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Effect of Protein, Fat, and Both Protein and Fat on the Glycemic Response of a High Glycemic Index Meal

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EFFECT OF PROTEIN, FAT AND BOTH PROTEIN AND FAT ON THE GLYCEMIC RESPONSE OF A HIGH GLYCEMIC INDEX MEAL

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DEDICATION

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Elevated blood glucose (BG) levels from a diet high in refined carbohydrates, even in the absence of diabetes, may increase the risk for chronic diseases including cardiovascular disease, type 2 diabetes, and obesity. The purpose of this study was to determine if supplementation of a high glycemic load breakfast with protein, fat, or a combination of the two attenuates the glycemic response in non-diabetic subjects. Thirteen healthy adults, age 24.7±4y, BMI 25.1±4.5 completed four trials, having fasted 8-12h for this randomized, double-blind crossover study.

Fasting BG was measured, then subjects consumed 2 slices of white bread and 250mL of apple juice (60g carbohydrate) alone (control), or with an added protein (100kcal egg white), fat (100kcal butter), or protein+fat (50kcal egg white and 50kcal butter) within 15min, then repeated BG measurement at 15, 30, 60, 90, and 120min after baseline. ANOVA indicated that there was a difference in the time that BG peaked among the groups. Tukey's post-hoc analysis indicated that BG peaked earlier for the added protein group compared to the added fat group (P=0.007). The spike was not significantly different between the control and the treatments, and there were no differences in BG at the time points measured between the control and the treatments. The spike in BG (peak minus baseline) was significantly lower with added fat compared to added protein group (69.0±15.4 vs 46.9±13.0, respectively, P<0.05); at 15 and 30 min, BG was higher in the added protein group compared to the added fat group. There was no significant difference

in iAUC among the control and the treatments. The results of this study indicate that 100kcal of added protein, fat, or protein+fat did not influence the spike in BG in response to a high refined carbohydrate meal, but an HGI meal with added protein in the form of egg white resulted in a greater spike in BG compared and HGI meal with added fat in the form of butter.

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In the United States, the dietary consumption of refined carbohydrate and simple sugar has increased over the past 100 years. This increased intake of processed carbohydrates parallels the increased prevalence of diabetes, cardiovascular disease (CVD), and obesity (1,2) as the inflammatory process increases. The ingestion of refined carbohydrates and foods high in sugar results in a greater "spike" in blood glucose (BG) and overall glycemic response than carbohydrates in their natural food form (3,4).

Glycemic index (GI) is the glycemic response (post-prandial blood glucose response) elicited by a portion of food containing 50 g of available carbohydrate relative to 50 g of glucose or a portion of white bread containing 50 g available carbohydrate. Many factors besides the total amount of available carbohydrate in the food affect the GI. The specific factors include the degree of gelatinization of starch, chemical composition of the starch or sugar, physical form of the food (e.g. liquid vs solid and particle size), type and amount of fiber, as well as the presence of other macronutrients including fat and protein. Glycemic load is a more realistic predicator of glycemic response since foods are not typically consumed in portions that contain 50 gm of carbohydrate. GL is the product of GI and the amount of available carbohydrate content in a given amount of food (GL = GI X Serving grams of carbohydrate). Refined carbohydrate sources that are low in fiber and high in sugar tend to have the highest GI and GL (5).

Low GI/GL diets are associated with improved blood lipid profiles, improved glycemic control, and reduced CVD and cancer risk, although not all studies agree (4,6,7,8,9,10,11). In the absence of diabetes, the postprandial BG is directly related to CVD, mortality, and obesity (12,13,14).

Since it is not realistic to expect that the majority of individuals will eliminate the intake of high GI/GL foods, given the modern food environment, strategies to mitigate the glycemic response to such foods would be useful. The addition of fat, protein, or fat and protein together to a high GI/GL meal has been shown to attenuate the glycemic response to the meal (15).

At this point, it is unclear if added protein, fat, or a combination of the two has the most beneficial effect on glycemic response. Because some may need to limit protein or fat in the diet, it would be useful to determine if the desired beneficial effect can be achieved by the addition of one of these macronutrients alone, or if a combination of the two is optimal. Recent research found that added fat and protein in the form of two Tbsp peanut butter attenuated the BG spike from a high GI breakfast consisting of two slices of white bread and 250 mL of apple juice (16).

The purpose of this study is to determine if added protein, in the form of egg whites, or added fat, in the form of butter, has the greater impact on the BG spike in response to the above meal, and if the simultaneous addition of egg white and butter has a synergistic effect.

Literature Review

Refined Carbohydrates

Carbohydrates are found in a wide variety of foods such as grains and starches (bread, rice, cereal, pasta, starchy vegetables, legumes), fruit and fruit juices, milk and yogurt, as well as dessert-type foods, candy, snack foods and other foods with added sugars. Non-starchy vegetables have a small amount of carbohydrates. Carbohydrates are one of the three primary macronutrients in the diet providing a source of fuel for the body in the form of glucose. The healthfulness of carbohydrate containing foods is variable; the unhealthier forms are highly processed foods, foods low in fiber, and those with added sugar. These highly refined

carbohydrate foods can have a negative health impact in part because they can be digested quickly, resulting in a dramatic elevation in blood glucose (17).

Over the last 150 years, industrialization has resulted in the development of a plethora of processed food items, resulting in a shift in the western diet from predominantly carbohydrate-rich foods, such as whole grains, and fruit high in fiber to processed grains, sugar-sweetened beverages, and processed foods high in refined carbohydrates and with added sugar. These processed and refined carbohydrate sources tend to have a higher GI or GL and a lower fiber content than other sources of carbohydrate and are generally lower in nutrient density and higher in kcal. The post-industrial change in the food supply has raised questions regarding the impact of foods, with a high GI, on health. Foods high in refined grains are quickly digested (due to grinding or milling that decreases the particle size and discards most of the bran and the germ) and absorbed, resulting in insulin spikes that are greater than foods in their natural form. If consumed frequently and in large amounts, spikes in glucose and insulin from refined and processed carbohydrates can have a variety of adverse consequences, increasing the risk for obesity, diabetes, and CVD, among other health issues. Because of the increased health risk associated with a diet high in refined carbohydrates, health officials are making efforts to encourage the consumption of diets that limit the amount of sugar and refined carbohydrates and consider ensure adequate fiber intake (3,4).

Refined carbohydrates (high GI foods) are related to diabetes development by the resultant increase in blood glucose and insulin levels (18). Elevated insulin over time from a habitual HGI diet can increase insulin resistance and eventually damage the beta cells that produce insulin (19).

For many decades, CVD, diabetes, and obesity were linked to high fat intake rather than refined carbohydrate intake. Mu and colleagues found that although replacing saturated fat with low GI carbohydrates was associated with a lower risk of myocardial infarction, replacing saturated fat with high GI carbohydrates was associated with a higher risk of myocardial infarction (3). In a commentary (20), the researchers concluded that although diets high in saturated fat or refined carbohydrate likely increase the risk for CVD, diets high in refined carbohydrates are more detrimental since these refined carbohydrates elevate the rate of inflammation.

In conclusion, diets are high in refined carbohydrates are linked to metabolic changes that can increase the risk of insulin resistance, elevated blood lipids (triglycerides), and chronic disease (1,5). Research has shown that processed foods and sugar will adversely affect the viability of the gut microbiome, weakening the immune system and increasing susceptibility to health issues (21).

Blood Glucose Response to Carbohydrate

Blood glucose levels are mainly controlled by the antagonistic actions of two hormones, insulin and glucagon, produced and secreted by the pancreas. Insulin's role in glucose homeostasis is to lower BG when BG is elevated. The role of insulin and glucagon is to maintain glucose levels in a normal range of 70-115mg/dL, preventing hyper- and hypo-glycemia (17). Hormones such as somatostatin, epinephrine, cortisol, growth hormone, and thyroxine also influence blood glucose, but unlike insulin and glucagon, these hormones' function is not to maintain glucose homeostasis.

When a food containing carbohydrate is consumed, it is digested to into monosaccharides including glucose. Glucose is then absorbed into the circulatory system causing an increase in

BG. This increase in BG stimulates the pancreatic beta cells of the Islet of Langerhans to produce and secrete the proteinaceous hormone insulin. Insulin binds to receptors on adipose and muscle cells, resulting in the translocation of GLUT4 glucose transport proteins from inside the cell to the cell surface. GLUT4 transports glucose into cells, thereby lowering BG. Insulin signals the liver to synthesize glycogen from glucose, storing glucose in the liver (22,23)

Although carbohydrate is the primary macronutrient that results in an elevation in blood glucose, protein can elevate BG after ingestion when glucogenic amino acids are converted to glucose in the liver. Dietary fat has a minimal impact on BG, but the glycerol portion of triglycerides can be converted to glucose. The effect of protein and fat on BG are most certainly not as profound as dietary carbohydrate but may act to attenuate the glycemic response to a carbohydrate load. This process will be discussed in detail elsewhere in this literature review.

In contrast to insulin, glucagon's main function is to elevate BG when BG levels are low. Glucagon is a proteinaceous hormone secreted by the alpha cells of the pancreas. Glucagon acts to increase BG in two primary ways. First glucagon is a catabolic hormone, it acts to increase BG by stimulating glycogenolysis, which is the catabolism of glycogen resulting in glucose being released from hepatocytes or being used for glycolysis in muscle. Glucagon elevates blood glucose levels using several metabolic pathways. Second glucagon increases the gluconeogenesis, the synthesis of glucose from glucogenic amino acids. Lactate and the glycerol portion of triglycerides as gluconeogenic precursors (22,23).

The insulin: glucagon ratio assists in controlling carbohydrate and lipid metabolism. These hormones work in a company with each other, even though in opposing directions, it may make sense to interpret the insulin: glucagon ration rather than assessing their absolute values. The insulin: glucagon changes inversely with the requirement for endogenous glucose

production. The ration has low-value in starvation and high-value during loading with exogenous carbohydrates (24). In the fasted state and between meals, as BG levels decline, glucagon is released to increase BG levels to maintain homeostasis (9,17).

It is not unusual for BG to increase to 140mg/dL after the consumption of a high carbohydrate food. Postprandial hyperglycemia is defined as an excessive elevation in the blood glucose after meal consumption. A postprandial BG of 160mg/dL or higher is considered postprandial hyperglycemia occurs in healthy individuals when a large amount of rapidly absorbed carbohydrate is consumed. In heathy individuals, the pancreas compensates for postprandial hyperglycemia by increasing insulin secretion, which lowers the BG by the mechanisms described earlier in this section. In individuals with diabetes, either the insulin action is impaired, and the adipocytes and myocytes, as well as the liver, become insulin resistant or insufficient insulin is released by the pancreas in response to an elevation in BG or both mechanisms resulting in hyperglycemia (25).

Recent studies suggest a direct relationship between hyperglycemia and CVD oxidative stress (1), endothelial dysfunction (2), thrombosis (26), and low-density lipoprotein oxidation (7) in those with and without diabetes (5,27). Controlling postprandial hyperglycemia reduces the oxidative stress which in turn reduces inflammation. Both oxidative stress and inflammation have been implicated in the etiology of many chronic diseases, including diabetes and CVD (28).

The International Diabetes Federation and American Diabetes Association emphasizes the importance of controlling postprandial blood glucose; specifically reducing the spike in BG from carbohydrate containing meals to achieve good overall glycemic control. One of their stated strategies for this is the consumption of a low GL or GI diet (8). GI/GL is a primary determinant

of postprandial BG associated with food or diets (29). Low GL/GI diets have resulted in dramatic reduction in risks for CVD, diabetes, and improved weight control over time through controlling postprandial hyperglycemia (30,31,32).

Implications of Glycemic Index and Glycemic Load

Glycemic response (GR) is the postprandial change in blood glucose concentration evoked when a meal that includes carbohydrate is consumed. Glycemic index (GI) is the GR of a portion of a specific food that has 50 gm of accessible carbohydrate compared to the GR of a portion of a standard food, such as white bread, that has 50 gm of accessible carbohydrate (28).

Glycemic load (GL) accounts for the actual portion of a food item consumed, and thus reflects both the GI and quantity of carbohydrate in the food. Specifically, GL is calculated by multiplying the GI of the food item by the carbohydrate content of the food item (3). High GI foods include foods that are digested and absorbed more quickly than foods with a lower GI, and thus result in a greater GR. High GI food are those with a GI of 70 or greater, medium GI foods are those with a GI of 56-69, and foods considered to have a low GI have a GI of 55 or less (33).

Since the first publication on GI in 1981 (25), the applicability of the GI to human health continues to be discussed. Increasingly, researchers have been finding potential benefits to a low GI/GL diet, specifically in relation to diabetes, CVD, cancer, and obesity.

In many studies, low GI foods improved lipid profiles and reduced risk for CVD, promoted weight loss (4,27), improved glycemic control in those with type 2 diabetes (26), and enhanced endothelial cell function in obese people without diabetes (8). Additionally, the utilization of low GI foods has been associated with diminished risks of diabetes, CVD, and certain cancers (5,18).

Investigators have found that lower GI foods result in less dramatic blood glucose fluctuations than high GI foods. Dramatic fluctuations (or spikes) in BG result in increased oxidative stress, which is implicated in inflammation-based diseases such as diabetes, CVD, and cancer (7).

In a study of free-living people with type 2 diabetes and obesity, a connection between the GI and GR was investigated utilizing a continuous glucose monitoring device and simultaneous 3-day food record. The dietary GI was positively associated with incremental area under the glucose curve (iAUC), and average blood glucose (28). Multiple meta-analysis studies have concluded that low GI foods remarkably enhanced glycemic control (4), LDL cholesterol level (29), plasminogen activator inhibitor-1(30,31), and C-reactive protein (32).

With consumption of a high GI/GL meal, blood glucose and insulin levels both elevate to a greater degree than after a low GI/GL meal, leading to stimulation of the cellular glucose uptake, restriction of hepatic glucose production, and inhibition of lipolysis. Both insulin and glucagon play a critical in controlling BG levels. When BG increases above normal levels, the insulin level increases to maintain BG within the normal range, while a drop in BG stimulates glucagon secretion to maintain BG within normal limits (23). A dramatic spike in blood glucose can be followed by low blood glucose (rebound hypoglycemia) (34). Hypoglycemia has been linked to uncontrolled hunger and overeating which induced weight gain (19).

Addition of slowly digested carbohydrate and fiber as legumes, and nuts to the diet has been shown to reduce the GI of the diet, giving these foods the potential to improve glycemic control (9, 35). As a result, all these studies shown the importance of an optimal postprandial blood glucose control in people with or without diabetes.

Elevated Chronic and Postprandial BG Relationship to Disease Risk

Diabetes is a metabolic condition characterized by high blood glucose levels due to inadequate insulin production, or peripheral tissue resistance to insulin (36). There are two primary types of diabetes: Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM). In T1DM, autoimmune destruction of pancreatic beta-cells causes insulin insufficiency. In contrast, T2DM is the result of a defect in the ability of insulin to stimulate glucose uptake by muscle and adipose cells, as well as hepatic uptake and storage of glucose, known as insulin resistance (37). Over time, pancreatic beta-cell destruction or decompensation also occurs in T2DM, contributing to hyperglycemia. Over time, hyperglycemia can lead to the long-term consequences of diabetes, including cardiovascular disease, retinopathy, neuropathy, nephropathy, dementia, infections and limb amputations (38). BG is controlled with diet, exercise, and medication: exogenous insulin in the case of insulin insufficiency, and with oral medications and/or other injectable medications to treat insulin resistance. In some cases of T2DM, diet and exercise alone may be sufficient to control BG, thus eliminating the side effects of medications (38).

Prediabetes is characterized by insulin resistance and elevated BG, but BG is not elevated to an extent that meets the diagnostic criteria for diabetes (36). Prediabetes dramatically increases a person's risk for T2DM, but progression to T2DM can be prevented with diet and lifestyle (39). The ADA's diagnostic criteria for diabetes and prediabetes are shown in Table 1.

Table 1. “Criteria for the Diagnosis of Prediabetes and Diabetes”

Criteria	Prediabetes	Diabetes
Fasting Plasma Glucose (FPG)	100 mg/dL	≥ 126 mg/dL
2 h PG during 75 g of OGTT	140 mg/dL	≥ 200 mg/dL
A1C	5.7-6.6%	≥ 6.5%

Note. This Graph was adapted from American Diabetes Association (40)

Meta-analyses confirm that low GI diets undoubtedly enhanced glycemic control for diabetic patients (8). In addition, such diets reduce LDL-cholesterol (11), reduce inflammatory factors, such as plasminogen activator inhibitor-1 (41,42) and C-reactive protein (43) especially at higher BMIs (>25 kg), which would reduce the risk for cardiovascular complications of diabetes. Low GI foods and diets diminished risk factors for the CVD, as well as certain cancers, including breast and colorectal in women (6). As described earlier in this literature review, the beneficial metabolic consequences of a low GI diet are likely due to the slowed absorption of carbohydrate seen with low GI foods.

The Nurses' Health Study (44,45) revealed that CHD risk was increased in those consuming higher GL foods, independent of BMI. Higher GI diets were associated with non-alcoholic fatty liver disease in those with insulin resistance (46). Epidemiological evidence indicates an association of postprandial glycemia with CHD risk and mortality in those with diabetes (47), as well as those without diabetes (12) among healthy individuals. A study conducted by Ferrannini (48) showed that at a higher BG (over 120 mg/dL) resulted in a decline in beta-cell function up to 60% in both lean and obese subjects. Loss of beta-cell function is a manifestation of insulin resistance, which later progresses to T2DM resulting in postprandial hyperglycemia (49). Elevated BG after eating increases production of acetyl CoA and activity of mitochondrial oxidative phosphorylation, resulting in an increase in free radical production. It is

proposed that excessive oxidative stress is part of the pathology of both diabetes and CHD (58,59).

People with diabetes are more prone to develop CVD at every life stage (52), due to injury to blood vessels that leads to dysfunction of the endothelial cells and atherosclerosis (53). Diabetes is a principal cause of CVD morbidity and mortality globally (49). Incidence of CVD, CVD-related death, and stroke is increased in diabetic patients in comparison with non-diabetic patients (54). Diabetes doubles the chance of metabolic dysfunctions leading to metabolic syndrome. Metabolic syndrome is characterized by abdominal adiposity, hyperglycemia, hypertension, and dyslipidemia. Metabolic syndrome greatly increases the risk of both diabetes and CVD (54) and increases the risk of premature mortality and medical expenses, while decreasing employee productivity and quality of life (36).

Epidemiological and clinical research have connected T2DM with increased risk of developing Alzheimer's disease (AD). AD and T2DM are both conditions associated with peripheral and central insulin signaling irregularities. Those with diabetes have a two-fivefold increased the risk of Alzheimer disease in comparison to those without diabetes (37).

High blood glucose (not related to T2DM) is associated with impaired cognitive function, especially older people free of dementia. T2DM is associated with increased brain atrophy, decline in cognitive function, and increased risk for dementia (55).

Effect of Protein on the Glycemic Response

Consuming protein with a carbohydrate increases insulin secretion by the pancreas greater than when carbohydrate is consumed alone, and this increase in insulin should result in a diminished spike in BG. One study observed that the consumption of 25 g of protein with a 50 g oral glucose solution increased plasma insulin by 2.5 and diminished the blood glucose by

almost 30 % response in patients with type 2 diabetes mellitus compared to the consumption of the glucose solution alone (56). Another study observed that given 28 g of protein mainly whey protein with 50 g of carbohydrates in a meal has been shown to decrease the glycemic response in comparison to the consumption of carbohydrate alone (57).

Adding protein to a carbohydrate load delays gastric emptying, via its stimulatory effect on the secretion of gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (58). It is well known that the rate of gastric emptying is a primary determinant of postprandial glycemic response. The addition of protein to a carbohydrate load would be presumed to delay the rate of gastric emptying and therefore absorption of glucose, attenuating the glycemic response (56). The insulinotropic impact of protein has been recognized primarily with the consumption of animal protein (such as whey), which are a significant source of branched-chain amino acids (BCAAs). Research suggests that the source of protein, whether animal or vegetable, and the amino acid composition of proteins influence the effect on blood glucose concentrations (59).

Whey protein which is a fast-absorbing protein increases insulin response to a greater degree than casein protein which is a slow-absorbing protein, indicating that the type of consumed protein influences insulin secretion (60).

When protein is added to a meal, it is a source of additional calories, which could be of concern in terms of weight gain. However, several sources of protein increase satiety, which may result in decreased food intake. The most extensively researched protein-containing food in relation to satiety and decreased glycemic response is milk. The whey protein in milk has been linked with beneficial effects on energy balance, appetite, and glucose metabolism. Whey is known as a fast-absorbing protein, since it moves quickly through the stomach without digestion,

whey remains mostly intact and is associated with a lower glycemic response, while casein is known as a slow-absorbing protein, since it moves slowly through the stomach and becomes hydrolyzed by stomach acid, causing it to precipitate. Whey also has a higher branched chain amino acids (BCAA) composition compared to casein, and it is believed that whey has a greater effect on satiety than casein; this could be because insulin is considered a satiety hormone. The only established information so far is that the addition of protein to a carbohydrate-containing meal decreases the postprandial glycemic response (58).

Effect of Fat on the Glycemic Response

Of the macronutrients, fat has the most profound effect on delaying gastric emptying. When fat is added to a carbohydrate-containing beverage or meal, or even is delivered directly into the small intestines, the rate of gastric emptying is slowed, and the blood glucose and insulin responses are attenuated. Fat stimulates GIP and GLP-1 secretion. The slowing of gastric emptying and stimulation of GIP and GLP-1 are reliant on the digestion of fat (triglycerides) to fatty acids in the digestive system. The beneficial effect of fat on glycemic response to a carbohydrate is greater if the fat is consumed before and alone, rather than with a carbohydrate-containing meal. Fat consumption before the meal ensures that the gut hormones that delay gastric emptying are released in time to slow the absorption carbohydrate as the carbohydrate enters the digestive tract. One study showed that the administration of olive oil as a preload to a carbohydrate meal slowed the postprandial increase and lowered the blood glucose spike compared to olive oil consumed with a meal (61). Furthermore, the type of fat performs a function in glycemic response. Researchers have found that saturated fatty acids such as butter diminish insulin sensitivity and reduce gastric emptying, while monounsaturated fatty acids

(MUFAs) promote better insulin sensitivity and stimulate GLP-1 secretion to a greater degree. In this study, the fat type was more important than the quantity on improving glycemic response.

The researchers recommended utilizing MUFAs before a carbohydrate meal to weaken the glycemic response (62).

Effect of Protein and Fat on the Glycemic Response

It is generally affirmed that the addition of fat and protein to a carbohydrate meal diminishes glycemic response by slowing gastric emptying and stimulating insulin secretion. Typical healthy meals include both fat and protein, which allows for a synergistic effect; however, there are many specialized foods available that are primarily protein or fat, not a combination. There is evidence, however, that fat and protein may not have the same effects in all circumstances. One study found that fat and protein decreased the glycemic response to an oral glucose load in healthy humans (63). Studies have revealed that the effects of protein and fat are autonomous of each other; however, gram-for-gram, protein has been shown to elicit a 2 to 3 times more prominent effect than fat. The greater capacity of protein to diminish the glycemic response compared to fat implies that the mechanisms by which fat decreases glycemic response differs from protein (64). Studies of protein and fat ingestion together on glycemic response are contradictory. One reasonable answer to explain the differences is that it is that the source of fat and protein could influence the response (65). A further explanation is that the source of carbohydrate in the meal could influence the effect of fat and protein (66). A study that utilized a liquid meal found that protein (soy protein) lowered the glucose response, while the added fat (corn oil) did not affect glucose response. This result was surprising because added fat typically reduces the glycemic response. Thus, meal consistency may be a factor in the response to added

fat and protein; a carbohydrate that is rapidly absorbed may not permit adequate time for fat to stimulate the secretion of hormones that delaying gastric emptying.

Another explanation is that fat and protein could separately attenuate the discharge of CCK and peptide YY; these are the two gut hormones that delay gastric emptying (65). A study has found that a diet high in whey protein (30% protein, 20% fat, and 50% carbohydrates) stimulated insulin to a greater degree in comparison to a diet high in fat (15% protein, 35% fat, and 50% carbohydrates) (59). The outcome was explained by the ability of protein to stimulate insulin secretion, whereas fat does not have this effect (59). Both fat and protein are considered to lessen the glycemic response by slowing gastric emptying, protein has the added advantage of stimulating insulin secretion (67). Interestingly, in one study, protein added to meal contained different concentration of fat and contact concentration of carbohydrates, lowered the blood glucose response regardless of whether the participants had a low, medium, or high fasting insulin level, in this study the BG measured at different intervals including the fasting BG (65).

Even though the research is not definitive, the majority of research has found that adding either fat or protein components alone or together to a carbohydrate meal results in an attenuation of the glycemic response (67).

Materials and Methods

This study was a randomized, double-blind crossover design and was approved by the Institutional Review Board at the University of the Incarnate Word. All participants provided informed, written consent, prior to data collection. See Appendix A for Consent Form.

Recruitment

Power analysis revealed that 12 participants were needed to detect a difference in the blood glucose spike and incremental area under the curve (AUC) among treatments.

A total of 14 participants were recruited from scripted announcements in nutrition classes at a university campus. One participant withdrew (due to family issues) [see Appendix B for script].

Inclusion criteria

To participate, volunteers had to be non-smoking young adults over 18 years of age and younger than 30 years of age who indicated they were in good health (provided by questionnaire). All participants had to have fasting BG values less than 100 mg/dL. Participants could have no allergies or intolerance to egg, butter (dairy), apple juice, or wheat. In addition, volunteers had to agree to fast for at least 8 hours prior to data collection and abstain from exercise on the days of the data collection.

Exclusion criteria

Volunteers under 110 lb and who were pregnant, or breastfeeding were excluded. Also excluded were individuals with medical conditions (including injuries that could cause physiological stress) and/or taking medications that would affect carbohydrate metabolism. (see appendix B for the inclusion and exclusion criteria).

Procedure

Participants were trained on the use of glucometers (FreeStyle Lite, Abbott Diabetes Care, Inc., Alameda, CA) prior to data collection. Glucometers were calibrated with test solution prior to data collection. Participants measured their own blood glucose, supervised by investigators who verified values before recording them on the data sheet. The percent coefficient of variation for the glucometers used was <1%. Participants wiped their fingers with an alcohol pad then allowed the finger to dry. Glucometers were numbered, and the participants used the same glucometer each time. The participants measured their blood glucose levels using the glucometer twice for each time point. If the two measures were not within 10 mg/dl they

measured a third time. The closest values were averaged to be used in data analysis. The participants brought smartphones to time glucose testing. Timers were provided for those who did not bring a smartphone.

On the first day of data collection, after having fasted for at least 8 hour and abstaining form exercise, the participants completed a questionnaire detailing demographic and anthropometric information including gender, age, and ethnicity. In addition, height and weight were measured, BMI was calculated, and waist circumference was measured at the umbilicus to the nearest 0.5 cm using a tape measure. The participants were given a coded data sheet to record blood glucose as well as a numbered glucometer. See appendix C

Participants received the control and the treatment meals in a randomized double-blind manner. Randomization was performed using computer generated random numbers.

Table 2. “Dietary Patterns”

Treatments	Diet	Kcal
Control	2 slices of white bread and 250 ml of apple juice	256 kcal from carbohydrate
Protein	2 slices of white bread served with 192 g of cooked egg white and 250 ml of apple juice	256 kcal from carbohydrate and 100 kcal from protein
Fat	2 slices of white bread served with 14 g of butter and 250 ml of apple juice	256 kcal from carbohydrate and 100 kcal from fat
Protein & Fat	2 slices of white bread served with 96 g of cooked egg white, 7 g of butter and 250 ml of apple juice	256 kcal from carbohydrate, 50 kcal from protein and 50 kcal from fat

Control treatment. After determining baseline blood glucose, participants consumed two slices of white bread and 250 ml of apple juice (Table 2). The participants sat quietly and repeated blood glucose measurement at 15, 30, 60, 90, and 120 minutes after the first bite of the

meal. Investigators recorded blood glucose values on participant's data sheets to ensure accuracy and collected the data sheets at the end of the data collection period.

Protein treatment. Participants measured their fasting blood glucose as described earlier using the same glucometer as day 1 (Table 2). After obtaining their baseline blood glucose value, they consumed the breakfast described for the first day with the addition of 192 g (providing 100 kcal) of cooked egg white, served between the slices of white bread. Participants sat quietly and measured blood glucose according to protocol followed on day 1.

Fat treatment. Participants measured their fasting blood glucose as described earlier using the same glucometer as day 1 and day 2 (Table 2). After obtaining their baseline blood glucose value, they consumed the breakfast described for the first day with the addition of 14 g (providing 100 kcal) of butter served between the slices of white bread. Participants sat quietly and measured blood glucose according to protocol followed on day 1 and 2.

Protein and Fat treatment. Participants measured their fasting blood glucose as described earlier using the same glucometer as days 1, 2 and 3 (Table 2). After obtaining their baseline blood glucose value, they consumed the breakfast descried in for the first day with the addition of 96 g of egg white (providing 50 kcal) and 7 g of butter (providing 50 kcal) served between the slices of white bread. Participants sat quietly and measured blood glucose according to protocol followed on days 1, 2 and 3.

Statistical Analysis

Glucose incremental area under the curve (iAUC) was calculated for each participant using the trapezoidal approximation method for all four days of data collection. All statistical analyses were completed using R statistics software. Descriptive statistics were performed on the demographic, anthropometric, BG values at each time point, spike in BG, and

iAUC. ANOVA was used to determine any differences in baseline, 15, 30, 60, 90, and 120 minutes blood glucose values, as well as the magnitude of the spike in BG and iAUC among the control and treatments. Tukey's post-hoc test was used to determine the level of significance.

Results

Participant Characteristics

A total of 13 healthy young adults completed the study. Specifically, there were 7 females and 6 males, 11 Hispanics, 1 non-Hispanic Caucasian, and 1 African American. See Table 3 for a summary of participant characteristics.

Blood Glucose Response

The BG values were not significantly different at 15, 30, and 60 mins for the treatment groups vs the control group. In fat treatment, the BG values were significantly lower compared to the protein treatment at 15 and 30 mins ($p<0.05$). However, there was no difference baseline at 90- and 120-mins BG values among the treatments and control groups. See Table 4 for BG values at each time interval.

The spike in BG (peak minus baseline) average was lower for the added fat treatment (59.87-33.97) compared to the control day, added protein, and added protein and fat treatments (74.57-41.35), (84.42-53.58), (75.15-42.85) respectively, however; the difference was not significant. The iAUC was lower on the fat treatment day compared to the protein day, but the difference was not significant. ANOVA indicated that there was a difference in the time that BG peaked. Tukey's post-hoc analysis showed that BG peaked earlier for the added protein group compared to the added fat group. ($p=0.007$) See Table 5

See Table 3, Table 4 and Table 5 for a comparison of BG parameters and iAUC, and Figure 1 and Figure 2 for a graphic representation.

Table 3. "Participant Characteristics"

Variable	Mean±SD (n=13)	Range
Age (yrs)	24.7±4	28.7-20.7
Height (cm)	166.1±10.6	176.7-155.5
Weight (kg)	69.5±15.1	84.6-54.4
BMI[a]	25.1±4.5	29.6-20.6

[a]BMI = Body Mass Index; weight in kilograms divided by height in meters squared

Table 4. "Blood Glucose Indicators in Response to a High Glycemic Index Meal on Control and Treatment Days"

Variable	Control Mean±SD (*n*=13)	Added Protein Mean±SD (*n*=13)	Added Fat Mean±SD (*n*=13)	Added Protein+Fat Mean±SD (*n*=13)
Fasting BG[a] (mg/dL)	86.77±8.52 [a]	85.62±7.71 [a]	87.08±7.58 [a]	83.63±5.01 [a]
15 min postprandial	135.3±14.79	142.77±13.73	126.35±9.97	129.96±8.89
30 min postprandial	138.04±20.73	150.38±21.82	126.5±17.23	137.88±19.79
60 min postprandial	114.15±13.78 [a]	111.27±19.76 [a]	116.69±13.78 [a]	115.73±15.65 [a]
90 min postprandial	88.58±7.47 [a]	91.35±10.54 [a]	96.92±14.41 [a]	91.88±10.67 [a]
120min postprandial	83.81±10.67 [a]	84.42±9.85 [a]	87.54±9.73 [a]	83.81±11.77 [a]

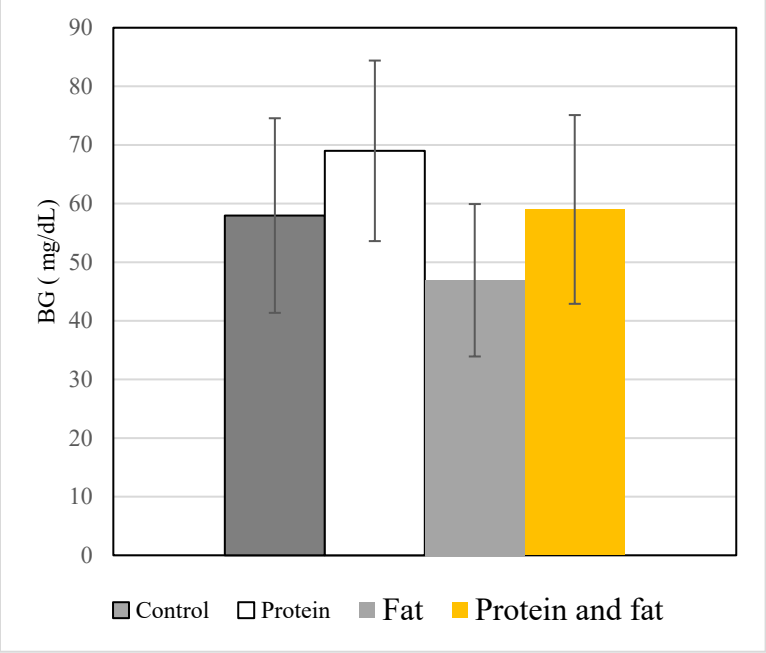
[a]BG = Blood Glucose (no significance differences among the groups)
Added protein was significantly higher than added fat ($p<0.05$)

Table 5. "Peak and Incremental Area Under the Curve in Response to a High Glycemic Index Meal on Control and Treatment Days"

Variable	Control Mean±SD (*n*=13)	Added Protein Mean±SD (*n*=13)	Added Fat Mean±SD (*n*=13)	Added Protein+Fat Mean±SD (*n*=13)
Peak – Baseline[b] (mg/dL)	57.96±16.61	69±15.42	46.92±12.95	59±16.15
iAUC (mg/dL/120min)[c]	151.12±80.50 [a]	175.31±76.80 [a]	147±91.21 [a]	163.26±72 [a]

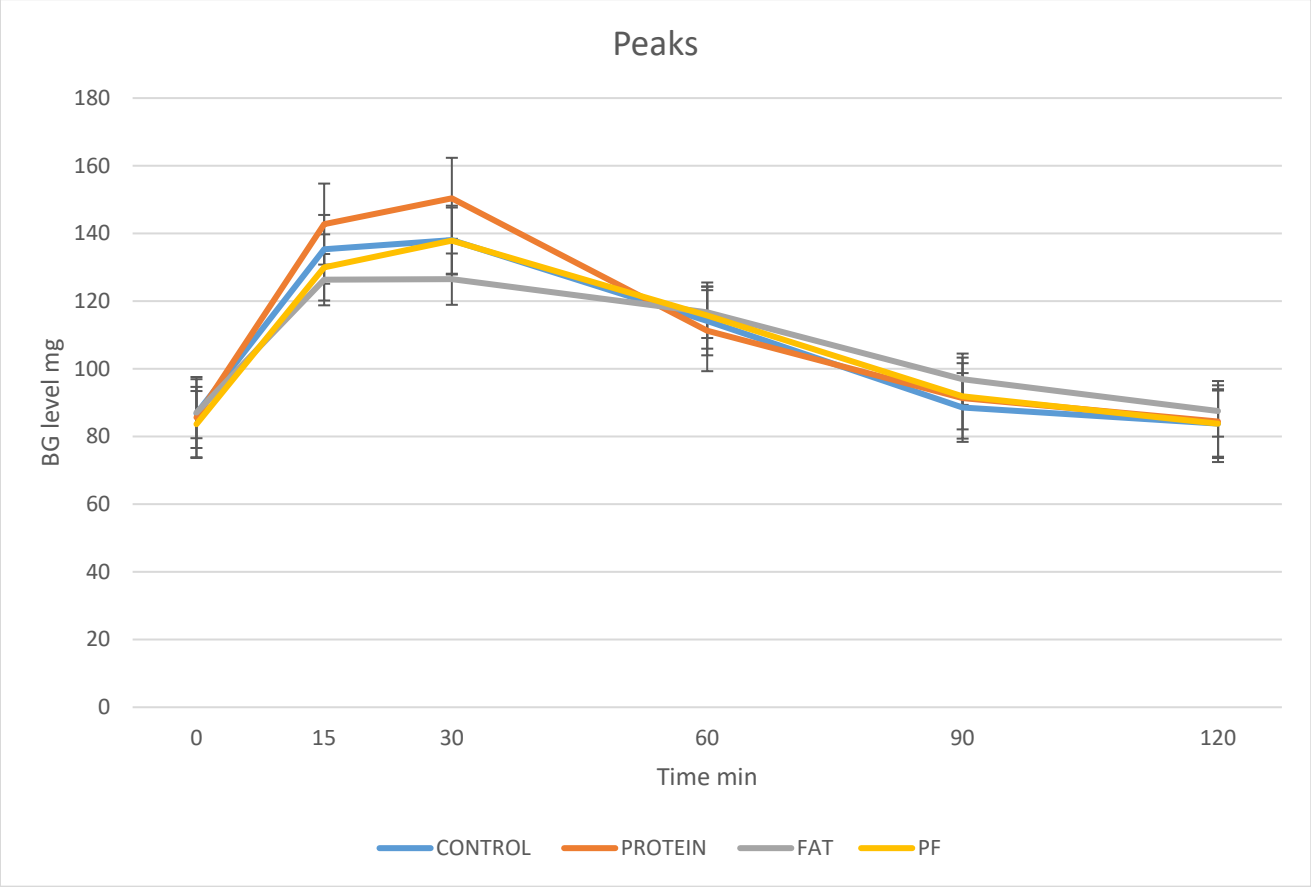
[a]BG = Blood Glucose (no significance differences among the groups)
[b]The difference between Peak BG and Baseline BG (spike)
[c]iAUC = Incremental Area Under the Glucose Curve

Figure 1. "Spike in Blood Glucose from the Control and Treatment (Added Protein, Added Fat, and Added Protein+Fat)"



N= 13. Data presented are means±SD.

Figure 2. "Glucose Response to the Control Breakfast and Control Plus Added Protein, Fat, and Added Protein+Fat[a]"



[a] Data presented are means±SEM. BG values were significantly lower ($p < 0.05$) for the added fat group compared to the added protein group at 15 and 30 min. There was no difference in BG among the groups at any other time.

Discussion

The results of the present study conflict with previous studies that found a beneficial effect of the co-ingestion of protein and fat on the glycemic response to a meal (64,66,67). It is generally believed that the addition of protein and fat to an HGI meal attenuates the glycemic response by stimulating insulin secretion and delaying gastric emptying. Nevertheless, a significant disparity exists in study design, which likely to explains, to some degree, the

variability in the magnitude of the effect of protein and fat on the glycemic response of a meal when consumed individually or together.

The literature indicates that fat and protein may not have the same results in all conditions. The origin and the quantity of protein or fat in the meal, as well as the food source of the carbohydrate, may influence the effect, making it difficult to not only generalize the effect of fat and protein on glycemic response, but also to identify the mechanisms for the effect. Slowed gastric emptying is generally considered to be the primary mechanism by which fat diminishes postprandial glycemic responses. Numerous factors seem to influence the effect of fat on the glycemic response to an HGI meal. The principal factors supported by research include the timing of fat consumption, the type of fat consumed, and the glycemic index of the carbohydrate in the meal. Concerning the timing of fat consumption, a researcher and his group (61) investigated the effect of ingestion of the olive oil as a preload, 30 minutes before, versus ingestion with an HGI meal. The consumption of the olive oil, as a preload, considerably delayed gastric emptying, delayed the postprandial rise in blood glucose and plasma insulin, and increased the levels of gut hormones that influence gastric emptying such as GIP and GLP-1 to a greater degree than when the olive oil was consumed with the meal. The researchers proposed that ingestion of the olive oil before the meal allowed for time for triglyceride digestion to occur and stimulate gut hormone secretion. In other words, the extent of the slowing of gastric emptying is likely to be more significant when the oil is consumed before rather than with an HGI meal because it takes approximately 30-40 min for small intestine feedback mechanisms produced by fat to take effect.

The present study revealed a trend for an earlier peak in blood glucose on the added protein treatment day compared to the control, fat, and both fat and protein treatments. This

could be because the source of the added protein, egg white, is quickly digested and absorbed and glucogenic amino acids may contribute to the increase in blood glucose. The fat and protein plus fat treatments may not have delayed the rise since they were consumed with the meal rather than as a preload, and thus there was not enough time for the triglycerides to be digested to fatty acids, to induce the hormonal response to slow gastric emptying. Moreover, there is evidence that the type of fat used as well as the glycemic index of the carbohydrate impact the overall effect of fat on glycemic response. Another study (62) with 13 patients, with type 1 diabetes mellitus, who consumed two sets of meals with an identical carbohydrate content but differing in glycemic index. The amount and type of added fat varied in the treatments. Both saturated fatty acids (SFA) in the form of butter, MUFAs in the form of extra virgin olive oil were examined.

The investigators concluded that MUFAs, in the form of extra virgin olive oil, enhanced postprandial insulin sensitivity and stimulated GLP-1 secretion while the SFAs in the form of butter lowered postprandial insulin sensitivity and slowed gastric emptying. Furthermore, they concluded that the glycemic index of the meal significantly influenced the effect of added fat on the glycemic response. Adding various types of fats to meals with a low glycemic index did not alter the postprandial blood glucose response, whereas, it did alter blood glucose response in HGI meals. For that reason, type of fat should be taken into consideration when the meal with an HGI is consumed. In the present study, the patients consumed an HGI meal containing two slices of white bread and a cup of apple juice. According to current research, under these conditions, the sources of fat and protein are an essential predictor of glycemic response. According to this study, the fat, butter, is used in this study, had the potential to decrease the postprandial insulin sensitivity and delay gastric emptying; however, the glycemic response of the added fat and added fat plus protein treatment did not differ from the control. An insufficient amount of fat

(1 Tbsp or 14.2g in the fat only treatment and 0.5 Tbsp or 7.1g in the fat plus protein treatment) could account for the lack of an effect.

Similar to research on fat's impact on the glycemic response to a meal, the mechanisms for the effect of protein on the glycemic responses are not fully understood. Protein's capacity to lessen the glycemic response to a carbohydrate load is purportedly due to stimulating insulin secretion as well as delaying the gastric emptying. In research, numerous factors have been linked to the variability of the effect of protein on glycemic response. The most widely accepted explanations are protein quality (animal vs. vegetable), the amino acid composition of the protein, and whether the protein is ingested in the liquid vs. solid form. A group of researchers (58) published a review about the impact of various protein sources on the glycemic and insulinemic responses to a carbohydrate load and determined that protein source (animal or vegetable) and the amino acid composition of proteins may result in different effects on the blood glucose levels. The consumption of high biological value proteins, such as animal origin foods, especially whey protein which is rich in branched chain amino acids (BCAAs), seem to have the most beneficial effect on the glycemic response; insulin secretion is enhanced, and therefore, glucose uptake by cells and glycogen synthesis is improved. Moreover, high concentrations of BCAAs contribute to the production of glucose in the liver (gluconeogenesis) through the alanine-glucose cycle, which also affects blood glucose. Lan-Pidhainy (65) hypothesized that some amino acids seem to compete with insulin by binding to insulin receptors on hepatocytes; as a result, less insulin is removed by the liver and peripheral insulin concentrations rise. Another study (60) discovered that the type of ingested protein influenced the insulin response and action in those with type 2 diabetes. Quickly absorbed protein, in the

form of whey, improved insulin secretion to a greater extent than slowly absorbed protein in the form of casein.

In this study the added protein did not reduce the glycemic response may be because of the type of protein used the (egg white) or insufficient amount of protein used.

Finally, the effects of the co-ingestion of protein and fat on postprandial blood glucose responses have not been examined systematically, and the response to various meals cannot be predicted with certainly (64). Most protein-containing foods also have fat, and it is not known if they act synergistically to influence blood glucose response to a carbohydrate load.

A longitudinal study (64) examined the dose-response consequences of protein and fat on the glycemic response of 20 healthy adults. Participants ingested 50 grams of glucose solution dissolved in 250 mL of water plus 0, 5, 10, or 30 gm of fat. At each level of fat, 0, 5, 10, or 30 gm protein were added; therefore, each level of fat was tested with each level of protein. They concluded that the effect of protein and fat were not related to each other, but gram-for-gram, the protein produced a two to three times greater effect than fat, with insignificant interaction between fat and protein. Fat decreased the glycemic responses to a broader range in participants who had low-level fasting plasma insulin, while the protein had an extra intense impact in the participants who had high waist circumference and a large intake of dietary fiber. These results indicate that there is a separate mechanism by which fat reduces glycemic response in comparison to protein.

A study (67) examined the glycemic and insulinemic responses to an HGI meal (mashed potatoes) when consumed with a high-fat food, rapeseed oil, and a high-protein food, chicken breast. The outcomes showed that adding fat alone, or protein alone, and the combination of both fat and protein together lowered the glycemic response. Gulliford (66) discovered that blood

glucose responses to various types of carbohydrate foods are not equally affected by the co-ingestion of protein and fat, which can explain the reason that the research suggests that the responses to different meals are not well predicted by glycemic index of carbohydrates.

In this present study, the consumption of egg white, or butter, or both did not result in a lower spike in blood glucose compared to the control when 2 slices of white bread and 250ml apple juice were consumed. This is in contrast with the studies described above which did find an effect. The discrepancy could result from the amounts of protein, fat, and protein plus fat used and needed to have an effect.

Despite the addition of protein, fat or both did not change the glycemic response, but the spike of added protein was higher compared to added fat, the reason beyond that the protein was absorbed quickly and glucogenic amino acids contributed to the risk in the blood glucose.

In interpreting the results of the present study, limitations must be recognized including small sample size with low power to determine a difference in AUC, venipuncture for glucose determination has better validity and reliability than a glucometer, and a dose-response effect was not examined. Additionally, results can only be generalized to the healthy, young, population, which are not as high risk for glucose intolerance. Results may have been different with a different HGI meal, so results cannot be generalized to all HGI meals.

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Appendix A

Code

Consent to Participate in a Research Study
Effect of protein, fat and protein+fat supplementation on the glycemic response to a high carbohydrate meal

You are being asked to participate in a research study conducted by Muhammed Taai, MD, and fellow graduate students, Lesley Lilly, MS and Sofia Maragoudakis. The purpose of this study is to determine if added protein, fat or protein+fat will blunt the blood glucose response to a high carbohydrate meal. We will also use data collected from this study to determine if added protein, fat or protein plus fat influences the satiety of a meal using a hunger/satiety scale.

For this study, you will complete a screening questionnaire to determine eligibility for the study. To be eligible to participate in the study, you must be a healthy adult over age 18, with no diseases or disorders that could affect blood glucose levels, who doesn't smoke. You must also be non-pregnant, over 110lb, with no allergies or intolerances to gluten (which is in the white bread), egg whites or butter and must be willing to consume 2 pieces of white bread, 1 cup apple juice, and on test days, either 100kcal (192gm) egg whites, 100kcal (14gm) butter or 50kcal (96gm) egg whites and 50kcal (7gm) butter. You must be willing to fast for at least 8 hours, but no longer than 12 hours, prior to data collection and not perform any physical activity on the mornings before data collection.

You understand that your participation in the study involves the following procedures:

You will be instructed on how to measure blood glucose using the glucometer provided for this study. The measurement includes wiping a finger with an alcohol pad, then allowing the finger to dry. A special lance is then used to prick the finger for a small drop of blood. There will be a "pin-prick" sensation that may result in some slight discomfort. A glucose test strip is placed at the finger where the small drop of blood appears, then placed in the glucometer for determination of blood glucose level. The finger is then wiped again with the alcohol pad. Lancets, glucose test strips, and alcohol pads will be disposed of in a sharp's container. Trained technicians and trained personnel will be available to help with the blood glucose measurement if needed. On each day of data collection, you will arrive having fasted for at least 8, but no more than 12 hours.

On day 1 of data collection, you will fill out a questionnaire that included demographic information, such as age, gender, ethnicity, and family history of diabetes. Your height, weight, and waist circumference will be measured. You will be given a data collection sheet with a code. You will need to record this code in your cell phone or on a card to keep in your wallet so we can give you the correct data collection form on the second and subsequent days of data collection. You will measure your blood glucose using a glucometer twice. If the two measures are not within 10mg/dL, you will measure a 3rd time. You will record all measurements on a form provided to you. After obtaining your fasting blood glucose, you will eat two pieces of white bread and drink 1 cup of apple juice. Then you will sit quietly and measure your blood glucose at 15, 30, 60, 90 and 120 minutes after you finished the meal. You will use a timer on your smartphone if you have one to time the measurements, or a timer will be provided for you. You will then give your data collection sheet to the investigator.

On day 2 of data collection, you will again measure your fasting blood glucose as described earlier, then consume two pieces of white bread, drink 1 cup of apple juice with 100g of added egg whites, 100g of

added butter OR 50g of added egg whites and 50g of added butter within 15 minutes. Immediately after consumption, you sit quietly for the remainder of the data collection. You will measure your blood glucose at 15, 30, 60, 90 and 120 minutes. You will then give your data collection sheet to the investigator.

On day 3 of data collection, you will again measure your fasting blood glucose as described earlier, then consume the breakfast described in day 2 (added egg whites, added butter or added egg whites and butter) that you did not consume on day 2. You will measure your blood glucose at 15, 30, 60, 90 and 120 minutes after you finished your meal. You will then give your data collection sheet to the investigator.

On day 4 of data collection, you will again measure your fasting blood glucose as described earlier, then consume the meal described in day 2 (added egg whites, added butter or added egg whites and butter) that you did not consume on day 2 or 3. You will measure your blood glucose at 15, 30, 60, 90 and 120 minutes after you finished your meal. You will then give your data collection sheet to the investigator.

Each day of data collection will require no more than three hours of your time. A possible benefit of this study is to see if eating a protein, fat or protein+fat can blunt the effect of glucose in response to carbohydrate intake. If this is the case, health benefits, including reduced inflammation (which has the potential of reducing the risk of many chronic diseases) and prevention of swings in blood glucose could occur. It is also important to determine if there is a correlation between hunger levels and blood glucose response.

We will input the data from your questionnaires into a spreadsheet for analysis. Your name will not be included in the data spreadsheet, only your study "code number." All data will be confidential, and no copies will be maintained by the study investigator. Your identity will be protected and any publication that follows this study will only display data of groups, not of individuals. Participation is voluntary and you have the right to refuse participation without penalty of any kind. You have the right, at the end of the study, to be informed of the findings of this study. If you have questions, please ask them at any time.

If you have additional questions later or you wish to report a problem that may be related to this study, contact:

Muhammed Taai, MD Phone: 405-388-7842 email: altaai@student.uiwtx.edu

To contact the University of the Incarnate Word committee that reviews and approves research with human subjects, the Institutional Review Board (IRB), and ask any questions about your rights as a research participant, call: UIW IRB, Office of Research Development (210) 805-3036.

If you completely understand the expectations and rights of participants in this study, all of your questions have been answered to your satisfaction, and you are willing to participate in this study please sign and date this consent form in the space provided. To sign this consent form, you must be 18 years old or older by today's date.

_____	_____	_____
Participant Name (printed)	Participant signature	Date Signed

_____	_____
Witness signature	Date Signed

Appendix B

Screening Questionnaire

Effect of protein, fat and protein+fat supplementation on the glycemic response to a high carbohydrate meal

Please answer the following questions by circling the appropriate answer.

1. Are you over the age of 18? Yes No
2. Are you pregnant or breastfeeding? Yes No
3. Do you weigh at least 110 pounds? Yes No
4. Are you in general good health? Yes No
5. Do you smoke? Yes No
6. Do you take any medications that can affect your blood glucose? Yes No
7. Do you have any medical disorder that can affect your blood glucose? Yes No
8. Do you have any allergies or intolerances that would make you unable to consume white bread which contains gluten, egg whites, butter or apple juice?

   Yes No
9. Are you willing to consume 2 slices of white bread and applie juice on four occasions?

   Yes No
10. Are you willing to fast for at least 8 hours prior to data collection, which will occur on 4 separate days?

    Yes No

If you meet the screening criteria for this study and would like to participate, please contact Muhammed Taai at altaai@student.uiwtx.edu for further instructions. You will need to sign the Informed Consent From prior to participation.

Glucometer number	Code

DATA SHEET

Effect of protein, fat and protein+fat supplementation on the glycemic response to a high carbohydrate meal

1. What is your gender? (circle one) male female
2. What is your age in years?_____
3. Do you have a family history of diabetes? (circle one) Yes No

   If yes, what type of diabetes? (circle one)

   Type 1 Type 2 Other (specify)_____

   What relative(s) have been diagnosed with diabetes (circle all that apply)

   Sibling Mother Father Grandparent (how many)_____
4. Are you Hispanic or Latino? Yes No Choose not to answer
5. Which one or more of the following would you say is your race/ethnicity? Select all that apply.
   _____Non-Hispanic Caucasian
   _____ Hispanic or Latino
   _____Black or African American
   _____Asian
   _____American Indian or Alaskan Native
   _____Native Hawaiian or other Pacific Islander
   _____Choose not to answer

Appendix C

Code

Anthropometric and Blood Glucose Data

Height_____ Weight_____ Waists circumference_____

Day 1: If first two measurements are within 10 units, you don't have to do a third…

Fasting Blood Glucose: 1_____ 2_____ 3_____

15 min blood glucose: 1_____ 2_____ 3_____

30 min blood glucose: 1_____ 2_____ 3_____

60 min blood glucose: 1_____ 2_____ 3_____

90 min blood glucose: 1_____ 2_____ 3_____

120 min blood glucose: 1_____ 2_____ 3_____

Day 2:

Fasting Blood Glucose: 1_____ 2_____ 3_____

15 min blood glucose: 1_____ 2_____ 3_____

30 min blood glucose: 1_____ 2_____ 3_____

60 min blood glucose: 1_____ 2_____ 3_____

90 min blood glucose: 1_____ 2_____ 3_____

120 min blood glucose: 1_____ 2_____ 3_____

Day 3:

Fasting Blood Glucose: 1_____ 2_____ 3_____

15 min blood glucose: 1_____ 2_____ 3_____

30 min blood glucose: 1_____ 2_____ 3_____

60 min blood glucose: 1_____ 2_____ 3_____

90 min blood glucose: 1_____ 2_____ 3_____

120 min blood glucose: 1_____ 2_____ 3_____

Day 4:

Fasting Blood Glucose: 1_____ 2_____ 3_____

15 min blood glucose: 1_____ 2_____ 3_____

30 min blood glucose: 1_____ 2_____ 3_____

60 min blood glucose: 1_____ 2_____ 3_____

90 min blood glucose: 1_____ 2_____ 3_____

120 min blood glucose: 1_____ 2_____ 3_____