denton

Re: Approval for Acute Effects of Dairy and Plant Proteins on Blood Amino Acids, Glucose, Hormones, and Metabolism (Protocol #: 18840)

The above referenced study was reviewed at a fully convened meeting of the Denton IRB (operating under FWA00000178). The study was approved on 1/28/2016. This approval is valid for one year and expires on 1/27/2017. The IRB will send an email notification 45 days prior to the expiration date with instructions to extend or close the study. It is your responsibility to request an extension for the study if it is not yet complete, to close the protocol file when the study is complete, and to make certain that the study is not conducted beyond the expiration date.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Dr. Shane Broughton, Nutrition & Food Sciences

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619
940-898-3378
email: IRB@twu.edu
http://www.twu.edu/irb.html

DATE: February 25, 2016

TO: Dr. Nancy DiMarco
Nutrition & Food Sciences

FROM: Institutional Review Board - Denton

Re: Notification of Approval for Modification for Acute Effects of Dairy and Plant Proteins on Blood Amino Acids, Glucose, Hormones, and Metabolism (Protocol #: 18840)

The following modification(s) have been approved by the IRB:

Extending the duration of testing by one hour each testing day.
Addition of PAR-Q screening survey attached
Modified Flyer attached.
Changes reflected on Consent Form

APPENDIX B

Informed Consent

TEXAS WOMAN'S UNIVERSITY
CONSENT TO PARTICIPATE IN RESEARCH

Project Title: Acute Effects of Dairy and Plant Proteins on Blood Amino Acids, Glucose, Hormones, and Metabolism

Principal Investigator: Nancy DiMarco, PhD, RDN, CSSD
Institute for Women's Health
Jordan Joy, MS

Investigators' email: ndimarco@twu.edu or jmjoyx@gmail.com

Investigators' Phone: 940-898-2792 or 845-XXX-XXXX

Purpose of the Research Study

The primary purpose of this investigation is to study how fast the building blocks of protein (amino acids) appear in the blood after drinking a partially broken down protein (whey-one of the major proteins in milk) or pea. The secondary purpose is to determine how whey or pea protein affects your calorie burn and also how hungry, sleepy, energetic, or bloated you feel. You will be randomized into one of the 10 protein supplement trials, either 1) whey protein, 2) pea protein, 3) hydrolyzed whey protein (partially digested protein), 4) hydrolyzed pea protein (partially digested protein), 5) whey protein + carbohydrate, 6) pea protein + carbohydrate, 7) hydrolyzed whey protein + carbohydrate, 8) hydrolyzed pea protein + carbohydrate, 9) 0.3g/kg muscle mass carbohydrate, or 10) 0.6g/kg muscle mass carbohydrate.
You were selected as a possible participant because you are a male aged 18 to 30 years, in good health, are a nonsmoker, consume fewer than 12 alcoholic beverages per week, have reported consistent exercise (minimum 2 days per week) for the past 1 or more years, and are willing to involve yourself in the testing outlined in this consent form. You also indicated you had no allergy to whey or pea protein.

Procedures

If you agree to be in this study, you will be asked to do the following:

- Participate in all testing sessions at their planned times. Testing times will all be during the morning Monday – Saturday and we will work to accommodate your schedule when possible. Each protein supplement trial, of which there are 12 will take 5 weeks to complete. Testing will occur once per week for 5 weeks, including the preliminary visit.

- Consume the test beverages, which will consist of **Protein supplements.** Each test beverage will consist of one of the following in a randomized order:
  - 0.3g whey protein hydrolysate per kg lean soft tissue (LST), a measure of muscle mass
  - 0.3g whey protein concentrate per kg LST
  - 0.3g PurisPea 870H pea protein hydrolysate per kg LST
  - 0.3g PurisPea 870 pea protein concentrate per kg LST
  - treatment #1 + 0.3g/kg LST maltodextrin (a form of carbohydrate)
  - treatment #2 + 0.3g/kg LST maltodextrin
  - treatment #3 + 0.3g/kg LST maltodextrin

- treatment #4 + 0.3g/kg LST maltodextrin
- 0.3g/kg LST maltodextrin
- 0.6g/kg LST maltodextrin

- Each of these formulas would be mixed in 16 ounces of water. You will possibly consume one of these protein supplements during each test visit in a randomized order. The amount of protein is adjusted for your individual muscle mass, but you will not consume less than 20g protein.

- As a part of screening, it will also be required that you consume a 75g carbohydrate drink to determine your glucose tolerance. After an overnight fast, you will come to the lab between 7 and 8 am and drink the carbohydrate drink. You will have blood drawn at 0, 15, 30, 45, 60, 90 and 120 minutes and each time ~10 mL of blood will be collected.

- Consume the same foods for 24 hours prior to each testing visit. A food record will be kept and analyzed to provide the diet you will consume prior to each trial.

- Blood samples will be collected at each visit using an indwelling catheter and correspond to immediately before and 10, 20, 30, 45, 60, 90, 120, and 180 minutes following protein supplement ingestion.

- Metabolic testing (resting metabolic rate, RMR) can only occur in the morning following a ten-hour, overnight fast. This test will occur during each visit before and 30, 60, 90, 120, 150, and 180 minutes following ingestion of the protein supplement. For this test, you will be asked to lie quietly on your back for 20 minutes, in a darkened room, while wearing a mask which will collect and analyze your inspired and expired air.

- **Questionnaires:** You will be asked to complete a health history questionnaire, demographic questionnaire, and visual analogue scale. These questionnaires will take approximately 15 minutes to complete. The purpose of these questionnaires is shown below.

  Health History Questionnaire: The purpose of the health history questionnaire is to identify individuals who may need physician clearance prior to participation. Additionally, the medical history questionnaire will be used to optimize safety during exercise testing and participation in the program.

  Par-Q: The purpose of the Par-Q (Physical Activity Readiness Questionnaire) is to screen for contraindications to exercise. Those who do not meet healthy criteria will be excluded from this study

  Demographic Questionnaire: The purpose of the demographic questionnaire is to characterize who is participating in the program.

  Visual Analogue Scales: The purpose of the visual analogue scales are to determine your subjective feelings of hunger, energy, sleepiness, and bloatedness

following supplement ingestion. These scales will be completed after each protein supplement trial.

Length of Participation

Total participation time will last about 24-44 hours over 5-11 weeks. During this time you will be asked to come in for the preliminary visit to ask questions, consent to begin additional screening procedures (height, weight, body fat, and glucose tolerance measures). The following 4 visits will consist of the protein supplement drinks, blood draws, metabolic testing, and visual analogue scales.

Potential risks: There exists the possibility of certain risks occurring during data collection and baseline measurements. They include blood draw discomfort of bruising and infection, loss of confidentiality, emotional discomfort in sharing personal information, embarrassment, hypoglycemia, nausea and fainting, latex allergy, and canopy discomfort.

Bruising: The risk of bruising resulting from blood draws is minimal due to this procedure being performed by trained personnel. Universal precautions and aseptic technique will be used during all blood draw procedures. To minimize bruising, pressure will be applied to the site for approximately five minutes after each blood draw.

Infection: The risk of infection resulting from blood draws is minimal due to the procedures being performed by trained personnel. Universal precautions and aseptic technique will be used during all blood draw procedures. Sites for blood draws will be cleaned with alcohol immediately prior to each venipuncture. Each new needle that is opened will be disposed of in biohazard boxes immediately after use.

There exists the possibility of the **loss of confidentiality** as a potential risk of participation in this study. Confidentiality will be protected to the extent that is allowed by law. To minimize this risk, all data will be kept in a locked file cabinet. Data collection forms will be coded with a numerical system rather than your name. A single identification form will be used to link names with a numerical code. There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions. This study is voluntary and you may discontinue at any time.

Emotional Discomfort in Sharing Personal Information: During the collection of personal information you may feel emotionally uneasy. To minimize emotional discomfort with the collection of this information, you will have the option to share this information with a research team member of the same sex.

Embarrassment: During the measurement of body composition, height and weight, you may feel embarrassed. To minimize embarrassment, you have the option to have measurements taken by a research team member of the same sex. Additionally, to ensure privacy, height and weight measurements will be conducted in a small private room located in the Pioneer Performance Clinic (HDB 011).

Hypoglycemia: Hypoglycemia (low blood sugar) may result from prolonged fasting. If you have signs of hypoglycemia during a testing session, the test will be terminated. Signs of hypoglycemia include tremors, cold sweat, low body temperature, headache,

confusion, hallucinations, bizarre behavior, convulsions, and coma. You will be given a glass of orange juice and monitored until the signs of hypoglycemia subside.

Nausea and Fainting: If you feel nauseous or faint due to prolonged fasting, you will also be asked to lie on your back on the floor with your feet elevated to alleviate these symptoms.

Latex Allergy: The phlebotomist will wear gloves during all blood draws. Prior to the first blood draw, you will be asked if you are allergic to latex. If you inform the phlebotomist that you are allergic to latex, non-latex gloves and tourniquet will be used.

Canopy Discomfort: During procedures that require the collection of CO_2 and O_2, the participant's head and face will be covered by a canopy which will be connected to the metabolic cart. The canopy will be placed such that least amount of air leaks in or out of the canopy. The participant will be expected to breathe normally/ quietly inside the canopy without falling asleep for 30 min. Breathing in a closed space may cause discomfort. To minimize discomfort, an appropriate amount of airflow will be maintained inside the canopy. Participant will be checked on time and again to ensure ease of breathing. Participants may experience anxiety while being inside the canopy. If they do, they may discontinue the study.

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Benefits of being in the study are:

Receiving free protein supplements in the form of whey protein or pea protein, information on your body composition, and metabolism. You will receive $100 in compensation at the conclusion of the study.

Voluntary Nature of the Study

Participation in this study is voluntary and as a participant, you have the right to withdraw from the study at any time without penalty. Should you desire to withdraw from the study at any point, you are entitled to be informed of any data collected from you that has been analyzed at any time point.

All data with any personal identifiers will be destroyed at the conclusion of the study. All identifiable data on paper will be shredded and data stored on the primary investigator's computer will be deleted from the hard drive.

Contacts and Questions

If you have concerns or complaints about the research, the researcher(s) conducting this study can be contacted at:

Nancy DiMarco
940-898-2792
ndimarco@mail.twu.edu

Jordan Joy
845-XXX-XXXX
jmjoyx@gmail.com

You will be given a copy of this signed and dated consent form to keep. If you are not given a copy of this consent form or lose it and would like a replacement, please request one. If you have any questions about the research study you should ask the researchers; their phone numbers are at the top of this form. If you have questions about your rights as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at 940-898-3378 or via e-mail at IRB@twu.edu.

Signature Date

If you would like to receive a summary of the results of this investigation, please provide an address or email address to which this summary should be sent:

_____
_____
_____

Participants Initials____

APPENDIX C

Health History Questionnaire

IWH Wellness & Sport Evaluation Program
Health Questionnaire

Name _____ **Date** ____/____/20_____
(Last) (First) (Middle)

		Circle One	Notes
(1)	**Have you been under the care of a physician during the past 2 years?**	**Yes No**	
(2)	**Are you allergic to penicillin, any drugs, medicine, or latex?**	**Yes No**	
(3)	**Have you ever had excessive bleeding which required special treatment?**	**Yes No**	
(4)	**Women: Is there a chance you might be pregnant?**	**Yes No**	
(5)	**Women: Are you taking any birth control medication?**	**Yes No**	
(6)	**Have you had adverse reaction to local anesthetics?**	**Yes No**	

(7) Do you use recreational drugs?_____ If yes, what?_____
(8) Do you use tobacco? _____ If so, what form? _____
(9) Date of last medical exam _____
(10) Circle Yes to any of the following which you have had or have at present. Circle No to those that you have not had.

Yes	**No**	**High Blood Pressure**	**Yes**	**No**	**Unexplained Shortness of Breath**
Yes	**No**	**High Blood Cholesterol**	**Yes**	**No**	**Chronic Cough or Bronchitis**
Yes	**No**	**Chest Pain or Pressure (Angina)**	**Yes**	**No**	**Tuberculosis (TB)**
Yes	**No**	**Heart Disease or Attack**	**Yes**	**No**	**Emphysema**
Yes	**No**	**Heart Pacemaker**	**Yes**	**No**	**Asthma**
Yes	**No**	**Heart Failure**	**Yes**	**No**	**Hay Fever**
Yes	**No**	**Heart Surgery**	**Yes**	**No**	**Allergies or Hives**
Yes	**No**	**Fainting or Lightheadedness**	**Yes**	**No**	**Sinus Trouble**
Yes	**No**	**Artificial Heart Valve**	**Yes**	**No**	**Cancer**
Yes	**No**	**Congenital Heart Lesions**	**Yes**	**No**	**Leukemia or Lymphoma**
Yes	**No**	**Mitral Valve Prolapse**	**Yes**	**No**	**Radiation or Chemotherapy**
Yes	**No**	**Stroke**	**Yes**	**No**	**Anemia**
Yes	**No**	**Transient Ishemic Attack**	**Yes**	**No**	**Bruise Easily**
Yes	**No**	**Lupus**	**Yes**	**No**	**Bleeding Disorders**
Yes	**No**	**Rheumatic Fever**	**Yes**	**No**	**Sickle Cell Disease**
Yes	**No**	**Scarlet Fever**	**Yes**	**No**	**Alcoholism**
Yes	**No**	**Chronic Fatigue**	**Yes**	**No**	**Drug Addiction**
Yes	**No**	**Artificial Joints**	**Yes**	**No**	**Blood Transfusion**
Yes	**No**	**Kidney Dialysis**	**Yes**	**No**	**Liver Disease**
Yes	**No**	**Kidney Disease**	**Yes**	**No**	**Yellow Jaundice**
Yes	**No**	**Eating Disorder**	**Yes**	**No**	**Hepatitis**
Yes	**No**	**Rheumatoid Arthritis**	**Yes**	**No**	**AIDS / HIV Infection**
Yes	**No**	**Arthritis**	**Yes**	**No**	**Cold Sores / Fever Blisters**
Yes	**No**	**Chronic Head, Neck, or Back Pain**	**Yes**	**No**	**Psychiatric Treatment**
Yes	**No**	**Diabetes Requiring Insulin**	**Yes**	**No**	**Depression / Bipolar**
Yes	**No**	**Diabetes Not Requiring Insulin**	**Yes**	**No**	**Nervousness / Anxiety**
Yes	**No**	**Hypoglycemia**	**Yes**	**No**	**Dizzy Spells**
Yes	**No**	**Hyperthyroidism (High)**	**Yes**	**No**	**Epilepsy or Seizures**
Yes	**No**	**Hypothyroidism (Low)**	**Yes**	**No**	**Condition Requiring Cortisone Medicine**
Yes	**No**	**Ulcers**	**Yes**	**No**	**Glaucoma**
Yes	**No**	**Pulmonary Disease**	**Yes**	**No**	**Spine or Hip Fractures**

Medication/Dosage/Date Started/Reason _____

Medication/Dosage/Date Started/Reason _____

Medication/Dosage/Date Started/Reason _____

Please list all non-prescription medication or vitamins or nutritional supplements you are currently taking.

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

Name/Dosage/Date Started/Reason _____

List all surgical procedures that you have had in the past.

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

Year _____ Type of Surgery/Reason _____

List all hospitalizations of 24 hours or more for any reason.

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Year _____ Reason for hospitalization _____

Other Health Information

Please use this space to record any other personal health information that was not listed above.

_____

_____

"I Attest To The Fact That The Information Given Above Is Correct And I Consent To Receive Clinical Services."

_____

(Parent or Guardian must sign for patient under age 18.)

APPENDIX D

Demographic Questionnaire

IWH Wellness & Sport Evaluation Program
Demographic Questionnaire

Name: _____ **Date:** _____/_____/20_____
(Last) (First) (Middle)

Phone: (_____) _____ **Email:** _____

Address: _____ **City** _____ **ST** _____ **Zip** _____

How would you prefer we contact you? ❑ Phone ❑ Email ❑ Mail

Date of Birth: _____ / _____ / _____ **Gender:** ❑ Male ❑ Female

Ethnicity: (Check all that apply)

❑ African American
❑ American Indian
❑ Asian/Pacific Islander
❑ Caucasian (non-Hispanic)
❑ Hispanic
❑ Scandinavian
❑ Other: _____

What is the highest level of education you have attained? (Please mark only one)

❑ Less than a high school diploma
❑ High school graduate
❑ Some college or technical training
❑ Associate's degree or equivalent
❑ Bachelor's degree
❑ Graduate degree

What is your present work situation? (Check all that apply)

❑ Employed full-time
❑ Employed part-time
❑ Semi-retired
❑ Fully-retired
❑ Self-employed
❑ Unemployed
❑ Homemaker
❑ Student
❑ On disability
❑ Other: _____

Are or were you a student at Texas Woman's University?

❑ Yes, I am a current student ❑ No ❑ I am a TWU alumnus

Are you a current employee of Texas Woman's University?
❑ Yes ❑ No

Emergency Contact:

Name: _____ **Phone:** (_____) _____

Please provide the name of your physician.

Name: _____ **Phone:** (_____) _____