

Effects of Postmeal Walking on Postprandial
Glucose Control and Oxidative Stress

by

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ABSTRACT

Background: Postprandial hyperglycemia can increase levels of oxidative stress and is an independent risk factor for complications associated with type 2 diabetes.

Purpose: To evaluate the acute effects of a 15-min postmeal walk on glucose control and markers of oxidative stress following a high-carbohydrate meal.

Methods: Ten obese subjects (55.0 ± 10.0 yrs) with impaired fasting glucose (107.1 ± 9.0 mg/dL) participated in this repeated measures trial. Subjects arrived at the laboratory following an overnight fast and underwent one of three conditions: 1) Test meal with no walking or fiber (CON), 2) Test meal with 10g fiber and no walking (FIB), 3) Test meal with no fiber followed by a 15-min treadmill walk at preferred walking speed (WALK). Blood samples were taken over four hours and assayed for glucose, insulin, thiobarbituric reactive substances (TBARS), catalase, uric acid, and total antioxidant capacity (TAC). A repeated measures ANOVA was used to compare mean differences for all outcome variables.

Results: The 2hr and 4hr incremental area under the curve (iAUC) for glucose was lower in both FIB (2hr: $-93.59 \text{ mmol} \cdot 120 \text{ min} \cdot \text{L}^{-1}$, $p = 0.006$; 4hr: $-92.59 \text{ mmol} \cdot 240 \text{ min} \cdot \text{L}^{-1}$; $p = 0.041$) and WALK (2hr: $-77.21 \text{ mmol} \cdot 120 \text{ min} \cdot \text{L}^{-1}$, $p = 0.002$; 4hr: $-102.94 \text{ mmol} \cdot 240 \text{ min} \cdot \text{L}^{-1}$; $p = 0.005$) conditions respectively, compared with CON. There were no differences in 2hr or 4hr iAUC for glucose between FIB and WALK (2hr: $p = 0.493$; 4hr: $p = 0.783$). The 2hr iAUC for insulin was significantly lower in both FIB ($-37.15 \text{ } \mu\text{U} \cdot \text{h}/\text{mL}$; $p = 0.021$) and WALK ($-66.35 \text{ } \mu\text{U} \cdot \text{h}/\text{mL}$; $p < 0.001$) conditions, compared with CON, and was significantly lower in the WALK ($-29.2 \text{ } \mu\text{U} \cdot \text{h}/\text{mL}$; $p = 0.049$) condition, compared with FIB. The 4hr iAUC for insulin in the WALK condition was significantly

lower than both CON ($-104.51 \mu\text{U} \cdot \text{h}/\text{mL}$; $p = 0.001$) and FIB ($-77.12 \mu\text{U} \cdot \text{h}/\text{mL}$; $p = 0.006$) conditions. Markers of oxidative stress were not significantly different between conditions.

Conclusion: A moderate 15-minute postmeal walk is an effective strategy to reduce postprandial hyperglycemia. However, it is unclear if this attenuation could lead to improvements in postprandial oxidative stress.

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CHAPTER 1

INTRODUCTION

The prevalence of impaired glucose regulation and type 2 diabetes (T2D) are rapidly increasing throughout the United States (US) and much of the developed world (Inzucchi, 2012). Indeed, approximately 382 million people worldwide were living with diabetes in 2013, and that number is expected to increase to 592 million people by 2035 (Guariguata et al., 2014). Type 2 diabetes is a major risk factor for blindness, end-stage renal failure, amputations and coronary artery disease (Bailey et al., 2014; Poulsen et al., 2013), and current estimates suggest that cardiovascular disease (CVD) is responsible for approximately 80% of deaths in patients with T2D (International Diabetes Federation (IDF), 2014). Consequently, the increasing prevalence of T2D imposes a substantial financial burden on healthcare costs and the US economy. In 2012, the total economic cost of diagnosed diabetes was estimated at \$245 billion, and current projections indicate that the economic liability of this disease will continue to increase, even after controlling for population growth and inflation (American Diabetes Association (ADA), 2013). As a result, identification of primary prevention or risk reduction strategies is necessary to attenuate the increasing burden of T2D and its complications.

Prediabetes is a condition characterized by elevated blood glucose levels (100-125mg/dL) that have not yet reached a diabetic range (Alberti et al., 1998). Prevalence of prediabetes has increased markedly among US adults in the last 20 years. Data from the National Diabetes Statistic Report 2014 indicate that 86 million Americans age 20 and older have prediabetes, resulting in approximately 1.7 million new cases of diabetes each year (CDC, 2014). Individuals with prediabetes are at greater risk of developing T2D

(DECODE, 2003), and the condition is an independent risk factor for CVD mortality (Barr et al., 2007; Meigs, Nathan, D'Agostino, & Wilson, 2002). Therefore, a unique opportunity exists within this group for targeted and effective prevention strategies.

Elevated blood glucose after a meal, termed postprandial hyperglycemia, may directly contribute to the progression of impaired fasting glucose (IFG) to T2D (Meigs et al., 2002) and might represent a key factor contributing to the increased risk of CVD observed in this population (O'Keefe & Bell, 2007). Epidemiological findings suggest that postprandial hyperglycemia is a critical component of CVD risk in T2D. The Diabetes Epidemiological Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) and Asia (DECODA) established that postprandial hyperglycemic values were better predictors of all-cause mortality and CVD than fasting glucose values (DECODE, 2001; Nakagami et al., 2006). Similarly, the Framingham Offspring Study showed that postchallenge hyperglycemia was a better predictor of ischemic events than glycated hemoglobin (HbA1C) and was an independent risk factor for CVD (Meigs et al., 2002).

Postprandial glucose spikes tend to generate reactive oxygen species (ROS), which can cause cellular oxidative stress (Phaniendra, Jestadi, & Periyasamy, 2015; Williamson & Cooper, 1980). This oxidative stress is now recognized as a key pathogenic factor leading to insulin resistance, beta cell dysfunction, impaired glucose tolerance, and eventual T2D (Ceriello & Motz, 2004). Additionally, oxidative stress plays a key role in the pathogenesis of both micro and macrovascular diabetic complications and is considered to be a unifying mechanism underlying the relation between acute hyperglycemia and the increase in cardiovascular risk (Brownlee, 2005). Studies

targeting postprandial hyperglycemia in individuals with impaired glucose tolerance (IGT) have demonstrated cardiovascular benefits. For example, the STOP-NIDDM group demonstrated a 49% reduction in the relative risk of new CVD events in individuals receiving a treatment targeted at lowering postprandial glucose spikes, compared with placebo after an average of 3.3 years of follow-up (Chiasson et al., 2003).

Since individuals spend most of their waking hours in the postprandial state, identifying ways to normalize the blood glucose response during this time could prove particularly efficacious. While drug therapies can be used to successfully treat specific problems of this disorder, a much more cost-effective and systemic solution may include a lifestyle approach. The Diabetes Prevention Program (DPP) has published several studies indicating that T2D risk can be attenuated by both diet and exercise (Knowler et al., 2002; Perreault et al., 2012). In 2002, the DPP investigated the comparative effectiveness of a lifestyle-intervention program or administration of metformin on development of T2D in a group of 3234 nondiabetic individuals with IFG and IGT (Knowler et al., 2002). The lifestyle intervention program included a low-calorie, low-fat diet and at least 150 minutes of moderate physical activity (brisk walking) per week. After 2.8 years of follow-up, the incidence of diabetes was 11.0 and 4.8 cases per 100 person-years in the placebo and lifestyle groups, respectively. The lifestyle intervention reduced T2D incidence by 58%, which was significantly more effective than metformin.

Exercise, either alone or in addition to other lifestyle modifications, is considered to be a principal prevention strategy in the management of T2D. The favorable effects of chronic exercise on insulin sensitivity and glucose levels are well established (Stanford & Goodyear, 2014). Furthermore, a number of lifestyle intervention trials in prediabetics

have shown a successful reduction in the incidence of T2D, as well as its associated risk factors (Knowler et al., 2002; Li et al., 2008; Ramachandran et al., 2006). However, many of these interventions recommend an exercise program that individuals do not generally adopt following study completion. To address this, some investigators have recently assessed the efficacy of low or moderate-intensity exercise protocols (walking) in mitigating metabolic risk factors.

Nygaard et al. (2009) investigated the effects of a 15-minute or 40-minute postmeal walk on blood glucose levels in 14 older (>50 years) healthy women. The walking was performed at a self-selected slow pace, corresponding to a rating of perceived exertion (RPE) of 9 on the 6-20 Borg scale. The authors showed that both walks were effective in significantly lowering average glucose concentration values, and the 40-minute walk significantly lowered glucose incremental area under the curve (iAUC) by 31.2%. The authors concluded that even slow short-term postmeal walking could reduce the blood glucose response to a high carbohydrate meal (Nygaard et al., 2009). Additionally, a recent study in individuals with IFG (blood glucose: 105-125mg/dL) found that 15-minute postmeal walks were significantly more effective at improving 24-hour glycemic control than 45-minutes of sustained morning or evening walking (DiPietro et al., 2013). This suggests that the postprandial period may represent a unique time period to target specific and adoptable preventive strategies, such as walking.

This emerging body of literature represents an exciting opportunity to present at-risk individuals with a realistic and adoptable strategy to control postprandial hyperglycemia. However, there are still very few studies that have examined the glycemic response to postmeal walking, and no study to my knowledge that has looked at

the effect of walking on oxidative stress resulting from a high glycemic meal. Therefore, the **primary aim** of this study is to evaluate the effects of a 15-minute moderate postmeal walk on glucose control and the postprandial incremental area under the curve (iAUC) for glucose and insulin in a group of at-risk individuals against a positive control (fiber supplementation). The **secondary aim** will be to assess the impact of postprandial hyperglycemia on markers of oxidative stress and antioxidant capacity. I **hypothesize** that the postmeal walk, similar to fiber, will attenuate the rise in blood glucose and insulin, as well as the iAUC of both, following a high glycemic meal. Additionally, I **hypothesize** that compared with control, markers of antioxidant status will increase and markers of oxidative stress will decrease for the fiber and walking trials, respectively.

CHAPTER 2

REVIEW OF LITERATURE

Health and Economic Burden of Type 2 Diabetes

The increasing prevalence of diabetes over the last few decades (Selvin, Parrinello, Sacks, & Coresh, 2014; CDC, 2012) has made it one of the most common and costly disorders in the US. In 2012, approximately 29.1 million Americans or 9.3% of the population had diabetes (CDC, 2014). Of this 29.1 million, approximately 8.1 million cases were undiagnosed. It is estimated that an additional 86 million Americans aged 20 years or older had prediabetes (fasting glucose 100-126 mg/dL) resulting in approximately 1.7 million new cases of diabetes each year, primarily in individuals aged 45-64 years. The percentage of Americans living with diabetes is approximately four times higher in individuals aged 45-64 (16.2%) and over six times higher in individuals 65 years or older (25.9%), compared with those who are 20-44 years of age (4.1%). In all adults aged 20 years or older, the prevalence of diabetes is 13.6% in men and 11.2% in women (CDC, 2014). Type 2 diabetes accounts for 90-95% of all diabetes cases in the US, and recent estimates suggest that as many as 1 in 3 US adults could have diabetes by 2050 if current trends continue (CDC, 2010).

Diabetes is the seventh leading cause of death in the United States (CDC, 2014), and is a major risk factor for CVD (American Diabetes Association [ADA], 2014). Indeed, more than 90% of diabetic patients are thought to be at risk for CVD, and have a worsened prognosis after experiencing a cardiac event than those without the disease (IDF, 2014). Additionally, diabetes is the leading cause of kidney failure, non-accident

leg and foot amputations, and new cases of blindness among adults under age 75 (CDC, 2010).

In addition to a significant healthcare issue, diabetes represents a major economic burden for the United States. Annual medical expenses for individuals with diagnosed diabetes are estimated to be more than twice as high, on average, than in those without diabetes (ADA, 2014). In 2012, the total economic cost of diagnosed diabetes was approximately \$245 billion, including \$176 billion in direct medical costs and \$69 billion in reduced productivity (ADA, 2014). This number represents a 41% increase in total estimated costs from 2007. It is estimated that individuals with diagnosed diabetes will spend nearly \$125,000 in excess medical expenses throughout the course of their lives, and that low-cost diabetes prevention could save this amount for each new case of diabetes prevented at age 40 years (Zhuo et al., 2014).

Type 2 Diabetes Pathology

While Type 1 diabetes results from an autoimmune destruction of pancreatic beta cells, T2D is a complex metabolic disorder characterized by hyperglycemia, insulin resistance, and relative impairment in insulin secretion. A prospective study by Weyer et al. (1999) investigated the time course for insulin resistance and reduced insulin secretory capacity during the progression from normal glucose tolerance (NGT) to T2D. Seventeen Pima Indians, who experienced a deterioration of glucose tolerance from normal to impaired to diabetic over an average of 5.1 years of follow up, were assessed. Insulin action (hyperinsulinemic, euglycemic clamp) and insulin secretion (intravenous glucose tolerance test) were measured. Additionally, body weight and percent body fat were assessed at each visit. Subjects were evaluated an average of 5.4 times throughout the

study. Body weight increased by 14% during the follow-up. Insulin-stimulated glucose disposal decreased by 12% in the transition of NGT to IGT and by an additional 19% in the transition of IGT to diabetes. Insulin secretion decreased by 27% during the transition from NGT to IGT, and a further 51% during progression from IGT to diabetes (Weyer, Bogardus, Mott, & Pratly, 1999). These data suggest that the deterioration of both insulin action and secretion occur early in the pathogenesis of diabetes. Similarly, a study of 6538 British civil servants with NGT, followed for an average of 8.2 years, indicated that changes in glucose concentrations, insulin sensitivity, and insulin secretion were evident in diabetic subjects 3-6 years before their diagnosis (Tabak et al. 2009).

In order to determine the relative contribution of insulin resistance and insulin secretory dysfunction at different developmental stages, Weyer, Tataranni, Bogardus, & Pratley (2001) analyzed prospective data from a cohort of Pima Indians who were followed for up to 13 years. They assessed the predictive effects of insulin resistance and early-phase insulin secretion separately for the progression from NGT to IGT and IGT to diabetes. Their findings indicated that insulin-stimulated glucose uptake and early-phase insulin secretion were independent and additive predictors of both the transition from NGT to IGT and the progression from IGT to diabetes. Haffner and colleagues (2009) then investigated the relative differences in insulin sensitivity and insulin secretion across distinct glucose tolerant categories in a group of 6414 Finnish men. Compared to those with normal glucose tolerance, insulin sensitivity was decreased by 26% in individuals with IGT and 46% in newly diagnosed diabetic individuals, and statistical significance was retained after adjusting for both age and BMI. Early-phase insulin release was decreased by 8% in individuals with IGT and 43% in newly diagnosed diabetics,

compared with normal glucose tolerant individuals (Stančáková, et al., 2009). The impairment of insulin sensitivity began at an early stage, while insulin secretory capacity was largely maintained. In contrast, the impairment of insulin secretion progressed considerably in the diabetic range of fasting glucose tolerance, suggesting that impaired insulin sensitivity may be a key feature in the early stages of metabolic dysfunction, while impaired early-phase insulin secretion may be a much larger feature later in the progression of the disease.

Beta Cell Dysfunction

The relative impact of beta cell dysfunction on the progression of impaired glucose tolerance and ultimately T2D is well understood (DeFronzo, Eldor, & Abdul-Ghani, 2013). A key feature of this dysfunction may be relative beta cell volume and frequency of beta cell apoptosis. In order to quantify this, Butler et al. (2003) examined pancreatic tissue from post mortem subjects with normal glucose tolerance, as well as those with IFG or T2D. Individuals with IFG demonstrated a 40% deficit in beta cell volume even before the onset of T2D, and T2D individuals exhibited a 63% deficit in beta cell volume. The mechanism underlying these deficits was shown to be increased beta cell apoptosis, which was increased 3-fold in the diabetic individuals (Butler et al. 2003). Later work by this group highlighted the importance of these finding, revealing a curvilinear relationship in humans between the relative beta cell volume and fasting glucose concentrations (Ritzel, Butler, A., Rizza, Veldhuis, & Butler, P., 2006). The authors indicated that below a threshold of about 1.1% (point of inflection between beta cell volume and fasting glucose) of pancreas volume, insulin sensitivity and functional defects in insulin secretion have a much greater impact on blood glucose concentrations.

These data suggest that beta cell apoptosis, along with beta cell dysfunction, are central events in the development of T2D.

Defronzo (2009) highlighted the following factors in the pathogenesis of beta cell dysfunction and failure observed at the stage of IGT and T2D:

- *Age*: The incidence of T2D increases with advancing age, which is associated with decrease beta cell function and mass.
- *Genes*: More than half of obese insulin-resistant individuals will never develop diabetes, underscoring a potential genetic link in beta cell dysfunction and loss. Genome-wide association studies have indicated that the majority of genes linked to T2D play a direct role in beta cell function or mass (Alejandro, Gregg, Blandino-Rosano, Cras-Meneur, & Bernal-Mizrachi, 2014). Transcription factors regulated by Wnt signaling, including the most influential variant in the TCF7L2 locus are considered potential risk factors for T2D (Frayling, 2007; Grarup, Sandholt, Hansen, & Pedersen, 2014).
- *Insulin resistance*: An insulin resistant state places an increased demand on the beta cell to hyper-secrete insulin, which may play an important role in the progressive beta cell failure observed in T2D. The beta cell has been shown to expand during conditions of insulin resistance in mice (Hull et al., 2005). Furthermore, this capacity to expand in response to metabolic stress decreases with age (Rankin & Kushner, 2009). The exact mechanism by which insulin resistance leads to beta cell failure is not fully understood; however, the expansion in beta cells that occurs in response to metabolic stress are thought to

- include signaling from growth factors (insulin) and nutrients, including glucose and amino acids (Alejandro et al., 2014).
- *Lipotoxicity*: Chronic elevations in plasma free fatty acid (FFA) levels overwhelm islet beta cells and further diminish beta cell function. While current evidence suggests that the damaging effects of lipids on beta cell function occurs predominately in the presence of concomitant hyperglycemia (Alejandro et al., 2014), studies have also shown deleterious effects of FFAs independent of high glucose. Dubois et al. (2004) isolated human islets from non-obese and non-diabetic donors and cultured them with 1 or 2 mmol/l non-esterified fatty acids. They found that the addition of non-esterified fatty acids at 1 or 2 mmol/l led to decreases in glucose-stimulated insulin secretion of 19.3% and 49.9% respectively, independent of glucotoxicity. More recently, lipid infusion has been shown to reduce insulin-stimulated whole body glucose disposal in non-diabetic adolescents, as measured by a hyperinsulinemic euglycemic clamp (Hughan, Bonadonna, Lee, Michaliszyn, & Arslanian, 2013), demonstrating an important role of lipids on insulin resistance and T2D risk.
 - *Glucotoxicity*: The inability of pancreatic beta cells to adapt to the high metabolic demand of T2D individuals results in hyperglycemia, which itself exerts damaging effects on beta cells, resulting in loss of beta cell function and mass (Bensellam, Laybutt, & Jonas, 2012). The current understanding of the mechanisms underlying the harmful effects of glucotoxicity on beta cells is based mainly on animal studies and *in vitro* experimentation on isolated islets and beta cells (Bensellam et al., 2012). Some proposed mechanisms include beta cell

overstimulation (Sempoux et al., 2001), oxidative stress (Kaneto et al., 1999), cellular endoplasmic reticulum stress (Osowski & Urano, 2010), hypoxia (Bensellam et al., 2012), and inflammation (Homo-Delarche et al., 2006).

- *Gut-derived Factors*: Incretins are metabolic hormones that stimulate a decrease in blood glucose levels, and abnormalities in the incretin axis may play an important role in progressive beta cell failure observed in T2D. Glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) are the two main molecules responsible for the incretin effect (DeFronzo, 2009). GLP-1 stimulates insulin secretion, while delaying gastric emptying and promoting satiety. Deficiency in GLP-1 is observed in individuals with IGT and worsens in those with T2D (Toft-Nielsen et al., 2001). Umpierrez et al. (2014) found that patients receiving a GLP-1 analogue, as opposed to metformin, experienced a 22% greater reduction in glycated hemoglobin (HbA1C), which was sustained over the entire 52-week study, indicating a significant impact of GLP-1 on glucose control. Conversely GIP is lowered in individuals with T2D and appears to be resistant to the stimulatory effects of GLP-1 on insulin secretion (DeFronzo, 2009).

Insulin Resistance

Insulin resistance is present when the biological effects of insulin, including glucose uptake at the skeletal muscle and endogenous glucose production in the liver, are less and more than expected, respectively. These abnormalities in insulin action often occur in individuals before the onset of frank T2D. Under normal conditions, insulin binds and activates the specific plasma membrane insulin receptor with tyrosine kinase activity, thus allowing the tyrosine phosphorylation of insulin receptor substrate (IRS)

proteins. These tyrosine phosphorylated sites serve as binding scaffolds for various adapter proteins, leading to a downstream signaling cascade that results in the translocation of glucose transporters to the cell surface for glucose uptake. Insulin resistance likely results from mechanisms blocking insulin signaling and is present namely at the level of the liver, muscle, and adipose tissue in T2D individuals.

Adipose tissue: Adipose tissue is the primary source of FFAs for triglyceride synthesis in the liver. Under normal conditions, the release of FFAs from the adipose is proportional to hepatic utilization and insulin plays a key role in inhibiting this release. However, under insulin resistant conditions, insulin fails to inhibit lipolysis, which leads to an oversupply of FFA to the liver, increased hepatic triglyceride synthesis, as well as increased intracellular accumulation of lipid products that impairs insulin signaling and activates inflammatory pathways (NFkB, JNK) that release adipocytokines, which can further induce insulin resistance in the liver and skeletal muscle (Cusi, 2012).

Liver: At rest, energy demands are met through the production of glucose by gluconeogenesis in the liver (small amount in the kidneys). This hepatic glucose production at rest has been shown to be markedly elevated in individuals with T2D. In the liver, insulin promotes glycogen synthesis and to a lesser extent, de novo lipogenesis, while also inhibiting gluconeogenesis. Under insulin resistant conditions, intramyocellular triglyceride concentrations accumulate and impair the ability of insulin to regulate gluconeogenesis and glycogen synthesis. At the same time, lipogenesis is unaffected by insulin defects and together with the increased delivery of dietary glucose to the liver, leads to an accumulation of FFAs (Samuel & Shulman, 2012).

Muscle: Skeletal muscle is the major site of glucose uptake in the postprandial state, responsible for ~80% of glucose uptake during normal postprandial conditions. Typically in the skeletal muscle, insulin promotes glucose uptake by activating a signaling cascade resulting in the translocation of the glucose transporter 4 (GLUT4) to the cell membrane. Initially, insulin binds to the insulin receptor leading to the phosphorylation of IRS-1 on tyrosine residues. Tyrosine phosphorylation of IRS-1 results in activation of phosphatidylinositol 3-kinase (PI3K and the downstream protein Akt/PKB, which enables the translocation of GLUT4 to the plasma membrane and subsequent entry of glucose into the cell. A defect in this insulin-signaling cascade, evident in individuals with metabolic dysfunction, occurs due to an impairment of insulin to phosphorylate tyrosine residues in IRS-1, which eventually impairs the entry of glucose into the muscle cell (Stanford & Goodyear, 2014).

Basal versus Postprandial Markers for Disease Prediction

Epidemiological findings suggest that postprandial hyperglycemia is an independent risk factor for complications of T2D. The Diabetes Epidemiological Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) and Asia (DECODA) found that postprandial hyperglycemia values are better predictors of all-cause mortality and CVD than fasting glucose values (DECODE Study Group, 2001; Nakagami et al., 2006). Similarly, the Framingham Offspring Study showed that postchallenge hyperglycemia is a better predictor of ischemic events than glycated hemoglobin (HbA1C) and is an independent risk factor of CVD (Meigs, Nathan, D'Agostino, & Wilson, 2002).

Glycated hemoglobin is a measure used to identify the average plasma glucose concentration over a prolonged period of time. Higher levels of HbA1C are associated with increased risk of CVD (Patel et al., 2008), and current recommendations indicate that HbA1C should be below 7.0% for most patients (ADA, 2009). Both fasting and postprandial hyperglycemia contribute to HbA1C, but plasma glucose is generally measured in the fasting state, and postprandial hyperglycemia usually remains a second or third line target in the treatment of hyperglycemia (Nathan et al., 2007). In 2003, Monnier, Lapinski, & Colette (2003) published a landmark study describing the relative contributions of both fasting and postprandial hyperglycemia to overall hyperglycemic exposure at varying levels of HbA1C. The analysis was based on a 1-day 4-period glucose profile in 290 non-insulin patients with T2D. Plasma glucose was measured at fasting, and during postprandial periods throughout the day, and the relative contribution of each to overall diurnal hyperglycemia was calculated. Data were then compared over quintiles of HbA1C. The authors showed that postprandial hyperglycemia accounted for ~70% of overall glycemic exposure in the lowest range of HbA1C (< 7.3%), and ~30% in the highest range of HbA1c (> 10.2%), suggesting that the relative contribution of postprandial hyperglycemia is predominant in fairly controlled patients, whereas the contribution of fasting hyperglycemia increases gradually as glucose control worsens (Monnier et al., 2003). This was important because not only did it reinforce the necessity of considering both fasting and postprandial hyperglycemia, the results also offered an explanation for the inconsistency of results from previous studies.

A recent study by this group assessed the composition of residual dysglycemia when HbA1c was between 6.5% and 7%, a range consistent with the current definition of

diabetes (Monnier, Colette, Dejager, & Owens, 2014). One hundred individuals with T2D were divided into either group one (HbA1c <6.5%) or group two (HbA1c 6.5-6.9%) and underwent continuous glucose monitoring (CGM) for three days. Postprandial hyperglycemia was measured on the first two days for two hours following each main meal. The authors found that in individuals under satisfactory glycemic control (HbA1c <7.0%), the differences between those exhibiting mild dysglycemia (6.5-6.9%) and those with better glycemic control (<6.5%) were due to greater contributions from postprandial hyperglycemia in the higher group (Monnier et al., 2014). The findings provide compelling evidence for the need to focus on decreasing postprandial glucose excursions for disease prevention.

Indeed, studies targeting postprandial hyperglycemia in individuals with IGT have shown cardiovascular benefits. The STOP-NIDDM group evaluated a total of 1368 patients with IGT that were randomized to a treatment (100mg acarbose 3/day at the start of meals) or placebo group. Individuals in the active treatment group experienced a 49% reduction in the relative risk of new CVD events compared with placebo after an average of 3.3 years of follow-up (Chiasson et al., 2003). Conversely, the HEART2D study found no significant difference between postprandial and basal insulin therapy and CVD outcomes after 2.7 years in patients with T2D (Raz et al., 2009). However, the study population represented an extremely high-risk group, as average HbA1c levels were well above 7.0% and all patients had already experienced an acute myocardial infarction in both groups. The inconsistency of these studies echo the findings from Monnier et al. (2014), indicating that interventions targeting postprandial hyperglycemia may need to

occur at an earlier stage of disease progression, and that the relative contribution of postprandial hyperglycemia to average glucose may decrease as hyperglycemia worsens.

Assessment of Postprandial Glucose Control

In healthy individuals, fasting plasma glucose concentrations typically range from 70-99 mg/dL and begin to rise ~10 minutes after meal consumption as a result of normal carbohydrate absorption from the gut. Thereafter, concentrations peak at ~30-60 minutes and return to normal fasting levels between 2 and 3 hours following the start of the meal. The magnitude of the rise in plasma glucose following the meal depends on many factors, including the timing, quantity, and composition of the meal. Glucose control in the postprandial period is determined by carbohydrate absorption, insulin and glucagon secretion, and their collective effects on glucose metabolism in the liver and peripheral tissues. In individuals with IGT or T2D, peak insulin levels are delayed and insufficient to control immediate postprandial glucose excursions. The enhanced glycemic response observed in these populations is the result of a number of metabolic abnormalities, including beta cell secretion, suppression of hepatic glucose uptake and production, and impaired peripheral glucose uptake at the skeletal muscle. The following are tests used to determine glucose concentrations and kinetics in the postprandial period.

Oral Glucose Tolerance Test

The oral glucose tolerance test (OGTT) is a simple test that is widely used in research and practice to identify impaired glucose tolerance, which is defined on the basis of abnormal plasma glucose following a 2-hour OGTT. Individuals without diabetes but with an OGTT 2-hour value of 140-199 mg/dL are considered to have IGT (ADA, 2014). Generally, a blood sample is taken following an overnight fast, after which subjects are

asked to consume a liquid containing a certain amount of glucose (usually 75 grams) in five minutes. After consumption, blood samples are generally taken at 30, 60, 90 and 120 minutes to determine glucose (and insulin) concentrations. Oral glucose tolerance reflects the efficiency of the body to handle an oral glucose load, but does not directly assess insulin sensitivity or resistance. While the test is commonly used in both research and clinical practice to determine glucose tolerance (ADA, 2014), there are some limitations of the test in research situations. These include variability in the rate of gastric emptying, which can cause variability and imprecision from the start, even in the same individual, as well as the limitation of a standard OGTT to identify the dynamics of glucose and insulin action (Patarrão, Lautt, and Macedo, 2015). The OGTT does not measure the relative contributions of beta cell function and insulin sensitivity to overall glycemic exposure, and results should be interpreted carefully.

Meal Tolerance Test

The meal tolerance test (MTT) is similar to the OGTT, but is more representative of physiological situations. The procedure includes a baseline blood draw following an overnight fast, after which subjects consume a liquid or solid mixed meal in a specified amount of time, and blood samples (usually glucose and insulin) are taken at 30-minute intervals over a two-hour period. The MTT has been frequently used in studies assessing the impact of an acute bout of exercise on glucose control in healthy (Heiss & Tollefson, 2014; Nygaard, Tomten, & Høstmark, 2009; Manohar et al., 2012), T2D (Colberg et al., 2009; Karstoft, Christensen, Pedersen, & Solomon, 2014), and IGT (DiPietro, Gribok, Stevens, Hamm, & Rumpler, 2013; Lunde, Hjellset, & Høstmark, 2012) individuals. Advantages of the MTT include use of a physiological stimulus more representative of

ordinary glycemic exposure and a more physiological stimulus for assessing an incretin response to a meal (Maki, McKenney, Farmer, Reeves & Dicklin, 2009). A recent review article by Patarrão et al. (2014) compared the OGTT to the MTT and indicated that the MTT is a simple procedure that is less unpleasant for the individual than a standard OGTT. The authors suggested that the beta cell response is generally stronger after a mixed meal than after an OGTT with equal carbohydrates, likely a function of the lower glycemic index of the meal or slower gastric emptying, and could lead to lower glucose excursions. The authors concluded that the MTT was more physiologically representative of the human diet and potentially useful in understanding beta cell function in the different categories of glucose intolerance, but not necessarily insulin sensitivity and/or resistance (Patarrão et al., 2014).

Glucose Tracer Techniques

Both the OGTT and MTT provide valid and reliable assessments of glucose tolerance following a meal, but do not provide evidence of glucose kinetics or relative impact of insulin secretion or resistance. In fasting conditions, the amount of glucose entering the blood is generally equal to the amount of glucose leaving (or clearing) the circulation. Glucose entering the blood under these conditions is primarily derived from the liver and measurement of appearance to clearance is straightforward. However, in the postprandial period, the amount of glucose in the blood is a function of gastric emptying, glucose absorption, appearance of ingested glucose, endogenous glucose production, as well as glucose disappearance, and an alteration in any of these processes can impact overall glucose tolerance. The use of a tracer has been employed in many acute exercise studies to assess the relative impact of each of these processes to overall postprandial

glycemic control (Knudsen et al., 2014; Larsen et al., 1997; Larsen et al., 1999). The dual-isotope tracer technique allows for the relative assessment of each of these variables. The approach utilizes two tracers (one infused and one ingested) to simultaneously measure the rate of glucose appearance from the meal and endogenous glucose production. The appearance of ingested glucose is calculated by multiplying the rate of appearance to the tracer-tracee ratio of the meal. Initial splanchnic (gut and liver) glucose uptake is calculated by subtracting the portion of the ingested glucose that reaches the circulation from the total amount of glucose ingested. Endogenous glucose production is calculated by subtracting the rate of appearance of ingested glucose from total glucose appearance. Finally, glucose disappearance is calculated by subtracting the change in glucose mass from the total rate of glucose appearance (Knudsen et al., 2014; Vella & Rizza, 2009). Although the dual-isotope method has been utilized in many studies, results have not always been consistent. Consequently, alternative glucose tracer techniques have been used, including a triple tracer technique, which includes a labeled tracer on the meal glucose and a second tracer infused intravenously to mimic the appearance of meal-derived glucose (Vella & Rizza, 2009). Despite respective limitations, both methods provide a more thorough model of relative substrate fluxes to determine postprandial glucose control.

Surrogate indices for insulin sensitivity and resistance

The hyperinsulinemic-euglycemic clamp technique is considered the gold standard measure of peripheral insulin sensitivity. However, this technique is expensive, time consuming, invasive, and not easy to assess in many cases. Therefore, surrogate measures of insulin sensitivity (from fasting and postprandial values) are utilized instead.

The homeostatic model assessment (HOMA) is a model used to quantify insulin resistance and beta cell function. It is calculated from fasting glucose and insulin concentrations and assumes a feedback loop between the liver and beta cell (Matthews, et al., 1985). The quantitative insulin sensitivity check index (QUICKI) also uses fasting glucose and insulin concentrations and is one of the most thoroughly evaluated and validated surrogate indices to determine insulin sensitivity (Otten, Ahrén & Olsson, 2014). Both the HOMA and QUICKI models are easy to employ, as they require a single blood draw from a fasting participant; however, both primarily reflect hepatic insulin sensitivity, and largely do not measure peripheral insulin sensitivity, an important consideration in the postprandial period.

Surrogate indices calculated from an OGTT may provide a better estimation of hepatic and peripheral insulin resistance during postprandial periods. These indices include the Cederholm and Wibell index, Gut index, Avignon index, Matsuda index, Belfiore index, Stumvoll index, McAuley index, and the oral glucose insulin sensitivity (OGIS) calculation (Patarrão et al., 2014). A recent review article by Otten et al. (2014) indicated that the OGTT-based surrogate markers with the strongest correlations to the clamp were the Stumvoll index, the OGIS, the Matsuda index, and the Gutt index. Furthermore, the revised QUICKI fasting surrogate marker appeared to be as good as the OGTT-based indices for estimating insulin sensitivity. The authors concluded that these measures were valid and appropriate for use in clinical studies (Otten et al., 2014).

Fiber and Postprandial Hyperglycemia

Dietary fibers are indigestible polysaccharides found naturally in plants. They are simplistically, but commonly, classified according to their solubility in water, as this

generally dictates their physiological action. However, evidence now suggests that classifying fibers by viscosity may be even more indicative of their proposed benefits (Chutkan et al., 2012). Both soluble and insoluble fibers have been shown to have different but noticeable health benefits including laxation, an attenuation of LDL cholesterol and variance in blood glucose levels, as well as a reduction of overall glucose and insulin levels. Soluble fibers dissolve in water and can be further divided into viscous (gel-forming) or nonviscous types. Viscosity refers to the fiber's ability to thicken and form gels when mixed with fluids in the small intestine. By forming these thick gels, soluble fiber delays gastric emptying, which can modulate blood sugar levels and insulin sensitivity (Slavin, Martini, Jacobs, & Marguart, 1999). Consequently, soluble viscous fiber, as opposed to non-viscous or insoluble, has demonstrated the greatest protection against postprandial hyperglycemia due to its delayed effect on gastric emptying (Slavin et al., 1999).

An early study by Jenkins et al. (1978) showed that a reduction in postprandial blood glucose was highly correlated with fiber viscosity and a delay in mouth to cecum transit time following an OGTT. The delay of gastric emptying and absorption of glucose from the small intestine was proposed as the mechanism for this attenuation of postprandial glucose. Since that time, these observations have been corroborated and extended in many studies of healthy subjects. In a double-blind crossover design, Rigaud et al. (1998) found that 7.4g of psyllium, a soluble viscous fiber, attenuated the postprandial rise of serum glucose, triglycerides, and insulin levels following both a liquid and solid meal. Although they did not find a delay in gastric emptying, they did conclude that the attenuation was due to a delay in mouth to cecum transit time (Rigaud,

Paycha, Meulemans, & Mignon, 1998). Additionally, Sierra et al. (2001) compared the postprandial effects of 50g of glucose load with either 10.5g of a soluble viscous fiber (psyllium), or 10.5g of a soluble non-viscous fiber (guar-gum), on glucose and insulin concentration in 10 healthy females. They found that both types of fiber significantly decreased serum insulin concentrations; however, only the soluble viscous fiber decreased both insulin and glucose postprandial concentrations (Sierra et al., 2001). Together, these studies provide convincing evidence for the beneficial role of soluble viscous dietary fiber in the attenuation of postprandial hyperglycemia.

Long-term Intervention Studies for T2D

Six large-scale longitudinal studies have investigated the impact of a lifestyle intervention program on delaying or preventing the incidence of T2D (Eriksson & Lindgärde, 1991; Knowler et al., 2002; Kosaka, Noda, & Kuzuya, 2005; Pan et al., 1997; Ramachandran, et al., 2006; Tuomilehto et al., 2001). These studies assessed the impact of a diet and exercise intervention versus the standard of care treatment on T2D in individuals with IGT in Sweden (Eriksson & Lindgärde, 1991; Eriksson & Lindgärde, 1998), Finland (Tuomilehto et al., 2001; Lindström et al., 2003; Lindström et al., 2006), United States (Knowler et al., 2002; Knowler et al., 2009), India (Ramachandran, 2006), China (Pan et al., 1997; Li et al., 2008), and Japan (Kosaka, 2005).

One of the earliest lifestyle intervention studies for the prevention of T2D was conducted from 1974-1985 in middle-aged men from Malmö, Sweden (Eriksson & Lindgärde, 1991). After screening, the authors assigned males, aged 47-49, who were part of a large five-year screening program, to one of four groups: 1) T2D with lifestyle intervention (n = 21), 2) IGT with lifestyle intervention (n = 181), 3) IGT with standard

care (n = 79), 4) NGT with standard care (n = 144). The lifestyle intervention was a randomized crossover design and included six months of supervised physical training and six months of dietary treatment. After the 12 months, subjects were instructed to continue following the protocol either in groups or on their own and attend regular check-ups for the remaining five years. At the end of the five years, glucose tolerance had improved in 75.8% and had normalized (2hr value < 7.0mmol/l) in 52.2% of cases in the IGT with lifestyle intervention group, compared with a deterioration of 67.1% in the IGT with standard care group. Additionally, diabetes incidence at five years was 10.6% in the IGT with lifestyle intervention group versus 28.6% in the IGT with standard care group. At the 12-year follow-up, there was no difference in mortality rates between men with NGT and the IGT men who underwent lifestyle intervention (Eriksson & Lindgärde, 1998). Furthermore, both groups had less than half the mortality rate after 12-years of follow-up than the IGT men who did not receive the lifestyle intervention.

The Finnish Diabetes Prevention Study was the first large randomized controlled trial to assess the impact of an intensive lifestyle intervention on T2D prevention in a group of high-risk individuals (Tuomilehto et al., 2001). The authors randomly assigned 522 middle-aged men and women with IGT (2hr glucose: 140-200 mg/dL) to either a lifestyle intervention or control group. The lifestyle intervention included individualized counseling aimed at a five percent weight reduction from dietary counseling and adherence, as well as moderate exercise for at least 30 minutes each day. After an average follow-up of 3.2 years, the incidence of diabetes in the intervention group was less than half (11% vs. 23%), compared with control. Furthermore, diabetes incidence was inversely associated with the number and magnitude of lifestyle changes made

(Tuomilehto et al., 2001). The relative risk of diabetes was 58% higher in individuals with IGT who did not receive the lifestyle intervention, compared with those who did. In 2006, Lindström et al. (2006) published the 7-year follow-up results. The authors indicated a 43% reduction in relative risk in the intervention group, compared with control, during the total follow up, which was significantly proportional to the attainment of the intervention goals, including weight loss, dietary adjustment, and increased physical activity. Furthermore, the intervention group maintained a 36% reduction in relative risk during the 3-year post-intervention period, demonstrating the maintenance of beneficial lifestyle changes in the intervention group even after study completion.

The Diabetes Prevention Program Research Group assessed the impact of a lifestyle intervention or treatment with metformin on preventing or delaying the onset of T2D in an at-risk population (Knowler et al., 2002). A group of 3,234 individuals with IFG and IGT (fasting: 95-125 mg/dL; post-load: 140-199mg/dL) were randomly assigned to one of three groups: 1) Placebo, 2) Metformin (850mg twice daily), or 3) Lifestyle modification program. The lifestyle modification included a 16-lesson curriculum discussing healthy eating, exercise, and behavior modification with the goal of achieving a weight reduction of seven percent of initial body weight, the adoption of a healthy low-calorie, low-fat diet, and at least 150 minutes of moderate intensity exercise each week. After an average follow-up of 2.8 years, the incidence of diabetes was 58% lower in the lifestyle intervention group and 31% lower in the metformin group, compared with placebo. Furthermore, the incidence of diabetes was 39% lower in the lifestyle intervention group than in the metformin group. The 10-year follow-up results were published in 2009 in a group of 2,766 enrolled participants. At follow-up, diabetes

incidence was reduced by 34% in the lifestyle group and 18% in the metformin group, compared with placebo (Knowler et al., 2009).

In The Indian Diabetes Prevention Program, the authors followed a similar design to assess the impact of lifestyle intervention and/or metformin on diabetes incidence (Ramachandran et al., 2006). A group of 531 men and women with IGT (2hr glucose: 140-199mg/dL) were randomized into one of four groups: 1) Control, 2) Lifestyle modification, 3) Metformin treatment (250mg or 500mg twice daily), and 4) Lifestyle modification plus metformin. After a median follow up period of 30 months, the incidence of diabetes was 55% in the control versus 39.3%, 40.5%, and 39.5% in groups 2-4 respectively. Compared with control, the relative risk reduction was 28.5% in the lifestyle modification group, 26.4% in the metformin group, and 28.2% in the lifestyle modification plus metformin group. Unlike the Diabetes Prevention Program in the United States (Knowler et al., 2002), the Indian group found no significant differences between the lifestyle modification and the metformin group. Furthermore, they found that both interventions combined did not elicit any additional benefits with respect to reducing T2D risk (Ramachandran et al., 2006).

The Da Qing IGT and Diabetes Study investigated the relative impact of a six-year diet or exercise intervention, as well as a combined program, on T2D incidence in individuals with IGT (Pan et al., 1997). A group of 577 individuals were randomized into one of four groups: 1) control, 2) diet only, 3) exercise only, and 4) diet plus exercise. At six years, there was an overall reduction in diabetes incidence of 33% in the diet-only group, 47% in the exercise only group, and 38% in the diet plus exercise group. Li et al. (2008) published the 20-year follow-up results, indicating that participants in the

combined lifestyle intervention groups had a 43% lower incidence of T2D over the 20-year period, after controlling for age and clustering by clinic. The 20-year cumulative incidence of T2D was 80% in the intervention groups and 93% in the control group, which translated to an average of 3.6 fewer years with T2D for those in the intervention group compared with those in the control group. Interestingly, there was no significant difference in the rate of cardiovascular events, as well as CVD or all-cause mortality between the intervention and control groups.

Kosaka et al. (2005) investigated the impact of a four-year intensive lifestyle intervention on decreasing T2D incidence in 458 men with IGT (2hr-glucose: 160-239 mg/dL on 100g OGTTs). Subjects were randomly assigned in a 4:1 ratio to either a standard intervention group (advice on diet, exercise, and weight loss) or an intensive lifestyle intervention group. Individuals in the intensive lifestyle intervention group were informed of their desirable body weight and advised to weigh themselves at least once per week. They also received diet and exercise instruction every 3-4 months at each hospital visit. After four years, the cumulative incidence of diabetes was 9.3% in the control group and 3% in the intensive lifestyle intervention group, translating to a 67.4% lower risk of diabetes incidence.

Acute Exercise and Postprandial Glucose Control (non-walking)

Exercise is considered to be a cornerstone in the management of diabetes, and regular physical exercise can delay or prevent the progression of this disease (Knowler et al., 2002; Li et al., 2008). A recent meta-analysis by Macleod, Terada, Chahal, & Boule (2013) examined the effect of acute and chronic exercise on glycemic control in individuals with T2D. The authors showed that single-bout exercise sessions, as well as

long-term exercise, could improve glucose control in T2D subjects. The authors reviewed 11 studies that examined the glycemic response to exercise, including eight short-term exercise trials (≤ 2 weeks) and three long-term exercise trials (> 2 months). Seven out of the eight acute studies examined the effect of a single exercise session on glucose alterations. The authors indicated that exercise reduced 24-hour average glucose concentrations by 14 mg/dL and reduced daily time spent above 180 mg/dL by 129 minutes. However, exercise did not affect changes in fasting glucose concentrations. The authors speculated that the non-significant effect on fasting glucose may be due to the fact that postprandial hyperglycemia is more closely associated with insulin resistance at the level of the muscle, as opposed to increased hepatic insulin resistance (Macleod et al., 2013). Since exercise has routinely been shown to improve peripheral insulin sensitivity without affecting hepatic insulin sensitivity, exercise would be expected to have a greater effect on hyperglycemia in the postprandial period.

Postmeal Exercise

The impact of acute exercise on overall postprandial glucose control is still unclear. While many studies indicate that acute exercise lowers postprandial glucose (Gillen et al., 2012; Heden et al., 2015; Larsen, Dela, Kjaer, & Galbo, 1997; Larsen, Dela, Madsbad, & Galbo, 1999; Poirier et al., 2000; van Dijk et al., 2012; Rynders et al., 2014), others show either mixed effects (Ho, Dhaliwal, Hills, & Pal, 2010; Manders, Van Dijk, & Van Loon, 2010; Oberlin et al., 2014) or no effect (Baynard, Franklin, Goulopoulou, Carhart Jr, & Kanaley, 2005; Gonzalez, Veasey, Rumbold, & Stevenson, 2013; Knudsen, Karstoft, Pedersen, van Hall, & Solomon, 2014; Morishima, Mori, Sasaki, & Goto, 2014; Rose, Howlett, King, & Hargreaves, 2001). The four studies that

have investigated the effects of acute aerobic (Larsen et al., 1997; Larsen et al., 1999; Poirier et al., 2001) or resistance (Heden et al., 2015) postmeal exercise on glucose control have all indicated a decrease in glucose and insulin in the postprandial period. Larsen and colleagues conducted a series of studies that examined the effects of moderate and intense postmeal exercise on glucose regulation in T2D patients (Larsen et al., 1997; Larsen et al., 1999). The first study examined the acute effects of 45 minutes of bicycle exercise at 50% VO_{2max} compared to a resting control condition. The exercise session was performed 45 minutes after the standardized breakfast meal. The authors showed that average glucose and insulin concentrations and AUC were reduced in the postprandial period following breakfast. Additionally, they found a significant reduction in the rate of glucose appearance and an increase in the rate of glucose disappearance in the exercise condition, measured by glucose tracer. Interestingly, these effects were not observed in the postprandial period following the subsequent lunch meal. In a similar follow-up study, Larsen et al. (1999) examined the effect of postmeal high-intensity exercise on postprandial hyperglycemia in patients with T2D. The exercise condition included four intermittent cycling bouts consisting of a three-minute warm-up at 50% VO_{2max} followed by four minutes at 100% VO_{2max} . The authors found that intermittent, high intensity exercise decreased average glucose and insulin concentrations by 22 mg/dL and 7 μ U/mL respectively and significantly decreased breakfast AUC for both glucose and insulin. The attenuation of glucose was likely the result of an increase in glucose clearance and disappearance observed using the isotopic tracer technique. Although, similar to their previous study, the authors showed a slight rebound increase of glucose following the

exercise period and no significant reductions in glucose or insulin following the subsequent main meal (Larsen et al., 1999).

Poirier et al. (2001) investigated the glucoregulatory and metabolic responses to postmeal aerobic exercise performed either in the fasted state or two hours after a meal in 10 patients with T2D. The exercise session consisted of cycling at 60% $\text{VO}_{2\text{peak}}$ for 60 minutes, and was performed either two hours following a standardized breakfast or in the fasted state. The authors indicated that while plasma glucose values were ~ 23 mg/dL higher at the start of exercise in the fed state, the average response of glucose to exercise was significantly attenuated even when adjusted for baseline glucose values. Plasma glucose concentrations decreased 40% in the fed state, but only 9% following exercise in the fasted condition. While hepatic glucose production was not assessed, the authors speculated that the higher insulin levels during exercise in the fed state likely partially blunted hepatic glucose production, thereby resulting in a greater attenuation of glucose levels following exercise in the fed state (Poirier et al., 2001).

A recent study by Heden et al. (2015) investigated the importance of exercise timing on glucose regulation and CVD risk factors. Thirteen patients with T2D completed three trials in random order including 1) no resistance exercise (control), 2) premeal resistance exercise, and 3) postmeal resistance exercise beginning 45 minutes after dinner. The resistance exercise included two sets of 10 repetitions at 50% of the measured 10-RM for eight exercises. The authors found that both pre and postmeal resistance exercise reduced postprandial glucose and insulin concentrations. The glucose iAUC was reduced by approximately 18% and 30% in both premeal and postmeal exercise, respectively. Furthermore, the insulin iAUC was reduced by 35% and 48% in

premeal and postmeal exercise, respectively, but via different mechanisms. Using model-based beta cell function parameters, the authors showed that premeal exercise enhanced estimated insulin clearance, while postmeal exercise reduced estimated insulin secretion and enhanced estimated insulin clearance (Heden et al., 2015). The enhanced attenuation of glucose from postmeal exercise is likely the result of a synergistic role between elevated plasma insulin following a meal, as well as skeletal muscle contractions during exercise. Of interest, blood glucose concentrations, similar to the studies by Larsen et al. (1997 & 1999), rebounded following the postmeal exercise session. The authors speculated that the rebound was likely due to a simultaneous reduction in skeletal muscle glucose uptake following exercise and a transient increase in hepatic glucose production (Heden et al., 2015).

Premeal Exercise

The beneficial effects of acute premeal exercise on the immediate postprandial period are unclear, but most studies report no change or exaggerated metabolic responses (Gonzalez et al., 2013; Knudsen et al., 2014; Morishima et al., 2014; Rose et al., 2001; Rynders et al., 2014). Rynders et al. (2014) examined the impact of an acute isocaloric bout of moderate and high-intensity exercise on the exercise, post-exercise, and three-hour OGTT metabolic responses in 18 prediabetic individuals. Subjects completed three conditions in a randomized order including 1) control (rest), 2) moderate intensity exercise, and 3) high-intensity exercise. Each condition was followed by a one-hour period of recovery and a subsequent three-hour OGTT. The authors showed that indices of insulin sensitivity were improved by 51% and 85% following moderate and high-intensity exercise, respectively. Additionally, while there was no difference in total AUC

for glucose and insulin, moderate-intensity exercise improved the late-phase postprandial insulin AUC and high-intensity exercise improved both the insulin and glucose late-phase AUC (Rynders et al., 2014), which likely indicated an impairment in first-phase insulin secretion. Similar to other studies (Morishima et al., 2014; Rose et al., 2001), Rynders et al. (2014) found that glucose levels peaked approximately 30 minutes following high-intensity exercise, returning to near baseline levels at one hour. While not directly measured, the authors suggested that circulating catecholamine concentrations were upregulated during exercise, potentially stimulating skeletal muscle glycogenolysis and inhibiting insulin secretion in the immediate post-exercise period. Therefore, the timing of exercise, particularly of higher intensity, in relation to meal ingestion appears to be an important consideration with potential implications for prediabetic individuals.

The kinetics underlying postprandial glucose tolerance following exercise was recently investigated in a study by Knudsen et al. (2014), which examined glucose concentrations and kinetics after a single bout of exercise in subjects across the entire glucose tolerance spectrum. Subjects included 24 men with NGT ($n = 8$), IGT ($n = 8$), or T2D ($n = 8$), based on the WHO definitions. Each subject completed an exercise and rest condition in randomized order, followed an hour later by a 180-minute OGTT with glucose tracers. The exercise condition included one hour of cycling at 50% W_{max} . The authors indicated that postprandial endogenous glucose appearance increased similarly and total glucose clearance increased similarly in all three groups following exercise, suggesting that endogenous glucose production and contraction-stimulated glucose disposal was similar between metabolic groups (Knudsen et al., 2014). The peak exogenous glucose value was ~20% higher following exercise in NGT individuals,

compared with IGT and T2D. This has been shown in other studies, which indicate that a single bout of exercise can increase the appearance of ingested glucose following exercise in healthy individuals (Rose et al. 2001). Of interest, the lack of an effect of exercise on postprandial hyperglycemia in IGT subjects in this study are in opposition to Rynders et al. (2014), who showed that high-intensity exercise significantly attenuated late-phase AUC for postprandial glucose. The authors suggested that these differences might be related to the lower exercise intensity and subsequent lower insulin dependent or independent glucose disposal observed (Knudsen et al., 2014), although, high-intensity exercise in other acute studies have similarly shown no immediate effect (Gillen et al., 2012; Manders et al., 2010).

The lack of observed effects in studies assessing the impact of a premeal exercise bout on glucose control in a single subsequent meal are potentially confounded by either a transient increase in catecholamine concentrations (Kjaer et al., 1990) and/or an immediate upregulation of exogenous glucose appearance (Knudsen et al., 2014; Rose et al., 2001), and do not account for the delayed and protracted benefits of exercise. For example, studies using continuous glucose monitors (CGM) to assess glucose kinetics over a 24-hour period have shown beneficial effects following both moderate (Manders et al., 2010; Oberlin et al., 2013; van Dijk et al., 2012) and high-intensity (Gillen et al., 2012; Manders et al., 2010) exercise before a meal. Of these, no study indicated an attenuation of blood glucose in the initial two-hour postprandial period. However, three of the four studies calculated average postprandial AUC for glucose and insulin, with two showing a decrease in total AUC (Gillen et al., 2012; Oberlin et al., 2013) and one showing no change in total AUC (Manders et al., 2010) over the 24-hour period.

Oberlin et al. (2013) investigated the effect of a single bout of exercise on 24-hour average and postprandial glucose excursion in nine individuals with T2D. Subjects consumed three standardized meals over a two-day period while wearing CGMs during either a control or exercise condition. The exercise bout included 60 minutes of aerobic exercise at 60% of heart rate reserve. The authors showed that a single bout of exercise decreased average blood glucose concentrations by 10% in the first 24-hours following exercise. Additionally, exercise significantly lowered total postprandial AUC for all six meals; however, pairwise comparisons revealed that only meal two was significantly lower in the exercise versus control condition and the effects of exercise were undetectable on the second day. Furthermore, two-hour postprandial AUC for glucose following exercise was not significantly different from control (Oberlin et al., 2013). Similar results were seen in a study by Gillen et al. (2011), which showed that one session of high-intensity training in T2D individuals significantly lowered postmeal peak glucose concentrations by 31 mg/dL, as well as the sum of the three-hour postprandial glucose AUCs. This reduction in postprandial glucose excursion resulted in a 65% reduction in overall time spent in hyperglycemia in the 24-hour post-exercise period, compared with control. While the mechanisms mediating the reductions were not assessed, the authors reasoned, similar to Oberlin et al. (2013), that exercise may have increased skeletal muscle insulin sensitivity, as well as potentially increasing GLUT4 content and translocation in the post-exercise period (Gillen et al., 2011).

Conversely, Manders et al. (2010) found no difference in average postprandial glucose AUC during the 24-hour period following exercise. The authors assessed the impact of a single bout of low and high-intensity exercise on 24-hour glucose control in a

group of nine patients with T2D. The low intensity exercise session included 60 minutes of cycling at 35% W_{max} , and the high intensity exercise included cycling for 30 minutes at 70% W_{max} . All meals were standardized and included three meals and three snacks for the 24-hour period. The authors found that average 24-hour glucose concentrations were reduced by ~29 mg/dL following the 60-minute low intensity exercise, but were not significantly reduced in the 30-minute high intensity exercise trial. These results are in opposition to those by Rynders et al. (2014), who showed that high-intensity exercise, but not moderate-intensity exercise resulted in an attenuated blood glucose response. One possible reason for this discrepancy is that the time course of acute glucose attenuation following exercise may be different between patients with T2D and the prediabetic population used in the study by Rynders et al. (2014). Additionally, postprandial hyperglycemia following the dinner meal was significantly reduced by ~50% following both exercise bouts, which resulted in a 50% reduction in total time spent in hyperglycemia (>180 mg/dL). In this study, low-intensity exercise was found to be superior to high-intensity exercise in improving 24-hour glucose control in T2D when corrected for energy expenditure. The authors speculated that in an untrained population, total energy expenditure might be more related to the beneficial effects of exercise on glycemic control than intensity. Similar to Larsen et al. (1997, 1999), the authors reasoned that the postprandial glucose-lowering effects of both low and high intensity exercise might have a delayed but protracted effect (Manders et al., 2010). In the studies by Larsen et al. (1997, 1999), postmeal exercise decreased postprandial glucose AUC for the meal immediately preceding exercise, but not for the subsequent lunch meal. Possibly, there would be an attenuating effect on later meals throughout the 24-hour

period as well; however no study to my knowledge has assessed the effect of postmeal exercise on 24-hour glucose kinetics.

Walking and Postprandial Hyperglycemia

While structured exercise programs of varying intensities have been shown to improve both acute and chronic glucose control, there is little evidence to show that these programs are adopted following study completion (Yates, Davies, Gorely, Bull, & Khunti, 2009). Walking might represent a more easily adoptable and sustainable mode of exercise for at-risk individuals, and has been shown to improve glucose control in acute and chronic exercise studies (Dunstan et al., 2012; Yates et al., 2009). The Prediabetes Risk Education and Physical Activity Recommendation and Encouragement (PREPARE) structured education program was designed to increase walking activity through pedometer use in 87 individuals with IGT (Yates et al., 2009). After one year, subjects in the pedometer group increased their activity by approximately 2,000 steps per day, which translated to ~140 minutes of moderate-intensity walking each week. Consequently, 2-hour postchallenge glucose decreased by 24 mg/dL and fasting glucose was approximately 6 mg/dL lower in the pedometer group, compared with the control group (Yates et al., 2009). Similarly, Swartz et al. (2003) showed that a walking program designed to increase step count to 10,000 steps per day for eight weeks was effective at improving glucose control in inactive women at risk for T2D. After eight weeks, daily step count increased 85%, resulting in a 14 mg/dL drop in 2-hour postchallenge glucose and a significantly lower AUC for glucose (Swartz et al., 2003). Both studies indicated improvements in glucose control without a concomitant change in body weight, body fat

percentage, or waist circumference, suggesting that metabolic benefits from walking occur independent of weight or fat loss.

Acute walking bouts might have an important effect on postprandial hyperglycemia and glucose control as well. Manohar et al. (2012) examined the effect of walking on glucose variability in healthy individuals and patients with Type 1 diabetes. Subjects were fitted with a CGM and admitted to a clinical research unit for three days and four nights. Each subject consumed three meals per day (one followed by a period of inactivity and two followed by a 33.5 minute walk at 1.2 miles per hour to simulate free living walking). Data were analyzed from 30 minutes before each meal until 4.5 hours after meal consumption. Walking lowered postprandial glucose AUC by 53% in healthy subjects and by 59% in patients with Type 1 diabetes, compared with the meals followed by inactivity. The authors concluded that performing low-grade walking activities, even as light in intensity as chores of daily living, immediately after a meal could be an important strategy for lowering postprandial glucose excursion (Manohar et al., 2012).

Recent studies utilizing a similar intermittent walking approach throughout the day have also shown improvements in glucose control (Bailey & Locke, 2014; Dunstan et al., 2012; Francois et al., 2014; Peddie et al., 2013). Francois et al. (2014) recently investigated the impact of small doses of high-intensity walking (exercise snacks) on blood glucose control in nine individuals with IFG. Thirty minutes of continuous walking at 60% of HRmax 30 minutes before the dinner meal was compared with an energy-matched protocol of six 1-minute bouts of walking at 90% of HRmax with 1-minute slow recovery before the breakfast, lunch, and dinner meals. The authors showed that the exercise snacks significantly lowered the 3-hour mean postprandial glucose following

breakfast and dinner, whereas continuous exercise had no effect. Moreover, only exercise snacking resulted in a significantly lower 24-hour glucose concentration compared with baseline, with no effect following continuous exercise. The authors concluded that brief and intense bouts of walking before main meals were a time-efficient and effective approach to improving glycemic control in at-risk individuals (Francois et al., 2014). Importantly, the continuous 30-minute exercise bout was completed at the end of the day, so the first two meals of the day acted as control meals for the exercise day. Additionally, the beneficial effects of exercise snacks on glucose control might have been more related to the intermittent nature of the walk, as opposed to the intensity of the walk, as others have shown that intermittent walking at low intensities elicits metabolic protection as well (Bailey & Locke, 2014; Dunstan et al., 2012; Peddie et al., 2013). For example, Dunstan et al. (2012) showed that two-minute bouts of walking every 20 minutes significantly reduced glucose and insulin iAUC, regardless of intensity. In this randomized repeated measures study, 19 overweight/obese subjects completed three different trials, including 1) uninterrupted sitting, 2) sitting + 2-minute bouts of light-intensity (~2.0 mph) walking every 20 minutes, 3) sitting + 2-minute bouts of moderate-intensity (~3.6-4.0 mph) walking every 20 minutes. Following completion of the three trials, the authors showed that relative to the uninterrupted sitting condition, the 5-hour iAUC for glucose and insulin was 24.1% and 23% lower with light-intensity intermittent walking and 29.6% and 23% lower with moderate-intensity walking, respectively (Dunstan et al., 2012). Furthermore there were no significant differences in any measure between the walking conditions, suggesting that walking can elicit metabolic protection at varying intensities.

Similar to intermittent walking, studies have shown that single bouts of walking before (Hasson, Freedson, & Braun, 2006; Karstoft, et al., 2014) and after (Hashimoto, Hayashi, Yoshida, & Naito, 2013; Heiss & Tollefson, 2014; Lunde et al., 2012; Nygaard et al., 2009) a meal can effectively reduce postprandial glucose and/or insulin. A recent study by Karstoft et al. (2014) investigated the differential effects of a single interval walking session versus an oxygen-consumption and time duration-matched continuous walking session on glycemic control in patients with T2D. Ten subjects completed each of the trials approximately 45 minutes before a MTT. The authors indicated that both interval and continuous walking decreased glucose concentrations similarly during exercise, but that mean incremental postprandial glucose concentration was decreased in the interval walking group only. Furthermore, using stable glucose isotopic tracers, the authors showed that the metabolic clearance rate of glucose during the meal was increased ~21% following interval walking and only ~15% in the continuous walking group, which likely contributed to lower mean postprandial glucose concentrations (Karstoft et al., 2014). The lack of effect following the continuous walking protocol is in opposition to many studies showing improvements in glucose control following a continuous walk of varying length (Haxhi, di Palumbo, & Sacchetti, 2013). It should be noted that Karstoft et al. (2014) used a continuous walking protocol 45 minutes prior to meal consumption, as opposed to a postmeal walk. A recent review article by Haxhi et al. (2013) assessed the current literature regarding the importance of exercise timing for improving metabolic control and concluded that aerobic exercise is more effective at reducing postprandial hyperglycemia when performed after a meal, in both healthy and diabetic patients. Furthermore, other studies assessing the impact of a premeal walk have

shown no benefits on glucose in the postprandial period (Hasson et al., 2006), suggesting that the timing, as opposed to the mode of the walk, was the reason for observed differences. Indeed, a study by Colberg et al. (2009) showed that a 20-minute self-paced postmeal walk was more effective at lowering glucose values in the postprandial period than a similar walk immediately preceding the meal in a group of T2D patients. Glucose concentrations were ~49 mg/dL lower at the end of the postmeal exercise session when compared with the premeal exercise session, although the study did not assess the impact of these exercise sessions on subsequent meals or 24-hour glucose control.

Light to moderate intensity walking has consistently improved glucose control in individuals when performed after a meal (Hashimoto et al., 2013; Heiss & Tollefson, 2014; Lunde et al., 2012; Nygaard et al., 2009). Hashimoto et al. (2013) investigated the acute effect of postprandial exercise on glucose metabolism after a meal in a group of 14 healthy but sedentary women. Twenty minutes following ingestion of a high glycemic test meal, subjects either rested (control) or walked for 30 minutes at ~50% VO_{2max} . The authors showed that compared with the control condition, the postmeal walk significantly lowered both the glucose and insulin 2-hour iAUC by 43% and 39%, respectively. Additionally, the walk significantly lowered the 6-hour iAUC for insulin by 42%, potentially demonstrating a powerful insulin sparing effect from exercise (Hashimoto et al., 2013). The authors speculated that the inhibition of insulin secretion was likely mediated by the activation of α -adrenergic receptors on pancreatic beta cells from an increase in sympathetic activity associated with exercise. These findings are in agreement with the idea that postprandial exercise may be the best time to lower postprandial glucose and improve glucose control (Haxhi, et al., 2013). Indeed, a recent study in

young men and women found that a similar bout of walking (30 minutes at 50-60% of maximal heart rate) immediately after consuming a candy bar, significantly blunted the glucose response and lowered glucose concentrations at 30 minutes by ~30 mg/dL, when compared with candy bar ingestion without walking (Heiss & Tollefson, 2014).

The efficacy of postmeal walking on attenuating postprandial glucose is likely due to a combination of factors, including higher insulin concentration at the start of the walk, which blunts endogenous glucose production, as well as increased muscle contraction leading to an enhanced insulin-independent glucose uptake at the skeletal muscle. As a result of the latter, there is likely a linear relationship between length of the postmeal walk and glucose attenuation. This has been assessed in two studies comparing the differential impact of both short and longer duration walks of equal intensity on postprandial glucose in both healthy (Nygaard et al., 2009) and IFG/IGT (Lunde et al., 2012) subjects. Nygaard et al. (2009) examined whether a 15-minute or 40 minute self-selected postmeal walk (mean RPE = 9) could blunt the increase in blood glucose following a high-carbohydrate meal. In a randomized crossover design, average postprandial glucose and glucose iAUC was assessed following either a 15-minute or 40-minute self-selected free-living walk. The walk began immediately following meal consumption, after which subjects rested for the remaining two hours. Both walks significantly lowered average glucose concentration values and the 40-minute walk significantly lowered glucose iAUC by 31.2%. While glucose iAUC decreased 11% following the 15-minute walk, this did not reach significance. Moreover, there appeared to be a linear relationship between time spent walking and the attenuation of blood glucose, indicating a dose-response influence of postmeal walking. The authors suggested

that the acute effects of moderate or low-intensity walking might be as beneficial as the effect of vigorous activity, as endogenous glucose production is generally not affected by plasma catecholamine levels at such a low intensity, although this was not directly assessed in this study.

Postprandial hyperglycemia is strongly associated with skeletal muscle insulin resistance, as opposed to hepatic insulin resistance. Since contraction-stimulated glucose uptake is generally not impaired in individuals with IGT, it is likely that the benefits of postmeal exercise are even greater in this population. Lunde et al. (2012) showed this in a study of 11 obese females with IGT and/or IFG. Subjects completed two walking protocols of varying length at a slow pace (RPE = 8) immediately following a high glycemic meal and 2-hour iAUC for glucose was assessed. The authors indicated that the 2-hour iAUC for glucose significantly decreased by 30.6% and 39% after the 20-minute and 40-minute walk, respectively. Furthermore, subjects with the largest iAUC on the control day (no walk) demonstrated the greatest reduction in postprandial glucose when walking for 40 minutes after the meal, suggesting that as individuals become more insulin resistant, the magnitude of postprandial hyperglycemic protection increases. Although this study was not designed to explore mechanisms, the authors suggested that walking might have stimulated GLUT4 translocation to the cell membrane independent of insulin, which could potentially offer insulin resistant individuals a distinctive benefit of walking after a meal (Lunde et al., 2012). Together, these results strongly suggest that postmeal walking, even at low or moderate intensities, can effectively attenuate postprandial hyperglycemia, and may represent a low-barrier activity that could have preventive

potential if routinely performed. However, long-term effects of repeated postmeal walks have not yet been assessed in IGT or T2D patients.

Mechanisms Regulating Exercise-Induced Glucose Uptake

It is well established that contraction-stimulated glucose uptake is normal or near normal in individuals with impaired insulin-stimulated glucose uptake (Knudsen et al., 2014). Contractions appear to stimulate GLUT4 translocation via activation of molecular signals independent from insulin signaling, although the precise mechanisms are not well understood. Indeed, exercise appears to have no effect on insulin receptor and insulin receptor substrate-1/2 tyrosine phosphorylation or on PI 3-kinase activity (Treadway, James, Burcel, & Ruderman, 1989), both critical steps in the classical insulin-signaling cascade. A recent review article by Stanford & Goodyear (2014) highlighted the molecular mechanisms that appear to mediate contraction-stimulated glucose uptake in skeletal muscle during exercise, including changes in energy status (AMP/ATP), increases in intracellular calcium (Ca^{2+}) concentration, increased ROS, and rearrangement of the actin cytoskeleton. The current understanding of the regulatory mechanisms involved in contraction-stimulated glucose uptake is depicted in Figure 1.

The energy charge of the muscle is decreased during intense or prolonged exercise, which leads to a decrease in intracellular ATP and an increase in AMP concentrations, subsequently activating the energy sensor AMP-activated protein kinase (AMPK) (Hardie, 2007). AMPK is a heterotrimeric protein composed of a catalytic α -subunit and regulatory β - and γ -subunits that plays a critical role in cellular energy homeostasis. Activation of AMPK is positively correlated with increased contraction-stimulated glucose uptake, and this function has been shown to be lost in mouse models

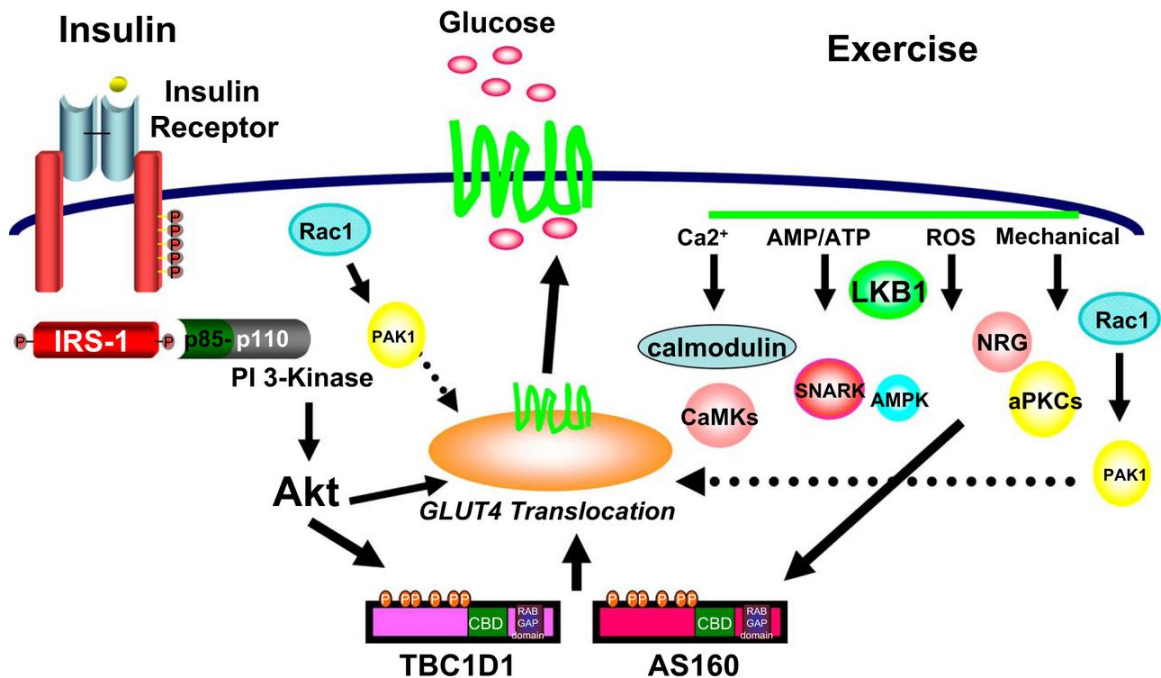


Figure 1. A proposed model of the signaling pathways underlying exercise and insulin regulation of glucose transport. (Adapted from Stanford & Goodyear, 2014).

deficient in subunits of AMPK (Daugaard & Richter, 2004). Furthermore, muscle specific knockout models of liver kinase B1 (LKB1), the upstream target of AMPK, have completely inhibited AMPK activation and severely blunted contraction-stimulated glucose uptake (Koh et al., 2006). Therefore, the increase in skeletal muscle glucose uptake during exercise could be dependent on activation of AMPK and its upstream target, LKB1. However, partial deficiency of AMPK in knockout mice models is associated with normal glucose uptake during muscle contraction (Barnes et al., 2004), and a recent study found that glucose uptake was normal or higher during treadmill running in LKB1 knockout mice, compared with control mice (Jeppesen et al., 2013). Taken together, these data suggest that both AMPK and LKB1 are important regulators in contraction-stimulated glucose uptake, but that there are likely other important mechanisms involved.

Increases in intracellular calcium (Ca^{2+}) might play an important role in glucose uptake during exercise. The binding of Ca^{2+} to its messenger protein, calmodulin, leads to the activation of Ca^{2+} /calmodulin-dependent protein kinase 1 (CAMK1). Studies have shown that incubation with a Ca^{2+} /calmodulin inhibitor decreased contraction-stimulated glucose uptake and significantly reduced CaMKII phosphorylation independent of AMPK inhibition (Wright, Hucker, Holloszy, & Han, 2004). Additionally, Ca^{2+} /calmodulin overexpression has been shown to increase glucose uptake in mouse skeletal muscle even in the presence of dead AMPK subunits (Witczak, Fujii, Hirshman, & Goodyear, 2007), suggesting a regulatory effect independent of changes in energy. However, other studies have observed an inhibition of contraction-stimulated glucose uptake following CaMK inhibition through an AMPK-dependent signaling pathway (Jensen, Scherling, Viollet, Wojtaszewski, & Richter, 2008). Consequently, the role of AMPK in the regulation of skeletal muscle glucose uptake is still unclear. However, it remains likely that Ca^{2+} has a somewhat indirect effect on muscle glucose uptake by activating the SERCA pump during muscle contraction, thereby causing a metabolic stress, which might activate AMPK and increase glucose uptake during exercise.

The small Rho family GTPase rac1 is a small signaling protein thought to be involved in both insulin and exercise-stimulated GLUT4 translocation and subsequent glucose uptake in skeletal muscle (SyLOW, Møller, Kleinert, Richter, & Jensen, 2014). A recent study found that chemical inhibition of rac1 in mouse muscle partially impairs contraction-stimulated glucose uptake (SyLOW et al., 2013). Due to its effect on both insulin and exercise-stimulated glucose uptake, rac1 is likely involved in a point of convergence between insulin and exercise stimulated glucose uptake (Stanford &

Goodyear, 2014). This downstream point of convergence likely involves the molecules AS160 (aka TBC1D4) and TBC1D1, which requires the involvement of Rab proteins. Rab proteins are members of the Ras small superfamily and are involved in numerous membrane trafficking events, including vesicle budding, tethering, and fusion, and consequently GLUT4 translocation (Richter & Hargreaves, 2013). Phosphorylation of specific residues on AS160 and TBC1D1 leads to activation of target Rab proteins and subsequent GLUT4 translocation (Richter & Hargreaves, 2013). Studies have shown that the phosphorylation of AS160 residues is enhanced after prolonged exercise in humans (Treebak et al., 2007), and mutations of AS160 phosphorylation sites inhibits both insulin and contraction-induced glucose uptake (Kramer et al., 2006). Additionally, mutations of TBC1D1 phosphorylation differentially regulate insulin and contraction-stimulated glucose uptake in skeletal muscle (Vichaiwong et al., 2010), which suggests that TBC1D1 regulate glucose uptake through distinct phosphorylation sites depending on the upstream signal. Consequently, it appears that both AS160 and TBC1D1, involving both Rab and rac1 proteins, may represent a downstream point of convergence for the regulation of GLUT4 translocation via both insulin and contraction-stimulated glucose uptake.

Oxidative Stress & Insulin Resistance

Oxidative stress is a primary mechanism for insulin resistance and subsequent T2D. It is defined as an imbalance between oxidants and antioxidants in favor of the oxidants (Sies, 1985), which ultimately leads to a disruption in redox signaling and control (Jones, 2006). Previous studies have demonstrated that levels of oxidative products are elevated in the muscles of diabetic mice (Bonnard et al., 2008), as well as in

patients with T2D (Al-Aubaidy & Jelinek, 2011; Lodovici et al., 2008; Tsikas et al., 2015), demonstrating a strong relationship between both oxidative stress and insulin resistance. Importantly, biomarkers of oxidative stress, including 8-OHdG and 8-iso-PGF_{2α}, have been shown to be elevated in individuals with prediabetes as well (Al-Aubaidy & Jelinek, 2014; Huang, Que, & Shen, 2014), suggesting the presence of oxidative damage early on in the cascade of metabolic dysfunction. Furthermore, while fat accumulation and obesity are associated with elevated levels (Matsuda & Shimomura, 2013), oxidative stress biomarkers are raised in normal weight individuals who exhibit insulin resistance (Katsuki et al., 2004), and the degree of oxidative stress amongst obese individuals is strongly associated with the degree of metabolic dysfunction and insulin resistance (Tinahones et al., 2009).

Insulin resistance results when stimulation by oxidants blocks insulin-stimulated glucose uptake and GLUT4 translocation by impairing upstream signaling. Presently, the phosphorylation of Ser/Thr instead of tyrosine phosphorylation within the insulin receptor and IRS1/2 represent the principal signaling disruptions leading to insulin resistance (Boura-Halfon & Zick, 2009). Numerous Ser/Thr kinases are likely to play a role in the disruption of molecular insulin signaling, including, c-Jun N-terminal kinase-1 (JNK1), inhibitory- κ B kinase β (IKK β), protein kinase C θ (PKC θ), and extracellular signal regulated kinase-1 (ERK1) (Boura-Halfon & Zick, 2009; Paz et al., 1999), although activation of JNK1 is likely the most important mechanism (Hotamisligil, 2006). This activation of JNK1 has been shown to directly inhibit tyrosine phosphorylation at IRS-1, thereby preventing recruitment of the protein to the activated insulin receptor (Aguirre et al., 2002; Fröjdö et al., 2011). The JNK1-stimulated

phosphorylation disrupts downstream signaling events, leading to impaired insulin signaling.

Although not fully understood, the most direct and convincing evidence implicating ROS in the disruption of insulin signaling come from cell cultures of muscle tissue. A steady increase in the generation of H_2O_2 was originally identified in insulin-responsive cell lines, including 3T3-L1 and L6 myotubes, when incubated with glucose and glucose oxidase (Maddux et al., 2001; Rudich, Tirosh, Potashnik, Khamaisi, & Bashan, 1999). The influx of H_2O_2 has frequently been shown to disrupt glucose transport activity by decreasing insulin sensitivity (Dokken, Saengsirisuwan, Kim, Teachey, & Henriksen, 2008; Maddux et al., 2001; Singh et al., 2008). Additionally, induction of insulin resistance in cell cultures via $TNF\alpha$ or palmitate treatment causes a significant rise in oxidative stress in cultured rat L6 muscle cells (Gao, Griffiths, & Bailey, 2009; Ishiki et al., 2013), and this effect is ameliorated when cells are treated with astaxanthin, a carotenoid known for its antioxidant properties (Ishiki et al., 2013). Furthermore, H_2O_2 impairs insulin signaling and glucose transport capacity in rat soleus muscle cultures (Archuleta et al., 2009). A study by Archuleta et al. (2009) demonstrated that the H_2O_2 -induced insulin resistance was associated with a degradation of IRS1 and IRS2 proteins, as well as distal impairments of Akt phosphorylation. Importantly, the loss of IRS proteins in rat soleus muscle was selective, as the protein expression at other crucial signaling steps was unaffected by the influx of H_2O_2 (Archuleta et al., 2009).

While several mechanisms have been proposed to explain the role of oxidative stress in insulin resistance, including activation of stress signaling pathways (Hotamisligil, 2006) and the upregulation of cytokines (Ishiki et al., 2013), the increased

stress is also associated with a reduced cellular capability to deal with enhanced oxidative products, including decreased antioxidant defenses. Both enzymatic and nonenzymatic antioxidants are reduced in individuals with T2D (Sedighi, Makhluh, Shokrzadeh, & Hoorshad, 2014), and insulin resistance is associated with reductions in total antioxidant activity (Tinahones et al., 2009), although this effect is not always shown (Stranges et al., 2007). Interestingly, beta cells appear to have a lower abundance of enzymatic antioxidants, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), compared to other body tissues (Lenzen, Drinkgern, & Tiedge, 1996), suggesting that insulin secretion is a primary oxidative target in times of increased ROS production. Furthermore, several antioxidants have been shown to improve insulin signaling for glucose uptake and attenuate the increase of oxidative products (Li, Zhang, Liu, Sun, & Xia, 2015; Maddux et al., 2001; Singh et al., 2008), although the mechanisms involved in these intracellular events are largely unknown. A recent study by Li et al. (2015) found that supplementing with anthocyanins, flavonoid pigments known to exhibit antioxidant effects, twice daily for 24 weeks effectively increased total radical-trapping antioxidant capacity and ferric ion reducing antioxidant power, measures of total antioxidant capacity, in individuals with T2D. Additionally, compared with placebo, individuals in the anthocyanin group exhibited reduced serum levels of oxidative markers, including 8-iso-PGF_{2α}, 13-hydroxyoctadecadienoic acid, and carbonylated proteins, indicating a powerful antioxidant effect.

In vivo models have shown that pretreatment with antioxidants can maintain antioxidant defenses in the presence of oxidative stress, and block the activation of serine kinases, associated with insulin resistance (Maddux et al., 2001; Rudich et al., 1999;

Wang et al., 2012). The overexpression of NYGGF4 has been shown to inhibit insulin-stimulated glucose transport and impair GLUT4 translocation in mature adipocytes by blocking signals in the classical insulin-signaling pathway (Wu et al., 2011). Consistent with this, Wang et al. (2012) showed that overexpressing the gene NYGGF4 in adipocytes, led to significantly increased levels of ROS and inhibition of glucose uptake into adipocytes. However, pretreatment with 100 or 200 mM of α -lipoic acid, a compound with antioxidant properties, considerably reduced the rise in ROS associated with NYGGF4 overexpression and enhanced glucose uptake by activating IRS-1 and Akt phosphorylation in NYGGF4 overexpression adipocytes. Furthermore, Singh et al. (2008) demonstrated that treatment with gamma-tocopherol, an isoform of vitamin E with powerful antioxidant effects, ameliorated the deleterious effects of oxidative stress on insulin sensitivity in cultured rat L6 myotubes. Interestingly, the insulin resistance induced by glucose oxidase, did not appear to be due to impairments in the classical insulin signaling cascade, suggesting that oxidative stress might impair insulin-stimulated glucose uptake via mechanisms unrelated to specific proteins in the insulin signaling cascade.

The pathophysiology of insulin resistance appears to involve a complex interaction between oxidative stress and inflammation, whereby both disorders mutually reinforce and exacerbate the effects of one another. For example, as an oxidative imbalance occurs, ROS activate stress-signaling pathways, including the JNK1 and IKK β kinases, increasing the expression of pro-inflammatory mediators (TNF α and IL-6) and causing an inflammatory response (Gloire, Legrand-Poels, & Piette, 2006). Conversely, the upregulation of TNF α and IL-6 independently trigger ROS production in many cell

types associated with insulin resistance (Gonzalez et al., 2012). Consequently, the inflammatory response is always associated with an increase in ROS production, which results in a greater inflammatory response as part of a damaging oxidative-inflammatory cycle.

Hyperglycemia & Oxidative Stress

Hyperglycemia can increase the production of free radicals by increasing the production of Acetyl-CoA and subsequently NADH as a consequence of increased substrate (glucose) into the citric acid cycle (Phaniendra et al., 2015). When excessive NADH cannot be dissipated by oxidative phosphorylation, single electrons are transferred to oxygen, leading to the production of reactive oxygen species, namely superoxide anion (Phaniendra et al., 2015; Williamson & Cooper, 1980). This overproduction of superoxide anion by the mitochondrial electron transport chain is considered the unifying theory to explain the mediating role of oxidative stress in hyperglycemic-induced diabetic complications.

Du et al. (2001) demonstrated this effect by exposing cells to a hyperglycemic environment, indicating an increase in voltage above the critical threshold to increase superoxide formation in exposed cells. In another study, the same research group depleted mitochondrial DNA from normal endothelial cells to form cells that lacked a functional mitochondrial electron transport chain. After removal, the effect of hyperglycemia on ROS production was completely lost and activation of the pathways responsible for diabetic complications (polyol pathway, AGE formation, and PKC) was completely inhibited (Brownlee, 2005). Furthermore, Nishikawa et al. (2000) looked at the effect of superoxide dismutase (SOD) overexpression on hyperglycemic-activated

pathways. SOD is an antioxidant enzyme known to play a key role in the detoxification of superoxide radicals. Aortic endothelial cells were incubated with various levels of glucose and/or the antioxidant SOD and intracellular formation of ROS were detected using a fluorescent probe. Hyperglycemia was shown to cause a significant increase in ROS generation. However, ROS production was completely inhibited when superoxide was degraded by infusion of SOD under hyperglycemic conditions (Nishikawa et al. 2000).

Evidence of hyperglycemic-induced oxidative stress has been shown in studies *in vivo* as well. Ceriello, et al. (1999) studied this phenomenon in 10 T2D patients who were given two distinct meals designed to induce different levels of postprandial hyperglycemia. The meals were administered in a randomized order and blood samples were taken at baseline, 60, and 120 minutes following the meals. Plasma glucose, insulin, cholesterol, triglycerides, and total radical trapping activity were measured. Measures of glucose, insulin, cholesterol and triglycerides all significantly increased and total radical trapping activity decreased following both meals. Moreover, all values were significantly more exaggerated following the meal that induced a greater degree of hyperglycemia (Ceriello et al., 1999), suggesting that postprandial hyperglycemia may be a major contributor of oxidative stress. In a different study design, Ceriello and colleagues demonstrated that markers of oxidative stress increase in response to an oral glucose tolerance test and a standardized meal in both normal and diabetic subjects. Additionally, antioxidant status, as well as radical trapping activity, were significantly reduced in both groups (Ceriello et al., 1998). The authors concluded that hyperglycemia might acutely induce oxidative stress.

A study by Gregersen, Samocha-Bonet, Heilbronn, and Campbell (2012) assessed the relative contributions of fat versus carbohydrate intake on the postprandial oxidative stress response in serum and skeletal muscle. A group of 15 healthy individuals completed two trials in a randomized order including 1) ingestion of a high-fat meal, of which 34% was saturated, and 2) ingestion of a high-carbohydrate meal. Samples were taken at baseline and three hours post-ingestion for plasma markers of oxidative stress, as well as muscle biopsies from the vastus lateralis. The authors indicated an elevation in serum and muscle expression of IL-6 in both conditions. Furthermore, plasma total antioxidant status and muscle SOD were decreased following the high-carbohydrate meal only. The authors concluded that a high-carbohydrate meal might evoke a greater postprandial oxidative stress response than a meal high in fat (Gregersen, 2012).

Monnier et al. (2006) assessed the impact of postprandial hyperglycemia on markers of oxidative stress in a study of 21 T2D patients and 21 age-matched controls. The researchers examined the association between oxidative stress and glucose kinetics by measuring urinary 8-iso PGF, an end product of lipid peroxidation, average 24-hour glucose fluctuations, HbA1c, and fasting glucose. Mean amplitude of glucose excursions explained 74% of the variation in 8-iso PGF and no markers of prolonged glucose exposure were significantly correlated with 8-iso PGF, indicating significant postprandial involvement (Monnier et al., 2006). Additionally, a study by Sampson, Gopaul, Davies, Hughes, & Carrier (2002) indicated a significant increase in 8-iso PGF during acute hyperglycemia following an oral glucose tolerance test in T2D patients. These findings provide evidence for an independent association between acute postprandial hyperglycemia and increased levels of oxidative stress.

Since oxidative stress is a precursor of inflammation, measures of inflammatory cytokines under hyperglycemic conditions also offer compelling evidence for their relationship. Several studies have indicated that chronic hyperglycemia causes a dramatic increase in the release of inflammatory cytokines, mediated by ROS generation (Yan, 2014). Esposito et al. (2002) assessed the role of glucose in the regulation of circulating levels of IL-6, TNF-alpha, and IL-1 in subjects with normal or impaired glucose tolerance. Plasma glucose levels were acutely raised and maintained for 5 hours while insulin secretion was blocked in both control and subjects with IGT. Plasma cytokine levels increased in both groups but were more pronounced in IGT subjects. On another occasion, subjects received the same glucose infusion while also being infused with the antioxidant glutathione. Plasma cytokine levels did not show a significant change from baseline in either group with the antioxidant infusion (Esposito et al., 2002). These findings suggest a causal role for hyperglycemia in the generation of oxidative stress.

Mechanisms of oxidative stress generation

Under hyperglycemic conditions, more glucose will flux through the glycolytic pathway, increasing the production of pyruvate and acetyl-CoA and subsequently excess NADH. As previously explained, excess NADH will cause an electron pressure on the mitochondrial electron transport chain, ultimately yielding superoxide anions (Phaniendra et al., 2015). Superoxide is a ROS, as well as the precursor to all ROS and can cause significant levels of oxidative stress when produced in excess (Yan, 2014). The ROS produced as a function of increased levels of NADH can impair and inhibit glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity, an essential enzyme necessary to catalyze the sixth step of glycolysis (Brownlee, 2001). As such, impairments

of GAPDH decrease the efficiency of glucose breakdown and entrance into the Krebs Cycle and lead to an accumulation of glyceraldehyde-3-P (G3P). This process may result in the accumulation of ROS and subsequent oxidative stress via the activation of several branching pathways, including the polyol pathway, hexosamine pathway, protein kinase-C (PKC) pathway, and increased intracellular formation of advanced glycation end products (AGEs) (Figure 1).

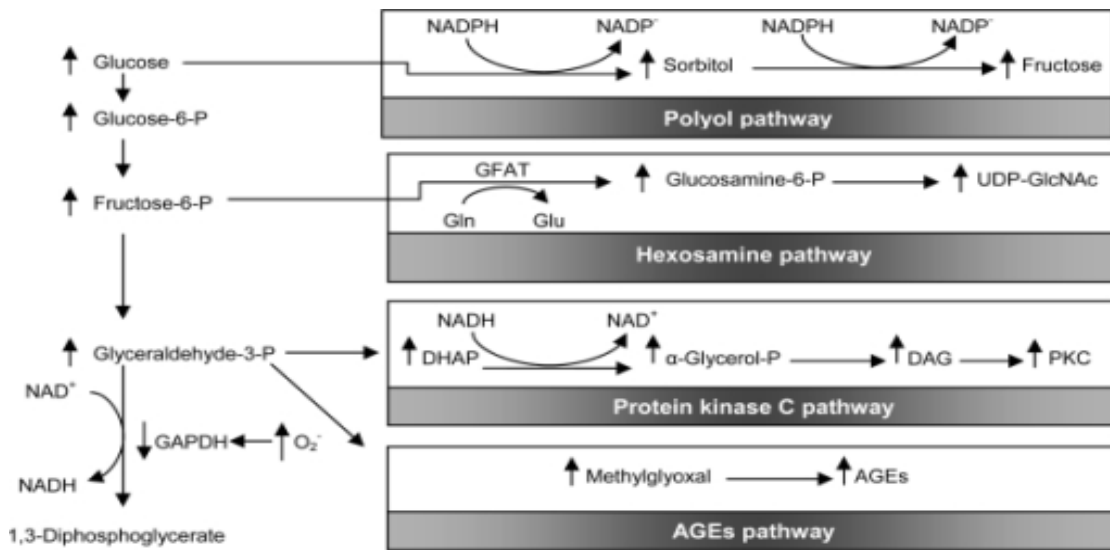


Figure 2: The branching pathways that are potentially activated when glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is partially inactivated by superoxide overproduction in response to hyperglycemia. The four pathways are linked to additional reactive oxygen species production. (Adapted from Ceriello & Testa, 2009).

Polyol pathway: In response to high blood glucose levels, activation of the polyol pathway causes the reduction of glucose to sorbitol via aldose reductase, which is further converted to fructose via sorbitol dehydrogenase (Vedantham, Ananthakrishnan, Schmidt, & Ramasamy, 2012). NADPH is consumed in the initial conversion of glucose to sorbitol as a consequence of polyol activation, which diminishes its availability to reduce glutathione (GSH) since glutathione reductase, which regenerates GSH from its

oxidized form, requires NADPH to function. This process results in decreased NAD^+/NADH and decreased levels of glutathione (GSH), which compromises cellular antioxidant capacity (Bravi et al., 1997). Conversely, aldose reductase inhibitors have been shown to block the metabolism of the polyol pathway and attenuate levels of oxidative stress, as well as diabetic complications (Hu et al., 2014; Srivastava et al., 2011; Vegantham et al., 2012).

Hexosamine pathway: During hyperglycemic conditions, excess substrate is shunted into the hexosamine pathway, which branches off from fructose 6-phosphate in the glycolytic pathway. Glucose 6-phosphate is converted to glucosamine 6-phosphate via glutamine-fructose 6-P amidotransferase (GFAT), the rate-limiting enzyme for this pathway (Buse, 2006). The final conversion of glucosamine 6-phosphate yields UDP-N-acetyl glucosamine, which is utilized as a substrate for the enzymatic glycosylation of transcription factors that catalyze posttranslational modifications of proteins on serine and threonine residues (Buse, 2006; Denzel & Antebi, 2014). As a result, increased glucose flux through this pathway has been found to be involved in enhanced production of ROS (Lima, Spitler, Choi, Webb, & Tostes, 2012), as well as a disruption in insulin signaling (Andreozzi et al., 2004) and diabetic complications (Semba, Huang, Luty, Van Eyk, & Hart, 2014).

Protein Kinase-C (PKC) activation pathway: High blood glucose levels promote the chronic increase of diacylglycerol (DAG) concentrations from the breakdown of fructose 1:6-biphosphate and glyceraldehyde-3-P. As an activator of PKC, the rise in DAG leads to an increase in PKC activation, which has been shown to induce ROS production by NADPH oxidase that catalyzes one electron reduction of molecular oxygen

to form superoxide (Giacco & Brownlee, 2010). Additionally, PKC activation can impair insulin signaling by inhibiting Akt-dependent nitric oxide synthase (Choi et al., 2014), as well as induce the overexpression of the fibrinolytic inhibitor, plasminogen activator inhibitor (PAI)-1, and NF- κ B (Giacco & Brownlee, 2010), which likely plays a major role in the accelerated vascular disease observed in diabetics.

Advanced Glycation End Products (AGEs): AGEs are formed under hyperglycemic conditions when high levels of intracellular glucose cause the auto-oxidation of glucose to eventually yield methylglyoxal from glyceraldehyde-3-P when GAPDH function is impaired (Brownlee, 2001; Wang et al., 2015). These compounds react with free amino groups of intracellular and extracellular proteins, forming AGEs, most notably glycosylated hemoglobin (HbA1c). The binding of AGEs to their receptors (RAGE) can induce the generation of ROS (Wang et al., 2015), as well as activate the redox-sensitive NF- κ B signaling pathway leading to chronic inflammation (Tobon-Velasco, Cuevas, & Torres-Ramos, 2014).

Assessment of Oxidative Stress

The accurate measurement of both ROS and antioxidants is necessary in order to assess the level of oxidative stress present in biological systems. However, while the short half-life of ROS makes them excellent signaling molecules, it also confounds their precise measurement (Strobel, Fassett, Marsh, & Coombes, 2011). Consequently, studies have focused on identifying stable markers in the circulation that may reflect systemic oxidative stress, including molecules that are modified by interactions with ROS (DNA, lipids, proteins), as well as molecules of the antioxidant system (Ho, Galougahi, Liu,

Bhindi, & Figtree, 2013). Common biomarkers used to assess acute changes in oxidative stress are described below.

Hydrogen Peroxide (H₂O₂)

H₂O₂ is a natural metabolic byproduct that serves as an important intracellular messenger, acting as a cell-signaling molecule (Ray, Huang, & Tsuji, 2012) and playing a key role in vascular function and homeostasis (Breton-Romero & Lamas, 2014).

Paradoxically, H₂O₂ at high concentrations is a molecule with strong oxidizing capacity that serves as a cytotoxic agent to cells in biological systems. It is produced *in vivo* by the dismutation of superoxide, and is readily converted to the reactive hydroxyl radical by interaction with transition metal ions, namely iron. Both catalase and glutathione peroxidase can catalyze the decomposition of H₂O₂ to H₂O and water, effectively precluding its deleterious effects. High levels of H₂O₂, functioning through NF-κB and various kinases, have been associated with diabetes, atherosclerosis, asthma, inflammatory arthritis, and other chronic diseases (Breton-Romero & Lamas, 2014; Ray et al., 2012). Studies utilizing cell culture models have shown that the addition of H₂O₂ to insulin-receptive cells causes a significant decrease in cellular response to insulin, predominately resulting from a disruption in insulin signaling at insulin receptor phosphorylation and more downstream at Akt phosphorylation (Archuleta et al., 2009; Dokken et al., 2008). This disruption of insulin signaling ultimately results in decreased glucose transport into the cell and higher levels of glucose in the blood. Additionally, H₂O₂ at high concentrations can induce the release of cytochrome c and apoptosis-inducing factor into the cytosol, where through the activation of caspase, they can lead to cell death (Yuana, Murrell, Trickett, & Wang, 2003). Consequently, while normal levels

of H₂O₂ appear to be important in the regulation of homeostatic cellular signaling, high levels result in an oxidized shift of the redox state, thereby inducing oxidative stress and subsequently activating stress-signaling pathways that can lead to additional cellular damage.

Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation is the oxidative degradation of lipids, resulting from increased oxidative stress in cells. The end products of lipid peroxidation are reactive aldehydes, namely malondialdehyde (MDA). Because ROS are difficult to assess directly, the end products of oxidative stress-induced damage have been used in a number of studies, including those involving diabetic rats (Ahmadvand et al., 2014) and humans (Gonzalez, Lucero, & Ojeda, 2015). A study assessing the effect of acute exercise on postprandial TBARS in prediabetic women found significantly higher serum levels of TBARS six hours postmeal, compared with premeal, but found no difference between conditions (Melton, Tucker, Fisher-Wellman, Schilling, & Bloomer, 2009), suggesting that the intensity and duration of exercise may not have been enough to attenuate a rise in MDA. Nevertheless, while TBARS is a well-established assay for screening MDA, controversy exists regarding the ability of TBARS to provide an accurate estimate of oxidative stress, as the assay only measures a single end product (Ho et al., 2013). Additionally, research has indicated that only certain lipid peroxidation products generate MDA, and MDA may not be generated exclusively through lipid peroxidation (Del Rio, Stewart, & Pellegrini, 2005). Because of this, TBARS should be measured in concert with additional markers of both oxidative stress and antioxidants to get a more complete idea of the risk for subsequent inflammation and CVD.

Uric Acid

Uric acid is an organic compound that exists as the final oxidation product of purine catabolism in humans. It is a powerful scavenger of free radicals, responsible for ~60% of free radical scavenging in plasma (Ames, Cathcart, Schwiers, Hochstein, 1981), and has long been considered an important antioxidant. However, there appears to be a paradoxical relationship between the antioxidant properties of uric acid, and its direct pro-oxidant role, as recent work has shown that high uric acid levels can directly induce insulin resistance (Zhu et al., 2014) and are associated with obesity (Tsushima et al., 2013). Although it is possible that high levels of uric acid observed in metabolically unhealthy populations is the result of an adaptive response to increased levels of oxidative stress, this has not been fully elucidated. A recent study by Fabbrini et al. (2014) evaluated whether alterations in uric acid concentrations would affect antioxidant capacity and markers of oxidative stress. The authors showed that acute uric acid reduction, by infusion with a recombinant urate oxidase, caused a 45-95% decrease in antioxidant capacity and a 25-40% increase in markers of oxidative stress. The authors concluded that uric acid is a major circulating antioxidant that might provide a protective mechanism against oxidative stress.

Total Antioxidant Capacity (TAC)

TAC considers the additive, synergistic, and/or antagonistic relationship between free radicals and antioxidants in a biological system. The most common methods developed to assess TAC include oxygen reducing absorbance capacity (ORAC) assay, the trolox-equivalent antioxidant capacity assay (TEAC), and ferric reducing ability assay (FRAP) (Fraga et al., 2014). These assessments are generally based on challenging a

substrate with an oxidant (i.e. peroxy radicals) to obtain a high level of oxidation either with or without the presence of the antioxidant-containing sample to be tested. TAC assays offer an advantage over measuring individual antioxidants, as they incorporate a variety of antioxidant actions of different compounds. However, controversy exists over the usefulness of TAC as a predictor or marker of disease (Fraga et al., 2014). Studies assessing the reproducibility and reliability of TAC have found that different TAC assays poorly correlate with one another (Cao & Prior, 1998; Fraga et al., 2014). Additionally, a recent review article by Fraga et al. (2014) concluded that while TAC is a quick and inexpensive assay frequently used in research, different TAC assays detect specific combinations of compounds, but ignore the contribution of other indirect antioxidant measures that likely affect the system. Nonetheless, many studies have found significant associations between elevated TAC levels and lower levels of inflammatory biomarkers (Wang et al., 2013), and the assay is currently the best measure of the synergistic effects of antioxidant defenses.

Catalase

Catalase is one of the main detoxifying enzymes required to neutralize ROS found in cells. It catalyzes the decomposition of H_2O_2 to water and oxygen, essentially mitigating the damaging effects of H_2O_2 . Several catalase gene polymorphisms have been identified and shown to be associated with diabetes (Banerjee & Vats, 2014), and catalase deficiency (acatalasemia) is a leading cause of oxidative stress and macrovascular complications (Banerjee & Vats, 2014; Petrovič, 2014). A recent study by Canale, Farney, McCarthy, & Bloomer (2014) assessed the impact of acute exercise on postprandial oxidative stress and showed a significant reduction in antioxidant defenses,

including catalase and superoxide dismutase, at 2 and 4 hours following a lipid-rich meal, although contrary to other studies (Michailidis et al., 2007), there was no difference in catalase between exercise conditions.

Exercise-induced Oxidative Stress

Dillard, Litov, Savin, Dumelin, & Tappel (1978) published the first evidence of exercise-induced oxidative stress over three decades ago revealing that 60 minutes of endurance exercise at 60% of VO_{2max} resulted in increased levels of lipid peroxidation, an end product of OS. Additionally, they showed that Vitamin E supplementation reduced this production. Although the source was unidentified, they concluded that exercise increased levels of oxidative stress, and that antioxidant supplementation could attenuate this response. Following this early work, numerous studies demonstrated that acute exercise elicits oxidative stress through excess production of ROS in both trained and untrained individuals. Ristow et al. (2009) demonstrated this in 20 previously untrained individuals. Following an acute 3-day exercise protocol, concentrations of TBARS (a well-established end product of lipid peroxidation) were significantly elevated within the skeletal muscle. In contrast, there was no increase in TBARS following exercise in individuals who were taking antioxidant supplements. As contraction-induced oxidative stress is apparent, recent work sought to uncover the sources and consequences of production, which are still debated today.

One of the major differences between exercise-induced oxidative stress and the stress generated from other damaging events is the source of free radicals. The majority of ROS generated at rest is from the mitochondrial electron transport chain; however, exercise-induced oxidative stress appears to be generated within the skeletal muscle

itself. Production of superoxide in skeletal muscle is the first upstream step in the ROS cascade, and this production is likely generated at multiple subcellular sites within the muscle fiber (Powers, Nelson, & Hudson, 2011). Growing evidence suggests that mitochondria during exercise likely contribute far less to the production of ROS than once believed. Currently, NADPH oxidase enzymes are considered a possible major contributor. Although not fully understood, studies have identified NADPH oxidase enzymes throughout the muscle, including the sarcoplasmic reticulum, T-tubules, and plasma membrane. Intracellular NADPH can act as a substrate for superoxide production both within and outside the cell and are likely activated during contractile activity, thereby generating ROS (Powers, et al., 2011).

Exercise-induced oxidative stress is now considered to be important in the upregulation of antioxidant defense systems, including SOD and glutathione peroxidase (Powers, et al., 2011). Hollander et al. (2001) demonstrated that an acute bout of exercise, through activation of NFκB, enhanced genes for SOD in rat skeletal muscle. Since the expression of SOD was dependent on NFκB, which is activated by ROS, the authors suggested that the adaptive process was induced by ROS. Contributing to this understanding, recent work has shown that antioxidant supplementation attenuates exercise-induced oxidative stress, as well as subsequent adaptations of the muscle. Michailidis et al. (2013) recently demonstrated this in 10 recreationally trained, healthy males. Subjects received either a NAC (thiol-based antioxidant) supplement or placebo following 300 eccentric contractions. They found that although supplementation attenuated oxidative damage (measured by TBARS) and inflammation (CRP & IL-1), mediated by an attenuation of NFκB, it delayed long-term recovery by interfering with

intracellular signaling pathways. These findings may help explain the connection between regular exercise and chronic disease risk reduction.

The hormesis theory suggests that biological systems respond to the exposure of chemicals and toxins with a bell-shaped (or inverted U-shaped) curve, a phenomenon characterized by a low dose of stimulation with high dose of inhibition. This theory of hormesis has been proposed to explain the role of exercise in modulating free radical production (Radak, Chung, & Goto, 2005). The most important effect of exercise on the body is the adaptation process, the effects of which are systemic and often specific. The original stress theory developed by Selye (1956) stated that for a chronic stressor, the body responds with a decreased reaction (i.e. decrease in antioxidants), and then an increased resistance (i.e. upregulation of antioxidants), followed by exhaustion. Therefore, chronic stress (physical inactivity or extremely long bouts of exercise) is potentially harmful, as the body does not have time to rest and therefore adapt.

Following the discovery of exercise-induced oxidative stress over 30 years ago, research has focused on the positive role that oxidative stress may play within the cells. While many mechanisms are still not fully understood, it is now believed that exercise-induced oxidative stress is important to upregulate antioxidant defense systems within the cells. It appears that this response follows a hormesis curve, whereby moderate levels of exercise with sufficient rest are optimal to allow for adaptation (upregulation) of antioxidant defense systems. Therefore, oxidative stress induced by exercise appears to be important and protective, whereas oxidative stress induced by other stressors (hyperglycemia), particularly when chronic, appear to be dangerous and damaging to the body.

Acute Exercise and Oxidative Stress

A review article by Bloomer and colleagues in 2008 highlighted the potential role of acute exercise in modulating postprandial oxidative stress in diabetic and prediabetic individuals (Tucker, Fisher-Wellman, & Bloomer, 2008). The authors outlined three distinct ways that exercise could potentially reduce postprandial oxidative stress. These included an increase in endogenous antioxidant defense systems, an improvement in blood glucose transport and clearance due to increased GLUT4 trafficking, and improved blood triglyceride clearance. With respect to improved blood glucose clearance, the authors identified two separate effects of acute exercise on skeletal muscle glucose transport, including an insulin-independent stimulation of glucose transport, as well as a transient increase in insulin sensitivity following exercise completion, although this is largely dependent on intensity and duration of the exercise bout. While no study at the time of this review had investigated the effect of acute exercise on modulating postprandial oxidative stress in metabolically impaired individuals, the authors concluded that acute exercise might be a favorable treatment option to reduce postprandial oxidative stress.

To date, the effect of acute exercise on postprandial oxidative stress has been assessed in four studies involving both healthy (Canale et al., 2014; Clegg et al., 2007; McClean et al., 2007) and prediabetic (Melton et al., 2009) individuals. All of the studies assessed the impact of a single aerobic exercise bout on markers of oxidative stress and/or antioxidant capacity following a high fat meal. Of these, two found a significant change in oxidative markers during the exercise condition, compared with the control condition (Clegg et al., 2007; McClean et al., 2007), while two showed no effect (Canale

et al., 2014; Melton et al., 2009). A series of studies by McClean and colleagues provided the first evidence that acute exercise could influence postprandial metabolism and markers of oxidative stress (Clegg et al., 2007; McClean et al., 2007). Clegg et al. (2007) assessed the efficacy of moderate exercise, performed prior to meal ingestion, on attenuating the rise in lipemia and oxidative stress after a high-fat meal. Eight young healthy males completed two protocols in randomized order including ingestion of a high-fat meal alone or ingestion of a high-fat meal, preceded by one hour of cycling at 60% of their age-predicted maximal heart rate. Measures of oxidative stress included serum lipid hydroperoxides (LOOH), a product of lipid peroxidation that has been implicated in the disruption of biological processes and protein/DNA damage. Venous blood samples were taken prior to and immediately following exercise, as well as 2, 4, and 6 hours post-ingestion. They showed that LOOH significantly increased two hours following the meal in both conditions, but concentrations were significantly elevated at four hours in the control condition only. The authors concluded that a single bout of moderate intensity exercise performed prior to meal ingestion was effective in attenuating the increase in lipid peroxidation, which could represent a decrease in levels of oxidative stress (Clegg et al., 2007).

A second study by this research group investigated the effect of an acute exercise bout performed after meal ingestion on markers of oxidative stress and antioxidant capacity in healthy trained males (McClean et al., 2007). Ten trained male subjects completed two randomly assigned conditions including a high-fat meal alone and a high-fat meal followed two hours later by one hour of treadmill exercise at 60% age-predicted maximal heart rate. Measures of oxidative stress, including LOOH and SOD were taken

at baseline, two, three, and four hours post ingestion. They showed that while LOOH concentrations increased in both conditions following meal ingestion, levels were significantly lower at three hours post ingestion during the exercise condition. Furthermore, SOD levels following the meal were only significantly reduced during the control condition and were significantly lower than in the exercise condition at three hours post-ingestion. While mechanisms were not addressed in either study, the authors indicated that markers of oxidative stress were likely mitigated due to the attenuation of postprandial plasma triglyceride levels observed during the exercise condition. The authors again concluded that a single bout of aerobic exercise could reduce levels of oxidative stress following a high fat meal.

Alternatively, previous work by Bloomer and colleagues has indicated no change in measures of postprandial oxidative stress following an acute exercise bout, when compared to a control condition (Canale et al., 2014; Melton et al., 2009). A recent study by Canale et al. (2014) assessed the impact of aerobic and anaerobic exercise bouts of varying intensities and durations on biomarkers of oxidative stress and antioxidant capacity following a high-fat meal. A group of 12 trained men underwent four conditions in a randomized order including 1) no exercise control, 2) 60 minutes of cycling at 70% heart rate reserve, 3) five 60-second sprints at 100% maximal capacity, and 4) ten 15-second sprints at 200% maximal capacity. A high-fat test meal was consumed one hour after exercise completion and blood samples were taken at baseline and two and four hours after meal ingestion. Measures of oxidative stress and antioxidant capacity included MDA, H₂O₂, advanced oxidation of protein products (AOPP), TAC, SOD, catalase, and glutathione peroxidase (GPx). The authors indicated a significant time effect for

measures of MDA, H₂O₂, AOPP, SOD, and CAT, with values higher at two and four hours post-ingestion for all biomarkers, indicating an upregulation of oxidative stress following ingestion of a high-fat meal. However, none of the exercise conditions appeared to impact or attenuate these increases. There was a significant condition effect observed for TAC, with values higher at two and four hours for the 15-second sprint condition than the control and 60-minute aerobic exercise session. This may represent a transient increase of oxidative products, and subsequent antioxidant defenses, which is often observed following high-intensity exercise. The authors concluded that acute exercise in the form of steady state aerobic exercise or high-intensity cycle sprints did not appear to attenuate the postprandial rise in markers of oxidative stress induced by a lipid-rich meal.

Melton et al. (2009) conducted the only study to assess the impact of an acute bout of exercise on postprandial oxidative stress in a prediabetic population. A group of 16 obese women with an average fasting glucose of 107 mg/dL consumed a high-fat meal with and without a 45-minute cycling bout at 65% heart rate reserve. The high-fat meal was consumed 15 minute following the completion of the exercise bout. Plasma markers of TAC, xanthine oxidase (XO), H₂O₂, MDA, triglycerides, and glucose were taken at baseline and at one, two four, and six hours following meal ingestion. They indicated a main time effect for XO, H₂O₂, MDA, and triglycerides, with values higher from one to six hours post-ingestion, and a time effect for TAC, with values lower four hours following ingestion of the meal. However, there was no difference in any of the concentrations following the meal between the exercise and control condition. The authors concluded that a single bout of exercise of the intensity and duration performed in

this study was not effective in attenuating markers of oxidative stress following a high-fat meal. Although not directly assessed, they reasoned that this was likely due to the failure of the exercise bout to attenuate the rise in postprandial triglycerides, which significantly increased during both conditions (Melton et al., 2009).

CHAPTER 3

METHODS

Subjects.

Subjects were recruited from the greater Phoenix area through fliers, emails, and online announcements. All subjects were obese ($BMI \geq 30 \text{ kg/m}^2$), nonsmoking, sedentary (fewer than two 20-min bouts of exercise per week during the previous three months) adults, between the ages of 35 and 70 years. Additionally, all subjects had a fasting capillary blood glucose that measured between 100-125 mg/dL. Individuals who answered “yes” to any of the questions on the “Par-Q & You” questionnaire or refused to discontinue antioxidant supplement use were excluded. Prescribed medication use was permitted as long as use was consistent for the three months prior to the study and intake remained consistent throughout the study duration. Individuals with food allergies or medical conditions that impacted normal functioning of the gastrointestinal tract (e.g. Crohn’s disease, Celiac sprue, short bowel syndrome, ostomy, lactose intolerance etc.) were also excluded. Eligible participants had to be able to walk on a motorized treadmill at a moderate pace and agree to follow the study protocol.

An a priori power analysis was performed to determine the sample size necessary to detect significant changes in postprandial glycemia. From previous glycemic data collected in our laboratory, it was determined that for a within-subjects repeated measures design, in order to detect a large effect size (Cohen $f=0.4$) (Cohen, 1988) in two-hour glucose incremental area under the curve (iAUC) (at a 0.05% significance level and power > 0.80 with an expected 20% dropout rate), 10 subjects would need to be recruited. For postprandial glycemic excursion, previous studies have achieved statistical

power for detecting differences in two-hour iAUC with 10-11 subjects (DiPietro et al., 2013; Lunde et al., 2012). The Arizona State University Institutional Review Board (IRB) approved this study (see Appendix A for IRB approval), and all volunteers were given a detailed description of the protocol and provided written informed consent prior to participation.

Experimental Design.

The complete study design is diagrammed in Figure 2. All subjects underwent three trials in a randomized order in this repeated measures design study. All trials were separated by approximately one week, a time period shown to be adequate for measuring

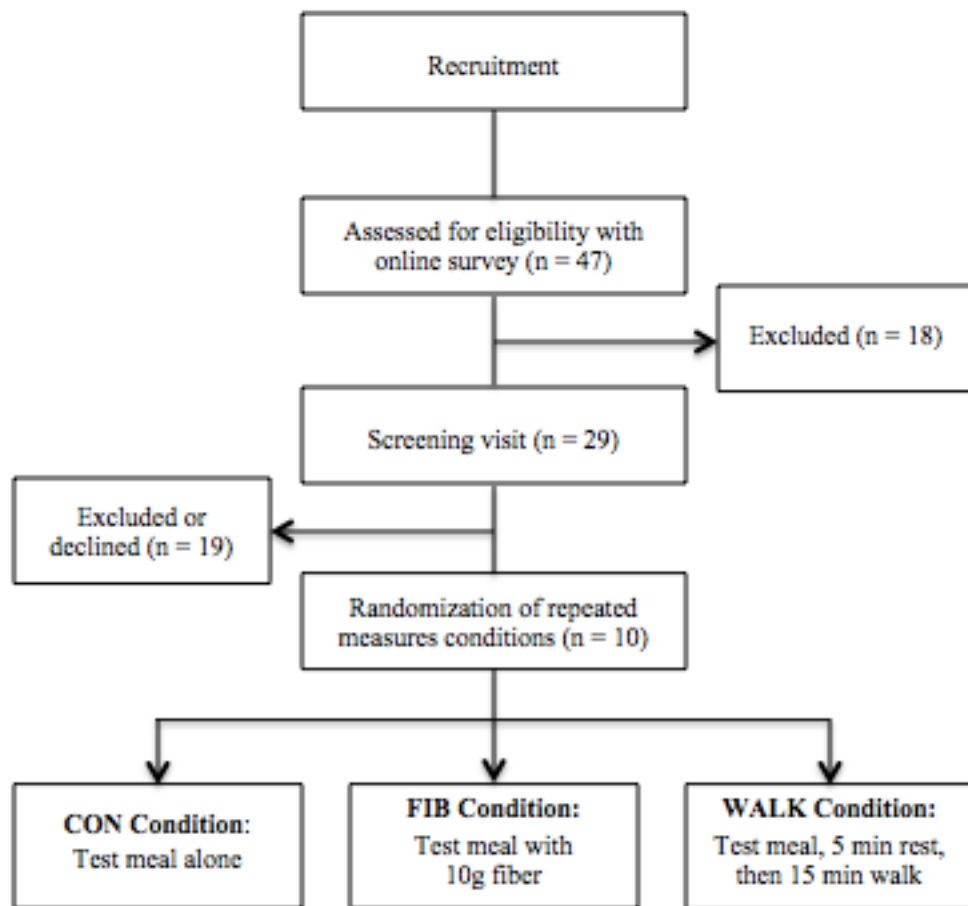


Figure 3: Study design from recruitment to trial implementation. Subjects completed three trials in a randomized order spaced one week apart.

differences in glycemic response without carryover effects. (Karstoft et al., 2014; Lunde et al., 2012). The three trials included: 1) Control session with no walking or fiber supplementation (CON); 2) Fiber session with 10g soluble viscous fiber consumed with test meal (FIB); 3) Walking session with a 15-minute moderate postmeal walk at the subject's preferred walking speed (WALK). Subjects received compensation on a graded scale for each phase of the crossover trial.

Screening Visit

Participants who met the inclusion criteria obtained from the online survey were scheduled to attend an initial screening visit. Subjects provided written consent and completed a medical history questionnaire including a PAR-Q in order to verify further inclusion criteria and the absence of any contraindications to the exercise and dietary protocols. Height of subjects was measured on a stadiometer and weight was assessed on a Tanita body composition analyzer (Tanita Corporation of America, Arlington Heights, IL). Waist circumference was measured according to American College of Sports Medicine [ACSM], 2013) guidelines. Subjects who met all preliminary inclusion criteria were assessed for additional body composition measures and fasting capillary blood glucose samples (fingerstick). Additionally, the preferred walking speed of each subject was calculated using a validated 10-meter walk test (Peters, Fritz, & Krotish, 2013; Wade, Maggs, & Hewer, 1987). Briefly, each subject was asked to walk at a comfortable, normal pace across a 10-meter measured area four different times. Only the middle six meters was measured to eliminate the effects of acceleration and deceleration. Start and stop performance time was recorded when the toes of the leading foot crossed the two-meter and the eight-meter mark, respectively. The number of seconds taken to complete

each of the last three walks was measured and entered into the calculation to determine preferred walking speed per mile.

Body composition.

Body mass index (BMI), total body fat percentage (BF%), and visceral adipose tissue were measured using the DXA (Lunar iDXA, General Electric Medical Systems, Madison, WI, USA) and analyzed using its enCORE™ software (platform version 13.6). Dual energy X-ray absorptiometry (DXA) can accurately predict relative contributions of subcutaneous and visceral adipose tissue in adults using methods previously described (Kaul et al., 2012). Standard warm-up procedures and calibration were performed prior to testing, and a certified radiation technologist conducted all measurements. Subjects were required to wear clothing that was free of any metal and were tested in a fasted state.

Meals.

Subjects consumed identical meals at each of the three visits (with the exception of a fiber supplement added to the FIBER trial). Subjects consumed the glycemic test meal upon arrival, following a fasting blood draw. The test meal was comprised of a common breakfast meal, including a bagel (Dunkin Donuts) with butter (20g), apple juice (240mL), and added sugar (24g). The meal was a total of 670 calories and the macronutrient composition was 70% carbohydrates, 23% fat, and 7% protein. Studies utilizing a similar macronutrient composition have observed significant postprandial glycemic spikes in both healthy individuals (Manohar et al., 2012) and those at risk for impaired glucose tolerance (DiPietro et al., 2013). Subjects were also asked to consume one plain bagel (consisting of 270 calories and 52g carbohydrates), provided at the previous visit, on the day before each of the three trials. Additionally, each subject was

asked to write down the dinner meal on the night before the first testing session to serve as a reminder of what they consumed. Subjects were then instructed to consume this meal prior to each subsequent testing period. The same trained researcher performed a 24-hour dietary recall on each visit to ensure similar dietary intake on the day preceding testing for each subject. Nutrient composition was analyzed by the same volunteer using the Food Processor Diet Analysis & Fitness Software (ESHA Research, Salem, OR).

Study Trials.

Three additional visits (~4 hours each) were scheduled following the baseline visit. Each visit occurred approximately one week apart and subjects were asked to arrive at the laboratory at the same time of day for each visit. The order of the three conditions was randomly determined for each individual. Subjects began testing between 7:00am and 8:00am following at least a 10-hour overnight fast. Subjects were instructed to remain sedentary on the previous day, as well as the morning of testing. The protocol for blood collection is depicted in Figure 3. Fasting baseline venous blood samples were taken upon arrival by a trained phlebotomist or research nurse. Subjects were then given

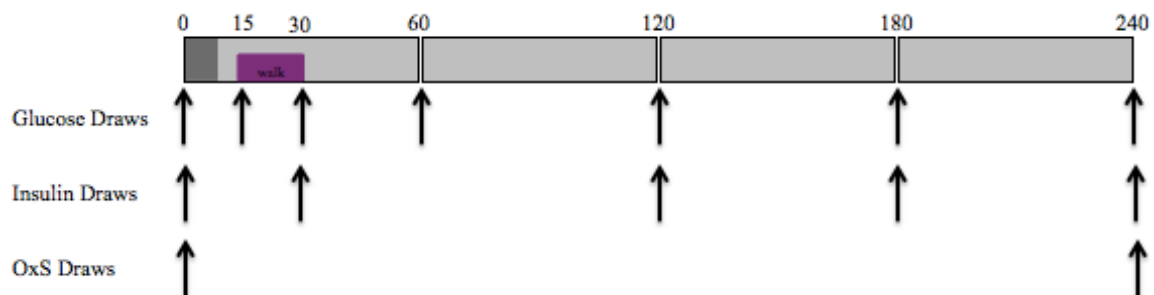


Figure 4. Protocol for blood draws from baseline to 4 hours. Baseline samples were taken immediately prior to meal consumption (denoted by dark grey shading). The walk (denoted by magenta box) occurred on the WALK trial only. OxS = markers of oxidative stress and antioxidant capacity. Time expressed as minutes.

10 minutes to consume the respective test meal, and asked to consume the beverage before eating the bagel. The four-hour clock began as soon as the subject took the first sip of the beverage. Blood glucose was taken at minutes 0, 15, 30, 60, 120, 180, and 240, insulin was taken at minutes 0, 30, 120, 180, and 240, and measures of oxidative stress and antioxidant capacity were taken at minutes 0 and 240. During this time, subjects were permitted to read, watch television, or work on the computer but were not provided with any additional food or beverages except water.

1. CONTROL: After consuming the test meal, subjects remained in the lab for blood testing with minimal activity for the remainder of the four hours.

2. FIBER: Ten grams of soluble viscous fiber was mixed with the apple juice in the metabolic kitchen immediately prior to consumption. Subjects consumed the fiber and juice, along with the rest of the test meal. Following consumption, subjects remained in the lab with minimal activity for blood testing for the remainder of the four hours.

3. WALK: After consuming the test meal, subjects were given five minutes to transition to the exercise physiology lab where blood glucose was measured immediately before beginning the walk. At minute 15, subjects began walking on a motorized treadmill at their calculated preferred walking speed (Mean = 3.0 mph). Heart rate was monitored continuously during exercise and was recorded every minute using a heart rate monitor (Polar Electro OY, Kempele, Finland). Rating of Perceived Exertion (RPE) was also recorded every three minutes. Venous blood glucose and insulin measures were taken immediately following the walk, after which subjects were asked to return to the phlebotomy lab where they rested with minimal activity for additional blood testing over the remaining 3.5 hours.

Blood Analyses.

Blood glucose samples were obtained at the prescreen visit from capillary blood using finger pricks and analyzed with a glucometer (Accu-Chek Aviva Plus, Roche Diagnostics, Indianapolis, IN, USA). The Accu-Chek Aviva Plus has been shown to be an accurate system when monitoring blood glucose, meeting all current International Organization for Standardization (ISO) criteria (Brazg et al., 2013). Whole blood was collected by venipuncture and centrifuged for 15 minutes at 3000 rpm. Plasma and serum was stored at -80° Celsius until analyzed for glucose, insulin and measures of oxidative stress. The positive iAUC for glucose and insulin was calculated according to the method of Wolever et al. (2004; Appendix D). The homeostatic model assessment (HOMA) was calculated according to the method of Levy et al. (1998) and used to assess insulin sensitivity (HOMA-IR). All samples were measured in duplicate and accepted if the intra- and interassay coefficient of variation (CV) were below 10% for each sample.

Glucose. Glucose was measured in plasma with an automated chemistry analyzer (Cobas C111; Roche Diagnostics, Indianapolis, IN) using colorimetric enzymatic reagents. Glucose was measured in plasma that had sodium fluoride added as a glycolysis inhibitor. The results were expressed in milligrams per deciliter (mg/dL).

Insulin. Insulin was measured in plasma using the ultrasensitive human radioimmunoassay kit (Millipore Corporation, Billerica, MA). A fixed concentration of labeled tracer antigen was incubated with antiserum to limit antigen-binding sites, and the amount of tracer bound to the antibody was measured using the double antibody/PEG technique (Morgan & Lazarow, 1962). The results were expressed in microunits per milliliter ($\mu\text{U/mL}$).

TBARS. TBARS were determined in EDTA plasma by spectrophotometry (ZeptoMetrix Corporation, Buffalo, NY). The samples were incubated with 30 μ L of sodium dodecyl sulfate and 70 μ L of TBA buffer reagent (solution of 212mg thiobarbituric acid, 20mL of acetic acid, and 20mL sodium hydroxide). After heating the mixture to 95°C for 60 minutes, the samples were put on ice for 10 minutes, and then centrifuged at 3,000rpm for 15 minutes at room temperature. The supernatant was removed and absorbance was read at 532 nm. The results were expressed as MDA equivalents in nmol/l by interpolation from the standard curve.

TAC. TAC was measured in plasma (heparin) using a colorimetric assay kit (Cayman Chemical Company, Ann Arbor, MI), and was based on the ability of antioxidants in the sample to inhibit the oxidation of ABTS radical cation by metmyoglobin. The oxidized ABTS was measured at 750nm, and the antioxidant capacity was compared with that of Trolox to yield a Trolox equivalent. The results were expressed as final antioxidant concentrations (mM) obtained from calculating the linear regression from the standard curve.

Catalase. Catalase was measured in EDTA plasma using a colorimetric assay kit (Cayman Chemical Company, Ann Arbor, MI) based upon the peroxidatic function of catalase for determination of enzyme activity. The results were expressed as catalase activity (nmol/min/ml) by multiplying the sample dilution by the quotient of the calculated formaldehyde of each sample and the incubation time (20 minutes).

Uric Acid. Remaining serum samples were pooled and sent out to an independent biochemical laboratory (Sonora Quest Laboratories, Tempe, AZ) for analysis. The results were expressed as milligrams per deciliter (mg/dL).

Statistical Analyses.

All analyses were conducted using the Statistical Package for Social Sciences (SPSS Version 22.0, Armonk, NY, USA). Data are reported as mean \pm standard deviation (SD) unless otherwise noted. Descriptive statistics were used to describe subject characteristics. All outcome variables were tested for normality using the Shapiro-Wilk test, homogeneity using Levene's test, and sphericity using Mauchly's test of sphericity and transformed when applicable. The main outcome measures were glucose, glucose iAUC, insulin, insulin iAUC, TBARS, TAC, catalase, and uric acid. Repeated measures ANOVA was used to determine the main effects of condition on glucose iAUC, insulin iAUC, and differences of TBARS, TAC, catalase, and uric acid between the three conditions. A two-way repeated measures ANOVA was used to calculate both main and interaction effects of condition and time on average glucose and insulin. If a main effect existed, subsequent post-hoc analyses were performed using the protected LSD post-hoc test. All P values were calculated assuming two-sided alternative hypothesis and $P < 0.05$ was considered statistically significant.

CHAPTER 4

RESULTS

Subject Characteristics

Forty-seven volunteers responded and completed the online initial eligibility survey. Of these, 29 qualified and came to the laboratory for a screening visit. Eighteen individuals were excluded at screening due to normal blood glucose concentrations, as measured by capillary blood (<100 mg/dL). One subject declined to participate due to scheduling conflicts. Consequently, 10 subjects (3 males, 7 females) were enrolled and completed all trials in the study.

Table 1: Physical characteristics of subjects at baseline.

	All Subjects (<i>N</i> = 10)	Male (<i>N</i> = 3)	Female (<i>N</i> = 7)
Age (yrs)	55.0 ± 10.0	59.3 ± 9.3	53.1 ± 10.4
Height (cm)	170.8 ± 10.5	183.5 ± 8.9	165.3 ± 4.7
Weight (kg)	105.8 ± 21.3	112.6 ± 3.6	102.8 ± 25.4
BMI (kg/m ²)	36.3 ± 7.4	33.7 ± 4.0	37.4 ± 8.5
Waist (cm)	114.0 ± 17.1	115.3 ± 6.5	113.4 ± 20.6
Body Fat (%)	46.6 ± 8.0	37.8 ± 3.5	50.4 ± 6.1
Visceral Fat (cm ³)	2856 ± 1268	3576 ± 1451	2547 ± 1157
Glucose (mg/dL)	107.1 ± 9.0	103.3 ± 2.3	108.7 ± 10.5
HOMA-IR*	5.3 ± 2.1	5.4 ± 1.6	5.3 ± 2.4
Walk Speed (mph)**	3.0 ± 0.3	3.2 ± 0.1	2.9 ± 0.2

Values represent mean ± SD.

* HOMA-IR = Homeostatic Model Assessment of Insulin Resistance, calculated according to the method of Levy et al. (2008).

**Calculated using the 10-meter walk test. Average of 3 trials to determine preferred walking speed of each subject.

Dietary Composition

Dietary intake analysis of the 24-hour dietary recalls for the day preceding each of the three trials are shown in Table 2. There were no significant differences in mean energy intake and intake of carbohydrates, fat, protein, sugar, or fiber between the three trials. On average, subjects consumed approximately 2291 calories per day as 49% of energy from carbohydrates, 35% of energy from fat, and 16% of energy from protein. Additionally, subjects consumed an average of 31g of saturated fat, 101g of sugar and 21g of fiber on the day before each study visit.

Table 2. Dietary composition on the day preceding each trial*.

	Control	Fiber	Walk	<i>P</i> value
Energy (kcal/d)	2073 ± 487	2396 ± 562	2403 ± 655	0.125
Carbohydrate (g/d)	254 ± 69	280 ± 72 ¹	299 ± 69	0.361
Carbohydrate (%)	49.3 ± 8.4	47.5 ± 8.8	50.7 ± 5.9	0.745
Fat (g/d)	77 ± 27	97 ± 29	95 ± 36	0.239
Fat (%)	33.4 ± 8.5	36.2 ± 6.9	34.7 ± 6.1	0.820
Protein (g/d)	91 ± 33	101 ± 38	93 ± 38	0.508
Protein (%)	17.4 ± 3.9	16.6 ± 3.9	15.5 ± 3.9	0.380
Sat Fat (g/d)	25 ± 11	35 ± 19	32 ± 13	0.285
Sugar (g/d)	89 ± 47	99 ± 45	116 ± 50	0.287
Fiber (g/d)	19 ± 7	22 ± 12 ¹	22 ± 10	0.655

Values represent mean ± SD. *P* values represent repeated measures ANOVA.

*Nutrient composition values were obtained from Food Processor Diet Analysis & Fitness Software (ESHA Research, Salem, OR).

¹Data transformed for analysis.

Postprandial Glucose and Insulin

As shown in Table 3, there were no statistical differences in fasting glucose (range: 101.5 – 102.7 mg dL⁻¹; p=0.927) concentrations at baseline across three conditions. Both FIB (-21.5 mg dL⁻¹; p = 0.003) and WALK (-33.25 mg dL⁻¹; p < 0.001) conditions, respectively, had lower glucose concentrations than did CON at min 30. There were no statistical differences in glucose concentration between WALK and FIB conditions at any time period. There was no significant trial x time interaction for glucose concentrations (p = 0.173). Figure 2 shows the pattern of blood glucose concentrations over the four-hour postprandial period, comparing the three conditions.

Table 3. Postprandial plasma glucose and insulin concentrations and incremental area under the curve (iAUC) at 2 and 4 hours for each condition.

	Control	Fiber	Walk	P value
<i>Glucose (mg/dL)</i>				
Baseline	101.5 ± 19.5	102.5 ± 15.4	102.7 ± 17.2	0.927
Minute 15	129.5 ± 18.0	115.5 ± 12.6	122.5 ± 20.6	0.102
Minute 30	155.6 ± 19.8 ^a	134.1 ± 14.5 ^b	122.4 ± 23.6 ^b	<0.001
Minute 60	146.2 ± 32.8	128.1 ± 29.0	140.15 ± 24.8	0.098
Minute 120	118.6 ± 33.8	112.0 ± 18.6	111.6 ± 24.5	0.604
Minute 180	96.3 ± 25.6	106.5 ± 21.7	92.4 ± 20.5	0.108
Minute 240	93.2 ± 19.7	95.1 ± 12.4	87.2 ± 14.1	0.271
2hr iAUC*	235.3 ± 89.7 ^a	141.7 ± 87.1 ^b	158.1 ± 82.6 ^b	0.009 [†]
4hr iAUC*	286.3 ± 133.6 ^a	193.74 ± 121.7 ^b	183.4 ± 82.6 ^b	0.021 [†]
<i>Insulin (μU mL⁻¹)</i>				
Baseline	20.9 ± 8.3	20.8 ± 4.8	20.1 ± 6.5	0.932
Minute 30	91.5 ± 20.9 ^a	66.6 ± 19.5 ^b	51.3 ± 20.8 ^b	0.001
Minute 120	132.2 ± 70.4 ^a	115.3 ± 47.5 ^{ab}	95.4 ± 45.1 ^b	0.042
Minute 180	60.1 ± 33.7 ^a	75.1 ± 27.8 ^a	43.0 ± 22.3 ^b	0.018
Minute 240	36.9 ± 23.8 ^a	42.5 ± 19.9 ^b	28.1 ± 17.1 ^a	0.038
2hr iAUC*	153.9 ± 58.1 ^a	116.8 ± 39.7 ^b	87.6 ± 43.2 ^c	0.001 [†]
4hr iAUC*	256.5 ± 126.5 ^a	229.1 ± 87.4 ^b	152.0 ± 84.5 ^c	0.001 [†]

Values represent mean ± SD.

^{a,b}Means with different superscripts are significantly different (repeated measures ANOVA; LSD post-hoc analysis; p < 0.05).

[†]P value for iAUC calculated using repeated measures ANOVA.

*Estimated according to the method of Wolever et al. (2004) and expressed in mmol·120 min·L⁻¹ for 2 hours and mmol·240 min·L⁻¹ for 4 hours.

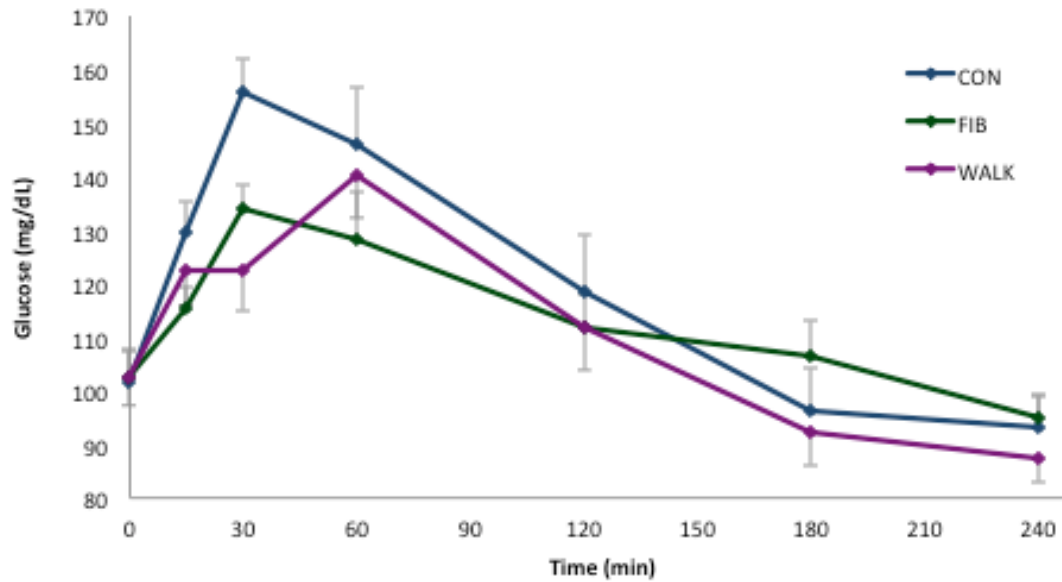


Figure 5. Change in postprandial plasma glucose concentrations at baseline and after meal consumption. Error bars represent ± 1 SEM. CON = control, FIB = fiber, WALK = walking.

There were no statistical differences in fasting insulin (range: 20.1 – 20.9 $\mu\text{U mL}^{-1}$; $p = 0.932$) concentrations at baseline across three conditions, as shown in Table 3. The FIB ($-24.89 \mu\text{U mL}^{-1}$; $p = 0.001$) and WALK ($-40.22 \mu\text{U mL}^{-1}$; $p = 0.002$) conditions had lower insulin concentrations than did the CON condition. The WALK condition remained significantly lower than CON at 120 minutes ($-36.83 \mu\text{U mL}^{-1}$; $p = 0.018$), was significantly lower than both the CON ($-17.03 \mu\text{U mL}^{-1}$; $p = 0.032$) and FIB ($-31.96 \mu\text{U mL}^{-1}$; $p = 0.004$) conditions at 180 minutes, and was significantly lower than the FIB ($-14.44 \mu\text{U mL}^{-1}$; $p = 0.008$) at 240 minutes. There was no significant trial x time interaction for insulin concentrations ($p = 0.054$).

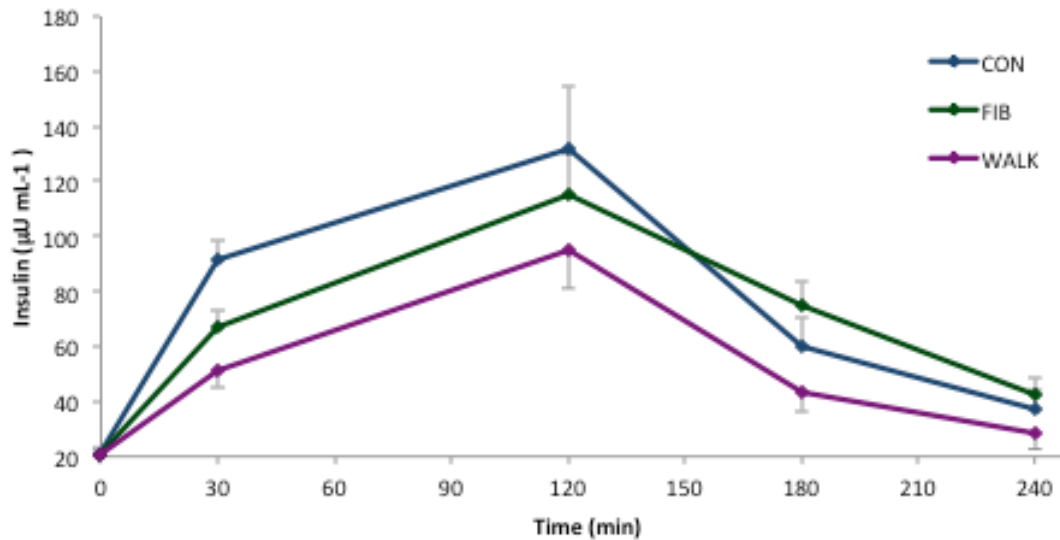


Figure 6. Change in postprandial plasma insulin concentrations at baseline and after meal consumption. Error bars represent ± 1 SEM. CON = control, FIB = fiber, WALK = walking.

Incremental area under the curve (iAUC) for both glucose and insulin are shown in Table 3. There was a significant main effect of condition on 2-hour and 4-hour iAUC for glucose (2hr: $p = 0.009$; 4hr: $p = 0.021$). Post hoc analysis documented that the 2-hour iAUC for glucose was significantly lower in both FIB ($-93.59 \text{ mmol}\cdot 120 \text{ min}\cdot \text{L}^{-1}$; $p = 0.006$) and WALK ($-77.21 \text{ mmol}\cdot 120 \text{ min}\cdot \text{L}^{-1}$; $p = 0.002$) conditions compared with CON condition (Figure 3). Similarly, the 4-hour iAUC for glucose was significantly lower in both FIB ($-92.59 \text{ mmol}\cdot 240 \text{ min}\cdot \text{L}^{-1}$; $p = 0.041$) and WALK ($-102.94 \text{ mmol}\cdot 240 \text{ min}\cdot \text{L}^{-1}$; $p = 0.005$) conditions compared with CON condition (Figure 4). There were no statistical differences in 2-hour or 4-hour iAUC for glucose between FIB and WALK conditions (2hr: $p = 0.493$; 4hr: $p = 0.783$). There was also a significant main effect of condition on 2-hour and 4-hour iAUC for insulin (2hr: $p = 0.001$; 4hr: $p = 0.003$). Post hoc analysis revealed that the 2-hour iAUC for insulin was significantly lower in both FIB ($-37.15 \mu\text{U}$

·h/mL; $p = 0.021$) and WALK ($-66.35 \mu\text{U} \cdot \text{h/mL}$; $p < 0.001$) conditions, compared with CON. Furthermore, 2-hour iAUC for insulin was significantly lower in the WALK ($-29.2 \mu\text{U} \cdot \text{h/mL}$; $p = 0.049$) condition, compared with FIB. The 4-hour iAUC for insulin in the WALK condition was significantly lower than both the CON ($-104.51 \mu\text{U} \cdot \text{h/mL}$; $p = 0.001$) and FIB ($-77.12 \mu\text{U} \cdot \text{h/mL}$; $p = 0.006$) conditions. There were no significant differences in 4-hour iAUC between the CON and FIB conditions ($p = 0.302$).

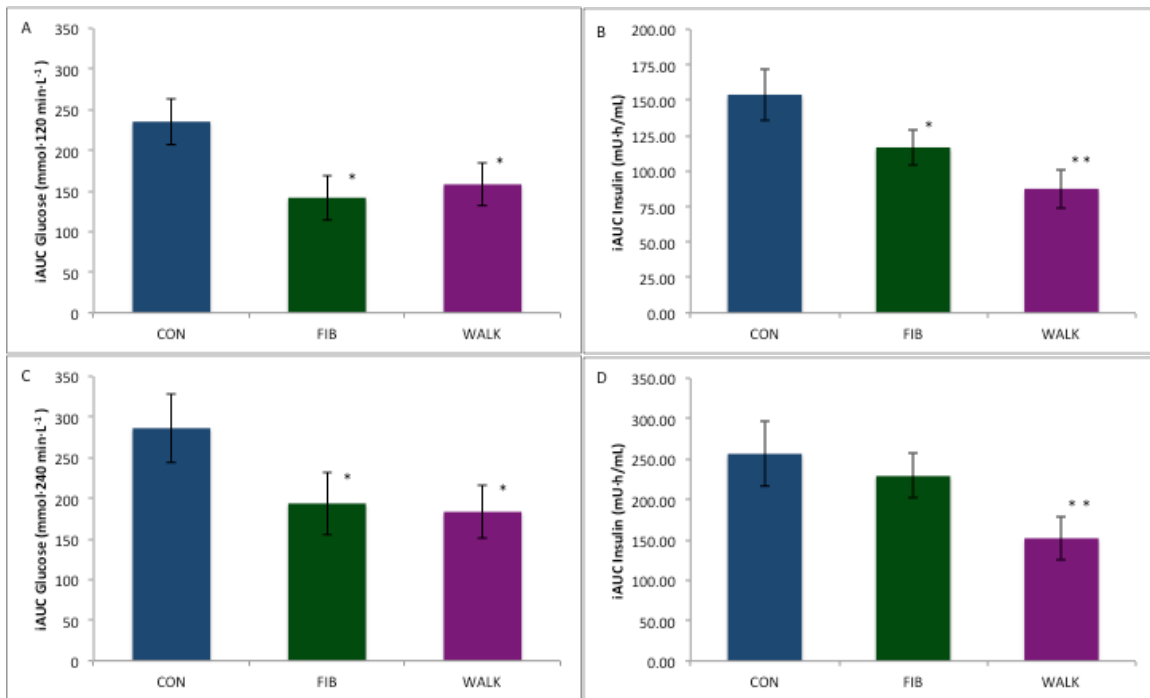


Figure 7. Comparison of incremental area under the curve (iAUC) for glucose and insulin between trials. Panel A: 2hr iAUC Glucose ($p = 0.009$; repeated measures ANOVA), Panel B: 2hr iAUC Insulin ($p = 0.001$; repeated measures ANOVA), Panel C: 4hr iAUC Glucose ($p = 0.021$; repeated measures ANOVA), Panel D: 4hr iAUC Insulin ($p = 0.001$; repeated measures ANOVA). Error bars represent ± 1 SEM. *Significantly different from control (CON). **Significantly different from both CON and fiber (FIB). CON = control, FIB = fiber, WALK = walking.

Antioxidant Capacity and Oxidative Stress

Baseline concentrations of thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC), catalase and uric acid were not different between conditions. Furthermore, biomarkers for TBARS, TAC, catalase and uric acid were unchanged in the four-hour postprandial period in all trials (Table 3).

Table 4. Effects of intervention on plasma markers of antioxidant activity and oxidative stress (M ± SD).

	Baseline	4 Hours	Difference	<i>P</i> value [†]
<i>TBARS</i>				0.267
CON	4.31 ± 1.21	4.13 ± 1.01	-0.18	
FIB	3.92 ± 0.91	4.14 ± 0.85	0.21	
WALK	4.41 ± 1.18	4.18 ± 0.88	-0.23	
<i>TAC</i>				0.138
CON	1.72 ± 0.56	2.13 ± 0.44	0.42	
FIB	1.95 ± 0.49	1.97 ± 0.36	0.02	
WALK	1.60 ± 0.50	2.10 ± 0.42	0.50	
<i>Catalase</i>				0.683
CON	7.86 ± 9.98	6.07 ± 2.87	-1.78	
FIB	7.33 ± 4.80	7.24 ± 4.88	-0.09	
WALK	8.26 ± 8.99	4.07 ± 1.43	-4.18	
<i>Uric Acid</i>				0.295
CON	5.55 ± 1.30	5.58 ± 1.46	0.03	
FIB	5.87 ± 1.31	5.69 ± 1.32	-0.18	
WALK	5.67 ± 1.23	5.61 ± 1.35	-0.06 ¹	

Values represent mean ± SD.

The absolute change data are reported as the difference. There were no differences between groups at baseline. TBARS are expressed as nmol/l, TAC is expressed as mM, catalase is expressed as nmol/min/ml, and uric acid is expressed as μM.

[†]*P* values calculated from difference scores (repeated measures ANOVA).

¹Data transformed for analysis

CHAPTER 5

DISCUSSION

The objective of this study was to evaluate the effects of a 15-minute moderate postmeal walk as compared to a positive control (fiber supplementation) or no-exercise, no fiber control condition on glucose regulation and markers of oxidative stress in obese sedentary individuals with IFG. Our major finding was that both the walking and fiber conditions were effective in reducing postprandial glucose and insulin concentrations when compared to the control condition; however the walking condition appeared to be more effective than fiber at sparing insulin secretion up to four hours after the meal. The 2-hour glucose iAUC was reduced by 40% and 33% in the fiber and walking conditions, respectively as compared to the control condition. This attenuation lasted through the 4-hour postprandial period, evidenced by a 32% and 36% reduction in 4-hour glucose iAUC in the fiber and walking conditions, respectively as compared to the control condition. At two hours, the fiber condition resulted in a 24% decrease in insulin iAUC, compared with control. Moreover, the walking condition was superior to both the control and fiber conditions, evidenced by a 43% and 25% reduction in 2-hour insulin iAUC, respectively. Insulin concentrations dropped considerably between two and three hours during the walking and control conditions, while there appeared to be a more gradual protracted decline of insulin in the fiber condition during this time. As such, the walking condition resulted in a 41% and 34% reduction in 4-hour insulin iAUC, when compared with the control and fiber conditions, respectively, while there was no difference in insulin iAUC between the fiber and control conditions at four hours. Therefore, the 15-

minute postmeal walk was superior to both the control and fiber conditions at sparing insulin secretion up to four hours following meal ingestion.

With respect to measures of oxidative stress and antioxidant capacity, neither the walking condition nor the fiber condition appeared to impact concentrations of TBARS, TAC, catalase, or uric acid four hours following the test meal. Furthermore, the test meal during the control condition did not have a significant impact on any of our chosen markers of oxidative stress or antioxidant capacity.

Postprandial Glucose and Insulin

This is the first study to show that a moderate 15-minute walk after a meal can effectively lower the postprandial glucose response for up to four hours in individuals with impaired fasting glucose. Previous work by Nygaard et al. (2009) examined whether a 15-minute or 40-minute self-selected postmeal walk (RPE = 9) could blunt the increase in blood glucose following a high carbohydrate meal in 14 metabolically healthy women. They indicated that only the 40-minute walk significantly lowered glucose iAUC by 31%. Despite an 11% reduction in glucose iAUC following the 15-minute walk, this did not reach significance. Moreover, there appeared to be a dose-response relationship between time spent walking and the attenuation of blood glucose. Since postprandial hyperglycemia is strongly associated with skeletal muscle insulin resistance, it is possible that the benefits of postmeal exercise are even greater in insulin resistant individuals. In the present study, 2-hour iAUC for glucose was reduced by 33% following a 15-minute walk in individuals with IFG, representing a much greater reduction in postprandial glucose than that observed in the study by Nygaard et al. (2009). This suggests that as

individuals become more insulin resistant, the magnitude of postprandial hyperglycemic protection increases.

Lunde et al. (2012) also investigated the impact of postmeal walking on individuals with impaired glucose regulation. On two different occasions, 11 obese women with IGT and/or IFG completed either a 20-minute or 40-minute slow walking bout (RPE = 8) immediately following a high-carbohydrate meal. In this study, compared with control, the 2-hour iAUC for glucose significantly decreased by 31% and 39% after the 20-minute and 40-minute walk, respectively. The superior attenuation of postprandial glucose in walks of longer duration is likely due to increased muscle contraction leading to enhanced insulin-independent glucose uptake at the skeletal muscle (Knudsen et al., 2014; Stanford & Goodyear, 2014). As a result, there appears to be a linear relationship between length of the postmeal walk and overall glucose attenuation. While the present study did not assess this, it is likely that our subjects would have experienced greater declines in postprandial glucose concentrations following a similar walking protocol that lasted longer than 15 minutes.

The present study indicated a greater reduction in 2-hour glucose iAUC following a 15-minute walk than the 20-minute walk in the study by Lunde et al. (2012), as well as the 15-minute walk in the study by Nygaard et al. (2009). Importantly, while the walk in the present study was self-selected by the individuals and calculated as preferred walking speed, the intensity was higher than those observed in previous studies (Lunde et al., 2012; Nygaard et al., 2009). The subjects in our study self-selected a walking speed of approximately 3.0mph, which resulted in an average effort of 68% HRmax and a self-reported average RPE of 12, which is higher than the reported RPE of 8 and 9 in the

Lunde and Nygaard studies, respectively. While no study to my knowledge has assessed the differential effect of postmeal walking at varying intensities on postprandial glucose control, it is possible that the walking condition in the present study elicited a greater attenuation in postprandial glycemia than previous trials because the intensity of the activity was greater. However, this could be due to an increase in overall energy expenditure, as opposed to a strictly intensity-dependent outcome. Indeed, Manders et al. (2010) assessed the impact of a single bout of low and high-intensity exercise on postprandial hyperglycemia and 24-hour glucose control in a group of nine patients with T2D. The low intensity exercise session included 60 minutes of cycling at 35% W_{max} , and the equicaloric high intensity exercise included cycling for 30 minutes at 70% W_{max} . The authors found that average 24-hour glucose concentrations were reduced by ~29 mg/dL following the 60-minute low intensity exercise, but were not significantly reduced in the 30-minute high intensity exercise trial. The attenuation of average glucose was influenced by a 50% reduction in postprandial glucose following the dinner meal, which resulted in a 50% reduction in total time spent in hyperglycemia (>180 mg/dL). In this study, low-intensity exercise was superior to high-intensity exercise in improving 24-hour glucose control in T2D when corrected for energy expenditure, suggesting that total energy expenditure might be more related to the beneficial effects of exercise on glycemic control than intensity (Manders et al., 2010).

In the present study, glucose concentrations increased markedly in both the control and walking conditions immediately following the meal. However, unlike the control condition, in which blood glucose continued to increase to hyperglycemic levels, concentrations leveled off from minutes 15 to 30 while subjects were walking and were

significantly lower 30 minutes following the meal than in the control condition. Of interest, blood glucose concentrations rebounded when postmeal exercise ceased and were no longer significantly lower than that of the control session 60 minutes following consumption of the meal. These findings are similar to a series of studies by Larsen et al. (1997, 1999), which showed that both moderate and high intensity exercise performed after a meal significantly reduced both glucose and insulin AUC. In both studies, glucose was attenuated during the exercise bout, resulting in an overall lower AUC, despite a rebound effect of glucose following exercise completion.

While the mechanisms responsible for this were not explored in the present study, there is evidence to suggest that the rebound of blood glucose following exercise observed in the studies by Larsen (1997, 1999) and others (Heden et al., 2015), as well as the present study, is likely due to a simultaneous reduction in skeletal muscle glucose uptake following exercise and a transient increase in hepatic glucose production (Kjaer, 1998). However, while increased levels of epinephrine immediately following exercise have been shown to increase the rate of hepatic glucose production and subsequently glucose appearance in the blood (Kjaer et al., 1990; Rynders et al., 2014), this was likely not the case in the present study. For a given exercise, the transient catecholamine response is closely related to the intensity of the effort and generally does not appear to increase considerably until around 75% of a maximal aerobic effort (Zouhal et al., 2008). While catecholamine levels were not measured in the present study, subjects were walking for a short period of time (15 minutes) at an average pace of 3.0mph and had a calculated average heart rate of 68% during the walk. Therefore, it is unlikely that the

increase in blood glucose immediately following exercise was due to a transient sympathetic response.

For the first time, the present study showed that a short 15-minute postmeal walk can significantly reduce plasma insulin concentrations for up to four hours, and was more effective than fiber at sparing insulin. Average plasma insulin concentrations were significantly lower during the walking condition than the control condition after four hours. Furthermore, 4-hour insulin iAUC was reduced by approximately 41% during the walking condition, compared to in the control condition, and although not significant, insulin concentrations remained lower during the walk than during the control condition at four hours. These observations are similar to a study by Hashimoto et al. (2013), which is the only other study, to my knowledge, to assess the effect of postmeal walking on postprandial insulin concentrations. The authors investigated the acute effects of walking for 30 minutes at $\sim 50\%$ VO_{2max} 20 minutes following a high glycemic meal on glucose control in 14 healthy but sedentary women. The authors indicated that the 30-minute postmeal walk significantly lowered the 6-hour iAUC for insulin by 42%, when compared with the control condition.

Conversely, the attenuation of insulin during the fiber condition was similar to the walking condition at 30 minutes, but this significance was lost at 120 minutes. After two hours, insulin concentrations during the fiber condition declined much more gradually and were higher than both the walk and control conditions at three and four hours, although only significantly higher than the walk condition. Soluble fiber, the type used in this study, forms thick gels when mixed with fluids in the small intestine, which subsequently delays gastric emptying. This delay of gastric emptying is known to reduce

postprandial glucose excursion due to a more gradual release of substrate into the circulation (Slavin et al., 1999). Although the present study was not designed to directly assess underlying mechanisms, it is likely that the initial attenuation of insulin during the fiber condition, which appeared to be similar during the walking protocol, was a consequence of a slower release of stomach contents into the small intestine for absorption (Sierra et al., 2001). Without fiber (control and walking protocols), the contents of the meal were likely digested and absorbed into the blood stream much more quickly, resulting in a quicker appearance of glucose in the blood. The decline in insulin concentrations observed during the walking and control conditions after 2 hours was likely the results of quicker glucose disposal, as the contents of the stomach were depleted. As a consequence of the gradual release of gastric contents due to the fiber, insulin levels remained elevated for a longer period of time. While insulin concentrations during the fiber condition never peaked to a level observed during the control conditions, the iAUC for insulin remained the same as control at four hours after the meal. However, insulin concentrations during the walk condition were significantly lower than both the control and fiber conditions throughout the 4-hour postprandial period, suggesting that walking might be an even better strategy than fiber to reduce postprandial glucose excursion in individuals with IFG.

The present study did not explore mechanisms for the improvements in postprandial glucose control following the walk. However, it is well established that contraction-stimulated glucose uptake is normal or near normal in individuals with impaired insulin-stimulated glucose uptake (Knudsen et al., 2014; Stanford & Goodyear, 2014). Contractions appear to stimulate GLUT4 translocation via activation of molecular

signals independent from insulin signaling, although the precise mechanisms are not well understood (Stanford & Goodyear, 2014). A second possible explanation for decreased glucose concentrations is that higher insulin levels following the meal resulted in decreased hepatic glucose production. Indeed, Poirier et al. (2001) indicated a 77% greater reduction in blood glucose following 60 minutes of cycling at 60% $\text{VO}_{2\text{peak}}$ in the fed state, compared with postabsorptive exercise. However, in the present study there were no significant differences in glucose concentrations at 15 minutes (start of the walk) between conditions. Therefore, greater plasma glucose attenuations during exercise cannot be ascribed to higher baseline glucose values and it is likely that the attenuation was a function of enhanced peripheral glucose uptake during and to a lesser extent, immediately after exercise.

Oxidative Stress and Antioxidant Capacity

While both the fiber and walking conditions significantly attenuated postprandial hyperglycemia, data from the present study indicate that neither condition had an effect on markers of oxidative stress or antioxidant capacity following a high-glycemic meal. Importantly, no time effects were observed for any of the biomarkers during any condition. The lack of observable effect of our test meal to induce oxidative stress during the control condition is unfortunate and makes it difficult to elucidate the potential impact of walking on attenuating the oxidative response. Many studies have indicated an increased oxidative response following ingestion of a mixed meal; however, the meal is often high in fat and designed to measure plasma triglyceride levels and subsequent oxidative stress (Canale et al., 2014; Clegg et al., 2007; McClean et al., 2007; Melton et al., 2009). Nevertheless, both hyperlipidemia and hyperglycemia in the postprandial

period are known to induce oxidative stress (Gergersen et al., 2012), and high glycemic meals have resulted in a rise of oxidative products in healthy (Ceriello et al., 1999; Gregersen et al., 2012; Kasuya et al., 2015) and diabetic (Ceriello et al., 1998) subjects. A study by Gregersen et al. (2012) assessed the relative contributions of fat versus carbohydrate intake on the postprandial oxidative stress response in serum and skeletal muscle in a group of 15 healthy individuals. Subjects consumed either a high-fat meal or a high-carbohydrate meal on two separate occasions. The authors reported an elevation in serum and muscle expression of IL-6 following both meals. Furthermore, plasma total antioxidant status and muscle SOD were decreased following the high-carbohydrate meal only, suggesting that a high-carbohydrate meal might evoke a greater postprandial oxidative stress response than a meal high in fat (Gregersen, 2012). Additionally, a study by Ceriello et al. (1999) evaluated the effects of two different meals designed to induce different levels of hyperglycemia on plasma oxidative status in a group of 10 individuals with T2D. The meals were administered in a randomized order and blood samples were taken at baseline, 60, and 120 minutes following the meals. Measures of glucose, insulin, cholesterol and triglycerides all significantly increased and total radical trapping activity decreased following both meals. Moreover, all values were significantly more exaggerated following the meal that induced a greater degree of hyperglycemia (Ceriello et al., 1999), suggesting that postprandial hyperglycemia may be a major contributor of oxidative stress.

The test meal in the present study was designed to mimic a standard breakfast and totaled 670 kcals including 118 grams of carbohydrates (70%), 17 g fat (23%), and 11 g of protein (7%). This is similar in composition to the meals used in the studies by

Gregersen et al. (2012), as well as a series of studies by Ceriello et al. (1997, 1998). Moreover, a recent study by Kasuya et al. (2015) indicated a decrease in antioxidant potential three hours following a meal of white rice totaling only 223 kcals and 50 grams of carbohydrates. Therefore, the meal used in the present study should have been adequate to elicit an oxidative response. Furthermore, the subjects in the present study had IFG, lower plasma antioxidants, and higher resting oxidative stress levels than what is commonly found in healthy populations (Canale et al., 2014). Since individuals with impaired glucose regulation are more susceptible to increased levels of postprandial oxidative stress (Tucker et al., 2008), it is difficult to explain the lack of observable effect of our test meal on markers of oxidative stress in the present study. Some potential explanations might include the timing of our blood draws and/or the measurement of improper biomarkers to ascertain the extent of postprandial oxidative stress. Although four hours is a commonly used measurement time to assess acute changes in oxidative stress, some studies have seen greater changes at two hours (Canale et al., 2014; Clegg et al., 2007) and three hours (McClellan et al., 2007) than when measured at four hours. Therefore, it is possible that because of the timing of our measurements (0 and 4 hours), we might have missed more transient changes in our chosen biomarkers. Additionally, the assessment of total oxidative stress, either acutely or chronically, poses many challenges. The direct measurement of oxidative stress is challenging, due to the short half-life and reactivity of ROS. Therefore, several indirect measurement techniques are utilized in research, including oxidative damage to lipids, proteins, DNA, as well as exogenous and endogenous antioxidant defense systems. While measurement of specific biomarkers is important to assess possible damage from free radical production or

reduced antioxidant status, the various interactive effects of these systems is likely not accounted for, and the numerous available biomarkers makes the comparison of oxidative stress between studies difficult.

While the present study showed that a moderate 15-minute postmeal walk could attenuate postprandial glucose and insulin concentrations following a high-carbohydrate meal, this did not translate to a change in markers of oxidative stress or antioxidant capacity. Only four studies to my knowledge have investigated the effects of exercise on postprandial oxidative stress (Canale et al., 2014; Clegg et al., 2007; McClean et al., 2007; Melton et al., 2009). Of these, two found a significant change in oxidative markers during the exercise condition, compared with the control condition (Clegg et al., 2007; McClean et al., 2007), while two showed no effect (Canale et al., 2014; Melton et al., 2009). Clegg et al. (2007) assessed the efficacy of one hour of cycling at 60% of HRmax, performed prior to meal ingestion, on attenuating the rise in postprandial lipemia and oxidative stress in eight healthy males. They showed that LOOH, a product of lipid peroxidation, significantly increased two hours following the meal in both conditions, but concentrations were significantly elevated at four hours in the control condition only, suggesting a protective role of premeal exercise. McClean et al. (2007) investigated the effect of a one-hour treadmill exercise session at 60% HRmax two hours after a meal on markers of oxidative stress and antioxidant capacity in trained males. The authors showed that while LOOH concentrations increased in both conditions following meal ingestion, levels were significantly lower at three hours post ingestion during the exercise condition and SOD levels following the meal were only significantly reduced during the control condition, indicating oxidative protection following exercise. Conversely, previous work

by Bloomer and colleagues has indicated no change in measures of postprandial oxidative stress following an acute exercise bout, when compared to a control condition (Canale et al., 2014; Melton et al., 2009). A recent study by Canale et al. (2014) assessed the impact of aerobic and anaerobic exercise bouts of varying intensities and durations on biomarkers of oxidative stress and antioxidant capacity following a high-fat meal in trained men. The authors indicated a significant time effect for measures of MDA, H₂O₂, AOPP, SOD, and CAT, with values higher at two and four hours post-ingestion for all biomarkers; however, none of the exercise conditions appeared to attenuate these increases. Interestingly, TAC values were higher at two and four hours for the 15-second sprint condition than the control and 60-minute aerobic exercise session, which might represent a transient increase of oxidative products, and subsequent antioxidant defenses, which is often observed following high-intensity exercise (Bessa et al., 2013).

In regards to the timing and intensity of the exercise bout, previous studies have seen an effect of an hour of moderate-intensity cycling or walking performed before (Clegg et al., 2007) or two hours after (McClellan et al., 2007) a test meal on markers of oxidative stress and antioxidant capacity. This was the first study to assess the impact of a short-duration walking bout immediately after meal ingestion on these markers. Since glycemic spikes in the postprandial period have been shown to independently induce oxidative stress (Ceriello et al., 1999; Phaniendra et al., 2015), we believed that attenuating this response could potentially reduce the oxidative stress associated with that meal. However, the present study failed to demonstrate an increase in any of the chosen oxidative markers following meal ingestion, and was unable to identify an effect of walking on attenuating this response.

Strengths

This study has several strengths. It is the first study to show that a moderate 15-minute postmeal walk can improve postprandial glucose control in individuals with IFG and the first study to assess the impact of immediate postmeal exercise on markers of oxidative stress and antioxidant capacity. The present study utilized a repeated measures crossover design where each subject served as his or her own control for each treatment, which greatly enhanced the internal validity and reliability of our findings. We objectively assessed dietary intake on the day preceding each study visit using a 24-hour recall by a trained nutrition professional and found no significant differences for any of the dietary variables between conditions. Therefore, it is reasonable to suggest that our findings were not impacted by changes in diet. Lastly, we assessed the impact of a potentially easily adoptable strategy on a specific and independent metabolic risk factor (postprandial glycemic excursion) in an at-risk population who might possibly benefit the most from targeted preventive measures.

Limitations

This study has some limitations. No objective measures were used to assess physical activity levels on the days preceding each visit. Although subjects were asked to refrain from exercise 24 hours prior to testing, even an acute bout of exercise has been shown to improve insulin sensitivity for up to 72 hours (Adams, 2013) and this should be considered in future studies. As discussed above, the timing of our blood draws and/or the chosen biomarkers may have been inadequate to ascertain the extent of postprandial oxidative stress. We measured for oxidative stress at baseline and four hours after the meal only. Some studies have seen greater changes in markers of oxidative stress at two

hours (Canale et al., 2014; Clegg et al., 2007) and three hours (McClean et al., 2007) than when measured at four hours, and it is therefore possible that we might have missed more transient changes in our chosen biomarkers. Finally, this study examined the acute effect of a single bout of postmeal walking, and the findings cannot be extrapolated to long-term exposure. Future studies should assess whether repeated daily exposure has a lasting effect on glucose control

Conclusion

This study suggests that a moderate 15-minute walk performed shortly after a meal may be an effective strategy to improve postprandial glucose control for up to four hours in individuals with impaired fasting glucose. This highlights the importance of considering exercise timing and not just duration and/or intensity for individuals with impaired glucose regulation. Given the independent risk associated with postprandial hyperglycemia and possible oxidative damage resulting from hyperglycemic excursion, there are immense health benefits to identifying easily adoptable exercise strategies for at-risk individuals. Whether a postmeal walk affects 24-hour glucose regulation or subsequent levels of oxidative stress remains to be evaluated in future studies.

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APPENDIX A

CONSENT FORM, IRB APPROVAL

Mealtime Walking Study

INTRODUCTON

The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS

Dr. Carol Johnston and Jessica Knurick, a doctoral student, at Arizona State University Downtown Campus, have requested your participation in a research study.

STUDY PURPOSE

The purpose of the research is to evaluate the effect of postmeal walking on markers of oxidative stress and glycemia in individuals at risk for diabetes.

DESCRIPTION OF RESEARCH STUDY

You have indicated to us that you are 55-75 years of age and generally sedentary. If female, you have no possibility of being pregnant and no longer have a menstrual cycle. This study will initially involve the completion of brief demographic and health history questionnaires to demonstrate the absence of conditions that may contraindicate health and blood assessments. This research entails that you visit our test facility on three additional occasions. At each of these occasions you will consume a test meal (bagel and apple juice), and provide blood samples from a central venous catheter.

At the first lab visit, you will be asked to complete a questionnaire about your general health. A fasting pre-screen blood sample from a finger prick will be obtained to determine inclusion in the study. Your height, bodyweight, waist circumference, and blood pressure will be measured. In addition, body composition (relative amounts of fat and lean tissue) will be determined by using an FDA-approved bone density measurement machine. The procedure is called Dual-energy X-ray Absorptiometry (DEXA). You will be asked to lie face up on an open, padded table for 7 minutes while the scanner arm of the DEXA machine passes over the entire body. You can wear regular clothing but any metal must be removed. You will be exposed to a small amount of radiation (1-4 microSieverts) that is within an acceptable range per the FDA. For comparison, you would be exposed to approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x-ray. (For test accuracy, you will be asked about test procedures using barium/isotopes in the recent past and be scheduled for visit 1 with an adequate lapse of time.) Lastly, you will be asked to walk across a short measured area to assess preferred walking speed. This visit will last ~1 hour. Three additional visits will be scheduled based on availability.

For the remaining 3 visits (~4 hours/visit) you will be asked to fast (no food or drink with the exception of water in the preceding 12 hours). You will also be instructed not to exercise the day prior to each visit. You will be provided with a subway gift card and asked to consume the same subway sandwich on the night before each lab visit. You will also be asked to consume 1 bagel the day prior to testing. The test meal will be consumed within 15 minutes of arrival. The three visits will be spaced approximately one week apart. At these visits you will consume the test meal with or without a postmeal treadmill walk. During the treadmill walk, we will record heart rate data from a monitor placed around the upper abdomen. For each session, a fasting blood sample will be obtained for determination of basal concentrations of blood glucose and markers of oxidative stress. You will then be given 15 minutes to eat a standardized meal (bagel and apple juice). Venous blood samples will be collected prior to meal ingestion and at 15, 30, 60, 120, 180 and 240 minutes post meal (<3 tablespoons total).

RISKS

A certified X-ray technician will complete all DEXA scans. A trained phlebotomist will perform blood draws under standard and sterile conditions, but temporary bruising of the skin or a feeling of faintness is possible at the time of the venous blood draw. For the blood pressure tests, a squeezing pressure will be applied to the upper arm for a short time. The test meal is a bagel and juice and contains gluten, which is unsuitable for individuals who have gluten intolerance. Treadmill walking will be at low-moderate intensity (2.5-3.0 mph) pace under the supervision of a trained exercise physiologist. We will ask that you bring appropriate walking shoes to this visit. You are asked to tell investigators to stop any testing at any time if desired.

BENEFITS

You will not benefit from this study, but you will be provided with all your health marker test results if desired including your bone mineral density, body fat composition, and fasting blood glucose and insulin concentrations. You will have the opportunity to attend a free healthy living strategies class after study completion offered by a diabetic educator.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators. Plasma from blood samples will be stored for 5 years in freezers in the laboratories of the Nutrition Program at Arizona State University after which time they will be disposed of as biohazard waste.

WITHDRAWAL PRIVILEGE

You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not affect you any manner.

COSTS AND PAYMENTS

The researchers want your decision about participating in the study to be absolutely voluntary. Yet they recognize that your participation may pose some costs related to time and travel. Participants will receive cash incentives (\$10, \$15, and \$20 [totaling \$45 for participation]) at lab visits 2, 3, and 4 to offset these costs.

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, 500 N. 3rd St., Phoenix, AZ 85004. [602-827-2265]

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

Subject's Signature

Printed Name

Date

Contact phone number

Email

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator _____

Date _____



APPROVAL FULL BOARD

Carol Johnston
SNHP - Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 2/19/2014 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Mealtime Walking Study to Improve Postprandial Metabolic Response
Investigator:	Carol Johnston
IRB ID:	STUDY00000563
Funding:	None
Grant Title:	None
Grant ID:	None
Documents Reviewed:	<ul style="list-style-type: none">• Consent, Category: Consent Form;• Protocol, Category: IRB Protocol;• Satiety scale, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);• Mood questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);• Calendar, Category: Participant materials (specific directions for them);• Recruitment flyer and email, Category: Recruitment Materials;• Survey monkey , Category: Recruitment materials/advertisements /verbal scripts/phone scripts;• Health screener, Category: Screening forms;• PAR-Q, Category: Screening forms;

The IRB approved the protocol from 2/12/2014 to 2/11/2015 inclusive. Before 2/11/2015, you are to submit a completed "FORM: Continuing Review (HRP-212)" and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 2/11/2015 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:

Jessica Knurick



APPROVAL:CONTINUATION

Carol Johnston
SNHP - Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 2/4/2015 the ASU IRB reviewed the following protocol:

Type of Review:	Continuing Review
Title:	Mealtime Walking Study to Improve Postprandial Metabolic Response
Investigator:	Carol Johnston
IRB ID:	STUDY00000563
Category of review:	(9) Convened IRB determined minimal risk
Funding:	None
Grant Title:	None
Grant ID:	None
Documents Reviewed:	<ul style="list-style-type: none">• consent, Category: Consent Form;• Consent, Category: Consent Form;• modification (9/10) clean, Category: Consent Form;

The IRB approved the protocol from 2/4/2015 to 2/10/2016 inclusive. Three weeks before 2/10/2016 you are to submit a completed "FORM: Continuing Review (HRP-212)" and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 2/10/2016 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

APPENDIX B
RECRUITMENT FLIER, ONLINE SCREENING SURVEY, SUBJECT
QUESTIONNAIRES



Mealtime Walking Trial

School of Nutrition
& Health
Promotion

THE NUTRITION PROGRAM AT ASU IS RECRUITING NON-SMOKING, SEDENTARY ADULTS. THIS STUDY WILL EXAMINE WHETHER A POSTMEAL WALK REDUCES THE METABOLIC IMPACT OF A STANDARD AMERICAN MEAL.

Participation will include:

- Enrolling in a 4-week trial including 4 visits to the Nutrition Laboratories at the downtown Phoenix campus (5th and Van Buren Streets)
- Providing blood samples at each visit spaced 1 week apart.
- Maintaining normal diet and activity patterns
- Incentives will be provided during the study, including \$50 cash, complete bone health & blood glucose and insulin results, and free enrollment in our healthy living strategies class after study completion

INTERESTED?? Please visit our recruitment site:

www.surveymonkey.com/s/mealstudy

Adults Needed for ASU Mealtime Walking Study

The ASU Nutrition Program is recruiting non-smoking, sedentary adults for a research trial. This 4-week trial will examine whether a postmeal walk will improve the metabolic impact of a standard American meal. If you are willing to visit the lab once per week for 4 weeks and provide blood samples at each visit, you may be interested in this trial. Incentives will be provided during the study, totaling \$50 cash, complete bone health & blood glucose and insulin results, and free enrollment in our healthy living strategies class after study completion for 4-weeks of participation.

For more information or to apply for the study, please visit our recruitment site:

www.surveymonkey.com/s/mealstudy

Survey Monkey Questionnaire

1. Please provide your email address:
2. Are you between the age of 55 and 75 years old? Yes No
3. Are you Male or Female? Male Female
4. What is your height?
5. What is your weight (best guess)?
6. Do you smoke? Yes No
7. If female, do you still have a menstrual cycle? Yes No
8. How often do you purposefully exercise (days/week) 0, 1-2, 3-4, 5+
9. Are you healthy and free of chronic disease? Yes No Unsure

If "No" please list:

10. Has your physician diagnosed you with diabetes? Yes No Unsure
11. Are you able and willing to walk on a treadmill at a brisk pace (~2.5-3 mph) for 15 minutes?
12. Has your physician diagnosed you with any gastrointestinal-related conditions or malabsorption disorder (such as Crohn's disease, Celiac sprue, polyps, irritable bowel syndrome, gluten intolerance, etc.)? Yes No Unsure

If "Yes" please list:

13. Do you have any food allergies?

If "Yes" please comment:

14. Would you be willing and able to visit the ASU Downtown Phoenix campus for 4 visits about 1 week apart for this study? (1st visit will take ~45 minutes; the remaining 3 visits will take ~4 hours) Yes No Unsure
15. Are you ok with giving blood samples? Yes No Unsure

HEALTH HISTORY QUESTIONNAIRE

ID# _____

1. Gender: M F

2. Age: _____

3. Have you lost or gained more than 10 lbs in the last 12 months? Yes No
If yes, how much lost or gained? _____ How long ago? _____

4. Ethnicity: (please circle) Native American African-American Caucasian
Hispanic Asian Other

5. Do you smoke? No, never _____
Yes _____ # Cigarettes per day = _____
I used to, but I quit _____ months/years (circle) ago

6. Have you ever been pregnant? _____
If yes, date of last pregnancy? _____

7. What were the dates of your last menstrual cycle? _____

8. Do you take any medications regularly? Yes No *If yes, list type and frequency:*

<u>Medication</u>	<u>Dosage</u>	<u>Frequency</u>

9. Do you currently take supplements (vitamins, minerals, herbs, etc.)? Yes No
If yes, list type and frequency:

<u>Supplement</u>	<u>Dosage</u>	<u>Frequency</u>

10. Have you ever been hospitalized? _____ If yes, for what?

11. Please ANSWER (YES/NO) if **you currently have** or if **you have ever** been clinically diagnosed with any of the following diseases or symptoms:

	YES	NO		YES	NO
Coronary Heart Disease			Chest Pain		
High Blood Pressure			Shortness of Breath		
Heart Murmur			Heart Palpitations		
Rheumatic Fever			Any Heart Problems		
Irregular Heart Beat			Coughing of Blood		
Varicose Veins			Feeling Faint or Dizzy		
Stroke			Lung Disease		
Diabetes			Liver Disease		
Low Blood Sugar			Kidney Disease		
Bronchial Asthma			Thyroid Disease		
Hay Fever			Anemia		
Leg or Ankle Swelling			Hormone Imbalances		
Eating Disorder			Depression		

Please elaborate on any condition listed above. _____

12. How would you rate your lifestyle?

Not active _____ Active _____
 Somewhat active _____ Very Active _____

13. Please circle the total time you spend in each category for an average **week**.

Light activities such as:

Slow walking, golf, slow cycling, doubles tennis, easy swimming, gardening

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Moderate activities such as:

Moderate walking, cycling, singles tennis, moderate swimming, weight lifting

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Vigorous activities such as:

Fast walking/jogging, fast cycling, court sports, fast swimming, heavy/intense weight lifting

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

14. How much alcohol do you drink? (average drinks per week) _____

15. Do you have any food allergies? Yes No If yes, explain: _____

16. The test shake contains dairy and gluten. Is this a problem? Yes No
If yes, explain: _____

APPENDIX C

AVERAGE WALKING INTENSITY DATA

Average walking intensity as a percentage of estimated maximal heart rate and rating of perceived exertion (RPE).

All Subjects (N = 10)

Heart Rate (bpm)*	113.6 ± 13.7
% HRmax*	68.6 ± 8.1
RPE	12.4 ± 2.0

Values represent mean ± SD.

*Heart rate data not available for one subject (n = 9).

APPENDIX D

CALCULATION FOR INCREMENTAL AREA UNDER THE CURVE

Incremental area under the curve (iAUC) was calculated using the trapezoidal method in accordance with the recommendations by Wolever (2004).

1. To calculate the area of a trapezoid: (start concentration + end concentration) x $\frac{1}{2}$ time (min).
2. Total iAUC: Sum of all time periods (trapezoids).
3. Baseline measures were subtracted from all subsequent readings before AUC was calculated to elicit an incremental area under the curve.
4. Only areas above the baseline value were considered, resulting in a positive iAUC, as values that fell below baseline were subtracted out