Glycemic response to tortilla consumption: Influence of physical activity and insulin resistance

BY

Justin Fletcher

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Chairperson

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Committee members

Date Defended: _____

The Thesis Committee for Justin Fletcher

certifies that this is the approved version of the following thesis:

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Chairperson

Date approved:_____

ABSTRACT

Insulin resistance (IR) reduces glucose uptake and leads to elevated glucose levels (EGL). Foods that limit postprandial glycemic response (PPGR) and exercising can help limit these EGL. 25 subjects consumed 6 different types of tortillas. Bean or nopal flour was added to corn flour tortillas to determine if these ingredients mitigated the glycemic response. We also investigated if individuals meeting the Center for Disease Controls' (CDC) physical activity (PA) recommendations had lower IR and if that influenced the PPGR. PPGR to different types of tortillas was measured for 3 hours. Participants also completed a physical activity questionnaire to determine PA levels. We found that substituting bean or nopal for corn in a tortilla did not reduce glycemic response. It was also found that those who met CDCs' recommendations had lower IR compared to those who did not and that this did significantly affect PPGR to 100% corn flour tortillas.

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LIST OF ABBREVIATIONS

2-DOG	2-Deoxyglucose
3MG	3-O-Methyl-D-Glucose
ANOVA	Analysis of Variance
AS	Available Starch
AUC	Area Under the Curve
BMI	Body Mass Index
CD	Control Diet
CVD	Cardiovascular Disease
FFA	Free Fatty Acid
GI	Glycemic Index
GIR	Glucose Infusion Rate
GL	Glycemic Load
GLUT-4	Glucose Transporter Type 4
HDL	High Density Lipoprotein
HFD	High Fat Diet
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IAUC	Incremental Area Under the Curve
IFG	Impaired Fasting Glucose
IGT	Imaired Glucose Tolerance
IKK	kB Kinase

JNK	c-Jun NH2-Terminal Kinase
LDL	Low Density Lipoprotein
NFG	Normal Fasting Glucose
NGT	Normal Glucose Tolerance
NIDDM	Non-Insulin Dependent Diabetes Mellitus
pGI	Predicted Glycemic Index
PKB	Protein Kinase B

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CHAPTER I

INTRODUCTION

BACKGROUND

It is important for individuals with diabetes or other metabolic conditions to maintain blood glucose at safe levels to prevent the progression of these conditions and diseases. In fact, the first goal listed in the American Diabetes Association's 2008 position statement is for those with diabetes to properly maintain blood glucose within safe ranges (7). It is estimated that there are currently 26.8 million individuals in the United States and 6.8 million individuals in Mexico with diabetes mellitus (74). The key abnormality behind type II diabetes is insulin resistance (63), which is a defect in the insulin signaling pathway, limiting glucose uptake and leading to higher postprandial glycemic responses. The pancreas tries to compensate for the insulin resistance by producing extra insulin to bring down the blood glucose levels, which results in an environment of hyperinsulinemia. Elevated blood glucose and hyperinsulinemia are two conditions that aid the progression of deleterious conditions, such as hypertension, coronary artery disease, neuropathy, renal failure, and blindness (63, 76). The pancreas can only compensate for so long, and if the pancreas continues to be challenged it will start to succumb and impaired glucose tolerance (IGT) and diabetes will ensue (63). An

individual with insulin resistance can make lifestyle changes to slow or prevent this progression, by eating healthy and being physically active (7).

Eating foods with a low glycemic index is one way to limit the impact a meal has on glycemia (37, 38). Another way to limit postprandial glycemic response is to pick foods that have fiber or carbohydrates that digest more slowly. Barcaridi-Gascon et al. (5) found that adding nopal, a prickly pear cactus pad, to breakfast meals reduced postprandial glycemic response. Adding nopal to meals significantly reduced the incremental area under the blood glucose response curve (IAUC) compared to meals without nopal. It also lowered the glycemic index of the meal. Kabir et al. (41) studied the effects of a waxy cornstarch diet and a mung bean diet on rats metabolism. They found that waxy cornstarch had a high glycemic index, was digested faster, and caused higher glycemic and insulinemic responses than the mung bean. Mung bean had a different ratio of certain carbohydrates in the starch causing it to digest slower, reducing its impact on the rats glycemia.

Once the glucose has reached the blood it is easier for an individual with normal or above normal insulin sensitivity to dispose of the glucose in the bloodstream than it is for someone who is more insulin resistant. This is shown by King et al. (45) who found that trained athletes where able to uptake more glucose in response to submaximal insulin concentrations than detrained athletes. However, there was no difference in glucose disposal between trained and detrained athletes in response to maximal insulin concentrations. The authors concluded that a trained individuals are more sensitive to insulin and are able to uptake the glucose more easily that those individuals who were detrained and had lower insulin sensitivity (45). This shows that an individual can help

control glycemia through increasing insulin sensitivity by getting adequate amounts of physical activity (30, 49). The U.S. Department of Health and Human Services recommend that people get 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity aerobic activity every week (1). Healthy People 2010 recommends 30 minutes of moderate-intensity aerobic activity 5 days a week, or 20 minutes of vigorous-intensity aerobic activity 3 days a week for health benefits (25). Both recommend some resistance training each week on top of the recommended aerobic training (1, 25).

STATEMENT OF THE PROBLEM

The carbohydrate content of a food plays a large role in determining how quickly the carbohydrate is digested and enters the bloodstream, and therefore the magnitude of the glycemic response. Both corn and bean contain starch, which is made up of amylose and amylopectin. Amylopectin is large and branched; making it easier for the digestive process to break down, while amylose is more linear, which makes it harder to break down. The ratio of amylose to amylopectin can affect the digestion rate of a particular food item, and this in turn affects the glycemic response (38).

Sayago-Ayerdi et al. (71) found that fresh tortillas had an available starch (AS), or the starch that is digested, content of 63.4%, where as cooked beans had 36.2% AS content. These two food items also differed in the amount of resistant starch, with beans containing a higher percentage. Resistant starch as defined by the authors, is "the sum of starch plus starch degradation products not absorbed in the small intestine of healthy individuals." When these food items were tested for rate of digestion, tortillas were hydrolyzed faster than bean. White bread was used as a reference for comparison. After

180 minutes white bread had a digestion value of 50%. Fresh tortilla had a digestion value of 40%, while beans only had a value of 11%. From these values one would expect the tortilla would cause a higher postprandial glycemic response than the beans. The authors calculated a predicted glycemic index (pGI) and the unstored beans did have a lower pGI of 27%, while fresh tortillas had a pGI of 75%. The higher the value, the greater the expectations are that the food will cause a greater glycemic response. Substituting portions of corn flour in a tortilla with bean (pulse) or nopal may slow the digestion process thereby diminishing the postprandial glycemic response.

The main purpose of this study is to compare the postprandial glycemic response of a tortilla containing 100% corn flour to tortillas containing different ratios of corn, nopal, and pulse (bean) flour. It is hypothesized that Tortillas containing different ratios of corn, nopal, and/or pulses will cause a lower glycemic response than tortillas containing 100% corn. A second purpose of this investigation is to determine if there is a correlation between insulin resistance and postprandial glycemic response to tortilla consumption, and how physical activity influences this relationship. It is hypothesized that those individuals who have lower insulin resistance (HOMA-IR) levels will have lower postprandial glycemic responses to tortilla consumption than those who have higher insulin resistance levels.

SIGNIFICANCE

This research will have important implications in the field of nutrition. There is little information published regarding the amount of tortillas consumed in Mexico. However, it has been reported that as much as 70% of the calories consumed on a daily

basis come from tortillas(71). The present study will investigate if replacing a portion of corn flour with nopal and/or pulse (bean) flour in tortillas will mitigate postprandial glycemic response compared to 100% corn flour tortillas. If tortillas containing nopal and/or pulse flour do reduce postprandial glycemic response, it could give people a healthier alternative to the usual corn flour tortilla. This study also will examine whether or not U.S. Department of Health and Human Services' physical activity recommendations were enough to improve glycemic control.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Insulin resistance is a condition where the body can no longer adequately dispose of glucose in the blood, which can lead to abnormally high postprandial glycemic responses. For those individuals experiencing these high postprandial glycemic responses, there are measures they can take to mitigate these conditions and their consequences. One way to reduce postprandial glycemic response is by adhering to a low glycemic index diet, along with consuming sufficient amounts of fiber(35, 68). Furthermore, avoiding high fatty foods and caffeinated products have been found to cause acute insulin resistance and help in managing glycemia. These products interfere with the insulin signaling pathway and glucose disposal, which can lead to higher glycemic response to meals. Participation in physical activity is another way to reduce postprandial glycemic response. An acute bout of exercise has been shown to increase insulin sensitivity for up to 2 days (54), while exercising consistently over a long period of time (200min/week/8 months) has been shown to increase insulin sensitivity for up to 15 days from the last bout of exercise (6) depending on the health of the individual and the type of exercise. One way in which physical activity is able to increase insulin

sensitivity is by helping individuals lose weight with fat loss. Obesity and excess adiposity are conditions that lead to higher plasma free fatty acids, which have been shown to cause insulin resistance. Eating healthy and getting the proper amount of exercise can help prevent individuals old or young, lean or obese from developing serious health conditions. Health conditions, such as diabetes and CVD can be avoided with proper diet and physical activity by maintaining or increasing insulin sensitivity and keeping postprandial glycemic response to meals down to more reasonable levels. The purpose of this literature review will be to describe how insulin resistance and the type of food consumed can affect postprandial glycemic response, and how insulin resistance is influenced by physical activity.

A. Carbohydrate Digestion

Digestion of carbohydrates starts in the mouth with salivary amylase. After the process of mastication, the food is swallowed and travels to the stomach where the food is digested and broken down before entering the small intestine. The majority of the digestion of starch and disaccharides occurs in the first segment of the small intestine called the duodenum (21). Starch, a common carbohydrate found in food, is made up of two components, amylose and amylopectin. Pancreatic amylase is an enzyme in the duodenum that helps break down amylose by breaking the α1-4 bonds that hold the glucose units together (28). It breaks amylose into two pieces; maltose, which is a disaccharide, and a trisaccharide known as maltotriose (21). Amylase is only able to partially break down amylopectin, because amylopectin not only contains the α1-4 bond,

but also the α1-6 bond, which amylase cannot break (28). Amylase hydrolyzes amylopectin into units called limit dextrins.

As the smaller pieces of carbohydrate move through the small intestine, they continue to be broken down by brush border enzymes. The enzyme maltase breaks up the disaccharide maltose into 2 monosaccharide glucose molecules. Sucrase, a second brush border enzyme, acts on sucrose and breaks it down into the monosaccharides glucose and fructose. A third brush border enzyme, lactase, acts on lactose to break it down into the monosaccharides glucose and galactose. Alpha dextrinase is able to break down limit dextrins into glucose monosaccharides. These monosaccharides are then absorbed by the wall of the small intestine (21, 36, 39, 89) and will enter the blood stream (36).

B. What is postprandial glycemic response?

The increase in blood glucose levels after a meal is termed postprandial glycemic response. Postprandial glycemic response is a measure that can be used to gauge the competency of the metabolic system. Essentially, postprandial blood glucose concentration is made up of two components. These two components are glucose appearance and glucose clearance (7, 29, 72, 75).

B.1 Appearance

Amount: The amount and type of carbohydrate in the meal can play a large role in the rate of appearance. A position statement by the American Diabetes Association titled Nutrition Recommendations and Interventions for Diabetes published in 2008 (7)

claims that often the biggest cause behind the level of postprandial glycemic response is the amount of carbohydrate consumed. Gannon et al. (18) demonstrated the importance of the amount of carbohydrate a meal contains on glycemic response. In their study, subjects (n=6) consumed 3 different mixed meals containing different amounts of carbohydrate, and postprandial glycemic and insulin responses were measured. One meal had a higher carbohydrate content (247g potential glucose, 32g fructose, and 12g galactose). The second meal was supposed to represent the normal American meal, and contained (189 g potential glucose, 19g fructose, and 22g galactose). Finally, the third meal, which was considered the experimental meal, had a lower amount of carbohydrate in the form of starch, and instead contained more sucrose (134g potential glucose, 75g fructose, and 7g galactose). They found that the high carbohydrate meal resulted in a slightly higher postprandial glycemic response than the American meal and that these two caused a much higher glycemic response than the experimental meal. When the glycemic responses to the three meals were compared using the mean 24-h integrated glucose area response, though no significant the high carbohydrate meal resulted in a 34% higher glucose concentration than the American meal. While the experimental meal was significantly lower than both of the other two meals. Insulin response was a little higher after consumption of the American meal than it was to the high carbohydrate meal, and the experimental meal had much lower insulin response than either of the other two meals. In contrast, Pearce et al. (60) conducted a study testing whether or not spreading the amount of carbohydrate in each meal evenly throughout the day was easier on glycemic response compared to consuming large amount of carbohydrate at a single meal (breakfast, lunch, or dinner). They found a weak correlation (r=0.40) between the amount

of carbohydrate in a mixed meal and postprandial peak glycemic response. They calculated that the amount of carbohydrate was only able to account for 16% of the variance seen in postprandial peak glucose response.

Type: The second factor affecting appearance is the type of carbohydrate consumed (7). The type, or source of carbohydrate that a food contains can affect the rate at which it is digested and therefore affect the glycemic response (38). For instance, waxy cornstarch, which had an amylose-amylopectin ratio of 5-995g/kg was digested faster in rats than mung bean that had an amylose-amylopectin ratio of 320-680 g/kg (41). The main cause for this difference in digestion rate was the fact that cornstarch had a lower amylose to amylopectin ratio. Amylopectin is a large, highly branched polymer, which gives it more surface area for enzymes to work on, and therefore it is broken down faster (85). Amylose is a linear starch that is harder for enzymes to breakdown for absorption (38, 85).

The physical form of the food also affects the digestion rate. Any industrial process applied to the ingredients in the foods in preparation, and the culinary method used to prepare the food, all influence the finished form of the food (4, 90). One study that clearly shows the importance of the type and form of carbohydrate to postprandial glycemic response examined the glycemic index and digestion rates of legumes and cereals (rice and spaghetti) both in-vitro and in-vivo (4). The in-vivo portion of the study involved 10 healthy men 21-24 years old. The subjects were fed two meals, one was lentil based and the other was bean based. Postprandial glycemic response to these meals was measured for 2 hours. The purpose of the in-vitro portion of the study was to determine the digestion rate of each food type. The lentils and beans were cooked

individually and in combination with the different cereals in a variety of manners to test different effects on digestion rates and glycemic response. This study found that legumes ground up in small particles for soups digested faster than legumes prepared as a whole grain. The authors suggest that this is caused by the grinding process, which increases the surface area for the enzymes to act on. This allows the enzymes to break the carbohydrates down faster and the rate of digestion goes up. The authors also found that the bean-spaghetti combination digested very slowly. This may be due to the high percentage of lente digestion starch found in spaghetti. Additionally, through combining data from the in-vivo and in-vitro portions of the study it was found that the glycemic index calculated from the bean based meal and the lentil-based meal was related to the rate at which they were digested.

It appears that the amount of carbohydrate, or at least the available carbohydrate, contained in a particular food is not the only factor affecting postprandial glycemic response. As mentioned earlier, the type of carbohydrate and preparation of the food also plays a large roll in the postprandial glycemic response. There have been attempts to create a method of measuring/predicting a food's ability to impact glycemic response (57). Jenkins et al. developed a concept known as the glycemic index (GI) to classify a food based on the postprandial glycemic response (37). Wolever et al. defines GI "as the incremental area under the glucose response curve for a 50-g carbohydrate portion of a food expressed as a percentage of that after 50g carbohydrate from white bread is taken by the same subject" (88). Said another way, GI is determined by measuring the increase in blood glucose concentration above fasting levels over a two-hour period after consuming a food that usually contains 50 grams of carbohydrate (57). Then this value is

divided by the glycemic response to a reference food such as 50 grams of glucose or white bread then multiplied by 100 (7, 29). There is another measurement that has been developed that takes into account both GI and the amount of carbohydrate in a food or meal. This system of measurement is known as the glycemic load (GL), which is calculated by taking the GI and dividing it by 100. This value is then multiplied by the amount of available carbohydrate, and then this value is multiplied by the total weight of the food (57). High postprandial glycemic responses can increase risk for developing cardiovascular abnormalities and diabetes (8). For this reason it is important to keep meals to a low GI and GL (27, 80). These systems of classifying foods based on their impact on postprandial glycemic response can be very helpful to diabetics who need to carefully monitor there blood glucose concentrations (8).

Wolever and Bolognesi (88) report that both the amount and type of carbohydrate are equally important in impacting postprandial glycemic response. Their study involved 7 individuals (3 females, 4 males) approximately 25 years of age. In this study, they measured postprandial glycemic response to barley, spaghetti, and potato at 3 different carbohydrate doses each. Those 3 carbohydrate doses were 25, 50, and 100 grams. They also tested white bread with each participant at 4 different carbohydrate doses of 25, 50, 75, and 100 grams. Postprandial glycemic response to white bread was used to calculate the glycemic index (GI) of each food type. They found that both the amount and type of carbohydrate significantly affected the areas under the curve for both capillary and plasma glucose concentrations. Furthermore, they both significantly affected plasma insulin concentration. The postprandial glycemic response was highest for the potato, and barley had the lowest response. Glycemic response also increased in response to

increasingly higher carbohydrate load (25-100g); however this increase was not linear. The amount of carbohydrate was responsible for 57% of the average change in glycemic response as measured from the capillary and 50% as measured from plasma glucose. They found that the GI values of the barley, spaghetti, white bread, and potato were significantly related to the glycemic and insulin responses to each food. The GI was responsible for 60% of the average change in glycemic response as measured from the capillary and 64% as measured by plasma glucose. When they combined the GI with the amount of carbohydrate of each food type, they found that it accounted for 85 to 94 percent of the average change in glycemic response and insulin response.

Being able to judge how an entire meal impacts a particular individual's postprandial blood glucose is not as simple as examining foods that are mostly, or entirely composed of carbohydrates. Food is composed of varying degrees of fat, protein, and carbohydrates and each individual has a different response to these foods. There are several factors both with the individual and with the food the consumed that influence the rate of digestion and therefore the glycemic response. One way that the type of carbohydrate affects appearance is through the rate of gastric emptying. The slower the gastric emptying, the lower the post meal blood glucose concentration (29). Even the state of an individual's glycemia can play a role on the rate of gastric emptying. For example, if an individual is hyperglycemic then that individual may have a slower gastric emptying rate after consuming food (29). Often, people eat meals that have numerous and mixed nutrients, which can affect digestion rate. The length of the postprandial glycemic response period depends on the macronutrient content of the food consumed. For

example, meals high in carbohydrate may have a postprandial period of 2-3 hours, while high fat meals may have a postprandial period lasting approximately 8 hours (23).

B.2 Clearance

A healthy human body is able to minimize postprandial glycemic responses and keep the glucose concentration in the blood at safe levels. An important process in removing glucose from the blood after a meal is the insulin stimulated insulin signaling pathway, which takes place in many different tissue types throughout the body. The most important tissue for glucose removal is skeletal muscle, which has been shown to be responsible for 75% of the body's insulin stimulated glucose clearance (52). As the concentration of glucose in the blood rises after a meal the beta cells in the pancreas releases insulin into the blood stream(44, 76). Insulin is an anabolic hormone, which causes carbohydrates, proteins, and lipids to be stored rather than broken down(69). Insulin will then bind to insulin receptors located on the tissues and cause the tissue cells to take in glucose and either metabolize it or store it (44, 76). This process keeps these macronutrients in the cells rather than in circulation (69). It only takes minutes for the body to respond to the increase in blood glucose after a meal. Once insulin has bound to it's receptor it activates the insulin signaling pathway and sends a protein called GLUT-4 to the membrane surface to transport glucose across the plasma membrane of the cell (65). GLUT-4 transporters are located in skeletal muscle, adipose tissue, and cardiac muscle (34, 69). During the basal state most of the GLUT-4 transporters are found within vesicles inside the cell. When activated, a large number of GLUT-4 transporters translocate to the plasma membrane to uptake glucose (14, 69, 78, 79). The cell needs

GLUT-4 transporters to transport carbohydrate across the plasma membrane, because the plasma membrane is made of a lipid bilayer. Carbohydrates cannot pass through this lipid barrier unaided (76). GLUT-4 transporters bring in the majority of the glucose into the muscle. The number of transporters that translocate to the plasma membrane is a major determining factor in how much glucose gets transported (58). It has been found that when previously endurance trained men, cease training for 10 days that GLUT-4 protein content decreases (52). This has also been found by Houmard et al. (32) who studied how 2 weeks of training cessation in exercise trained subjects affected GLUT-4 content. They found that 2 weeks of training cessation reduced GLUT-4 content down to baseline values before 12 weeks of exercise training. This reduction in the amount of GLUT-4 correlated with reductions in insulin action, which also returned to baseline values. The authors believe that the reduction in the amount of GLUT-4 plays a role in the decreased insulin action. The insulin signaling pathway and glucose metabolism process are extremely complex, because there are many factors that can affect and interfere with the way they function, such as the foods we consume, amount of physical activity performed, body composition, and even the aging process.

C. Insulin Resistance

The term insulin resistance is used to describe a problem with glucose uptake as it pertains to the insulin signaling pathway. There are multiple causes of insulin resistance, and there are certain causes that only influence insulin resistance for a short while. These causes can often be something that is consumed, such as caffeine, or high fat foods. Other

causes of insulin resistance are more chronic and are usually the result of some condition or disease such as obesity or the aging process.

C.1 Acute

Caffeine: It's estimated that 80% of Americans consume some form of caffeine on a daily basis. It is the number one choice of drug in the world (24). In western society the average individual consumes 200-400mg of caffeine in a day (64). With caffeine being such a popular stimulant, many researchers have conducted studies examining its effects on glycemic response, insulin sensitivity, and glucose disposal. Caffeine has been found to acutely decrease glucose disposal (22, 43, 84). Battram et al. (10) measured glucose kinetics with the isoglycemic-hyperinsulinemic clamp technique so they could measure the acute effects of caffeine on glucose metabolism while holding plasma glucose and insulin concentrations constant. Using this method they found that caffeine acutely reduces insulin stimulated glucose disposal, but had no affect on endogenous glucose production (10). The effect of caffeine goes beyond just glucose disposal. In a study by Greer et al., the effects of caffeine (5mg/kg body wt) on whole-body glucose disposal using the hyperinsulinemic-euglycemic clamp technique were tested. Subjects were lean, sedentary men aged 25± 0.5 years. They found that whole body glucose disposal was 24% less after caffeine consumption compared to a placebo. The caffeine treatment also caused the skeletal muscle to store 35% less carbohydrate than the placebo (22). Caffeine also has been shown to amplify the effects of a meal that normally would not cause a large glycemic response. Moisey et al. demonstrated this (56), by comparing caffeinated and decaffeinated coffee to examine the effects on postprandial glucose and

insulin responses. The subjects consumed meals that had both high and low glycemic indexes. They found that caffeinated coffee decreased insulin sensitivity by 29%. Caffeinated coffee compared to decaf greatly increased glucose and insulin area under the curves. Even after consuming a low glycemic meal, the differences in response between decaffeinated and caffeinated coffee in glucose area under the curve was 41 mmol•2h/L compared to 131 mmol•2h/L respectively (56).

High fat foods: Another consumable item that can acutely cause insulin resistance are foods with high fat content. It has been found that feeding rats a high fat diet (HFD) leads to insulin resistance(19). It is believed that the free fatty acids activate c-JUN NH_2-terminal kinase (JNK) and inhibitor kB kinase (IKK) through an signaling pathway in adipose tissue that ultimately leads to the inhibition of the IRS-1 receptor (19). HFDs have even been shown to reduce the effects of exercise on insulin sensitivity. Tanaka et al. (82) studied rats that were fed either a HFD or a control diet (CD) for 4 weeks. Upon completion of the four week diet the rats either remained sedentary or they completed a 1 hour bout of aerobic exercise. They found that the HFD impaired insulin dependent and insulin independent glucose tolerance compared to the CD. The HFD decreased the effect of exercise on insulin-stimulated 3-*O*-methyl-D-glucose (3MG) by 25%. The HFD also affected insulin dependent 3MG uptake in sedentary animals, decreasing uptake by 59% compared to CD rats. While exercise significantly increased glucose uptake in both treatment groups compared to the sedentary control, the increase was significantly less in the HFD group. The mechanisms behind this finding are not known.

High fat diets also have been shown to cause insulin resistance in humans. Homko et al. (31) investigated how the acute (4hrs) elevation of FFA levels into high physiological ranges affected insulin sensitivity in healthy normal weight women. They found that the elevated FFA concentration decreased insulin sensitivity, inhibiting insulin stimulated glucose disposal by 40%. Elevated FFAs also can interfere with insulin's ability to suppress endogenous glucose production. This study shows that elevated FFAs not only cause peripheral insulin resistance, but also hepatic insulin resistance (31).

C.2 Chronic

BMI: One chronic condition that has been associated with insulin resistance and a whole host of other physiological abnormalities is that of obesity. As already discussed in this section, FFAs floating around in the blood stream have been shown to cause insulin resistance. There is a correlation between increasing levels of adiposity and increasing concentrations of free fatty acids in the blood. Obesity is a condition that is striking people of all ages, all over the United States (73). There is a great deal of scientific evidence showing just how deleterious this condition can be to a human's health (2, 12).

Many studies have examined the association between higher amounts of body fat and insulin resistance. Abbasi et al. (2) examined three different weight classes: normal weight (18.5-24.9), overweight (25.0-29.9), and obese (30.0-34.6). They found that the higher the BMI the higher the steady state plasma glucose concentration and insulin resistance. The authors stated that while a higher BMI does lead to higher insulin resistance people can be overweight or obese and still be insulin sensitive, just as those people at a normal weight can be insulin resistant (2). Boden et al. (12) found that FFA

inhibited total insulin stimulated glucose disposal by 40-50% in subjects with non-insulin dependent diabetes mellitus (NIDDM) with an isoglycemic clamp. FFA also interfered with other insulin stimulated actions such as glycogen synthesis, glycolysis, and carbohydrate oxidation. Inhibition of glucose uptake seemed to be dependent upon the concentration of free fatty acids in the plasma. When FFA concentrations were raised to high physiological levels this inhibition grew to nearly 100%, showing that insulin action was affected in a dose dependent manner by FFA (12).

Acipimax, a drug that is able to lower FFA levels in the blood, may help investigators determine the noxious health consequences that FFAs have on the body. The effects of Acipimax were studied in an investigation by Santomauro et al. (70) using 9 healthy non-obese and 34 obese subjects. Some obese subjects had diabetes and some had impaired glucose tolerance. Acipimax lowered plasma FFA levels approximately 60% in lean individuals compared to taking a placebo. Compared to taking the placebo, Acipimax lowered FFAs by 57.9±4.1, 56.5±6.8, and 70.4±3.4% in obese non-diabetics, obese impaired glucose tolerant, and obese diabetic subjects, respectively. Obese individuals with impaired glucose tolerance and diabetes had significantly higher basal insulin levels after taking the placebo than lean subjects, but all four groups had significant insulin reductions of 50% after taking Acipimax. Acipimax also lowered fasting glucose concentrations and resulted in a lower area under the glucose curve during a 2-hour oral glucose tolerance test in all groups, especially in the diabetic group that had moderately elevated basal glucose levels. Obese subjects had 50% lower glucose infusion rates (GIR), which is reflective of insulin stimulated glucose uptake, than lean subjects after taking a placebo. Obese individuals with impaired glucose tolerance and

diabetes had 70% lower GIR compared to the lean subjects. Acipimax increased GIRs by 23±4% in lean control subjects, 131±13% in obese individuals, and 103±27% in diabetic subjects. That is a two-fold increase in insulin stimulated glucose uptake. They found an inverse linear relationship between FFA levels and GIR. Overall, Acipimax lowered FFA concentration and this decrease was associated with an increase in insulin stimulated glucose uptake. Authors believe that the 131% increase in insulin stimulated glucose uptake was enough to implicate FFAs as the cause of insulin resistance. However, the increases in the impaired glucose tolerant and diabetic groups were not high enough. They still remained below 50% of that of the lean individual's insulin stimulated glucose uptake measured values. As a result, the authors attribute the majority of the insulin resistance found in these two groups to FFA (70).

Age: The aging process is often associated with an increased level of insulin resistance, although it is difficult to decipher the reason behind this association. It may be due to the aging process itself or the possibility that aged individuals tend to be more sedentary and have increased percentages of body fat (15). In 2003 Short et al. (77) conducted a study investigating the effects of a 5 day a week, 16 week, moderate intensity aerobic exercise program on young and old participants (21-87 years old, n=65) compared to a control (n=37). At baseline they found that body fat increased with increasing age, and insulin sensitivity decreased by 8% for every ten years in age. Insulin sensitivity increased with the exercise program, but as the age of the subjects progressed, the increase in insulin sensitivity became smaller. For example, there was a 72% increase in the younger group, a 20% increase in the middle aged group, while the older group

only experienced an insulin sensitivity increase of 5%. This study examined many possible causes for these differences in insulin sensitivity including changes in visceral and subcutaneous fat, mitochondrial oxidative capacity, and changes in the levels of GLUT-4 in the muscle. It was determined that none of these variables were responsible for these differences, and that the response seen in insulin sensitivity to exercise was age-dependent (77). Dela et al. found that insulin stimulated glucose uptake was similar between the different age groups once corrected for fat free mass and that the rate of glucose uptake responded to training similarly in both old and young subjects (15). This finding is in agreement with Karakelides et al. (42) who studied insulin sensitivity, body composition, and protein in the muscles of both lean and obese elderly individuals. These results were then compared to the same measurements taken on young controls. They found that insulin sensitivity was lower in obese subjects compared with lean individuals independent of age. Independent of obesity, they found that there was not a difference in insulin sensitivity between the old participants and young controls. It was postulated that declines in insulin sensitivity associated with aging is really due to decreases in physical activity and increases in adiposity (42). This decline in insulin sensitivity experienced by elderly affects their postprandial glycemic response to food as shown in a study by Basu et al. (9). They studied postprandial glycemic responses and insulin sensitivity in 67 healthy elderly (70.1±0.7 years) subjects as well as in 21 young (23.7±0.8 years) subjects. The elderly group had a higher BMI and higher percentage of body fat compared to the younger controls. Peak glucose response to both the mixed meal and the glucose injection were higher in the elderly group compared to the young. Fasting and peak insulin responses were similar between the two groups, but overall insulin response was greater

in the elderly group. The authors determined that problems in insulin secretion, insulin action, and clearance lead to a decrease in glucose tolerance seen in the aging. In this study, insulin action post-meal and in response to glucose injection was lower in the elderly group compared to the controls. The strongest determinant of insulin action in this study was body fat. The deficiencies in insulin secretion occurred with both the meal and glucose injections, which indicates that it is a problem with β-cell function. In summary, insulin sensitivity and glucose tolerance decrease with aging, which seems to be caused by multiple factors, including increased adiposity interfering with insulin action and altered β-cell function in response to foods (9).

D. Pathophysiology

There is a stage between normal glucose tolerance and diabetes termed pre-diabetes, which has 2 subcategories. One is impaired fasting glucose, which is when an individual has elevated plasma glucose levels after an overnight fast. Concentration levels of (100-125 mg/dL) are classified as impaired fasting glucose (IFG). Impaired glucose tolerance is the second subcategory and is defined as having an above normal glucose response to carbohydrates consumed. An individual who participates in a 2-hour glucose tolerance test and has a glycemic response of (140-199mg/dl) will be classified as impaired glucose tolerant (IGT). Either IGT or IFG can increase risk for diabetes and they both seem to have independent associations with health abnormalities (53). Meyer et al. (53) studied 402 non-diabetic individuals, who after an oral glucose tolerance test were put into the following groups/categories: normal fasting glucose (NFG)/normal glucose tolerance (NGT), impaired fasting glucose (IFG)/NGT, NFG/impaired glucose

tolerance (IGT). Of the 3 groups IFG/NGT had the highest fasting glucose. The NFG/IGT group had significantly higher fasting plasma glucose concentrations than the NFG/NGT group, although this difference was still small. However, the NFG/IGT group had the highest plasma glucose concentration during the oral glucose tolerance test. Fasting insulin was similar in all 3 groups. In addition, both conditions affect pancreatic insulin secretion. While IFG is associated with reduced first phase insulin secretion, IGT is associated with reduced first and second phase insulin secretion. However, IGT is associated with decreased insulin sensitivity while IFG is not. Overall, the authors found that IFG and IGT have different and separate health consequences, and they believe that the consequences of these two conditions are additive for those who have both.

Measuring IFG and IGT is very important in gauging an individual's risk for other diseases. There is an association between IGT and cardiovascular disease (CVD), and this association is stronger than the association between IFG and CVD (72). Tominaga et al. (86) has described IGT as being a risk factor for CVD. IGT is diagnosed via oral glucose tolerance test, while measuring IFG is as simple as taking a blood sample after an overnight fast (86). Tominaga found that individuals with IGT had significantly lower survival rates from CVD compared to those with normal glucose tolerance. There was no difference between IFG and NFG when examining survival rates of those with CVD. This study done by Tominaga was conducted before the American Diabetes Association lowered the bottom half of its criteria range for IFG from 110 mg/dL to 100mg/dL (86). Tia et al. (81) believes that lowering the criteria for diagnosis with IFG will help identify those with IGT. It may also help identify those with increased risk of diabetes. But, even with lowering the criteria for IFG, it still is not as good a predictor of

disease as IGT. The authors believe that lowering the limit is causing younger individuals to be diagnosed with IFG and is lowering the predictive power for ischemic heart disease. In their study 63.5% of those with IFG also had at least one factor of metabolic syndrome (81). Ilany et al. (33) also found a link between IGT and the cardiovascular abnormalities. They examined patients admitted to their intensive coronary care unit at Sheba Medical Center in Israel for defects in glucose metabolism and insulin action. Each participant in the study had an oral glucose tolerance test to assess abnormalities related to glucose metabolism. They found that more than 80% of those patients with acute CAD also had some sort of glucose metabolism abnormality. These authors also found that IGT and IFG are both insulin resistant conditions when compared to those with normal glycemia. However, IGT is a more insulin resistant condition than IFG. Also, diabetes mellitus and IGT have been found to be risk factors for CAD and in this study these two conditions were found in cardiac patients far more commonly than they are found in the normal population. The investigators conclude that the majority of their patients with CAD also have some form of dysglycemia, and that these conditions may be risk factors of CAD. For this reason they believe it is important to control postprandial glycemic response to prevent cardiovascular disease (33).

One way to control postprandial glycemic response is to maintain a diet with low GI and/or low GL values. This was found to be important in a meta-analysis by Barclay et al. (8), where 37 prospective cohort studies were analyzed to determine if there is an association between chronic disease and glycemic index (GI), and/or glycemic load (GL). They found that there is a positive association between risk of developing type 2 diabetes, and diets that have higher GI or GL values. They also found that these two measurements

also are independently associated with heart disease. In prevention of certain diseases such as type 2 diabetes the authors found that low GI or GL diets are just as important, maybe more so, than those diets that emphasize high fiber or whole grains. The authors claim that the data from this meta-analysis supports their hypothesis that elevated postprandial glycemia is a contributing factor to chronic disease (8). While Barclay et al. note that low GI/GL diets are just as important as diets with high fiber, Salmeron et al. (68) found that combining the two also can prevent disease. Salmeron et al. analyzed the diets and health of 42,759 men in a population study. A follow up study was conducted 6 years later to determine health and incidence of type 2 diabetes mellitus. There were 523 cases of type 2 diabetes that developed over that 6-year time span. After analyzing diets they found that total fat intake and total fiber intake was not associated with risk of diabetes. However, cereal fiber was inversely associated with risk for diabetes. It also was found that total carbohydrate was not associated with the risk of developing type 2 diabetes, but the GI of foods consumed was associated after adjusting for consumption of cereal fiber. Diets that had high glycemic loads along with lower cereal fiber were identified as risks for type 2 diabetes mellitus. The authors mention that hyperinsulinemia is one of the best predictors of type 2 diabetes. And diets with higher glycemic index require more insulin production, especially in insulin resistant individuals such as those with diabetes. The authors believe that diets with high glycemic loads and low amounts of cereal fiber are more taxing on the pancreas, which has to produce more insulin. Glucose tolerance appears normal as long as the pancreas can meet the demands and keep compensating for the insulin resistance. Eventually, diabetes develops when the pancreas can no longer meet these demands (68).

E. Physical Activity

The consequences of unhealthy eating and obesity have already been discussed in this review. Sedentary behavior is one contributing factor of obesity, which, in turn, can lead to multiple health abnormalities including metabolic syndrome. Dunstan et al. (17) conducted a study investigating the associations between television viewing time and glycemic abnormalities. This study involved 8,357 (3,781 men; 4,576 women) non-diabetic adults in Australia. A questionnaire was used to assess physical activity and television viewing time. They found an inverse relationship between physical activity and risk of glucose metabolism abnormalities in both men and women. Furthermore, in women there was a significant association between television viewing time and abnormalities in glucose metabolism. After adjusting for waist circumference, the association between TV time and problems with glucose metabolism lessened in women, but the association was still stronger than it was in men. IGT is one abnormality shown to have a positive association with TV time in women, but not in men. IFG on the other hand did not show any significant association with TV time in either gender. The authors found that getting 150 minutes or more of physical activity a week significantly reduced women's risk of developing IGT. On the other hand, men and women who watched more than 14 hours of TV a week had significantly higher risk of developing type 2 diabetes. The authors suggested that insulin resistance in the muscle was responsible for the elevated 2-hour post-challenge plasma glucose levels seen in individuals who watched more TV. This is possible because sedentary individuals don't use their muscles as often

or as strenuously as active people, and the muscle is responsible for a large portion of postprandial glucose uptake.

It appears that physical activity cessation has a major impact on insulin sensitivity and it begins to decrease within days of physical activity cessation. Kump and Booth investigated the effects of decreasing the amount of exercise on insulin sensitivity in rats and the mechanisms behind it. Rats had running wheels in their cages for 3 weeks. Rats were divided into four different treatment groups, and then different groups of rats had their wheels locked at different time periods. The four treatment groups were as follows: a sedentary group, a group that had wheels locked for 5 hours before being killed, another for 29 hours, and a final group that had locked wheels for 53 hours before being killed. After dissecting the epitrochlearis muscle it was incubated in a solution to measure 2-Deoxyglucose (2-DOG) uptake with sub-maximal insulin stimulation. The rat group with the wheel locked for 53 hours showed similar decreases in 2-DOG insulin stimulated uptake as the sedentary group. Insulin receptor binding had similar values between the 5 and the 29-hour rats, but decreased to the same levels as the sedentary group by the 53 hour training cessation mark. They also found that GLUT-4 levels decreased to that of the sedentary values at some point between 29 and 53 hours of training cessation (48). In another rat study conducted by Gollisch et al. (20) rats were fed a high fat diet for 4 weeks. In this study the trained rats gained less weight, and had lower blood glucose concentrations compared to the sedentary control. The trained group not only avoided the weight gain, but they also did not gain the adiposity that the sedentary group gained. This adiposity gained in the sedentary group includes the number of adipose cells, cell size, and fat pad mass. The HFD also caused insulin resistance in the sedentary rats, but not in

the trained rats. The authors believe that the development of insulin resistance may have been caused by increased adiposity. Exercise training improved insulin sensitivity in trained rats, but not in sedentary rats. Similar results also have been seen in human studies. A study conducted by Krogh-Madsen et al. (47) examined how reducing the amount of an individual's ambulatory activity affects insulin sensitivity. They found that reducing subjects' number of daily steps by 85 percent significantly decreased peripheral insulin sensitivity. Insulin sensitivity is improved by various activities and exercises, but it is unclear which type of exercise is most effective at increasing insulin sensitivity. Exercise takes on a large variety of activities. Two large categories could be resistance training and anaerobic activities, and the other could be aerobic activities, such as jogging or biking.

E.1 Weight Training/Anaerobic

Since skeletal muscle is already one of the largest sites for glucose disposal (52), one would think that making this area even larger via resistance training would thereby improve glucose disposal. Although resistance training has not been investigated nearly as much as endurance training, it has been studied in a variety of ways. Koopman et al. (46) simply wanted to see if there was an acute effect of resistance training on insulin sensitivity. Their investigation was comprised of 12 healthy, normal weight males approximately 23 years of age. They found that one bout of exercise involving 5 different activities can improve whole body insulin sensitivity by as much as 13 percent and that this affect lasts at least 24 hours. Holten et al. (30) wanted to examine what effects a longer resistance training program had on insulin sensitivity. They tested 10 subjects with

type 2 diabetes mellitus (62±2 years of age) and 7 healthy controls (61±2 years of age). Subjects worked out one leg 30 minutes a day, 3 times a week, for 6 weeks with high repetitions using light weight. Insulin sensitivity was tested 16 hours after the last bout of training. They found an increase in insulin sensitivity in both groups and they don't believe that it is entirely due to an increase in fat free mass. Muscle biopsies showed increases in GLUT-4 proteins, insulin receptor protein expression, and increased levels of PKB in both groups. They believe that the increase in insulin action was caused by multiple factors including increases in these muscle proteins. In another study Miller et al. (55) examined the effects of a resistance training program in 11 healthy males between the ages of 50 and 65. Subjects resistance trained 3 times a week for 16 weeks. Participants increased their fat free mass and experienced a 1.6% decrease in body fat. In addition, glucose disposal improved by more than 20% after the weight training intervention. Training also caused fasting plasma insulin levels and insulin responses to oral glucose tolerance test to decrease despite fasting glucose levels staying approximately the same. Although the cause of the improvement is unknown, the authors do not believe the small changes in body fat, or gains in fat free mass are entirely responsible for the increase in insulin action. In a final example, Polak et al. (62) studied 12 obese men approximately 50 years of age. This group included 5 diabetics, 4 individuals with impaired glucose tolerance, and 3 subjects with normal glucose tolerance. These subjects trained 1 hour a day, 3 days a week, for 12 weeks and performed 12-15 reps at 60-70% of their 1RM. However, subjects did not see changes in their weight or body composition. After correcting for kg of fat free mass they found that subjects had increased glucose disposal by 24.4% and their insulin sensitivity by 30.8%.

Again, the authors are not sure what exactly is responsible for these improvements. These studies show that relatively moderate intensity resistance training only 3 days a week can improve glucose disposal and insulin action. This is an important finding, which could enable many overweight, previously sedentary individuals to make improvements in their health without performing strenuous activity.

E.2 Aerobic Training

Aerobic activity is another popular form of exercise that has been shown to be advantageous for glycemic health. LeBlanc et al. (49) compared a highly trained group that had a VO_2 max above $60ml \bullet kg^{-1} \bullet min^{-1}$ to a group that performed below this level. The trained group had significantly lower basal glucose and insulin levels than the lesser trained group, as well as significantly lower blood glucose levels during the first 20 minutes of an intravenous glucose tolerance test. However, during the subsequent 100 minutes there was not a difference between groups. In addition, during the glucose tolerance test the peak concentration of plasma insulin levels in the more highly trained group was less than half of that of the peak of the second group (30 mμ/ml vs 75 mμ/ml, respectively). The trained group had significantly greater insulin binding than the lesser trained group. The authors found a negative relationship between percentage of body fat and percentage of insulin bound. The lesser trained group had 14.8% body fat, while the higher trained group only had 4.4%. There also was a correlation between VO_2 max and percentage of insulin bound, with this correlation becoming significant with the less trained subjects. LeBlanc and colleagues (49) believe the higher level of training decreases body fat, and therefore increases insulin sensitivity. Heath et al. (26) came to a

different conclusion regarding what is responsible for changes in insulin sensitivity with training. They investigated the effects of training cessation on insulin sensitivity in individuals that normally trained at least 45 minutes a day, 5-7 days per week for at least 6 months prior to the sedentary period. The subject's glucose tolerance decreased significantly over the 10-day sedentary period. However, after 10 days of training cessation, just one bout of exercise on day 12 increased glucose tolerance enough that levels nearly returned to those seen during training. They show that training cessation caused insulin sensitivity and insulin binding to decrease significantly, while there were no significant changes that took place in either VO_2 max or in body fat. For this reason, the authors do not believe either VO_2 max or body fat was responsible for the increases seen in insulin sensitivity with training. Heath et al. (26) suggested there were two possibilities for the blunted insulin response to glucose loads after training. One reason may be due to the pancreas limiting its insulin secretion to the same glucose load more so than it did before training or during training cessation. As mentioned earlier, LeBlanc et al. also found lower insulin secretion in trained subjects (49). Heath et al. (26) believed a second reason was that there was a training adaptation in the skeletal muscle and other tissues that allowed for faster rate of glucose uptake, even with reduced insulin secretion levels so that more insulin was not necessary. One bout of exercise after 10 days of inactivity nearly restored the insulin binding, and insulin response to glucose loads to trained levels.

A more recent study (66) examined the differences in beta cell function and insulin sensitivity between a group of female endurance athletes (18-69 yrs. old) and a control group of sedentary women (18-50 yrs. old). All subjects had a BMI level below

25 kg/m², although the athletes had significantly lower body fat percentages and body weight, and significantly higher fat free mass compared to the sedentary control group. The trained group, on average, trained 5 to 6 days a week for a total of 12 hours a week. To test β-cell function and glucose disposal, the hyperglycemic clamp and hyperinsulemic-euglycemic clamp were used. This investigation found that there was an association between the amount of body fat a subject had and their insulin response during a state of hyperglycemia. Physical activity helped preserve the first and second phases of insulin secretion, and insulin sensitivity throughout the different age groups. It also was found that there was a positive correlation between the amount of muscle mass a subject had and glucose uptake, while there was a negative relationship between percentage of body fat and glucose uptake. Compared to the control group, the trained group had greater glucose disposal and this increased ability to uptake glucose was similar between the different age groups within the trained group. The athletes preserved or increased their insulin sensitivity, pancreatic function, and glucose uptake compared to the sedentary controls because they maintained fat free mass and decreased body fat through physical activity. This was especially true in the older athletes compared to the older sedentary controls. This study demonstrates that being physically fit cannot only give an individual better insulin sensitivity and glucose disposal, but that these benefits will last throughout a lifetime if physical fitness is maintained.

E.3 Aerobic vs. Resistance Training

It is important to understand how aerobic and resistance training activities affect insulin sensitivity. It would be beneficial to know if one type of activity is more effective

at increasing insulin sensitivity than the other, or if a combination of the two is most effective. Poehlman et al. (61) conducted a study that examined the effects of both types of activity on insulin sensitivity. They studied the effects of resistance training and endurance training on sedentary women aged 18-35 with a BMI less than 26. Women participated in a 6 month resistance training program 3 days a week, or a 6 month endurance training program 3 days a week at an intensity of 75-95% of their max heart rate for 20-60 minutes. Body weight, BMI, and fat free mass all increased in the resistance trained women, but not in the endurance or control groups. Glucose disposal increased in both endurance (16%) and resistance (9%) trained groups, but not in the control group. The authors suggested that the improvement in glucose disposal seen in the resistance trained group was probably due to the increase in muscle mass, because once these values were corrected for kg per fat free mass no improvement was seen. It is not believed that the increase was due to any changes that would increase the muscle's sensitivity to insulin. The authors hypothesized that the endurance group would increase their insulin sensitivity by decreasing body fat percentage. This was not the case, however, instead the endurance group saw glucose metabolism improvements, without changes in body fat. The authors stated that this improvement seen in the endurance group was due to changes within the muscle that improved glucose metabolism. On the other hand, Ahmadizad et al. (3) saw decreases in insulin resistance through resistance and endurance training without significant changes in body weight, hip to waist ratio, or BMI. Twenty-four obese sedentary men approximately 40 years old participated in this study. The resistance training group performed circuit training for 50-60 minutes a day, 3 days a week, for 12 weeks. They performed 4 sets of 11 different exercises at 50-60% of

their 1 RM. The endurance training group trained for 20-30 min a day, 3 days a week for 12 weeks at 75-85% of their HR max. Although these interventions did not result in changes in body weight or BMI, subjects did see a significant drop in percentage of body fat. Insulin resistance as assessed by HOMA-IR decreased by 35.7% in the endurance group and 38.5% in the resistance training group.

E.4 Intensity/Duration

The type of training may not be the most important influencer on insulin sensitivity; intensity or duration of exercise may also be critical factors. Bajpeyi et al. (6) investigated the effects of different exercise prescriptions on insulin sensitivity and related variables. Their study used sedentary overweight to obese individuals who were randomized into one of three groups: low-volume/moderate intensity, low-volume/vigorous intensity, and high-volume/vigorous intensity. The aerobic training intervention lasted 8 months and then participants were asked to cease training for 15 days. Insulin sensitivity increased in all three groups. They found that after 15 days from the last bout of training, the low-volume/moderate intensity and high-volume/vigorous intensity groups still had significantly higher insulin sensitivity (30%) compared to baseline levels, where as the low-volume/vigorous intensity group did not. These results may be due to the 2 groups having a training regime that required higher volume and duration (3-4 times a week of 60 minute sessions compared to 3 times a week of 45 minute sessions). The authors believed the extra training duration might have been behind the improved insulin action lasting longer. This is especially so, considering the low-volume/moderate intensity had the same volume as the low-volume/vigorous

intensity group, but the low volume/moderate intensity group saw more improvement in insulin sensitivity than the low volume/vigorous intensity group. Because they were training at a moderate intensity, it took them longer to complete the low-amount of volume compared to the vigorous group (6). These findings contrast those of DiPietro et al. (16) who compared low (50% VO_2 peak), medium (65% VO_2 peak), and high (80% VO_2 peak) intensities at a given volume (300 kcal/session) for 9 months in healthy non-obese women (≥60 years of age). They found that at a given volume the high intensity produced the biggest gains in insulin sensitivity (21% compared to 16% with moderate intensity). These increases in insulin sensitivity persisted in these older women for up to 72 hrs after the last bout of exercise. This training induced elevated insulin sensitivity occurred without changes in body composition or VO_2 peak.

More than half of Americans fail to get the minimal amount of recommended physical activity for health benefits regardless of the intensity of the physical activity (50). The 2001 Behavioral Risk Factor Surveillance System (11) is a telephone survey that inquires about people's physical activity. More specifically, it looks at moderate and/or vigorous intensity physical activity used in everyday living. Macera et al. (50) analyzed the data from this survey to gain an understanding of people's physical activity during non-work hours and whether it is sufficient to obtain health benefits. This survey questioned 82,834 men and 120,286 women 18 years of age and older in the United States. They used the 1996 Surgeon General's report and guidelines (1) as their standard to measure people against. This report stresses the importance of moderate intensity activities, such as yard work, fast paced walks, etc., to promote good health. This level of intensity has been shown to lower all cause mortality, improve quality of life, and sustain

cardiovascular health (50). They recommend 30 minutes of moderate level activity per a day 5 or more days per a week, or 20 minutes of vigorous intensity physical activity 3 or more days per week. They found that men and women (32%) were similar in meeting the recommendations for moderate physical activity. However, a higher percentage of men (29%) than women (20%) respondents participated in vigorous physical activity during non-working hours. Not only did they examine gender differences, but also difference between age groups. Meeting the recommendations for physical activity declined with age. For instance 50% of the women between the ages of 18 and 29 met guidelines, but only 27% of women who were older than 75 got enough physical activity. For men it was 58% and 38% respectively. Another finding is that far fewer obese men and women met recommendations for physical activity compared to healthy weight men and women. They found that 50% of healthy weight women met physical activity guidelines while only 33% of obese women did. The authors conclude that more than half of the adults in the United States fail to get the recommended amount of activity to obtain health benefits despite all the expansions of definitions for physical activity (50). As this section has shown, exercise has a large impact on health, and glucose metabolism. If people would make exercise more of a priority they could spare themselves the increased risk of developing type 2 diabetes, cardiovascular problems, or health problems associated with metabolic syndrome.

Conclusion

The purpose of this literature review was to describe postprandial glycemic response and several factors that influence this response. Postprandial glycemic response is composed of two components, appearance and disappearance. The key to maintaining glycemic health is to limit the magnitude of the appearance, and to improve or maintain the body's ability to dispose of glucose in the blood stream. Research shows that the amount and type of carbohydrate in a food heavily influences the degree of the glycemic response. Knowing the type of carbohydrate a food contains is important, because not all carbohydrates are digested at the same rate. Foods with a low glycemic index will often have carbohydrates that digest slower and will mitigate the glycemic response. In addition, avoiding certain foods, such as caffeine and high fatty foods that acutely cause insulin resistance also is important. Foods that cause insulin resistance have been found to interfere with the body's ability to dispose of glucose in the blood stream causing elevated blood glucose levels. Research has also shown that getting proper amounts of physical activity (resistance training and/or aerobic activity) every week increases insulin sensitivity. This is beneficial because an increase in insulin sensitivity has been shown to increase the body's ability to dispose of glucose in the blood. It also helps the individual lose weight, which is helpful because obesity and excess adiposity are associated with increased levels of free fatty acids in the blood stream, which cause insulin resistance. Eating a healthy diet and exercising on a regular basis will help maintain insulin sensitivity, reduce postprandial glycemic response to foods, and reduce the risk of type II diabetes mellitus, cardiovascular disease, and other health abnormalities.

CHAPTER 3

METHODOLOGY

Experimental subjects

A total of 25 subjects (13 males, 12 females) participated in this study. Inclusion criteria required that subjects have a body mass index (BMI) of 20-32 (body weight (kg)/height (m^2)), and were between the ages of 20 and 65 years old. The subjects lipid profile had to meet the following criteria: total cholesterol <240 mg/dL, triglycerides <600 mg/dL, low-density lipoprotein (LDL) <170 mg/dL, and high-density lipoprotein (HDL) >40mg/dL. A DinaMap monitoring system (ProCare 100, GE Medical Systems, Milwaukee, WI), was used to measure blood pressure. Blood pressure had to be <140 systolic and <90 diastolic. Fasting glucose levels had to be 100 mg/dL or less. Individuals were excluded from the present study if they had a history of diabetes, thyroid or heart disease, or hypertension. Subjects were also excluded if they had a history of chronic alcohol use, smoke, or took any of the following substances: cortisone, corticotrophin, or phenytoin. In addition, subjects could not be gluten intolerant/celiac disease or adhere to any specialized diet regimes. The Human Subjects Committee at the University of Kansas approved the experimental protocol. Subjects provided written informed consent.

HOMA-IR

Subjects had their blood drawn during the screening process for lipid, fasting blood glucose, and insulin levels. Blood measures (lipids, fasting glucose, fasting insulin) were all analyzed at Lab Core/Quest located on the University of Kansas campus. HOMA-IR was calculated as fasting insulin (pmol/l) X glucose (mmol/l)/135 (51, 83).

Physical Activity Questionnaire

This investigation did not have physical activity inclusion or exclusion criteria. Therefore, subjects participating had a wide range of physical activity levels including sedentary individuals. Subject's physical activity was assessed via the Seven-Day Physical Activity Recall (67). This questionnaire was completed at baseline, and at visits 2, 5, and 7. Physical activity questionnaires were completed throughout the course of the study to confirm that physical activity levels remained consistent throughout the course of the study. Participants were considered physically active in this study if they met the aerobic physical activity criteria set by the U.S. Department of Health and Human Services (25). This criteria includes a minimum of 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity physical activity a week. They also were considered physically active if they met a combination of the two intensities totaling 150 minutes of physical activity a week. Subjects were asked to refrain from exercising for 24 hours before their testing time.

Study Design

Subjects participated in this study for a total of 7 weeks, and reported to the lab one day a week during this time. During the first visit, the subjects tasted each of the 6

different types of tortillas and rated them based on appearance, texture, flavor, and general acceptability. The tortillas that were used in the study are as follows: 1. Control, commercially available semi-refined corn flour tortilla 2. Commercially available semi-refined corn flour (90%) + Nopal flour (10%) tortilla 3. Commercially available semi-refined corn flour (80%) + Nopal flour (20%) 4. Commercially available semi refined corn flour (90%) +whole bean (phaseolis) flour (10%) 5. Commercially available semi refined corn flour (80%) + Nopal flour (10%) + whole bean (phaseolis) flour (10%) 6. Blue aleurone corn flour tortilla.

During each of the 6 subsequent visits, subjects were randomly assigned one of the six different types of tortillas to consume. Subjects reported to the lab fasted between 7 and 9am in the morning and were allotted 20 minutes to consume 180g of each type of tortilla for their breakfast meal. Subjects drank water with their tortillas ad libitum and total water intake was recorded. During each of these visits, subjects had their basal blood glucose levels measured via finger prick using the Accu-Chek Compact Plus Diabetes Monitoring Kit (Roche Diagnostics 9115 Hague Road Indianapolis, IN). One basal blood glucose measurement was taken via finger prick prior to consumption of the tortillas. Postprandial blood glucose measurements were then taken via finger prick at 30, 60, 90, 120, and 180 minutes.

Preparatory dietary and physical activity control

Participants were asked to abstain from alcohol 48 hours before each visit, as alcohol has been shown to increase insulin sensitivity (40). Participants were given a list of foods that contained nopal or pulses, and were asked to avoid eating these foods 48

hours prior to testing. In the 24-hour period before testing, subjects were asked to abstain from physical activity and to have no more than one serving of caffeine. Caffeine has been shown to inhibit insulin sensitivity and glucose uptake (22, 56). Subjects were also asked to fast for 12 hours before testing with the exception of water.

Statistics

To evaluate postprandial glycemic reponse to tortillas, area under the curve (AUCs) was calculated using the trapezoid method in Microsoft Excel 2007. Peak glycemic values in response to each tortilla, for each participant, was defined as the highest glycemic measurement from one of the five time points (30, 60, 90, 120 and 180). Differences in AUC and peak glycemic measurements between the different types of tortillas were evaluated using repeated measures analysis of variance (ANOVA) in SPSS software, ver. 17.0 (SPSS Inc, Chicago, IL). To control for Type 1 error in pairwise comparisons a Bonferroni approach was used. In analyzing AUC the sphericity assumption was violated in the AUC analyses, the Greenhouse-Geisser was used to adjust the degrees of freedom.

To investigate differences in the time it took for participants to eat each tortilla a repeated measures one-way ANOVA was calculated using SPSS software ver. 17.0 (SPSS Inc, Chicago, IL). In this calculation the sphericity assumption was violated and the Greenhouse-Geisser was used to adjust for the degrees of freedom.

To evaluate HOMA-IRs influence on postprandial blood glucose response a bivariate correlation (Pearson correlation coefficient) was performed to compare the AUC for the control tortilla to the participants HOMA-IR value. A bivariate correlation

(Pearson correlation coefficient) also was used to assess the relationship between HOMA-IR and physical activity. Both calculations were conducted using SPSS software, ver. 17.0 (SPSS Inc, Chicago, IL). BMI and gender also were assessed in this model to determine if either variable influences HOMA-IR. Each participant's physical activity level was assessed (Microsoft Excel 2007) by averaging the minutes of moderate and vigorous physical activity from the four completed physical activity questionnaires. The average minutes of vigorous physical activity was then multiplied by 2 and added to the average minutes of moderate physical activity providing the total physical activity for each participant. To determine if the U.S. Department of Health and Human Services guidelines (Center for Disease Control) for physical activity are sufficient enough to impact HOMA-IR a t-test (Microsoft Excel 2007) was run on HOMA-IR levels between those who met the recommendations and those who did not. T-tests in Microsoft Excel were also used to assess gender differences in HOMA-IR, physical activity, fasting glucose, and fasting insulin concentrations.

CHAPTER 4

RESULTS

Participant's baseline characteristics are presented in Table 1. Participants were stratified based on BMI and gender. BMI was stratified into two categories; normal weight (20- 24.9), and overweight (25- 29.9). No significant differences were found between genders on HOMA-IR, physical activity, fasting glucose, or fasting insulin ($P>0.05$).

There were no significant differences in total AUC results between the different types of tortillas $F(2.727, 65.437)= 1.080$, $p=0.360$ (Figure 1 and Table 2). There also were no significant differences in the peak postprandial glycemic values between the different types of tortillas $F(5, 120)= 1.792$, $p= 0.120$ (Table 2). Furthermore, there were no significant differences in the amount of time it took participants to consume the different types of tortillas $F(2.978, 71.475)= 1.150$, $p=0.335$ (Figure 2).

No significant correlation ($p < 0.05$) existed between HOMA-IR and the AUC for the 10% nopal flour ($r=0.183$, $p=0.190$), blue corn flour ($r=0.027$, $p=0.449$), 20% nopal flour ($r=0.303$, $p=0.070$), 10% nopal-10%bean flour ($r=0.263$, $p=0.102$), or the 20% bean

Table 1.
Participant characteristic

	Male (n=13)	Female (n=12)	Total (n=25)
BMI (kg/m^2)	25.02 ± 2.60	24.09 ± 3.23	24.58 ± 2.90
Age (yrs)	26.92 ± 6.49	32.83 ± 12.17	29.76 ± 9.89
HOMA-IR	1.26 ± 0.47	1.54± 0.73	1.39 ± 0.61
PA (min)	318.70 ± 359.30	350.30 ± 332.10	333.90 ± 339.67
Fasting Glucose (mg/dl)	85.62 ± 4.05	81.42 ± 6.95	83.6 ± 5.91
Fasting Insulin (uIU/ml)	5.12 ± 1.91	5.65 ± 3.10	5.82 ± 2.60

Values are means ± SD

Table 2.

Postprandial glycemic response to tortilla consumption

	10% Nopal	100% Corn	Blue Corn	20% Nopal	10% Nopal,10% Bean	20% Bean
Postprandial Maximal Glucose (mg/dL)	139.16 ± 4.36	143.76 ± 5.11	134.28 ± 5.27	141.12 ± 4.21	141.72 ± 4.39	132.68 ± 3.21
Postprandial Glucose Spike (mg/dL)	53.48 ± 22.92	58.44 ± 26.09	48.44 ± 25.02	55.64 ± 19.38	56.96 ± 21.12	45.48 ± 17.59
Postprandial AUC (mg/dL)	311.29 ± 31.57	309.59 ± 30.01	312.2 ± 47.44	307.14 ± 25.43	307.78 ± 31.49	298.89 ± 24.34

AUC, area under the curve

Values are means ± SD

flour (r=0.314, p=0.063) tortillas. However, there was a significant correlation between HOMA-IR and the control (100% corn flour) tortilla (r=0.360, p=0.039). There also were no significant correlations between HOMA-IR and BMI (r=0.112, p=0.297), or HOMA-IR and gender (r=0.239, p=0.125). However, there was a significant inverse correlation between physical activity and HOMA-IR (r= -0.553, p=0.002; Figure 3).

Figure 1. The average area under the curve for each type of tortilla.

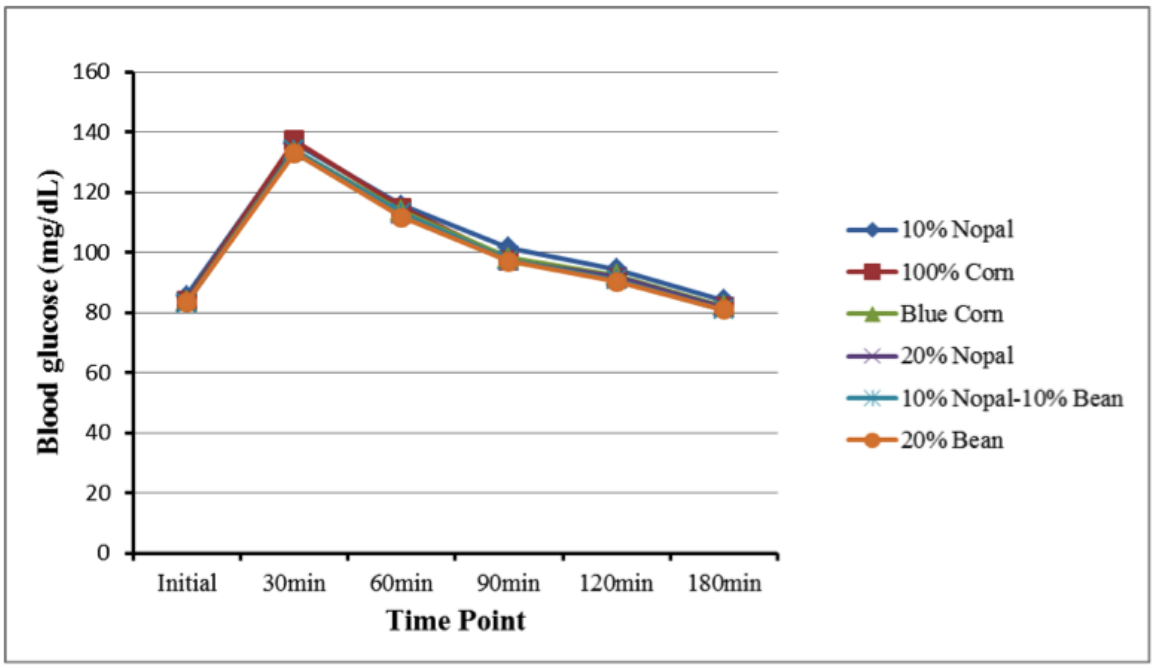


Figure 2. The average time it took for participants to consume each type of tortilla**.**

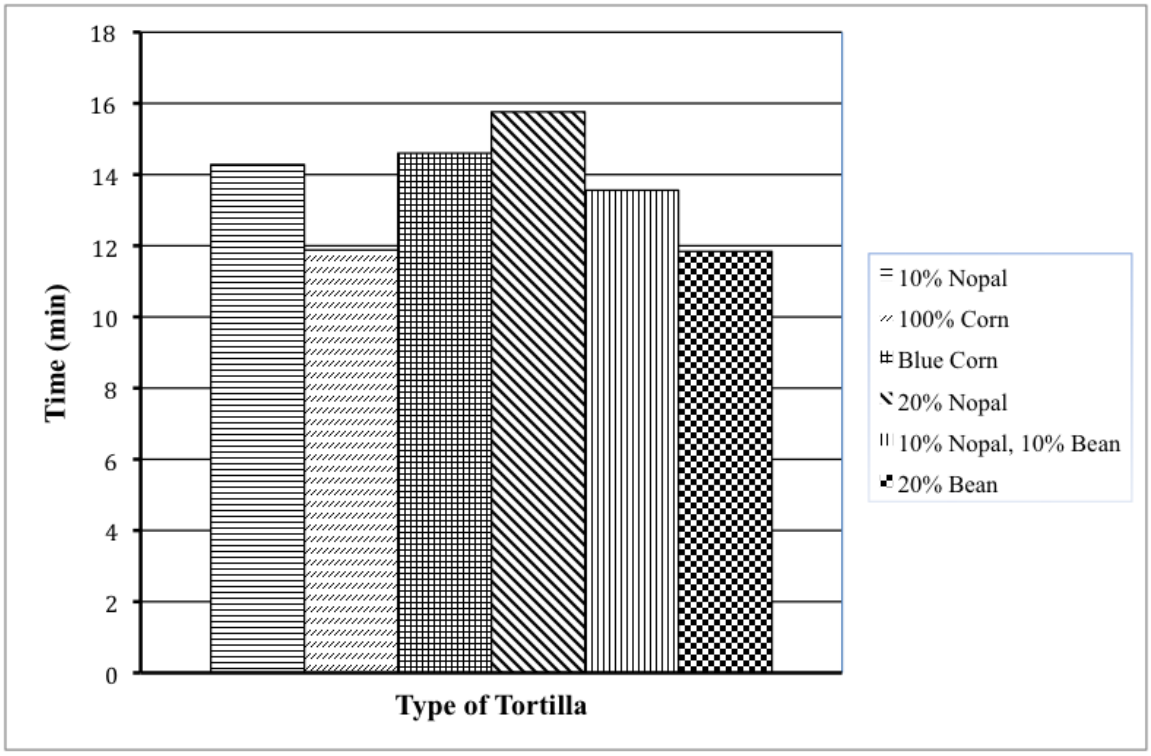
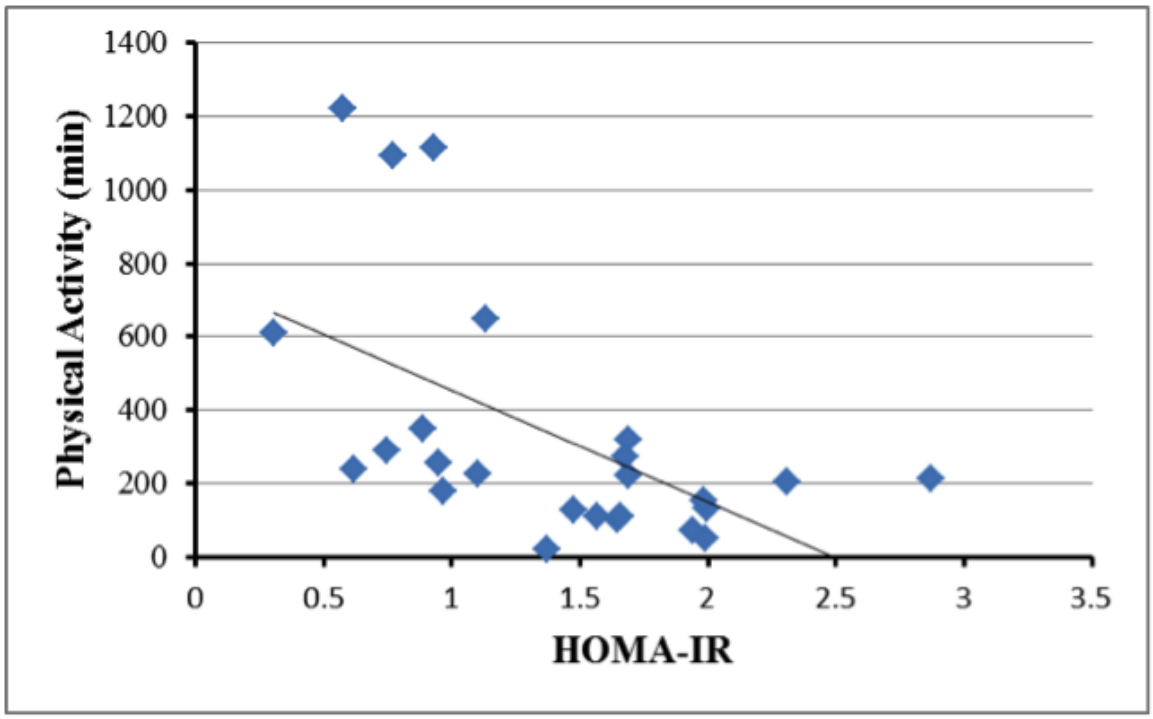


Figure 3. Scatterplot shows the relation between HOMA-IR values and average weekly physical activity values in minutes.



CHAPTER 5

DISCUSSION

The primary aim of the current investigation was to determine if substituting bean and/or nopal flour for a portion of the corn flour in a tortilla reduces the postprandial glycemic response compared to the 100% corn flour tortilla. A secondary aim was to determine if there was a correlation between HOMA-IR values and glycemic response to tortilla consumption, and if physical activity influenced this relationship.

The present investigation showed that substituting bean and/or nopal flour for a portion of the corn flour in a tortilla did not significantly reduce the postprandial glycemic response. This finding contradicts those of Bacardi-Gascon et al. (5) who found that adding nopal to common Mexican breakfast meals significantly reduced the postprandial glycemic response. In another study that investigated how food items may reduce glycemic response, Kabir et al. (41) compared the effects of mung bean starch and waxy cornstarch in mixed meals on blood glucose concentrations in rats. They found rats that were fed mung bean starch had lower glucose and insulin peak values than rats that were fed cornstarch. Calculated glycemic and insulemic indices also were lower for the mung bean starch than for the waxy cornstarch. These two studies used mixed meals, while the current investigation used only plain tortillas, which could explain the discrepancy in the findings.

The variability in macronutrient content of the meals examined also could play a role in why previous studies have observed decreases in glycemic response with nopal and bean while the current investigation did not. Other studies (71, 87) have found that black beans also have the ability to lower postprandial glycemic response when combined with other food items. Black beans have lower amounts of available starch (digestible starch) and higher amounts of resistant starch than commercial tortillas, which results in black beans having a significantly lower hydrolysis index and a lower predicted glycemic index. When black beans and tortillas were combined, the hydrolysis index values were reduced to those of black beans alone and the predicted glycemic index decreased as well. This shows how influential the different starches found in bean are on digestion and glycemic response when mixed with other meals.

The method of food preparation or other factors that influence the physical form of the food can change the rate at which the food is digested and therefore the postprandial glycemic response (4, 90). Araya et al. (4) found that ground up beans were digested faster than beans served as whole grains. In fact, the starch digestion index values were highest for beans prepared as flour as opposed to beans served as a whole grain or served with spaghetti, indicating that the flour digested faster. Jenkins et al. (38) found a highly significant correlation between the digestibility of a food item and the impact that food item had on glycemic response. When beans and/or nopal are ground up into flour it is possible that they lose some of their effectiveness in mitigating the postprandial glycemic response.

Insulin sensitivity also influences the glycemic response to a particular food item. It is well established that insulin sensitivity is improved by physical activity (47, 54, 61,

77). King et al. (45) found that during a euglycemic clamp procedure individuals in a detrained state showed reductions (up to 23%) in glucose uptake in response to submaximal insulin concentrations compared to when the same participants were in a trained state. Conversely, there was no significant difference in glucose uptake between trained and detrained in response to maximal insulin concentrations, indicating that trained individuals are more sensitive to insulin and have greater glucose uptake than untrained individuals. Furthermore, Nowak et al. (59) found that in response to a three month training program women's HOMA-IR values significantly decreased. In addition, women's glucose and insulin responses to an oral glucose tolerance test also significantly decreased. These results agree with the findings of the current investigation, as the present study found an inverse correlation between physical activity and insulin resistance (HOMA-IR), which indicates that as physical activity levels increase, insulin resistance decreases.

It was hypothesized that this decrease in insulin resistance would impact postprandial glycemic response to tortilla consumption, causing a smaller AUC and peak glycemic value. A significant relationship between HOMA-IR values and AUC for the 100% corn flour (control) tortilla was found. However, this relationship did not extend to the other types of tortillas despite the fact that there was not a significant difference in glycemic response between the different types of tortillas. Perhaps this discrepancy is due to variability in macronutrient content in the different types of tortillas, however, macronutrient content was not provided for the tortillas used in the current investigation.

Additional limitations in this study include the sample size, inclusion criteria, and the method of assessing glycemic measurements. Due to restricted amounts of supplies,

this study only evaluated 25 subjects (13 males, 12 females), however, had a larger sample size been possible it would allow for more statistical power to investigate the effects of BMI, gender, physical activity on HOMA-IR and differences in glycemic response between different tortillas. Statistical power was further weakened by nonrestrictive inclusion criteria, as the criteria allowed for participants to be between the ages of 20 and 65. Insulin resistance has been shown to increase with age (9), so decreasing the ceiling of the age limit approximately 20 years may decrease the confounding affect on insulin resistance. This would allow for better analysis of physical activities effects on HOMA-IR. Inclusion criteria also specified a BMI range of 20-32; with a sample size of 25 it would have been better to use 29.9 as the upper limit of this range. Then it would be possible to analyze the relationship between BMI and HOMA-IR in normal weight and overweight individuals, where normal weight individuals would serve as a control. Another limitation was the use of the Accu-chek compact plus to measure blood glucose levels. Although a specific meter was assigned to each participant to be used for all testing in order to control for variability between measurements, the Accu-chek compact plus was not intended to be used for scientific purposes and its accuracy may be a considerable limitation. An alternative may have been to use the finger prick technique, collect blood using Eppendorf tubes, and then measure blood glucose levels with an automatic spectrophotometric analyzer as done by Brand-Miller et al. (13).

The amount of each type of tortilla that was given to the participants may have been another limitation. Tortillas were frozen prior to arrival at the Energy Balance lab at the University of Kansas and it is possible that the tortillas lost water content during the

process of freezing and thawing. Participants were given exactly 180.0 grams of each tortilla, which was determined by using a digital scale. The actual tortilla count differed slightly between tortilla types. One reason behind this difference in tortilla weight may have been water content. If one type of tortilla lost more water than another type then it would take more tortillas to weigh 180 grams. Thus, some participants may have consumed larger amounts of a particular type of tortilla depending on the amount of water lost as a result of the freezing and thawing process. An extra amount of tortillas may have also made it harder for some participants to finish the given amount of tortillas. Participants were unable to consume the tortillas within 20 minutes 13% of the time. However, the average time for tortilla consumption was not significantly different between tortillas types (Figure 2). Using fresh tortillas rather than tortillas that have been frozen may have prevented water loss from the tortillas and participants would not have had to eat so many tortillas. Another possibility for the variability in tortilla counts could be that some of the tortillas simply have a higher density due to certain ingredients, which causes them to weigh more.

In summary, the results of the present study demonstrate that substituting nopal or bean flour for corn flour in tortillas does not significantly decrease the postprandial glycemic response. It was also found that those individuals who met or exceeded the U.S. Department of Health and Human Service's physical activity recommendations had significantly lower HOMA-IR values. In addition, the HOMA-IR values were found to significantly correlate with the postprandial glycemic response (AUC) of the control tortilla. Even though a healthier alternative to the 100% corn flour tortilla was not found

in this study, it was found that there is incentive for people to meet the physical activity recommendations of the U.S. Department of Health and Human Services.

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APPENDIX A

Informed Consent

Approved by the Human Subjects Committee University of Kansas, Lawrence Campus (HSCL). Approval expires one year from 9/18/2009. HSCL#18188

CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

TITLE
Palatability and acute glycemic and satiety responses to tortillas containing blends of bean and corn flour

INTRODUCTION
The Center for Physical Activity and Weight Management at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you wish to participate in the present study. You may refuse to sign this form and not participate in this study. You should be aware that even if you agree to participate, you are free to withdraw at any time. If you do withdraw from this study, it will not affect your relationship with this unit, the services it may provide to you, or the University of Kansas.

PURPOSE OF THE STUDY
The purpose of this project is to measure the blood sugar (glucose) response to 6 different corn and bean flour tortillas in healthy volunteers. A secondary objective will be to assess the appearance, taste, and texture of each tortilla, as well as level of fullness after consumption of the tortilla.

PROCEDURES
You will be one of approximately 50 participants in this study. You will be required to report to our Energy Balance Laboratory on the KU campus a total of 7 times during an 7 week period. There will be a minimum 6 day period between each visit. During the first visit you will be asked to taste test all seven products and rate each of them based on appearance, taste, and texture. After the initial visit, study subjects will be asked to consume 1 of 6 different tortillas on 6 separate days. Each of these visits will require that you abstain from alcohol for 48 hours. In addition, you will be required to fast for 12 hours prior to arrival at the Energy Balance Lab. Before participating in this study you will be asked to complete screening assessments. The following is a description of each assessment that will occur in the study.

Screening Assessments:

1. Screening Questionnaire: You will be asked to complete a questionnaire that will provide us with your health and demographic information. More specifically, the questionnaire contains questions regarding medication use, pre-existing health problems, and drug and alcohol use. The demographic portion of the questionnaire will ask you about your age, gender, race, and ethnicity. The health information will be used to determine if you are eligible to participate. The demographic information will be used to describe the study group. The screening questionnaire is completed during the initial screening process only and should take about 5 minutes to complete.

2. Screening Blood Chemistry: You will be asked to report to Watkins Health Center between 8 and 10am after a 12 hour fast (except water). A blood sample (about 5 teaspoons) will be taken from a vein in your arm by a trained phlebotomist to evaluate blood lipids, glucose, and insulin to determine if you are eligible to participate in this study. This test will occur during the initial screening process only and will require approximately 10 minutes to complete.

3. Screening Blood Pressure: You will be asked to report to the Energy Balance Lab between 7 and 9am after a 12 hour fast (except water). Your blood pressure will be measured using a FDA certified DinaMap unit and standard blood pressure cuff to determine if you are eligible to participate in the study. The assessment will occur during the initial screening process only and will take about 10 minutes to complete.

4. Screening Height and Weight: You will be asked to report to the Energy Balance Lab between 7 and 9am after an overnight fast (except water). You will be weighed in a standard hospital gown using a digital scale. Your height will be measured using a stadiometer. Your height and weight will be used to determine if you are eligible to participate in this study. The height and weight assessment will happen during the initial screening process only and will take about 5 minutes to complete.

If you are eligible to continue with the study, you will be asked to participate in the following assessments:

1. Physical Activity Questionnnaire: You will be asked to complete a questionnaire that will help us determine your lifestyle activity patterns. The questionnaire will happen at baseline (screening process), and after visits 2, 5, and 7. This questionnaire will take about 20 minutes to complete.

2. Taste Test Questionnaires: You will be asked to report to the Energy Balance Lab between 6 and 9am after a 12 hour fast (except water). You will be asked to taste 6 different tortillas and rate each of them based on acceptability of appearance, texture, flavor, and general acceptability. The assessment will happen at baseline only and will take about 60 minutes to complete.

3. Glucose Measurement and Hunger Assessment: You will be asked to report to the Energy Balance Lab between 6 and 9am after a 12 hour fast (except water) on 6 separate days. On each of these 6 days, you will eat eight, 4-inch tortillas of identical composition. At each subsequent visit you will consume tortillas of different corn and bean flour ratios. You will have your blood glucose levels checked via finger prick prior to eating the tortillas and again 30, 60, 90,120, and 180 minutes after you have finished eating. At these same minute time points we will ask you to complete a questionnaire to assess how full you are. The process will take about 3.5 hours each day and will occur on 6 separate days.

RISKS

Blood Samples: A small chance of infection exists with a blood draw. Bruising and pain at the site of sampling is also possible.

BENEFITS

Upon completion of the project, you will receive valuable information regarding your blood lipids, glucose, and insulin values. Your blood glucose responses will differ between the various tortillas. Upon completion of the study, you will receive information regarding your blood glucose response to each type of tortilla. In addition, your data will make an important contribution to the scientific literature and you will be financially rewarded for successful participation.

PAYMENT TO PARTICIPANTS

You will be paid $400 total upon completion of all required assessments and study obligations. You will be paid $100 upon completion of all assessments on the second visit, $100 upon completion of all assessments on the fifth visit, and $200 upon completion of the study. Study obligations include, but are not limited to, attending 100% of the visits, consumption of all tortillas provided, and completion of all questionnaires. Investigators may ask for your social security number in order to comply with federal and state tax and accounting regulations.

PARTICIPANT CONFIDENTIALITY

Your name will not be associated in any way with the information collected about you or with the research findings from this study. The researcher(s) will use a study number instead of your name. The information collected about you will be used by Dr. Bryan Smith, his co-investigators, and officials at KU that oversee research, including committees and offices that review and monitor research studies. In addition, we may share information with colleagues who are conducting similar research studies but will not share any information that would identify an individual. The researchers will not share information about you unless required by law or unless you provide written permission. Permission granted on this date to use and disclose your information remains in effect indefinitely. By signing this form you give permission for the use and disclosure of your information for purposes of this study at any time in the future.

INSTITUTIONAL DISCLAIMER STATEMENT
In the event of injury, the Kansas Tort Claims Act provides for compensation if it can be demonstrated that the injury was caused by the negligent or wrongful act or omission of a state employee acting within the scope of his/her employment.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION
You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, you cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION
You may withdraw your consent to participate in this study at any time. You also have the right to cancel your permission to use and disclose information collected about you, in writing, at any time, by sending your written request to: Dr. Bryan Smith, Energy Balance Laboratory, Schiefelbusch Institute for Life Span Studies, 100 Robinson Center, Lawrence, KS 66045. If you cancel permission to use your information, the researchers will stop collecting additional information about you. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

QUESTIONS ABOUT PARTICIPATION
Questions about procedures should be directed to the researcher(s) listed at the end of this consent form.

PARTICIPANT CERTIFICATION:
I have read this Consent and Authorization form. I have had the opportunity to ask, and I have received answers to, any questions I had regarding the study. I understand that if I have any additional questions about my rights as a research participant, I may call (785) 864-7429 or (785) 864-7385, write the Human Subjects Committee Lawrence Campus (HSCL), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7563, or email mdenning@ku.edu.

I agree to take part in this study as a research participant.

_____ _____
Type/Print Participant's Name Date

_____
Participant's Signature

Researcher Contact Information

Bryan Smith, Ph.D.
Research Scientist
Energy Balance Laboratory
Schiefelbusch Institute for Life Span Studies
100 Robinson Center
Lawrence, KS 66045
(785) 864-0782

Joseph E. Donnelly, ED.D.
Professor/Director
Energy Balance Laboratory
Schiefelbusch Institute for Life Span Studies
100 Robinson Center
Lawrence, KS 66045
(785) 864-0797

Debra Sullivan, Ph.D., R.D.
Chair, Department of Nutrition, 1012 Delp
KU Medical Center
3901 Rainbow Blvd
Kansas City, KS 66160
(913) 588-5357

APPENDIX B

Health History/Initial Eligibility Questionnaire

Tortilla Study
Initial Eligibility Questionnaire/Health History Form

Name:_____

Gender: ☐ M (1) ☐ F (2) Age:_____ Height: ft____in____
Weight:_____

BMI:_____(Official Use ONLY)
Ethnicity: _____ Hispanic
_____ Not Hispanic
_____ Other/Unknown
Race: _____ American Indian or Alaskan Native
_____ Asian
_____ Pacific Islander
_____ Black, Not of Hispanic Origin
_____ White, Not of Hispanic Origin
_____ More than one race
_____ Unknown

Home/Cell Phone: (____)_____Email:_____

Medical History of Participant:

1. Medications (Especially those containing cortisone, corticotrophin or phenytoin)?

☐ Yes ☐ No Type:_____

2. Do you have known:

Diabetes? ☐ Yes ☐ No Heart Disease? ☐ Yes ☐ No

High Blood Pressure? ☐ Yes ☐ No Thyroid Condition? ☐ Yes ☐ No

3. Do you use tobacco products of any kind? ☐ Yes ☐ No

Other Questions:

4. Do you have Celiac disease/ gluten intolerance? ☐ Yes ☐ No

5. Do you participate in any **specialized diet regimes**? ☐ Yes ☐ No
(Vegetarian, Vegan, etc.)

How did you hear about the study?
_____ (1) Newspaper Advertisement _____ (4) Flyer, Bulletin Board
_____ (2) Other Participant _____ (5) Other
_____ (3) Orientation

APPENDIX C.

Diet Instruction

Tortilla Study Diet Instructions

Thank you for your participation in the study. You will be eating tortillas on a weekly basis for a total of 8 weeks. You will have an appointment on the same day of the week, unless previously scheduled. Below are detailed instructions on what you can and cannot do during the course of the study. It is very important to follow these directions in order not to contaminate the results.

48 Hours before Testing Time

-No Alcohol can be consumed in the 48 hour window before the test
-Refrain from eating the following foods: Nopal (Fleshy part of a Prickly Pear)
Pulses (beans) – which include the following:

1. Dry beans (*Phaseolus spp.* including several species now in *Vigna*)
   - Kidney bean, haricot bean, pinto bean, navy bean
   - Lima bean, butter bean
   - Azuki bean, adzuki bean
   - Mung bean, golden gram, green gram
   - Black gram, Urad
   - Scarlet runner bean
   - Ricebean
   - Moth bean
   - Tepary bean

2. Dry broad beans
   - Horse bean
   - Broad bean
   - Field bean

3. Dry peas (*Pisum spp.*)
   - Garden pea
   - Protein pea
4. Chickpea, Garbanzo, Bengal gram (*Cicer arietinum*)
5. Dry cowpea, Black-eyed pea, blackeye bean (*Vigna unguiculata*)
6. Pigeon pea, Arhar /Toor, cajan pea, congo bean (*Cajanus cajan*)
7. Lentil (*Lens culinaris*)
8. Bambara groundnut, earth pea (*Vigna subterranea*)
9. Vetch, common vetch (*Vicia sativa*)
10. Lupins (*Lupinus spp.*)

11. Minor pulses include:
    - Lablab, hyacinth bean
    - Jack bean
    - sword bean
    - Winged bean
    - Velvet bean, cowitch
    - Yam bean

24 Hours before Testing

-Only one serving of caffeine can be consumed within 24 hours of your testing. Servings are one small (8 fluid ounce) cup of coffee or tea OR one can of regular soda. Energy Drinks are not to be consumed within 24 hours of your testing.

12 Hours before Testing

-No food or beverages can be consumed
-Water is ok
-Try to avoid eating a meal that is high in fatty foods:
    -Avoid adding butter/margarine/oils to foods
    -Avoid fried foods
    -Avoid high fat cuts of meat
-Try to eat foods that are highly processed grains and refined sugars

Morning of the test –

-Make sure to arrive to your appointment on time
-Remember not to eat any food items or drink anything other than water.

APPENDIX D.

Seven-day Physical Activity Recall

7-Day Physical Activity Recall SSN

PAR#: 1 2 3 4 5 6 7 Participant_____

Interviewer_____ Today is_____ Today's Date_____

1. Were you employed in the last seven days? 0. **No** (Skip to **Q#4**) 1. **Yes**
2. How many days of the last seven did you work? ____ days
3. How many total hours did you work in the last seven days? ____ hours last week
4. What two days do you consider your weekend days? _____, _____ (mark days below with a squiggle)

WORKSHEET

		DAYS						
	SLEEP	**1 __**	**2 __**	**3 __**	**4 __**	**5 __**	**6 __**	**7 __**
MORNING	**Moderate**							
	Hard							
	Very Hard							
AFTERNOON	**Moderate**							
	Hard							
	Very Hard							
EVENING	**Moderate**							
	Hard							
	Very Hard							
Total Min Per Day	**Strength:**							
	Flexibility:	____	____	____	____	____	____	____

4a. Compared to your physical activity over the past 3 months, was last week's physical activity more, less, or about the same?

1. More 2. Less 3. About the same

6. Do you think this was a valid PAR Interview?

1. Yes 0. No
If NO, go to the back and explain.

5. Were there any problems with the PAR interview?

0. No 1. Yes
If YES, go to the back and explain.

7. Were there any special circumstances concerning this PAR ?

0. No 1. Yes, If YES, what were they?(circle)

1. Injury all week 2. Illness all week 3. Illness part week
4. Injury part week 5. Pregnancy 6. Other:

APPENDIX E

Glycemic Response to Each Type of Tortilla (mg/dL)

Nopal 10%

Initial	30min	60min	90min	120min	180min
76	121	94	93	87	80
73	109	84	77	99	87
99	141	106	89	82	81
91	111	99	93	81	81
73	143	145	130	119	80
74	137	160	117	113	81
88	122	126	100	92	85
93	135	76	102	95	93
97	204	153	105	75	74
91	121	91	79	85	85
86	121	120	123	101	92
94	123	109	85	106	96
84	132	109	85	75	85
85	145	149	119	97	77
96	154	103	93	89	91
87	137	94	93	100	83
90	157	110	90	84	89
95	126	107	122	110	103
86	126	91	93	78	90
62	152	105	92	74	67
84	163	147	99	101	83
82	124	118	111	83	74
83	167	131	123	108	76
87	119	157	141	139	97
86	116	110	88	90	77

100% Corn

Initial	30min	60min	90min	120min	180min
78	102	98	79	81	81
88	163	119	89	87	83
91	101	105	82	80	77
73	110	91	71	76	75
88	135	133	108	107	81
91	133	113	97	96	91
97	131	101	108	89	99
87	188	136	114	78	82
85	147	96	98	89	78
88	129	119	106	95	71
94	154	118	86	90	104
84	130	111	95	90	88
80	164	167	116	99	71
85	131	109	90	89	84
83	136	130	111	109	84
92	143	105	96	92	84
92	129	138	114	133	87
87	160	108	87	88	90
77	153	93	58	77	70
85	222	148	59	84	85
81	139	110	85	70	81
80	149	122	128	111	81
79	165	153	129	90	66
88	127	124	110	96	87

Blue Corn

Initial	30min	60min	90min	120min	180min
83	106	92	93	100	77
73	94	101	98	94	75
91	151	105	81	92	90
87	105	93	104	77	85
84	146	165	180	155	109
82	134	96	92	83	78
94	133	133	122	109	90
90	129	83	104	94	92
95	172	110	101	91	83
83	117	90	88	80	80
89	148	158	105	85	94
92	117	112	104	104	96
84	110	94	96	83	84
83	156	137	106	101	82
94	164	148	125	105	84
81	124	122	99	107	79
97	124	110	95	89	91
85	108	113	99	109	110
83	124	109	84	89	86
70	111	84	74	57	60
89	191	138	81	90	83
81	129	117	89	91	71
80	133	106	112	107	75
84	147	172	159	161	99
92	103	98	87	89	81

20% Nopal

Initial	30min	60min	90min	120min	180min
83	132	105	81	92	86
78	112	103	83	92	77
88	160	98	87	102	98
87	89	93	99	80	80
81	155	137	112	115	77
91	155	129	118	107	95
94	139	99	88	110	96
91	178	128	95	94	75
90	142	101	96	88	85
90	146	155	113	86	90
95	130	98	108	95	100
83	136	117	90	101	89
78	122	110	85	94	73
93	120	112	99	90	77
85	134	118	108	92	78
93	190	94	68	71	82
90	105	95	144	113	92
95	168	115	90	105	99
79	142	94	89	78	70
86	143	155	90	90	79
83	126	113	85	72	72
72	147	123	110	83	81
81	130	112	103	111	73
73	101	111	87	77	71
78	146	136	109	87	70

10% Nopal/10% Bean

Initial	30min	60min	90min	120min	180min
81	124	88	103	93	88
76	99	99	78	93	75
92	152	105	83	91	93
81	122	93	97	78	85
82	137	167	108	83	67
82	138	100	97	73	81
91	123	102	101	111	88
86	148	128	97	87	90
87	166	123	95	79	82
89	130	99	93	91	91
89	181	136	120	104	93
99	137	97	109	92	93
83	127	96	95	92	91
82	168	144	125	105	60
88	171	101	91	75	71
78	122	109	110	89	80
92	141	111	81	69	76
92	130	128	115	104	84
89	129	90	84	90	94
73	97	82	78	69	83
84	157	150	117	84	83
81	136	157	109	97	72
75	159	151	127	106	71
85	143	160	139	116	73
82	138	97	79	76	76

20% Bean

Initial	30min	60min	90min	120min	180min
76	118	89	91	88	86
81	100	109	89	85	75
95	130	87	97	89	83
77	149	97	92	96	76
82	117	119	102	91	84
89	135	110	97	94	77
101	131	112	90	91	88
89	123	113	75	89	88
92	152	96	81	79	89
94	112	90	84	88	92
92	126	113	103	86	83
107	126	100	111	91	99
89	129	110	97	90	87
84	151	144	108	89	72
90	137	92	96	87	80
93	136	118	114	105	83
83	107	87	76	79	77
91	139	103	138	114	85
85	150	99	89	84	81
75	135	105	67	62	78
89	170	101	87	90	89
88	144	116	91	84	78
77	139	139	127	113	73
81	144	146	137	92	81
80	95	104	87	91	80

APPENDIX F

Area Under The Curve For Each Type Of Tortilla

10% Nopal	100% Corn	Blue Corn	20% Nopal	10% Nopal/ 10% Bean	20% Bean
278.25	287.5	279.75	291.75	291.5	277
271	260.25	272.75	276	264.25	270.5
294.75	314.25	305.25	320	307.75	289
275.5	265.25	273	262.25	277.25	298.25
356.5	248.75	437.25	347	322.25	299.75
350.75	330.75	282.75	351.5	283.25	302.25
307.5	311.75	344.25	317	313	304
297.5	310.5	297	331.25	318.25	288.5
348.5	340.25	325	300.5	314	291.25
274.5	297.5	268.25	339	297	278.5
325.25	305.75	338.5	313	365.25	300
309.5	322	315.5	312.5	311.75	313
282.75	300.5	275.25	285	294.25	301.25
339	353.25	337	294.75	347.75	325.25
311.25	295	362.75	309.25	295.25	290.25
300.25	333	312.5	293.5	296.75	327.5
308.5	306	301	325.25	279.25	253.5
335.25	356.75	318	338.5	329.5	340.75
280	310.25	289	275.75	288.25	293.75
279	264	224.75	322.5	240	257.75
342.75	341.25	336.25	272.75	337.5	313.25
296.25	280.25	291.5	310.75	330	299.5
350.25	343.25	313.25	312.5	352.25	343
383	343.75	430.25	261	365.75	343.25
284.5	318	274.25	315.25	272.5	271.25

APPENDIX G

Maximal Glycemic Values for Each Type of Tortilla

10% Nopal	100% Corn	Blue Corn	20% Nopal	10%Nopa/10%Bean	20% Bean
121	137	106	132	124	118
109	102	101	112	99	109
141	163	151	160	152	130
111	105	105	99	122	149
145	110	180	155	167	119
160	135	134	155	138	135
126	133	133	139	123	131
135	131	129	178	148	123
204	188	172	142	166	152
121	147	117	155	130	112
123	129	158	130	181	126
123	154	117	136	137	126
132	130	110	122	127	129
149	167	156	120	168	151
154	131	164	134	171	137
137	136	124	190	122	136
157	143	124	144	141	107
126	138	113	168	130	139
126	160	124	142	129	150
152	153	111	155	97	135
163	222	191	126	157	170
124	139	129	147	157	144
167	149	133	130	159	139
157	165	172	111	160	146
116	127	103	146	138	104

APPENDIX H

Participants Average Minutes of Physical Activity a Week

Minutes of Physical Activity for Each Participant

127.75
257.50
1115.00
1092.50
612.50
132.50
321.25
20.00
227.50
651.25
206.25
288.33
220.00
1221.25
101.67
215.00
271.25
153.00
240.00
178.25
50.00
70.00
112.50
110.00
351.25

APPENDIX I

Participants HOMA-IR Values

HOMA-IR
1.475
0.948
0.933
0.773
0.306
1.996
1.690
1.372
1.105
1.136
2.308
0.745
1.689
0.578
1.645
2.873
1.681
1.982
0.616
0.970
1.991
1.943
1.567
1.658
0.887