THE INDIVIDUAL AND COMBINED EFFECTS OF WHEY PROTEIN AND ACUTE AEROBIC EXERCISE ON GLYCEMIC CONTROL

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DEDICATION

To my wife, Meg Castleberry, thank you for

all your patience, love, and support.

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ABSTRACT

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A large issue with glycemic control can be attributed to postprandial hyperglycemia. The purpose of this study was to evaluate the combined effect of acute aerobic exercise and whey protein, on plasma glucose, insulin, gastric inhibitory peptide (GIP), glucagon like peptide 1 (GLP-1), and glucagon in normal, healthy men. Eleven males (mean ± SD age: 24.3 ± 5.4 years; BMI: 26.0 ± 5.3 kg/m^2; HbA1c: 5.2 ± 0.2 %; VO_2 max: 38.3 ± 6.1 ml/kg/min) completed four randomized trials consisting of: aerobic exercise only (EX), aerobic exercise combined with 50 g whey (EXW), no exercise and whey protein (W), or no exercise and no whey protein (R). Aerobic exercise was completed 12-14 hr prior to a 75 g oral glucose tolerance test (OGTT). Whey protein was administered 30 min prior to the OGTT. Total area under the curve (AUC) for glucose was significantly lower for EXW and W compared to EX and R. Insulin AUC was significantly higher for W and EXW compared to EX and R. GIP, GLP-1, and glucagon significantly increased in both EXW and W trials compared to R and EX. There were no significant differences found in insulin sensitivity using the Matsuda index. This study suggest that postprandial hyperglycemia can be alleviated by consumption of 50 g of

whey protein prior to a 75 g glucose challenge. However, an acute bout of exercise did not confer any additional benefit.

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CHAPTER I

INTRODUCTION

In the United States, 22 million Americans have been diagnosed with diabetes and approximately 86 million Americans are estimated to have prediabetes based on impaired fasting glucose or impaired glucose tolerance (CDC, 2017). There are many strategies currently used to treat insulin resistance that leads to diabetes including a combination of medication(s), diet, exercise, and weight loss (Bosello, Armellini, Zamboni, & Fitchet, 1997; Boule et al., 2011; Hamdy, Goodyear, & Horton 2001). The American College of Sports Medicine (ACSM) recommends a total of 150 min per week of physical activity for general health purposes without specific guidance of order or frequency which that amount of time should be completed (ACSM, 2015). Certain aspects of exercise are focused on the prevention and treatment of type 2 diabetes (T2D) such as endurance exercise versus a combined resistance and endurance training program (Cuff et al., 2003; Maiorana, O'Driscoll, Goodman, Taylor, & Green, 2002; Marcus et al., 2008), long-term training studies (Castaneda et al., 2002; Sigal et al., 2007), as well as volume compared to intensity of exercise on insulin sensitivity and glucose control (Houmard et al., 2004).

Significant reductions in fasting plasma glucose and insulin concentrations post 3 consecutive days of exercise in healthy young women have been reported (Jankowski, Ben-Ezra, Gozansky, & Scheaffer, 2004). In healthy older men and women, the effects of consecutive days of exercise on glycemic control indicate 20% reductions ($p < .05$) in

insulin response to a glucose challenge post 7 days of exercise, compared to a single bout of exercise that resulted in smaller insulin decrements without significance (Cononie, Goldberg, Rogus, & Hagberg, 1994). In other studies, a single bout of exercise lowered insulin responses 12-24 hr post exercise (Douen et al., 1990; Hubinger, Franzen, & Gries, 1987; Jankowski, Ben-Ezra, Kendrick, Morriss, & Nichols, 1999; Larsen, Dela, Kjaer, & Galbo, 1997).

Aerobic exercise contributes to the alleviation and prevention of type 2 diabetic symptoms. More recently, other methods have gained ground in the literature by using different mechanisms to assist with glycemic control, such as whey protein. People with T2D respond to whey protein, either taken as a preload (Jakubowicz et al., 2014; Ma et al., 2009; Ma et al., 2015) or with a meal (Ang, Muller, Wagenlehner, Pilatz, & Linn, 2012; Frid, Nilsson, Holst, & Bjorck, 2005; Mortensen et al., 2009), with decreased glucose and increased insulin responses (Jakubowicz et al., 2014; Ma et al., 2015; Ma et al., 2009); no difference in glucose response and higher insulin (Ang et al., 2012; Frid et al., 2005); or decreased glucose and no difference in insulin response (Mortensen et al., 2009). All studies used a single dose of whey protein that ranged from 21-27 g (Ang et al., 2012; Frid et al., 2005; Ma et al., 2015) and 45-55 g (Jakubowicz et al., 2014; Ma et al., 2009; Mortensen et al., 2009).

Whey protein ingestion either before a meal or taken with a meal significantly reduced glycemic responses in healthy individuals (Akhavan, Luhovyy, Brown, Cho, & Anderson, 2010; Akhavan et al., 2014; Gunnerud, Ostman, & Bjorck, 2013; Petersen et

al., 2009), individuals with T2D (Jakubowicz & Froy, 2013; Jakubowicz et al., 2014; Ma et al., 2009) and individuals with prediabetes (Hoefle et al., 2015). Whey protein acts by inducing the secretion of incretin hormones (glucagon-like peptide-1, GLP-1; glucose-dependent insulinotropic peptide, GIP) that stimulate insulin release, and slows gastric emptying (Salehi et al., 2012). Although the mechanism has not been completely elucidated, incretins account for ~50–70% of insulin secretion during carbohydrate feeding in healthy individuals (Holst & Deacon, 2013), while incretin response is blunted in people with T2D and prediabetes (Bagger et al., 2011). When consumed prior to or with a meal, 50 g of whey protein has been shown to augment insulin and incretin secretion, while reducing peak glucose and glucose area under the curve (AUC) in people with T2D (Ma et al., 2009), and doses as low as 9 g of whey protein have similar responses in healthy adults (Gunnerud et al., 2013). Clifton, Galbraith, and Coles (2014) showed that 17 g of whey protein prior to a mixed meal decreased average blood glucose concentration (0.8 mmol/L over 3 hr) and peak blood glucose concentration (2.1 mmol/L) in both people who have prediabetes and diabetes. However, insulin was not measured.

In contrast to the above mentioned studies, Hoefle et al. (2015) found a reduced effect on overall blood glucose response in people with prediabetes over 240 min when 50 g of whey was taken with 50 g of maltodextrin; yet peak plasma glucose was significantly lower ($p < .0001$) with whey versus maltodextrin alone (Hoefle et al., 2015). There was also an increase in plasma insulin concentration by 96% (Hoefle et al., 2015).

In summary, the peak plasma glucose concentration response to whey protein in individuals with prediabetes still decreases with a significant increase in insulin.

The deviations in responses could be related to multiple reasons including mixing people with T2D with people who have prediabetes, only people with diet-controlled T2D (Frid et al., 2005; Ma et al., 2015; Ma et al., 2009); people taking medications (sulfonylurea or metformin) and/or diet controlled (Ang et al., 2012; Jakubowicz et al., 2014; Mortensen et al., 2009). In addition, the aforementioned studies had a mixed participant composition of men (n = *3*-9) and women (n = 1-12) and did not identify sex-based responses likely due to the small overall number of participants (range 8-20; mean 12).

Whey protein seems to increase the secretion of insulin from the pancreas, while aerobic exercise is suggested to decrease insulin secretion while improving sensitivity in skeletal muscle cells. The combination of the two has not been examined. The investigators hope that aerobic exercise will stimulate an increase in insulin sensitivity, while whey protein will cause an increase in insulin secretion. Therefore the combination of the two treatments should lower glycemic response to a glucose challenge.

Problem Statement

The purpose of this study is to compare the effects of whey protein coupled with acute exercise on glycemic responses. The participants were recruited from Texas Woman's University and the community of Denton, Texas. Inclusion criteria were male, age 18-44, healthy, sedentary individuals with no known dyslipidemia or heart disease.

Participants completed height, weight, BMI, and dual energy x-ray absorptiometry scans. All participants completed four trials: (1) exercise + 50 g whey; (2) exercise without whey; (3) 50 g whey only; and (4) no exercise and no whey. The single bout of aerobic exercise consisted of walking on the treadmill for 60 min at 75% VO2 max. Approximately 12-14 hr post exercise, the participant will complete a 75g oral glucose tolerance test (OGTT) after each trial. Blood samples will be taken over the course of three hr for the analysis of glucose, insulin, c-peptide, GIP, and GLP-1. Participants will be asked to keep a three day diet record and asked to consume the same diet prior to the OGTT.

Total AUC will be used for comparisons of all dependent variables. All dependent variables will be statistically analyzed using a repeated measures ANOVA for the four treatments, as well as time-point variations between insulin and glucose across all timepoints (-30, 0, 15, 30, 60, 90, 120, and 150 min).

Hypothesis

This study will examine the dual effects of whey protein and an acute bout of exercise on glycemic control compared to whey only, exercise only, and no exercise or whey protein.

The hypotheses that will be tested include:

1. Exercise will stimulate a decrease in plasma insulin concentration and plasma glucose concentration 12-24 hr post exercise.

2. Whey protein will stimulate an increase in plasma insulin concentration through an increase in incretins concentration (GLP-1, GIP) and a decrease in plasma glucose concentration.
3. There will be increased plasma insulin concentration and C-peptide concentration, along with increased clearance, with a decrease in plasma glucose concentration following exercise and whey protein.

Definitions

C-peptide: A part of pro-insulin, secreted in equal amounts as insulin. A marker of insulin production from the beta cells of the pancreas (Leighton, Sainsbury, & Jones, 2017).

Gastric Inhibitory Polypeptide (GIP): A hormone secreted by the intestine in response to nutrient intake. Has significant effect on insulin secretion from the pancreas (Hansen, Tencerova, Frolich, Kassem, & Frost, 2017).

Glucagon-Like Peptide-1 (GLP-1): A hormone secreted by the intestine in response to nutrient intake. Has significant effect on insulin secretion from the pancreas (Hansen et al. 2017).

Glut-4 Protein: Glucose transporter responsible for moving glucose from the blood, into the cell, specifically skeletal muscle cells. Insulin is a strong stimulant for Glut-4 translocation to the cell membrane (Ferrier, 2017).

Impaired Glucose Tolerance: A two hr plasma glucose concentration of ($\geq$ 140 and < 200 mg/dl) following a 75 g glucose load on an OGTT (Genuth et al., 2003).

Impaired Fasting Glucose: Plasma glucose ranging from normal to diabetic ($\geq$ 100 and < 126 mg/dl) (Genuth et al., 2003).

Insulin: A hormone produced by the beta cells of the islets of Langerhans located on the pancreas. This hormone is responsible for decreasing blood glucose levels (CDC, 2017)

Oral Glucose Tolerance Test (OGTT): A clinical test used for the test of diabetes or prediabetes. Can also be used to determine medication regimen and disease status (CDC, 2017)

Type II Diabetes Mellitus: A diagnosis when a person's blood glucose concentration is above a healthy level. Can be a marker of pancreatic function and skeletal muscle function (CDC, 2017)

Limitations

Limitations for this study include:

1. This study will only examine the acute effects of a single bout of exercise and 50g of whey protein in men aged 18-44 yr.
2. This study does not examine sex differences.
3. This study does not examine a dose effect of whey protein or aerobic exercise.

Significance

There is a positive influence of an acute bout of moderate to intense exercise on glycemic response (Douen et al., 1990; Rogers, 1989; Hubinger et al., 1987). An acute bout will elicit an improvement in insulin sensitivity at the skeletal muscle cell, decrease insulin secretion from the beta cells of the pancreas, and increase hepatic insulin

extraction, while still significantly lowering plasma glucose concentration. Whey protein, when ingested prior to or with a meal, can elicit an increase in plasma insulin concentration, while also decreasing plasma glucose concentration (Jakubowicz et al., 2014; Ma et al., 2009; Ma et al., 2015).

CHAPTER II

REVIEW OF THE LITERATURE

The purpose of this study is to examine the effects of whey protein coupled with aerobic exercise on glycemic responses. Specifically, this study will determine differences in plasma glucose, insulin, and insulin sensitivity to either aerobic exercise, whey protein, or the combination of the two.

The following literature review will evaluate the existing research related to whey protein as well as acute aerobic exercise on glycemic responses. PubMed was used in the literature review search. Key words searched included glycemic response, acute exercise, glucose, insulin, and insulin sensitivity. Relevant studies, along with reference lists from those studies were used in the production of this literature review.

Outline

1. Role of Insulin Sensitivity on glycemic control
    a. Increases, Decreases, Skeletal muscle
2. Effects of Acute Exercise on Glucose Concentration
3. Exercise Intensity and Duration on Glycemic Control
4. Incretins
    a. GIP, GLP-1
5. Exercise and Incretins
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Role of Insulin Sensitivity on Glycemic Control

Insulin sensitivity manifests in different tissues including adipose, liver, and the pancreas. Insulin's action on adipose tissue consists of inhibiting hormone-sensitive lipase (responsible for release of fatty acids), as well as increasing transport of glucose into adipocytes by means of triacylglycerol synthesis (Ferrier, 2014). It is also responsible for the delivery of triglycerides into adipose tissue. Adipose tissue itself is responsible for providing fatty acids to the blood for transport to working muscles to be used for energy. Therefore, an increase in capillary density is seen in subcutaneous adipose tissue in healthy, regularly exercising individuals (Frayn & Karpe, 2014). Blood flow increases postprandially in subcutaneous adipose tissue to increase delivery of products to be stored. This has also shown an increase in lipoprotein lipase activity which is responsible for storage in adipose tissue (Frayn & Humphreys, 2012). Blood flow increases 2-4 times in abdominal and lower body fat depots in healthy subjects with a peak approximately 1 hr post feeding, which seems to coincide with insulin production (Sotornik et al., 2012). Ye and Gimble (2011) explain that people who are obese or have T2D may experience hypoxia due to the decreased adipose tissue blood flow, while regularly exercising individuals will have normal or increased blood flow to adipose tissue (Ye, 2011).

Individuals with diabetes have a significantly lower blood flow increase post-prandially (Coppack et al., 1990; Jansson, Larsson, & Lonnroth, 1998; Jansson, Larsson, Smith, & Lonnroth, 1992). These participants also showed increases in the rate of lipolysis (opposite of what insulin should do) which leads to increased FFAs in the blood.

Postprandial adipose tissue blood flow is decreased due to the downregulation of the adrenergic receptor during chronic sympathetic stimulation in a milieu of long-standing hyperinsulinemia (Sotornik et al., 2012). Figure 1 shows a relationship of insulin impairment, blood flow, and plasma glucose.

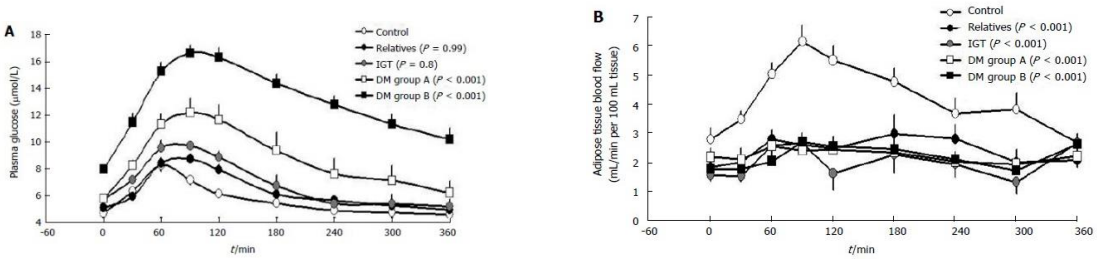

Figure 1. Glucose control relative to blood flow (Dimitriadis et al., 2008)

In metabolically healthy individuals, exercise and insulin promote capillary recruitment in skeletal muscle and subcutaneous adipose tissue. However, this has been shown to be impaired in individuals with T2D and early phase insulin resistance. Previous research has even shown this decreased blood flow in individuals with pre-diabetes (Lambadiari, Triatafyllou, & Dimitriadis, 2015). Insulin sensitivity is decreased in obese populations, due to the increase in fat deposits outside of subcutaneous and visceral adipocytes (Hardy, Czech, & Corvera, 2012). Hepatic insulin sensitivity is positively correlated with fasting insulin concentrations and lipocytes in the liver (Jung & Choi, 2014; Yki-Jarvinen, 2015). Figure 2 depicts how excess lipid can be stored in separate tissues.

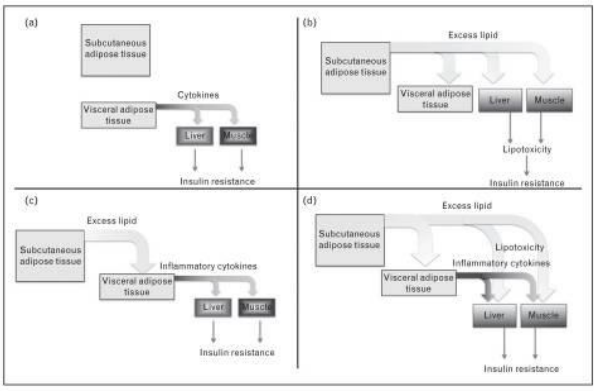

Figure 2. Excess lipid storage (Hardy, Czech, & Corvera, 2012). This figure illustrates the location of lipid storage once excess lipids are consumed.

Multiple studies have shown correlations between adiponectin content and liver adiposity (Bajaj et al., 2004; Johanson et al., 2003; Pajvani et al., 2004; Sutinen et al., 2003; Tiikainen et al., 2004). Higher amounts of adiponectin are negatively correlated with liver adiposity. Increased adipocytes in the liver (lipotoxicity in the liver) can be the main cause of decreased hepatic insulin sensitivity. Insulin suppresses hepatic glucose production by more than 50%, while also inhibiting its counterpart, glucagon (Cherrington, Lacy, & Chiasson, 1978). Therefore, decreased levels of adiponectin can have a downstream effect on hepatic insulin sensitivity, glucagon concentration, and overall, blood glucose concetrations.

An article published in *Nature* elucidates the mechanisms by which decreased hepatic insulin sensitivity is caused from increased fat, inflammatory signaling, and overall, a cause for T2D. Hyperglycemia can also cause an increase in the production of diacylglycerols (Ramana et al., 2005). The increased diacylglycerols (represented as DAG in the figure below) cause PKC activation and translocation to the membrane which

inhibits IRS-2. As shown in Figure 3, this leads to a downstream cascade that results in decreased glycogen synthesis and increased gluconeogenesis.

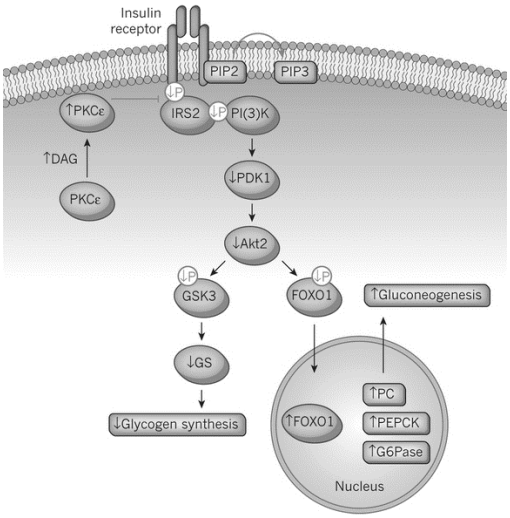

Figure 3. Insulin signaling (Perry, Samuel, Petersen, & Shulman, 2014)

Since insulin is secreted from the beta cells of the pancreas, beta cell dysfunction is a common occurrence in individuals with T2D. The first signs of this are seen with decreased first-phase insulin production and a decline in the second phase (Porte, 1990). Even during beta cell failure, non-glucose secretagogues, such as GIP, and GLP-1, are able to stimulate an insulin response that ultimately keeps insulin secretion "normal." GIP, and GLP-1 act on specific receptors to upregulate cAMP as a second messenger, activating protein kinases, ultimately leading to depolarization of the cell that will cause release of insulin through exocytosis (Baggio & Drucker, 2007).

Hyperglycemia may not be the result of decreased beta cells in the pancreas. Autopsies of human and animals with T2D have shown a 20-50% reduction in beta cells

of the pancreas. However, in multiple animal models, partial pancreatomies show a compensation of the remaining beta cells and are reported to be more sensitive to glucose and resulted in normo-glycemia, even though there was a 75% reduction in maximum insulin response (Johnston et al., 1987; McCulloch et al., 1988; Ward, Wallum, Beard, Taborsky, & Porte, 1988).

One possible cause of beta cell dysfunction could be related to amylin. Amylin is produced by the beta cells of the pancreas with insulin and is responsible for delaying gastric emptying and promoting satiety. It has also been found to aggregate itself into pancreatic islets, therefore becoming known as amyloid deposits. These deposits take over the beta cells where they will cease to function. Amyloid deposits have been used to induce hyperglycemia in past studies, therefore suggesting it may be a cause to insulin resistance in the pancreas (Hoppener et al., 2008; Soeller et al., 1998; Verchere, D'Alessio, Palmiter, & Doe, 1996). A high fat diet (enough to double the weight of the mice) led to a greater rate of amyloid deposits, further linking obesity and T2D (Verchere et al., 1996).

Beta cells may also experience apoptosis caused from chronic stress to the endoplasmic reticulum, which can be overstimulated by high rates of insulin production (Cernea & Dobreanu, 2013). Another cause of apoptosis are high levels of LDL which disrupt JNK pathways in the beta cell, leading to islet inflammation, and resulting in cell death over time. This is also the result of excess triglyceride accumulation (Listenberger et al., 2003).

Effects of Acute Exercise on Glucose Concentration

There are many reasons glucose uptake increases in skeletal muscle during and post exercise. One is with the increase in blood flow. The increase in blood flow increases the "chances" of glucose binding to a Glut-4 protein and being taken up by the cell (Richter & Hargreaves, 2013). Other ways include moving the Glut-4 vesicle to the membrane. Insulin mediated Glut-4 translocation occurs throughout its own pathway. However, exercise induced translocation does use the same method towards the end of its pathway. Insulin binds to a tyrosine kinase receptor, which autophosphorylates the β subunit. Insulin receptor substrates activate PI3K with PIP2 to generate PIP3. This activates PDK1 that upregulates Akt. Akt upregulates AS160 that activates TBC1D4 which is where the exercise induced process meets this process and follows the same pathway to move Glut-4 vesicles to the membrane (Turcotte & Fisher, 2008).

Exercise causes an increase in muscle contractions which can lead to Glut-4 translocation. Muscle contractions cause an increase in calcium which is also leaked from the sarcoplasmic reticulum. Calcium stimulates calcium/calmodulin dependent kinase. This has been shown in rat models to activate protein kinase C and diacylglycerol. Richter and Hargreaves (2013) believe this causes metabolic stress to the cell which stimulates AMPK. This positive stress can be related to not only the calcium influx stimulating AMPK, but also a decrease in ATP, as well as an increase in AMP, which can also be influenced by exercise. This causes a downstream activation of TBC1D4 and TBC1D1 activity which causes Glut-4 translocation with Rab GTP (Richter &

Hargreaves, 2013). Calcium will continue to be present due to the SERCA pump that is responsible for sequestering calcium and releasing it as needed. It is well understood that an increase in AMPK activity increases insulin sensitivity, due to the fact that more Glut-4 translocation will improve glucose removal from the blood for up to 18 hr (Holloszy, 2005; Lee et al., 2005; Yang et al., 2012). The AMP-activated protein kinase (AMPK) signaling pathway coordinates cell growth, autophagy, & metabolism.

Multiple studies have examined the effect of aerobic exercise on Glut-4 activity. Douen et al. (1990) studied the effect of acute exercise on rat hind limb muscles. The rats performed 45 minutes of treadmill running on a 15% grade. The first 5 minutes was performed at 20 m/min and the following 40 minutes were faster at 30m/min. Rats were fasted overnight before the exercise bout. Immediately following the exercise bout, muscles were removed for analysis. Douen et al. found that exercise increased Glut-4 on the cell membrane of skeletal muscle 3.2 times that of non-exercised rats. Henriksen (2002) in a mini-review reported that normal rodent models performing moderate or high intensity exercise can improve glucose tolerance and insulin action on glucose transport. The main factor being Glut-4 expression is shown to be increased post exercise for 18 hr (Henriksen, 2002; Young, Garthwaite, Bryan, Cartier, & Holloszy, 1983).

Studies have also examined aerobic exercise and glycemia the following morning. Rogers et al. in 1988 showed that 1 week of intense exercise (50-60 minutes at 68% VO_2 max) in seven individuals with NIDDM and three males with impaired glucose tolerance that plasma glucose AUC reduced 36% at 120 min post OGTT the morning after the last

exercise bout. Plasma insulin decreased 32% accordingly (Rogers et al., 1988). Seven days of aerobic exercise (walking for 50 minutes at 65% HRR) in 12 obese African American women with hypertension completed an IVGTT the morning following the last exercise bout. Insulin sensitivity increased from 2.68 to 4.23 min-1/pmol/L. Fasting insulin and blood glucose both decreased (Brown, Moore, Korytkowski, McCole, & Hagberg, 1997).

Literature also suggests improvements in glycemic control within hr post exercise. Hubinger (1987) had 16 T2D, 6 with basal hyperinsulinemia and 10 without, cycle for 1 hr at a HR of 120 BPM and ~48 and ~52 watts respectively. Glucose and insulin were measured for 7 hr post exercise. Glucose decreased significantly in both groups. Insulin decreased significantly in the second group (Hubinger et al., 1987). Nine individuals with NIDDM completed a post-prandial study. Subjects ate a standardized breakfast, followed by a standardized lunch 4 hr later. Larsen et al. found that 45 minutes of cycling at 53% VO_2 max 45 minutes after a breakfast meal significantly reduced glucose AUC, insulin, and C-peptide 4 hr after breakfast. Measurements were taken post-standardized lunch (Larsen et al., 1997).

Nagasawa, Sato, and Ishiko (1991) examined rats after one hr of treadmill running. Glucose infusion rate was measured using the euglycemic insulin clamp 1 hr, 3 hr, 6 hr, and 24 hr post exercise. Significant increases in glucose infusion rate was found for both the 6hr and 24hr post exercise clamp. Nagasawa et al. (1991) hypothesize this delay in response could be from the suppression of glucose uptake mechanisms caused

from catecholamines during exercise. To further elucidate this response, it has been suggested that epinephrine increases Glut-4 phosphorylation without inhibiting insulin-stimulated transport (Lee et al., 1997).

Magkos, Tsekouras, Kavouras, Mittendorfer, and Sidossis, (2008) suggests a curvilinear relationship in improvements of insulin sensitivity based on exercise and energy expenditure. Magkos et al. examined 30 non-obese, recreationally active men who exercised at 60% VO2 max ranging from 30 minutes to 120 minutes. The results showed a curvilinear relationship in insulin sensitivity based on caloric expenditure. A few flaws in the study were no direct measurement of their kcal meals prior to the blood sampling the next morning, and it was not repeated measures (Magkos, Tsekouras, Kavouras, Mittendorfer, & Sidossis, 2008).

During moderate intensity exercise, glycolysis and beta oxidation are the primary sources of energy. Once exercise stops, the rate of glycolysis decreases immediately. However, glucose is still being taken up by the skeletal muscle cells due to Glut-4 translocation and the increase in blood flow. Blood flow in humans has been shown to diminish between 15 and 45 min post-exercise, dependent on workload (Bangsbo et al., 1990). A review article by Borghouts and Keizer (2000) stated that swimming rats can have increased blood flow for 2 hr post exercise (Borhouts & Keizer, 2000). That same article also stated that the glucose uptake time frame post exercise is also dependent on muscle fiber type.

Goodyear et al. (1990) stated that exercise stimulated Glut-4 translocation returns to resting values 2 hr post exercise (Goodyear et al., 1990). Ren, Semenkovich, Gulve, Gao, and Holloszy (1994) studied rats 16 hr after exercise and found that insulin stimulated an increase in Glut-4 translocation (Ren, Semenkovich, Gulve, Gao, & Holloszy, 1994). Kim et al. (1995) performed a study with gene expression in rats after endurance training and found an increase in gene expression of IRS-1 and MAP kinase (Kim et al., 1995). It could be hypothesized that this affect could be seen post an acute bout of exercise. A review article about the potential mechanisms in skeletal muscle that are related to insulin resistance and exercise states that the combinations of mechanisms causing an increase in Glut-4 translocation are "redundant" and they all play a role. Those mechanisms include increases in calcium levels, activation of AMPK, and increased blood flow (Holloszy, 2005; Lee et al., 2005; Turcotte & Fisher, 2008; Yang et al., 2012).

Another potential mechanism from AMPK activation post an acute bout of exercise is an increase in fatty acid oxidation. Exercise inhibits fatty acid synthesis and increases fatty acid oxidation by inhibiting the actions of acetyl CoA carboxylase (ACC). ACC, when deemed inactive by AMP dependent kinase, inhibits malonyl CoA, which is directly involved in fatty acid synthesis. When these processes are put into motion, the opposite processes are upregulated, meaning an increase in fatty acid oxidation (Turcotte & Fisher, 2008). This effect has been seen in multiple studies (Abu-Elheiga, Oh, Kordari, & Wakil, 2003; Abu-Elheiga, Matzuk, Abo-Hashema, & Wakil, 2001; Harwood et al.

2003; Patil, Kallqvist, Olsen, Vogt, & Gislerod, 2007). The increase in AMPK activation leads to Glut 4 translocation, improving glucose uptake for up to 18 hr (Holloszy, 2005; Lee et al., 2005; Yang et al., 2012).

Exercise Intensity and Duration on Glycemic Control

Many studies have given thought to knowing the most important factor in exercise in relation to glycemic control. Houmard et al. (2004) examined volume and intensity of exercise training. Houmard et al. compared low volume/mod intensity (12 miles 40-55% VO_2 peak per week), low volume/high intensity (12 miles 65-80% VO_2 peak per week), high volume/high intensity (20 miles 65-80% VO_2 peak per week), and a control group. Insulin sensitivity was calculated from an IVGTT. All exercise groups improved insulin sensitivity significantly over the 6 month study while the control group decreased their sensitivity. The biggest take-away from this study is that the low volume/moderate intensity group and the high volume/high intensity groups had the greatest increments in sensitivity. Both groups also exercised for approximately 170 minutes per week while the low volume/high intensity group only exercised for 115 minutes per week (Houmard et al., 2004).

A large cohort study published in 1998 by Mayer-Davis et al. examined frequency of vigorous exercise over a year in 1,467 subjects. Results suggested estimated energy expenditure correlated with frequency of vigorous exercise and those that participated in at least 5 days a week had the most positive increases in insulin sensitivity. These results

were also equal after evaluating for different diabetic statuses, such as healthy, diabetic, or pre-diabetic (Mayer-Davis et al., 1998).

DiPiertro et al. (2006) compared the effects of 9 months, 4 days per week of high intensity aerobic training (80% VO_2 peak) and moderate intensity (65% VO_2 peak) in healthy, non-obese older women. This study kept volume the same for each session at 300 kcals. Insulin sensitivity was calculated from the euglycemic-hyperinsulinemic clamp. Interestingly, only the high intensity training group improved insulin sensitivity significantly (21%), while the moderate intensity group did not reach significance (16%) (DiPietro et al., 2006).

Contrary to the previous study mentioned, Braun, Zimmerman, and Kretchmer (1995) examined insulin sensitivity after 2 days of exercise in a repeated measures study of low intensity (50% VO_2 peak) and high intensity (75% VO_2 peak) in women who were non-insulin dependent diabetic. The duration of exercise was adjusted to account for equal kcal expenditure for the different exercise conditions. The clamp was used on Day 3 post exercise and the results showed equal insulin sensitivity responses in both exercise groups. This may need to be further studied as the insulin sensitivity response may be insignificant after two acute bouts of exercise, but over time, higher intensity may be more beneficial. This could be due to the participants' diabetic status, however, the Mayer-Davis study suggested no differences, regardless of their diabetic status (ie., healthy, pre-diabetic, or diabetic) (Braun et al., 1995).

Kang et al. studied the effect of 7 days of cycling in a repeated measures design in men who were obese and obese with diabetes. The 2 trials consisted of cycling for 70 min at 50% VO_2 peak and 50 min at 70% VO_2 peak, keeping Kcal expenditure the same. The study resulted in that only the obese group found a decreased AUC for insulin post 70% VO_2 peak exercise. The group with diabetes did not see changes. It is hypothesized that this could be from the group with diabetes already being hypoinsulinemic (Kang et al., 1996). One recent study by Rynders et al. in 2014 compared moderate intensity (200 kcal at lactate threshold), and high intensity (200 kcal at 75% difference of lactate threshold and max) with a 3 hr OGTT 1 hr post exercise. Improvements were seen in both groups for insulin sensitivity with no differences between groups. This study used prediabetic adults (Rynders et al., 2014).

There is no definitive answer on the "best" combination of exercise for glycemic control. However, previous studies have shown that certain regimens can result in improvements in glycemic control with either decreased insulin, decreased glucose, or both. As mentioned before, caloric expenditure is the main element for glycemic improvements to glucose and insulin. However, exercise duration and intensity are also important factors. Many early studies only examined intensity such as Douen et al. (1990) which showed 45 min. of high intensity improved Glut 4 translocation, and Rogers et al (1988) that 50-60 min of 68% VO_2 max decreased both glucose and insulin. Below is a list of a few studies and the results of each to help determine what works "best" for glycemic control.

Table 1

Exercise Responses

Author	Intensity	Duration	Training	Result
Douen et al. (1990)	Very high (rats)	45 min	Single bout	Glut-4 translocation
Rogers et al. (1988)	68% VO2 max (T2D)	50-60 min	7 days	↓ glucose ↓ insulin
Brown et al. (1997)	65% HRR (Healthy)	50 min	7 days	↓ glucose ↓ insulin
Hubinger et al. (1987)	120 bpm (52 & 48 watts) (T2D)	60 min	Single bout	↓ glucose ↓ insulin
Larsen et al (1997)	53% VO2 max (T2D)	45 min	Single bout	↓ glucose ↓insulin
Nagasawa et al. (1991)	Running (rats)	60 min	Single bout	↓ insulin sensitivity
Magkos et al (2008)	60% VO2 max (Healthy)	30-120 min	Single bout	Curvilinear relationship of insulin sensitivity

There is not a certain amount of exercise, whether that be an intensity, duration, energy expenditure, or mode that will elicit results in all individuals. However, individuals who are obese, and have NIDDM will probably elicit a healthy response to the lowest dose of exercise shown in the literature. On the other hand, previous data in our own lab has shown that healthy individuals may not show a significant change in glycemic response to a similar bout of exercise for 60 min at 70% VO_2 max (Castleberry et al. 2017). Regardless, it looks like 45 minutes at a moderate intensity (53% VO2 max) is enough to show an acute change in either fasting insulin concentration, fasting glucose concentration, or the insulin and glucose response to a glucose challenge. Based on the previous studies mentioned and prior research, energy expenditure is the primary variable

in insulin sensitivity (Audelin et al., 2012). If volume/EE is kept the same, then both duration (Houmard et al., 2004; Bajpeyi et al., 2009) and intensity (DiPiertro, Dziura, Yeckel, & Neufer, 2006) become secondary variables that can also have a positive effect on sensitivity.

Not all studies show an improvement in glycemic response when the participants are healthy individuals. Previous studies at TWU (Castleberry et al., 2017), in addition to Cononie et al. (1994) suggests that healthy individuals may not show a response after a single bout of exercise lasting approximately 1 hr at an intensity around 70% VO_2 max. Other studies, such as Rynders et al. (2014) mentioned earlier, have shown that expending 200 kcals, regardless of time or intensity, can improve insulin sensitivity in individuals with pre-diabetes. Healthy subjects who burn more than 200 kcals in our studies did not show a significant improvement in insulin $_i$AUC, or insulin sensitivity. However, published literature states that a single bout of aerobic exercise with an intensity > 60% VO_2 max for 60 minutes will elicit an improved glycemic response (Brown et al., 1997; Magkos et al., 2008).

Incretins

Incretins have been shown to be an important factor in insulin secretion, accounting for 50-70% of secretion following a meal (Nauck et al., 1986). Recently, evidence has shown there is a reduction in incretin response in those with reduced insulin sensitivity. Visboll, Krarup, Deacon, Madsbad, and Holst (2001) published a cross sectional study examining GLP-1 and GIP in healthy and people with T2D. Both

incretins measured were found to be significantly lower for the group with T2D following a mixed meal breakfast (Visboll et al., 2001). Toft-Nielsen (2001) found similar results in GLP-1 and GIP. This study examined 54 people with T2D, 33 healthy controls, and 15 individuals with impaired glucose tolerance. The results can be seen in Figure 4 below.

	T2DM	NGT	IGT	T2DM *vs.* NGT	ANOVA correcting for covariates
Glucose AUC (mmol/liter·240 min)	3289 ± 152	1452 ± 24	1591 ± 45	<0.001	$<0.001^{sex,\ a}$
Insulin AUC (10^c× pmol/liter·240 min)	30.9 ± 3.9	31.1 ± 3.1	57.7 ± 9.8	0.13	$0.016^{BMI,\ b}$
C-peptide AUC (10^c× pmol/liter·240 min)	315 ± 23	327 ± 20	442 ± 45	NS	$0.096^{BMI,\ b}$
Glucagon AUC (pmol/liter·240 min)	3585 ± 174	2386 ± 230	3108 ± 372	<0.001	$<0.001^{BMI,\ a}$
PP AUC (10^c× pmol/liter·240 min)	29.6 ± 2.9	35.0 ± 3.9	29.1 ± 4.1	NS	NS^{age}
GIP AUC (10^c× pmol/liter·240 min)	13.4 ± 0.7	16.0 ± 1.3	15.0 ± 1.4	0.047	$0.095^{BMI,\ sex,\ b}$
GLP-1 AUC (pmol/liter·240 min)	2482 ± 145	3101 ± 198	2765 ± 185	0.024	$0.011^{BMI,\ sex,\ a}$
GLP-1 AUC, incremental (pmol/liter·240 min)	907 ± 92	1927 ± 177	1587 ± 185	<0.001	$<0.001^{BMI,\ sex,\ a}$

Figure 4. Glycemic control in various health disparities

There was a pattern in GIP and GLP-1 response for type 2, impaired glucose tolerance, and healthy, showing decreases in GIP and decreases in GLP-1 concentrations based on degree of disease progression (Toft-Nielsen, 2001). Similar responses were found for GLP-1, but on the contrary, GIP was increased for T2D in another study

comparing the same variables (Muscelli et al., 2008). Those results showing increases for GIP based on disease progression, and decreases in GLP-1 based on disease progression can be seen below.

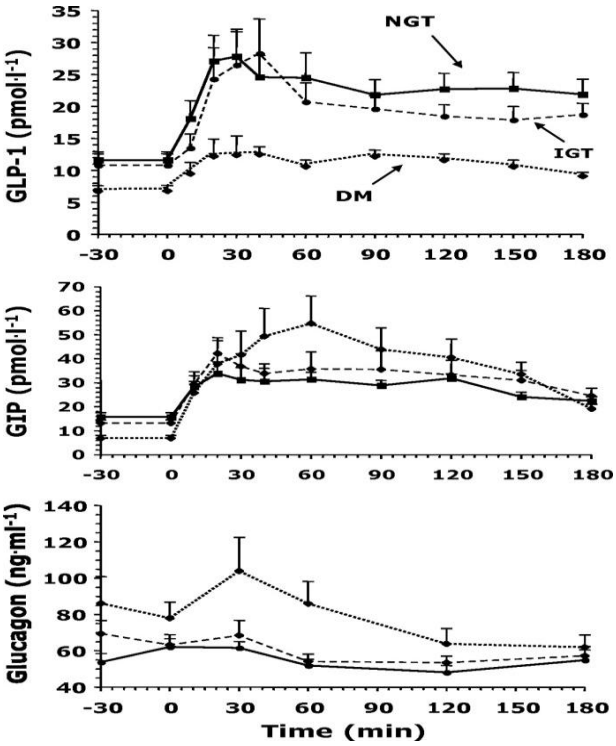

Figure 5. Incretin response for diabetes

Jensen et al. (2012) found similar results as Muscelli et al. (2008) in that GIP slightly increased while GLP-1 was decreased in individuals with impaired glucose tolerance (Jensen et al. 2012). This study used both individuals with normal glucose tolerance (NGT) and impaired glucose tolerance. The impaired glucose tolerance group has the lower GLP-1 values following a 75 g OGTT. However, another study by Bagger et al. (2011) (also in the lab of Vilsboll and J.J. Holst) found decreased GIP and GLP-1 values post an OGTT in individuals with type 2 diabetes (Bagger et al., 2011). It seems

most studies are in agreement that GLP-1 is decreased with a reduction in insulin sensitivity.

Another mechanism that may cause a dysfunction in the incretin response could be related to Dipeptidyl-peptidase 4 (DPP4). Dipeptidyl-peptidase 4 is responsible for the breakdown of GIP and GLP-1. Rohrborn, Wronkowitz, and Eckel (2015) stated that expression of DPP4 is dysregulated in disease conditions such as inflammation, cancer, obesity, and diabetes. DPP4 binds to adenosine deaminase (ADA), which causes the breakdown of incretin hormones such as GIP, GLP-1, and PYY. Individuals who are obese and those with T2D have increased ADA acitivity as a result of chronic inflammation and therefore higher amounts and faster breakdown of incretin hormones. This reduces the amount of insulin secretion (Rohrborn, Wronkowitz, & Eckel, 2015). It also re-emphasizes that incretin hormones are responsible for 50% of postprandial insulin secretion. This can better explain the importance of incretin hormones and the ability to lower ADA and DPP4 expression (Rohrborn et al., 2015).

Exercise and Incretins

Martins, Morgan, Bloom, and Robertson (2007) measured PYY, GLP-1, and PP post a meal and post a meal plus exercise. Subjects cycled for 60 minutes at 65% of their max HR. Exercise increased PYY, GLP-1, and PP levels one hr post exercise. There was also no change in ghrelin levels post exercise. This is evidence that satiety is increased post exercise while ghrelin is either decreased or unchanged (Martins, Morgan, Bloom, & Robertson, 2007). Kelly et al. (2009) examined older obese adults with impaired glucose

tolerance. Subjects performed 12 weeks of aerobic training, 5 d/w, 60 min, 755 VO_2 max, with either a eucaloric diet or hypocaloric diet. This was followed by a 3 hr, 75 g OGTT. Insulin and GIP were both decreased while glucose had no significant change. PYY also increased. The diet and exercise group had the largest changes but both groups still saw significant changes (Kelly et al., 2009).

Contradictory to the above study, Heden et al. (2013) conducted an acute bout of exercise in obese and lean individuals (walking for 1 hr at 55-60% VO2 peak) prior to a 12 hr fast and a 4 hr mixed meal test. Heden et al. found a decreased insulin response in the obese exercise trial with no changes from any trial in glucose, C-peptide, GIP, and GLP-1 (Heden et al., 2013).

Glycemic Responses from Whey Protein

Whey protein induces the secretion of incretin hormones (glucagon-like peptide-1, GLP-1; glucose-dependent insulinotropic peptide, GIP) that stimulate insulin release and slows gastric emptying. Although the mechanism has not been completely elucidated, incretins account for ~50–70% of insulin secretion during carbohydrate feeding in healthy individuals (Holst & Deacon, 2013), while incretin response has suggested to be blunted in people with prediabetes and even more so in people with T2D (Bagger et al., 2011). When consumed prior to or with a meal, 50 g of whey protein has been shown to augment insulin and incretin secretion, while reducing peak glucose and glucose AUC in individuals with T2D (Ma et al., 2009), and doses as low as 9 g of whey protein have similar responses in healthy adults (Gunnerud et al., 2013). Whey protein

has a higher proportion of branched chain amino acids compared to casein (Hall, Millward, Long, & Morgan, 2003), and has also been shown to digest at a faster rate (Petersen et al., 2009).

Clifton et al. (2014) showed that 17 g of whey protein prior to a mixed meal decreased average blood glucose concentrations (.8 mmol/L over 3 hr) and peak glucose (2.1 mmol/L) in people with either prediabetes or diabetes, however insulin was not measured. This study also combined the whey protein with guar gum, which is a known fiber that reduces gastric emptying. Therefore one cannot discern the changes in glucose related to guar gum, whey protein, or insulin (Clifton et al., 2014).

Whey protein ingestion either 30 minutes before a meal or taken with a meal significantly reduced glycemic responses in healthy (Petersen et al., 2009; Akhavan et al., 2010; Akhavan et al., 2014; Gunnerud et al., 2013), and individuals with T2D (Jakubowicz & Froy, 2013; Jakubowicz et al., 2014; Ma et al., 2009). Hoefle et al. (2015) suggests 50 g maltodextrin plus 50 g of whey significantly decreased peak and mean blood glucose responses over 240 minutes. Blood insulin concentration was increased ($p = .0008$) and the insulin index (Insulin/Glucose) increased 274% ($p < .05$). This study used individuals with prediabetes and healthy individuals, however did not measure a dose response (Hoefle, et al., 2015).

In healthy people (7 women; 5 men) with normal fasting blood glucose concentration, 9 g and 18 g of whey protein combined with 25 g of glucose (Gunnerud et al., 2013) reduced the incremental AUC ($_{i}$AUC) for blood glucose concentration

compared to the control condition (25 g of glucose alone) by 37% and 46% respectively; however these decreases were not significantly different from each other. In addition, peak blood glucose concentration was also significantly lower in both whey protein trials compared to the control. There was also a significant increase in both $_{i}$AUC (64%) and peak (est. 74%) blood insulin concentration during the 18 g whey protein trial. Therefore, it can concluded that 9 g of whey protein may be enough to see a significant decrease in blood glucose concentration with an increase in blood insulin concentration in healthy men. Nevertheless, the dose response was limited to only 18 g of whey, and not measured in individuals with T2D or those at risk of developing T2D.

Akhavan et al. (2010) used 10-40 g doses of whey protein as a pre-load drink prior to a meal in normal healthy men showed a decrease in $_{i}$AUC for blood glucose concentration for all protein doses. It should be noted, blood glucose $_{i}$AUC concentration for 10-20-30 g of whey was not different from each other, however the 40 g dose produced the greatest reduction in blood glucose concentration and was significantly lower than all doses (Akhavan et al., 2010). In a subsequent study, Akhavan et al. (2014) found both 10 g and 20 g of whey protein pre-meal decreased blood glucose concentration responses in young healthy men. There was also an incretin response (increased GLP-1, increased PYY). Even with the incretin increase, there was little blood insulin concentration response to explain the lower glycemic response (Akhavan et al., 2014).

Petersen et al. (2009) found glucose $_{i}$AUC decreased in a dose response manner (7.6%, 13.3%, and 37.5%) using a 50 g glucose drink combined with 5, 10, or 20 g of whey protein compared to glucose alone in healthy men and women. They also found peak blood glucose concentration decreased by more than 2 mmol/L during the 20 g whey protein load over the 120 minute response period. Based on these results, Petersen et al (2009) determined the average reduction in blood glucose $_{i}$AUC concentration was 4.6 mmol.min/L/gram of ingested whey. This study used both men and women, did not measure insulin, and did not use individuals with prediabetes or T2D (Petersen et al., 2009).

In contrast to the above mentioned studies, Hoefle et al. (2015) found no effect on overall blood glucose concentration response in people with pre-diabetes over 240 minutes when 50 g of whey was taken with 50 g of maltodextrin; yet peak plasma glucose concentration was significantly lower ($p < .0001$) with whey vs maltodextrin alone. There was also an increase in plasma insulin concentration by 96%. In summary, the glycemic response to whey protein in individuals with pre-diabetes appears weakened with a significant increase in insulin, but still has a significant effect on glycemic response to a load (Hoefle et al., 2015).

People with T2D respond to whey protein, either taken as a pre-load (Ma et al., 2009; Ma et al., 2015; Jakubowicz et al., 2013) or with a meal (Mortensen et al., 2009; Frid et al., 2005; Ang et al., 2012), with decreased blood glucose concentration and increased blood insulin responses (Ma et al., 2009; Ma et al., 2015; Jakubowicz et al.,

2014); no difference in blood glucose response and higher blood insulin (Frid et al., 2005; Ang et al., 2012); or decreased blood glucose and no difference in blood insulin response (Mortensen et al., 2009). All studies used a single dose of whey protein that ranged from 21-27 g (Ang et al., 2012; Ma et al., 2015; Frid et al., 2005) and 45-55 g (Ma et al., 2009; Jakubowicz et al., 2014; Mortensen et al., 2009).

The deviations in responses could be related to multiple sources/reasons including only people with diet-controlled T2D (Ma et al., 2009; Ma et al., 2015; Frid et al., 2005); people taking medications (sulfonylurea or metformin) and/or diet controlled (Ang et al., 2012; Jakubowicz et al., 2014; Mortensen et al., 2009). In addition all these studies had a mixed participant composition of men (3-9) and women (1-12) and did not identify gender responses likely due to the small overall number of participants (range 8-20; mean 12).

Dipeptidyl-peptidase 4 (DPP4) is responsible for the breakdown of GIP and GLP-1. Rohrborn et al (2015) review states that expression of DPP4 is dysregulated in disease conditions such as inflammation, cancer, obesity, and diabetes. DPP4 binds to adenosine deaminase (ADA) which causes the breakdown of incretin hormones such as GIP, GLP-1, and PYY. Obese individuals and those with type 2 diabetes have increased available ADA (linked with chronic inflammation) and therefore higher amounts and faster breakdown of incretin hormones. This reduces the amount of insulin secretion (Rohrborn et al., 2015). Also stated in the review article is that incretin hormones are responsible for

50% of postprandial insulin secretion. This can better explain the importance of incretin hormones and the ability to lower ADA and DPP4 expression (Rohrborn et al., 2015).

Whey protein not only increases blood glucose uptake by way of increased insulin production, it also plays a role in skeletal muscle cells. Kakigi et al. (2014) conducted a whey protein ingestion study where 15 male subjects performed 4x6 knee extensions. Seven of the subjects consumed either 10 g or 20 g of whey protein immediately upon completion of the exercises. Muscle biopsies were taken 1 hr post exercise and measured Akt and mTOR expression. There was no increase in the phosphorylation of Akt or mTOR, but the whey protein increased in both in a dose dependent manner (Kakigi et al., 2014). As discussed previously, an increase in Akt leads to a downstream cascade of reactions that eventually lead to Glut-4 translocation to the membrane.

Morato et al. (2013) examined whether whey protein or whey protein hydrolysate improved Glut-4 expression on the plasma membrane. Wistar rats were fed different chow (casein, whey, whey hydrolysate, casein w/ exercise, whey w/ exercise, and whey hydrolysate w/ exercise) for 9 days before measuring Glut-4 and Akt activity. Glut-4 and Akt expression increased in the whey groups for exercise and sedentary rats. The interesting part about this study is that blood insulin concentration did not increase in any of the 6 trials (Morato et al., 2013). This study did not measure glucose.

CHAPTER III

METHODS

Study Overview

This study examined the independent and combined effects of whey protein and an acute bout of exercise on glycemic control. This study determined if there was a combined effect in controlling plasma glucose responses and insulin secretion in healthy men.

Participants

Participants for this study were 11, apparently healthy, sedentary males between the ages of 18 and 44 years who did not smoke, have diabetes, cardiovascular disease, or other metabolic abnormalities. Exclusion criteria also included taking medications that influence blood glucose concentration or blood pressure response, and a recorded answer of "Yes" on a PAR-Q assessment. Sedentary status was defined by the ACSM guidelines as participation in exercise less than 3 days per week or less than 150 min of aerobic physical conditioning for three months. A medical history questionnaire, physical activity readiness questionnaire (PAR-Q), and an informed consent was completed by all participants. The procedures were explained verbally, and copies of the informed consent given to the participants. The protocols for this study were approved by the Institutional Review Board of Texas Woman's University.

Procedures

Data collection took place over the course of 4-5 weeks. Participants were recruited from Texas Woman's University and the surrounding Denton, Texas, area. Participants were screened for hyperglycemia prior to beginning the trials.

Screening

A blood sample was drawn from individuals 10-12 hr after fasted using a sterile butterfly needle from an antecubital vein and analyzed for plasma glucose concentration, hemoglobin A1C, metabolic panel, and lipid panel. Blood was placed into a 6ml serum separator vacutainer and a 6ml EDTA vacutainer to be sent to a third-party laboratory for analysis. Participants with a blood glucose value $\geq$ 100 mg/dl were excluded from participation. Participants were also screened for alcohol consumption, which is known to have effects on glucose tolerance. Participants kept three day diet records of food and drink prior to every trial, and were asked to consume the same dinner meal (large sub sandwich) at the same time the night prior to all OGTTs.

Anthropometrics

Following the glucose assessment, body composition was measured using dual-energy x-ray absorptiometry (DXA; Lunar Prodigy; GE Healthcare, Madison, WI). In addition to the DXA, height, weight, and body mass index (BMI) were measured and calculated for descriptive variables. Height was measured using a stadiometer (Perspective Enterprises; Kalamazoo, MI) and weight was measured to the nearest 0.1 kg

using a digital scale (Tanita Corp.; Arlington Heights, IL). Both measurements were taken without shoes. BMI was calculated using the following equation (Equation 1):

$$BMI = kg/m^2 \qquad \text{Equation 1.}$$

Maximal Aerobic Capacity Test

Participants underwent a maximal oxygen uptake test on a Quinton ST65 motor driven treadmill (Seattle, Washington) to determine maximal VO_2 (VO_2 max) During this test, the participants walked on the treadmill at a speed of 3.5 miles per hr (brisk walking pace). At the start of the test, the elevation of the treadmill was set at 0.0 % and then increased 2% every minute until VO_2 max was reached. During this test, heart rate was continuously monitored using a Quinton Q Stress 12 lead electrocardiograph. Heart rate was recorded during the last 10 seconds of each minute as well as throughout the recovery cool down period. Expired air was collected continuously during this test using a Parvomedics Oxygen Uptake System. A fitted rubber mouthpiece attached to a Rudolph R2700 two way breathing valve was used to collect expired gas. Thirty second averages of expired gasses will be measured continuously through indirect calorimetry (TrueOne 2400; ParvoMedics, Sandy, UT). After completion of the test, heart rate was monitored until it returned to 120 beats per minute or less. Criteria to determine a VO_2 max was a plateau in VO_2 with an increase in workload, heart rate within 10 bpm of age-predicted HR_{max}, or RER >1.10. The peak VO_2 value from the last minute was used as the VO_2 max.

Approximately 7-10 days after the test, participants returned to the laboratory at Texas Woman's University for the first of four treatment sessions. The four treatments were: rest (no exercise) with 50 grams of whey (W), an exercise session with 50 grams of whey (EXW), an exercise session with only water (EX), and no exercise and only water (R).

Whey Protein Pre-load

Fifty g of whey protein isolate (Isopure Unflavored WPI, Nature's Best, Hauppauge, NY) or water, was administered 30 minutes prior to an oral glucose tolerance test (OGTT) as a pre-load. The pre-load drinks consisted of 250 ml of water for the water only treatments, and 250 ml of water mixed with the whey protein. At least 1 week elapsed between the end of one treatment session and the beginning of the next treatment session. The order of experimental treatment sessions was randomized using a letter selection (A, B, or C) from someone not affiliated with the study. Each letter corresponds to a treatment that the person selecting did not know.

Exercise or Rest Protocol

Each exercise session entailed walking for 60 minutes at 3.5 mph. at 70% of VO_2 max, as determined by previous maximal testing. VO_2 was measured during the first 10 minutes of exercise and treadmill elevation was adjusted to achieve the prescribed exercise intensity for each participant. VO_2 was again measured during the last 10 minutes of the exercise session and averaged with the first 10 minutes for average exercising VO_2. Participants were given practice workloads at the beginning of the

exercise session to verify exercise intensity and to increase familiarity. During exercise, heart rate was continuously monitored. During the non-exercise trials, the participant came to the lab and sat for 60 minutes in a chair.

Oral Glucose Tolerance Test

Approximately 12-14 hr following the treatment session, as described above, the participant visited the lab for an oral glucose tolerance test (four OGTTs total over the course of the treatment sessions). This procedure required the following: fasting for 10-12 hr; placement of a venous catheter in a hand or forearm vein by a trained phlebotomist; consuming either the 50g whey with water or water only 30 minutes prior to drinking a commercial glucose tolerance test drink (75 grams of glucose); 8 blood samples (~40 ml total) were taken at -30 min, 0, 15, 30, 60, 90, and 120 min post, taken over the course of 3 hr while resting upright in a bed or recliner chair. A sterile saline drip was used to flush the catheter following every blood draw, along with a drip rate of 1 drop per 4 seconds. Following all glucose tolerance tests, participants were fed breakfast consisting of a banana and granola bar.

Blood Collection

Each blood sample was collected in a 5ml syringe, and then transferred to a 5ml EDTA vacutainer with a protease inhibitor mixture. Samples were centrifuged immediately at 3000 rpm at 4^{o}C for 10 minutes for the separation of plasma and red blood cells. Plasma was then aliquoted into pre-labeled cryogenic vials and frozen at -80^{o}C until further analysis.

Inhibitors

Recommended doses of serine protease inhibitor Pefabloc® SC (#11429876001), Sigma-Aldrich Protease Cocktail Inhibitor (#P2714-1BTL), and Sigma-Aldrich Dipeptidyl Peptidase IV (#D4943 Sigma) were brought to room temperature, mixed in a test tube, and placed into each EDTA vacutainer used for blood collection. Vacutainers were kept on ice till needed for use.

Biochemical Analyses

Plasma glucose was measured using a YSI 2900 Bioanalyzer (YSI Life Science, OH, USA) through the use of enzyme electrode technology. Plasma hormone concentrations of C-peptide, GIP, GLP-1, insulin, and glucagon were analyzed using a Luminex MagPix® System. A Luminex Human Metabolic Hormone multiplex assay (HMHEMAG-34, EMD Millipore, Billerica, MA) was used to analyze 25 µl of each sample in duplicate.

Statistical Analyses

Total area under the curve for glucose, insulin, and C-peptide was calculated using the trapezoidal method. Differences in all dependent variables were analyzed by 2x2 repeated-measures analysis of variance (RM ANOVA) with an alpha level of 0.05. Baseline (-30 min) through the 0 min time point was analyzed by one way RM ANOVA. All SPSS output can be found in Appendices I and J. Insulin sensitivity was calculated using the Matsuda index (Equation 2).

$$\frac{100000}{\sqrt{\frac{(fasting\ glucose * fasting\ insulin)}{(mean\ glucose * mean\ insulin)}}}$$

Equation 2.

CHAPTER IV

PRESENTATION OF FINDINGS

Participant Characteristics

Twelve participants were recruited from Texas Woman's University and the surrounding Denton, Texas, area. The recruitment was conducted through word-of-mouth and flyers posted around the Denton campus. Recruitment criteria required the participants to be male, sedentary, age 18-40, who did not smoke, with no known history of cardiovascular disease, diabetes, or other metabolic abnormalities. One participant did not complete the study due to failure to comply with study guidelines. Table 2. describes the participants. All data are presented as mean ± standard deviation (SD).

Table 2

Participant Descriptive Characteristics

	Males (n=11)
Age (y)	24.3 ± 5.4
Height (cm)	179.3 ± 5.4
Weight (kg)	84.3 ± 19.9
BMI (kg/m^2)	26.0 ± 5.3
HbA1c (%)	5.2 ± 0.2
Fasting Plasma Glucose (mg/dl)	85.2 ± 6.6
VO_{2max} (ml/kg/min)	38.3 ± 6.1
VO_{2max} (L/min)	3.1 ± 0.5
Average Exercise HR (EX) (bpm)	160.3 ± 12.6
Average Exercise HR (EX/W) (bpm)	160.0 ± 16.0
Average Exercise VO2 (EX) (L/min)	2.2 ± 0.3
Average Exercise VO2 (EX) (L/min)	2.2 ± 0.5

Data are presented as mean ± SD.

Sedentary was defined by structured exercise or physical activity $\leq$ 3 days per week or $\leq$ 150 min/week (American College of Sports Medicine, 2015). The average BMI resulted in the participant population being considered overweight. Average hemoglobin A1c (5.2%) and fasting plasma glucose concentration (85.2 mg/dL) were in normal ranges for healthy individuals. Exercising heart rate was 81.1% of their age predicted maximum heart rate, while exercising VO_2 was 70.6% of average VO_2 max.

Glucose Response

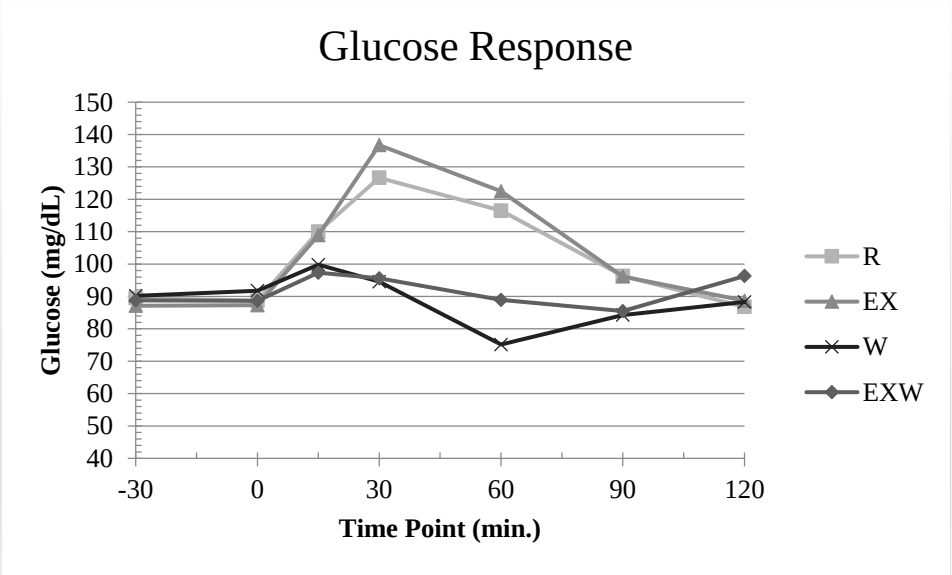

Figure 6. Average glucose responses during OGTT. Values are represented as mean glucose (mg/dL).

As shown in Figure 6, participants averaged near baseline for W (W_{mean} = 89.1 mg/dL, baseline = 90.2 mg/dL) while also maintaining glucose homeostasis for EXW (EXW_{mean} = 91.6 mg/dL, baseline = 88.9 mg/dL), despite consuming a 75 g glucose

beverage. The participants' responses for glucose in both R and EX never reached above 140 mg/dL.

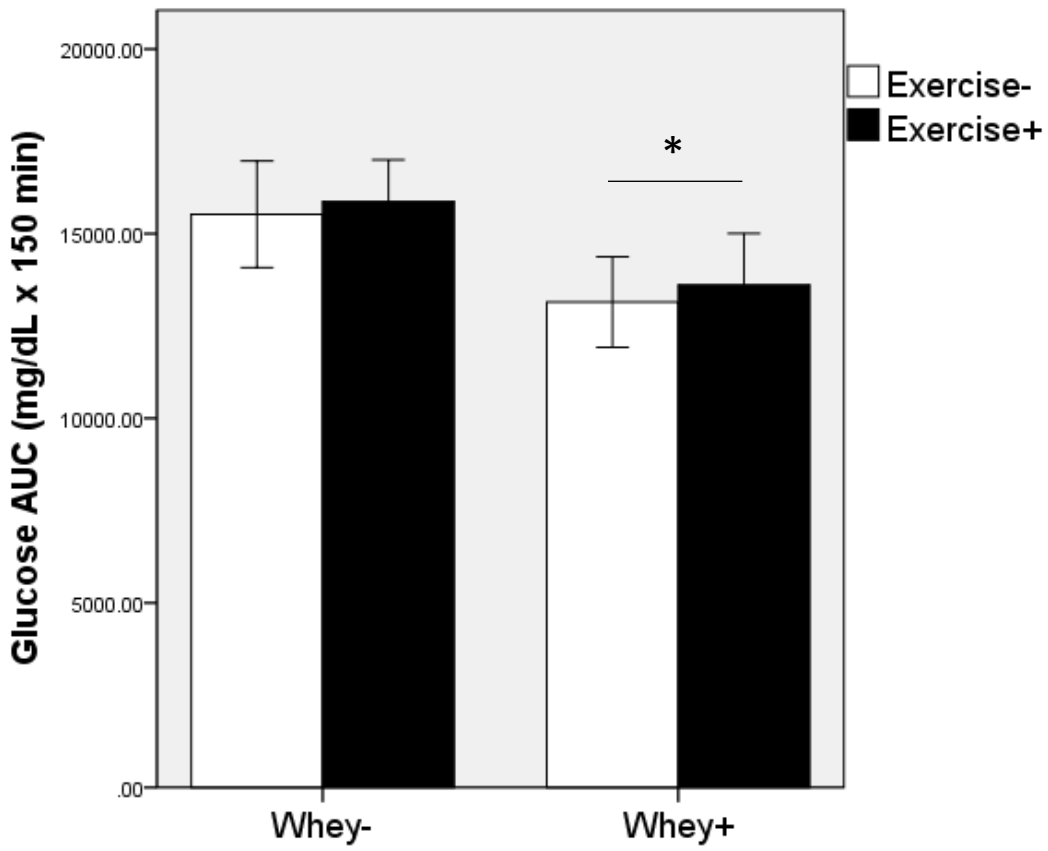

Figure 7. Glucose AUCs. Data are presented as mean ± SD. R = 15524 ± 722, EX = 15871 ± 564, W = 13149 ± 613, EXW = 13614 ± 695. * Denotes main effect for whey.

As shown in Figure 7, there was a main effect for glucose AUC for both W and EXW. Glucose AUC for W was significantly decreased compared to R (*$p < 0.01$, 15.3%). EXW decreased plasma glucose AUC from EX ($p < 0.01$, 14.2%). No differences were found between R and EX, or between W and EXW. Whey protein seems to have a strong impact on glucose AUC without regards to acute aerobic exercise.

Insulin Responses

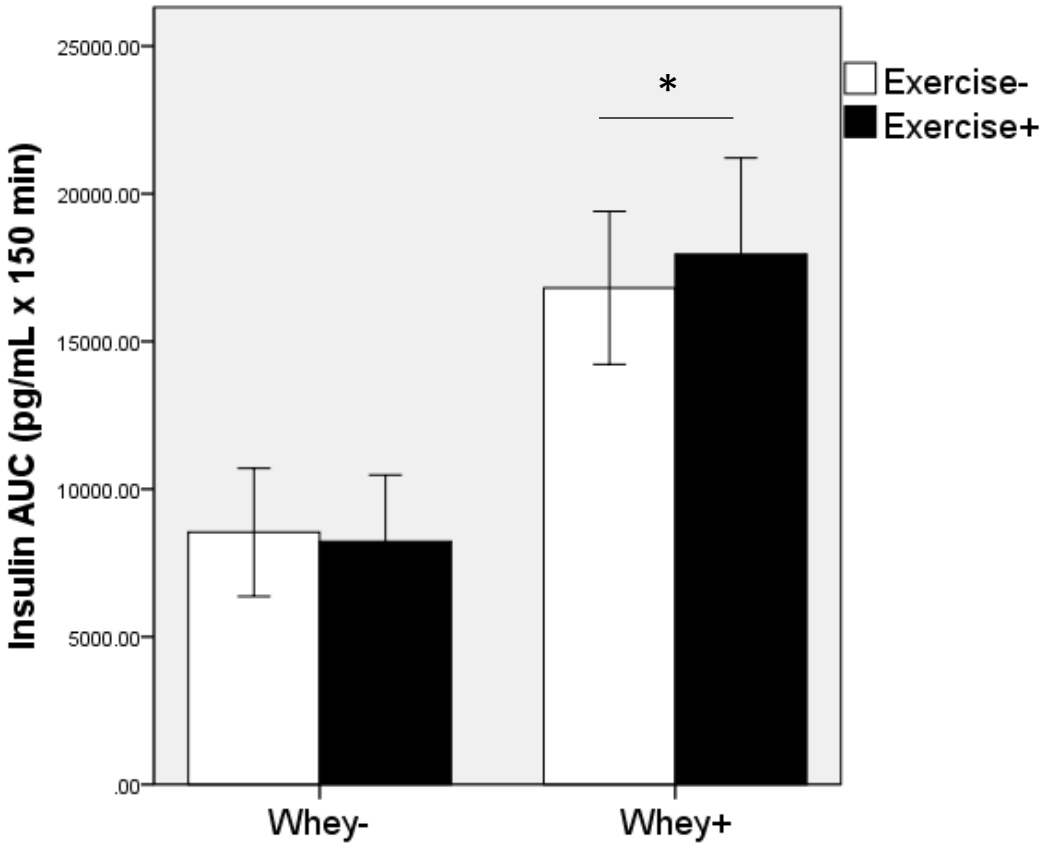

Figure 8. Insulin AUCs. Data are presented as mean ± SD. R = 414656 ± 78256, EX = 352804 ± 57553, W = 526376 ± 72688, EXW = 558892 ± 116296. * Denotes main effect for whey.

As shown in Figure 8, insulin AUC significantly increased from R to W (p = 0.014, 21.2%). EXW significantly increased plasma insulin AUC compared to EX (p = 0.028, 36.9%). There were no differences between R and EX, or between W and EXW.

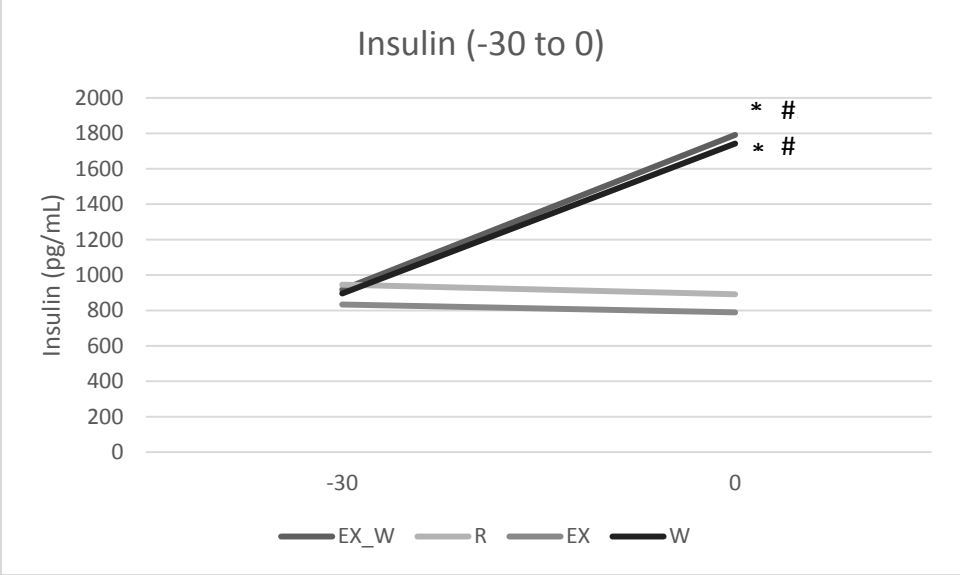

Figure 9. Insulin response 30 minutes after preload. * Denotes significant differences from R, # denotes significant differences from EX.

As shown in Figure 9, insulin significantly increased 30 minutes post ingestion of whey for EXW compared to R (*p = 0.01, 50.3%) and EX (#p = 0.01, 55.9%). Insulin increased for W compared to R (*p < 0.01, 48.8%), and EX (#p < 0.03, 54.7%).

C-peptide Responses

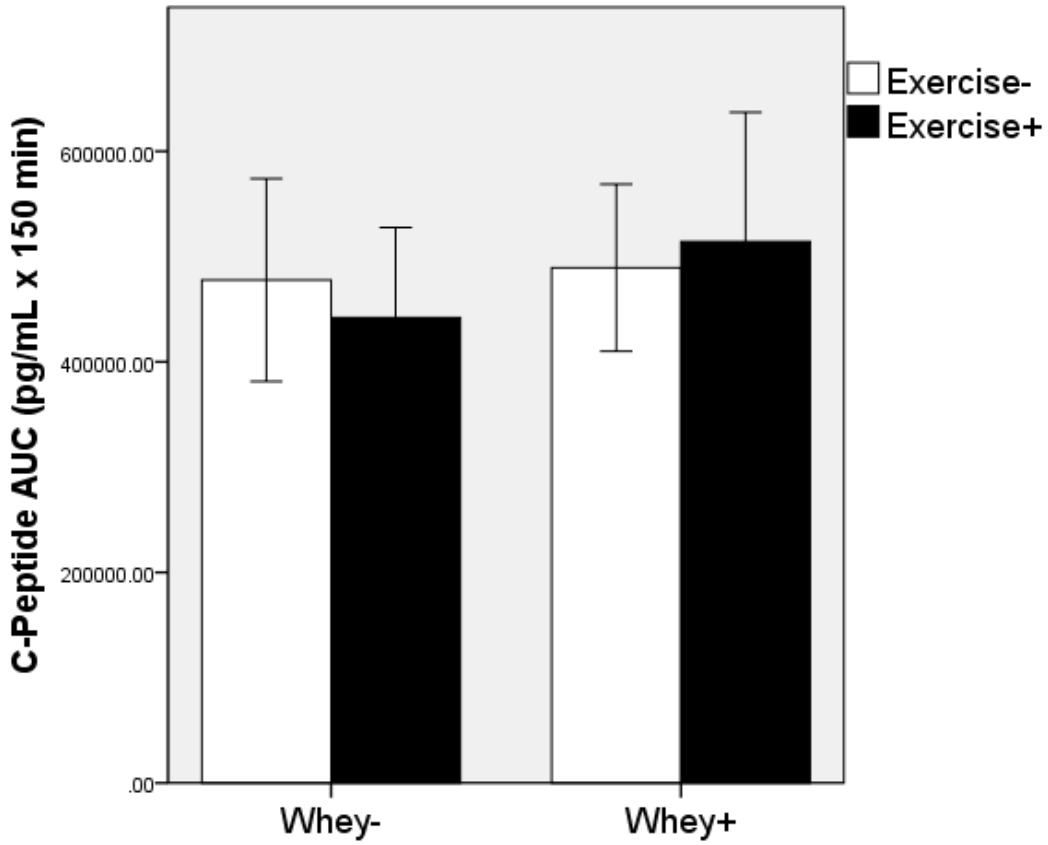

Figure 10. C-peptide AUCs. Data are presented as mean ± SD. R = 477676 ± 48195, EX = 441972 ± 42779, W = 489362 ± 39689, EXW = 514268 ± 61413.

As shown in Figure 10, no significant differences were observed for C-peptide across all four trials. C-peptide was 14.1% greater following EXW compared to EX but was not found to be significant (p = 0.15).

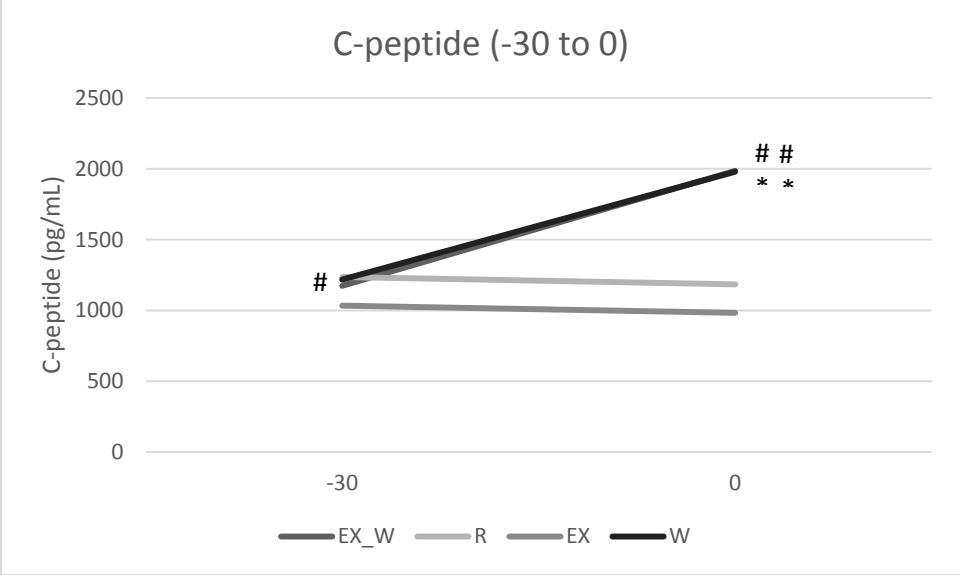

Figure 11. C-peptide response 30 minutes after preload. * Denotes significant differences from R, # denotes significant differences from EX.

As shown in Figure 11, C-peptide was significantly increased at -30 for W compared to EX (#$p = 0.02$). C-peptide increased from R to EXW (*$p < 0.01$, 40.4%), and R to W (*$p < 0.01$, 40.2%). C-peptide also increased from EX to EXW (#$p < 0.01$, 50.5 %) and EX to W (#$p < 0.01$, 50.4%).

GIP Response

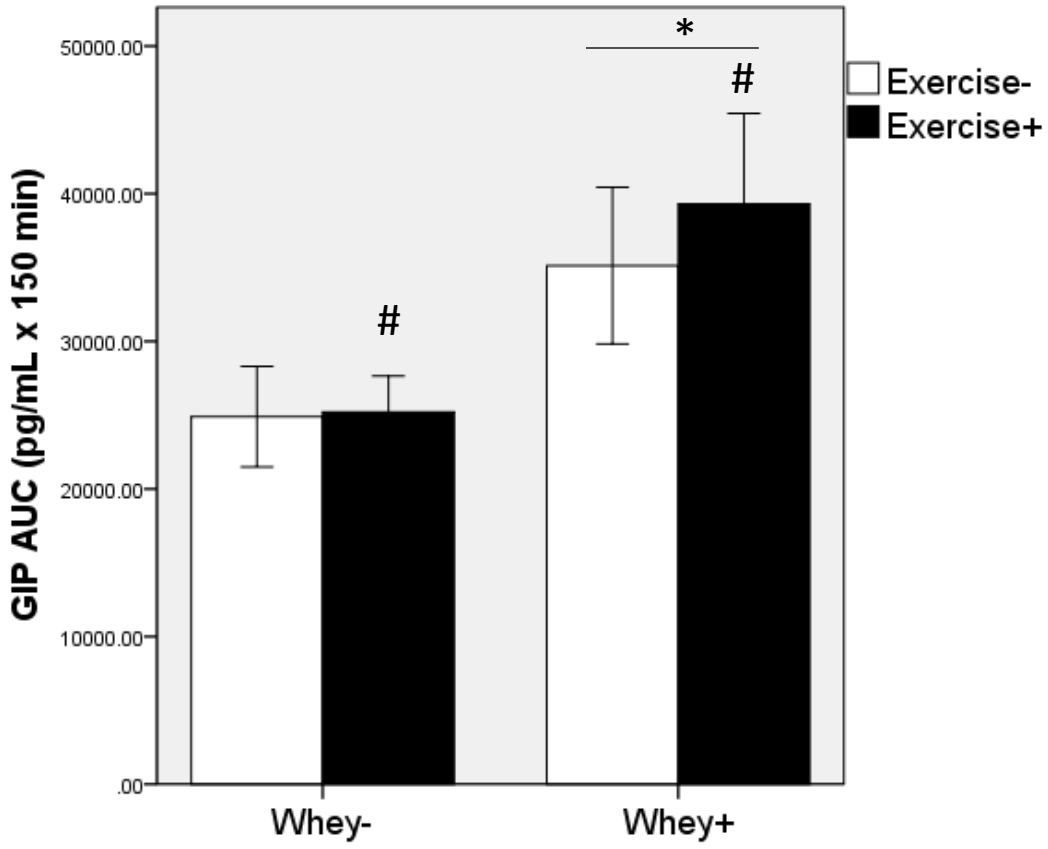

Figure 12. GIP AUCs. Data are presented as mean ± SD. R = 24893 ± 1704, EX = 25208 ± 1217, W = 35126 ± 2654, EXW = 39306 ± 3065. * Denotes main effect for whey, # denotes main effect for exercise.

As shown in Figure 12, GIP significantly increased from R to W ($p < 0.01$, 29.1%). EXW significantly increased from EX ($p < 0.01$, 35.9%). EXW increased beyond W by 10.6% but failed to reach significance. Whey protein had a significant effect on GIP, potentially causing an increase in insulin.

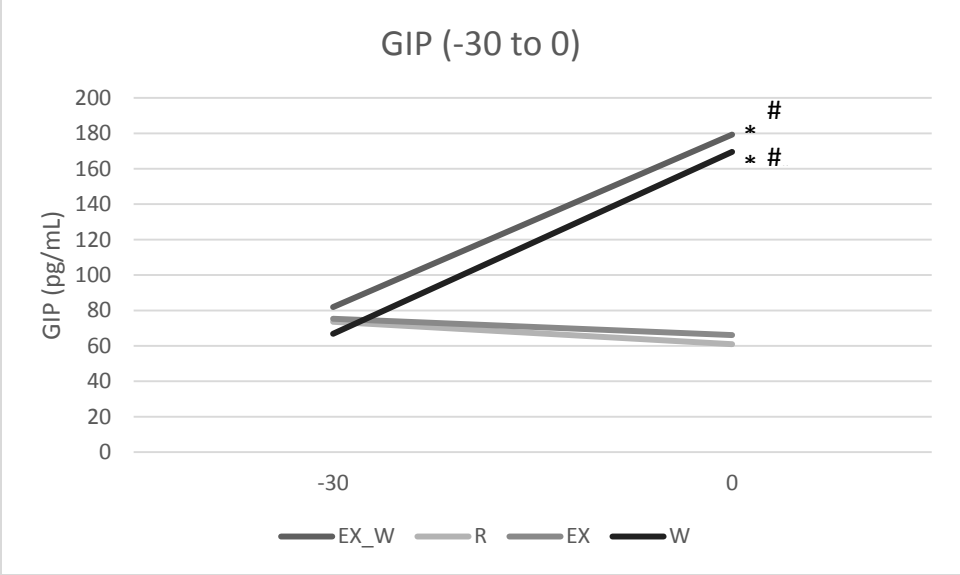

Figure 13. GIP response 30 minutes after preload. * Denotes significant differences from R, # denotes significant differences from EX.

As shown in Figure 13, no differences were found at -30 between the four trials. GIP significantly increased from R to EXW (*$p < 0.01$, 66.5%), and from R to W (*$p < 0.01$, 64.5%). There were also increases in GIP from EX to EXW (#$p < 0.01$, 63.1%), and from EX to W (#$p < 0.01$, 60.9%).

GLP-1 Response

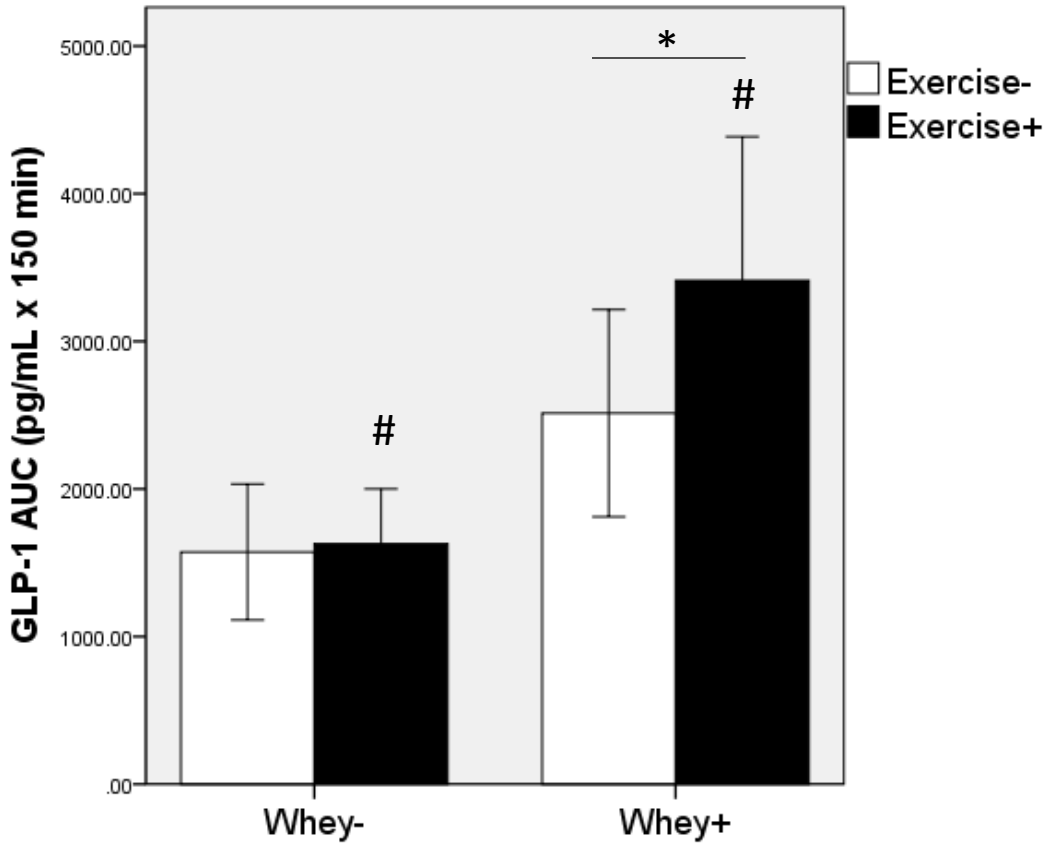

Figure 14. GLP-1 AUCs. Data are presented as mean ± SD. R = 1573 ± 230, EX = 1628 ± 187, W=2514 ± 351, EXW = 3412 ± 487. * Denotes main effect for whey, # denotes main effect for exercise.

As shown in Figure 14, there was a significant interaction for the combination of whey and exercise treatments for GLP-1. W was significantly higher than R ($p < 0.01$, 37.4%). EXW significantly increased from EX ($p < 0.01$, 52.3%), and from W ($p < 0.01$, 26.3%). EXW showed the highest increase, despite EX not increasing compared to R. Whey protein may enhance the GLP-1 response post exercise.

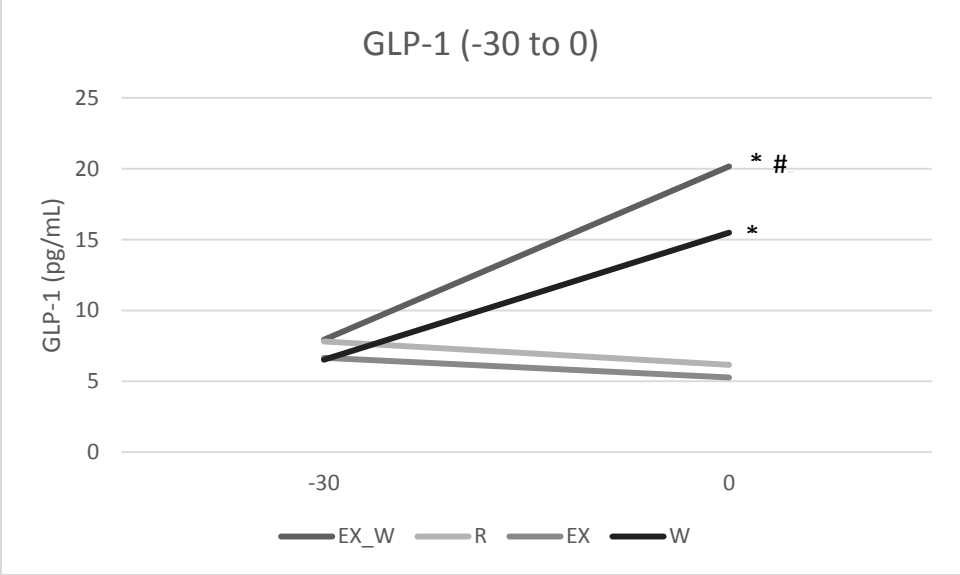

Figure 15. GLP-1 response 30 minutes after preload. * Denotes significant differences from R, # denotes significant differences from EX.

As shown in Figure 15, no differences were found at -30 across all trials. GLP-1 increased from R to EXW ($p < 0.01$, 70%), but not from R to W (60%). There was a significant increase from EX to EXW ($p < 0.01$, 75%), and from EX to W ($p = 0.03$, 67%).

Glucagon Response

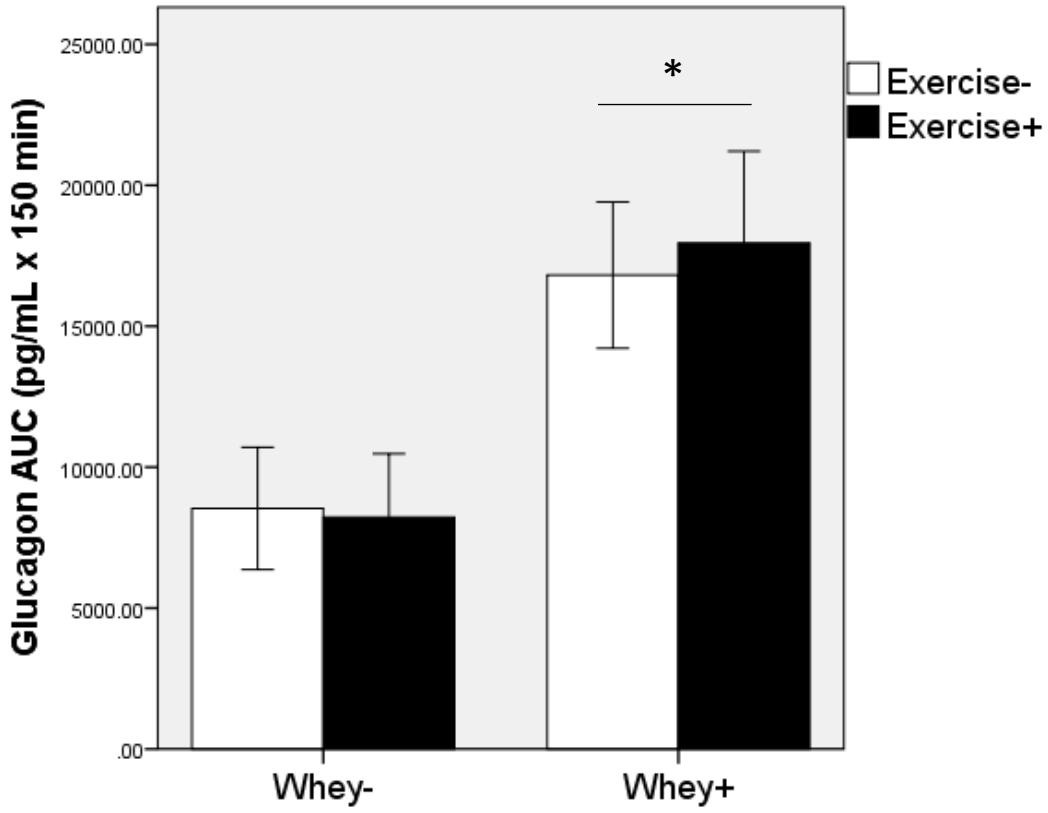

Figure 16. Glucagon AUCs. Data are presented as mean ± SD. R = 8536 ± 1085, EX = 8227 ± 1126, W = 16814 ± 1297, EXW = 17953 ± 1628. * Denotes main effect for whey.

As shown in Figure 16, W significantly increased from R ($p < 0.01$, 49.2%). EXW increased from EX ($p < 0.01$, 54.2%). No differences between R and EX, or between W and EXW. Whey protein shows a stimulation of glucagon, with exercise not showing an effect.

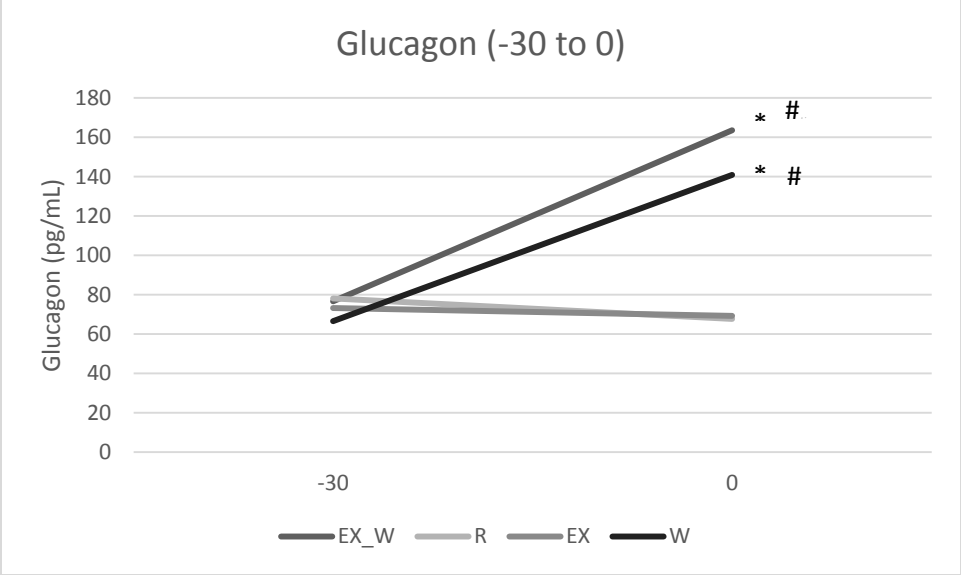

Figure 17. Glucagon response 30 minutes after preload. * Denotes significant differences from R, # denotes significant differences from EX.

As shown in Figure 17, no differences were found at -30 between the four trials. EXW increased in glucagon compared to R (*$p < 0.01$, 58.9%), and EX (#$p < 0.01$, 57.7%). Glucagon for W increased compared to R (*$p < 0.01$, 52.1%), and EX (#$p < 0.01$, 50.7%).

Insulin sensitivity was calculated using the Matsuda Index. There were no significant differences found in insulin sensitivity. This could be due to the fact that with a decrease in plasma glucose AUC, there was a comparable but opposite effect in insulin AUC.

CHAPTER V

DISCUSSION AND SUMMARY

Aerobic exercise is well understood to contribute to the alleviation and prevention of type 2 diabetic symptoms, such as impaired glucose tolerance and decreased insulin sensitivity. It has been suggested that blood glucose fluctuations, such as postprandial hyperglycemia from T2D, are positively correlated with coronary artery disease (Zhang, Xu, Jiao, Wu, Zhou, & Lv, 2013). In healthy individuals, aerobic exercise has been shown to decrease insulin response to a glucose challenge, while maintaining or decreasing glucose $_{i}$AUC. Other methods, besides aerobic exercise, such as whey protein, have reduced glycemic responses in healthy individuals (Akhavan et al., 2010; Akhavan et al., 2014; Gunnerud et al., 2013; Petersen et al., 2009), while stimulating an increase in insulin secretion.

The purpose of this study was to examine the independent and combined effects of both acute aerobic exercise, and whey protein, on glycemic responses in healthy men. The major findings of this study were the significant decreases in plasma glucose AUCs to W, and EXW, along with the increases in GIP and GLP-1 in the same trials.

Exercise Responses

Exercise has been shown to increase GLP-1 response one hr after exercise of 60 minutes at 65% max HR (Martins et al., 2007). However, this study did not show changes in GLP-1 AUC from EX compared to R, 12 hr post exercise. This is likely due to the participants consuming dinner between exercise and the OGTT. We did show similar responses to Heden et al. that an acute bout of exercise in lean and obese individuals walking for one hr at 55-60% VO_2 peak 12 hr prior to a mixed meal test showed no changes in glucose, C-peptide, GIP, or GLP-1, compared to no exercise (Heden et al., 2013). This has also been shown in previous literature that up to six hr post exercise to exhaustion, GIP response was not different than no exercise prior to a glucose challenge (Blom, Hostmark, Flaten, & Hermansen, 1985). The current study suggests that exercise does not alter fasting GIP or GLP-1 plasma concentrations, or AUC responses from a 75 g OGTT.

Acute aerobic exercise has also been shown to stimulate a glucagon response during exercise (Galbo, Holst, & Christensen, 1975). However, due to the prolonged amount of time between exercise and the OGTT the following morning, there were no changes seen between EX, and R, especially since there was a meal between exercise and the OGTT.

C-peptide concentration did not show any significant differences across the four trials. Accordingly, insulin did not significantly decrease with EX. This data is contradictory to previous studies that a single bout of aerobic exercise can either decrease

plasma glucose concentration, decrease insulin concentration, or the combination (Brown et al., 1997; Hubinger et al., 1987; Larsen et al., 1997; Rogers, 1989). Nevertheless, not all studies have shown a decrease in plasma insulin post an acute bout of aerobic exercise. Cononie et al. (1994) did not show significant changes in plasma glucose concentration or insulin concentration (15%) after an acute bout of aerobic exercise for 50 minutes at 70% VO_2 max. Cononie used eight men and one woman between the ages of 61 and 82 yrs. Young, healthy participants in previous studies within our lab (Ben-Ezra et al., 1995; Castleberry et al., 2017) have shown similar results as Cononie et al., with the current study showing a decrease in insulin concentration of 14.9 % for EX compared to R, however, this did not reach significance.

The current study did not show significant changes in plasma glucose AUC between R and EX. This is likely the result of the participants' fitness level, and the exercise intensity. Literature is contradictory with regard to glucose responses. Some studies show a decrease in plasma glucose response post exercise (Rogers, 1989; Brown et al., 1997; Larsen, et al., 1997), while others show no change in plasma glucose response (Ben-Ezra et al., 1995; Jankowski et al., 2004; Cononie et al., 1994). Many different variables could help try to explain this such as health status (T2D or healthy), fitness level of the participants, exercise time, intensity. Mode may also play a role since some studies such as Hubinger et al. (1987) used cycling ergometry, while others used treadmill walking such as Ben-Ezra et al. 1995, and Jankowski et al. 2004.

The current study did not find any significant differences in insulin sensitivity using the Matsuda index. This is contradictory to other studies, such as Young et al. (1989), where well trained and untrained males completed cycling for 40 minutes at 40% and 80% VO_2 max. Fourteen hr post exercise, subjects completed an OGTT, and the untrained group decreased plasma insulin total AUC by 40% with no change in plasma glucose AUC across the trials (Young, Enslin, & Kuca, 1989). This study also found One potential explanation for the current study not observing differences in insulin sensitivity is that exercise intensity was not high enough since we only used 70% and not 80% max VO_2. However, Young et al. still observed changes with 40% effort. Another possible explanation is the mode of exercise.

While Young et al. and the current study based exercise on percent VO_2 max, cycling results in 7% lower values than treadmill (Loftin, Sothern, Warren, & Udall, 2004). However, the percent VO_2 max efforts were not different. There were also no differences in muscle activation based on EMG analysis between cycling and treadmill walking (Prosser, Stanley, Norman, Park, & Damiano, 2011). However, Prosser et al. (2011) did not increase the grade on the treadmill. Other potential explanations could have been kcal expenditure or deficit. It is suggested that energy expenditure is the strongest determinant of insulin sensitivity (Audelin et al., 2012). Although Audelin et al. (2012) was a training study, exercise decreased average caloric intake, while simultaneously increasing caloric expenditure. However, previous literature shows that

when workload is matched for energy expenditure, intensity was the determining factor for decreasing insulin responses to an OGTT (Ben-Ezra et al., 1995).

Whey Protein Responses

A significant increase in both GIP, and GLP-1 AUC responses to the trials including whey protein (W and EXW) were observed following combination of the preload and 75 g OGTT. Lower doses of whey protein administered either with glucose, or as a pre-load, have not consistently shown significantly decreases in plasma glucose concentration. In healthy individuals, 9 and 18 g of whey combined with 25 g of glucose did not reduce $_{i}$AUC significantly for blood glucose (Gunnerud et al., 2013). Whey protein doses of 10-30 g administered as a pre-load had similar responses in normal, healthy men (Akhavan et al., 2010). However, 40 g or more of whey protein ingestion has continually resulted in significant decreases in plasma glucose following a meal test (Akhavan et al., 2010), 50 g (Ma et al., 2015), and 55 g (Ma et al., 2009). Interestingly, the current study suggested whey protein stimulated an increase in GIP, GLP-1, insulin, and glucagon, prior to glucose ingestion. These results are similar in response to individuals with T2D. Ma et al. (2009), observed increased GIP and GLP-1 30 minutes post 55 g whey protein ingestion in individuals with type 2 diabetes. One difference in the current study we observed significant increases in insulin between -30 and zero, where Ma et al. (2009) did not.

The current study also observed a significant decrease in plasma glucose AUC, similar to Ma et al. 2009. Fifty g of whey protein administered 30 minutes prior to a 75 g

OGTT with both whey protein alone, and combined with acute aerobic exercise decreased plasma glucose AUC. The GIP and GLP-1 responses were also similar to previous literature where whey protein induced a greater secretion of incretins (Holst & Deacon, 2013), while also significantly increasing insulin responses. Other studies have also replicated these results with whey protein promoting an increase in GIP, GLP-1, and insulin, while also significantly decreasing plasma glucose concentration (Baggio & Drucker 2007; Phillips & Prins, 2011; Theodorakis et al., 2006).

Glucagon showed similar increases as the GIP and GLP-1 responses with the whey trials. R and EX trials were preloaded with water only, therefore, no increase in GIP, GLP-1, insulin, C-peptide, or glucagon were found. Glucagon has been shown to be enhanced through increases in GIP, however, it is also inhibited by GLP-1 (Seino, Fukushima, & Yabe, 2010). Since the incretins have opposing effects on glucagon, it should be expected that the glucagon response for W and EXW would be similar to R. This study still showed increases in glucagon response to whey protein, despite similar increases in both GIP and GLP-1. It can be speculated that GIP has a stronger upregulation effect on glucagon than GLP-1 on inhibition as our results were similar to Seino et al. Another explanation could be the bioavailability of GIP. The absolute amount of GIP concentration measured in the plasma was more than 10x that of GLP-1 concentration. Even with the differences in amount of GIP and GLP-1, the incretin response significantly increased with W and EXW, stimulating an increase in insulin (Muscelli et al., 2008; Vollmer et al., 2008).

Insulin AUC increased for W compared to R, and EXW compared to EX. This is similar to previous studies using whey protein doses as large as 40 g (Akhavan et al., 2010; Ma et al., 2009; Ma et al., 2015). Akhaven et al. observed a 33.3 % increase in the 30 minutes post ingestion of whey alone, which was similar to our response of 48.8 %. Akhaven et al. used a meal test, while the current study used a glucose beverage.

Glucagon is known to increase blood glucose concentration while insulin is known to decrease blood glucose concentration. Since these are opposing actions, we suspected glucagon to be inhibited with a rise in insulin. Insulin is known to inhibit glucagon secretion (Kaneko et al., 1999). Therefore, the rise in incretins should have stimulated an increase insulin, ultimately decreasing plasma glucagon and glucose concentrations. Significant decreases in plasma glucose AUC for W were observed, despite significant increases in glucagon concentration, and plasma insulin AUC. Since GIP and GLP-1 both promote insulin secretion, GIP is known to stimulate glucagon as well, while GLP-1 inhibits glucagon secretion (Baggio & Drucker, 2007). To speculate, we believe that GIP has a stronger effect to promote an increase in glucagon, than GLP-1 has on inhibition of glucagon. We also believe that insulin may play a stronger role in decreasing plasma glucose than glucagon has on raising it. Insulin and glucagon concentrations were both significantly increased 30 minutes post ingestion of whey protein, while plasma glucose concentration remained unchanged, prior to glucose ingestion. However, another explanation for the decrease in glucose is insulin was 30x higher in absolute concentration than glucagon. This could not be compared to other

studies as the current study was the first to use the same units of measure for both insulin, and glucagon. It has also been shown that GLP-1 has an effect on slowing gastric emptying, leading to a decrease in plasma glucose concentration (Imeryuz et al. 1997).

Exercise and Whey Responses

The combined effect of both acute aerobic exercise and whey protein preload prior to an OGTT resulted in a similar increase in GIP concentration and insulin concentration, while GLP-1 concentration was augmented with the combination of exercise and whey, and not exercise alone. This has not been previously studied, but as mentioned earlier, GIP and GLP-1 concentrations were not affected by exercise alone.

The insulin response was significantly increased for both W and EXW. We speculated that since acute aerobic exercise and whey protein have opposing actions on insulin, insulin response should not differ for EXW compared to R. However, there was a significant increase in plasma insulin AUC for both whey trials. Plasma glucose AUC was significantly lower for both W and EXW. Future studies may want to examine skeletal muscle function, Glut-4 translocation, and glucose uptake on skeletal muscle, as this may provide a better explanation of decreased plasma glucose.

Conclusion

Whey protein provided a greater reduction in postprandial hyperglycemia that naturally occurs during an OGTT in normal, healthy males, than exercise alone. Exercise did provide additional benefit by significantly decreasing plasma insulin AUC, despite no changes in insulin sensitivity, or by decreasing plasma glucose AUC. Overall, the

combined effect of acute aerobic exercise and whey protein did not provide additional benefits to postprandial hyperglycemia, insulin, or glucose response during a 75 g OGTT compared to whey protein alone.

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APPENDIX A

Institutional Review Board 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: October 27, 2017

TO: Mr. Todd Castleberry
Kinesiology

FROM: Institutional Review Board (IRB) - Denton

Re: *Approval for The Effect of Whey Protein in Addition to Aerobic Exercise on Glycemic Control (Protocol #: 19806)*

The above referenced study was reviewed at a fully convened meeting of the Denton IRB (operating under FWA00000178). The study was approved on 10/26/2017. This approval is valid for one year and expires on 10/26/2018. 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.

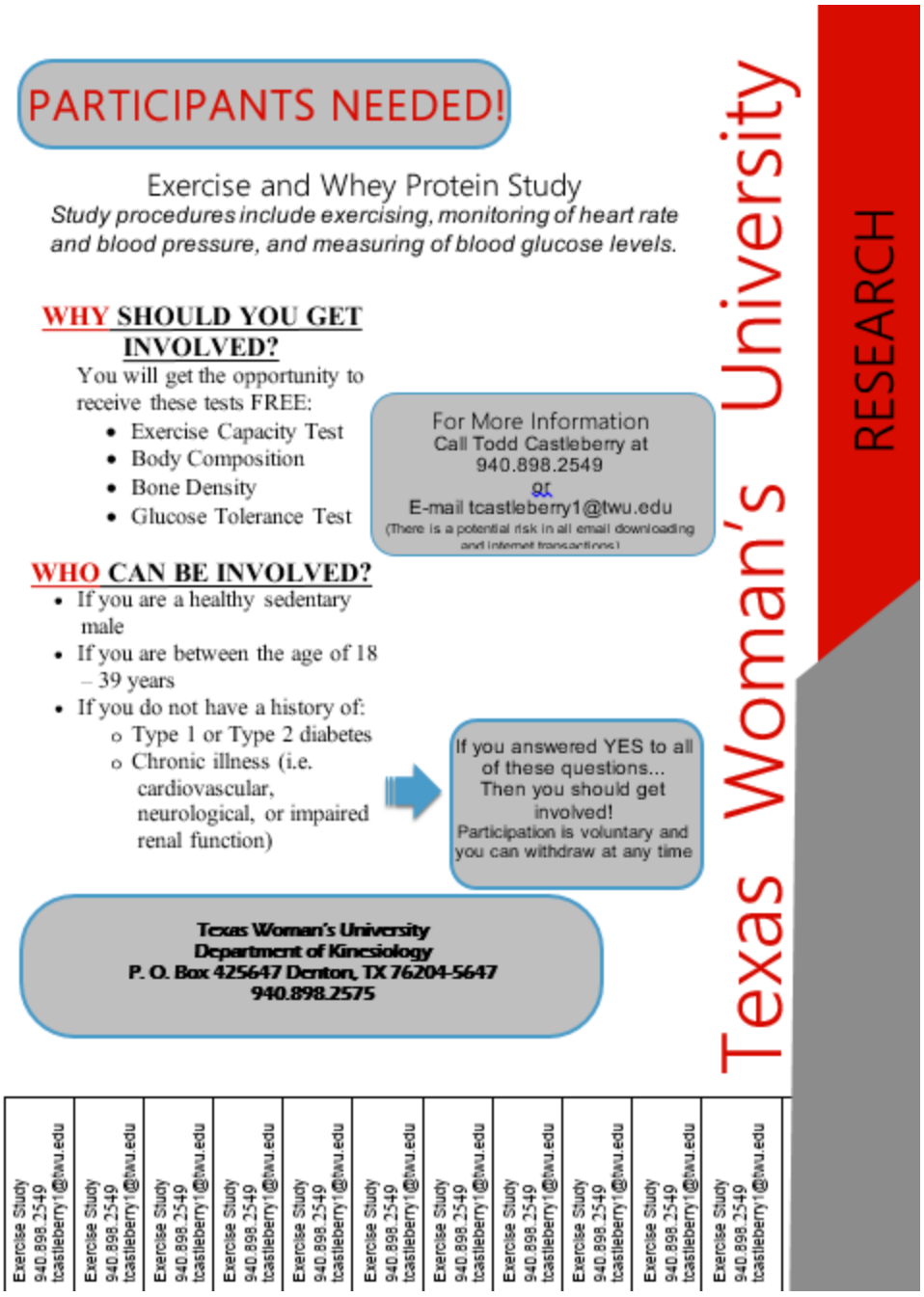
If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your 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. David Nichols, Kinesiology
Dr. Vic Ben-Ezra, Kinesiology
Graduate School

APPENDIX B

Participant Recruitment Flyer


PARTICIPANTS NEEDED!
Exercise and Whey Protein Study
Study procedures include exercising, monitoring of heart rate and blood pressure, and measuring of blood glucose levels.
WHY SHOULD YOU GET INVOLVED?
You will get the opportunity to receive these tests FREE:
Exercise Capacity Test
Body Composition
Bone Density
Glucose Tolerance Test
For More Information
Call Todd Castleberry at 940.898.2549
or
E-mail tcastleberry1@twu.edu
(There is a potential risk in all email downloading and internet transactions)
WHO CAN BE INVOLVED?
If you are a healthy sedentary male
If you are between the age of 18 – 39 years
If you do not have a history of:
Type 1 or Type 2 diabetes
Chronic illness (i.e. cardiovascular, neurological, or impaired renal function)
If you answered YES to all of these questions... Then you should get involved!
Participation is voluntary and you can withdraw at any time
Texas Woman's University
Department of Kinesiology
P. O. Box 425647 Denton, TX 76204-5647
940.898.2575
Texas Woman's University
RESEARCH
Exercise Study 940.898.2549 tcastleberry1@twu.edu

APPENDIX C

Informed Consent

TEXAS WOMAN'S UNIVERSITY
CONSENT TO PARTICIPATE IN RESEARCH

Title: The Effect of Whey Protein in Addition to Aerobic Exercise on Glycemic Control

Principal Investigator: Todd Castleberry..... tcastleberry1@twu.edu 940-898-2549
Faculty Advisor: Vic Ben-Ezra ..... vbenezra@twu.edu 940-898-2597

You may call the study investigators during regular office hours at 940-898-2459.

Instructions:
Please read this consent form carefully and take your time making a decision about whether to participate. As the researchers discuss this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. The purpose of the study, risks, inconveniences, discomforts, and other important information about the study are listed below. If you decide to participate, you will be given a copy of this form to keep.

Why is this study being done?
This study is being done as a dissertation to investigate the effect of a whey protein and exercise on glucose control and gut hormone responses. Normal blood glucose levels need to be maintained. Blood glucose levels that are too high or low can cause health problems such as diabetes, stroke, and cardiovascular disease. This study will investigate the effects of whey protein and exercise on glucose metabolism.

Why am I being asked to take part in this research study?
You are being asked to take part in this study because you are:

- Male
- Between the ages of 18 and 34 years
- Have not been diagnosed with Type 2 Diabetes Mellitus

You are not eligible in this study if you:

You need to be free of cardiovascular disease, diabetes, and hypertension. In addition, you cannot be taking medication that may influence glucose or blood pressure responses as this may interfere with the variables of interest (glucose, insulin) in the study. Exclusion criteria will include current smokers (within the past year). Par-Q will be given to you to ensure your safety and to address any health issues. You will be excluded if you answer yes to any of the Par-Q questions. You will be exercising at a vigorous intensity during the study and need to be free of known risk factors. You cannot have had surgeries or injuries within the past 6 months or food allergies specific to the whey protein.

Additional exclusions include: alcohol consumption of more than 12 drinks per week/2 drinks per night, lactose intolerance, an irregular diet or sudden change in diet or weight, and a body mass index (BMI) $\geq$ 30.

Do I have to take part in this research study?"
No. You have the right to choose whether you want to take part in this research study. If you decide to participate and later change your mind, you are free to stop participation at any time.

What is involved in the study?
If you volunteer to take part in this research study, you will be asked to sign this consent form and complete the tests and procedures that are explained later in this consent form. All tests and procedures are done solely for the purpose of the study and are not intended to diagnose or treat medical problems.

To complete this project, information will be collected during 9 different days. If you agree to participate in the study, you will be required to visit the laboratory up to 9 times. The first 3 days will involve screening procedures and preliminary testing. You will be asked to come in on 2 other days, 7-14 days apart, and walk on a treadmill for 60 minutes. The following morning after exercise, you will drink 50g of whey protein mixed in 250 ml of water, or 250 ml of water alone. You will also complete another trial of whey mixed with water and water alone without exercise. Each of these drinks will be followed by an oral glucose tolerance test (OGTT) drink that contains 75 grams of glucose (sugar). The order of these drinks will be randomly assigned. You will also be asked to complete a food/sleep log prior to each OGTT.

Study Summary: Whey protein drink and oral glucose tolerance test (OGTT)
You will undergo 4 different trials: **CON** (no exercise and water only taken 30 minutes before the OGTT); **EX** (exercise and water only water taken 30 minutes before the OGTT);
WHEY (no exercise and 50 grams whey protein in water taken 30 minutes before the OGTT); and **EXWHEY** (exercise and 50 grams whey protein in water taken 30 minutes before the OGTT). Blood samples will be taken before the water or whey protein drink and 30 minutes after the drink. You will then drink a 75 grams glucose drink and blood samples will be taken at the following times after the drink: 15, 30, 60, 90, 120, and 150 minutes.

1. Procedure

Screening Procedures
To help decide if you qualify for this study, the researchers will ask you questions about your health, including medications you take and any surgical procedures you have had.

You will also complete these procedures:

- Medical history Questionnaire;
- Dual Energy X-Ray Absorptiometry (DXA)
- Oral ingestion of 50g of whey protein
- Oral glucose tolerance test drink (50 grams of glucose)

If you decide to participate in the study, you are committing to approximately 20 hours of testing and having approximately 1/3 pint or 160 ml of blood drawn. This study involves 9 total visits to the laboratory. These visits include three preliminary/familiarization session (3), exercise sessions (2), the OGTT preceded with just water (2), and two other visits with an OGTT preceded by 50g (2) of whey protein mixed in water.

During the course of this study you will have the following tests and procedures:

Preliminary session:
Description of Procedure: In this first session, you will provide informed written consent, fill out a medical history questionnaire and have the following measurements taken: height, weight,

and waist circumference. Anthropometric measures (height, weight, and waist circumference) will be taken once prior to beginning the four trials (**CON, EX, WHEY, EXWHEY**). Height will be measured with a stadiometer while in bare feet or wearing socks. Weight will be measured with a standard weighing scale while wearing gym shorts, socks, and a shirt. Waist circumference will be measured with a measuring tape at approximately one inch above the umbilicus (belly button). All anthropometric measures will be taken in a private room by an investigator of the same sex as you. These measurements should take approximately 10 minutes to complete. In addition to anthropometric measurements, a baseline finger-tip blood sample will be collected. It will consist of a trained phlebotomist using a lancet to poke a tiny hole in your finger to draw 1 drop of blood.
Body composition (fat free mass and fat mass) will be measured with DXA (Dual X-ray Absorptiometry) while lying supine and wearing gym shorts, shirt and socks. This procedure will take place in the Human Development Bldg (HDB) in the Institute for Women's Health located in the Human Development Building; rooms 013 and 010. This procedure will be performed by a trained technician.

Body Weight: Weight will be measured with a standard weighing scale while wearing gym shorts, socks, and a shirt.
Duration of Procedure: Each waist measurement will take about 30 seconds.

Waist Circumference:
Description of Procedure: To determine your waist circumference, you will stand with your arms at your sides, feet together, and abdomen relaxed. A horizontal measure will be taken at the narrowest part of your torso above the bellybutton.
Duration of Procedure: Each waist measurement will take about 30 seconds.

Body Height:
Description of Procedure: You will be asked to stand on a scale which will measure your height.
Duration of Procedure: Each height measurement will take about 30 seconds.

Maximal Oxygen Uptake Test (Max Test):
Description of Procedure: This test measures your body's peak ability to use oxygen. The test involves exercising on a treadmill beginning at a light/easy intensity and gradually increasing to a point at which you can no longer continue. The intensity of the exercise will get harder every couple of minutes. You will breathe regular room through a snorkel-like mouthpiece or facemask during this test, and air that you breathe out will be analyzed by a computer.
Duration of Procedure: The total duration of the test will be about 30 min (this includes10-15 min of warm-up).

Submaximal Exercise and Rest Treatments:
Description of Procedure: Approximately 7-10 days after the max test you will return to the laboratory for the first of four treatments. The treatments are: rest (no exercise) with 50 grams of whey, an exercise session with 50 grams of whey, an exercise session with only water, and no exercise and only water. At least one week will elapse between the end of one treatment and the beginning of the next treatment. The whey will be administered 30 minutes prior to the oral glucose tolerance test for all trials and mixed with 250 ml of water. At least one week will elapse between the end of one treatment and the beginning of the next treatment. The order will be randomized. You will be walking at 75% of your maximum ability for 60 minutes during each exercise session. Everyone completes a total of seven exercise sessions. Sessions involve walking on the treadmill at 3.5 mph (similar to a brisk walk). The elevation of the treadmill will be adjusted for each

individual in order to achieve the desired exercise intensity. You will be given practice workloads at the beginning of the exercise sessions to verify exercise intensity. During exercise, your heart rate will be monitored by ECG or Polar heart rate monitors. In addition, there will be a five minute break in each exercise session at the thirty minute mark.

Duration of Procedure: The maximum total duration of this exercise day will be approximately 60 minutes for each exercise session.

Electrocardiogram:
Description of Procedure: Sticky patches will be applied to your skin to measure the heart's electrical signals. It is possible that a small amount of chest hair may need to be shaved to get the patch to stick.
Duration of Procedure: The electrocardiogram will be measured during the entire experiment (approximately 1 hour).

Rating of Perceived Exertion:
Description of Procedure: You will be asked to rate on a standardized scale how hard you feel you are exercising.
Duration of Procedure: You will be asked several times throughout the study to rate your perceived exertion.

Heart rate:
Description of Procedure: Heart rate will be measured by wearing a strap placed around your chest that transmits electrical signals.
Duration of Procedure: Heart rate will be measured during the entire experiment (approximately 1 hour).

Blood pressure:
Description of Procedure: Your blood pressure will also be monitored using a cuff placed on your upper arm that is inflated and deflated periodically during the study. A 24 hr blood pressure device will be given to you and will inflate and deflate periodically over 24 hrs.
Duration of Procedure: The cuff will be on your upper arm during the measurement of blood pressure. We will take blood pressure measurements at different time points during the experiment. Each measurement will last approximately 30 seconds.

Oxygen Consumption:
Description of Procedure: This measures your body's ability to use oxygen. This involves breathing regular room air through a snorkel-like mouthpiece or facemask during the experiments, and air that you breathe out will be analyzed by a computer.
Duration of Procedure: The total duration of the test will be intermittently over the duration of the experiment (5minute intervals over 60minutes).

Oral Glucose Tolerance Testing (OGTT): You will have a total of four oral glucose tolerance tests that each take approximately 3-3.5 hours to complete.

Peripheral intravenous catheter during oral glucose tolerance test:
Description of Procedure: A sterile catheter, which is a thin flexible plastic tube, will be inserted into an arm vein so that blood can be taken several times without having multiple sticks with a needle. A phlebotomist will draw blood samples for various chemicals in the body glucose (sugar in the blood), insulin, C-peptide, glucagon, GLP-1, and GIP (intestinal hormones).

Duration of Procedure: The catheter will be in place for the entire experiment (approximately 3.5 hours).

Oral Glucose Tolerance Test

Description of Procedure: During this study, you will undergo an oral glucose tolerance test. This is a three hour test that involves several blood samples. On the night before your test, you will eat your last meal and should not eat or drink anything other than water for at least 10 to 12 hours after. For this test, you will have a peripheral intravenous catheter (thin plastic tube described above) inserted into a large vein in your arm (procedure described above). The investigator will then give you a sugary substance to drink (75 gram glucose) over a short period. Blood samples will be drawn 10 minutes after the drink and then at 30 minute intervals for 180 minutes. The time at which you begin drinking the beverage will count as "0 minute." The phlebotomist will take a total of small samples (4 mL each) of blood from your vein during the 3 hour test (two times before you drink the beverage and 7 after you drink the beverage). The total amount of blood taken during each test will be approximately 36 ml or ~3 tablespoons. The results of the oral glucose tolerance test will allow the researchers to observe how your body reacts to a glucose load.

Duration of Procedure: Blood samples will be drawn every 30 minutes for 180 minutes. The oral glucose tolerance test will take approximately 3 hours.

Whey Protein + Glucose Tolerance Test

Description of Procedure: During this study, you will drink a whey protein drink two different times. When you come to the lab in the morning for this test, you will have a peripheral intravenous catheter (thin plastic tube described above) inserted into a large vein in your arm. The researchers will then give you 50g of whey protein mixed in water on each visit. After 30 minutes, a 75g glucose drink (approximately 125 ml of fluid), will be consumed. Blood samples will be drawn as described above for the OGTT. The time at which you begin drinking the beverage will count as "0 minute." The phlebotomist will take a total of 9 small samples (4 mL each for a total of 36 ml or ~2.5 tablespoon) of blood from your vein during the 3 hour test (one time before you drink the water or whey protein drink, two before the 75 gram OGTT drink and 6 after you drink the beverage). The results of the oral whey protein concentration test will allow the researchers to observe how your body reacts to whey protein and its effects on glucose metabolism.

Duration of Procedure: Blood samples will be drawn every 30 minutes for 180 minutes. The oral glucose tolerance test will take approximately 3 hours.

Potential Risks

Loss of Confidentiality: It is possible that there might be a loss of participant confidentiality in emails, other internet communications and data stored offline. To minimize this risk all data will be stored in a password-protected computer and a locked cabinet in PI's office. All data forms collected will be coded using alphabets and numbers. A single identification form linking names with their respective codes will be kept in a separate folder from the other data. Persons not associated with the study will have no access to the folders (soft or hard copies).

Fainting: During exercise testing heart rate and RPE will be monitored by a trained professional. While blood is being drawn through the catheter the participants will be sitting and also provided a snack after the necessary data has been gathered.

Muscle/Ligament Pulls: You will be asked to stretch or do a few warm up exercises prior to beginning exercise. These include walking toe touches, walking quad stretches, slow jog, jumping jacks, and high knees.

Radiation Exposure: The risks associated with a DXA scan include exposure to small amounts of radiation. DXA scanning utilizes radiation to obtain an image of your body. Everyone receives a small amount of unavoidable radiation from the environment each year. Some of this radiation comes from space and some from naturally-occurring forms of radioactive water and minerals. he DXA scan technique gives your body the equivalent of about 4 extra days' worth of this Natural radiation. The dose to patient from DXA is considered small (0.08–4.6 μSv (Njeh, 1999) The radiation dose we have discussed is what will be received from this study only and does not include any exposure you may have received or will receive from other tests. It is possible that having several of these tests may add to possible risk of injury or disease. To minimize this risk only 1 scan will take place at the preliminary visit for this research project.

Embarrassment: There is a risk of embarrassment for the body weight and waist circumference measurements. To minimize this risk, trained technicians of the same gender as the participant will perform these measurements.

Max exercise test-heart attack, stroke, or death: Max exercise test-heart attack, stroke, or death: Exercise rarely causes any problems in normal subjects, and the risks are no greater in obese individuals. However, as with any vigorous exercise test, the risk of cardiac events vary and are directly associated with the incidence of cardiovascular disease. Exercise testing may occasionally be accompanied by abnormal blood pressure, nausea, fainting, muscles soreness, joint and bone injury, and in rare instances, heart attack, stroke, or death. During exercise stress test, the risk of having a heart attack or even dying goes up slightly. However, the risk of sudden death during any exercise bout is low: 1/10,000 tests (0.01%), heart attack: 4/10,000 tests (0.04%), and as are the complications requiring hospitalization: 10/10,000 (0.2%) (American College of Sports Medicine. et al., 2010). There are no additional risks involved with completing the graded exercise test beyond that associated with exercise and hard physical exertion. Every precaution will be taken to minimize the risk by closely monitoring vital signs (blood pressure, heart rate and rhythm) throughout the exercise. These tests will be stopped if signs or symptoms of cardiac ischemia develop, or if arrhythmias occur during exercise. American College of Sports Medicine guidelines will be followed regarding physician attendance for graded maximal exercise tests (American College of Sports Medicine. et al., 2010). All members of the research study team will be certified in CPR and AED (automated external defibrillators). If the participants are at a high risk of these serious cardiovascular events, they will not be admitted into the study. Signs and symptoms for high risk include, but are not limited to ECG abnormalities; pain or discomfort in the chest, neck, jaw, arms, or other areas that may result from ischemia; shortness of breath at rest or with mild exertion; dizziness or loss of consciousness; dyspnea (abnormally uncomfortable awareness of breathing); ankle edema; palpitations or tachycardia (forceful or rapid beating of heart); known heart murmur; or unusual fatigue or shortness of breath with usual activities. If it is suspected that serious risks are occurring, emergency medical help will be called immediately. In addition, an AED is available in the exercise physiology laboratory (PH 116).

Peripheral Venous Catheter Placement and bruising: There is risk of bruising with venous catheter placement. A trained phlebotomist will place the catheter and use pressure and gauze to remove it to reduce the risk of bruising. The risks of collecting a blood sample will include the possibility of requiring more than one attempt to obtain the blood sample, local discomfort (pinch when the needle enters your skin), minor bruising or bleeding at the site (10%), or possible temporary lightheadedness, infection (<0.01%), or development of a blood clot (< 0.01%). These risks are slightly increased compared to a standard blood draw. The amount of blood being withdrawn for 8 visits over the course of the study is about ~20 tablespoons or ~300 mL and will not affect your ability to participate in normal daily activities. One single donation of blood is roughly a pint (1 pint = 450mL, American Red Cross). A trained and an experienced individual will perform the technique and your blood will be collected in a hygienic setting with sterile materials and biohazard protection measures to minimize these risks.

Hypoglycemia (low blood glucose): Some people feel sick after drinking the glucose liquid and may vomit. In our lab this has occurred twice in 20 years (over 500 OGGTs). It is possible that blood glucose levels may drop very low toward the end of the test. Symptoms of low blood glucose include weakness, hunger, sweating, and feeling nervous or restless. If levels are very low, the test will be stopped. Hypoglycemia (low blood sugar, (<4mmol/L or <72mg/dL) may result from prolonged fasting. If the participants show signs of hypoglycemia during the testing session, the test will be terminated. Signs of hypoglycemia include headache, confusion, hallucinations, bizarre behavior, tremors, cold sweat, low body temperature, blurry vision, shaking or trembling, fast heartbeat, sweating, tiredness/ weakness, convulsions, and coma. Participants will be given a glass of orange juice or carbohydrate rich food and monitored in the lab until signs of hypoglycemia subside.

Hyperglycemia (high glucose): The participant may find it difficult to drink the extremely sweet glucose (sugary) liquid. Some people feel sick after drinking the glucose liquid and may vomit. In our lab this has occurred twice in 20 years (over 500 OGGTs). It is possible that blood glucose levels may drop very low toward the end of the test. Symptoms of low blood glucose include weakness, hunger, sweating, and feeling nervous or restless. If levels are very low, the test will be stopped. Hypoglycemia (low blood sugar, (<4mmol/L or <72mg/dL) may result from prolonged fasting. If the participants show signs of hypoglycemia during the testing session, the test will be terminated. Signs of hypoglycemia include headache, confusion, hallucinations, bizarre behavior, tremors, cold sweat, low body temperature, blurry vision, shaking or trembling, fast heartbeat, sweating, tiredness/ weakness, convulsions, and coma. Participants will be given a glass of orange juice or carbohydrate rich food and monitored in the lab until signs of hypoglycemia subside. Glucose will be continuously monitored and checked throughout the test to minimize the risk of hyperglycemia.

Loss of Time: You will be allocating time to be available. To minimize unwanted loss of your time during the study schedules will be made and given to both you and the research team. These schedules will inform both parties of the day and time of day that you are scheduled to be in the lab. These schedules will also outline what you will be doing for the day. This will allow the research team to plan in advance to ensure that everything is performed and completed in the available time frame.

Infection and blood clot, and breakage of catheter: There is also a small risk of the catheter perforating (going through) the vein or not being inserted into a blood vessel. Also, participants may experience discomfort, bleeding, and/or bruising. On a rare occasion, they may feel dizzy or faint. The likelihood of these complications is very remote (about 1 in 10,000) when the procedure is carried out by trained personnel and proper equipment is used, as it will be in this case. Universal precautions 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. A registered nurse or a trained phlebotomist will obtain these blood samples.

Discomfort and Fatigue: Discomfort and fatigue may occur during the aerobic exercise testing and exercise bouts. To insure participant safety trained professionals will be on hand monitoring heart rate and RPE. Participants may stop procedures at any time.

Latex Allergy: The phlebotomist will wear non-latex gloves during all blood draws. Prior to each blood draw participants will be asked if they are allergic to latex. If participants inform the phlebotomist that they are allergic to latex another type of tourniquet will be used.
Participants will be allocating time to be available. To minimize unwanted loss of the participants' time during the study schedules will be made and given to both the research team and the participants. These schedules will inform both parties of the day, and time of day that the participants' are scheduled to be in the lab. These schedules will also outline what the participants will be doing for the day. This will allow the research team to plan in advance to ensure that everything is performed and completed in the available time frame.

How long can I expect to be in this study?
You will be asked to complete nine visits in a one-two month period. You will need to come into the lab for three preliminary visit (approximately 1-1.5 hrs), 2 exercise sessions, and 4 visits for the OGTT (approximately 4 hrs each visit).

2. Benefits

Following completion of the study, you will have the results of the study and your individual results. You will receive information regarding your glucose tolerance, body composition, bone density, and how your body reacts to whey protein. This information is supplied free of charge and will increase your awareness of your personal health

In case of a medical emergency, the fire department's Emergency Medical Team will be alerted. Telephones are available in the testing laboratories.

We will try to prevent any problem that could result from this research. Please let us know at once if there is a problem and we will help you. You should understand, however, that TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research. The investigators are prepared to advise you in case of adverse effects, which you should report to them promptly. Phone numbers where the investigators may be reached are provided in this form.

To reduce the possibility of improper disclosure, your name will be kept confidential and will not be associated with the data in any presentation of results. All subjects will be given a code number. Your data will be kept on file in a locked cabinet for a maximum of 5 years after the data are published. All data will be destroyed (shredded) after the five-year period. All data will be discarded in the recycle bin or regular trash, making sure that your name is completely obliterated on any documents or data files.

You will be given a copy of this signed and dated consent form to keep. 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 of Participant Date

*If you would like to know the results of this study tell us where you want them to be sent:

Telephone number:_____
Or
Mailing Address:
_____

Approved by the
Texas Woman's University
Institutional Review Board
Approved: October 26, 2017
Modifications Approved:
November 10, 2017

APPENDIX D

Health History Form

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 Ischemic 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

List all prescription medications that you are currently taking.

Medication/Dosage/Date Started/Reason _____
Medication/Dosage/Date Started/Reason _____
Medication/Dosage/Date Started/Reason _____
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.)

This section for office use only:

Comments:

_____

_____

_____

_____

_____

APPENDIX E

Basic Questionnaire

QUESTIONNAIRE

How much alcohol do you consume? Per night_____

Per week_____

Are you lactose intolerant? _____

Are you currently participating in a controlled diet regimen?

_____

If so, please explain

_____

_____

Have you gained or lost more than 5lbs in the last 2 months? _____

APPENDIX F

Data Collection Forms

TC DISSERTATION

Cover Sheet

Checklist:

_____ Anthropometrics
_____ DXA
_____ Diet questionnaire
_____ My fitness pal
_____ Informed consent
_____ Health history
_____ Fasting glucose x2
_____ VO2max test

Anthropometric Measures:

Name: _____ Height: _____

Age: _____ Sex: _____ Weight: _____

Fasting glucose: _____ _____

TC DISSERTATION

VO2 max Data Collection

Participant: _____ TRIAL: _____ Date: _____

Stage (1 min each)	Speed (mph)	Grade (%)	HR (bpm)	VO2 (L/min)	BP (mmHg)
0 (rest)	-----	-----			
1	3.5	0			-----
2	3.5	2			-----
3	3.5	4			
4	3.5	6			-----
5	3.5	8			-----
6	3.5	10			
7	3.5	12			-----
8	3.5	14			-----
9	3.5	16			
10	3.5	18			-----
11	3.5	20			-----
12	3.5	22			
13	3.5	24			-----
14	3.5	25			-----

- 30 sec VO2 averages

TC DISSERTATION

OGTT Data Collection

Participant: _____ TRIAL: _____ Date: _____

- Inhibitors
- Whey amount _____

- Cath time of stick _____
- Drink time (whey) _____
- Drink time (gluc) _____

	Time Points (min)	**Stopwatch Time**	**Time of Day (clock)**	**Glucose (mg/dL)**
	-30			
Glu Consmp	------			
	0			
	15			
	30			
	60			
	90			
	120			
	150			

Notes:

_____
_____
_____
_____
_____

TC DISSERTATION

Exercise Data Collection

Participant: _____ TRIAL: _____ Date: _____

Time Points (min)	HR (bpm)	VO2 (L/min)
0		
5		
10		
25		
26		
27		
28		
29		
30		
60		
61		
62		
63		
64		
65		

Notes:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

APPENDIX G

3-Day Diet Record

Day 1: 24 Hour Dietary and Sleep Record

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening/Bed time Snacks**	**Serving Size**
Number of hours of sleep last night					

Day 2: 24 Hour Dietary and Sleep Record

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening/Bed time Snacks**	**Serving Size**
Number of hours of sleep last night					

Day 3: 24 Hour Dietary and Sleep Record

Breakfast	Serving Size	Lunch	Serving Size	Dinner	Serving Size
Morning Snacks	**Serving Size**	**Afternoon Snacks**	**Serving Size**	**Evening/Bed time Snacks**	**Serving Size**
Number of hours of sleep last night					

APPENDIX H

Participant Descriptives

participant	sex	height (cm)	weight (kg)	age (yrs)	body fat %	ethnicity	waist cir (cm)	BMI	HbA1c	FPG (mg/dl)	VO2 max (L/min)	VO2 max (ml/kg/min)	70% VO2 (L
101	male	176.53	67.77	24	14.6	asian		21.7	5.1	80	2.9	42.5	2.03
102	male	177.8	64.77	21	18.4	asian		20.5	5.3	91.2	2.39	36.7	1.673
103	male	175.26	100.24	32	39	hispanic		32.6	5.4	95	2.83	29.3	1.981
104	male	177.04	66.86	35	23.3	other		21.3	5.1	81.8	2.77	42.6	1.939
105	male	182.88	97.98	26	21.8	black		29.3	5.4	95	3.66	38.3	2.562
106	male	170.18	73.48	21	19.1	asian		25.4	5.2	75.5	3.48	47.6	2.436
108	male	190.5	123.83	20	31	white		34.1	4.9	86.4	3.85	32.6	2.695
109	male	181.61	63.78	20	22.3	asian		19.6		81.6	2.57	39.7	1.799
110	male	184.15	103.42	28	36.5	white		30.5	5.2	86.75	3.24	31.4	2.268
111	male	176.53	76.2	21	25.5	asian		24.5	4.8	86	3.48	46.1	2.436
112	male	180.3	89.5	19					5.2	78	3.04	33.9	2.128
AVG		179.3436364	84.34818182	24.272727	25.15			25.95	5.16	85.20454545	3.11	38.24545455	2.177
std		5.387888775	19.89724595	5.3682569	7.966771268			5.3196178	0.195505	6.557837088	0.468380187	6.069656273	0.3278661

APPENDIX I

Glucose Data

		Glucose							
Participant	**Time (min)**	**-30**	**0**	**15**	**30**	**60**	**90**	**120**	**150**
101		82.95	86.1	99	104.5	83.45	92.65	132.5	129
102		93.6	88.65	87.85	73.75	54.2	67.55	66.45	72.8
103		106	103.5	114.5	111	86.8	102	119	114
104		90.75	90	96.85	96.6	73.95	86.1	108	100
105		81.4	79.35	88.85	69.25	50.8	51.9	51.15	72.25
106		82.15	87.45	119.5	108	78.2	88	87.05	62.6
108		94.25	99.85	109	95.5	72.35	112.5	84.6	113
109		93.7	96.6	102.5	111.5	99.65	73.65	76.7	87.35
110		104	102	111	117	85.4	86.75	106	93.25
111		77.85	92.85	94.5	89.7	89.65	108.5	91.9	110.5
112		85.5	83.05	74.25	63.4	52.2	57.35	47.9	81.15
Avg.		90.195455	91.76364	99.8	94.563636	75.15	84.26818	88.295455	94.172727
St Dev		9.219611	7.912019	13.320998	18.475634	16.42341	19.89186	26.9258	21.005456

	Glucose								
Participant	Time (min)	-30	0	15	30	60	90	120	150
101		81.85	75.4	118	133.5	100.5	71.75	77.95	60.1
102		90.15	92.75	108.5	133	98.1	72.3	61.55	63.3
103		87.5	92.6	98.1	124.5	126.5	110.5	95.3	105.5
104		83.7	80.9	104.5	124.5	129.5	116	96.95	67.8
105		88.7	87	110	143	131	96.55	99.6	60.3
106		77.25	79.6	108	158	139	112.5	66.1	62.9
108		95.95	94.45	125	150.5	128	94.65	108	90.35
109		91.5	91.5	109	168	189	118	107.5	104.5
110		97.3	99.05	107	136	137	98.7	86.8	89.6
111		81.3	81.65	112	122.5	84.8	97.3	85.3	73.55
112		83.15	85.5	98.05	110.5	83.9	69.5	90.4	83.9
Avg.		87.12273	87.3091	108.923	136.727	122.4818	96.15909	88.67727	78.34545
St Dev		6.317648	7.36022	7.81759	16.9519	30.10458	17.97033	15.29007	17.26477

	Glucose								
Participant	**Time (min)**	**-30**	**0**	**15**	**30**	**60**	**90**	**120**	**150**
101		80.9	81.75	103.5	131	124.5	80.65	63.3	72.75
102		87.35	84.75	98.15	123	83.2	67.15	61.3	64.15
103		97.55	98.7	113	139.5	152.5	139	126.5	115.5
104		90.55	90.5	109.5	130.5	90.7	81.2	76.1	58.3
105		83.5	79.3	92.45	109	89.45	77.75	90.2	72.6
106		78.05	76.8	108.5	134.5	137.5	111	77.55	47
108		99.65	98.9	126.5	155	99.85	87.6	97.65	90.3
109		97.7	98.6	135	163	174.5	135	95.9	89.8
110		98.1	99.45	98.65	111	143.5	102.5	97.2	101.5
111		86.35	82.4	127	101	97.85	95.85	81.25	64.65
112		84.55	84.45	98.05	95.65	88	81.7	87.6	45.6
Avg.		89.477273	88.69091	110.02727	126.65	116.5045	96.30909	86.7773	74.740909
St Dev		7.6826546	8.789079	14.000149	21.3874	31.36483	23.51876	18.2492	22.266138

	Glucose								
Participant	**Time (min)**	**-30**	**0**	**15**	**30**	**60**	**90**	**120**	**150**
101		79.65	82.35	90	85.75	64.05	59.65	75.15	72.65
102		88.55	83.4	90.65	68.45	64.7	77.55	80.2	74.5
103		92.3	97.6	102.5	108	98.75	114.5	131.5	122
104		90.6	88.85	92	85.35	74.15	71.3	78.2	83.2
105		87.75	87.15	85.85	73.65	80.05	67.5	94.55	81.25
106		76.25	75.9	99.55	110	95.85	101.5	115	76.4
108		94.55	97.2	121	136.5	107.5	110.5	116.5	88.6
109		86.45	91	116	137	136	90.3	75.85	94.55
110		101	102.5	107	109	105.5	101.5	133.5	111
111		91.1	89.45	82	65.05	95.55	93.7	78	80.4
112		88.75	79.75	84.05	72.75	56	52.2	81.05	79.85
Avg.		88.81364	88.65	97.32727	95.59091	88.91818	85.47273	96.3182	87.67273
St Dev		6.712641	8.139441	13.05344	26.08426	23.6874	21.0576	23.2454	15.72355

APPENDIX J

Insulin, C-peptide, Glucagon, GIP, GLP-1 Active Data

19: C-peptide 20: Ghrelin 21: GIP 22: GLP-1 33: Glucagon 36: Insulin
53: PP 54: PYY

Whey (W)

Whey					-30				
	participant	Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
	1	713.3025	229.9067	56.19964	7.776263	114.163	171.0271	60.21285	227.5833
	2	1322.608	104.668	56.81788	6	67.04081	1571.986	36.85255	201.325
	3	2053.312	67.80251	27.76415	6	68.13249	1367.849	33.394	77.97545
	4	822.5949	195.3043	58.73326	6	58.04205	674.052	78.49966	173.6989
	5	1161		43.87	3.05	63.44	1452		
	6	1073.971		97.71059	2.171755	60.73055	1003.414		
	8	2837.306		116.4001	8.678936	101.5921	1611.724		
	9	507.3048		66.70364	4.885554	37.06293	912.7258		
	10	986.371		77.44785	10.17401	55.59812	302.0949		
	11	860.86		45.09	6	28	328.12		
	12	1056		87.97	11.02	78.41	459.38		
	ave	1217.694	149.4204	66.79156	6.52332	66.56473	895.8521	52.23977	170.1457
	std	669.2109	75.82353	26.02917	2.730238	24.95958	543.4618	21.17453	65.26665

					0				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		1608.149	163.5225	232.5583	21.52314	203.2446	1344.527	74.69605	228.6307
		1860.845	85.20972	133.207	4.347669	113.1184	1926.019	55.9	202.49
		3181.035	68.63187	115.158	13.69056	142.0998	3056.764	186.096	82.69881
		1475.024	144.8572	147.495	14.83495	140.993	1080.077	117.4008	184.4458
		1509		83.56	9.65	123.47	1737		
		2385.503		233.4413	28.10023	181.2661	1807.799		
		3726.44		159.727	11.02271	174.918	3142.081		
		810.1941		173.6925	20.07287	94.0296	1481.883		
		1106.507		309.1396	14.09796	107.8168	441.729		
		2138		138.78	4.71	91.46	1357		
		1968		137.69	28.33	177.15	1787		
	ave	1978.973	115.5553	169.4953	15.4891	140.8697	1741.989	108.5232	174.5663
	std	862.7175	45.76066	64.63194	8.254163	38.47111	789.4169	57.76337	63.87471

					15				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		2734.598	117.9591	418.2723	41.1149	187.4263	3065.315	84.55471	222.2645
		2626.721	69.08073	191.5574	11.16221	116.2399	2695.84	75.48696	206.2364
		4754.501	48.08361	212.1676	17.65619	164.1155	7136.184	148.5492	97.26603
		2723.43	86.69571	200.7695	19.01931	101.7163	2298.911	116.3635	187.8794
		5147		209.56	39.83	150.9	7136		
		5323.957		370.1173	53.02238	126.4049	6485.219		
		5390.672		336.5804	10.00866	161.6653	7156.342		
		1349.981		238.7292	25.5097	100.4769	2709.458		
		1705.575		403.1999	25.70601	151.1362	984.8592		
		3685		203.91	5.26	76.25	2794		
		3932		279.74	42.58	150.3	5357		
	ave	3579.403	80.45477	278.6003	26.44267	135.1483	4347.193	106.2386	178.4116
	std	1453.733	29.56789	87.60937	15.64412	33.51319	2323.868	33.2092	55.89138

					30				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3835.183	92.01796	427.4104	26.93859	164.5602	4729.123	70.35453	228.4532
		3975.34	50.83375	226.919	9.297084	108.991	3980.512	66.43416	208.6475
		6282.916	41.3013	252.0676	19.86658	176.4632	11978.55	115.631	101.3232
		3573.754	68.29204	170.3706	11.49954	78.6259	2981.115	81.73998	179.1701
		5849		211.72	18.58	113.71	8950		
		6700.442		309.8401	27.80862	106.3306	7506.999		
		5560.465		273.7386	8.261852	137.9488	7609.161		
		2415.904		355.6914	26.87108	86.00861	5369.479		
		2615.832		458.5069	29.59471	166.9611	2372.896		
		4361		195.64	22	58.31	3463		
		6276		291.6	44.51	128.43	9657		
	ave	4676.894	63.11126	288.5004	22.29346	120.5763	6236.167	83.53992	179.3985
	std	1525.226	22.27741	93.26751	10.59197	38.45663	3112.284	22.35725	55.84999

					60				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3273.852	83.14746	293.7947	19.03498	184.77	3322.094	69.25139	251.4399
		3277.843	55.69408	299.5	11.56729	132.4779	2845.012	83.94482	203.7795
		4645.345	42.51422	171.2967	6.213368	117.6004	7542.698	66.303	88.48548
		3155.333	79.66166	174.6863	14.0867	80.54339	2267.082	104.1182	191.1824
		3732		199.97	11.13	167.26	3892		
		3809.861		247.4071	18.72273	130.4317	2263.177		
		3775.453		200.207	14.42966	134.6634	3105.84		
		3565.253		411.7023	16.20536	91.31265	7500.195		
		3170.596		525.7733	19.47074	141.4158	2091.447		
		3512		228.3	14.85	30.32	2021		
		5084		330.55	42.75	130.85	6275		
	ave	3727.412	65.25436	280.2898	17.1328	121.9677	3920.504	80.90437	183.7218
	std	617.2441	19.46147	109.6764	9.363507	42.27293	2146.893	17.29278	68.58977

					90				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		2479.218	119.434	248.9025	21.52314	143.4347	1633.425	93.99733	223.312
		3717.371	51.07052	280.7385	6.071569	105.9432	3327.606	82.72769	201.9225
		4544.646	54.52768	177.9198	5.27026	101.4465	6308.299	166.4369	77.97545
		3499.69	91.1232	176.59	9.163343	64.07495	2267.126	107.5398	189.5463
		2785		210.49	35.48	122.74	2790		
		3940.565		322.5091	24.70374	65.23613	2302.198		
		5785.314		263.2817	9.598395	112.3256	8646.835		
		2246.034		331.245	6.433867	61.40077	3795.324		
		1678.356		243.7836	14.78866	92.14308	871.9298		
		4655		262.6	7.94	102	2478		
		4090		300.06	39.53	153.84	5614		
	ave	3583.745	79.03885	256.1927	16.40936	102.235	3639.522	112.6754	173.1891
	std	1208.483	32.45932	52.35262	12.23483	30.8084	2316.083	37.24876	64.99003

					120				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3323.932	132.7029	339.2678	17.75603	117.6244	2283.049	113.6367	235.675
		3789.857	58.42617	301.24	5.613049	78.52118	3247.045	96.78458	198.7185
		5293.59	57.32042	193.9975	8.020645	65.75693	8162.108	216.626	83.76212
		4308.861	98.46586	184.5762	5.02468	47.37308	2788.544	86.45636	178.2702
		1790		169.58	19.6	84.63	1961		
		3655.987		230.1907	2.320598	49.38107	1543.002		
		4211.072		191.0755	16.61311	105.4447	3448.229		
		1497.278		308.4254	16.76376	46.57948	2105.416		
		1852.565		261.87	10.66384	55.54469	1157.773		
		3482		238.32	2.52	78	1658		
		2225		181.88	14.63	135.89	2021		
	ave	3220.922	86.72885	236.4021	10.86597	78.61323	2761.379	128.3759	174.1065
	std	1222.066	36.13527	58.85573	6.467355	30.18788	1923.475	59.89043	64.74533

Exercise (EX)

Exercise					-30				
	participant	Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
	1	580.5716	168.6477	91.73983	11.78589	99.843751	415	73.41378	215.285
	2	1150.175	75.91131	79.97388	6	74.207293	1549.4114	67.78515	211.0564
	3	1631.357	74.01668	40.14577	6	72.219132	1009.62723	38.61541	104.9156
	4	668.4558	189.2761	50.60634	3.049733	87.771395	643.290334	62.62004	182.7117
	5	1179		57.33	6.14	50.02	1499		
	6	975.8612		46.32786	5.993599	73.531795	1010.8299		
	8	2361.379		84.26784	11.30722	134.48025	1270.2405		
	9	543.6183		93.33477	6	32.880174	982.275644		
	10	777.1363		65.98751	2.817839	62.682221	228.113923		
	11	592.95		48.73	8	28.41	171.52		
	12	915.4		169.91	6.14	89.55	389.17		
	ave	1034.173	126.963	75.30489	6.657663	73.236001	833.498085	60.60859	178.4922
	std	548.72	60.63588	36.67347	2.824091	30.375397	494.390886	15.31036	51.13856

					0				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		574.5203	198.4411	61.34056	17.32352	150.2541	195.0993	103.589	283.7675
		998.4382	98.91749	58.76318	5	63.67028	1517.805	39.48422	208.691
		1656.65	75.43724	47.09632	5	57.44205	1047.109	84.31182	72.18877
		519.5065	178.1902	46.10329	3.049733	66.32108	544.658	101.4693	175.5504
		1086		61.59	3.15	51.46	1459		
		829.7686		93.00355	1.570266	62.98334	942.8212		
		2233.262		58.26614	7.834374	116.8921	1160.326		
		485.6424		87.8313	0.155444	31.29325	897.6361		
		839.7177		44.45986	5	33.6242	207.7672		
		613.26		45.05	5	33.99	225.28		
		973.76		123.34	4.82	93.33	482.09		
	ave	982.775	137.7465	66.07675	5.26394	69.2055	789.0538	82.21359	185.0494
	std	532.6964	59.74855	25.10589	4.490297	37.28182	488.0829	29.76516	87.81029

					15				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		1338.865	179.9946	242.0175	34.39563	121.9312	1156.093	203.7634	237.643
		2017.76	78.44635	125.7029	30.61899	58.77077	2038.783	70.3035	219.0256
		2020.796	69.27726	97.28356	25	54.69859	1628.974	69.77556	64.31057
		1457.711	137.3357	120.7548	15.94082	43.57651	1074.117	128.5772	187.8794
		2774		170.87	13.28	47.06	2846		
		2134.02		205.9301	29.97431	45.0273	1771.896		
		4373.005		358.24	36.54289	99.88972	5078.618		
		722.2706		181.4661	8.684925	12.58568	1281.223		
		1055.189		114.3394	6.16671	50.75963	375.7428		
		2667		233.5	46.49	15.47	1961		
		2062		202.62	35.21	72.92	1888		
	ave	2056.602	116.2635	186.6113	25.66403	56.60813	1918.222	118.1049	177.2146
	std	995.9352	52.10097	75.4176	12.95897	32.36423	1226.768	63.42381	78.01882

					30				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		1804.987	153.9354	264.4573	26.40971	147.5398	1567.356	121.8208	275.38
		3435.475	56.35085	152.6625	11.79686	46.26691	2717.119	44.94105	214.513
		4228.103	59.74642	154.3797	7.972549	45.13728	5076.381	77.26431	83.76212
		2594.171	104.9251	147.5966	10.96285	35.7222	1645.476	87.41537	186.1799
		5168		227.02	8.51	32.18	5208		
		4541.193		246.1789	22.3528	40.42426	3075.772		
		6241.857		300.2265	17.83329	83.03922	8630.553		
		1329.624		263.4177	9.020536	57	1839.229		
		1929.375		242.1969	11.26632	24.73258	928.0043		
		4358		173.9	19.86	57	2594		
		3985		218.56	26.75	62.19	3958		
	ave	3601.435	93.73944	217.3269	15.70317	57.38475	3385.445	82.86037	189.9587
	std	1537.777	45.83343	52.5894	7.18522	33.97271	2234.986	31.66412	79.98272

					60				
		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3175.376	134.4854	209.2443	10.11079	91.37121	1803.503	83.59627	230.5957
		4878.077	53.3992	181.7365	9.426228	45.13728	2986.816	50.8102	209.7994
		5219.595	48.26959	135.7035	9	49	7372.248	66.90245	77.97545
		4625.129	90.01048	178.8897	7.256027	37.67729	3106.692	64.9802	184.4458
		6204		189.32	8.28	26.44	5591		
		5113.673		235.995	16.17122	35.13943	2806.304		
		6689.794		196.9408	4.024832	71.42611	6801.439		
		2196.216		274.9033	1.216078	49	2496.607		
		2725.286		226.0593	7.337487	20.34041	1611.089		
		3136		220.77	8.62	49	1350		
		3619		253.76	23.68	64.68	2633		
	ave	4325.65	81.54117	209.3929	9.556605	49.01925	3505.336	66.57228	175.7041
	std	1458.278	39.89064	38.80512	5.972824	20.51749	2097.429	13.42762	67.83038

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3043.797	151.3554	212.5485	19.47417	76.09887	1658.584	82.67671	213.2616
		4507.047	54.58663	205.408	13.03507	53.28383	2689.711	47.38576	213.9491
		5192.816	56.46193	148.415	8	20.76658	7136.193	58.50855	83.76212
		5519.405	103.4986	191.9708	4.16113	31.97855	3746.927	56.91828	180.9409
		4828		214.45	6.03	24.41	3918		
		4539.103		206.3825	7.355053	31.48187	1988.964		
		4027.556		140.1811	2.332471	67.54527	2337.417		
		1874.832		194.5944	8	42	1925.059		
		3027.085		158.0167	8	15.91036	1125.718		
		3627		224.14	4.5	42	1492		
		1975		221.76	10.11	59.62	914.85		
	ave	3832.876	91.47564	192.5334	8.272536	42.28139	2630.311	61.37232	172.9784
	std	1235.93	45.88716	29.95803	4.733373	19.75907	1779.117	15.0282	61.43907

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		2670.213	166.4205	231.5007	13.78882	108.4055	1337.852	96.8623	246.5949
		3749.116	67.42274	212.1031	8.930271	53.28383	2228.088	64.93749	209.8631
		4740.153	64.05802	128.0886	9	28.7527	6294.907	67.21407	82.47015
		4788.633	118.3773	178.5439	5.686742	29.79454	2668.468	49.50915	180.041
		3826		200.8	18.25	30.32	2724		
		2257.52		112.9971	9	37.04727	1112.026		
		4120.626		146.7237	0.684998	54.66508	2985.066		
		1678.021		210.4947	5.708763	46	1890.919		
		2152.89		193.4427	9	12.58568	638.9732		
		3035		235.88	7.6	46	1439		
		1969		236	12.27	60.91	1271		
	ave	3180.652	104.0696	189.6886	9.0836	46.16042	2235.482	69.63075	179.7423
	std	1120.583	48.42953	43.29647	4.604622	24.99445	1541.657	19.7847	70.32896

Exercise/Whey (EXW)

Exercise/Whey									
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participant	Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
1	887.327888	221.808564	188.307564	33.4549536	120.102206	415.240437	118.324709	229.299286
2	1127.53365	90.3591154	42.6471357	4.76406577	97.4373058	1558.58018	47.6884767	225.362296
3	1824.38441	68.1169471	29.265703	2.04005288	110.201502	1278.64205	29.9186386	56.4323577
4	831.510502	130.475151	39.6442727	7.40786237	60.5647358	682.048361	62.6181006	189.546314
5	1141		55.55	6.48	45.54	1384		
6	1001.07529		102.813571	2.99957921	97.5411371	1097.87304		
8	2775.07002		86.5822913	4.97875703	116.87793	1604.01466		
9	397.330626		74.3732316	1.9579822	24.4868568	912.56296		
10	878.387718		74.83647	6	54.4111464	293.172165		
11	856.4		58.41	6	26.44	377.15		
12	1191		148.46	11.13	89.55	493.32		
ave	1173.7291	127.689944	81.8991127	7.92847755	76.6502563	917.873077	64.6374813	175.160063
std	633.310037	67.8444413	48.6430426	8.85994726	35.5390173	493.181859	38.2058596	81.1470926

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		1427.71075	191.03499	232.466622	32.1946282	260.679911	1053.2041	107.310892	239.990779
		1726.36681	64.7533491	166.345399	17.8452191	170.935016	1844.18325	92.14327	235.09598
		3100.41003	67.516101	192.335019	16.592782	217.036911	3430.9625	180.440557	83.7621186
		1531.73062	94.5319097	146.563559	14.7115351	164.733821	1121.1352	146.674125	191.245853
		1693		109.75	18.95	114.1	1993		
		1665.01127		227.440107	21.3335873	172.336599	1365.1926		
		3872.70416		128.024943	11.6214244	198.025393	3724.24079		
		605.394704		208.179308	27.7176173	94.0807968	1153.38525		
		1483.80586		182.202249	18.5223786	121.708946	726.331019		
		2031		192.94	6.36	121.28	1328		
		2691		185.72	35.89	164.01	1966		
	ave	1984.37584	104.459088	179.269746	20.1581065	163.538854	1791.42134	131.642211	187.523682
	std	907.369436	59.2600645	38.8453278	8.75226956	49.4223389	971.080206	39.8305429	72.5631595

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		2442.1195	103.851317	346.108631	36.2548238	147.124939	2121.17224	113.08053	197.040843
		3208.55149	48.1230038	255.211349	25.4481597	148.922246	3183.28672	96.4032154	221.218359
		3539.18093	47.9506708	218.758001	14.9610654	157.016323	4993.72752	91.6039044	72.1887742
		2184.96424	81.8759751	245.880389	17.3642869	120.11391	2115.35796	158.948294	234.413257
		2065		126.96	31.69	118.3	2344		
		4104.75688		333.790461	64.5652918	114.841113	4148.28014		
		6917.80526		330.923668	16.2419314	191.476697	10550.4795		
		1059.79606		394.755696	42.7496437	89.1922725	2242.66289		
		2123.14974		266.187229	26.3123743	121.241146	1497.75361		
		3581		287.27	18.58	85.64	2969		
		4573		383.02	54.73	148.5	5625		
	ave	3254.48401	70.4502416	289.896857	31.7179615	131.124422	3799.15642	115.008986	181.215308
	std	1593.31099	27.3916984	78.5847593	16.4871126	31.0855878	2588.79946	30.7048004	74.3135217

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3251.4352	117.396616	380.300481	45.8078898	212.671366	2837.62875	90.1368405	215.664872
		4344.72168	36.5628889	259.245205	18.6451345	151.946068	3991.4892	90.6266482	228.601192
		5151.41156	43.1447858	245.260018	14.0191208	157.157397	9061.9026	86.1122777	88.4854841
		3365.90076	46.7817715	197.232011	18.4337795	87.9593747	2734.25462	89.6129285	225.80806
		1874		120.09	37.54	131.19	2184		
		5856.1399		316.829494	29.6018443	81.1415254	5529.94291		
		8924.80096		420.967802	13.9610554	182.366442	17394.0699		
		1836.93488		393.096883	47.9715865	53.7041115	4062.54782		
		3054.7062		351.969205	22.7371978	129.75547	2712.63322		
		3872		251.09	8.57	78.41	2749		
		6142		341.56	54.24	127.03	7588		
	ave	4334.00465	60.9715155	297.967373	28.3206917	126.666523	5531.40628	89.1221737	189.639902
	std	2087.47784	37.8537278	91.8654979	15.7282359	48.2919999	4515.73419	2.04884577	67.664942

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3725.6463	87.3950282	500.052896	37.6474781	154.40349	3409.78351	73.9297815	215.627061
		3048.69252	40.0991373	304.016978	23.3786085	178.640942	2689.43363	94.6769063	220.121981
		5160.10284	31.7553791	222.766803	12.0249826	142.136817	9162.15803	77.7681792	83.7621186
		3409.55626	53.018531	236.109445	20.7308208	86.9048251	2737.94449	138.564635	204.10914
		3127		191.51	27.35	126.68	3993		
		5263.28654		270.597827	31.8235998	79.2957955	3673.24988		
		7316.92429		322.504123	11.2115775	150.699768	11148.0525		
		3515.84814		503.461572	29.9614608	63.45036	8190.50315		
		3530.8714		361.626193	14.0481507	103.793453	2548.46043		
		3448		308.02	17.49	75.16	2132		
		4844		400.2	44.01	123.83	4754		
	ave	4217.26621	53.0670189	329.169622	24.5160617	116.817768	4948.96233	96.2348756	180.905075
	std	1303.34189	24.5001124	104.481814	10.7290835	37.7174175	3087.51467	29.6241564	65.1121204

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		2676.12209	127.530919	446.238268	42.0974581	183.24633	1726.08653	99.7911441	242.072593
		3842.19244	43.562951	339.627968	17.2374882	141.13141	3586.07048	114.863072	249.199792
		5261.0609	40.478195	277.447973	19.4274672	166.047413	8031.0724	175.956295	88.4854841
		3759.00087	53.7723676	209.311727	7.25602737	56.8466728	2649.99577	104.118249	191.245853
		1802		168.03	27.89	141.7	1879		
		5303.82333		257.37059	43.0200298	55.7448137	3104.748		
		7471.64868		367.125719	10.2528188	102.162611	11789.7906		
		2737.63524		386.173479	9.846372	66.3849604	4231.43386		
		2700.86451		228.313437	20.0458979	69.9177056	1082.41216		
		4066		248.09	8.28	116	2349		
		2777		231.05	39.26	185.54	1551		
	ave	3854.30437	66.3361081	287.161742	22.2375963	116.792902	3816.41907	123.68219	192.750931
	std	1629.64163	41.1902124	85.8071828	13.7919405	50.0542637	3260.5765	35.4207511	74.1454629

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3422.69741	130.300142	400.709524	29.8527841	134.357956	2117.67943	103.095592	239.46378
		4105.44351	43.8379019	345.679296	20.1307787	100.03709	3069.62037	92.6405684	228.453152
		6847.49589	44.0149545	315.965814	10.7419331	126.434758	11777.7761	170.127799	97.2660336
		3729.98788	64.3091355	208.415234	6.87388896	47.3730785	2149.67542	103.179247	189.546314
		2771		160.79	22.44	85.64	2687		
		5504.62687		236.252718	20.9343453	43.580221	3143.5716		
		7958.02004		214.158355	8.174545	84.7277801	10279.2299		
		1335.2559		448.250169	57.9612127	112.844535	1893.12768		
		3285.05704		347.907385	17.1675648	59.1853936	1877.01971		
		3321		261.52	5.37	33.99	1446		
		4311		360.94	37.9	124.19	3861		
	ave	4235.59859	70.6155335	300.053499	21.5951866	86.5782557	4027.42729	117.260802	188.68232
	std	1884.80754	40.9335051	90.0839629	15.6757822	36.143072	3545.31134	35.590348	64.5965934

Control (R)

Control *R					-30				
	participant	Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
	1	880.92278	232.426311	98.66545753	18.5504634	151.960701	559.789743	80.4949387	248.8644
	2	801.11092	92.6980482	72.56501576	3.69532797	89.4973898	1480.80247	84.6735425	213.3804
	3	1931.916	70.0998656	59.78292338	2.44814515	98.1023323	1334.97219	43.673861	104.9156
	4	857.37303	170.825525	48.22785656	5.02468034	59.3693569	689.297741	55.6859491	189.5463
	5	1525		51.28	22.28	74.05	1682		
	6	1069.8519		71.14724228	2.74300455	84.748073	1062.02371		
	8	2879.3614		78.93747636	11.5456723	104.876554	1576.70014		
	9	423.00743		57.7265101	3.22951605	22.7301717	882.241698		
	10	1268.083		71.22700441	6	52.7431433	428.613872		
	11	939.17		41.89	6	28.41	328.12		
	12	1015		157.76	4.39	92.4	377.15		
	ave	1235.5269	141.512438	73.56449876	7.80970998	78.080702	945.610142	66.1320729	189.1767
	std	674.80082	74.4024856	32.16007979	6.76044079	36.7371572	508.175707	19.6940837	61.23319

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		767.217816	237.439094	67.41250196	8.35020468	122.42474	195.09933	70.912749	208.6475
		791.014637	104.563518	46.96246968	7.04886173	107.74719	1515.4558	105.3287	245.9228
		1889.56738	75.1374583	53.95508349	2	75.615155	1345.4166	61.610759	95.30542
		659.657668	190.10999	38.56847124	2.24092	45.133784	599.31195	57.523055	175.5504
		1467		39.9	22	68.3	1639		
		897.390162		63.4765051	0.75878874	67.366987	980.9326		
		2950.66659		69.76279199	6.15969521	94.029603	1574.5661		
		407.142081		65.45640209	6	20.727758	819.51646		
		1195.84163		46.67036987	0.47417383	38.343641	375.74277		
		829.5		44.1	6	19	303.01		
		1166		133.87	6.7	85.64	459.38		
	ave	1183.72709	151.812515	60.92132686	6.15751311	67.66626	891.58469	73.843815	181.3565
	std	715.114817	75.0780475	26.72055951	5.92454128	34.096753	548.0217	21.724823	64.16671

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		1324.456	194.1541	213.477	24.82448	135.3471	1095.11	90.38652	249.6577
		1513.101	90.18717	133.5629	29.274	124.4569	1910.652	104.6855	270.7808
		3426.642	63.2927	216.8936	10.81597	61.03124	3598.271	66.4113	100.8584
		1492.782	145.0305	137.2574	17.01828	41.12606	1049.902	187.4447	189.4483
		2953		117.39	8.06	59.62	3154		
		2651.609		183.4174	23.62019	49.38107	1954.805		
		4834.126		283.7333	15.7179	73.64437	4803.144		
		639.296		195.1104	20.79647	72	1317.458		
		1197.373		68.50706	20	40.83799	375.9005		
		3430		242.11	17.6	72	2426		
		2654		219.95	36.11	64.68	2143		
	ave	2374.217	123.1661	182.8554	20.34884	72.19316	2166.204	112.232	202.6863
	std	1256.145	58.28032	62.59428	8.006955	30.87058	1283.626	52.56952	76.13001

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		2556.949	164.9791	262.9526	20.38871	90.96199	2198.489	70.28167	216.6307
		3965.969	57.31731	174.7273	18.73104	46.26691	2940.094	70.33584	219.5983
		5273.546	48.70684	191.3622	11.79467	56.0273	7821.957	63.97608	108.5079
		2535.177	105.3057	146.0583	10.14346	35.88873	1612.247	115.8574	187.8794
		5543		125.23	6.25	40.8	7051		
		3651.76		196.7866	9.41976	29.35822	2083.78		
		7993.696		307.8674	11.8588	74.3555	10481.47		
		1184.274		226.8539	14.23978	12.58568	1762.487		
		1672.769		209.836	8.43141	35.70174	814.1547		
		4045		240.84	3.05	49	2455		
		5761		229.32	26.4	68.3	7906		
	ave	4016.649	94.07725	210.1667	12.7916	49.02237	4284.244	80.11275	183.1541
	std	2018.597	53.42572	51.99112	6.761858	22.27294	3340.341	24.01606	51.77915

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		4350.614	133.9792	283.3799	15.8113	99.48891	3770.38	64.632	223.2303
		5450.989	62.4441	197.1561	14.12641	50.24634	3696.823	59.64554	228.6307
		6120.755	49.0753	151.5422	7	31.64099	9954.432	68.84312	93.20885
		3554.344	109.7214	137.3862	4.15682	31.74963	1837.017	95.31419	177.1175
		5667		110.37	7	32.18	6080		
		5527.514		196.6738	9.312727	24.7893	3184.788		
		6299.942		202.228	6.437434	61.65868	5576.618		
		1826.804		218.9534	6.879622	47	2507.849		
		3528.842		245.5026	5.805229	18.31906	2432.051		
		3571		208.3	3.97	47	1405		
		2345		268.5	27.35	75.16	1350		
	ave	4385.71	88.80499	201.8175	9.804504	47.20299	3799.542	72.10871	180.5468
	std	1534.854	39.79761	53.32263	6.931099	24.0355	2564.266	15.92053	62.64616

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		3467.536	135.1447	304.1935	19.33387	98.80162	2286.436	74.34032	228.6307
		5704.888	57.11099	214.9921	18.05484	54.69859	3145.811	68.9823	226.5509
		5948.937	46.93849	176.5382	10	20.76658	10094.61	83.66439	83.76212
		3714.842	112.7063	157.2517	4.684324	35.7222	1846.942	109.3557	194.5488
		3545		126.08	10	37.48	3104		
		5171.975		203.8058	4.557387	31.48187	2655.713		
		4353.195		222.0971	7.57676	64.34882	3490.24		
		2190.055		231.5748	3.120854	46	2639.535		
		3373.243		191.909	5	9.261006	1541.718		
		3685		198.32	5.59	46	1778		
		2714		263.33	28.03	63.44	1810		
	ave	3988.061	87.97513	208.1902	10.54073	46.18188	3126.637	84.08568	183.3731
	std	1191.441	42.713	48.74826	7.933394	24.24794	2399.014	17.90564	68.2149

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		Analyte 19	Analyte 20	Analyte 21	Analyte 22	Analyte 33	Analyte 36	Analyte 53	Analyte 54
		2188.713	180.4915	236.2924	17.64496	135.9542	1247.034	104.2381	259.4266
		4710.71	69.41171	202.0418	11.22067	54.69859	2655.807	80.51168	225.5034
		5962.003	52.82421	149.5717	7	57	9549.45	81.97492	83.76212
		3815.819	142.5061	156.975	6.483272	29.79454	1969.14	84.10075	189.5463
		4728		113.46	7	26.44	4805		
		3263.88		119.4051	1.819624	40.20323	1157.335		
		4527.253		143.5057	4.122687	56.50565	3057.023		
		1502.504		207.6507	3.324664	57	1644.268		
		3404.631		197.3287	7	57	1453.851		
		2771		180.51	7	57	1020		
		3685		234.71	10.68	55.64	3515		
	ave	3687.229	111.3084	176.4955	7.572352	57.02148	2915.81	87.70636	189.5596
	std	1270.615	60.37476	42.97099	4.358423	28.63099	2492.835	11.11922	76.0843

APPENDIX K

MAGPIX Procedures

Thaw samples 30 minutes

Bring kits to room temperature: 30 minutes

Sonicate bead vials 30 seconds

vortex beads 1 minutes

add 150 µL of beads to mixing bottle

add appropriate amount of bead diluent to bring mixing bottle to 3 mL, vortex 30 seconds

add 250 µL D.I. water to QC1 and 250 µL to QC2, invert, vortex, let sit 5 minutes

mix 10x wash buffer by adding 60 mL of buffer with 540 mL D.I. water

add 1 mL of D.I. water to serum matrix, vortex, let sit 10 minutes

add 250 µL D.I. water to standard #7, invert and vortex 10 seconds, let sit 5 minutes

add 200 µL assay buffer to 6 prelabeled micrfuge tubes

complete serial dilution starting with #7, adding 100 µL of 7 to 6, mix, 100 µL of 6 to 5, and so on.

0 pg/mL standard (background) is assay buffer

add 200 µL of assay buffer to each well on plate

seal, mix on plate shaker for 10 minutes at room temp. (300 rpm

decant 3x in sink and tap on absorbent towel till dry

add 25 µL of assay buffer to background wells

add 25 µL of standards to appropriate wells

add 25 µL of controls to appropriate wells

add 25 µL of assay buffer to sample wells

add 25 µL of matrix to background, standards, controls

add 25 µL samples to appropriate wells

vortex mixing bottle 30 seconds

add 25 µL of beads to each well, revortex beads every 3 columns

seal plate, wrap with foil, uncubate and agitate in refrigerator 16 hr (3.5 speed on shaker)

unwrap plate, place plate on magnet for 60 seconds, decant in sink 2x, no tapping

remove magnet from plate, place plate on magnet plate washer for 60 seconds

wash plate 3x (whey dose protocol)

add 50 µL of detection antibodies to each well

seal plate, cover with foil, incubate and agitate for 1 hr at room temp (300 rpm)

do not aspirate or decant

add 50 µL of streptavidin-phycoerythrin to each well

seal plate, cover with foil, incubate and agitate for 30 minutes at room temp. (300 rpm)

unwrap plate, place plate on magnet for 60 seconds, decant once in sink, no tapping

remove magnet, place plate on washer magnet for 60 seconds

wash plate 3x (whey dose protocol)

no decant

add 100 µL of drive fluid to all wells

shake plate for 5 minutes, 80 rpm at room temp

run plate

APPENDIX L

SPSS Output

Glucose

Descriptive Statistics

	Mean	Std. Deviation	N
R	15523.8068	2393.29903	11
W	13149.2727	2034.50636	11
EX	15870.8864	1869.36321	11
EX_W	13614.0341	2305.81475	11

Tests of Within-Subjects Effects

Measure: glucose

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
exercise	Sphericity Assumed	1812485.570	1	1812485.570	1.062	.327	.096	1.062	.154
	Greenhouse-Geisser	1812485.570	1.000	1812485.570	1.062	.327	.096	1.062	.154
	Huynh-Feldt	1812485.570	1.000	1812485.570	1.062	.327	.096	1.062	.154
	Lower-bound	1812485.570	1.000	1812485.570	1.062	.327	.096	1.062	.154
Error(exercise)	Sphericity Assumed	17068878.52	10	1706887.852					
	Greenhouse-Geisser	17068878.52	10.000	1706887.852					
	Huynh-Feldt	17068878.52	10.000	1706887.852					
	Lower-bound	17068878.52	10.000	1706887.852					
whey	Sphericity Assumed	58986784.04	1	58986784.04	38.492	.000	.794	38.492	1.000
	Greenhouse-Geisser	58986784.04	1.000	58986784.04	38.492	.000	.794	38.492	1.000
	Huynh-Feldt	58986784.04	1.000	58986784.04	38.492	.000	.794	38.492	1.000
	Lower-bound	58986784.04	1.000	58986784.04	38.492	.000	.794	38.492	1.000
Error(whey)	Sphericity Assumed	15324553.64	10	1532455.364					
	Greenhouse-Geisser	15324553.64	10.000	1532455.364					
	Huynh-Feldt	15324553.64	10.000	1532455.364					
	Lower-bound	15324553.64	10.000	1532455.364					
exercise * whey	Sphericity Assumed	38084.778	1	38084.778	.038	.850	.004	.038	.054
	Greenhouse-Geisser	38084.778	1.000	38084.778	.038	.850	.004	.038	.054
	Huynh-Feldt	38084.778	1.000	38084.778	.038	.850	.004	.038	.054
	Lower-bound	38084.778	1.000	38084.778	.038	.850	.004	.038	.054
Error(exercise*whey)	Sphericity Assumed	10105850.10	10	1010585.010					
	Greenhouse-Geisser	10105850.10	10.000	1010585.010					
	Huynh-Feldt	10105850.10	10.000	1010585.010					
	Lower-bound	10105850.10	10.000	1010585.010					

Pairwise Comparisons

Measure: glucose

exercise	(I) whey	(J) whey	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	1	2	2374.534*	518.779	.001	1218.623	3530.445
	2	1	-2374.534*	518.779	.001	-3530.445	-1218.623
2	1	2	2256.852*	439.590	.000	1277.384	3236.320
	2	1	-2256.852*	439.590	.000	-3236.320	-1277.384

Pairwise Comparisons

Measure: glucose

whey	(I) exercise	(J) exercise	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
1	1	2	-347.080	476.771	.483	-1409.392	715.233
	2	1	347.080	476.771	.483	-715.233	1409.392
2	1	2	-464.761	516.503	.389	-1615.601	686.078
	2	1	464.761	516.503	.389	-686.078	1615.601

Insulin, C-peptide, GIP, GLP-1, and Glucagon

Descriptive Statistics

	Mean	Std. Deviation	N
R_INSULIN	414655.8630	259544.34170	11
W_INSULIN	526375.6579	241080.34586	11
EX_INSULIN	352803.6725	190882.31297	11
EX_W_INSULIN	558891.9153	385710.19695	11
R_CPEP	477676.1626	159843.89848	11
W_CPEP	489361.9995	131634.87748	11
EX_CPEP	441971.8998	141881.57109	11
EX_W_CPEP	514267.8538	203685.44897	11
R_GIP	24893.4435	5649.92934	11
W_GIP	35126.2892	8802.86220	11
EX_GIP	25208.4433	4037.63091	11
EX_W_GIP	39305.5181	10166.11027	11
R_GLP1	1572.6757	763.40110	11
W_GLP1	2513.8520	1164.24768	11
EX_GLP1	1627.7136	619.07679	11
EX_W_GLP1	3412.0071	1616.40021	11
R_GLUCAGON	8536.4704	3598.07764	11
W_GLUCAGON	16813.5105	4301.90824	11

EX_GLUCAGON	8227.3681	3733.60900	11
EX_W_GLUCAGON	17953.2351	5398.57696	11

Tests of Within-Subjects Contrasts

Source	Measure	exercise	whey	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
exercise	insulin	Linear		2366641677	1	2366641677	.131	.725	.013	.131	.062
	cpeptide	Linear		320665467.0	1	320665467.0	.113	.743	.011	.113	.061
	gip	Linear		55544753.79	1	55544753.79	5.837	.036	.369	5.837	.587
	glp1	Linear		2498586.696	1	2498586.696	14.824	.003	.597	14.824	.933
	glucagon	Linear		1897316.898	1	1897316.898	1.030	.334	.093	1.030	.151
Error(exercise)	insulin	Linear		1.810E+11	10	1.810E+10					
	cpeptide	Linear		2.828E+10	10	2828339298					
	gip	Linear		95163107.72	10	9516310.772					
	glp1	Linear		1685489.550	10	168548.955					
	glucagon	Linear		18419382.71	10	1841938.271					
whey	insulin		Linear	2.778E+11	1	2.778E+11	10.841	.008	.520	10.841	.842
	cpeptide		Linear	1.940E+10	1	1.940E+10	1.998	.188	.167	1.998	.249
	gip		Linear	1627848827	1	1627848827	43.092	.000	.812	43.092	1.000
	glp1		Linear	20427509.41	1	20427509.41	19.193	.001	.657	19.193	.976
	glucagon		Linear	891287821.4	1	891287821.4	117.430	.000	.922	117.430	1.000
Error(whey)	insulin		Linear	2.562E+11	10	2.562E+10					
	cpeptide		Linear	9.709E+10	10	9709347169					
	gip		Linear	377757954.7	10	37775795.47					
	glp1		Linear	10643233.29	10	1064323.329					
	glucagon		Linear	75899437.89	10	7589943.789					
exercise * whey	insulin	Linear	Linear	2.449E+10	1	2.449E+10	1.403	.264	.123	1.403	.189
	cpeptide	Linear	Linear	1.010E+10	1	1.010E+10	1.842	.205	.156	1.842	.233
	gip	Linear	Linear	41063733.69	1	41063733.69	1.633	.230	.140	1.633	.212
	glp1	Linear	Linear	1954828.167	1	1954828.167	6.346	.030	.388	6.346	.623
	glucagon	Linear	Linear	5772523.804	1	5772523.804	1.961	.192	.164	1.961	.245
Error(exercise*whey)	insulin	Linear	Linear	1.745E+11	10	1.745E+10					
	cpeptide	Linear	Linear	5.485E+10	10	5484965192					
	gip	Linear	Linear	251415014.9	10	25141501.49					
	glp1	Linear	Linear	3080224.357	10	308022.436					
	glucagon	Linear	Linear	29434590.89	10	2943459.089					

Pairwise Comparisons

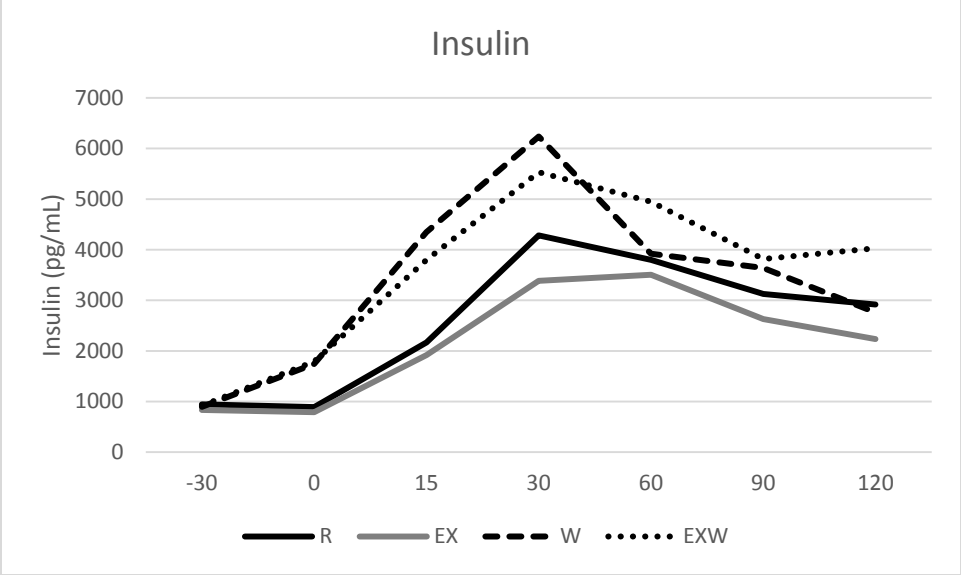
Measure	exercise	(I) whey	(J) whey	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
							Lower Bound	Upper Bound
insulin	1	1	2	-111719.795*	37582.493	.014	-195458.807	-27980.783
		2	1	111719.795*	37582.493	.014	27980.783	195458.807
	2	1	2	-206088.243*	80116.127	.028	-384598.097	-27578.388
		2	1	206088.243*	80116.127	.028	27578.388	384598.097
cpeptide	1	1	2	-11685.837	25069.242	.651	-67543.589	44171.916
		2	1	11685.837	25069.242	.651	-44171.916	67543.589
	2	1	2	-72295.954	46196.703	.149	-175228.622	30636.714
		2	1	72295.954	46196.703	.149	-30636.714	175228.622
gip	1	1	2	-10232.846*	2243.291	.001	-15231.210	-5234.481
		2	1	10232.846*	2243.291	.001	5234.481	15231.210
	2	1	2	-14097.075*	2531.235	.000	-19737.019	-8457.131
		2	1	14097.075*	2531.235	.000	8457.131	19737.019
glp1	1	1	2	-941.176*	277.927	.007	-1560.437	-321.915
		2	1	941.176*	277.927	.007	321.915	1560.437
	2	1	2	-1784.293*	415.059	.002	-2709.102	-859.485
		2	1	1784.293*	415.059	.002	859.485	2709.102
glucagon	1	1	2	-8277.040*	853.806	.000	-10179.439	-6374.641
		2	1	8277.040*	853.806	.000	6374.641	10179.439
	2	1	2	-9725.867*	1089.118	.000	-12152.574	-7299.160
		2	1	9725.867*	1089.118	.000	7299.160	12152.574

Pairwise Comparisons

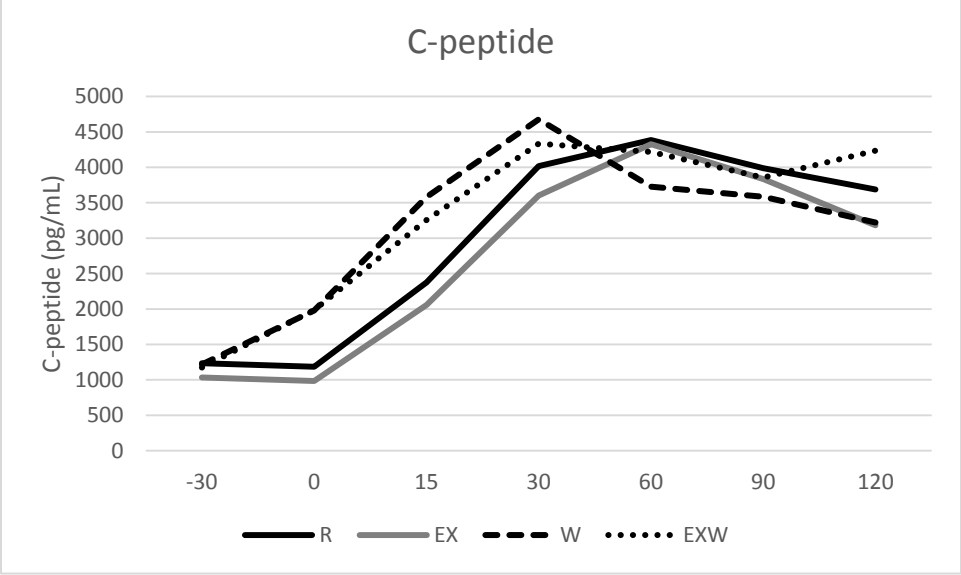
Measure	whey	(I) exercise	(J) exercise	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
							Lower Bound	Upper Bound
insulin	1	1	2	61852.191	30861.790	.073	-6912.162	130616.543
		2	1	-61852.191	30861.790	.073	-130616.543	6912.162
	2	1	2	-32516.257	74236.894	.671	-197926.366	132893.851
		2	1	32516.257	74236.894	.671	-132893.851	197926.366
cpeptide	1	1	2	35704.263	17759.216	.072	-3865.735	75274.261
		2	1	-35704.263	17759.216	.072	-75274.261	3865.735
	2	1	2	-24905.854	34584.970	.488	-101965.970	52154.262
		2	1	24905.854	34584.970	.488	-52154.262	101965.970
gip	1	1	2	-315.000	1257.822	.807	-3117.603	2487.603
		2	1	315.000	1257.822	.807	-2487.603	3117.603
	2	1	2	-4179.229	2172.396	.083	-9019.628	661.171
		2	1	4179.229	2172.396	.083	-661.171	9019.628
glp1	1	1	2	-55.038	156.629	.733	-404.028	293.953
		2	1	55.038	156.629	.733	-293.953	404.028
	2	1	2	-898.155*	249.232	.005	-1453.480	-342.831
		2	1	898.155*	249.232	.005	342.831	1453.480
glucagon	1	1	2	309.102	357.516	.408	-487.493	1105.698
		2	1	-309.102	357.516	.408	-1105.698	487.493
	2	1	2	-1139.725	861.542	.215	-3059.360	779.910
		2	1	1139.725	861.542	.215	-779.910	3059.360

APPENDIX M

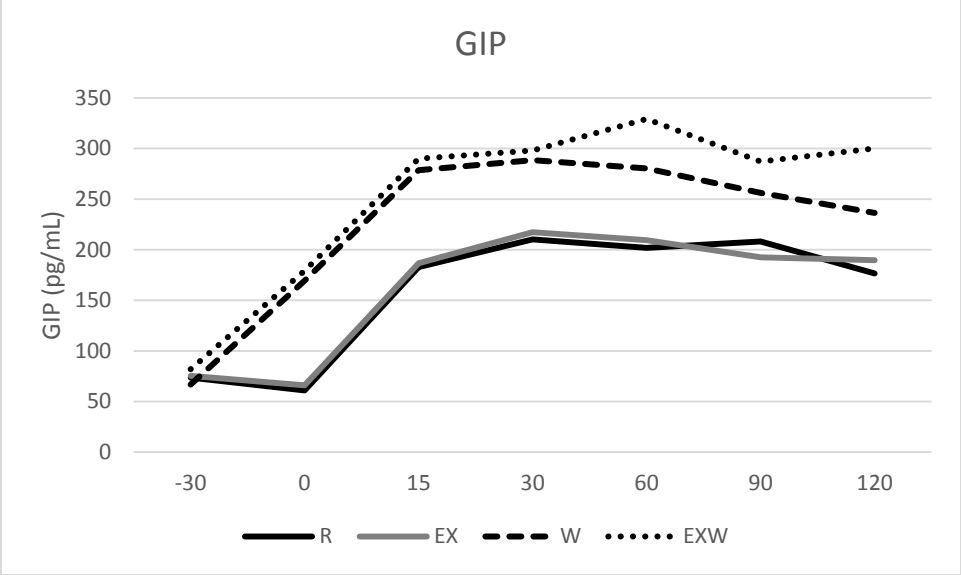
Timepoint Data for OGTT



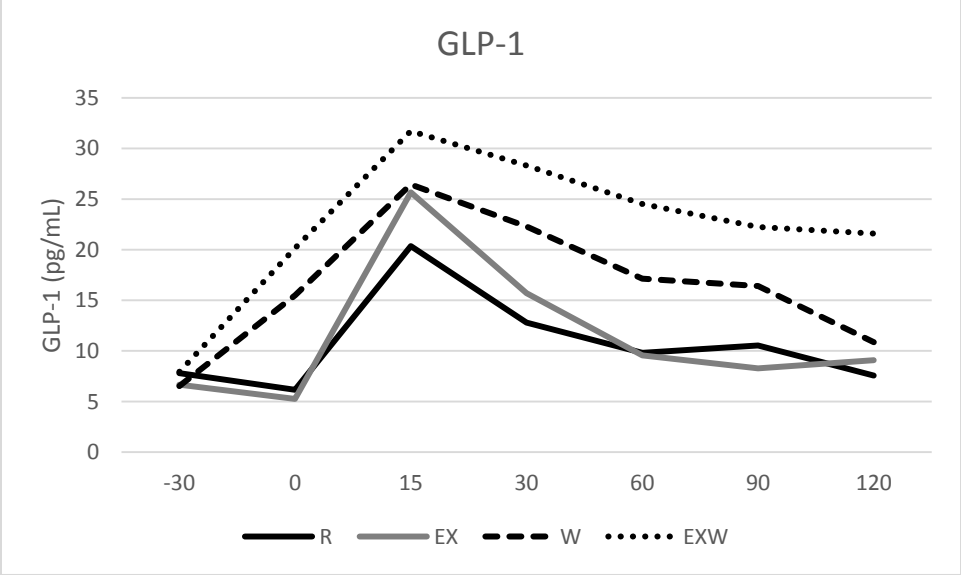
Average insulin responses during OGTT. Values are represented as mean insulin pg/mL.



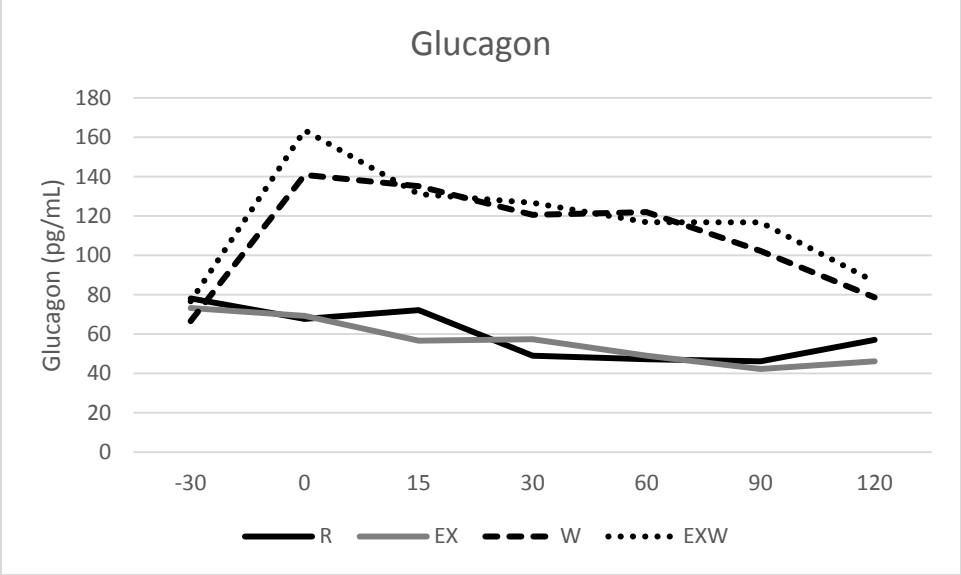
C-peptide responses during OGTT. Values are represented as mean C-peptide pg/mL.



Average GIP responses during OGTT. Values are represented as mean GIP pg/mL.



Average GLP-1 responses during OGTT. Values are represented as mean GLP-1 pg/mL.



Average GLP-1 responses during OGTT. Values are represented as mean Glucagon pg/mL.

APPENDIX N

Dietary Data

TC Diss Diet Logs Data (Day prior to OGTT)				
	R/R	EX/R	R/W	EX/W
1	1,255 kcal for day, 61 g CHO for dinner	2,080 kcal for the day, 163 g CHO for dinner	1,780 kcal for the day, 52 g CHO for dinner	3,127 kcal for the day, 155 g CHO for dinner
2	1,533 kcal for the day, 66 g CHO for dinner	2,176 kcal for the day, 66 g CHO for dinner	2,493 kcal for the day, 66 g CHO for dinner	1,953 kcal for the day, 66 g CHO for dinner
3	1400 kcal for the day, 78 g CHO for dinner	1400 kcal for the day, 78 g CHO for dinner	1400 kcal for the day, 78 g CHO for dinner	1440 kcal for the day, 81 g CHO for dinner
4	1,722 kcal for the day, 75 g CHO for dinner	1,712 kcal for the day, 76 g CHO for dinner	2,076 kcal for the day, 126 g CHO for dinner	2,649 kcal for the day, 144 g CHO for dinner
5	1,586 kcal for the day, 126 g CHO for dinner	1,796 kcal for the day, 126 g CHO for dinner	1,796 kcal for the day, 126 g CHO for dinner	1,586 kcal for the day, 126 g CHO for dinner
6	2,109 kcal for the day, 84 g CHO for dinner	2,109 kcal for the day, 84 g CHO for dinner	2,109 kcal for the day, 84 g CHO for dinner	2,109 kcal for the day, 84 g CHO for dinner
8	2,188 kcal for the day, 79 g CHO for dinner	2,958 kcal for the day, 79 g CHO for dinner	3,035 kcal for the day, 79 g CHO for dinner	2,192 kcal for the day, 79 g CHO for dinner
9	1,600 kcal for the day, 100 g CHO for dinner	1,690 kcal for the day, 128 g CHO for dinner	2,872 kcal for the day, 158 g CHO for dinner	2,110 kcal for the day, 170 g CHO for dinner
10	3,224 kcal for the day, 164 g CHO for dinner	3,000? kcal for the day, 164 g CHO for dinner	3,659 kcal for the day, 164 g CHO for dinner	3,043 kcal for the day, 164 g CHO for dinner
11	1,520 kcal for the day, 92 g CHO for dinner	1,340 kcal for the day, 109 g CHO for dinner	1,565 kcal for the day, 92 g CHO for dinner	1,270 kcal for the day, 92 g CHO for dinner
12	1,970 kcal for the day, 176 g CHO for dinner	1,257 kcal for the day, 123 g CHO for lunch	1,174 kcal for the day, 108 g CHO for dinner	2,850 kcal for the day, 66 g CHO for dinner