COMPARATIVE INSULIN AND GLYCEMIC RESPONSE TO DIETARY PROTEIN INTAKE IN HEALTHY MALES

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BY

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DEDICATION

To my parents, I never once thought there was anything in this world I couldn't do because you made sure I knew anything was possible. Thank you for always allowing me to chase my dreams. Without your love and dedication to my education and my dreams I would not have made it this far.

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ABSTRACT

TATYANA D. LONG

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The aim of this study was to compare plasma amino acid (AA) concentrations, insulin, and glycemic response to an intake of whey protein concentrate (WPC) or chicken protein isolate (CPI). Twenty-eight healthy males were assigned to a treatment drink of WPC, glucose, or CPI. WPC and glucose intake caused an insulinogenic effect followed by a significant drop below baseline at 180-minutes leading to blood glucose changes with similar characteristics as rebound hypoglycemia. CPI resulted in a rise in insulin ($p < .05$) much lower than that seen in the other treatment groups. Plasma glucose levels remained within a normal range for chicken ingestion and did not show a significant decrease below baseline. Whey led to a higher early AA response whereas chicken had a longer-term sustained response. In conclusion, CPI may induce a more favorable insulin response in combination with a steady moderate range of postprandial blood glucose compared to WPC.

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CHAPTER I

INTRODUCTION

Problem Statement

Dietary proteins, specifically branched chain amino acid (BCAA), are important for the control of postprandial blood glucose (PPG) concentrations (Farnfield et al., 2009; Stevenson & Allerton, 2018). Numerous studies have classified whey protein as an ideal protein for assisting in maintaining adequate glucose levels due to the higher level of BCAAs in whey (Almeida et al., 2016; Gunnerud et al., 2012, Hidayat et al., 2019). The benefits of other dietary protein sources, such as chicken, have not been thoroughly examined in relation to PPG and insulin concentrations. This study is the first to compare whey protein concentrate (WPC) to chicken protein isolate (CPI) in terms of their impact on PPG concentrations and the insulin response in healthy males. This study was reviewed and approved by the Texas Woman's University Institutional Review Board (IRB), Denton, Texas. This is a follow-up report using the data collected from the original study.

Hypotheses

This study compared plasma amino acid (AA) concentrations, and the insulin and glycemic response to an intake of WPC or CPI in healthy males. The parameters measured were insulin, glucose, and AA levels.

Hypotheses tested for this study were:

1) A single dose of CPI will elicit a similar insulin response as whey protein concentrate in healthy males. CPI will promote control of PPG concentrations in healthy males.

Limitations/Delimitations

1) Inclusion in the study was based on being in good health, regularly engaging in exercise, males only, and between the age of 18 and 25 years.
2) This study was not designed to present any long-term effects of the dietary proteins used on the participants.
3) Smokers and alcoholics were excluded from the study.

Significance of Study

The significance of this study was to provide more insight into the role of protein in the control of PPG in Type 2 diabetes mellitus (T2DM). This is also essential for healthy individuals due to the rise of prediabetes cases, which play a role in T2DM development (Akhavan et al., 2020; Sartorius et al., 2019). Understanding the glycemic response to proteins will help educate people with diabetes, or those at risk of diabetes and increase the protein options for people in this population who may not like whey protein, have milk allergies, or are just looking for alternatives. Also understanding the proteins effect on insulin and PPG can help people with diabetes have more options for better blood glucose control.

Definitions

1) Antecubital vein: The antecubital or median cubital vein is a vein commonly cannulated and used for intravenous access on the arm just below the bend at the elbow (Hacking, n.d.).
2) Gas chromatography mass spectrometry (GC/MS): An instrumental technique involving a gas chromatograph and a mass spectrometer with the ability to separate, identify, and quantify chemical mixtures. This technique is useful for analyzing low molecular weight compounds (University of Bristol, 2008).
3) Insulinotropic: A component that stimulates or has an effect on the production of insulin (Merriam-Webster, 2020).
4) Incretins: A group of hormones that are secreted to stimulate insulin release that then lowers blood glucose (Kim & Egan, 2008).
5) Gastric Emptying rate: The rate at which food enters and exits the stomach into the small intestine (Marathe et al., 2013).

CHAPTER II

REVIEW OF LITERATURE

Populations of Importance

Cardiovascular disease (CVD) has been associated as a cause of both morbidity and mortality for those with diabetes in the United States (Emerging Risk Factors Collaboration et al., 2010; Raghavan et al., 2019). In the US, 34.2 million people have been diagnosed with diabetes and 88 million people, 18 years or older, have been diagnosed with prediabetes (Centers for Disease Control and Prevention [CDC], 2020). High blood glucose levels associated with diabetes predispose people to CVD (Bailes, 2002). Insulin secretion defects and PPG are components associated with those at risk of T2DM (American Diabetes Association [ADA], 2020) and CVD (Hershon et al., 2019; National Institute of Health [NIH], 2020). Although the study focus is on diseases that are associated with impaired blood glucose control, it is important to note that diabetes mellitus is a major cause of other diseases such as myocardial infarction, chronic kidney failure, lower limb amputation, stroke, and blindness (ADA, 2009).

Having proper glycemic control for healthy individuals is crucial to help with prevention of diabetes (ADA, 2020) and CVD (Hershon et al., 2019; Ceriello et al., 2004). Hemoglobin A1C is an index used to measure overall glycemic control during the previous 2–3 months. PPG significantly contributes to A1C and is an independent risk factor for CVD. Therefore, increased levels of A1C are associated with increased risk of CVD (Hershon et al., 2019). There is increasing evidence to support the importance of

food for prevention and management of both T2DM (Hidayat et al., 2019) and CVD (Zhubi-Bakija et al., 2021). Considering the economic burden of diabetes mellitus, anything that can slow the progression of the disease and delay complications has significant clinical and public health implications (Skyler, 2000).

Diabetes consists of three main types. Type 1 diabetes mellitus is a chronic autoimmune disease where T cells gradually destroy insulin-producing beta cells ultimately creating insulin deficiency (ADA, 2009). T2DM occurs when pancreatic beta cells are unable to secrete enough insulin to keep up with the body's need due to increased insulin resistance (Skyler et al., 2017). Last, gestational diabetes is glucose intolerance during pregnancy that may be a result of underlying beta cell dysfunction and lead to insulin resistance, which is not categorized as overt diabetes and may resolve after birth (ADA, 2013). Of the 30 million people in the US diagnosed with diabetes, approximately 95% of them have T2DM (Levy, 2019; Skyler et al., 2017).

In healthy individuals, when food is ingested it causes a rise in plasma glucose. This rise elicits a postprandial increase in insulin released by pancreatic beta cells (Hershon et al., 2019). Insulin resistance occurs when the body requires more insulin to bring glucose into the cells then normal (Wilcox, 2005). T2DM occurs when dysfunctional beta cells cannot keep up with extra insulin needs due to insulin resistance leading to hyperglycemia (Hershon et al., 2019). Improving glucose homeostasis, reducing plasma glucose, and managing obesity through diet and exercise may be able to prevent T2DM (Chatterjee et al., 2017).

Insulin Response to a Meal

The historical recommendation for ideal dietary management of T2DM has involved high carbohydrates (CHO), consisting of 55–60% of a person's daily energy requirements (ADA, 2020). More recently, a shift in beliefs has found that higher protein and lower CHO may provide better glycemic and weight control for T2DM (Layman et al., 2008). This meal composition has been found to help with weight loss in obese individuals and may also be beneficial for healthy individuals in disease risk prevention (Lasker et al., 2008). The insulin response varies based on meal contents. Sun et al. (2014) evaluated different meal compositions using rice and a combination of chicken, fat, and/or vegetable in healthy participants. The meal that incorporated both rice and chicken breast led to a significantly increased insulin response compared to white rice alone. The insulinemic index for the meal with chicken was significantly higher than other meals at a value of 89 compared to a meal of glucose alone as a control with a value of 100 or rice alone with a value of 64. This study identified differences in the insulin response to glucose alone when compared to a CHO source with protein in healthy individuals.

In another study looking at T2DM patients, the insulin response was greater in individuals that ingested protein (free AA/protein mixture) with CHO compared to CHO alone (Van Loon et al., 2003). It is the insulin secretory response to carbohydrates not AA induced insulin secretion that has been determined to be blunted in T2DM. Therefore, it is proposed that increasing the AA composition of a meal that contains CHO can have a greater effect on the postprandial insulin response (Manders et al., 2014;

Hidayat et al., 2019). In a study done by Nilsson et al. (2004), which looked at healthy male participants, a meal with whey protein had a significantly higher insulin response when compared to milk, cod, or cheese as the protein source. This study concludes that milk proteins have insulinotropic properties in healthy males as well.

The insulin response may correlate with plasma AA concentrations. A protein hydrolysate induces a greater insulin response than intact protein due to a more rapid increase in plasma AA concentrations (Manders et al., 2014), but the difference may not be significant enough to warrant the use of hydrolysate over intact protein. It is also likely that the protein source may have a significant impact on the insulin response (Nilsson et al., 2004). Dietary protein sources vary in their AA composition, digestion, and absorption kinetics (Manders et al., 2014).

Incretins

When food is ingested hormones are released into the bloodstream from the gut (Edholm et al., 2010; Kim & Egan, 2008). These hormones are known as incretins. The incretins regulate the insulin secretory response to the food. This response is known as the incretin effect and accounts for 50–70% of the total insulin secreted after glucose ingestion (Edholm et al., 2010). The first incretin, glucose-dependent insulinotropic peptide (GIP), is synthesized by the enteroendocrine cells (K cells) within the duodenum and jejunum (Edholm et al., 2010; Kim & Egan, 2008). GIP levels in the body are low during fasting; after eating, GIP is released into the bloodstream within minutes of meals containing glucose or fat, with a weaker response to meals containing AAs. This GIP-mediated insulin secretion is mostly glucose dependent. In those with T2DM the GIP-

mediated insulin secretion is deficient, although there may be no change to plasma concentrations of GIP (Kim & Egan, 2008).

The second incretin, glucagon-like peptide-1 (GLP-1) is produced in the enteroendocrine (L cells) among the enterocytes of the small bowel and ascending colon (Kim & Egan, 2008). Similar to GIP, GLP-1 is secreted into the bloodstream in response to ingestion of food. This incretin is also known for stimulating postprandial insulin secretion as a result of glucose intake. This incretin also decreases food intake, slows the rate of endogenous glucose production, inhibits the secretion of glucagon, and inhibits gastric emptying (GE). These affects should result in a lower blood glucose in those with T2DM (Kim & Egan, 2008).

PPG Concentration After Protein Intake

Fasting plasma blood glucose (FPG) has been a marker of concern for those with T2DM. Unlike FPG, PPG is associated with increased risk of CVD, CVD mortality, and all-cause mortality (Hershon et al., 2019). In both healthy subjects and those with T2DM, a diet consisting of higher protein and decreased CHO has been associated with reduced PPG response, reduced HbA1C, and improved modification of insulin response (Gannon et al., 2003; Layman et al., 2008). A study performed by Gannon et al. (2003) followed a group with T2DM for 5 weeks that consumed a high-protein (30%) and low-CHO (40%) diet. Compared to the control (15% protein and 55% CHO), there was a reduction in PPG and improved glycemic control. During the course of the study HbA1c also had a significant decrease. The 5-week study design was long enough to see a 50% change in HbA1c and consisted of mixed meal composition including milk, beef, and chicken

proteins. A similar study done by Manders et al. (2014) saw a 23% reduction in PPG after ingesting 28 g of casein protein with a 65 g CHO meal.

GE and PPG

GE is the time it takes for the contents of the stomach to pass through and into the small intestine (Marathe et al., 2013). The GE rate depends on the composition, macronutrient, and energy contents of a meal. GE is different for solid meals versus liquids. When a solid meal is consumed first there is a "lag phase" that involves the breakdown of the solids, then a "linear emptying phase" follows (Lupoli et al., 2019). GE with liquids is directly connected to the volume of the stomach contents. The meal composition can have a drastic impact on GE. If a meal contains fat, protein, low GI foods, and dietary fibers then GE is slower (Lupoli et al., 2019). GE is delayed in those who have had T2DM for a long duration and often increased in those with early T2DM. It is theorized that GE affects PPG levels, therefore modifying the GE rate could improve PPG control (Marathe et al., 2013).

Blood AA Concentration After Intake of Protein

As previously stated, the AA concentration of a meal has an effect on the body's insulin response (Hidayat et al., 2019; Manders et al., 2014) and PPG response (Gannon et al., 2003; Layman et al., 2008). Whey protein isolate has a very favorable AA profile (Brennan et al., 2019). In a study completed by Nilsson et al. (2004), the AA profile of meals containing cod, whey, cheese, and milk were analyzed. The meal containing whey had postprandial blood containing leucine, lysine, valine, isoleucine, proline, alanine, and threonine with the highest peaks. The cod meal had lower levels of all AA concentrations

except alanine and lysine which peaked after 120 mins. The cheese meal had its highest AA peaks for proline and alanine. Milk had the highest response of proline, isoleucine, alanine, glutamine, valine, lysine, and leucine (Nilsson et al., 2004).

BCAAs in high concentrations have contributed to glucose production in the liver through the process of gluconeogenesis, which contributes to homeostasis of the body's blood glucose (Esteves de Oliveira et al., 2011). The BCAAs are valine, leucine, and isoleucine, and they are powerful insulin secretagogues (Esteves de Oliveira et al., 2011). Although plant proteins have different AA compositions, there is typically less leucine found in plant-based protein sources; consequently, they must be consumed in higher doses to match the content that can be found in other sources such as dairy. It is also important to note that plant-based proteins, even when matched, are not bio-equivalent to animal proteins (Brennan et al., 2019).

Van Loon et al. (2003) reported in T2DM patients an AA mixture with free phenylalanine, leucine, and other essential AAs, have had an elevated insulinotropic potential. Valine, leucine, isoleucine, and lysine had the greatest correlation between postprandial insulin and early rises in plasma AAs (Nilsson et al., 2004). Dietary AAs, glycine and leucine, simulated the release of insulin from the pancreas. Leucine also regulated the intracellular insulin signal in the adipose tissue and skeletal muscle (Layman et al., 2008). Other AAs have been known for stimulating the incretins, GIP and GLP-1, leading to reduced gastric leakage, inhibited glucagon secretion, and stimulation of insulin secretion (Esteves de Oliveira et al., 2011).

In conclusion, whey protein has key features that have made it a great choice for those looking to increase insulin secretion and decrease PPG. This study is needed to assess the use of CPI as another protein option for PPG control. The need for proper glycemic control is becoming an increasingly important form of prevention to reduce the risk factors of prediabetes, T2DM, and CVD in the United States population.

CHAPTER III
METHODS

Participants

This study was approved by the IRB at Texas Woman's University in Denton, TX (see Appendix A). The participants were voluntary and received compensation for the time they provided. All participants signed a written informed consent after the nature of the study and possible risk were explained. There was a total of 34 subjects originally recruited for this study with 28 completing. The inclusion criteria for all groups were male, aged 18–25 years old, in good health, nonalcoholic, nonsmokers, who engaged in regular exercise. Good health was assessed through pre-exercise testing health status questionnaires. All participants were required to be recreationally active with a blood glucose < 100 mg/dL pre OGTT, 200 mg/dL 1 hour post OGTT, and 140 mg/dL 2 hours post OGTT. Participants could not have dairy allergies, were unable to consume alcohol or whey protein supplements 24 hours before treatments and no vigorous exercise 48 hours or any exercise 24 hours prior to visits. Participants were assigned to four treatment groups consisting of WPC, whey protein hydrolysate, CPI, or maltodextrin.

The exclusion criteria for participants were a history of medical events that could significantly affect the study outcome, such as CVD, renal, metabolic, musculoskeletal, and hepatic disorders. Participants were excluded if they used nutritional supplements or medicines that could affect the study outcome. Other exclusion criteria were alcohol consumption $\geq$ 12 servings per week, or if they had a BMI $\geq$ 35 kg/m^2 or $\geq$ 30 kg/m^2 with a body fat percentage $\geq$ 25%.

Study Design

The original study was conducted as a randomized, between-subjects design. Each participant consumed a control diet and completed an overnight 10–12 hour fast prior to the treatment visit. The following morning, subjects in the whey protein arm of the study were assigned to one of three treatments. Each participant completed three treatments that were assigned at random and separated by 1–2 weeks. On the chicken arm of the study each participant only completed one treatment. All treatment visits were alike except for the treatment drinks given. The treatment drinks consisted of 0.3 g/kg lean soft tissue WPC, whey protein hydrolysate, CPI, or maltodextrin. Participants were not identical in both arms of the study, but there was some crossover. For this study, only the whey concentrate, chicken protein, and maltodextrin data are discussed. At the laboratory, participants presented food records to monitor diet compliance. As participants were to consume their same respective meals in the 24 hours preceding blood extraction, food records indicated that meal compliance was maintained. Participants were also screened for consumption of whey protein supplements and alcohol over the 24 hours previous to the treatment visit. Baseline weight, metabolic rate measurements, and a blood sample were taken before any treatments began. Immediately after baseline measurements were taken, treatment beverages were consumed followed by another blood sampling. Each participant had blood samples and metabolic measurements repeated over the course of 3 hours via a cannula in the antecubital vein. Blood was drawn at baseline then repeated at 0, 10, 20, 30, 45, 60, 90, 120, and 180 min following treatment drink consumption.

The first blood sample involved flushing the cannula set with 10 ml of saline, all other samples collected 6 ml of discard before flushing. Blood was stored in vacutainer tubes for serum or plasma (heparin) collection. For serum, 10–20 min was allowed for clotting, followed by a 15-min centrifuge spin at 1500 g at which time the supernatant was transferred and stored at -80°C until analyses. An EZ: fast AA analysis kit (Phenomenex Inc., Torrance, CA, USA) was used to isolate the free AAs using solid phase extraction for subsequent AA analysis using GC/MS. Shimadzu Analysis Software was used to analyze the chromatographs for AA concentrations.

Calculations/Statistical Analysis

The results were analyzed with SPSS 19.0 software (IBM Corp., Armonk, NY). Repeated measures analysis of variance (ANOVA) was used to measure differences in how groups changed over time. The ANOVA was used to measure blood glucose levels, insulin levels, and AA levels. A multivariate analysis of variance (MANOVA) was used for the BCAAs to determine group differences by time. The MANOVA expressed how the three groups changed in their composite score across 10 time points over time. Then 10 repeated measures MANOVA was run on the composite scores at each time point for the BCAAs. Differences among the BCAAs can only be done by running the composite scores since there are 10 time points allowing us to better visualize how the groups differ. A comparative analysis of the three groups: WPC, CPI, and maltodextrin, was conducted. This analysis identifies significant differences between the three groups. A Tukey honest significant difference (HSD) post hoc analysis was used to determine differences in mean glucose, insulin concentrations, BCAA and other blood AAs at each blood collection

time point among treatments. Power calculations were performed to identify chance of type II error. Data was reported as mean ± standard error. A p-value ≤ .05 was considered statistically significant.

CHAPTER IV

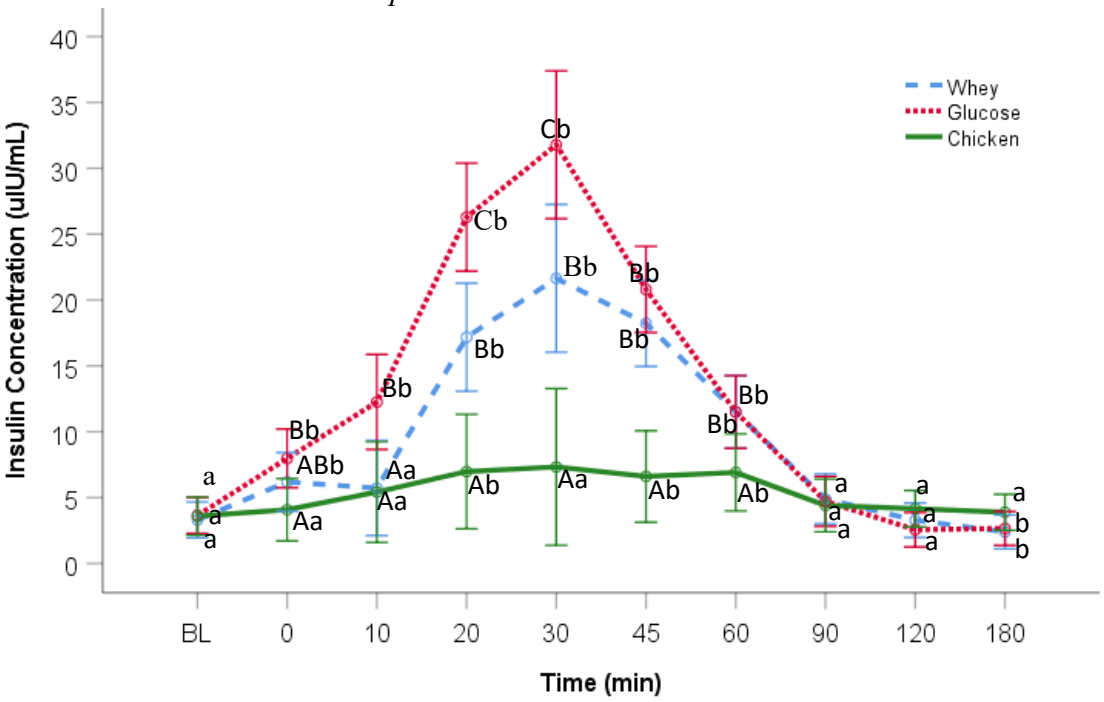
RESULTS

Insulin Response

The insulin response to different protein treatments is presented in Figure 1. A repeated measures ANOVA revealed a significant change in insulin over time regardless of group ($F = 58.606$, $p < .001$) and a significant change in insulin based on time by group interaction ($F = 10.887$, $p < .001$). A Tukey HSD post hoc analysis detected a significant difference between CPI and WPC treatments ($p = .004$), between CPI and glucose ($p < .001$) and between WPC and glucose ($p = 0.030$). WPC and glucose were significantly different at 10, 20, and 30 min with glucose being significantly higher till reaching its peak at 30 min. CPI and WPC were significantly different at 20, 30, 45, and 60 min, with WPC having a higher insulin release. CPI and glucose were significantly different at 0, 10, 20, 30, 45, and 60 min with glucose inducing a higher insulin response. There was no significant difference between groups at baseline (BL) or at 90, 120, and 180 min. There was an expected insulinogenic effect by both whey protein and glucose intake as shown in Figure 1. CPI produced only a small elevation in insulin over time. Figure 2 presents insulin area under the curve (AUC). Test for outliers removed one data point from the CPI and glucose groups. No significant difference was seen between groups.

Figure 1

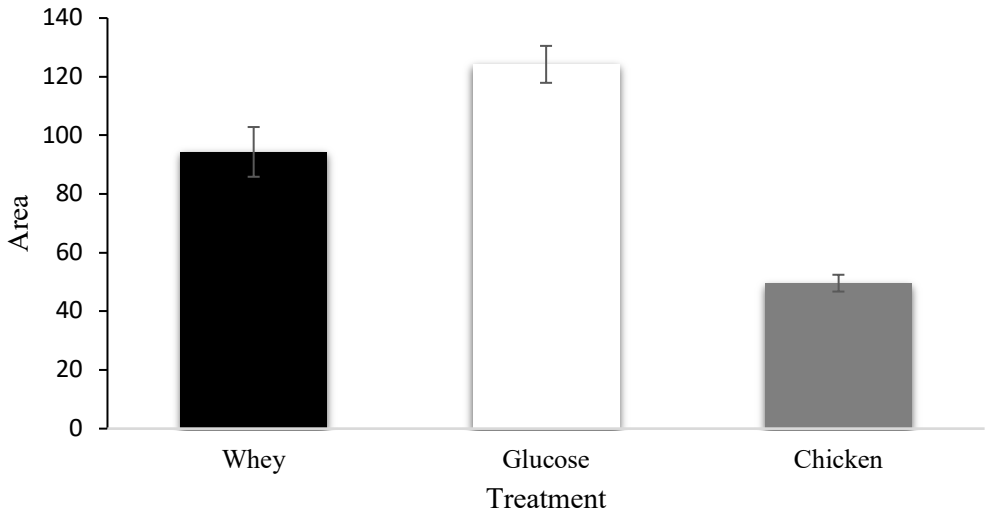
Insulin Concentration in Response to Treatment



Note. Capital letters (i.e., ABC) indicate significant difference between treatment groups ($p \leq .05$). Lower case letters (i.e., abc) indicate significant difference from baseline compared to another point within the same treatment group. Data is mean ± SEM.

Figure 2

Insulin AUC



Note. Mean ± SEM.

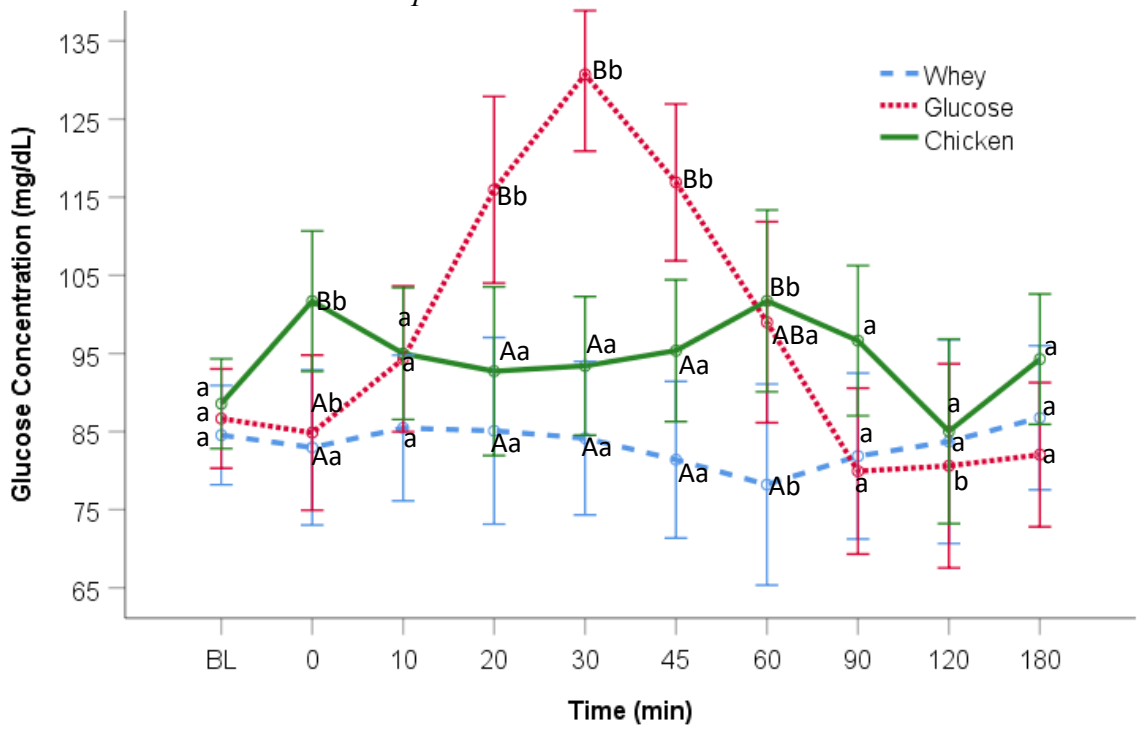
Glucose Response

The glucose response to treatment is presented in Figure 3. There was a significant change in glucose over time regardless of group ($F = 8.845$, $p < .001$) and a significant change in glucose based on time by group interaction ($F = 8.957$, $p < .001$). A Tukey HSD post hoc analysis detected a significant difference ($p = .047$) between WPC and glucose. There was no significant difference ($p = .866$) between CPI and glucose nor ($p = .104$) between CPI and WPC treatments. The plasma glucose concentrations were lowest with the WPC compared to CPI but both proteins had lower peak responses compared to glucose ingestion. Differences existed between treatment groups at specific time points. WPC and glucose were significantly different at 20, 30, and 45 min with a greater increase for the glucose treatment group. CPI and WPC were significantly

different at time point 0 and at 60 min, with CPI having higher glucose concentrations. CPI and glucose were significantly different at time 0, 20, 30, and 45 min. There was no significance between groups at baseline (BL), 10, 90, 120, and 180 min. Glucose concentration for the CPI group remain between 88–102 mg/dL with a steady blood glucose range. Glucose concentrations for the glucose group peaked as high as 130 mg/dL and as low as 79 mg/dL. The WPC group had steady glucose concentrations throughout the treatment times with a low of 78 mg/dL and a standard error reaching 65 mg/dL reaching levels that may signify hypoglycemia. Figure 4 presents blood glucose AUC. In a test for outliers there were two data points removed from the CPI group. No significance was seen between groups.

Figure 3

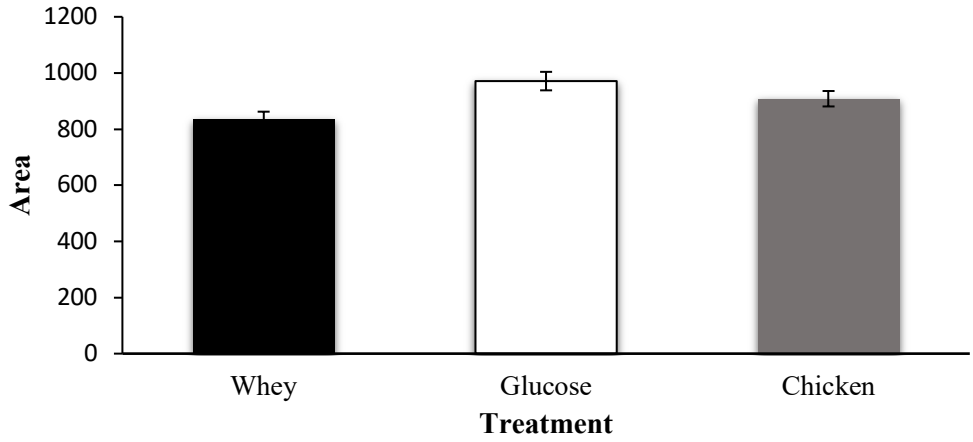
Glucose Concentrations in Response to Treatment



Note. Capital letters (i.e., ABC) indicate significant difference between treatment groups ($p \leq .05$). Lower case letters (i.e., abc) indicate significant difference from baseline compared to another point within the same treatment group. Data is mean ± SEM.

Figure 4

Blood Glucose AUC



Note. Mean ± SEM.

AA Response

Table 1 and Table 2 present AA concentration in response to the treatment groups. Baseline AA concentrations were not similar among groups, therefore AA concentrations were unable to be compared across groups. The CPI group tended to have a lower concentration at baseline. AA levels were analyzed based on differences from baseline within groups. AA response to whey protein ingestion resulted in an increase in levels that reached a peak within the 30-to-60-min time frame followed by a steady decline back to baseline or below by 180 min. AA concentrations in response to chicken resulted in steady increase in levels throughout the time frame, with many AA levels decreasing to levels at or above baseline at 180 min. None of the AA levels were significantly below baseline at 180 min in the CPI group.

Table 1

Amino Acid Concentration in Response to Treatment

Amino Acid	Time									
nmol/ml	BL	0	10	20	30	45	60	90	120	180
GLU										
Whey	35.38 ± 2.15[a]	34.87 ± 2.40[a]	41.13 ± 3.28[a]	47.67 ± 3.36[b]	54.65 ± 4.69[b]	58.48 ± 6.42[b]	51.76 ± 3.36[b]	50.27 ± 3.68[b]	44.01 ± 2.02[b]	38.56 ± 2.26[a]
Glucose	35.63 ± 2.08	34.04 ± 2.46	36.28 ± 2.51	39.98 ± 4.27	38.17 ± 3.14	38.21 ± 3.06	36.75 ± 2.24	36.64 ± 3.13	38.27 ± 2.84	35.67 ± 3.09
Chicken	32.3 ± 4.43	31.41 ± 4.36	29.86 ± 3.61	30.96 ± 6.69	39.36 ± 8.01	38.83 ± 7.78	40.09 ± 8.28	38.21 ± 7.54	37.24 ± 7.32	28.97 ± 4.21
ALA										
Whey	412.03 ± 34.14[a]	356.83 ± 39.95[a]	349.66 ± 50.39[a]	391.52 ± 44.33[a]	429.10 ± 50.42[a]	434.83 ± 56.18[a]	423.56 ± 34.28[a]	419.49 ± 59.61[a]	366.10 ± 36.83[a]	308.12 ± 43.41[b]
Glucose	380.16 ± 20.50[a]	411.32 ± 23.87[b]	396.53 ± 26.56[a]	364.08 ± 29.24[a]	329.30 ± 32.10[c]	362.74 ± 32.06[a]	339.85 ± 30.40[c]	346.67 ± 34.93[a]	344.94 ± 26.94[c]	364.30 ± 35.02[a]
Chicken	445.96 ± 44.38[a]	458.77 ± 46.75[a]	467.59 ± 54.78[a]	486.50 ± 48.84[b]	495.66 ± 57.69[b]	481.41 ± 44.80 [a]	479.08 ± 52.42[a]	476.88 ± 58.32[a]	516.31 ± 68.35[a]	440.29 ± 49.58[a]
PRO										
Whey	231.95 ± 22.79[a]	211.67 ± 17.11[a]	230.48 ± 23.92[a]	280.43 ± 21.31[b]	315.86 ± 23.88[b]	308.15 ± 30.30[b]	281.67 ± 13.90[a]	267.28 ± 31.47[a]	238.17 ± 10.76[a]	199.28 ± 21.65[a]
Glucose	224.75 ± 19.92 [a]	231.4 ± 19.35[a]	229.84 ± 22.61[a]	214.95 ± 20.40[a]	201.77 ± 22.90[b]	210.53 ± 21.35[a]	191.21 ± 21.51[b]	184.79 ± 21.48[b]	191.10 ± 17.66[b]	202.88 ± 20.63[a]
Chicken	192.63 ± 15.27[a]	201.29 ± 20.06[a]	202.72 ± 17.72[a]	216.96 ± 17.05[b]	229.21 ± 24.71[b]	230.11 ± 18.75[b]	225.97 ± 20.56[b]	214.92 ± 21.18[a]	232.06 ± 25.86[b]	202.56 ± 16.47[a]
PHE										
Whey	70.95 ± 3.72[a]	65.33 ± 3.63[a]	69.83 ± 2.90[a]	83.11 ± 4.75[a]	90.5 ± 4.62[b]	82.36 ± 6.29[a]	74.88 ± 4.82[a]	69.28 ± 6.20[a]	62.86 ± 3.38[a]	52.09 ± 4.25[c]
Glucose	67.23 ± 2.33[a]	67.29 ± 3.27[a]	65.77 ± 3.31[a]	61.96 ± 3.40[b]	57.56 ± 3.65[b]	60.92 ± 3.77[b]	54.92 ± 2.44[b]	55.08 ± 4.75[b]	56.12 ± 2.50[b]	57.60 ± 3.82[b]
Chicken	63.75 ± 2.71[a]	63.63 ± 2.47[a]	64.73 ± 3.46[a]	65.23 ± 3.40[a]	69.67 ± 4.88[b]	70.02 ± 2.55[b]	69.00 ± 3.81[a]	67.5 ± 3.50[a]	70.94 ± 3.88[b]	64.38 ± 2.56[a]
TRP										
Whey	72.01 ± 2.80[a]	66.03 ± 3.28[a]	68.47 ± 3.14[a]	75.15 ± 4.57[a]	82.05 ± 5.98[a]	81.30 ± 6.42[a]	78.67 ± 3.74[a]	73.51 ± 5.76[a]	70.81 ± 4.37[a]	60.50 ± 4.30[b]
Glucose	68.63 ± 2.19[a]	69.66 ± 3.35[a]	65.76 ± 2.11[b]	62.71 ± 5.96[a]	64.65 ± 4.87[a]	66.69 ± 4.91[a]	61.90 ± 4.63[c]	59.41 ± 4.21[c]	61.62 ± 3.27[c]	60.70 ± 3.55[c]
Chicken	44.36 ± 2.01[a]	48.76 ± 2.55[a]	47.95 ± 2.36[a]	53.75 ± 3.46[b]	50.73 ± 3.46[a]	52.34 ± 3.00[b]	52.77 ± 2.58[b]	51.39 ± 3.52[a]	56.53 ± 3.74[b]	47.20 ± 2.79[a]
TYR										
Whey	67.49 ± 3.66[a]	59.75 ± 4.12[a]	63.20 ± 3.53[a]	77.20 ± 6.56[a]	87.85 ± 7.14[b]	86.89 ± 8.37[b]	84.77 ± 8.96[a]	77.33 ± 8.53[a]	65.54 ± 5.06[a]	52.43 ± 3.96[c]
Glucose	63.71 ± 4.00[a]	63.36 ± 4.45[a]	62.58 ± 4.12[a]	59.66 ± 4.49[a]	55.49 ± 5.01[b]	57.08 ± 4.24[b]	49.56 ± 3.24[b]	49.20 ± 4.45[b]	51.05 ± 3.71[b]	52.00 ± 4.62[b]
Chicken	60.96 ± 3.03[a]	60.11 ± 3.53[a]	60.26 ± 4.00[a]	60.90 ± 3.52[a]	64.59 ± 3.96[a]	67.03 ± 2.37[b]	66.32 ± 3.6[a]	63.49 ± 2.8[a]	64.79 ± 3.11[a]	59.51 ± 2.98[a]

Note. Table of mean AA concentrations at each time point for each treatment group ± SEM. Means with different superscripts differ at the $p = .05$ level.

[a] baseline of treatment group. [b, c] significantly different from baseline within the same treatment group.

Table 2

Amino Acid Concentration in Response to Treatment

Amino Acid	Time									
nmol/mL	BL	0	10	20	30	45	60	90	120	180
GLY										
Whey	328.64 ± 30.42[a]	293.22 ± 26.66[a]	296.54 ± 28.58[a]	303.50 ± 25.30[a]	312.33 ± 30.85[a]	290.36 ± 33.11[a]	269.60 ± 19.98[a]	264.96 ± 30.21[b]	260.70 ± 24.26[b]	243.1 ± 29.88[b]
Glucose	311.82 ± 20.91[a]	321.11 ±24.39[a]	319.44 ± 24.95[a]	297.35 ± 24.48[a]	268.79 ± 19.34[b]	294.85 ± 25.78[a]	272.19 ± 23.31[b]	269.92 ± 26.34[a]	280.43 ± 20.62[b]	303.61 ± 26.33[a]
Chicken	356.72 ± 33.92[a]	359.66 ± 32.40[a]	365.19 ± 32.60[a]	400.34 ± 43.07[a]	434.07 ± 52.71[b]	402.96 ± 36.94[b]	388.97 ± 33.74[a]	388.44 ± 40.53[a]	439.98 ± 55.22[b]	387.68 ± 43.67[a]
THR										
Whey	145.00 ± 11.12[a]	132.78 ± 12.17[a]	152.21 ± 12.15[a]	200.13 ± 20.02[b]	250.49 ± 22.77[b]	255.00 ± 28.56[b]	231.62 ± 12.50[b]	214.48 ± 28.03[b]	188.31 ± 15.60[b]	150.90 ± 15.88[a]
Glucose	168.51 ± 36.66[a]	170.08 ± 34.60[a]	168.59 ± 32.48[a]	161.08 ± 32.66[a]	150.34 ± 28.46[a]	160.50 ± 33.55[a]	137.55 ± 27.67[b]	136.18 ± 28.59[b]	144.89 ± 26.87[a]	150.04 ± 29.94[a]
Chicken	195.37 ± 19.09[a]	202.47 ± 17.65[a]	215.00 ± 23.05[a]	225.80 ± 25.03[a]	252.04 ± 25.78[b]	247.15 ± 17.42[b]	265.94 ± 29.45[b]	250.26 ± 24.96[b]	272.42 ± 25.50[b]	222.79 ± 19.72[b]
SER										
Whey	112.15 ± 11.44[a]	101.07 ± 9.39[a]	122.93 ± 10.14[a]	164.75 ± 14.18[b]	186.49 ± 20.71[b]	184.89 ± 19.57[b]	148.71 ± 9.22[b]	137.69 ± 20.29[a]	120.34 ± 11.31[a]	104.78 ± 12.93[a]
Glucose	119.66 ± 6.43[a]	122.54 ± 6.02[a]	120.22 ± 5.86[a]	118.04 ± 4.88[a]	107.89 ± 5.15[a]	117.36 ± 7.09[a]	99.85 ± 4.45[b]	101.44 ± 6.84[a]	112.93 ± 5.30[a]	117.52 ± 5.53[a]
Chicken	150.37 ± 13.58[a]	146.98 ± 12.40[a]	158.07 ± 7.45[a]	165.21 ± 13.71[a]	195.97 ± 15.46[b]	188.12 ± 12.27[b]	191.22 ± 16.12[b]	172.20 ± 16.44[a]	190.22 ± 16.50[b]	148.99 ± 12.81[a]
ASN										
Whey	67.00 ± 8.16[a]	58.82 ± 6.74[a]	63.73 ± 6.02[a]	86.82 ± 9.48[b]	106.98 ± 9.15[b]	99.65 ± 7.96[b]	91.95 ± 7.65[b]	80.49 ± 9.57[a]	64.67 ± 5.22[a]	54.8 ± 4.68[a]
Glucose	68.12 ± 7.77[a]	68.62 ± 7.26[a]	67.13 ± 6.63[a]	64.31 ± 7.40[a]	58.23 ± 7.14[b]	61.48 ± 6.81[a]	54.01 ± 6.38[b]	52.83 ± 7.08[b]	58.31 ± 6.98[b]	61.98 ± 6.10[a]
Chicken	43.33 ± 15.37	65.04 ± 16.60	51.04 ± 12.83	50.07 ± 14.61	53.57 ± 13.15	42.3 ± 7.06	43.09 ± 8.32	38.96 ± 5.77	53.08 ± 9.34	35.03 ± 7.69
GLN										
Whey	713.47 ± 55.72	629.47 ± 77.86	635.36 ± 88.23	624.70 ± 106.67	779.51 ± 95.03	767.57 ± 99.43	660.5 ± 55.37	649.2 ± 125.54	693.2 ± 68.61	628.83 ± 63.31
Glucose	721.01 ± 51.16[a]	756.96 ± 41.91[a]	720.62 ± 36.26[a]	652.11 ± 77.89[a]	678.56 ± 38.25[a]	731.55 46.93[a]	607.56 ± 33.96[b]	669.86 ± 56.02[a]	714.8 ± 29.90[a]	702.78 ± 71.77[a]
Chicken	353.36 ± 32.08[a]	423.68 ± 26.65[b]	457.35 ± 41.77[b]	495.84 ± 50.80[b]	509.18 ± 51.97[b]	490.41 ± 55.81[b]	500.48 ± 39.55[b]	463.84 ± 50.48[a]	515.94 ± 41.83[b]	461.08 ± 41.84[b]
LYS										
Whey	262.72 ± 24.48[a]	235.40 ± 18.30[a]	264.25 ± 12.07[a]	365.19 ± 21.42[b]	466.60 ± 29.87[b]	447.00 ± 27.15[b]	406.73 ± 22.36[b]	365.33 ± 35.14[b]	279.64 ± 24.12[a]	245.56 ± 23.54[a]
Glucose	259.67 ± 19.69[a]	296.36 ± 19.61[a]	267.15 ± 17.35[a]	240.88 ± 31.01[a]	228.35 ± 23.80[a]	241.97 ± 20.86[a]	211.74 ± 15.72[b]	221.41 ± 24.90[a]	238.40 ± 20.31[a]	247.23 ± 29.45[a]
Chicken	125.17 ± 5.23[a]	122.38 ± 5.65[a]	128.56 ± 8.40[a]	139.60 ± 8.26[b]	155.31 ± 12.12[b]	167.21 ± 9.15[b]	160.70 ± 10.27[b]	162.85 ± 10.91[b]	169.93 ± 11.15[b]	145.26 ± 6.97[b]
HIS										
Whey	111.55 ± 4.20[a]	102.88 ± 6.42[a]	105.61 ± 4.87[a]	112.18 ± 7.54[a]	125.63 ± 7.29[a]	120.20 ± 9.86[a]	118.34 ± 4.35[a]	114.62 ± 11.15[a]	106.64 ± 6.19[a]	100.87 ± 5.43[b]
Glucose	110.84 ± 3.43[a]	116.66 ± 5.91[a]	108.87 ± 4.77[a]	111.04 ± 5.62[a]	104.31 ± 5.71[a]	110.60 ± 6.35[a]	98.95 ± 4.24[b]	98.72 ± 6.45[a]	108.63 ± 4.81[a]	105.76 ± 9.51[a]
Chicken	68.86 ± 3.16[a]	69.09 ± 3.74[a]	70.14 ± 4.58[a]	71.44 ± 4.03[a]	75.35 ± 3.72[b]	78.32 ± 2.82[b]	74.20 ± 3.05[a]	74.82 ± 3.53[a]	76.81 ± 3.46[b]	73.53 ± 3.06[a]

Note. Table of mean AA concentrations at each time point for each treatment group ± SEM. Means with different superscripts differ at the $p = .05$ level.

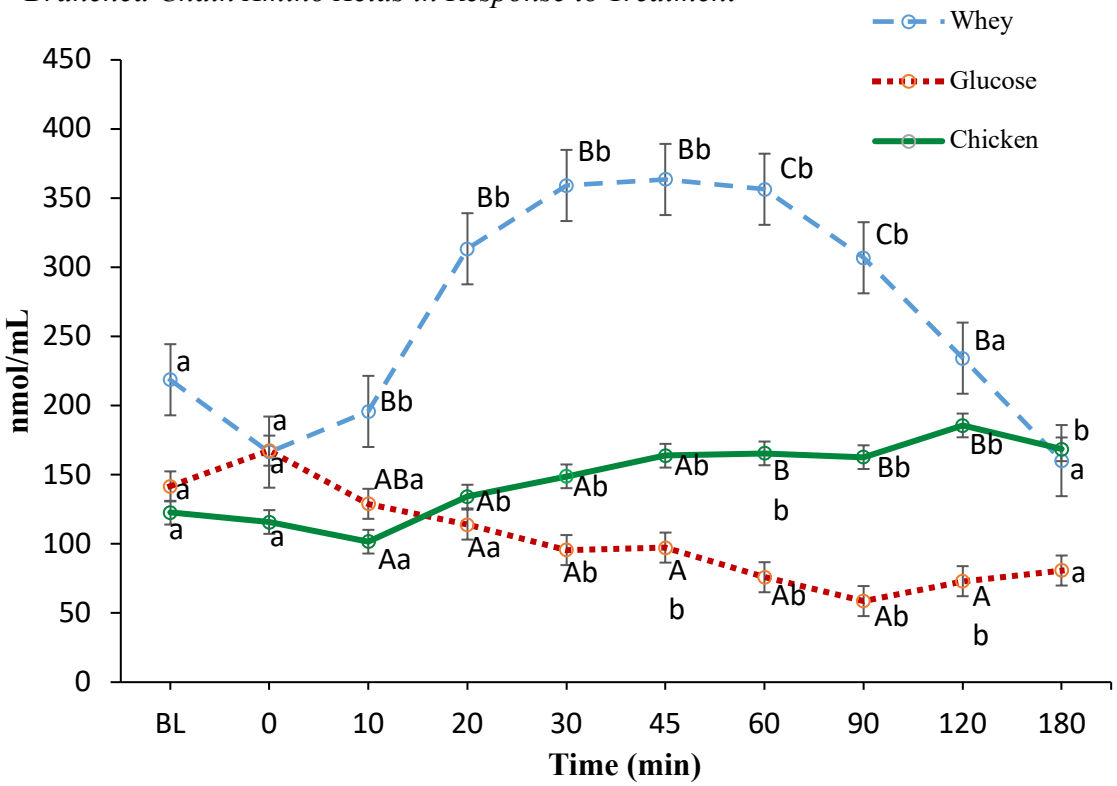
[a] baseline comparison. [b, c] significantly different from baseline within the same treatment group.

BCAAs

BCAAs in response to treatment is presented in Figure 5. A significant change in BCAAs based on treatment group and time was identified ($F = 8.360$, $p < .001$). A Tukey HSD post hoc analysis detected a significant difference ($p = .002$) between CPI and WPC treatments and a significant difference ($p < .001$) between WPC and glucose. Differences existed between treatment groups at specific time points. WPC had a significantly higher amount of BCAAs at time points 20, 30, 45, 60, and 90 min compared to CPI and glucose. There was no significant difference between groups at 180 min but the value of WPC was below the baseline value. CPI had a steady increase of BCAAs over time with a significant increase at 180 min compared to baseline. Figure 6 presents BCAAs AUC. A test for outliers was run and two data points were removed from the CPI group. No significance was seen between groups.

Figure 5

Branched Chain Amino Acids in Response to Treatment



Note. Capital letters (i.e., ABC) indicate significant difference between treatment groups ($p \leq .05$). Lower case letters (i.e., abc) indicate significant difference from baseline compared to another point within the same treatment group. Data is mean ± SEM.

Figure 6

BCAA AUC

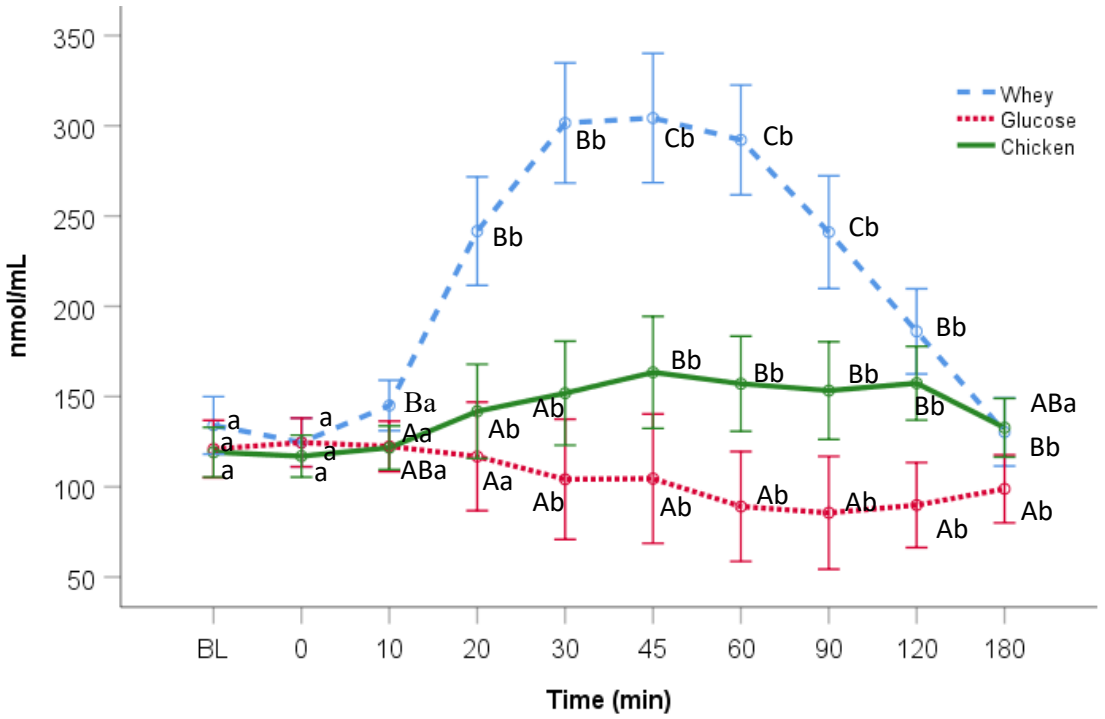
Note. Mean ± SEM.

Individual BCAAs: Leucine, Isoleucine, and Valine

Figure 7, 8, and 9 represent the leucine, isoleucine, and valine response to treatment. There was a significant difference between all three groups at 45, 60, and 90 min with whey having the greatest increase in leucine over that time. Although the BCAAs decrease in all three treatment groups at 180 min, chicken has a significant amount above baseline of all three BCAAs. Whey and glucose are either similar to baseline or significantly below baseline at 180 min.

Figure 7

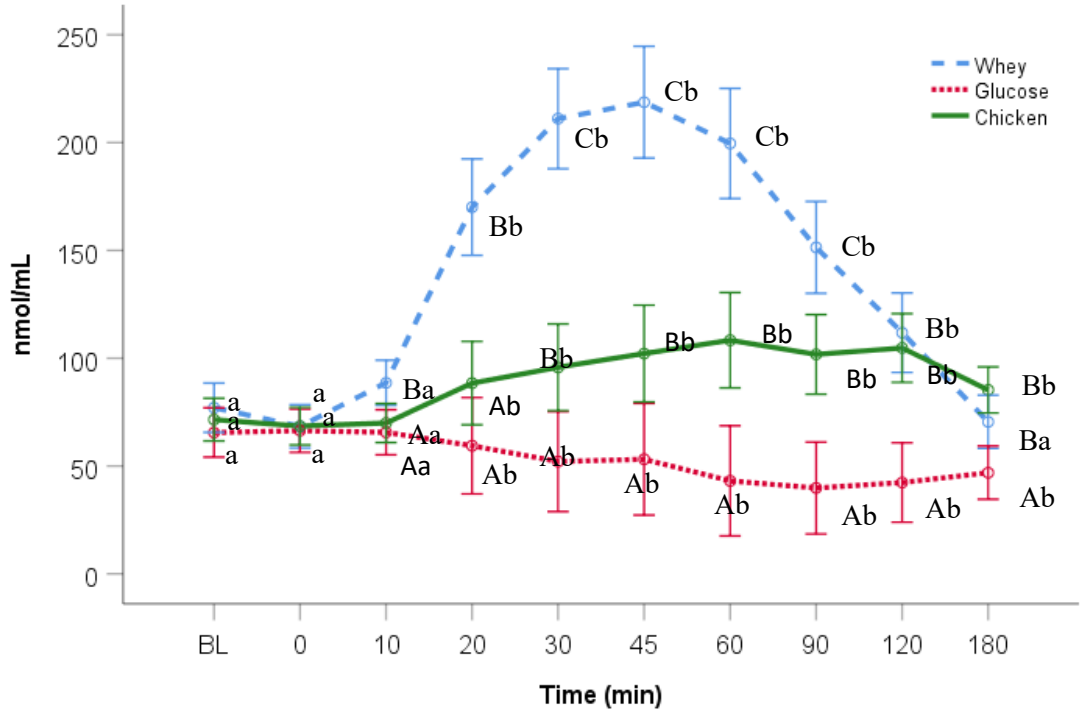
Leucine



Note. Capital letters (i.e., ABC) indicate significant difference between treatment groups ($p \leq .05$). Lower case letters (i.e., abc) indicate significant difference from baseline compared to another point within the same treatment group. Data is mean ± SEM.

Figure 8

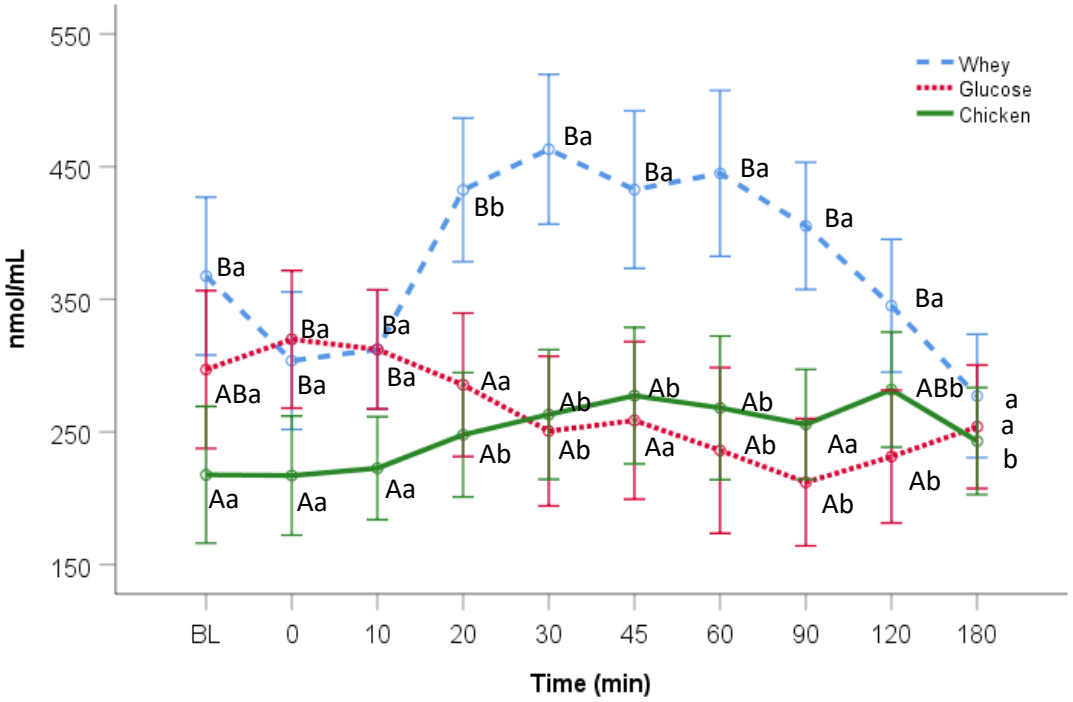
Isoleucine



Note. Capital letters (i.e., ABC) indicate significant difference between treatment groups ($p \leq .05$). Lower case letters (i.e., abc) indicate significant difference from baseline compared to another point within the same treatment group. Data is mean ± SEM.

Figure 9

Valine



Note. Capital letters (i.e., ABC) indicate significant difference between treatment groups ($p \leq .05$). Lower case letters (i.e., abc) indicate significant difference from baseline compared to another point within the same treatment group. Data is mean ± SEM.

CHAPTER V

DISCUSSION

The present study is the first to compare the impact of whey protein versus chicken protein on blood insulin, glucose, and AA profiles and concentrations across time. It was hypothesized that CPI would elicit a similar insulin response as WPC. In addition, it was proposed that CPI would promote control of PPG concentrations as effectively as WPC and better than glucose ingestion alone. Overall, the results of the study demonstrated that both WPC and glucose intake caused an insulinogenic effect with both reaching a peak at 30 min and then dropping below baseline at 180 min. While chicken consumption resulted in a significant rise in insulin, it was much lower than that seen in the other treatment groups. Both the chicken and whey treatment groups demonstrated better control of blood glucose compared to the glucose treatment group.

Plasma glucose concentrations were significantly increased after chicken protein intake compared to whey protein intake at time point 0 and 60 min. Though plasma glucose levels were increased with chicken intake, they still remained within a normal range and did not show the significant decrease below baseline as was induced by whey ingestion. The insulin response to glucose and whey ingestion led to blood glucose changes that could be characteristic of rebound hypoglycemia. In a study looking at four protein meals: whey, tuna, turkey, and egg albumin, in healthy men, there was a significant decrease in blood glucose after ingestion of a whey protein meal compared to the other three meals after a large postprandial insulin response (Pal & Ellis, 2010).

Similarly, Silva Ton et al. (2014) compared whey protein, egg white, and soy protein drink consumption in healthy individuals. Whey protein had a lower glycemic response than the other two protein drinks for the first 45 min but then there was no significant difference at 60, 90, or 120 min. Although this study implied it was the postprandial insulin secretion that caused this response in whey it was not directly measured. Contrasting this to a study in T2DM individuals with mixed meals, one meal containing whey and one containing ham as the protein source, the postprandial glucose was not significantly different between meals but the postprandial insulin was significantly higher for whey (Frid et al., 2005). Our study similarly saw WPC having a lower glycemic response and CPI had a similar response to other proteins such as ham and soy with the glycemic response slightly higher than WPC, with WPC having the higher postprandial insulin. The insulin and postprandial glucose response are similar in the studies with T2DM and healthy individuals.

Previously proteins have been thought to reduce the glucose response following ingestion, an effect mediated through an increase in insulin secretion (Hätönen et al., 2011). In the present study WPC ingestion, through its stimulated insulin release, resulted in low levels of glucose and by 180 min, induced a significant drop below baseline. Conversely CPI, resulted in consistently lower levels of insulin with two significant blood glucose peaks the highest reaching 102 mg/dL and had no values dropping significantly below baseline. In a study by Hätönen et al. (2011), when chicken breast was ingested with mashed potatoes in healthy individuals there was an increased insulin response compared to ingestion of mashed potatoes alone. A similar insulin response was

seen by Paterson et al. (2016) when combining chicken breast with rice in a study evaluating individuals with T1DM. Hätönen et al. (2011) reported a significantly decreased glycemic response with the mashed potato and chicken breast meal compared to mashed potatoes alone at the peak 30-min mark ($p = 0.04$). The study by Paterson et al. (2016) conveyed a similar glucose response with the 25 g protein drink but in contrast to the previous study the 20 g glucose drink reached a peak at 60 min and remained significantly increased till 120 min. This study was a little longer and resulted in the whey isolate protein powder, 75g and 100g, having significantly higher glycemic excursions than the glucose control at the 240- and 300-min interval. Although this is a phenomenon typically reported in T1DM as seen by Paterson et al (2016), the grams of protein in the current study did not reach the levels that typically see higher postprandial glycemic responses to compare the response in T1DM to healthy individuals. In the current study there began a rise in plasma glucose at 180 min in the WPC and CPI groups. The rise was not significant and it was unclear if it would continue past that time point. These studies including the current study suggest that the consumption of protein alone or in mixed meals will produce a lower glucose response compared to drinks or meals without protein whether or not there is a high insulin response.

It is important to note that hyperinsulinemia that follows hyperglycemia may be a risk factor for diseases associated with the metabolic syndrome; however, there is a lack of evidence on how hyperinsulinemia in the long-term, even without hyperglycemia, may affect health (Frid et al., 2005). CPI resulted in steady insulin control, compared to whey and glucose ingestion. The studies that have looked into whey protein as an insulin

secretagogue have not considered whether or not there are negative ramifications for this. The long-term effects of non-glucose-induced hyperinsulinemia have yet to be fully investigated, and there is less understanding on what it means for a protein to have a hyperinsulinemia response versus a moderate or low one. A review looking at the effects of hyperinsulinemia on the body has made connections with hyperinsulinemia preceding insulin resistance, moderate to high insulin levels promoting lipogenesis/obesity, also insulin therapy associated with increased risk of cardiovascular events or death for individuals with T2DM (Kolb et al., 2020). Although interesting for further research in diet induced hyperinsulinemia not related to hyperglycemia, many studies in this review have looked at hyperglycemia in relation to insulin therapy or in vitro. Hyperinsulinemia and its affect stretch past diabetes and can be an area of concern for metabolic syndrome and Alzheimer's disease, although there seems to be many direct and indirect mechanisms (Crofts et al., 2015).

The insulinogenic effect seen after whey ingestion is considered to be due to the significant BCAA rise in the blood (Gunnerud et al., 2012; Mignone et al., 2015; Pal & Ellis, 2010). This was also noticed in the present study. In the WPC group BCAAs increased from 0 min until a peak was reached at 45 min with a slow decrease to baseline, conversely insulin increased till it peaked at 30 min and quickly declined below baseline. When Gunnerud et al. (2012) evaluated human milk, bovine milk, and casein drinks in healthy individuals all protein drinks had a lowered glycemic response compared to the control. There also seemed to be a positive correlation between increased plasma AA levels and insulin secretion similar to what the current study saw in response to whey.

AA concentration difference from baseline was assessed as baseline levels tended to be lower prior to ingestion of chicken protein compared to glucose or whey. Whey ingestion led to a higher early AA response whereas chicken had a longer-term sustained response. AA concentrations at 180 min were at or above baseline for the CPI group whereas the whey group had levels at or below baseline at 180 min. The CPI group also had higher levels of BCAA at the 180-min mark, which was significantly higher than baseline whereas whey had a large rise in BCAAs early on and then dropped to below baseline by 180 min. In a postprandial study done by Samman et al. (2014), comparing chicken meal with a pork meal in healthy participants, they also reported a significant increase in BCAAs at 180 min after the ingestion of the chicken meal. A significant difference was also seen at 240 min for leucine and isoleucine compared to the pork meal indicating a need for a longer study to better assess the effects of chicken in the current study. Other studies have noted the higher BCAA concentrations seen after the ingestion of whey protein, but to our knowledge only the previously mentioned study has recorded the affects after 180 min (Nilsson et al., 2004).

When addressing AAs, other than BCAA, the focus has been on what AAs may affect insulin secretion. Research has been limited on what other postprandial AA concentrations mean for insulin and blood glucose. As previously mentioned BCAA support gluconeogenesis (Esteves de Oliveira et al., 2011) but other AAs support gluconeogenesis such as glycine. Increases in glycine may be directly linked to increases in insulin secretion and glucose production via gluconeogenesis. In a study on healthy individuals by Gannon et al. (2002), there was a significantly larger glucose area

response when glycine was ingested with glucose versus ingesting glycine alone which produced a negative glucose area response. The insulin area response mildly elevated when ingesting glycine alone and significantly increased with glycine ingested with glucose. In the current study glycine concentrations significantly increased over time in the CPI group before returning to baseline. In the whey group, glycine remained mostly constant and eventually was significantly below baseline by 90 min and continued to decrease by 180 min. It is possible that another mechanism is at play with oral ingestion of glycine that affects gluconeogenesis (Gannon et al., 2002).

Phenylalanine, arginine, and leucine have been associated with insulin secretion but not significantly higher than other protein intake (Van Loon et al., 2000). Alanine, glutamine, and lysine have been claimed as insulinotropic but some AAs are insulinotropic due to effects on beta cell function and others based on increased incretin secretion (Tricò et al., 2019). In the current study there was an increase in phenylalanine, alanine, glutamine, and lysine from baseline in both the WPC and CPI groups. Lower values of phenylalanine and higher values of alanine were seen in both groups compared to the other AAs. Although these AAs may add to the insulinogenic response to protein intake it may only be a mild affect compared to what is seen with BCAAs.

Possible Limitations

There were some limitations of the present study that need to be acknowledged. The study only included male participants in good health, which limited our ability to observe effects on metabolic responses. The length of the study was not designed to present long-term effects of the dietary proteins. Not all components reached baseline or

below by 180 min, there is no way to determine what would have happened with these components after minute 180 from the present study. Also, the levels of incretins were not measured to determine the affect they may have had on insulin release.

Future Research

There is a high need for more studies. Further investigation on different types of protein forms (concentrate, isolate, hydrolysate) on insulin response, PPG, and AA concentration is needed. There has been an increase in ready to drink protein shakes on the market and therefore a need to assess both whole food mixed meals and different liquid protein drinks. Research is needed on other population groups such as individuals with T2DM, females, and overweight and obese individuals to compare the response to healthy males. It is unclear how the metabolic response may differ in these other groups.

Conclusion

It has been demonstrated that a WPC ingestion leads to a hyperinsulinemia response with a low PPG response in healthy individuals. Chicken protein does not express a similar insulin response as WPC but may induce a more favorable consistent response in combination with a steady moderate range of PPG. BCAA concentrations are favorable for both proteins compared to glucose with whey having an early response and chicken protein an extended response. Therefore whey protein is one option for protein intake to help with blood glucose control, but chicken protein may be another good option especially when considering insulin concentrations in healthy individuals who may or may not be at risk of diseases.

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APPENDIX A

Institutional Review Board (IRB) Approval Letter

Texas Woman's University
Institutional Review Board (IRB)
irb@twu.edu
https://www.twu.edu/institutional-review-board-irb/

December 15, 2020

Shane Broughton
Nutrition and Food Sciences

Re: Exempt - IRB-FY2020-416 Comparative Insulin and Glycemic Response to Dietary Protein Intake in Healthy Males

Dear Shane Broughton,

The above referenced study has been reviewed by the TWU IRB - Denton operating under FWA00000178 and was determined to be exempt on December 15, 2020.

Note that any modifications to this study must be submitted for IRB review prior to their implementation, including the submission of any agency approval letters, changes in research personnel, and any changes in study procedures or instruments. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All modification requests, incident reports, and requests to close the file must be submitted through Cayuse.

On December 14, 2021, this approval will expire and the study must be renewed or closed. A reminder will be sent 45 days prior to this date.

If you have any questions or need additional information, please contact the IRB analyst indicated on your application in Cayuse or refer to the IRB website at http://www.twu.edu/institutional-review-board-irb/.

Sincerely,

TWU IRB - Denton