Effects of Resistance Exercise on Postprandial Metabolism in Obese Men with Prediabetes

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Effects of Resistance Exercise on Postprandial Metabolism in Obese Men with Prediabetes
by
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Prediabetes is a metabolic condition defined by elevated fasting (impaired fasting glucose (IFG)) and/or postprandial (impaired glucose tolerance (IGT)) glucose. Prediabetes affects nearly 86 million adults in the United States, with most (up to 70%) progressing to type 2 diabetes within as little as one year. Recent studies have indicated that elevated post-prandial glycemia and hypertriglyceridemia are early indicators of prediabetes, and are major risk factors for complications of prediabetes, including cardiovascular disease (CVD), stroke, elevated blood pressure, and obesity. Resistance exercise is a central component of exercise recommendations for individuals with type 2 diabetes and prediabetes, but the effect of resistance exercise on postprandial lipid and glucose metabolism are not known in this population.

In Chapter 2, we examine the effects of a single bout of resistance exercise on post-prandial glucose kinetics, insulin sensitivity, and carbohydrate oxidation after consumption of a mixed meal. Our results suggest that a single session of resistance exercise increases glucose
tolerance, reduces post-prandial insulin levels, increases insulin sensitivity and glucose clearance, and increases carbohydrate oxidation.

In Chapter 3, we examine the effects of a single bout of resistance exercise on post-prandial exogenous and endogenous lipid handling and partitioning into plasma chylomicrons, VLDL, and free fatty acids (FFA), as well as lipoprotein kinetics, FFA kinetics, and lipid oxidation. We found that a single session of resistance exercise reduces exogenous and endogenous lipid contributions to circulating lipoprotein and FFA pools, increases lipoprotein turnover, escalates FFA clearance rates, and improves lipid oxidation.

In Chapter 4, we examine the effects of a single bout of resistance exercise on skeletal muscle mitochondrial respiration and the expression of key genes known to regulate lipid and glucose metabolism. We also identify changes the expression of genes known to influence lipid and glucose metabolism in subcutaneous abdominal adipose tissue after the single session of resistance exercise. Our results suggest that a single session of resistance exercise increases skeletal muscle mitochondrial lipid oxidation, coupled respiration, and uncoupled respiration concurrently with increases in the expression of genes that increase lipid oxidation. We also found that genes involved in adipose tissue lipolysis and glyceroneogenesis were significantly up-regulated in adipose tissue after the resistance exercise session.

Overall, these results provide evidence for the beneficial effects of resistance exercise on postprandial glucose and lipid metabolism in obese men with prediabetes after consumption of a mixed meal. Improvements in postprandial lipid and glucose responses could reduce the risk for progression to type 2 diabetes, as well as the risk for complications of prediabetes.
Chapter 1: Background and Significance
1.1 What is Prediabetes?

Prediabetes is a state of elevated fasting or post-prandial glucose (impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), respectively) that develops as a result of the onset and progression of peripheral insulin resistance. Typically, insulin resistance manifests within a constellation of metabolic abnormalities including dyslipidemia, obesity, and hypertension collectively known as the “metabolic syndrome.” In 2017, the CDC estimated that 1 in 3 adults (roughly 84 million Americans) have prediabetes. Unfortunately, previous studies have also estimated that 90% of these individuals are unaware of their condition. The lack of patient awareness of prediabetes is a major health concern given that prediabetes increases one’s risk for life-threatening co-morbidities, including cardiovascular disease (CVD), chronic kidney disease, and overt type 2 diabetes. Indeed, 70% of individuals with prediabetes will go on to develop type 2 diabetes highlighting the importance of developing effective treatment strategies for this condition. The window to intervene, however, may be limited. For example, the Finnish Diabetes Prevention Study showed that 23% of participants with impaired glucose tolerance developed overt diabetes within 4 years. Likewise, the Diabetes Prevention Program Research Group has found that 11% of those with prediabetes developed type 2 diabetes in less than 3 years.

1.2 Complications of Prediabetes

While nephropathy and chronic kidney disease are commonly recognized as complications of type 2 diabetes, previous studies have found that individuals with prediabetes...
also present with elevated incidence of albuminuria and glomerular hyperfiltration (early markers of kidney disease). Microalbuminuria (a marker of microvascular injury) is found in twice as many patients with prediabetes as those with normoglycemia. Moreover, the National Health and Nutrition Examination Survey found 17.7% of subjects with prediabetes had chronic kidney disease, compared with only 10.6% of those without diabetes or prediabetes. While the exact mechanism linking prediabetes and chronic kidney disease is not known, studies suggest chronic hyperglycemia causes an increase in the absorption of glucose and sodium in the proximal tubule of the kidney, thereby reducing sodium delivery to the macula densa, which disrupts tubule-glomerular feedback mechanisms designed to regulate glomerular pressure and filtration.

Another common complication of prediabetes is neuropathy, as evidenced by increased prevalence of reduced heart rate variability, reduced postural-associatedin heart rate changes, and reduced sympathetic and parasympathetic function. There is also evidence of early sensorimotor neuropathy in individuals with IGT secondary to demyelination of small nerve fibers. Prediabetes also increases the risk for retinopathy, and facilitates structural changes in the retina, including reduced arteriole-to-venule ratio, and increased retinal arteriole or venular caliber. The Diabetes Prevention Program identified diabetic retinopathy in 7.9% of individuals with impaired glucose tolerance, suggesting retinopathy is an early complication of diabetes that begins to develop in the pre-diabetic state.

Most importantly, prediabetes increases the risk for macrovascular disease, including coronary artery disease, peripheral arterial disease, and cerebrovascular disease. The DECODE study found IGT to be associated with increased risk of coronary death and total cardiovascular death, independent of the level of FPG. Likewise, Huang et al. (2016) found that the risk for
cardiovascular disease increased by 13-30% in individuals with IFG or IGT based on the plasma glucose cutoffs used (American Diabetes Association vs. World Health Organization).\(^\text{16}\) Multiple studies have implicated the development of hyperglycemia, central obesity, high blood pressure, and elevated triglycerides and lipoproteins (particularly in the postprandial period as discussed below) as contributing factors to the elevated risk for macrovascular disease in prediabetes.\(^\text{17}\) In the United States, heart disease is the leading cause of death, while stroke and diabetes are the 5\(^\text{th}\) and 7\(^\text{th}\) leading causes of death, respectively.\(^\text{18}\) Therefore, it is imperative that individuals with prediabetes take the necessary steps to mitigate the severity of their condition and reduce their risk for these life-threatening complications.

1.3 Pathophysiology of Prediabetes

1.3.1 Progression from Normoglycemia to Prediabetes

In 2009, Tabak et al. used results from the Whitehall II Study to define identifiable changes in fasting glucose, oral glucose tolerance, insulin sensitivity, and beta cell function that precede diagnosis of type 2 diabetes. Specifically, the authors found that among individuals who did not develop diabetes over a 13-year follow-up, plasma glucose increased linearly at a rate of \(0.004\) mmol/L/year. In contrast, individuals who developed overt diabetes demonstrated a linear increase in plasma glucose of \(0.028\) mmol/L/year beginning 13 years prior to diagnosis, followed by a rapid, quadratic increase in plasma glucose 3 years before diagnosis.\(^\text{19}\) When looking at changes in 2-hour oral glucose tolerance test results, individuals developing type 2 diabetes demonstrated a linear increase in post-load glucose from 13 years to 6 years before diagnosis,
with a sudden rapid, cubic increase in plasma glucose starting 6 years before diagnosis.\textsuperscript{19} Similar results were found for changes in insulin sensitivity and beta cell function, with rapid declines identified \textasciitilde4-5 years before diagnosis.\textsuperscript{19}

The changes identified in the Whitehall II Study coincide closely with the work by Weir et al (2004), who defined five stages in the progression from normal glucose tolerance to type 2 diabetes.\textsuperscript{20} Stage one was defined as a compensatory period in which beta cells increase insulin secretion (secondary to an increase in beta cell mass) to maintain plasma glucose concentrations in the face of increasing insulin resistance.\textsuperscript{20} In Stage two the body begins to struggle to maintain plasma glucose concentrations, with plasma glucose rising to the upper end of the normal range. Early elevations in plasma glucose are caused by reductions in beta cell mass due to beta cell apoptosis and diminished glucose-stimulated insulin secretion in remaining beta cells.\textsuperscript{20} The second stage is also defined by a loss of acute insulin secretion, which further exacerbates the struggle for glucose homeostasis.\textsuperscript{20} Eventually, in Stage three, the individual loses the ability to compensate for the rising insulin resistance and beta cell dysfunction, causing plasma glucose levels to rise rapidly (as identified in the British Whitehall Study outlined above).\textsuperscript{20} Stage four also marks the onset of prediabetes, with the final two stages (four and five) characterized by further decompensation and progression to overt type 2 diabetes.\textsuperscript{20}

\subsection*{1.3.2 Insulin Resistance}

In the fasted and fed state, insulin serves as a master regulator of nutrient metabolism. Derangements of normal insulin regulation are a central component of the pathophysiology of both prediabetes and type 2 diabetes. This section includes a brief outline of the regulation of
insulin action and regulation, followed by a discussion of how insulin resistance leads to the metabolic abnormalities identified in individuals with prediabetes.

Insulin synthesis in the pancreas is controlled by multiple factors, including the uptake and metabolism of glucose, which stimulates insulin gene transcription and translation, and promotes transcript stability. Glucose enters the beta cell through the GLUT2 transporter via facilitated diffusion where it is metabolized, thereby increasing the intracellular ATP/ADP ratio and stimulating insulin secretion. In addition to glucose stimulation, insulin secretion can be directly or indirectly stimulated by specific combinations of amino acids (glutamine and leucine), fatty acids (fatty acid deficiency reduces glucose-stimulated insulin secretion), and a number of hormones (eg. estrogen, melatonin, GLP-1, leptin, growth hormone). In the post-absorptive period, pancreatic beta cells (which connect glucose uptake and metabolism with insulin secretion) reduce insulin secretion in parallel with falling glucose levels. Plasma insulin concentrations are further suppressed by the secretion of glucagon from pancreatic alpha cells, and by catecholamines (secreted from the sympathetic nervous system) that further augment glucagon release. Moreover, a transient state of insulin resistance develops in the post-absorptive state as a result of increased fatty acid oxidation (which can negatively affect signaling from the insulin receptor) in skeletal muscle, as well as the influence of hormones like growth hormone and cortisol; both which can contribute to the development of chronic insulin-resistance.

In the post-prandial period, glucose levels rise quickly. In response to the increase in plasma glucose, pancreatic beta cells increase their glucose uptake and ATP production, thereby increasing insulin secretion – a process further facilitated by declines in glucagon concentrations. Insulin secretion is also augmented by: 1) the parasympathetic nervous system
through acetylcholine release, which activates protein kinase C to enhance insulin secretion in beta cells, and 2) enteroendocrine L and K cells secretion of incretin hormones (GLP-1 and GIP, respectively) which have been shown to improve beta cell glucose sensitivity, reduce hepatic glucose production, and diminish adipose tissue lipolysis. Additionally, as plasma glucose and insulin concentrations rise, skeletal muscle glucose uptake and metabolism increase, which inhibits fatty acid oxidation and improves insulin sensitivity. 

The nature of insulin resistance in prediabetes has been shown differ between those with impaired fasting glucose (IFG, fasting glucose >100 but <125 mg/dL) and those with impaired glucose tolerance (IGT, 2-hour oral glucose tolerance test post-load plasma glucose 140-200 mg/dL). Using the oral glucose tolerance test (OGTT) model, previous studies have found that individuals with IFG demonstrate reductions in hepatic insulin sensitivity, as well as defective early insulin secretion from pancreatic beta cells (those vesicles that are already docked to the beta cell membrane and are released within 30 minutes of ingesting an oral glucose load). In contrast, the late phase of insulin secretion, as well as skeletal muscle insulin sensitivity, are normal or nearly normal, allowing plasma glucose to return to fasting levels within 2 hours during the OGTT. In contrast, individuals with IGT are characterized by skeletal muscle insulin resistance, and impaired late insulin secretion responses during the OGTT. Because fasting glucose primarily reflects hepatic glucose production (through glycogenolysis and gluconeogenesis), fasting glucose concentrations are normal, while post-prandial glucose excursions are elevated and sustained for a longer duration.

Insulin resistance has been shown to develop in conditions characterized by activation of the innate immune system. TNF alpha (α) secretion from activated macrophages in adipose tissue of obese subjects was associated with insulin resistance, which was improved with
declines in TNFα expression after weight loss. Likewise, cytokines levels are elevated in obese subjects, which have been shown to increase adipose tissue lipolysis. Increases in adipose tissue lipolysis can contributing to ectopic lipid accumulation and impaired insulin signaling. Moreover, the work by Nunez Lopez et al. (2016) suggests different cytokines may be expressed at different stages of disease, suggesting that changes in cytokine expression may contribute to the progression to prediabetes and type 2 diabetes. The development of insulin resistance has also been linked to deficits in glycogen synthesis, glucose transport, and hexokinase II activity, as well as genetic or epigenetic changes inherited from parents who are diabetic.

1.3.3 Beta Cell Dysfunction

In addition to changes in insulin sensitivity, two stages of beta cell function have been identified in prediabetes. Using the Lepr<sup>db</sup> mouse model of type 2 diabetes, Do and colleagues (2016) found that the early stages of prediabetes are characterized by increased insulin secretion secondary to increases in islet size and insulin content with concomitant reductions in alpha cell content. As the severity of prediabetes progresses, beta cells demonstrate reduced exocytic capacity – an adaptation driven by reduction in vesicle fusion, defective intracellular calcium responses (needed to stimulate exocytosis), and declines in insulin sensing capacity. The reduction in glucose sensing could be attributed to declines in Slc2a2 mRNA content, which codes for the integral GLUT2 transporter.

Multiple molecular mechanisms have implicated in the progression of beta cell dysfunction. Exposure to chronic hyperglycemia, often occurring as a result of a poor diet, induces oxidative stress and inflammation in beta cells. Activation of inflammatory pathways
involving a number of different cytokine responses can damage mitochondria and other vital organelles, induce protein, lipid, and nucleic acid damage, and promote beta cell apoptosis. Specifically, reactive oxygen species facilitate uncoupling of glucose sensing and insulin secretion that occurs in prediabetes (exacerbated by the low intrinsic expression of antioxidant enzymes in beta cells). Moreover, prediabetes often develops in those with obesity – a condition defined by the secretion of damaging adipokines (such as resistin) that: 1) interfere with insulin signaling and thereby inducing beta cell stress, and 2) reduce beta cell viability and enhances cell death. Previous studies have also provided evidence to support the role of pancreatic adiposity in genetic reprogramming that further exacerbates beta cell dysfunction. Moreover, beta cell necrosis secondary to macrophage infiltration and impaired autophagy (a process that clears the beta cell of defective organelles, including mitochondria) contributes to a loss of beta cell mass in prediabetes.

1.3.4 Impaired Incretin Responses

Incretin hormones are secreted from enteroendocrine L and K cells in response to the entry of glucose, amino acids, and fatty acids into the small intestine after a meal. The major incretins are Glucagon-Like Peptide-1 (GLP-1, secreted from K cells of the small intestine) and Glucose-dependent Insulinotropic Peptide (GIP, secreted from L cells of the small intestine) – both known to reduce plasma glucose concentrations by increasing insulin secretion and reducing gastrointestinal motility. Evidence for the role of incretins in glucose homeostasis was demonstrated by comparing the amount of glucose needed to be given orally vs. intravenously to produce the same plasma glucose concentration. Nauck et al. demonstrated that to achieve
similar plasma glucose concentrations, 25 grams of glucose needed to be administered orally vs. only 19 grams of glucose intravenously, indicating activation of a orally-induced glucose disposal mechanism that increases glucose uptake by ~24%. This mechanism, now identified as the incretin effect, is attributed to the secretion of incretin hormones immediately before, and during, the meal.

Given the role of incretin hormones in glucose regulation, it is not surprising that deficits in incretin secretion have been found in individuals with insulin resistance. Rask et al. (2001) found that in 35 nondiabetic men, the degree of insulin resistance was directly related to the area under the curve of postprandial GLP-1 and GIP concentrations. Likewise, in the Danish ADDITION-PRO study of 755 subjects, individuals with prediabetes demonstrated lower GLP-1 responses (up to 121% lower) to an OGTT than individuals with normal glucose tolerance, even after controlling for age and obesity. This study also identified that obesity alone was associated with further reductions in GLP-1 responses (by 20% compared to normal weight individuals), providing another potential mechanism contributing to reduced incretin responses in this condition.

The rate of gastric emptying is a tightly regulated process designed to control gastrointestinal motility to generate a relatively constant rate of nutrient passage from the stomach to the small intestine. Previous studies have demonstrated that the rate of gastrointestinal motility is regulated by plasma glucose concentrations through stimulation of incretin secretion. For example, food passage from the stomach to the small intestine stimulates a constant incretin response that lasts until the stomach is completely emptied. Moreover, a truncated form of GLP-1 has been found to inhibit the rate of gastric emptying, indicating a feedback loop between gastric contents, plasma glucose concentrations, and gastric motility.
through central inhibition of parasympathetic outflow. This feedback loop is an essential mechanism of controlling blood sugar because the rate of gastric emptying affects the duration of the small intestine’s exposure to the contents of the meal. The slowing influence of GLP-1 serves to reduce blood glucose concentrations, with some studies suggesting the glucose-lowering influence of GLP-1 may be attributed more so to its influences on gastric emptying than its influence on beta cell insulin secretion. Moreover, Boronikolos et al. (2015) found that the rate of gastric emptying increased with severity (Hba1c) and duration of diabetes, suggesting a direct role in the rate of nutrient absorption in the pathophysiology of glucose dysregulation.

1.3.5 Lipotoxicity

Multiple studies have implicated the development of lipotoxicity in the pathophysiology of prediabetes and its progression to overt diabetes. It is important to acknowledge that prediabetes often occurs in individuals who are obese. In obesity, adipocytes become enlarged to accommodate the increased demand for triglyceride deposition. Initially, adipocytes can accommodate these increased levels of triglycerides (secondary to an increased activity of triglyceride synthesis pathways) while maintaining a normal level of lipolysis. Over time, however, the ability of the adipocyte to continue to accommodate increased triglycerides slows and their signaling functions becomes altered. Hypertrophied adipocytes secrete chemoattractants that enhance macrophage infiltration of the adipocyte, which has been shown to increase lipolysis and reduce triglyceride synthesis. The ensuing inflammatory response negatively affects adipocyte function, and is commonly found in individuals with obesity.
These adipocyte adaptations lead to increased levels of circulating plasma free fatty acids and triglycerides, which can be taken up and accumulate in non-adipose tissues including skeletal muscle and the liver after hydrolysis by endothelial lipoprotein lipase. Several studies suggest that the accumulation of fatty acids in skeletal muscle is caused by an imbalance between free fatty acid delivery/availability, free fatty acid uptake, and elimination (re-esterification to intramyocellular triglycerides or oxidation). The “mitochondrial lipid overflow” hypothesis posits that excess delivery of fatty acids to mitochondria leads to incomplete beta oxidation and the accumulation of lipid intermediates, including acetyl-coA (produced by the repetitive splitting of 2 carbon subunits from the larger fatty acyl-coA in the mitochondria), NADH, diacylglycerols, cytosolic citrate, and long-chain acyl carnitine CoA: all which negatively regulate insulin signaling and glycolytic enzyme activity through Randle Cycle inhibition. The inability to completely oxidize fatty acids directed toward mitochondria also promotes mitochondrial stress and depletion of organic acid intermediates of the TCA cycle.

In addition to excess skeletal muscle and hepatic delivery of fatty acids, studies have demonstrated that processing of intramyocellular triglycerides (IMTG) may be an important determinant of insulin sensitivity. Perreault et al. (2010) found that individuals with prediabetes had a trend toward reduced IMTG synthesis rates at rest, and failed to suppress IMTG synthesis during exercise – suggesting inflexibility of the IMTG pool during progression to type 2 diabetes. In support of this hypothesis, Sparks et al. (2014) found reduced free fatty acid incorporation into IMTG in skeletal muscle and primary myoblasts in individuals with diabetes compared to BMI-matched controls. Further evidence for the importance of improving IMTG synthesis to slow the progression of insulin resistance comes from Liu et al (2007). These authors overexpressed diglyceride acyltransferase 1 (DGAT1, catalyzes the formation of
triglyceride from diglyceride) in differentiated C2C12 cells, causing a 6-fold increase in IMTG and a 50\% reduction in lipotoxic diacylglycerols.\textsuperscript{53} And in a separate study, mice overexpressing DGAT1 in skeletal muscle were able to store 12\% more IMTG and prevent diet-induced insulin resistance.\textsuperscript{53}

As identified by Schenk and Horowitz (2007), IMTG serve as a reservoir for fatty acids that is protective from the development of insulin resistance.\textsuperscript{54} Therefore, a loss of flexibility to store fatty acids as IMTG may be a catalyst for increasing insulin resistance in prediabetes. However, the derangement in IMTG handling may be different in individuals with IGT vs. IFG. Goossens et al. (2016) found that individuals with impaired glucose tolerance had reduced fractional synthetic rates of IMTG, with increased saturation of the free fatty acid pool and retention of saturated fatty acids than those with impaired fasting glucose.\textsuperscript{55} Individuals with prediabetes may not maintain the oxidative capacity to match the elevated rate of free fatty acid delivery to the mitochondria, as suggested by the work of Fabri et al. (2017).\textsuperscript{56} In this study, the authors assessed 248 individuals from the Baltimore Longitudinal Study of Aging and found that a longer duration of prediabetes and more severe hyperglycemia were associated with longer phosphocreatine recovery (a measure of mitochondrial oxidative metabolism capacity, longer denotes worse capacity) using 31P MRS.\textsuperscript{56} Moreover, Straczkowski et al. have demonstrated that ceramide levels are elevated in skeletal muscle of individuals with IGT and offspring of diabetic parents.\textsuperscript{57} Ceramide is a sphingolipid widely distributed in the cell membrane that can interfere with insulin signaling and has been implicated in the development of insulin resistance.\textsuperscript{57} Taken together, these results suggest that a failure to maintain balance between free fatty acid entry into skeletal muscle, and its removal through oxidation or IMTG synthesis, leading to the accumulation of lipotoxic intermediates that exacerbate insulin resistance. Worse still, pre-
diabetic muscle may have a reduced capacity to protect itself from lipotoxic damage, as evidenced by reduced levels of uncoupling protein 3 – a protein known to protect the mitochondrial matrix from lipid-induced damage.\textsuperscript{58} Specific deficits in oxidative capacity will be discussed below.\textsuperscript{58}

1.3.6 Mitochondrial Abnormalities

As indicated above, the development of lipoxicity may be related to deficits in skeletal muscle oxidative capacity. Because mitochondria are responsible for oxidative metabolism, several studies have probed for abnormalities in mitochondrial function in skeletal muscle from individuals with insulin resistance. In 2005, Sparks et al. found that a high fat diet reduced the expression of genes related to oxidative phosphorylation, proteins composing mitochondrial complex I, proteins in mitochondrial complex II, mitochondrial carrier protein SLC25A12, PGC1alpha, and PGC1beta in skeletal muscle.\textsuperscript{59} These changes could be driven by epigenetic mechanisms leading to repression of key mitochondrial proteins. For example, Zheng et al. found that DNA methylation of NADH Dehydrogenase subunit 6 (suppressing transcription of the gene coding for this subunit) increased with increasing severity of BMI, as well as insulin sensitivity (HOMA-IR) in early stage prediabetes.\textsuperscript{60}

In addition to changes in protein content and gene expression, several studies have implicated reactive oxygen species in the pathophysiology of prediabetes and overt diabetes. Hayder Al-Aubaidy et al. found that serum 8-OHdG, a marker of DNA damage secondary to oxidation of deoxyguanosine, was markedly elevated in both prediabetes and type 2 diabetes.\textsuperscript{61} Moreover, Bhansali et al. (2017) found that individuals with prediabetes and type 2 diabetes
presented with elevated reactive oxygen species in peripheral blood mononuclear cells concordant with their level of glucose dysregulation (prediabetes < newly diagnosed type 2 diabetes < advanced type 2 diabetes). Moreover, the authors found that mRNA expression for mitophagy-related genes were elevated in prediabetes, but down-regulated in type 2 diabetes – suggesting a potential compensation that delays the onset of type 2 diabetes by eliminating mitochondria damaged by oxidative stress. The impairment of mitophagy in more advanced disease may lead to the accumulation of dysfunctional mitochondria and worsening insulin sensitivity. Additionally, in obese Zucker rats (a model frequently used to study prediabetes), the administration of antioxidants R-Alpha Lipoic Acid and pyridoxamine reduced the accumulation of advanced glycation end products, reduced muscle protein carbonyls and urine conjugated dienes (markers of oxidative damage), and improved both fasting glucose and insulin sensitivity.

1.4 Postprandial Lipid Metabolism in Prediabetes

1.4.1 Physiology of Post-prandial Lipid Metabolism

Based on the literature presented above, it is clear that derangements in lipid metabolism contribute to the onset and progression of insulin resistance and diabetes. While lipid metabolism is important during the fasted state, recent studies have suggested that postprandial lipid responses may contribute more strongly to prediabetes and its associated comorbidities. Postprandial changes in plasma triglycerides reflect a complex interaction between multiple metabolic and hormonal influences. Plasma triglycerides are carried in lipoprotein particles
called triglyceride-rich lipoproteins (TRLs).\textsuperscript{64} TRL particles consist of chylomicrons (produced by enterocytes in the small intestine), very low density lipoproteins (VLDL, produced in the liver), and their remnant particles: low density, intermediate density, and high density lipoproteins.\textsuperscript{64}

After ingestion, meal triglycerides are emulsified by bile salts to formed mixed micelles, which are subsequently hydrolyzed by enzymes in brush border membrane of the small intestine to release free fatty acids and monoacylglycerol for uptake by enterocytes.\textsuperscript{65} In the enterocyte, free fatty acids and monocacylglycerol are re-esterified to triglycerides and packaged with cholesterol, fat soluble vitamins, and intracellular lipid droplets (formed from excess triglycerides absorbed during previous meals) into chylomicron particles in the Golgi apparatus.\textsuperscript{65} The chylomicrons contain the structural protein Apolipoprotein B\textsubscript{48} and AI (acting as a stabilizing agents and receptor ligands), an inner core of triglycerides and cholesterol ester, and an outer core of phospholipid and free cholesterol.\textsuperscript{66} While enterocytes continuously releases chylomicrons throughout the day, chylomicron concentrations rise sharply after the meal, as does their size (driven by the fusion of meal triglycerides with triglycerides left over from previous meals).\textsuperscript{64} These chylomicron lipoproteins are secreted into the lymphatic system, ultimately entering the plasma compartment through the thoracic duct. Once in the circulation, chylomicron ApoA-I dissolves and is replaced by Apo-Cs, which facilitates the unloading of packaged triglycerides after hydrolysis by the enzyme lipoprotein lipase (LPL) in tissue endothelium.\textsuperscript{64}

In contrast to chylomicrons, VLDL particles are formed through the sequential lipidation of the apoB-100 protein in the liver – a process initiated by the microsomal triglyceride transfer protein in the rough endoplasmic reticulum.\textsuperscript{64} The size and maturation of the VLDL particle is highly dependent on the accumulation of cytosolic triglyceride in the hepatocyte (a feature
pointing to the importance of fatty acid delivery to the liver from adipose tissue, and from chylomicron remnants incompletely hydrolyzed after the meal). After secretion from the liver, VLDL, like chylomicrons, are hydrolyzed by lipoprotein lipase, forming intermediate density lipoproteins (IDL). IDL can be further hydrolyzed in the circulation to low density lipoprotein (LDL), which is primarily removed from the circulation in the liver (through LDL receptor) and peripheral tissues where cholesterol is needed for biosynthetic purposes.

It is important to note that both chylomicrons and VLDL compete for the hydrolysis reactions driven by lipoprotein lipase. Previous studies have shown that endogenous TRLs (VLDL) accumulate in the plasma after fat ingestion due to a delay in their lipolysis secondary to competition for LPL with chylomicrons. This delay has been attributed to LPL’s preference for larger lipoproteins, and for a reduced efficiency of hydrolyzing VLDL triglycerides – both of which lead to a preference for chylomicrons. The competition for LPL is so strong, that some authors suggest postprandial triglyceride concentrations are driven primarily by VLDL secretion responses.

The metabolism of post-prandial lipoprotein triglycerides, like glucose, are closely regulated by hormones like insulin and incretins. In the liver, insulin suppresses VLDL concentrations by: 1) promoting apoB degradation to promote triglyceride storage; 2) inhibition of adipose tissue lipolysis, thereby reducing the availability of plasma free fatty acids from as a substrate for triglyceride synthesis (known as de novo lipogenesis which also contributes to elevations in chylomicron production in insulin resistance); 3) activation of lipoprotein lipase for triglyceride hydrolysis; and 4) suppression of apo CIII secretion (an inhibitor of lipoprotein lipase). The incretin GLP-1 has been found to reduce chylomicron production and post-prandial triglycerides. While the exact mechanism of this reduction is not completely understood, the
beneficial effects of GLP-1 on postprandial triglycerides could be related to the inhibition of gastric emptying, which may reduce the efficiency of dietary fat absorption or processing.\textsuperscript{71} GLP-1 may also improve lipid metabolism by increasing insulin secretion, reducing lymph output (reducing chylomicron secretion into the plasma), reducing circulating plasma free fatty acids, reducing apoB48 production, and/or reducing the triglyceride concentration of individual lipoprotein particles.\textsuperscript{72}

### 1.4.2 Derangements of Post-prandial Lipid Metabolism in Prediabetes

One of the hallmark features of prediabetes is the development of elevated post-prandial lipemia, which is defined as an exaggerated rise in plasma triglycerides and TRLs after consuming a mixed meal.\textsuperscript{73} A number of metabolic abnormalities in post-prandial lipid metabolism have been identified in prediabetes that may contribute to elevated lipemia, including increased TRL-TG production rates, reduced expression of lipid oxidative genes, and reduced intramyocellular partitioning of non-esterified fatty acids (NEFA) toward re-esterification (see “Lipotoxicity,” above) – an important intracellular mechanism of clearing free fatty acids liberated from triglyceride hydrolysis\textsuperscript{74,75} This is evidenced by Shojaee-Moradie et al.(2013) who reported elevated triglyceride levels, larger VLDL and chylomicrons pool sizes, and lipoprotein production rates in individuals with prediabetes compared to lean controls.\textsuperscript{76} Importantly, clearance rates were not different between groups, indicating an imbalance between lipoprotein production and clearance promotes elevated lipemia in the postprandial state in prediabetes.\textsuperscript{76}
Previous studies have also reported that insulin resistance is associated with elevations in ApoB-48 particles (chylomicrons), which may be exacerbated by abdominal obesity.\(^\text{77}\) Compared to nondiabetic women, women with diabetes demonstrated elevated Apo-B 48 particles and reduced fractional catabolic rate of chylomicron remnants.\(^\text{78}\) Furthermore, Duez et al. (2006) demonstrated that ApoB-48 particle production rates were significantly higher in hyperinsulinemic, insulin resistant men in the absence of a difference in clearance rates -- again suggesting a mismatch between lipoprotein production and clearance favoring lipoprotein accumulation.\(^\text{79}\)

Like chylomicrons, impairments in VLDL metabolism have also been identified during the post-prandial period in obesity, prediabetes, and type 2 diabetes. In 2002, Pont et al. reported that insulin resistant, abdominally obese women have elevated ApoB-100 particle production rates (the apolipoprotein in VLDL particles), as well as IDL and LDL production rates without changes in their clearance rates compared to healthy controls.\(^\text{80}\) This study also demonstrated increased hepatic production of large VLDL particles using \(^3\)H glycerol and VLDL exogenously labeled with iodine.\(^\text{80}\) Using a dual tracer approach, Adiels et al. (2006) found that liver fat content and plasma glucose predicted VLDL production rate, which was elevated in diabetic men.\(^\text{81}\) The presence of abdominal obesity alone may disturb lipid metabolism, as Chan et al. (2002) found that individuals with abdominal obesity presented with reduced uptake of VLDL, LDL, and IDL, which may further contribute to the accumulation and prolonged residence time of these lipoprotein particles.\(^\text{82}\)

These imbalances in post-prandial lipid metabolism are important because it places individuals with prediabetes at a greater risk for cardiovascular disease. Several large cohort studies have found that postprandial dyslipidemia is associated with an increased risk for
myocardial infarction, ischemic heart disease, death, and ischemic stroke in both men and women. The Women’s Health Study, which followed nearly 27,000 women for more than 11 years, demonstrated that higher non-fasting triglyceride levels were strongly associated with an increased risk of future cardiovascular events, independent of baseline cardiac risk factors. In contrast, fasting triglyceride levels showed little independent association with cardiovascular events, leading the authors to hypothesize that cardiovascular disease may be a post-prandial phenomenon.

1.5 Effects of Exercise on Substrate Metabolism in Prediabetes

1.5.1 General Benefits of Exercise on Disease Progression

Despite the myriad of metabolic abnormalities that contribute to the pathophysiology of prediabetes, individuals can have a meaningful impact on their skeletal muscle health, and whole-body function, through regular exercise. Fortunately, multiple studies have suggested that prediabetes is a reversible with lifestyle interventions utilizing exercise regimens. The Da Qing IGT and Diabetes Study evaluated 577 subjects with IGT who were randomized to a control group or to one of three active treatment groups (diet only, exercise only, or diet plus exercise). After a 6-year follow-up, the study found that the diet, exercise, and diet-plus-exercise interventions were associated with a 31%, 46%, and 42% reduction in risk of developing diabetes, respectively. Likewise, the Finnish Diabetes Prevention Study (DPS) showed that the combination of diet and exercise in individuals with IGT (n=522) reduced the risk of diabetes by
58% (with a cumulative incidence of type 2 diabetes of 11% in 4 years vs. 23% in the control group). Moreover, the Diabetes Prevention Program (DPP) Research Group demonstrated that after randomizing 3,234 subjects with IFG or IGT into groups receiving placebo, metformin, or a lifestyle intervention, the lifestyle intervention group had the lowest incidence of diabetes (4.8 per 100 person-years) compared to the other groups. Taken together, these results support for the potential for exercise interventions to reduce the risk for disease progression, as well their use for primary prevention of type 2 diabetes.

### 1.5.2 General Benefits of Aerobic Exercise

Aerobic exercise is traditionally utilized in lifestyle interventions for individuals with obesity and metabolic conditions as a means to increase skeletal muscle fatty acid oxidation and oxidative capacity. The term “aerobic” is used to describe repetitive, low resistance, submaximal exercise lasting several minutes to hours at various intensities. Aerobic exercise has been shown to increase muscle mitochondrial content and respiration, fatty acid oxidative capacity, resting energy level, oxidative enzyme activity, and insulin sensitivity. Increases in mitochondrial content are achieved through mitochondrial biogenesis – a process relying on coordination between transcription factors expressed by the mitochondria and the nucleus. Contractile activity has a direct effect on the expression of nuclear-encoded transcription factors, which are expressed to elicit specific phenotypic changes in skeletal muscle (e.g., enhanced oxidative capacity, hypertrophy, improved metabolic flexibility). For example, Tfam expression (a transcription factor encoded by the nucleus that translocates to the mitochondria to regulate the transcription of ETC chain complex IV) is up-regulated following muscle contraction.
Likewise, reductions in the expression of mitochondrial transcription factors correlated negatively with aerobic exercise capacity, suggesting that exercise can have positive effects on mitochondrial gene transcription.\(^9^0\)

Further adaptations in mitochondrial content are driven by mitochondrial fusion and fission reactions in skeletal muscle. The fusion of dysfunctional and healthy mitochondria facilitates the sharing of proteins, lipids, and RNA, and the elimination of DNA mutations through cross-complementation.\(^9^1\) Likewise, mitochondrial fission involves the replication of mitochondrial DNA and subsequent mitochondrial division. In 2005, Cartoni et al. reported increases in muscle mitofusin 1 and 2 (involved in fusion) expression measured 24 hours after cycling exercise.\(^9^2\) Furthermore, Garnier et al., (2005) demonstrated mRNA levels of Drp1 (involved in fission) increased proportionately with the VO2max of 18 individuals.\(^9^3\)

One of the most highly investigated regulatory proteins associated with aerobic exercise is PGC-1alpha (peroxisome proliferator-activated receptor gamma coactivator 1-alpha). PGC-1alpha is a transcriptional co-activator involved in a number of metabolic functions through its regulation of transcription factor activation (including estrogen-related receptor alpha, thyroid receptor, nuclear respiratory factor 1, NRF2, myocyte enhancer factor 2) and mitochondrial-nuclear cross-talk.\(^9^4\) PGC-1alpha expression is associated with enhanced mitochondrial biogenesis, angiogenesis, phenotypic switching to a more oxidative muscle phenotype (ie. increasing type 1 muscle fiber content), enhancement of GLUT4 translocation, increased fatty acid oxidation, reduced accumulation of lipid metabolites (cerimide, diacylglycerol, long chain fatty-acyl-coAs), and increased metabolic flexibility (or the ability of skeletal muscle to switch between substrates based on their abundance).\(^9^5\) Aerobic exercise has been associated with increases in multiple regulators of PGC-1alpha expression, including Ca\(^{2+}\)/calmodulin-dependent
serine/threonine protein phosphatase calcineurin (CnA), Ca\textsuperscript{2+}/calmodulin-dependent protein kinases (CaMKs), AMPK, nitric oxide synthase, and hydrogen peroxide.\textsuperscript{96-98} Finally, aerobic exercise is associated with the nuclear export, and inactivation of class IIa histone deacetylase (HDAC)\textsubscript{4} and HDAC\textsubscript{5}, which normally inhibits MEF2 and prevent PGC-1alpha transcription in skeletal muscle.\textsuperscript{99}

Aerobic exercise has also been shown to reduce body fat mass, increase insulin sensitivity, increase glucose tolerance, and augment glucose uptake via stimulation of GLUT4 transcription as well as translocation of intracellular GLUT4 to the sarcolemma in healthy and prediabetic populations.\textsuperscript{100-102} Moreover, a recent meta analysis found that aerobic exercise reduces HbA1c, fasting plasma glucose, LDL, plasma triglycerides, BMI, and systolic blood pressure while improving VO\textsubscript{2}peak in individuals with type 2 diabetes. (Yang, 2014).\textsuperscript{103} Taken together, these findings highlight the ability of aerobic exercise to target the metabolic impairments characteristic of the prediabetic population.

1.5.3 General Benefits of Resistance Exercise

In contrast to aerobic exercise, resistance training is characterized by the use of short bouts of very intense muscle contraction, which relies primarily on the use of phosphocreatine and glycolytic ATP production. Chronic resistance training is associated with muscle hypertrophy (especially in type II fibers), increased motor unit activation, stimulation of muscle protein synthesis, reductions in the rate of protein degradation, increased basal metabolic rate, and improved insulin sensitivity and glucose tolerance.\textsuperscript{104}
Additionally, several studies have presented strong evidence for the ability of resistance exercise to improve lipid metabolism, particularly in the hours after the exercise session. Hunter et al. (2004) found that 26 weeks of resistance training increased strength, lean mass, resting energy expenditure by 6.8%, and reduced the resting respiratory exchange ratio (RER) from 0.86 to 0.83 – indicative of a higher contribution of fatty acid oxidation to energy production.\textsuperscript{105} Similar results have been reported by Kirk et al. (2009) after 6 months of resistance training performed 3 times per week in sedentary young adults, and Van Etten et al. (1995) after 12 weeks of resistance training in young males.\textsuperscript{106,107} Gillette et al. (1994) demonstrated that resistance exercise caused a large decline in post-exercise RER during the first hour of recovery in males aged 25-35.\textsuperscript{108} Osterberg & Melby (2001) reported that a single bout of resistance training (100 minutes) increased resting fat oxidation rate, indicating that strenuous resistance exercise is capable of producing modest and prolonged elevations in fatty acid oxidation in young women.\textsuperscript{109} Jubrais et al. (2001) found that resistance training resulted in a 57% increase in oxidative capacity, as well as a 31% increase in mitochondrial volume density and muscle size in older adults.\textsuperscript{110} Magkos et al. (2008) found that a single session of resistance training significantly increased fatty acid oxidation the morning after exercise in untrained young men.\textsuperscript{111} Shepherd et al. (2014) reported increases in peak \(O_2\) uptake, cytochrome c oxidase protein content, and intramuscular triglyceride breakdown after 6 weeks of resistance training, which were associated with improved insulin sensitivity in sedentary young men.\textsuperscript{112} Finally, Meex et al. (2010) found that a combined program of aerobic and resistance exercise significantly increased fatty acid oxidation after 12 weeks of training in individuals with type 2 diabetes.\textsuperscript{113} These studies provide strong evidence for the capacity for resistance exercise to improve lipid oxidation and mitochondrial function in healthy subjects and in those with type 2 diabetes.
In addition to resting measurements of fatty acid oxidation, multiple studies have shown that resistance training promotes significant increases in excess post-exercise oxygen consumption (EPOC) – a prolonged increase in energy expenditure after exercise needed to restore myocyte homeostasis (eg. the removal of lactate). EPOC mainly involves oxidative metabolism, with multiple studies reporting lower respiratory quotients during recovery from exercise than at rest.\textsuperscript{114} For example, Binzen et al. reported elevated fatty acid oxidation following a single bout of resistance exercise, and an 18.6\% increase in oxygen consumption above rest.\textsuperscript{114} Likewise, Osterberg & Melby (2001) reported elevated EPOC after a single bout of resistance training, with a lower RER during EPOC than rest.\textsuperscript{109} And Farinatti et al (2016) also found lower RERs and higher fatty acid oxidation during EPOC following a single session of resistance exercise when compared to resting RER.\textsuperscript{115}

The meta analysis conducted by Yang et al. (2014) also showed that resistance exercise effectively reduces HBA1c, fasting blood glucose, insulin resistance, LDL, total triglycerides, body weight, body fat, and systolic blood pressure.\textsuperscript{103} Moreover, Yardley et al. (2013) found that resistance exercise produced greater reductions in plasma glucose than aerobic exercise during a one-hour recovery period in individuals with type 1 diabetes, suggesting resistance exercise may have a stronger impact on blood sugar control than aerobic exercise.\textsuperscript{116} Taken together, these studies highlight the potential for resistance exercise to improve both lipid and glucose metabolism, and the major metabolic derangements in prediabetes.

1.5.3.1 Examples of Resistance Exercise Prescriptions Studied

It is important to note that the studies identified above primarily utilize traditional
resistance training prescriptions. They did not require special modifications to make them more aerobic in nature, highlighting the intrinsic ability of resistance exercise to improve lipid metabolism. For example, the study by Magkos et al. utilized 80% maximum torque production for 3 sets of 10 on 12 exercises on a biodex isokinetic dynamometer.\textsuperscript{111} Shepherd et al., utilized 3 sets of 12 reps at 80% 1RM; Gillette et al. (1994) utilized five sets of 8-12 reps at 70% 1RM on 10 different exercises; Osterberg et al. (2000) used five sets of 10 exercises each performed for 10-15 reps at 70% 1RM; and Kirk et al. (2009) used one set of 9 exercises at 85-90% 1RM.\textsuperscript{106-112}

\section*{1.5.4 General Benefits of Concurrent Training}

Currently, exercise programs combining aerobic and resistance exercise are recommended for the management of metabolic conditions over either mode alone. The clinical trial by Siagl et al. (2008) found that the combination of aerobic and resistance training produced significantly greater reductions in HbA1c than aerobic or resistance training in isolation after 22 weeks in individuals with type 2 diabetes.\textsuperscript{117} Similarly, Yavari et al. (2012) found that combined training for 52 weeks promoted additional benefit in reducing HbA1c, plasma triglycerides, body fat percentage, and muscular percentage when compared to aerobic or resistance training alone in diabetes.\textsuperscript{118} In 2009, Marcus et al. demonstrated that a program combining aerobic and eccentric resistance exercise produced greater gains in thigh lean mass and reductions in BMI than aerobic exercise alone after 16 weeks.\textsuperscript{119} However, the use of concurrent training over either aerobic or resistance training alone may depend on the goals of the exercise regimen. For example, Kraemer et al. found that performing concurrent running and strength training produced less muscle hypertrophy when compared to strength training alone.\textsuperscript{120} Furthermore, Monteiro et al.
(2015), found equivalent reductions in body fat after 20 weeks of either aerobic exercise or combined aerobic and resistance exercise. Further research is needed to determine when, and how best, to combine aerobic and resistance exercise to maximize metabolic benefit.

1.5.5 Optimal Lifestyle Intervention Study

We recently completed a study assessing the effects of a 32-week lifestyle intervention combining diet and structured exercise to reduce the severity of type 2 diabetes. Fifteen (n=15) individuals with obesity and prediabetes were randomly assigned to the lifestyle intervention (ILT, n=8) or standard of care (SC, n=7). The ILT group received physical therapist supervised structured exercise sessions (1-hour) four times per week. The exercise consisted of 1 hour of interval aerobic exercise at 80-100% HR_{max} twice per week, 1 hour of resistance exercise (80-90% 1RM, 2 sets, 6 exercises, momentary muscular failure) once per week, and functional/free-weight training once per week in addition to 1-2 hours of unsupervised physical activity (walking, core stability, body weight exercises) on the weekend. Participants in the ILT group were instructed to maintain a high-protein, low-carbohydrate diet throughout the study. In contrast, participants in the SC received a one time information session discussing lifestyle modifications for individuals with type 2 diabetes, and informational materials that encouraged them to increase their physical activity and that contained information regarding current ADA dietary recommendations. Participants in the SC had follow-up visits once per month over the course of 32 weeks to track progress.
Fig. 1 Lipid handling after 32 weeks of ILT vs SC. A. Participants had a significant reduction in plasma triglycerides after ILT but not after SC. B. Participants tended to reduce plasma free fatty acids after ILT, but it was not significant (p>.05). C. ILT reduced resting respiratory quotient (RQ), whereas there was no change after SC. † significant interaction group x time. * Significant difference pre-post. § Significant effect of time.

In response to regular exercise and diet, individuals in the ILT demonstrated significantly greater improvements in lipid metabolism including reductions in total plasma triglycerides and resting respiratory quotient (indicating a greater contribution from lipid oxidation to resting energy expenditure).
Likewise, individuals in the ILT presented with significantly improved glucose metabolism (reduced areas under the glucose and insulin concentration curves on a modified 5-hour OGTT) compared to SC. These reductions were mediated by significant improvements in hepatic insulin sensitivity and insulin-stimulated glucose disposal (determined using the hyperinsulinemic-euglycemic clamp technique). These results suggest that exercise can improve glucose and lipid metabolism in individuals with type 2 diabetes. However, all measurements were taken in the fasted state. The next section will discuss current evidence for the benefits of exercise on post-prandial metabolism.

1.5.6 Effects of Aerobic Exercise on Post-Prandial Lipid and Glucose
Metabolism

In individuals with prediabetes -- a population with marked derangements in lipid metabolism -- a single bout of aerobic exercise has been shown to attenuate post-prandial lipemia and improve insulin sensitivity.\textsuperscript{122} For example Ho et al. (2012) utilized a cross-over design to investigate the effects of a 30 minute bout of aerobic or resistance exercise on post-prandial lipemia (participants exercised in the evening prior to a breakfast test meal).\textsuperscript{123} The authors found that aerobic exercise reduced postprandial plasma triglycerides by 8%, while resistance exercise reduced postprandial insulin levels by 30%.\textsuperscript{123} While few studies have directly compared aerobic vs. resistance exercise on post-prandial substrate responses, significant insight can be gained from studies assessing each mode of exercise individually.

1.5.7 Influence of Aerobic Exercise Prescription on Post-prandial Glucose and Lipid Metabolism

Previous studies have demonstrated that improvements in post-prandial substrate metabolism in response to aerobic exercise are influenced by a number of factors, including age, gender, exercise intensity, exercise duration, and the timing of exercise. Cox-York et al. (2012) found that 60 minutes of aerobic exercise did not effectively alter postprandial triglyceride responses to a series of moderate fat meals given throughout the day after a morning exercise bout in men or women with and without metabolic syndrome.\textsuperscript{124} However, women had a lower postprandial triglyceride response than men, with the greatest difference between men and women with metabolic syndrome.\textsuperscript{124} These results suggest that women and men metabolize
meal-derived triglycerides differently and could be an important variable to control for in future exercise studies. With regard to exercise intensity, Rynders and colleagues (2014) compared the effects of moderate vs. high intensity aerobic exercise (one hour) followed by an oral glucose tolerance test in prediabetic men and women. Both intensities resulted in improvements in insulin sensitivity, but only the high intensity condition resulted in significant reductions in glucose, insulin, and c-peptide areas under the curve -- indicating postprandial responses may be larger after higher intensity exercise. To determine the optimal duration of exercise needed to reduce hypertriglyceridemia in men with prediabetes, Zhang et al. (2007), compared the effects of 30, 45, and 60 minutes of aerobic exercise at 60% of their VO2peak. The authors demonstrated that while 30 minutes of exercise was sufficient to promote improvements in insulin sensitivity, 45 minutes were required to see reductions in postprandial triglyceride concentrations. Evidence also suggests that exercise performed prior to eating a meal may be more effective than exercising in the postprandial period. Derave et al. (2007) had participants with prediabetes perform aerobic exercise for 45 minutes at 60% of their VO2 max before eating, or after eating, breakfast. The authors found that exercising prior to eating breakfast resulted in a higher fat oxidation rate (determined with indirect calorimetry) than exercising after breakfast. Similarly, the glycemic response was improved more when exercising before compared with when exercising after breakfast. Zhang et al. (2004) reported similar results, adding that the acute benefits of aerobic exercise on glucose metabolism may be short lived (lasting ~12 hours) and dissipate by 24 hours.

1.5.7 Effects of Resistance Exercise on Post-Prandial Lipid and Glucose
Metabolism

Like aerobic exercise, evidence supports the ability of resistance exercise to improve post-prandial lipid metabolism. In 2007, Burns et al. found that repeated resistance exercise bouts at relatively low intensity (5 bouts, 5 exercises, 30-40% 1RM) reduced postprandial triglycerides by 12% in healthy subjects.\(^{128}\) Similarly, using a stable isotope tracer approach, Magkos et al. (2008) found that high intensity resistance exercise (3 sets of 10 repetitions for 12 exercises at 80% of peak torque production) reduced VLDL triglyceride concentrations, increased VLDL plasma clearance rates, and shortened the VLDL mean residence time compared to rest in healthy men.\(^{111}\) Moreover, these reductions were greater after the resistance exercise session than an equivalent bout of aerobic exercise.\(^{111}\) These results are further supported by the work of Davitt et al. (2013) – one of the few studies to evaluate the effects of resistance exercise outside of a healthy population.\(^{129}\) Davitt et al. found that resistance exercise reduced total and endogenously-derived triglyceride (triglycerides not coming from the meal) content, and increased total lipid oxidation rate in obese women.\(^{129}\) However, the authors also found that exogenously-derived triglyceride concentrations increased after both aerobic and resistance exercise, and that the reductions in postprandial lipemia are driven primarily through reduced concentrations of endogenous triglycerides rather than through enhanced clearance.\(^{129}\) Multiple studies have confirmed that resistance exercise can improve post-prandial triglycerides and improve fat oxidation for up to 16 hours after a single exercise session in healthy and populations.\(^{130}\)

However, not all studies have reported positive effects of resistance exercise on post-prandial metabolism. Burns et al. (2006) found that a single session of resistance exercise
completed the same day prior to a test meal increased postprandial triglyceride concentrations linked to the onset of post-exercise muscle damage in healthy males. Additionally, Burns et al (2005) found that resistance exercise had no effect on postprandial triglyceride concentrations or area under the curve (however, participants exercised the afternoon before the trial, thereby negating any acute benefits from this type of exercise). These inconsistencies require further study.

1.5.8 Effects of Resistance Exercise Prescription on Post-prandial Metabolism

Similar to aerobic exercise, there is evidence to suggest the benefits derived from resistance exercise are linked to gender, intensity, volume, type of resistance exercise, and previous training status. In 2005, Shannon et al. compared different volumes of resistance exercise (1, 3, and 5 sets) on postprandial substrate metabolism following a high fat meal. While no significant effects on post-prandial triglycerides or in insulin or glucose areas under the curve were found for any exercise volume in men and women combined, women demonstrated significantly lower postprandial triglycerides than men after exercise suggesting a potential effect of gender on postprandial lipid responses to exercise. Other studies examining the influence of training volume in healthy young men have also found that both high volume and low volume resistance exercise reduced postprandial triglyceride areas under the curve, with greater improvements coming after high volume vs. low volume prescriptions. However, the effects of a single bout of resistance exercise may dissipate over time like aerobic exercise, as Correa et al. failed to find differences in post-prandial triglycerides when consuming a meal 16 hours after the exercise session.
The intensity of resistance exercise has also shown to have an effect on postprandial substrate metabolism. Singhal et al. (2009) compared no exercise, moderate intensity resistance exercise (50% 8RM), and high intensity resistance exercise (100% 8RM) on postprandial triglyceride responses. The study found that only high intensity resistance exercise significantly reduced fasting and post-prandial triglyceride areas under the curve, and increased postprandial fat oxidation for up to 3 hours compared to the no-exercise condition. However, both moderate and high intensities improved insulin sensitivity. In addition to exercise prescription, the training status of the participant may also impact post-prandial responses. Thyfault et al. (2004) found that individuals with a history of resistance training demonstrated lower fat oxidation normalized for lean body mass in response to high carbohydrate and high fat meals, but higher carbohydrate oxidation per lean body mass after a high carbohydrate meal. Moreover, the resistance training group demonstrated higher diet-induced thermogenesis and reduced postprandial insulin and glucose areas under the curve, indicating differences in meal responses based on previous lifting history. Finally, even the type of resistance exercise may affect exercise responses. Pafili et al. (2009) found that eccentric leg press exercise reduced postprandial triglyceride area under the curve by 12% 16 hours after the training session compared to a no-exercise control condition. Similar to previous studies, these benefits were shown to disappear by 40 hours after exercise. However, differences between concentric and eccentric training, machines vs. free weights, or whole body vs. upper or lower body training have received little investigative attention.

1.6 Knowledge Gaps in Current Literature
In light of the evidence presented above, the following knowledge gaps need to be addressed:

1. Exercise non-responsiveness in Prediabetes

There is significant evidence to suggest that a large number of individuals with prediabetes are do not demonstrate improvements in glucose and lipid metabolism (i.e. non-responders) following exercise or lifestyle interventions. In the Finnish Diabetes Prevention Study, despite significant reductions in fat mass, the number needed to treat was 7 (meaning 7 individuals needed to be treated in order to prevent the development of type 2 diabetes in one participant). Further evidence suggests that individuals with both IFG and IGT demonstrate blunted responses to exercise interventions, even after high volume or high-intensity exercise and weight loss. These blunted responses could apply to post-prandial metabolism as well. Further research is needed to determine if individuals with prediabetes respond to a single bout of exercise. Moreover, additional research is needed to determine the exercise type, timing, and prescription that optimizes lipid and glucose metabolism in the post-prandial period – particularly with regard to resistance exercise, which has reported conflicting results in previous studies.

2. Influence of resistance exercise on post-prandial glucose metabolism in prediabetes

Most studies measure the effects of resistance exercise on fasted outcomes, or after an oral glucose tolerance test and have reported generally favorable results for glucose and insulin areas under the curve, insulin sensitivity, and glucose clearance. However, investigating the effects of resistance exercise on mixed meal (composed of glucose, fat and protein) glucose metabolism would more accurately reflect free-living conditions. Additionally, the
mechanisms of any corrective influence of resistance exercise on post-prandial glucose metabolism needs to be identified, including changes in beta cell function, insulin sensitivity, hepatic insulin sensitivity, and skeletal muscle glucose oxidation in this population. Skeletal muscle is a major source of glucose disposal after exercise, but the fate of post-prandial glucose absorbed by muscle (eg. oxidation vs. storage) after resistance training hasn’t been studied.

3. Influence of resistance exercise on post-prandial lipid metabolism in prediabetes

Resistance exercise is a central component of exercise recommendations for individuals with type 2 diabetes and prediabetes, but its effects on post-prandial lipid metabolism are unclear. Some studies have reported reduced triglycerides, VLDL triglyceride concentrations and mean residence time, improved fatty acid oxidation rates, and increased IMTG turnover in both the fasted and post-prandial state in healthy and obese men and women. However, other studies reported no benefit of resistance exercise in these populations. The effects of resistance exercise on post-prandial lipid metabolism in those with prediabetes hasn’t been studied. Moreover, the potential mechanisms of improved post-prandial lipid handling after resistance exercise remain unknown, including changes in exogenous or endogenous lipid metabolism, lipid oxidation, lipoprotein turnover and clearance, and FFA kinetics.

4. Influence of resistance exercise on skeletal muscle oxidative capacity and gene expression

The mitochondrial oxidative responses (lipid and carbohydrate) to an acute bout of resistance exercise have received little investigative attention despite the central role of skeletal muscle oxidation in post-prandial substrate clearance. This includes changes in the expression of key genes involved in lipid and glucose metabolism and how skeletal muscle substrate oxidation changes after resistance exercise and after consumption of a mixed meal.
1.7. Purposes and Specific Aims

The primary purposes of this dissertation are to determine the influence of an acute bout of resistance exercise on: 1) post-prandial lipid absorption and clearance, 2) post-prandial glucose absorption, clearance, and insulin sensitivity, and 3) skeletal muscle mitochondrial oxidative capacity, and muscle and adipose tissue gene expression and signaling in obese men with prediabetes. This study may help to improve the exercise prescription recommendations for individuals with prediabetes, and clarify how resistance exercise may reduce the risk for disease progression (to overt type 2 diabetes) and life-threatening complications (e.g. CVD).

Specific Aim 1 (Chapter 2). To characterize the effect of acute resistance exercise on basal and post-prandial glucose metabolism

Hypothesis 1a. Resistance exercise will decrease basal glucose production rate due to increased hepatic insulin sensitivity

Hypothesis 1b. Resistance exercise will increase post-prandial glucose disposal rate due to increased insulin sensitivity

Specific Aim 2 (Chapter 3): To characterize the effect of acute resistance exercise on basal and post-prandial lipid metabolism compared to rest
Hypothesis 2a. Resistance exercise will reduce adipose tissue lipolytic rate during the basal period due to increased adipose tissue insulin sensitivity

Hypothesis 2b. Resistance exercise will increase postprandial total lipid oxidation and meal-derived TRL-TG clearance rate

Hypothesis 2c. Resistance exercise will reduce post-prandial TRL-TG, chylomicron TG, and VLDL-TG concentration

Specific Aim 3 (Chapter 4). To characterize the effect of acute resistance exercise on skeletal muscle mitochondrial respiration, and skeletal muscle and subcutaneous adipose tissue gene expression

Hypothesis 3a. Resistance exercise will increase skeletal muscle mitochondrial lipid- and carbohydrate-supported oxidation

Hypothesis 3b. Resistance exercise will increase skeletal muscle PGC-1alpha mRNA content, as well as adipose tissue FOXO1 expression
1.8 References


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Chapter 2: Acute Effects of Resistance Exercise on Postprandial Glucose Metabolism in Obese Men with Prediabetes
Introduction

Prediabetes is a metabolic condition defined by elevated fasting (impaired fasting glucose (IFG)) and/or postprandial (impaired glucose tolerance (IGT)) glucose.¹ Prediabetes affects nearly 86 million adults in the United States, with most (up to 70%) progressing to type 2 diabetes within as little as one year.² While evidence suggests that type 2 diabetes can be reversed, most cases of remission involve invasive treatment strategies, including bariatric surgery to promote weight loss, or extreme lifestyle changes (eg. severe caloric restriction) that have poor long-term adherence.³⁴ Therefore, preventing the progression of prediabetes to type 2 diabetes would have profound health implications globally.

Previous studies have shown that lifestyle interventions combining diet and exercise can reverse prediabetes and restore normoglycemia. In 2002, the Diabetes Prevention Program Research Study demonstrated that lifestyle interventions aiming for 7% weight loss and 150 minutes per week of physical activity reduced the incidence of type 2 diabetes by 58% and was significantly more effective than treatment with metformin alone in individuals with prediabetes.⁵ Likewise, the Finnish Diabetes Prevention Study found that a lifestyle intervention focusing on weight loss, reducing total fat intake, and physical activity reduced the incidence of type 2 diabetes by 58% in obese men and women with impaired glucose tolerance.⁶ In light of these large-scale studies, the American Diabetes Association currently recommends individuals with prediabetes improve their diet and increase their physical activity levels (150 min/week of physical activity) to prevent or delay the onset of type 2 diabetes.⁷ However, recommendations regarding the use of resistance exercise for the prevention of prediabetes and type 2 diabetes are weakly supported (evidence grade B or C) and understudied.⁷
Several studies have demonstrated that resistance exercise improves post-prandial glucose metabolism in healthy individuals and in those with type 2 diabetes, but most of these studies simply assessed changes in glucose concentration, providing little insight into the mechanisms of these improvements.\textsuperscript{14-19} Investigating the effects of resistance exercise on mixed meal glucose metabolism more accurately reflects free-living conditions as most meals consist of a mixture of carbohydrates and lipid.\textsuperscript{20} Additionally, since most of the day is spent in the postprandial state after consumption of mixed meals, changes in postprandial glucose metabolism after a mixed meal might be expected to best elucidate the benefits of resistance exercise for glucose control and disease progression.\textsuperscript{21} Indeed, recent evidence suggests that postprandial hyperglycemia is a major, independent risk factor for cardiovascular disease (CVD) and other comorbidities associated with prediabetes, including hypertension, BMI, intraocular pressure, progression to type 2 diabetes, and all cause mortality.\textsuperscript{22,23}

Despite the strong connection between postprandial hyperglycemia and disease severity, no studies have evaluated the influence of pre-meal resistance exercise on postprandial glucose metabolism after consumption of a mixed meal in individuals with prediabetes. Moreover, the mechanisms for the potential effects of resistance exercise on postprandial glucose metabolism including beta cell function, insulin sensitivity, hepatic insulin sensitivity, and skeletal muscle glucose oxidation in individuals with prediabetes, are not known.\textsuperscript{24} Therefore, the purpose of this study was to determine the effects of a single bout of resistance exercise on postprandial glucose metabolism following a mixed-meal in obese, sedentary men with prediabetes.

\textbf{Methods}
Participants

Participants were recruited from the Washington University School of Medicine Diabetes Clinic, Washington University’s Volunteers for Health, the Center for Community Based Research, and from the surrounding St. Louis Community. Participants were included if they were male, aged 30-65 years with a BMI of 28-45 kg/m², and diagnosis of prediabetes (HbA1c >5.7 but <6.5%, or fasting plasma glucose >100 mg/dl, or 2-hour OGTT >140 mg/dL but <200 mg/dL).

Participants were excluded for having diagnosis of type 2 diabetes, use of insulin, participation in regular exercise (≥ 2x/week) within the previous 6 months; criteria meeting Class C or Class D of the American Heart Association Risk Stratification; history or evidence of serious pulmonary or cardiovascular disease including acute coronary syndrome, heart failure requiring medications, or New York Heart Association class III heart failure (patients with marked limitation of activity and who are comfortable only at rest) or IV heart failure (patients who should be at complete rest, confined to bed or chair and who have discomfort with any physical activity); coagulation disorders (platelets <100,000, PT >2 seconds above control or INR >1.5); anemia (hemoglobin <10.0 g/dL); any orthopedic, neurologic, metabolic or other medical condition that would prohibit the ability to perform resistance exercise; or joint replacement within the last year. Each participant read and signed an IRB-approved protocol and informed consent that was approved by the Human Research Protection Office at Washington University in St. Louis, MO.

Study Protocol
This study utilized a randomized crossover design to assess changes in postprandial glucose metabolism after a single bout of resistance exercise versus an equivalent period of rest. Participants who qualified for the study were then randomly assigned to one of two treatment orders: a rest visit followed by an exercise visit, or an exercise visit followed by a rest visit. In both groups, participants completed the rest and exercise visits on separate days >1 week apart.

Screening Visit: Participants were screened prior to enrollment to confirm diagnosis of prediabetes and absence of exclusion criteria. After an overnight fast, participants reported to the Washington University Clinical Research Unit (CRU) where their height, weight, BMI, and body composition were measured (using dual x-ray absorptiometry, DXA) and a medical history and physical were performed by the study physician. An intravenous (IV) catheter was inserted into the antecubital vein to collect fasting blood samples, which were analyzed for plasma glucose, HbA1c, a lipid panel, a comprehensive metabolic panel (CMP), and a complete blood count (CBC).

Body Composition

Following admission, each participant received whole-body dual energy X-ray absorptiometry (DXA) scans (Hologic Discovery GDR 1000/W, software version 12.6.2 OD; Waltham, MA) to assess regional and composite lean and fat mass in grams. Image analysis and sub-region (thigh, leg, trunk, upper extremities) composition quantification were performed following the guidelines provided by Hologic GDR software.
Oral Glucose Tolerance Test (OGTT)

Following the collection of fasting blood samples a two-hour, 75-gram OGTT was performed after a 12-hour overnight fast. All OGTTs were started between 7am and 9am. Plasma glucose was measured by the glucose oxidase method (YSI Stat Plus, Yellow Springs, OH) and insulin by double antibody radioimmunoassay.

Strength Testing

One repetition maximum (1RM) assessments were performed at the end of the screening visit on a Hoist single pod machine (San Diego, CA, USA) according to guidelines established by the American College of Sports Medicine. The maximum weight lifted through the full range of motion with proper form was recorded for the leg press, bench press, biceps curl, seated row, pull down, knee extension, and shoulder press.

Accelerometry

Daily physical activity over one-week was determined using a ActiGraph GT3X+ accelerometer (ActiGraph LLC, Pensacola, FL). The GTX3+ was placed on the non-dominant at the end of the screening visit wrist with Actigraph non-removable wristband. The wristbands were cut off by the study team when they returned for their rest or exercise visit to ensure the accelerometers were worn for the entire data collection period. ActiGraph data was collected for seven consecutive days at 30 Hz. The accelerometer output was sampled by a 12-bit analog-to-digital
converter. The percentage of time spent sedentary as well as the amount of time spent participating in different categories of physical activity including light, lifestyle, moderate, and vigorous were calculated using algorithms from Freedson in the ActiGraph software.\(^{26}\)

Rest Visit: The day prior to the rest visit, subjects consumed a standardized breakfast (522 Kcal), lunch (722 Kcal, snack 180 Kcal), and dinner (779 Kcal) provided by the Washington University Institute of Clinical and Translational Sciences Bionutrition Service. Participants also consumed a liquid formula (Ensure; Ross Laboratories, Columbus, OH) containing 250 kcal (40 g carbohydrates, 6.1 g fat, and 8.8 g protein) to ensure complete filling of hepatic glycogen stores as a snack before bedtime. Participants then fasted overnight (10hrs) with nothing taken by mouth except water and reported to the CRU at 0730 the following morning.. After admission, height and weight were measured using a standard scale and stadiometer, and an IV catheter was inserted into the antecubital vein to collect baseline fasting blood samples. Fasting blood samples were collected in heparinized tubes for glucose, insulin, c-peptide, citrate-lined tubes for PT-INR, and EDTA lined tubes for lipid kinetics analysis.

Following collection of baseline blood samples, participants rested quietly in the semi-recumbent position for 1 hour. At the end of the rest period, a second catheter was inserted into a hand vein on the contralateral arm, with the hand warmed to 55°C using a thermostatically controlled hot box to obtain arterialized blood samples. Thirty minutes after the rest period, a primed, constant infusion of [6,6-2H2]glucose (28 \(\mu\)mol/kg prime and 0.28 \(\mu\)mol\(\cdot\)kg\(-1\)\(\cdot\)min\(-1\) continuous infusion, Cambridge Isotopes, Tewksbury, MA), was administered for 180 minutes to
achieve isotopic steady state. Blood samples were collected at -10, 140, 160, and 180 minutes from the start of the tracer infusion to quantify basal (i.e. fasting) glucose metabolism kinetics.

Following the 180-minute baseline period, participants consumed a liquid study meal consisting of 20 Kcal/FFM, with a composition of 47.8% CHO, 36.1% fat, and 16.1% protein. To ensure individuals meal consistency for both visits, one large mixture was prepared and then split in half (each half consumed during the rest and exercise visit). Participants were asked to consume the liquid meal within 16 minutes (1/4 of the meal provided every 4 minutes). The sides of the liquid meal container were rinsed with 10 ml of water and the rinse was also consumed to ensure participants ingested the entire meal. Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 minutes post-meal ingestion. Isolated plasma was analyzed for glucose and insulin concentration, as well as glucose tracer enrichment. Samples of the liquid test meal, as well as the tracers used, were collected for enrichment analysis. The infusion of [6,6-$^{2}$H$_{2}$]glucose was continued throughout the postprandial period. An overview of the study protocol is provided in Figure 1. The [6,6-$^{2}$H$_{2}$]glucose was purchased from Cambridge Isotopes (Tewksbury, MA).

Exercise Visit: The exercise visit was identical to the rest visit except participants performed ~1 hour of moderate intensity resistance exercise rather than rest. The exercise session consisted of 8 lifts (bench press, pull down, seated row, biceps curl, knee extension, leg press, and overhead press) performed for 3 sets of 8-12 reps at 80% of their measured 1RM until volitional fatigue. Participants were given no more than 1 minute of rest between sets, and no more than 3 minutes rest between exercises. All exercise sessions were supervised by a licensed physical therapist.
Indirect Calorimetry

Whole body carbohydrate oxidation was measured using indirect calorimetry (Parvo Medics, Sandy, UT) and the ventilated hood technique prior to, and throughout, the postprandial period. VO$_2$ and VCO$_2$ were measured continuously for 15 minutes for the determination of resting energy expenditure, respiratory exchange ratio and carbohydrate oxidation while the participant laid quietly in bed. Measurements were taken at baseline (prior to exercise/rest period, 120 minutes after the start of the exercise/rest period, and then at 30, 60, 150, 210, 270, and 330 min after consuming the liquid test meal. Total carbohydrate oxidation rate (grams/minute) was calculated as previously described.$^27$

Muscle Biopsies

Muscle biopsy samples (~200 mg) were obtained from the vastus lateralis under local anesthesia (lidocaine, 2%) 90-minutes after consuming the liquid test meal using Tilley-Henkel forceps. One sample was immediately transferred into ice-cold relaxing medium (BIOPS) containing 10 mmol/l Ca$^{2+}$/EGTA buffer, 20 mmol/l imidazole, 50 mmol/l K$^+$-4-morpholinoethanesulfonic acid (Mes), 0.5 mmol/l dithiothreitol, 6.56 mmol/l MgCl$_2$, 5.77 mmol/l ATP; 15 mmol/l phosphocreatine at pH 7.1 for mitochondrial respirometry analysis. A second samples was flash frozen in liquid nitrogen and stored at -80°C until further analysis.

Mitochondrial Respiration

Subsamples of 5 mg wet weight ($W_w$) were transferred into BIOPS onto a small petri dish on an ice-cold metal plate and separated using forceps with sharp tips. To ensure complete permeabilization the fibers were incubated by gentle agitation at 4°C in BIOPS solution
containing 50 μg/ml saponin for 20 min. Fibers were washed for 15 min at 4°C in ice-cold mitochondrial respiration medium [MiR06; 0.5 mM EGTA, 3 mM MgCl₂, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH₂PO₄, 20 mM HEPES, 110 mM sucrose and 1 g/l BSA essentially fatty acid free, adjusted to pH 7.1, 2,800 units/mg solid catalase lypophilized powder], and wet weight of the fibers was measured on a microbalance (Mettler Toledo, Greifensee, Switzerland). With this method, the cholesterol-rich plasma membrane is selectively permeabilized, leaving intracellular membrane structures, such as mitochondria intact, and the entire mitochondrial population can be studied in the muscle sample.

Three milligrams (3 mg) wet weight was used per respirometer chamber (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) containing 2.090 ml MiR06 at 37°C. Oxygen concentration (μM = nmol/ml) and oxygen flux (pmol·s⁻¹·mg⁻¹; negative time derivative of oxygen concentration, divided by muscle mass per volume) were recorded using DatLab software (Oroboros Instruments). The Oxygraph-2k is a two-chamber titration-injection respirometer with a limit of detection of volume-specific oxygen flux of 1 pmol·s⁻¹·ml⁻¹. Instrumental background oxygen flux was corrected online, accounting for sensor oxygen consumption and oxygen diffusion between the medium and the chamber boundaries. The oxygen concentration in the chamber was maintained between 150 and 400 μM to avoid oxygen limitation of fiber respiration. Intermittent re-oxygenations were achieved by injecting 0.5 mL oxygen.

In the substrate-uncoupler-inhibitor titration protocol the following substrates were added (final concentrations): palmitoylcarnitine (0.05 mM), L-carnitine (5 mM), and malate (0.5 mM) to support electron entry from fatty acid β-oxidation through electron-transferring flavoprotein (ETF) and Complex I (CI) to coenzyme Q. Prior to the addition of ADP, oxygen utilization
occurs due to proton slip across the inner mitochondrial membrane (LEAK respiration). ADP (4 mM) was then added to stimulate fatty-acid supported oxidative phosphorylation (ETF + Cl)\textsubscript{Lip}.

The subsequent addition of pyruvate (10 mM) stimulates glycolytic oxidative phosphorylation. Glutamate (10 mM) was then added, followed by succinate (10 mM) to recapitulate the TCA cycle and stimulate maximal oxidative phosphorylation [(ETF + CI + CII)\textsubscript{Lip} + Pyr] through both ETC complex I and complex II. Cytochrome c (10 μM) is added to test the integrity of the outer mitochondrial membrane. The increase of flux with cytochrome c was on average 3.3 ± 0.4%.

Electron transfer system capacity (CI+II\textsubscript{E}) was reached by stepwise (.5 μM) addition of the uncoupler carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP). Finally, the addition of rotenone (0.5 μM), an inhibitor of complex I was used measure succinate-supported ETS capacity (CII\textsubscript{E}). A measure of pyruvate-supported respiration was determined as maximal oxidative phosphorylation [(ETF + CI + CII)\textsubscript{Lip} + Pyr] - lipid supported oxidative phosphorylation (ETF + CI)\textsubscript{Lip}.

Sample Analyses

Blood for plasma hormone and metabolite analysis was collected from an antecubital vein, immediately placed in chilled EDTA tubes, centrifuged at 2000xg for 10 minutes, and the supernatant collected and frozen at -80°C until analysis. Fasting samples were analyzed were used for comprehensive metabolic panel, lipid panel, CBC, and HbA1c by the Washington University Core Lab for Clinical Studies. Plasma glucose was measured using an automated glucose analyzer (Yellow Spring Instruments, Yellow Springs, OH). Plasma insulin concentration was measured by radioimmunoassay.
Calculations

The tracer-to-tracee ratio (TTRs) for plasma $[^2]H_2$glucose was quantified using capillary GC-MS (Agilent 6890N gas chromatograph and Agilent 5973N mass selective detector; Agilent, Palo Alto, CA), as previously described. For $[^2]H_2$glucose enrichment quantification, plasma proteins were precipitated with cold acetone, lipids were extracted into hexane, and the aqueous phase was dried (Labconco, Kansas City, MO). The heptafluorobutyric derivative of glucose was formed, and $[^3]H_2$glucose enrichment was quantified using GC-electron ionization-MS and selective ion monitoring (mass/charge ratio [m/z] 519 and 521).

From the plasma glucose and insulin values on the OGTT, an index of insulin resistance (IR), beta cell function (HOMA-%B), and insulin sensitivity (HOMA-%S) were calculated using the Homeostatic model assessment (HOMA) 2 using the HOMA2 calculator.

During the last 300 minutes of the baseline period (160-, 170-, and 180-min), a physiological and isotopic state was reached, and therefore Steele’s equation for steady state was used to calculate glucose flux (rate of appearance [Ra] and rate of disappearance [Rd]). Ra was calculated by dividing the [6,6-2H2]glucose infusion rate (umol/min) by the plasma tracer enrichment (TTR$_{6,6}$, mean of all three measurements). Glucose Rd was calculated as glucose Ra + [6,6-2H2]glucose infusion rate. Ra and Rd were normalized per kg of fat-free mass (FFM) from DXA. The hepatic insulin resistance index (HIRI) was calculated as the product of basal glucose Ra and plasma insulin.

After mixed meal ingestion, the total glucose rate of appearance (Ra$_{TOT}$) was calculated from TTR$_{6,6}$using Steele’s equation:
\[ Ra_{TOT} = \frac{IR - C(t) \times V \times \frac{dTTR_{6.6}(t)}{dt}}{TTR_{6.6}(t)} \]

where IR is the 6.6-[\textsuperscript{2}H\textsubscript{2}]glucose infusion rate, C is the plasma glucose concentration, and V is the volume of distribution of glucose (55 ml/kg, fixed).\textsuperscript{31} Before applying Steele’s equation, plasma TTR\textsubscript{6.6} data were smoothed using a spline fitting approach to stabilize the calculation of the derivative of enrichment. Total Ra represents the splanchnic Ra of glucose from ingested glucose, the liver and potentially some glycogenolysis and gluconeogenesis from the kidneys.

Rate of disappearance (Rd) was calculated as:

\[ Rd(t) = Ra_{TOT}(t) - \frac{dC(t)}{dt} \times V \]

Both Ra and Rd were normalized to kg FFM from DXA.\textsuperscript{31} The glucose metabolic clearance rate (MCR) was estimated as the Rd during the meal divided by the average plasma glucose. A measure of peripheral insulin resistance was then calculated by dividing the MCR by average plasma insulin as described by Gastaldelli et al. (2007).\textsuperscript{31} Rd(t) was also divided by plasma insulin at each point during the postprandial period to track changes in insulin sensitivity over time. The total area under the curve was also calculated for total Ra and total Rd using the trapezoid rule.

Areas under the curve, and incremental area under the curve for plasma glucose and insulin were calculated using the trapezoid rule. Oral insulin sensitivity was estimated using the oral glucose insulin sensitivity (OGIS) index from 3-h glucose and insulin values using methods described by Mari et al. (2001) accounting for the meal glucose load (90.3 grams).\textsuperscript{32} Postprandial glucose-stimulated insulin secretion (insulinogenic index, IGI) was calculated from the ratio of
total insulin secretion during the 6-hour postprandial period (AUC Insulin0-360), by the total plasma glucose load during the postprandial period (AUC Glucose0-360). Glucose tolerance was calculated according to the method proposed by Sluiter et al. (1976) as:

\[
GT = \frac{10^6}{(Gp)^2 * (Gp - 70)}
\]

where Gp is the peak plasma glucose during the postprandial period. Finally, the oral disposition index was calculated as AUCins/glu from 0 to 120 min*Matsuda ISI as described by Bi et al. (2012).

Statistical Analysis

Participant demographics were analyzed using mean ± SEM. Data were analyzed for normality using the Shapiro-Wilk test. Differences between rest and exercise conditions on measures of plasma glucose concentration, insulin concentration, insulin sensitivity, and glucose kinetics were assessed using a 2x2 mixed, repeated measures ANOVA with treatment (rest or exercise) and time (time the sample was collected in the study) entered as repeated measures, and order (rest>exercise or exercise>rest) as a between subjects factor to rule out confounding by carryover effects (to determine if the washout period between the rest and exercise visits was sufficient). The assumption of sphericity was assessed using Mauchly’s test. Huyn-feldt adjusted F values were used in cases of the violation of the assumption of sphericity. Significant interactions between treatment and time were further analyzed using paired t-tests. The Benjamini–Hochberg procedure was used to control for multiple comparisons. The two-way interaction between time and order, treatment and order, and the three-way interaction between
treatment, time, and order were tested to identify the presence of a carry-over effect. Areas under
the curve between conditions were analyzed according to the procedures outlined by Wellek and
Blettner (2012) for crossover designs, testing first for carry-over effects, followed by testing for
treatment effects. An alpha level \( p = 0.05 \) was used for significance.

Sample Size

Breen et al. (2011) reported an effect size of 2 for an increase in the rate glucose
disappearance after a single session of resistance exercise in healthy men. Therefore, a
conservative effect size of 1.5 was expected due to the potential blunted responses to resistance
exercise in men with prediabetes. Therefore, our anticipated sample size was seven to achieve
90% power to detect differences on a paired t-test for glucose disposal (at a two-tailed alpha
level of .05).

Results

Participant Characteristics

Nine male \( (n=9) \) participants were enrolled in the study. Participants were middle-aged,
obese, and sedentary. All participants were prediabetic, with the group means for HbA1c, 2-hour
plasma glucose of the OGTT, and fasting plasma glucose all within the prediabetic range (Table
1). Four \( (n=4) \) participants had HbA1c values \( \geq 5.7\% \), \( n=6 \) had fasting plasma glucose values
\( >100 \text{ mg/dL} \ (5.5 \text{ mmol/L}) \), and \( n=4 \) participants had 2-hour OGTT values \( >140 \text{ mg/dL} \ (7.8
\text{ mmol/L}) \). The average % body fat was in the 75th percentile for individuals in their age group as
established by the NHANES. Participants also demonstrated insulin resistance according to the HOMA2 model (HOMA2-IR, HOMA2-%S), with compensatory increases in beta cell function (HOMA2-%B). Participants also presented with elevated total triglycerides (>150 mg/dL), LDL >100 mg/dL, and slightly lower HDL (<40 mg/dL,) (all Table 1).

Plasma Glucose Concentration

Plasma glucose concentrations were significantly elevated immediately after resistance exercise (-10 min in Fig. 1, p=0.008) and at 170 minutes (p=0.016), but significantly lower at 270 minutes (p=0.034), and 330 minutes (p=0.049) compared to the rest condition (Figure 2). There were no interactions between treatment and order (p=.379), time and order (p=.540), or treatment, time and order (p=.945) indicating absence of a carry-over effect between treatments. Plasma glucose concentrations returned to baseline after the 3-hour basal period after the exercise/rest session prior to meal consumption (Figure 2). The postprandial glucose area under the curve (AUC), and integrated AUC (iAUC), were both significantly lower following resistance exercise compared to rest (p=0.046, p=0.021 respectively). No carry-over effect was found for either AUC analysis (total AUC: p=.356, iAUC: p=.136).

Plasma Insulin Concentration

There was a main effect of time (p<0.001) and a main effect of treatment (F (1,7)=6.065, p=0.045), but no interaction between treatment and time for plasma insulin concentration (Figure 2). There were no significant interactions between order and time (p=0.948), order and treatment
(p=0.170), or order treatment, and time (p=0.145) indicating the absence of a carry-over effect for plasma insulin concentration. The postprandial insulin AUC and iAUC was significantly lower after resistance exercise (p=0.037 and p=0.046 respectively) (Figure 2). There were no carry-over effects identified for total AUC or iAUC (p=0.226, p=0.213 respectively).

Insulin Sensitivity and Beta Cell Function

Oral glucose insulin sensitivity (OGIS) tended to increase following resistance exercise (Rest: 280.7 ± 10 ml•min⁻¹•m⁻², Exercise: 307.6 ± 18 ml•min⁻¹•m⁻², p=0.071), without evidence for a carry-over effect (p=0.506). Total glucose-stimulated insulin secretion (Insulinogenic Index) tended decline after resistance exercise versus rest (Rest: 13.4 ± 1.2 umol/uU, Exercise: 11.4 ± 1.4 umol/uU, t(8)=−2.14, p=0.063), with no carry-over effect (t(8)=−.709, p=0.498). Glucose tolerance was significantly greater after the resistance exercise session compared to rest (Rest: arbitrary units.49 ± .16 arbitrary units, Exercise: 65 ± .20, t(8)= -2.509, p=.036) without a carry-over effect (t(8)=−.618, p=0.553). Finally, the Oral Disposition Index was not different after the resistance exercise session (Rest: 7.18 ± 2.26, Exercise: 5.75 ± 1.86, t(8)=.685, p=.512) compared to rest, and no cross-over effect was found (t(8)=−.802, p=.445).

Glucose Kinetics

Glucose rate of appearance (Ra) was significantly higher after the resistance exercise session measured at the end of the basal/recovery period, as was the rate of disappearance (Rd) compared to rest (steady state, Figure 3). In the post-prandial period, glucose Rd normalized to
plasma insulin tended to be higher at the end of the basal period (measured from time points 160-180 min), suggesting a trend toward improved insulin sensitivity after the exercise session before the meal (Rest: 1.05 ± .15 umol/kg FFM/min/uU/ml, Exercise: 1.41 ± .26 umol/kg FFM/min/uU/ml, t(8)=1.96, p=0.085). Additionally, there was a significant main effect of treatment for glucose Rd normalized for plasma insulin concentration (F (1,8)=19.172, p=0.002), suggesting a greater rate of glucose clearance per unit of insulin during the overall postprandial period. There were no crossover interactions in the Rd/insulin analysis (see Figure 3). After the resistance exercise session, there were no effects of treatment on total glucose Ra or Rd, or the Ra and Rd AUC (no interaction between treatment and time, or main effects of treatment, all p>.05). The glucose metabolic clearance rate (p=.035) was significantly elevated, as was peripheral insulin sensitivity (p=.008) after training (Table 2).

Carbohydrate Oxidation Rate

There was a significant interaction between treatment and time for the respiratory exchange ratio (RER) (F (7, 56)=2.746, p=.016). Main effects analysis indicated the RER was significantly reduced after resistance exercise at time point 140 min (p=.029), indicating a reduction in carbohydrate oxidation (possibly due to increased glycogen replenishment) with a trend toward elevated RER at time point 390 minutes (p=.091). There was only a main effect of time (F(1,7)=5.378) for carbohydrate oxidation rate (grams/min), but no interaction between treatment and time (F(8,49)=1.457, p=.205, Figure 4). However, total post-prandial carbohydrate oxidation iAUC was significantly elevated in the postprandial period after resistance exercise compared to rest with no evidence of a carry-over effect (t(8)=.801, p=.446). Concurrent with the
improvements in postprandial carbohydrate oxidation, skeletal muscle mitochondrial respiration analysis indicated a significant increase in pyruvate-supported respiration ($t(7)=2.799$, $p=.012$), as well as maximal oxidative phosphorylation capacity ($t(7)=13.48$, $p<.001$) following resistance exercise compared to rest. No carry-over effects were found for either outcome.

Discussion

The effects of an acute bout of resistance exercise on postprandial glucose metabolism in individuals with prediabetes are poorly understood.\textsuperscript{16,17} Previous studies have reported that post-meal resistance exercise in individuals with prediabetes/type 2 diabetes reduces postprandial glucose excursions.\textsuperscript{15,37} To our knowledge, only one study has evaluated the effect of pre-meal resistance exercise on post-prandial glucose metabolism, which effectively reduced postprandial glucose and insulin iAUC in individuals with type 2 diabetes.\textsuperscript{15} We have confirmed these previous studies but expanded on them to demonstrate, for the first time, that a single bout of resistance exercise performed 4.5 hours before a mixed meal reduces postprandial glycemia and insulin concentrations, and that these changes were associated with improvements in peripheral insulin sensitivity, glucose clearance rates, and whole-body and skeletal muscle mitochondrial carbohydrate oxidation in obese men with prediabetes. Furthermore, we found that a single resistance exercise bout had no effect on beta cell function or Disposition Index in men with prediabetes, which suggests repeated bouts of exercise (ie. training) may be required to elicit positive adaptations in beta cell function in these individuals.

Previous studies have found that the contribution of postprandial glycemia to plasma HbA1c increases as the degree of glycemic control increases.\textsuperscript{38,39} Therefore, in individuals with
prediabetes who have only marginally impaired glycemic control, reducing the degree of
postprandial glycemia could be an integral component of their treatment. In individuals with
obesity and type 2 diabetes, chronic resistance exercise training has been shown to improve
postprandial glycemia following an OGTT.\textsuperscript{14-19} Our results extend these findings to men with
prediabetes after consumption of a mixed meal, which more closely approximates free-living
conditions. In our study, postprandial glucose concentrations were significantly lower following
acute resistance exercise by 90 minutes after meal consumption – a reduction that persisted to
150 minutes post-meal. In fact, plasma glucose was, on average, lower throughout the 6-hour
postprandial period after resistance exercise compared to rest. This is reflected by the reduction
in the total and integrated area under the postprandial glucose concentration curve; a finding
consistent with those from Andersen et al. (2007) and Lopez et al. (2014).\textsuperscript{40,41}

Plasma glucose concentration did however, spike immediately following resistance
exercise (at -10 min) significantly relative to the rest condition. Previous studies have found that
high intensity aerobic exercise increases glucose production 7-8 fold, while glucose clearance
only increased 3-4-fold, thereby resulting in a transient increase in plasma glucose.\textsuperscript{42} This rise in
plasma glucose we found this this study could be caused by a transient increase in hepatic insulin
resistance (in combination with the influence of a number of other hormones (e.g. cortisol,
growth hormone)), hepatic glucagon sensitivity, or increased gluconeogenesis in response to
increased availability of substrate (lactate, free fatty acids), to provide glucose to the recovering
muscle for glycogen replenishment and energy.\textsuperscript{42} In support of this notion, we found that hepatic
insulin resistance tended to increase following resistance exercise when measured at the end of
the basal period, and that this corresponded with a significant increase in endogenous glucose
production (Figure 3). The increase in plasma glucose following acute resistance exercise is
typically matched by a similar spike in plasma insulin to restore normoglycemia and promote insulin uptake. While not significantly different than baseline, we did identify a spike in insulin after the resistance exercise session at the same time as the spike in plasma glucose. Additionally, plasma glucose concentrations typically return to baseline within 1-2 hours of resistance exercise cessation. In our study, although plasma glucose and insulin concentrations returned to baseline by the end of the basal period, the total glucose rate of appearance remained significantly elevated over 3 hours after exercise. The return of plasma glucose to baseline appeared to be due to a concomitant, significant increase in plasma glucose rate of disappearance, and a trend toward increased insulin sensitivity (rate of disappearance per unit of insulin) that occurred post-exercise.

In contrast to the basal period, postprandial total glucose Ra and Rd were not significantly different compared to rest. However, when the rate of glucose disappearance was normalized to the plasma glucose concentration (i.e. the metabolic clearance rate), postprandial glucose clearance was significantly elevated after resistance exercise compared to rest. In addition, peripheral insulin sensitivity (expressed as both the metabolic clearance rate per unit of plasma insulin, and as the Rd normalized to plasma insulin) was increased following resistance exercise compared to rest. Therefore, improvements in postprandial plasma glucose following acute resistance exercise are most likely related to increases in glucose clearance rather than reductions in glucose appearance (the combination of glucose absorption and endogenous glucose production). Additional studies examining the effect of resistance exercise on endogenous versus exogenous glucose kinetics during the postprandial period following resistance exercise are needed to confirm this. Modulating postprandial glucose metabolism appears important in individuals with type 2 diabetes as postprandial suppression of endogenous
glucose production is delayed.\textsuperscript{43,44} Also, the rate of glucose clearance in those with type 2 diabetes is impaired, suggesting that interventions designed to suppress endogenous glucose production and improve glucose clearance (such as resistance exercise) could be highly effective in preventing the progression of prediabetes to overt type 2 diabetes.

Concurrent with declines in postprandial glucose concentrations, postprandial insulin concentrations were reduced after acute resistance exercise (main effect of treatment and reduced AUC and iAUC). Reductions in plasma insulin following resistance exercise suggest improved skeletal muscle insulin sensitivity or increases in GLUT4 (a known response to resistance exercise in skeletal muscle).\textsuperscript{45,46} This could also be driven by the reduction in plasma glucose (ie. less insulin needed), or changes in beta cell insulin secretion. Importantly, we only found a trend toward a reduction in the insulinogenic index after resistance exercise, with no change observed in the disposition index, indicating a decline in peripheral insulin resistance. Consistent with this finding, Heden et al (2013) found that one hour of treadmill walking (55-60\% VO\textsubscript{2peak}) significantly reduced the postprandial insulinogenic index in obese subjects compared to no-exercise obese controls.\textsuperscript{15} Moreover, insulin secretion rates were shown to be lower than non-obese controls following a single session of moderate-intensity aerobic exercise.\textsuperscript{47} Slentz et al. (2009) found that after vigorous, high-intensity aerobic exercise (65-80\% VO\textsubscript{2peak}) the insulin sensitivity index increased, with compensatory declines in the acute insulin response to intravenous glucose in sedentary, overweight adults.\textsuperscript{48} The mechanisms for the decline in insulin secretion after aerobic and resistance exercise are poorly understood, but could be related to declines in circulating free fatty acids or amino acids, declines in insulinotropic hormones such as GIP or GLP-1, or changes in adrenergic activation.\textsuperscript{49} Future studies are needed to elucidate the effects of resistance exercise on beta cell function.
The Disposition Index is a ratio of insulin secretion to insulin resistance and has been shown to be a significant predictor of the development of type 2 diabetes. Lorenzo et al. (2010) found that a one standard deviation increase in the Disposition Index reduced the odds of developing type 2 diabetes by 32% in 1,625 prediabetic individuals in the Insulin Resistance Atherosclerosis study. Heden et al. demonstrated that a single bout of moderate intensity aerobic exercise improved the Disposition Index by increasing insulin sensitivity without reducing glucose-stimulated insulin secretion. We found the a single bout of resistance exercise did not significantly affect Disposition Index in obese, prediabetic men. Additional studies are needed to determine if resistance exercise can improve the disposition index by modifying the prescription parameters (eg. intensity, time, free-weights vs. machines). Individuals with prediabetes may be more resistant to exercise adaptations than the healthy population studied by Heden et al. If so, a single bout of resistance exercise may not be enough to improve beta cell function in prediabetes – repeated bouts might be necessary (ie. training).

After the meal, the RER and total carbohydrate oxidation rate increased in both the rest and exercise conditions, but to an even greater extent after resistance exercise (indicated by the greater postprandial AUC for carbohydrate oxidation). The signaling mechanisms responsible for the transition between lipid and glucose oxidation is not completely understood, but has been linked to glucose-induced malonyl-coA production via supression of 5’ AMPK, which normally inhibits acetyl-CoA carboxylase and activates malonyl CoA decarboxylase. Malonyl-CoA has been shown to bind to and inhibit CPT-1, thereby reducing fatty acid oxidation. This shift in substrate oxidation manifests as a rise in the RER and total rate of carbohydrate oxidation. The greater increase in carbohydrate oxidation during the postprandial period could be due to increased removal of lipid intermediates that interfere with insulin signaling and glycolysis.
during the recovery period (evidenced by the decline in the RER we identified in our study).

The lipid overload hypothesis posits that excess delivery of fatty acids to mitochondria leads to incomplete beta oxidation and the accumulation of lipid intermediates, including acetyl-coA, NADH, diacylglycerols, cytosolic citrate, and long-chain acyl carnitine CoA, which negatively regulate insulin signaling and glycolytic enzyme activity through Randle Cycle Inhibition.

Previous studies have reported elevated levels of these toxic intermediates in skeletal muscle of individuals with prediabetes, suggesting that the increased clearance of these byproducts in the recovery period from exercise could enhance postprandial glucose oxidation.

Coincident with greater postprandial carbohydrate oxidation, using high-resolution respirometry of permeabilized muscle fibers collected 90 minutes after meal consumption, we found elevated rates of pyruvate-supported mitochondrial respiration and total oxidative phosphorylation following resistance exercise compared to rest. While we did not measure changes in protein phosphorylation, the enhanced rates of mitochondrial private-supported oxidation could be due to changes in glycolytic enzyme function, or other changes in kinase activity. This notion is supported by Perry et al. who demonstrated that two hours of cycling increased respiratory sensitivity to pyruvate and glutamate, suggesting complex I of the electron transport chain may become more sensitive to NADH following exercise. Further research is needed to determine if mitochondria prefer pyruvate after resistance exercise as opposed to other substrates (like free fatty acids), or of the increase in pyruvate oxidation was driven more-so by increases in overall respiration, and not specifically glucose oxidation.

Limitations
There are several limitations to this study. First, while we used an infused glucose tracer to track dilution from meal glucose, we could not distinguish exogenous from endogenous glucose production, which would be important to determine changes in hepatic insulin sensitivity as opposed to meal glucose rate of appearance. This study only enrolled adult men thus the results cannot be generalized to women and children. Additionally, the study sample size was based on anticipated differences in glucose rate of disappearance following resistance exercise and not powered to detect differences in other variables. Despite this, we found differences in peripheral insulin sensitivity, carbohydrate oxidation, and glucose tolerance following resistance exercise.

Acknowledgements

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University Clinical Translational Science Award, PI: Evanoff) and the Cambridge Isotope Laboratories Research Grant Program.
Works Cited


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Table 1. Participant Characteristics (N=9)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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</tr>
<tr>
<td>Height (cm)</td>
<td>178 (5.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>104.5 (9.9)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>33 (3)</td>
</tr>
<tr>
<td>WB Fat (kg)</td>
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</tr>
<tr>
<td>WB Lean (kg)</td>
<td>66.3 (1.5)</td>
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<tr>
<td>% Fat</td>
<td>31.8 (1.4)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.7 (.2)</td>
</tr>
<tr>
<td>2-hr OGTT (mmol/L)</td>
<td>9.0 (.5)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.9 (.4)</td>
</tr>
<tr>
<td>Fasting Insulin (uU/mL)</td>
<td>22 (2)</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>2.6 (.4)</td>
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<tr>
<td>HOMA2-%B</td>
<td>134.4 (17)</td>
</tr>
<tr>
<td>HOMA2-%S</td>
<td>75.6 (37)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>162.7 (7.5)</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>184.5 (11.4)</td>
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<tr>
<td>HDL (mg/dL)</td>
<td>38.4 (1.7)</td>
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<tr>
<td>LDL (mg/dL)</td>
<td>117.9 (10.3)</td>
</tr>
<tr>
<td>% Time Sedentary</td>
<td>69 (6.1)</td>
</tr>
<tr>
<td>% Time Light Activity</td>
<td>21 (3.6)</td>
</tr>
<tr>
<td>% Time Moderate Activity</td>
<td>10 (3)</td>
</tr>
<tr>
<td>% Time Vigorous Activity</td>
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</tr>
</tbody>
</table>

Values are presented as mean (standard error). % of time spent in each activity were derived from accelerometry data (see methods). WB = whole body. 2-hr OGTT is plasma glucose at 2-hours after a 75 gram oral glucose challenge. HOMA2 calculated using the HOMA2 calculator accessed from (http://www.dtu.oc.ac.uk).
Table 2. Postprandial Glucose Kinetics

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Rest</th>
<th>Exercise</th>
<th>Significance</th>
<th>Carry-over Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ra (umol/ kg FFM/min)</td>
<td>--</td>
<td>--</td>
<td>Tx: F(1,8) = .508, p=.496</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time: F(14,112) = 12.928, p&lt;.001*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Interaction: F(14,112) = .869, p=0.594</td>
<td></td>
</tr>
<tr>
<td>Time: F(14,112) = 12.928, p&lt;.001*</td>
<td></td>
<td></td>
<td>Interaction: F(14,112) = .869, p=0.594</td>
<td></td>
</tr>
<tr>
<td>Total Rd (umol/kg FFM/min)</td>
<td>--</td>
<td>--</td>
<td>Tx: F(1,8) = 1.134, p=.318</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time: F(14,112) = 28.339, p&lt;.001*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Interaction: F(14,112) = 1.183, p=0.298</td>
<td></td>
</tr>
<tr>
<td>Ra AUC (umol/kg FFM/min * 360 min)</td>
<td>8685.6 (195.5)</td>
<td>8709.5 (274.4)</td>
<td>p=0.921</td>
<td>NS</td>
</tr>
<tr>
<td>Rd AUC (umol/kg FFM/min * 360 min)</td>
<td>8506.5 (137.6)</td>
<td>8503.2 (235.8)</td>
<td>p=0.988</td>
<td>NS</td>
</tr>
<tr>
<td>Metabolic Clearance Rate (ml/kg FFM/min)</td>
<td>2.92 (.10)</td>
<td>3.41 (.11)</td>
<td>p=0.035*</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral Insulin Sensitivity (ml/kg FFM/min/uU)</td>
<td>.031 (.003)</td>
<td>.038 (.004)</td>
<td>p=0.008*</td>
<td>NS</td>
</tr>
</tbody>
</table>

Ra = glucose rate of appearance. Rd = glucose rate of disappearance. AUC = area under the curve determined using the trapezoid rule. Values are mean (SE). A 2x2 mixed repeated measures ANOVA was used to determine main effects of treatment (rest vs. resistance exercise), time (time-point in the postprandial period), and the interaction between treatment and time. Carry-over effects for total Ra and Rd were determined by assessing for interactions between treatment, time, and study order (rest>exercise or exercise>rest). * denotes significance at p<.05. NS denotes not significant.
Figure 1. Study Protocol

Day Before: Consume standardized breakfast, lunch, and dinner. Fast overnight 10-12 hours

Meal

Exercise Or Rest

Recovery/Basal Period

Post-prandial Period

[6,6-\text{H}_2]\text{glucose constant infusion (initiated 180 min before meal)}

Indirect Calorimetry

Blood Sample

Adipose and Muscle Biopsy

Meal: 35% fat, 49% carbohydrates, 16% protein. Total of 15-16 Kcal/kg FFM up to 800 Kcal separated into 4 aliquots consumed every 4 minutes.
Plasma glucose and insulin concentrations measured throughout the study (mean (SE)). The time 0 corresponds to the start of the glucose tracer. A. plasma glucose (mmol/L). There was a significant interaction between treatment and time (p=.001) with significantly elevated plasma glucose at -10 minutes (post-exercise blood draw) and 180 minutes (end of basal/recovery period), and significantly lower plasma glucose at 270 and 300 minutes. Postprandial total and integrated AUC were significantly reduced in the resistance exercise condition compared to rest (p<.05). B. Plasma insulin concentrations measured throughout the study. There was a main effect of treatment on plasma insulin, indicating overall plasma insulin levels were reduced in the resistance exercise condition compared to the rest condition. Postprandial total and integrated AUC were both significantly reduced after resistance exercise (p<.05)
Figure 3. Basal Glucose Kinetics and Postprandial Insulin Sensitivity

All values are mean (SE). A. Basal glucose rate of appearance (Ra) measured at the end of the basal/recovery period (160-180 min) just prior to eating the standardized mixed meal. B. Hepatic insulin resistance index tended to be increased after resistance exercise, but did not reach significance. C. Basal glucose rate of disappearance (Rd). D. Postprandial insulin sensitivity determined by normalizing plasma glucose Rd (calculated from Steele’s non-steady state equations) to plasma insulin. There was a main effect of treatment (p=.002), indicating that peripheral insulin sensitivity was significantly elevated after the resistance exercise session compared to rest. There were no carry-over effects for any of these measures.
Figure 4. Carbohydrate oxidation and mitochondrial respiration

A. Total carbohydrate oxidation (grams/min) determined using indirect calorimetry. There were no differences between the resistance exercise condition and rest at any time-points, but the integrated area under the curve in the postprandial period (180-540 min) was significantly reduced after resistance exercise compared to rest. B. Pyruvate-supported mitochondrial respiration was significantly elevated after resistance exercise, as was total oxidative phosphorylation (D). Maximal oxidative phosphorylation. * denotes significant difference between rest and exercise at p<.05.

All values are presented as mean (SE).
Chapter 3: Acute Effects of Resistance Exercise on Postprandial Lipid Metabolism in Obese Men with Prediabetes
Introduction

Prediabetes is an interim state of elevated blood glucose above normal levels, but below the levels diagnostic of type 2 diabetes. Prediabetes is highly prevalent in the United States - affecting nearly one in three US adults (> 84 million).\textsuperscript{1} In addition, it’s estimated that <12% of those with prediabetes are aware of their condition.\textsuperscript{1} Considering that prediabetes has been shown to increase the risk for life-threatening complications including nephropathy, neuropathy, cardiovascular disease (CVD), stroke, and type 2 diabetes, there is an urgent need for treatment approaches capable of restoring normoglycemia in these individuals.\textsuperscript{2,3}

Postprandial glycemia is thought to contribute to up to 70% of daytime hyperglycemia, and is a better predictor of glycemic control than pre-meal glycemia.\textsuperscript{4,5,6} Moreover, postprandial hyperglycemia increases the risk for CVD, elevated blood pressure, increased intraocular pressure, obesity, inflammation, and all cause mortality.\textsuperscript{7-10} However, the postprandial period is not solely characterized by elevations in plasma glucose. Plasma triglycerides (in the form of chylomicrons and very low density lipoproteins (VLDL)) are elevated after a meal and can contribute to CVD risk if the response is exaggerated or prolonged (termed postprandial dyslipidemia).\textsuperscript{11} Large cohort studies have confirmed that postprandial dyslipidemia increases the risk for myocardial infarction, ischemic heart disease, cardiac death, and ischemic stroke in both men and women independent of fasting triglyceride concentration.\textsuperscript{11,12}

In human and rodent studies of prediabetes, hypertriglyceridemia is identified as a major, early indicator of disease progression.\textsuperscript{13,14} Elevations in postprandial triglycerides, VLDL and chylomicrons pool sizes (collectively termed triglyceride-rich lipoproteins (TRL-TG)), and increased lipoprotein production rates have all been identified in individuals with prediabetes.
compared to lean, healthy controls.\textsuperscript{15} An imbalance between lipoprotein production and clearance promotes elevated lipemia, which can contribute to ectopic fat accumulation in non-adipose tissues (e.g. liver, skeletal muscle) and reduce insulin sensitivity.\textsuperscript{16,17,18} Individuals with prediabetes also have abnormalities in skeletal muscle lipid handling, including deficiencies in intramyocellular triglyceride (IMTG) synthesis (an important route of fatty acid clearance), prolonged retention of intramyocellular fatty acids, reduced beta oxidation capacity, lipid-induced oxidative stress, and mitochondrial DNA damage: all contributing to dyslipidemia.\textsuperscript{19-22}

Resistance exercise is a central component of exercise recommendations for individuals with type 2 diabetes and prediabetes, but the effect of resistance exercise on postprandial lipid metabolism is not known. There is some evidence in healthy lean and healthy obese men and women demonstrating that regular physical activity can improve postprandial lipid metabolism.\textsuperscript{23} For example, in healthy, obese men and women, acute resistance exercise reduced triglyceride concentration, VLDL triglyceride concentrations and mean residence time, improved fatty acid oxidation rate, and increased IMTG turnover in both the fasted and postprandial state\textsuperscript{24-28}; however, not all studies found these improvements.\textsuperscript{29,30} In addition, one study in individuals with type 2 diabetes found no effect of pre-meal and a modest effect of post-meal resistance exercise on postprandial triglyceride area under the curve.\textsuperscript{31} Thus, evidence in healthy and diabetic populations suggests that resistance exercise might improve postprandial lipid metabolism in individuals with prediabetes, however this has not been previously studied. Moreover, the potential mechanisms of improved postprandial lipid handling after resistance exercise in those with prediabetes (or other populations) remain unknown. Therefore, the overall aim of this study was to determine the acute effects of resistance exercise on postprandial lipid metabolism in overweight/obese men with prediabetes. We hypothesized that resistance exercise would reduce
the concentration of exogenous lipid to circulating TG pools (chylomicrons, VLDL, FFA), and this reduction would be linked to improvements in whole-body lipid oxidation and.

Methods

Participants

Participants were recruited from the Washington University School of Medicine Diabetes Clinic, Washington University’s Volunteers for Health, the Center for Community Based Research, and from the surrounding St. Louis Community. Inclusion criteria were: 1) male sex, 2) age 30-65 years, 3) BMI: 28-45 kg/m², 4) sedentary (routine exercise ≤ 2x/week within the previous 6 months) and 5) diagnosis of prediabetes (HbA1c >5.7 but <6.5%, or fasting plasma glucose >100 mg/dl, or 2-hour OGTT >140 mg/dL but <200 mg/dL). Exclusion criteria included: 1) diagnosis of type 2 diabetes, 2) use of insulin, 3) significant history of CVD, 4) coagulation disorder (platelets <100,000, PT >2 seconds above control or INR >1.5), 5) anemia (hemoglobin <10.0 g/dL), and 6) any orthopedic (including joint replacement within past year), neurologic or metabolic condition that would limit the ability to perform resistance exercise. Each participant read and signed an IRB-approved protocol and informed consent that was approved by the Human Research Protection Office at Washington University in St. Louis, MO.

Study Protocol

This study employed a randomized crossover design to assess changes in postprandial lipid metabolism after a single bout of resistance exercise versus an equivalent period of rest.
Participants who qualified for the study were then randomly assigned to one of two treatment orders: a rest visit followed by an exercise visit, or an exercise visit followed by a rest visit. In both groups, participants completed the rest and exercise visits on separate days >1 week apart.

Screening Visit: Participants were screened prior to enrollment to confirm diagnosis of prediabetes and absence of exclusion criteria. After an overnight fast, participants reported to the Washington University Clinical Research Unit (CRU) where their height, weight, BMI, and body composition were measured (using dual x-ray absorptiometry, DXA) and a medical history and physical were performed by the study physician. An intravenous (IV) catheter was inserted into the antecubital vein to collect fasting blood samples, which were analyzed for plasma glucose, HbA1c, a lipid panel, a comprehensive metabolic panel (CMP), and a complete blood count (CBC).

Body Composition

Each participant received whole-body dual energy X-ray absorptiometry (DXA) scans (Hologic Discovery GDR 1000/W, software version 12.6.2 OD; Waltham, MA) to assess regional and composite lean and fat mass in grams. Image analysis and sub-region (thigh, leg, trunk, upper extremities) composition quantification were performed following the guidelines provided by Hologic GDR software.

Oral Glucose Tolerance Test (OGTT)
A two-hour, 75-gram OGTT was performed after a 12-hour overnight fast. All OGTTs were started between 7am and 9am. Plasma glucose was measured by the glucose oxidase method (YSI Stat Plus, Yellow Springs, OH) and insulin by double antibody radioimmunoassay.

Strength Testing

One repetition maximum (1RM) assessments were performed on a Hoist single pod machine (San Diego, CA, USA) according to guidelines established by the American College of Sports Medicine. The maximum weight lifted through the full range of motion with proper form was recorded for the leg press, bench press, biceps curl, seated row, pull down, knee extension, and shoulder press.

Accelerometry

Daily physical activity over one-week was determined using a ActiGraph GT3X+ accelerometer (ActiGraph LLC, Pensacola, FL). The GTX3+ was placed on the non-dominant wrist with Actigraph non-removable wristbands following the OGTT. The wristbands were cut off by the study team when they returned for their rest or exercise visit to ensure the accelerometers were worn for the entire data collection period. ActiGraph data was collected for seven consecutive days at 30 Hz. The accelerometer output was sampled by a 12-bit analog-to-digital converter. The percentage of time spent sedentary as well as the amount of time spent participating in different categories of physical activity including light, lifestyle, moderate, and vigorous were calculated using algorithms from Freedson in the ActiGraph software.
Rest Visit: The day prior to the rest visit, subjects consumed a standardized breakfast (522 Kcal), lunch (722 Kcal, snack 180 Kcal), and dinner (779 Kcal) provided by the Washington University Institute of Clinical and Translational Sciences Bionutrition Service. Participants also consumed a liquid formula (Ensure; Ross Laboratories, Columbus, OH) containing 250 kcal (40 g carbohydrates, 6.1 g fat, and 8.8 g protein) to ensure complete filling of hepatic glycogen stores as a snack before bedtime. Participants then fasted overnight (10hrs) with nothing taken by mouth except water and reported to the CRU at 0730 the following morning. After admission, height and weight were measured using a standard scale and stadiometer, and an IV catheter was inserted into the antecubital vein to collect baseline fasting blood samples. Fasting blood samples were collected in heparinized tubes for glucose, insulin, c-peptide, citrate-lined tubes for PT-INR, and EDTA lined tubes for lipid kinetics analysis.

Following collection of baseline blood samples, participants rested quietly in the semi-recumbent position for 1 hour. At the end of the rest period the antecubital catheter was prepared for the administration of a prime-constant infusion of stable isotope tracers. Additionally, a second catheter was inserted into a hand vein on the contralateral arm, with the hand warmed to 55°C using a thermostatically controlled hot box to obtain arterialized blood samples. 30 minutes after the rest period, a primed, constant infusion of [6,6-2H2]glucose (28 μmol/kg prime and 0.28 μmol•kg-1•min-1 continuous infusion), was administered for 180 minutes to achieve isotopic steady state (data not presented in this paper). 60 minutes after the start of the glucose, a primed, constant infusion of NaH13CO3 (1 umol/kg priming dose), and [U-13C16]palmitate (.0053 μmol•kg-1•min-1 continuous infusion) were administered for 120 minutes to achieve isotopic
steady state. Blood samples were collected at -10, 160, 170, and 180 minutes from the start of the glucose tracer infusion to quantify basal (i.e. fasting) free fatty acid kinetics. The constant infusion of \([U-^{13}\text{C}_{16}]\text{palmitate}\) was continued through the postprandial period.

Following the 210 minute Baseline Period, participants consumed a liquid test meal consisting of 20 Kcal/FFM, with a composition of 47.8% CHO, 36.1% fat, and 16.1% protein. 10 mg/kg body weight of \([1,1,1,2,2,3,3,4,4,4-^{13}\text{C}_{12}]\text{tripalmitin}\) was added to the test meal and thoroughly mixed. The tripalmitin was emulsified in lecithin and vegetable oil, which was microwaved for 5 minutes to warm. The emulsified mixture was then added to Boost Plus, microwaved, and sonicated to evenly distribute the tracer. To ensure meal consistency for both visits, one large mixture was prepared and then split in half (each half consumed during the rest and exercise visit). Participants were asked to consume the liquid meal within 16 minutes (1/4 of the meal provided every 4 minutes). The sides of the liquid meal container were rinsed with 10 ml of water and the rinse was also consumed to ensure participants ingested the entire study meal. Lastly, 30 minutes after consuming the meal, a 75 umol/kg bolus infusion of \((1,1,2,3,3-^{2}\text{H}_5)\text{glycerol}\) was initiated to quantify lipoprotein kinetics. Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 minutes post-meal ingestion. Samples of the liquid test meal, as well as the tracers used were collected for enrichment analysis. Water was consumed ad libitum during the basal period, but aside from the test meal, study participants consumed no other beverages and no food until the 360-min postprandial measurement period ended. An overview of the study protocol is provided in Figure 1. All stable isotope tracers were obtained from Cambridge Isotope Laboratories (Tewksbury, MA).
Exercise Visit: The exercise visit was identical to the rest visit except participants performed ~1 hour of resistance exercise rather than rest. The exercise session consisted of 8 lifts (bench press, pull down, seated row, biceps curl, knee extension, leg press, and overhead press) performed for 3 sets of 8-12 reps at 80% of their measured 1RM until volitional fatigue. Participants were given no more than 1 minute of rest between sets, and no more than 3 minutes rest between exercises. All exercise sessions were supervised by a licensed physical therapist.

Indirect Calorimetry

Whole body carbohydrate oxidation was measured using indirect calorimetry (Parvo Medics, Sandy, UT) and the ventilated hood technique prior to, and throughout, the postprandial period. VO$_2$, and VCO$_2$ were measured continuously for 15 minutes for the determination of resting energy expenditure, respiratory exchange ratio and carbohydrate oxidation while the participant laid quietly in bed. Measurements were taken at baseline (prior to exercise/rest period, 120 minutes after the start of the exercise/rest period, and then at 30, 60, 150, 210, 270, and 330 min after consuming the liquid test meal. Total carbohydrate oxidation rate (grams/minute) was calculated as previously described.$^{37}$

Sample Processing

Blood samples were placed in chilled tubes containing EDTA and placed on ice. Plasma was recovered by low speed centrifugation within 30 min of sample collection. An aliquot of plasma (3 ml) was kept in the refrigerator for subsequent isolation of lipoprotein fractions, and the remaining plasma was frozen at -80C for further processing.
The TRL fraction was isolated by ultracentrifugation. Two milliliters of each plasma sample was transferred into Optiseal tubes (Beckman Instruments, Inc., Palo Alto, CA), covered with a saline solution (d=1.006 g/ml), and centrifuged in an ultracentrifuge (Beckman Instruments, Inc.) for 16 h at 45000 rpm and 8°C in a 50.4 Ti rotor. The top layer, containing TRL, was removed by tube slicing (Beckman Instruments, Inc.). The exact volume that was recovered (~1.3 ml) was recorded, and the samples were stored at -80°C. Chylomicrons were recovered by over layering 1 ml of plasma with the same saline solution, followed by ultracentrifugation for 35 minutes at 25000 rpm, 16°C, in a SW41 swinging bucket rotor (Beckman Instruments, Palo Alto, CA).

TRL-TG and chylomicrons were isolated by thin layer chromatography (TLC) on LK6D silica gel plates (Whatman, Clifton, NJ) using heptane-diethyl ether-formic acid (80:20:2, v/v/v) and visualized with 0.01% rhodamine 6G. TLC scrapings containing TGs were recovered, extracted with chloroform-methanol (3:1, v/v), transferred to 13 mm screw-top vials and dried under vacuum. Fatty acid methyl esters (FAMEs) were prepared by adding 0.5 ml of 10% acetyl chloride in methanol, capping the tubes with Teflon-lined caps, incubated at 70°C for 30 min, and dried under vacuum. The glycerol liberated by the transmethylation reaction was derivatized by the addition of 200 ul of 5% heptafluorobutyric (HFB) anhydride in ethyl acetate. Capped vials were incubated at 70°C for 30 min. After drying under vacuum, 100 ul ethyl acetate was added and samples were transferred to autosampler vials for GC-MS analysis of HFB- glycerol and palmitate methyl ester in the same run.

Plasma glycerol and palmitate were recovered from 0.25 ml plasma after precipitation of plasma proteins with acetone and extraction of the aqueous phase with hexane. The aqueous
phase containing glycerol was dried under vacuum (SpeedVac) and derivatized with 100 ul of HFB anhydride-ethyl acetate (1:1, v/v) at 70°C for 10 min. After drying in a SpeedVac, the samples were dissolved in 100 ul ethyl acetate. The tracer-to-tracee ratio (TTR) of all oral and infused tracers ($^{13}$C$_4$-palmitate, $^{13}$C$_{16}$-palmitate, and $^3$H$_5$-glycerol) in all TG and FFA pools was determined using gas chromatography–mass spectrometry (GC-MS) (Hewlett-Packard MSD 5973 system with capillary column) as previously described.  

Sample Analysis

Blood for plasma hormone and metabolite analysis was collected from an antecubital vein, immediately placed in chilled EDTA tubes, centrifuged at 2000xg for 10 minutes, and the supernatant collected and frozen at -80°C until analysis. Fasting samples were analyzed were used for comprehensive metabolic panel, lipid panel, CBC, and HbA1c and plasma insulin concentration was measured by radioimmunoassay; all performed HbA1c by the Washington University Core Lab for Clinical Studies. Total plasma, TRL, and chylomicron triglyceride concentrations were measured using an in vitro colorimetric assay (Wako L-Type TG Kit, Wako Diagnostics). VLDL concentrations were measured by subtracting chylomicron TG content from TRL-TG content. Selected ion abundances were compared against external standard curves for calculation of FFA concentration using quantitative GC/MS as previous described.  

Calculations

The concentration of $^{13}$C$_4$-palmitate and $^{13}$C$_{16}$-palmitate in TRL-TG, chylomicrons, and plasma free fatty acid (FFA) were calculated using the following equation:
The concentration of $^{13}$C$_4$-palmitate and $^{13}$C$_{16}$-palmitate in VLDL was calculated by subtracting tracer concentrations in chylomicrons from TRL-TG (chylomicrons + VLDL).

During the last 300 minutes of the baseline period (160, 170, and 180 minutes), a physiological and isotopic state was present, and therefore Steele’s equation for steady state was used to calculate free fatty acid flux (rate of appearance [Ra] and rate of disappearance [Rd]). Ra was calculated by dividing the $^{13}$C$_{16}$-palmitate infusion rate (umol/min) by the plasma tracer enrichment (TTR, mean of all three measurements). FFA Rd was calculated as FFA Ra + $^{13}$C$_{16}$-palmitate infusion rate. Ra was normalized to kg FM, while Rd was normalized per kg of fat-free mass (FFM) derived from DXA. The adipose tissue insulin resistance index (ATIRI) was calculated as the product of basal FFA Ra and plasma insulin concentration.

After mixed meal ingestion, the total FFA rate of appearance (Ra$_{total}$) was calculated from $^{13}$C$_{16}$-palmitate TTR using Steele’s equation:

$$ Ra_{TOT} = \frac{IR - C(t) * V * \frac{d 13C16 TTR(t)}{dt}}{13C16 TTR(t)} $$

where IR is the $^{13}$C$_{16}$-palmitate infusion rate, C is the plasma FFA concentration, and V is the volume of distribution (55 ml/kg, fixed). Before applying Steele’s equation, plasma $^{13}$C$_{16}$ TTR data were smoothed using a spline fitting approach to stabilize the calculation of the derivative of
enrichment. Rate of disappearance (Rd) was calculated as:

\[ Rd(t) = Ra_{TOT}(t) - \frac{dC(t)}{dt} \cdot V \]

Ra was normalized to kg FFM, and Rd was normalized to kg FFM. The FFA metabolic clearance rate (MCR) was estimated as the average Rd during the meal divided by the average plasma FFA concentration. Rd(t) was also divided by plasma insulin at each point during the postprandial period to track changes in insulin sensitivity over time. The total area under the curve was also calculated for total Ra and total Rd using the trapezoid rule. $^{13}$C$_4$-palmitate and $^{13}$C$_{16}$-palmitate absorption in each TG or FFA pool was also measured during the ascending portion of the concentration curves (a period when absorption outpaces clearance and is the primary contributor to the concentration), which corresponded to the period from 180-360 minutes (from the start of the meal to 3 hours post-meal). We identify this period as the “Absorptive Period,” in the results below.

The fractional turnover rate (FTR) of TRL-TG and chylomicrons was determined using the monoexponential approach.\(^4\) The plasma clearance rate of TRL-TG and chylomicrons (ml/min), which is an index of the efficiency of TRL-TG and chylomicron removal from the circulation via all possible routes, was calculated as the FTR times plasma volume. The mean residence time (MRT) of TRL-TG and chylomicrons in the circulation (min) was calculated as 1/FTR as described previously.

From the plasma glucose and insulin values on the OGTT, an index of insulin resistance (IR), beta cell function (HOMA-%B), and insulin sensitivity (HOMA-%S) were calculated using the Homeostatic model assessment (HOMA) 2 using the HOMA2 calculator.\(^4\)
Statistical Analysis

Participant demographics were analyzed using mean ± SE. Data were analyzed for normality using the Shapiro-Wilk test. Differences between rest and exercise conditions on measures of tracer concentrations, TTR, and FFA kinetics were assessed using a 2x2 mixed, repeated measures ANOVA with treatment (rest or exercise) and time (time the sample was collected in the study) entered as repeated measures, and order (rest>exercise or exercise>rest) as a between subjects factor to rule out confounding by carryover effects (to determine if the washout period between the rest and exercise visits was sufficient). The assumption of sphericity was assessed using Mauchly’s test. Huynfeldt adjusted F values were used in cases of the violation of the assumption of sphericity. Significant interactions between treatment and time were further analyzed using paired t-tests. The Benjamini–Hochberg procedure was used to control for multiple comparisons. The two-way interaction between time and order, treatment and order, and the three-way interaction between treatment, time, and order were tested identify the presence of a carry-over effect. Areas under the curve between conditions were analyzed according to the procedures outlined by Wellek and Blettner (2012) for cross-over designs, testing first for carry-over effects, followed by testing for treatment effects. An alpha level = .05 was used for significance. All analyses were completed in SPSS version 25.

Results

Participants
Nine (n=9) male participants were enrolled in the study. Participants were middle-aged, obese, and sedentary (Table 1). All participants were prediabetic, with the group means for HbA1c, 2-hour plasma glucose from the OGTT, and fasting plasma glucose all within the pre-diabetic range (Table 1). Four (n=4) participants had HbA1c values ≥ 5.7%, six had fasting plasma glucose values >100 mg/dL (5.5 mmol/L), and n=7 participants had 2-hour OGTT values >140 mg/dL (7.8 mmol/L). The average % body fat was in the 75th percentile for individuals in their age group as established by the NHANES. Participants also demonstrated insulin resistance according to the HOMA2 model (HOMA2-IR, HOMA2-%S), with compensatory increases in beta cell function (HOMA2-%B). Participants also presented with elevated total triglyceride (>150 mg/dL), LDL (normal: >100 mg/dL), with slightly reduced HDL (<40 mg/dL) concentrations (Table 1).

Total Plasma Triglyceride and Free Fatty Acid Concentration

Following resistance exercise, total postprandial integrated area under the curve (iAUC) for total plasma triglyceride concentration (p=.012), chylomicron triglyceride concentration (p=.036), and TRL-TG concentration (p=.0004) were significantly reduced. VLDL concentration iAUC (p=.065) tended to be reduced compared to the resting condition (Figure 2). Resistance exercise also tended to reduce postprandial plasma FFA concentration (p=.068) compared to rest.

Exogenous Lipid Metabolism Kinetics
\(^{13}\)C\(_4\)-palmitate (isotopically labeled palmitate from the Tripalmitin tracer added to the test meal) kinetics in chylomicrons, VLDL, TRL-TG, and plasma FFA were quantified throughout the postprandial period to determine meal-derived (exogenous) lipid handling. There was a significant interaction between treatment (i.e. exercise/rest) and time (p=.017) for exogenous palmitate concentration in chylomicrons and TRL-TG (p=.046) (Figure 3). Time-points that were significantly different between exercise and rest are indicated in Figure 3. There were no carry-over effects identified for any of these measures (all p>.05). There was only a main effect of time for the exogenous palmitate concentration in VLDL (p<.001). The concentration of exogenous palmitate in plasma FFA tended to be reduced after resistance exercise (main effect treatment: (p=.071), and also showed a main effect of time (p<.001). Resistance exercise significantly reduced the total AUC for exogenous in TRL-TG (p=.043), chylomicrons (p=.049), and tended to reduce exogenous palmitate AUC in VLDL-TG (p=.078) (Figure 3). The resistance exercise session also tended to reduce the total AUC for \(^{13}\)C\(_4\)-palmitate in plasma FFA (p=.078) compared to rest.

Endogenous Lipid Metabolism Kinetics

\(^{13}\)C\(_{16}\)-palmitate (an infused isotopically labeled fatty acid) concentration and enrichment in chylomicrons, VLDL, TRL-TG, and plasma FFA were quantified throughout the postprandial period to determine endogenous lipid handling. There was a significant interaction between treatment and time for both chylomicrons (p=.012) and TRL-TG (p=.035) for the concentration of endogenous palmitate (Figure 4). The time-points significantly different between rest and resistance exercise for chylomicrons and TRL-TG are shown in Figure 4. We also found a main
effect of treatment and a main effect of time for the concentration of endogenous palmitate in plasma FFA (Treatment: p=.049, time: p<.001), indicating that the resistance exercise session reduced postprandial endogenous palmitate concentrations overall compared to the rest session (Figure 4). We did not find any treatment effects for endogenous palmitate concentration in VLDL-TG (p=.168) due to large standard deviations for this measure. We also found that resistance exercise reduced the AUC for endogenous palmitate in TRL-TG (p=.041), chylomicrons (p=.024), and plasma FFA (p=.009), with no difference in the endogenous palmitate AUC for VLDL (p=.127) (Figure 4).

Absorptive Period Lipid Kinetics

Exogenous palmitate and endogenous palmitate absorption in each TG or FFA pool was also measured during the ascending portion of the concentration curves (a period when absorption outpaces clearance and is the primary contributor to the concentration), which corresponded to the period from 180-360 minutes (i.e. from the start of the meal to 3 hours post-meal). Similar to the total AUC, the absorptive AUC of exogenous palmitate was significantly reduced after resistance exercise in chylomicrons (Rest: 993.1 ± 353 umol/L•180 min vs Ex: 542.9 ± 209.7 umol/L•180 min, t(8)=2.37, p=.045) and TRL-TG (Rest: 1437.9 ± 313.2 umol/L•180 min vs Ex: 1020.2 ± 261.8 umol/L•180 min, t(8)=3.29, p=.044), and tended to reduce the AUC in plasma FFA (Rest: .42 ± .08 umol/ml•360 min, Ex: .29 ± .06 umol/ml•180 min, t(8)= 2.20, p=.058). There were no differences in the exogenous palmitate concentration AUC in VLDL from 180-360 minutes (p=.203). Likewise, we also found significant reductions in the absorptive phase of the endogenous palmitate AUC for chylomicrons (Rest: 141.7 ± 43.2
umol/L at 180 min vs Ex: 103.1 ± 34.8 umol/L at 180 min, t(8)=2.82, p=.022, TRL-TG (Rest: 328.9 ± 51.2 umol/L at 180 min vs Ex: 274.9 ± 36.4 umol/L at 180 min, t(8)=2.86, p=.021), but not for VLDL (p=.236), or plasma FFA (p=.319).

Basal/Recovery Period Free Fatty Acid Kinetics

One hundred eighty minutes (180 min) following the exercise/rest session, a steady state of U\textsuperscript{13}C palmitate was reached and U\textsuperscript{13}C rate of appearance and disappearance and adipose tissue insulin resistance index, were determined (Table 2). Resistance exercise resulted in a significant increase in U\textsuperscript{13}C palmitate acid rate of appearance and disappearance compared to rest (Table 2). There was also a trend (p=0.07) toward an increase in adipose tissue insulin resistance following resistance exercise compared to rest (Table 2).

Postprandial Free Fatty Acid Kinetics

There was a significant interaction between treatment and time for total FFA Rd (Table 2). Simple main effects analysis revealed total FFA Rd was significantly elevated after resistance exercise at time points 180 min, 196 min, and 480 min. We also found a trend toward an interaction between treatment and time for postprandial FFA total Ra (Table 2). However, postprandial FFA metabolic clearance rate, AUC for postprandial FFA total Rd, or the AUC for FFA total Ra were not different following exercise and rest.
Lipoprotein Turnover and Clearance Kinetics

Using incorporation and clearance of the $^2$H$_5$-glycerol in chylomicrons and TRL-TG, fractional turnover rate, mean residence time, and clearance rate for TRL-TG and chylomicrons were quantified (Table 3). Resistance exercise significantly reduced the TRL-TG mean residence time, and tended ($p=0.083$) to increase the fractional turnover rate and clearance rate ($p=0.074$). Additionally, we found significant reductions in the chylomicron mean residence time and clearance rates, with a trend ($p=0.054$) toward an increase in the fractional turnover rate (Table 3).

Lipid Oxidation

There was a significant interaction between treatment and time for lipid oxidation rate ($p=.007$) (Figure 5). Simple main effects demonstrated that lipid oxidation was significantly elevated at 140 minutes ($p=.014$) and 210 minutes ($p=.015$) post exercise/rest, with trends toward significant elevations at time points 240 ($p=.092$) and 330 ($p=.072$) above the rest condition after a single bout of resistance exercise. Lipid oxidation AUC was significantly greater after the resistance exercise session compared to rest ($p=.048$).

Discussion

Elevated postprandial lipemia is an early feature of prediabetes and contributes to an increased risk for cardiovascular disease and progression to type 2 diabetes.$^{15}$ Evidence from other populations suggest that resistance exercise may improve postprandial lipid metabolism,
however the effect of resistance exercise in individuals with prediabetes is unknown.\textsuperscript{24,25} Our study demonstrated the novel finding that a single bout of resistance exercise, performed 210 minutes before consuming a mixed carbohydrate and lipid meal, reduced total plasma TG, chylomicron and TRL-TG concentrations, and tended to reduce VLDL plasma FFA concentrations in men with prediabetes. In addition, our study revealed for the first time, that declines in plasma triglyceride concentration following acute resistance exercise were due to reductions of both exogenous (meal-derived) and endogenous (originating internally) triglycerides and fatty acids. Moreover, acute resistance exercise increased lipoprotein turnover, FFA clearance rate, and postprandial lipid oxidation rate. These data support the use of resistance exercise as a non-invasive treatment approach to reduce postprandial lipemia in men with prediabetes.

Chylomicron Metabolism

Postprandial chylomicron concentrations were reduced after resistance exercise compared to rest. This reduction was caused by: 1) a significant decline in the content exogenous triglycerides (reflected by reduced $^{13}$C\textsubscript{4}-palmitate concentration); and 2) a decline in endogenous FFA content (reflected by the reduced $^{13}$C\textsubscript{16}-palmitate concentration). The decline in exogenous triglyceride content following resistance exercise could be caused by a lower amount of meal-TG absorption or delayed meal-TG absorption. Aerobic exercise has been shown to reduce splanchnic blood flow by 45-80\% depending on the duration and intensity of the exercise session.\textsuperscript{43} Reductions in splanchnic blood flow reduce gastrointestinal motility and gastric emptying and could therefore change amount or timing of absorption of meal-derived TG or
Aerobic exercise has also been shown to increase the expression of genes that control enterocyte lipid anabolism, fatty acid oxidation, and mitochondrial content.\textsuperscript{44,45} Increases in enterocyte fatty acid oxidation have been shown to limit the triglyceridemic response to a meal by reducing the substrate available for triglyceride synthesis and chylomicron secretion.\textsuperscript{45,46,47} If similar to aerobic exercise, these data suggest that resistance exercise may have modulated exogenous lipid absorption, enterocyte metabolism, or lipid handling (e.g., changes in triglyceride synthesis or packaging of chylomicrons, changes in cytosolic lipid droplet kinetics). However, we did not measure these factors directly, and their role in the changes we observed after resistance exercise requires further investigation.

In addition to reductions in exogenous lipid contribution to chylomicrons we also found evidence that resistance exercise altered endogenous lipid contributions to these lipoproteins. Endogenous FFA can be taken up by the enterocyte from the portal system, re-esterified, and packaged into chylomicrons.\textsuperscript{48} Therefore, the reduction in $^{13}$C\textsubscript{16}-palmitate in chylomicrons in our study could have been caused by a reduction in the amount of endogenous palmitate in the circulation. Since $^{13}$C\textsubscript{16}-palmitate was infused at a constant rate, declines in $^{13}$C\textsubscript{16}-palmitate concentrations in the plasma could have been caused by an increase in plasma FFA clearance. Indeed, using non-steady state modeling, we found that the FFA Rd was elevated during the postprandial period after resistance exercise compared to rest, suggesting the $^{13}$C\textsubscript{16}-palmitate might have been cleared before it had a chance to be re-esterified by the enterocyte. The decline in $^{13}$C\textsubscript{16}-palmitate concentration could also have been due to undefined changes in the handling of endogenous FFA by the enterocyte (i.e., redirecting endogenous FFA toward storage as rather than re-esterification into chylomicrons), reductions in splanchnic blood flow after resistance exercise (thereby limiting the availability of endogenous FFA for enterocyte uptake), or
enhanced VLDL clearance prior to meal consumption (which would reduce the amount of endogenous lipid available for enterocyte uptake). However, we did not measure these factors directly, so they warrant further investigation.

Finally, there was a significant reduction in the chylomicron mean residence time and an increase in the chylomicron clearance rate after resistance exercise, which might have been caused by an increase in chylomicron hydrolysis by lipoprotein lipase. However, we did not measured LPL activity and thus further research is needed to explore the contribution of these various mechanisms to the decline in both exogenous and endogenous lipids to chylomicrons after resistance exercise. Overall, postprandial chylomicron exposure (represented by the AUC) following resistance exercise was reduced, which is important in decreasing postprandial lipemia, reducing body weight, and reducing the risk for severe complications such as CVD.

VLDL Metabolism

Unlike chylomicrons, we found only a trend toward reduction in the amount of exogenous palmitate in VLDL after resistance exercise. The appearance of exogenous palmitate in VLDL is likely from spillover of meal palmitate liberated after chylomicron hydrolysis but not taken up by skeletal muscle or adipose tissue. Meal-derived FFA can be taken up by the liver, re-esterified, and incorporated into VLDL. The spill-over of dietary FFA has been shown to account for ≥ 25% of postprandial FFA flux and could therefore be an important contributor to CVD risk and prediabetes. Mitigating meal FFA spill-over to the liver may subsequently lower VLDL production by reducing substrate for lipidation of apoB-100. It may also promote weight
loss by reducing the amount of FFA available for adipose tissue esterification in the postprandial period.

In contrast to exogenous lipid contributions to VLDL, endogenous palmitate in VLDL did not significantly change following resistance exercise. However, the total AUC for the amount of endogenous palmitate in VLDL tended to be lower (by ~ 60 umol/L). Resistance exercise may not have a strong effect on hepatic uptake of plasma FFA, evidenced by the similarity between endogenous palmitate concentration in VLDL between rest and exercise conditions. The lack of a difference in endogenous palmitate in VLDL might also be explained by a reduction in the availability of meal-derived FFA as a substrate for VLDL synthesis. With reduced exogenous FFA substrate, VLDL particles produced in the liver could be expected to have a larger contribution from endogenous FFA, thereby masking any true differences between rest and exercise conditions. Of the two sources of FFA for VLDL synthesis, the results of our study suggest reductions in the availability of exogenous FFA may more strongly contribute to reductions in postprandial VLDL concentrations after resistance exercise. Our results agree with those reported by Davitt et al. (2013), who found that resistance exercise performed 30 minutes prior to a meal reduced exogenous FFA concentrations but not endogenous FFA.32

Several previous studies have also confirmed that VLDL and chylomicrons compete for lipoprotein lipase in the postprandial period, and that lipoprotein lipase tends to prefer chylomicrons rather than VLDL for hydrolysis.50 Previous studies have shown that VLDL particles accumulate in the plasma after lipid ingestion due to a delay in their lipolysis, which can be attributed to LPL’s preference for larger lipoproteins, and to their reduced efficiency at hydrolyzing VLDL.51,52 If so, the trend toward reduction in VLDL concentration might be due to a reduction of available substrate or reductions in VLDL secretion rate more-so than clearance.
TRL-TG Metabolism

Further support for the diminished clearance of VLDL comes from partitioning of exogenous and endogenous lipids in TRL-TG - the combination of VLDL and chylomicrons. In TRL-TG we found significant reductions in both exogenous palmitate and endogenous palmitate, with greater reductions in chylomicrons than VLDL. When assessing TRL-TG turnover, we found only a significant reduction for mean residence time following resistance exercise. The difference between chylomicron clearance and TRL-TG clearance could be attributed to the VLDL contribution to the TRL-TG pool. Since chylomicron clearance rate was increased, and VLDL clearance was not significantly increased after resistance exercise, the effect size for lipoprotein clearance of TRL-TG was likely diluted by the smaller changes in VLDL metabolism.

Pre-Meal Free Fatty Acid Metabolism Kinetics

Following resistance exercise, pre-meal FFA Ra and Rd were increased and adipose tissue insulin resistance index tended to increase. FFA Ra from adipose tissue lipolysis has been shown to increase ~5 fold above resting conditions after moderate intensity aerobic exercise, and this increase can persist for 3-6 hours to increase the supply of FFA for recovering muscle in healthy subjects. Elevated levels of catecholamines and reductions in insulin are thought to contribute to the stimulation of adipose tissue lipolysis, which could be further augmented by increases in adipose tissue blood flow. We did not measure plasma catecholamines, but we did find that plasma insulin was reduced after the resistance exercise session (previously described).
The slight increase identified in adipose tissue insulin resistance (Table 2) would also serve to augment adipose tissue lipolysis and increase circulating FFA in the post-exercise period.

While we did not find a significant reduction in total postprandial plasma FFA (Figure 2) following resistance exercise, we did find significant reductions in endogenous FFA and a trend toward reduced exogenous FFA in plasma. The reduction in endogenous FFA suggests that plasma FFA clearance is elevated after resistance exercise, which coincides with our measure of increased postprandial FFA rate of disappearance (Table 2). FFA metabolic clearance rate and total FFA Rd AUC tended to increase following resistance exercise, which, taken together, suggests that resistance exercise improves postprandial plasma FFA clearance. Finally, we likely failed to find a difference in total FFA iAUC due to the large standard deviations in this measure, or because of increases in plasma FFA Ra (which tended to increase, though it did not reach significance). The increases in Ra could negate reductions in total FFA concentration that would have been driven by an unopposed increase in FFA Rd.

Lipid Oxidation

Whole-body lipid oxidation rate was significantly elevated after resistance exercise, that this increase persisted for more than 5 hours (2.5 of which were after the meal), and that participants oxidized significantly more lipid across the entire study compared to their rest visit. It is well-established that aerobic exercise increases FFA skeletal muscle uptake and oxidation in healthy subjects.\textsuperscript{58} In our study, we provide the novel evidence that resistance exercise can promote the similar effects in prediabetes, which might have important implications for long-term weight loss.
It also is important to note that lipid oxidation rate returned to baseline levels 3.5 hours into the postprandial period. At this point, we identified a rapid increase in the RER, indicating a shift toward greater use of carbohydrates for energy in the later half of the postprandial period. Previous studies have shown that consumption of a mixed meal results in a rapid switch to carbohydrate oxidation, even in high-fat meals.\textsuperscript{59} Previous studies have also shown that the acute effects of aerobic exercise on postprandial lipid metabolism dissipate over time lasting from 12-16 hours.\textsuperscript{60,26} The dissipation in effectiveness of a single bout of resistance exercise on postprandial lipid metabolism could have been due to meal-induced switching of primary fuel sources. Further investigation is needed to elucidate the effects of chronic resistance exercise (i.e. training) on postprandial lipid metabolism.

Conclusion

In our study, we found that a single bout of resistance exercise reduced postprandial lipemia in obese men with prediabetes. Using a stable isotope tracer approach, we found that resistance exercise lowers exogenous and endogenous lipid contributions to both plasma FFA, chylomicrons, and VLDL, increased plasma FFA rate of disappearance, and increased lipoprotein clearance rates – all of which contributed to reduced plasma TG concentrations. Moreover, we found that acute resistance exercise increased total lipid oxidation, the lipid oxidation rate, and the contribution of energy derived from lipid oxidation. Aerobic exercise may be difficult for many sedentary, obese individuals who are just beginning an exercise-training program or for those with balance or gait abnormalities. Resistance exercise is often more accommodating for individuals with little exercise experience, and may be easier to adhere to
due to the intermittent nature of the exercise and the lower time commitment needed to derive benefit. We provide evidence to suggest that resistance exercise can be highly beneficial for individuals with prediabetes, and may help reduce their risk of CVD and other for life-threatening complications associated with derangements in postprandial lipid metabolism.

Limitations

There are several limitations to this study. We did not isolate VLDL lipoproteins independent of chylomicrons. Therefore, we could not calculate the TTR or concentration of 13C4 or 13C16-palmitate in VLDL directly. Likewise, we also could not measure VLDL concentration directly. This study only enrolled adult men thus the results cannot be generalized to women and children. Additionally, the study sample size was limited and should be expanded to increase the validity of these findings.


16. van Herpen NA, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav. 2008 May 23;94(2):231-41


Table 1. Participant Characteristics (N=9)

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Values are presented as mean (standard error). % of time spent in each activity were derived from accelerometer data (see methods). WB = whole body. 2-hr OGTT is plasma glucose at 2-hours after a 75 gram oral glucose challenge. TG = triglycerides. HOMA2 calculated using the HOMA2 calculator accessed from (http://www.dtu.oc.ac.uk).
Table 2. Free Fatty Acid Kinetics

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<td>Basal FFA Ra (umol/kg FM/min)</td>
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<tr>
<td>ATIRI (umol/min/mU/ml)</td>
<td>2.45 ± .44</td>
<td>3.23 ± .76</td>
<td>t(8)=2.091, p=.070</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial FFA total Rd (umol/kg FFM/min)</td>
<td>--</td>
<td>--</td>
<td>F(11,77)=2.49, p=.01* 180 min (p=.043)‡ 196 min (p=.016)‡ 480 min (p=.031)‡</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial FFA total Ra (umol/kg FM/min)</td>
<td>--</td>
<td>--</td>
<td>F(12,84)=1.736, p=.073</td>
<td>NS</td>
</tr>
<tr>
<td>FFA Metabolic Clearance Rate (L/kg FFM/min)</td>
<td>2.98 ± .40</td>
<td>3.84 ± .67</td>
<td>t(8)=2.01, p=.079</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial FFA total Rd AUC (umol/kg FFM/min*360 min)</td>
<td>532.3 ± 128.1</td>
<td>908.6 ± 256.6</td>
<td>t(8)=2.03, p=.076</td>
<td>NS</td>
</tr>
<tr>
<td>Postprandial FFA total Ra AUC (umol/kg FM/min)</td>
<td>1029.9 ± 223.8</td>
<td>1830.4 ± 767.5</td>
<td>t(8)=1.44, p=.187</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SE. ATIRI = Adipose tissue insulin resistance index (higher = more insulin resistant). Ra=rate of appearance. Rd=rate of disappearance. FM=Fat mass. FFM= Fat-free mass. * denotes significant at p<.05 level. ‡ denotes exercise condition is greater than rest condition at that time-point. NS = not-significant
Table 3. Lipoprotein Kinetics

<table>
<thead>
<tr>
<th>Measure</th>
<th>Rest</th>
<th>EX</th>
<th>Sig.</th>
<th>Carry-over Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRL-TG FTR (pools/min)</td>
<td>-0.004 (0.0006)</td>
<td>-0.007(0.0014)</td>
<td>.083</td>
<td>NS</td>
</tr>
<tr>
<td>TRL-TG Mean Residence Time (min)</td>
<td>255.5 (34.1)</td>
<td>174.8 (24.1)</td>
<td>.020*</td>
<td>NS</td>
</tr>
<tr>
<td>TRL-TG Clearance Rate (ml/min)</td>
<td>-16.1 (2.1)</td>
<td>-25.1 (4.5)</td>
<td>.074</td>
<td>NS</td>
</tr>
<tr>
<td>Chylo FTR (pools/min)</td>
<td>-0.007 (0.0006)</td>
<td>-0.010 (0.001)</td>
<td>.054</td>
<td>NS</td>
</tr>
<tr>
<td>Chylo Mean Residence Time (min)</td>
<td>153.5(14.0)</td>
<td>109.5 (11.03)</td>
<td>.035*</td>
<td>NS</td>
</tr>
<tr>
<td>Chylo Clearance Rate (ml/min)</td>
<td>-24.6 (2.05)</td>
<td>-35.1 (3.4)</td>
<td>.048*</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SE. FTR=Fractional turnover rate. Chylo=chylomicron. TRL-TG=Triglyceride-rich lipoprotein triglycerides. * denotes significant at p<.05 level. NS = not-significant
Figure 1. Study Protocol

Day Before: Consume standardized breakfast, lunch, and dinner. Fast overnight 10-12 hours

Meal

[6,6-\text{H}_2]\text{glucose constant infusion} (initiated 180 min before meal)

[13C16\text{palmitate}] constant infusion (initiated 120 min before meal)

[\text{H}_2]\text{glycerol bolus injection}

Exercise Or Rest
Recovery/Basal Period
Post-prandial Period

Indirect Calorimetry

Adipose and Muscle Biopsy

Blood Sample

Meal: 35% fat, 49% carbohydrates, 16% protein. Total of 15-16 Kcal/kg FFM up to 800 Kcal separated into 4 aliquots consumed every 4 minutes.
Figure 2. Change in total plasma triglycerides and free fatty acids

Values are mean ± SE. A. Postprandial chylomicron iAUC in the rest and resistance exercise conditions. B. Postprandial VLDL iAUC in the rest and resistance exercise conditions. C. Postprandial TRL-TG iAUC in the rest and resistance exercise conditions. D. Postprandial total plasma TG iAUC in the rest and resistance exercise conditions. E. Postprandial FFA iAUC in the rest and exercise conditions. * Denotes significant difference between rest and exercise at p<.05. § Denotes trend toward significant difference between rest and exercise.
Figure 3. Exogenous Lipid Metabolism Kinetics
Values are mean ± SE. Black circles = rest condition. Gray circles = exercise condition. A. $^{13}$C$_4$-palmitate tracer to trace ratio (TTR) in chylomicrons, with the area under the curve (AUC) shown in the bar graph to the right throughout the study. There was a significant interaction between treatment and time for TTR of $^{13}$C$_4$-palmitate in chylomicrons. B. $^{13}$C$_4$-palmitate concentration in chylomicrons, with the area under the curve (AUC) shown in the bar graph to the right. There was a significant interaction between treatment and time for the amount of $^{13}$C$_4$-palmitate in chylomicrons, and the total area under the curve for the amount of $^{13}$C$_4$-palmitate in chylomicrons was significantly reduced after the resistance exercise session. C. $^{13}$C$_4$-palmitate TTR in TRL-TG, with the area under the curve (AUC) shown in the bar graph to the right. There was a significant interaction between treatment and time for TTR of $^{13}$C$_4$-palmitate in TR-TG. D. $^{13}$C$_4$-palmitate concentration in TRL-TG, with the area under the curve (AUC) shown in the bar graph to the right. There was a significant interaction between treatment and time for the amount of $^{13}$C$_4$-palmitate in TRL-TG, and the total AUC was significantly reduced after resistance exercise compared to rest. E. $^{13}$C$_4$-palmitate TTR in plasma FFA, with the area under the curve (AUC) shown in the bar graph to the right. There was a main effect of treatment for the amount of $^{13}$C$_4$-palmitate in plasma FFA (exercise lower than rest), and there was a trend toward a reduction in the total $^{13}$C$_4$-palmitate AUC in plasma FFA. F. $^{13}$C$_4$-palmitate concentration in plasma FFA, with the area under the curve (AUC) shown in the bar graph to the right. There was a main effect of treatment for the amount of $^{13}$C$_4$-palmitate in plasma FFA (exercise lower than rest), and there was a trend toward a reduction in the total $^{13}$C$_4$-palmitate AUC in plasma FFA. G. $^{13}$C$_4$-palmitate concentration in VLDL, with the area under the curve (AUC) shown in the bar graph to the right. There was a trend toward a reduction in the total $^{13}$C$_4$-palmitate AUC after resistance exercise compared to rest. Meal indicates timing of mixed meal consumption. * Denotes significant difference between rest and exercise (p<.05). § Denotes trend toward significant difference between rest and exercise.
Figure 4. Endogenous Lipid Metabolism
Values are mean ± SE. Black circles = rest condition. Gray circles = exercise condition. A. $^{13}\text{C}_{16}$-palmitate tracer to trace ratio (TTR) in chylomicrons, with the area under the curve (AUC) shown in the bar graph to the right throughout the study. There was a main effect of treatment (exercise lower than rest) for the TTR in chylomicrons. B. $^{13}\text{C}_{16}$-palmitate concentration in chylomicrons, with the area under the curve (AUC) shown in the bar graph to the right. There was a significant interaction between treatment and time for the amount of $^{13}\text{C}_{16}$-palmitate in chylomicrons, and the total area under the curve for the amount of $^{13}\text{C}_{16}$-palmitate in chylomicrons was significantly reduced after the resistance exercise session. C. $^{13}\text{C}_{16}$-palmitate TTR in TRL-TG, with the area under the curve (AUC) shown in the bar graph to the right. D. $^{13}\text{C}_{16}$-palmitate concentration in TRL-TG. There was a significant interaction between treatment and time for the amount of $^{13}\text{C}_{16}$-palmitate in TRL-TG, and the total AUC was significantly reduced after resistance exercise compared to rest. E. $^{13}\text{C}_{16}$-palmitate TTR in plasma FFA, with the area under the curve (AUC) shown in the bar graph to the right. F. $^{13}\text{C}_{16}$-palmitate concentration in plasma FFA, with the area under the curve (AUC) shown in the bar graph to the right. There was a main effect of treatment for the amount of $^{13}\text{C}_{16}$-palmitate in plasma FFA (exercise lower than rest), and there was a significant reduction in the total $^{13}\text{C}_{16}$-palmitate AUC in plasma FFA. G. $^{13}\text{C}_{16}$-palmitate concentration in VLDL, with the area under the curve (AUC) shown in the bar graph to the right. Meal indicates timing of mixed meal consumption. * Denotes significant difference between rest and exercise (p<.05). § Denotes trend toward significant difference between rest and exercise.
Figure 5. Lipid Oxidation

A. Shows the rate of lipid oxidation (grams per minute) in the rest (black solid circles) and exercise (gray circles) conditions. Meal indicates timing of mixed meal consumption. Gray box labeled “Rest/Exercise” denotes timing of the rest or exercise session. Box labeled “Recovery Period” denotes timing of the 180 minute recovery period. B. Total AUC for lipid oxidation rate in A. There was a significant interaction between treatment and time for lipid oxidation rate (see Results for details). * denotes significantly different between rest and exercise. § Denotes trend toward significant difference between rest and exercise.

Values are mean ± SE.
Chapter 4: Acute Effects of Resistance Exercise on Skeletal Muscle Mitochondrial Respiration, and Skeletal Muscle and Adipose Tissue Gene Expression in Obese Men with Prediabetes
Introduction

Prediabetes is a metabolic state characterized by elevated fasting and/or postprandial glucose that develops in response to the onset and progression of peripheral insulin resistance.\textsuperscript{1} One of the major contributors to insulin resistance is excessive delivery of triglycerides and fatty acids to non-adipose tissues such as skeletal muscle and liver (termed lipid overload).\textsuperscript{2} Prediabetes is also characterized by elevated fasting and postprandial lipemia, which may develop secondary to a poor diet, changes in adipose tissue lipolytic activity, reductions in mitochondrial oxidative capacity, and deficits in fatty acid and lipoprotein clearance across multiple organs.\textsuperscript{3} Alterations in lipid metabolism typically antedate the onset of prediabetes and type 2 diabetes, and should, therefore, be a major focus of treatment.\textsuperscript{4}

Recently, Straczkowski et al. found that skeletal muscle from individuals with impaired glucose tolerance, and from offspring of diabetic parents, had elevated levels of ceramide.\textsuperscript{5} Accumulation of ceramide and other lipid intermediates such as acetyl-coA, NADH, diacylglycerols, cytosolic citrate, and long-chain acyl carnitine CoA have been shown to induce insulin resistance in skeletal muscle through inhibition of key protein kinases in the insulin signaling pathway.\textsuperscript{6,7} Overabundance of these intermediates as seen in prediabetic skeletal muscle could be caused by an imbalance between free fatty acid delivery (elevated lipolytic rate and lipemia) and clearance (lower mitochondrial beta oxidation or re-estherification as intramyocellular triglyceride).\textsuperscript{8} Similarly, deficits in oxidative enzyme activity, oxidative phosphorylation pathway activity, and intramyocellular triglyceride synthesis have been identified in obesity and in non-diabetic individuals with a family history of type 2 diabetes – providing additional evidence for the role of lipid imbalance in disease progression.\textsuperscript{9}
While the mechanisms of diminished oxidative capacity in prediabetes are not fully understood, previous studies have shown that reactive oxygen species induce mitochondrial structural alterations, mitochondrial DNA damage, and mitochondrial dysfunction.\textsuperscript{10} Markers of mitochondrial DNA damage (8-OHdG, a marker of oxidation of deoxyguanosine) and reactive oxygen species are known to be elevated in prediabetes, along with reductions in uncoupling protein 3 – a protein known to protect the mitochondrial matrix from lipid-induced damage.\textsuperscript{11,12} Taken together, these studies suggest that reductions in mitochondrial oxidative capacity, driven by lipid overload, excessive reactive oxygen species production, mitochondrial DNA damage, and a reduced protective capacity, contribute to insulin resistance and the development of prediabetes. Therefore, treatment approaches that increase mitochondrial oxidative capacity and lipid clearance are potentially important in the treatment of prediabetes and prevention of type 2 diabetes.

Physical inactivity is known to reduce insulin sensitivity and reduce mitochondrial respiration in skeletal muscle.\textsuperscript{13} In contrast, increasing physical activity can increase skeletal muscle oxidative capacity and improve lipid metabolism in healthy lean and obese populations.\textsuperscript{14-18} Much less is known regarding the benefits of exercise on skeletal muscle oxidative capacity in those with prediabetes and type 2 diabetes. In particular, the mitochondrial response to resistance exercise has received little investigative attention despite being a central component of exercise recommendations for these individuals. Therefore, the purpose of this study was to investigate the impact of a single bout of acute resistance exercise on skeletal muscle mitochondrial oxidative capacity and skeletal muscle and adipose tissue gene expression in the postprandial state in obese, sedentary, prediabetic males. We hypothesized that acute resistance exercise would improve measures of skeletal muscle mitochondrial lipid and pyruvate
respiration, and that these changes would be accompanied by increases in gene expression associated with lipid mobilization and oxidation.

Methods

Participants

Participants were recruited from the Washington University School of Medicine Diabetes Clinic, Washington University’s Volunteers for Health, the Center for Community Based Research, and from the surrounding St. Louis Community. Participants were included if they were male, aged 30-65 years with, a BMI of 28-45 kg/m², and diagnosis of prediabetes (HbA1c >5.7 but <6.5%, or fasting plasma glucose >100 mg/dl, or 2-hour OGTT >140 mg/dL but <200 mg/dL). Participants were excluded for having diagnosis of type 2 diabetes, use of insulin, participation in regular exercise (≥ 2x/week) within the previous 6 months; history or evidence of serious pulmonary or cardiovascular disease; coagulation disorders (platelets <100,000, PT >2 seconds above control or INR >1.5); anemia (hemoglobin <10.0 g/dL); any orthopedic, neurologic, metabolic or other medical condition that would prohibit the ability to perform resistance exercise; or joint replacement within the last year. Each participant read and signed an IRB-approved protocol and informed consent that was approved by the Human Research Protection Office at Washington University in St. Louis, MO.

Study Protocol
This study utilized a randomized crossover design to assess changes in skeletal muscle mitochondria lipid and pyruvate respiration and gene expression after a single bout of resistance exercise vs. an equivalent period of rest. Participants who qualified for the study were then randomly assigned to one of two treatment orders: a rest visit followed by an exercise visit, or an exercise visit followed by a rest visit. In both groups, participants completed the rest and exercise visits on separate days >1 week apart.

Screening Visit: Participants were screened prior to enrollment to confirm diagnosis of prediabetes and absence of exclusion criteria. After an overnight fast, participants reported to the Washington University in St. Louis Clinical and Translational Research Unit (CTRU) where height (stadiometry), weight, BMI, and body composition were measured and a history and physical were performed by a licensed endocrinologist. An intravenous catheter was inserted into the antecubital vein to collect baseline fasting blood samples, which were analyzed for plasma glucose, HbA1c, a lipid panel, a comprehensive metabolic panel (CMP), and complete blood count (CBC).

Body Composition

Each participant received whole-body dual energy x-ray absorptiometry (DXA) scans (Hologic Discovery GDR 1000/W, software version 12.6.2 OD; Waltham, MA) to assess regional and composite lean and fat mass in grams. Image analysis and sub-region (thigh, leg, trunk, upper extremities) composition quantification was carried out following the guidelines provided by Hologic GDR software.
Oral Glucose Tolerance Test (OGTT)

Two-hour, 75-gram OGTTs were performed at baseline after a 12-hour overnight fast during the screening visit. All OGTTs were started between 7am and 9am. 2-hour plasma glucose values were used to identify individuals with or without prediabetes. Plasma glucose was measured with the glucose oxidase method (YSI Stat Plus, Yellow Springs, OH) and insulin by double antibody radioimmunoassay. An index of insulin resistance (IR), beta cell function (HOMA-%B), and insulin sensitivity (HOMA-%S) were calculated using the Homeostatic model assessment (HOMA) 2 using the HOMA2 calculator.19

Strength Testing

One repetition maximum (1RM) assessments were performed on a Hoist single pod machine (San Diego, CA, USA) according to guidelines established by the American College of Sports Medicine. The maximum weight lifted through the full range of motion with proper form was recorded for the leg press, bench press, biceps curl, seated row, pull down, knee extension, and shoulder press.

Accelerometry

Daily physical activity of participants was determined by actigraphy (ActiGraph GT3X+ accelerometer, ActiGraph LLC, Pensacola, FL). The GTX3+ was placed on the non-dominant
wrist with Actigraph non-removable wristbands at the completion of the Screening visit. The wristbands were cut off by the study team when participants returned for their second visit to ensure the accelerometers were worn for the entire data collection period. ActiGraph data was collected for seven consecutive days at 30 Hz. The accelerometer output was sampled by a 12-bit analog-to-digital converter. The percentage of time spent sedentary as well as the amount of time spent participating in different categories of physical activity including light, lifestyle, moderate, and vigorous were calculated using algorithms from Freedson in the ActiGraph software.

Rest and Exercise Visits: Figure 1 depicts the study schedule. For the rest and exercise visits, participants reported to the CTRU the morning after an overnight fast (10 h) with nothing taken by mouth except water. Study participants were instructed to maintain their current dietary and activity habits throughout the entire duration of the study. The day prior to the study visit, subjects were provided a standardized meal for breakfast (522 Kcal), lunch (722 Kcal, snack 180 Kcal), and dinner (779 Kcal) made by the Washington University CTRU Metabolic Kitchen. Participants were also instructed to consume a liquid formula (Ensure; Ross Laboratories, Columbus, OH) containing 250 kcal (40 g carbohydrates, 6.1 g fat, and 8.8 g protein) to ensure complete filling of hepatic glycogen stores as a snack before bedtime. After admission, height and weight was measured using a standard scale and stadiometer, and an IV catheter was inserted into the antecubital vein to collect a baseline fasting blood samples. Fasting blood samples were collected in heparinized tubes for glucose, insulin, and c-peptide, citrate-lined tubes for a PT-INR (for safety of muscle biopsy), and EDTA-lined tubes for lipid kinetics analysis.

One hour after the baseline IV placement and blood draws, participants completed either one hour of resistance exercise, or one hour of rest. During the rest visit, participants laid in bed
for 1 hour without physical activity. During the exercise visit, participants completed 1 hour of resistance exercise. The exercise session consisted of 8 exercises (bench press, pull down, seated row, biceps curl, knee extension, leg press, and overhead press) all performed for 3 sets of 8-12 repetitions at 80% of their measured 1RM until volitional fatigue. Participants were given no more than 1 minute of rest between sets, and no more than 3 minutes rest between exercises. All exercise sessions were supervised by a licensed physical therapist.

Following completion of the exercise or rest session, the antecubital catheter was prepared for the administration of prime-constant infusions of stable isotope tracers. Additionally, a second catheter was inserted into a hand vein on the contralateral arm, with the hand warmed to 55°C using a thermostatically controlled hot box to obtain arterialized blood samples. The catheter was kept patent throughout the study day, using a 0.9% saline solution. 30 minutes after the rest or exercise session, a primed, constant infusions of [6,6-2H2]glucose (28 μmol/kg prime and 0.28 μmol•kg⁻¹•min⁻¹ continuous infusion), was administered for 180 minutes to achieve isotopic steady state (data not presented in this paper). 90 minutes after the rest or exercise session, primed, constant infusions of [NaH¹³CO₃] (1 μmol/kg priming dose), and [U⁻¹³C₁₆]palmitate (.0053 μmol•kg⁻¹•min⁻¹ continuous infusion) were administered for 120 minutes to achieve isotopic steady state. Blood samples were collected at -70, 100, 110, and 120 minutes from the start of the palmitate tracer infusion. Blood was collected in EDTA tubes for lipid kinetics analysis.

Following the 210-minute Baseline Period, participants consumed a liquid test meal consisting of 20 Kcal/FFM, with a composition of 47.8% CHO, 36.1% fat, and 16.1% protein. 10 mg/kg body weight of [1,1,2,2,2,3,3,4,4,4-¹³C₁₂]tripalmitin was added to the test meal and thoroughly mixed. The tripalmitin was emulsified in lecithin and vegetable oil, which was
microwaved for 5 minutes to warm. The emulsified mixture was then added to Boost Plus, microwaved, and sonicated to evenly distribute the tracer. To ensure individuals ate from the same meal on each visit, one large mixture was prepared and then split in half (each half consumed during the rest and exercise visit). Participants were asked to consume the liquid meal within 16 minutes for consistency (1/4 of the meal provided every 4 minutes). The sides of the container were rinsed with 10 ml of water and the rinse was also consumed to ensure participants ingested the whole meal. Finally, 30 minutes after consuming the meal, a 75 umol/kg bolus infusion of (1,1,2,3,3-2H5)glycerol was initiated to track lipoprotein turnover kinetics. The infusions of the [U-13C16]palmitate and [6,6-2H2]glucose were continued throughout the postprandial period. Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 minutes post-meal ingestion. Water was consumed ad libitum during recovery, but aside from the test meal, study participants consumed no other beverages and no food until the 400-min postprandial measurement period ended. Isolated plasma was analyzed for free fatty acid, triglyceride, glycerol, and glucose tracer enrichment. Samples of the liquid test meal, as well as the tracers used were collected for enrichment analysis. All tracers were purchased from Cambridge Isotopes (Cambridge, MA).

Sample Analysis

Blood for plasma hormone and metabolite analysis was collected from an antecubital vein, immediately placed in chilled EDTA tubes, centrifuged at 2000xg for 10 minutes, and the supernatant collected and frozen at -80°C until analysis. Fasting samples were analyzed were used for comprehensive metabolic panel, lipid panel, CBC, and HbA1c. Plasma insulin concentration was measured by radioimmunoassay. The plasma insulin assay range is 2–300
µU/mL and the interassay coefficient of variation is 4% in the low (10.5 uU/mL) and high insulin concentration range (55.1 uU/mL).

Tissue Biopsies

**Skeletal Muscle**

Muscle biopsy samples were obtained from the vastus lateralis under local anesthesia (lidocaine, 2%) 90 minutes after consuming the liquid test meal using a Tilley-Henkel forceps. One sample was immediately transferred into ice-cold relaxing medium (BIOPS) containing 10 mmol/l Ca2+/EGTA buffer, 20 mmol/l imidazole, 50 mmol/l K+-4-morpholinoethanesulfonic acid (Mes), 0.5 mmol/l dithiothreitol, 6.56 mmol/l MgCl2, 5.77 mmol/l ATP; 15 mmol/l phosphocreatine at pH 7.1 for mitochondrial respirometry analysis. A second sample was flash frozen in liquid nitrogen and stored at -80 until further analysis.

**Subcutaneous Adipose Tissue**

Abdominal adipose tissue was obtained by needle aspiration. The biopsy site was anesthetized with 1% lidocaine, and adipose tissue was aspirated with a 10-mL syringe and a 14-gauge needle. Tissue samples were vigorously irrigated with iced saline and flash frozen in liquid nitrogen. Adipose samples were stored at -80 until analysis.

Skeletal Muscle Mitochondrial Respiration

Subsamples of 5 mg wet weight (W_w) were transferred into BIOPS onto a small petri dish on an ice-cold metal plate and separated using forceps with sharp tips. To ensure complete permeabilization the fibers were incubated by gentle agitation at 4°C in BIOPS solution containing 50 µg/ml saponin for 20 min. Fibers were washed for 10 min at 4°C in ice-cold
mitochondrial respiration medium [MiR06; 0.5 mM EGTA, 3 mM MgCl₂, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH₂PO₄, 20 mM HEPES, 110 mM sucrose and 1 g/l BSA essentially fatty acid free, adjusted to pH 7.1, 2,800 units/mg solid catalase lyophilized powder], and wet weight of the fibers was measured on a microbalance (Mettler Toledo, Greifensee, Switzerland). With this method, the cholesterol-rich plasma membrane is selectively permeabilized, leaving intracellular membrane structures, such as mitochondria intact, and the entire mitochondrial population can be studied in the muscle sample.

Three milligrams (3mg) wet weight was used per respirometer chamber (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) containing 2.090 ml MiR06 at 37°C. Oxygen concentration (µM = nmol/ml) and oxygen flux (pmol·s⁻¹·mg⁻¹; negative time derivative of oxygen concentration, divided by muscle mass per volume) were recorded using DatLab software (Oroboros Instruments). The Oxygraph-2k is a two-chamber titration-injection respirometer with a limit of detection of volume-specific oxygen flux of 1 pmol·s⁻¹·ml⁻¹. Instrumental background oxygen flux was corrected online, accounting for sensor oxygen consumption and oxygen diffusion between the medium and the chamber boundaries. The oxygen concentration in the chamber was maintained between 150 and 400 µM to avoid oxygen limitation of fiber respiration. Intermittent re-oxygenations were achieved by injecting .5 mL oxygen.

The substrate-uncoupler-inhibitor titration protocol (Table 1) was as follows (final concentrations): palmitoylcarnitine (0.05 mM), L-carnitine (5 mM), and Malate (.5 mM) to support electron entry from fatty acid β-oxidation through electron-transferring flavoprotein (ETF) and Complex I (CI) to coenzyme Q. Prior to the addition of ADP, oxygen utilization occurs due to proton slip across the inner mitochondrial membrane (LEAK respiration). ADP (4
mM) was then added to stimulate fatty-acid supported oxidative phosphorylation (ETF + CI)$_{Lip}$. The subsequent addition of pyruvate (10 mM) stimulates glycolytic oxidative phosphorylation. Glutamate (10 mM) was then added, followed by succinate (10 mM) to recapitulate the TCA cycle and stimulate maximal oxidative phosphorylation [(ETF + CI + CII)$_{Lip+Pyr}$] through both ETC complex I and complex II. Cytochrome c (10 μM) is added to test the integrity of the outer mitochondrial membrane. The increase of flux with cytochrome c was on average 3.3 ± 0.4%. Electron transfer system capacity (CI+II$_E$) was reached by stepwise (.5 μM) addition of the uncoupler carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP). Finally, the addition of rotenone (0.5 μM), an inhibitor of complex I was used measure succinate-supported ETS capacity (CII$_E$). Figure 2 and Table 1 outlines the titration protocol used in this study, and the respiratory states included in this analysis. Table 3 outlines the flux control and substrate control ratios calculated in this study.

Real-Time Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from skeletal muscle and subcutaneous adipose tissue samples using Trizol reagent (#15596018; Invitrogen, Carlsbad, CA) and RNeasy mini kit (#74104; Qiagen, Valencia, CA) respectively. Total RNA was reverse transcribed into cDNA by using the High-Capacity cDNA Reverse Transcription Kit (#4368813; Invitrogen). Relative gene expression was determined by using an ABI 7500 real-time PCR system (Invitrogen) and SYBR Green Master Mix (Invitrogen) as previously described (PMID: 29634313; 29294006; 27498863; 27732859; 26916363; 29687616). The expression of each gene was determined by normalizing the Ct (cycle threshold) value of each sample to the housekeeping control gene, ribosomal protein ($RPLP0$). Primer details are listed in Table 2.
Statistical Analysis

Participant demographics were analyzed using mean ± SE. Data were analyzed for normality using the Shapiro-Wilk test. Differences between rest and exercise on respiration and qPCR outcomes were analyzed according to the procedures outlined by Wellek and Blettner (2012) for cross-over designs, testing first for carry-over effects, followed by testing for treatment effects. An alpha level = .05 was used for significance. All analyses were completed in SPSS version 25.

Results

Participant Characteristics

Nine male participants were enrolled in the study. Participants had a mean age of 50, were obese (mean BMI: 33 ± 3), and mostly sedentary (mean % time sedentary: 69 ± 6.1%). All participants were pre-diabetic, with the group means for HbA1c, 2-hour plasma glucose of the OGTT, and fasting plasma glucose all within the pre-diabetic range (Table 1). Four (n=4) participants had HbA1c values ≥ 5.7%, n=6 had fasting plasma glucose values >100 mg/dL (5.5 mmol/L), and n=4 participants had 2-hour OGTT values >140 mg/dL (7.8 mmol/L). The average % body fat was in the 75th percentile for individuals in their age group as established by the NHANES. Participants also demonstrated insulin resistance according to the HOMA2 model (HOMA2-IR, HOMA2-%S), with compensatory increases in beta cell function (HOMA2-%B). Participants also presented with elevated total triglycerides (normal: <150 mg/dL, participant mean: 162.7 mg/dL), LDL (normal: <100 mg/dL, participant mean: 117.9 mg/dL), with slightly reduced HDL (normal: >40 mg/dL, participant mean: 38.4 mg/dL) (Table 4).
Mitochondrial Respiration

Muscle biopsies were obtained from n=8 participants. Acute resistance exercise significantly increased maximal lipid oxidation (t(7)= 4.49, p=.003), maximal complex I supported lipid + pyruvate oxidation (t(7)= 6.93, p<.001), maximal oxidative phosphorylation through complex I and II (t(7)= 5.64, p<.001), maximal electron transport capacity (t(7)= 3.31, p=.012), and maximal complex II supported respiration (t(7)= 3.51, p=.009). Leak respiration was not significantly elevated following the exercise session (t(7)= 1.24, p=.254) compared to the rest condition. No carry-over effects were identified for any of the variables (Figure 3).

Skeletal Muscle Mitochondrial Flux and Substrate Control Ratios

Following acute resistance exercise, OXPHOS control ratio and the respiratory control ratio significantly increased compared to the rest condition, however phosphorylation control ratio or leak control ratio were not different between acute resistance exercise and rest conditions (Table 5). Complex I supported lipid respiration normalized to maximal oxidative phosphorylation, complex I supported lipid respiration normalized to maximal electron transport capacity, the lipid to glucose oxidation ratio, and the free oxidative phosphorylation capacity following acute resistance exercise significantly increased compared to rest (Table 5).

Skeletal Muscle Gene Expression

RNA was isolated in muscle biopsy samples from n=8 participants. However, RNA in n=1 sample was degraded therefore data was analyzed from samples for qPCR analysis in n=7 participants. Following acute resistance exercise, expression of PGC1alpha increased ~1.8 fold
(p=0.014), PDK4 increased 4.8-fold (p=0.006) compared to the rest condition, FOXO1 increased 1.62-fold above rest (p<0.05), and MURF1 increased 2.46 above rest (p<0.05) (Figure 4). Lipoprotein lipase (LPL) expression tended to increase after resistance exercise (1.8-fold above rest condition, p=0.120) (Figure 4). SREBF1 (SREBP1) expression decreased after resistance exercise compared to the rest condition (p=0.016) (Figure 4). Expression of fatty acid synthase (FASN, p=.455), acetyl-CoA Carboxylase alpha (ACACA, p=.580), carnitine palmitoyltransferase 1 beta (CPT1B, p=.0195), carnitine palmitoyltransferase 1 alpha (CPT1A, p=0.847), uncoupling protein 3 (UCP3, p=0.321), or CD36 (p=.0502) were not different between resistance exercise and rest.

Abdominal Subcutaneous Adipose Tissue Gene Expression

Abdominal subcutaneous tissue biopsies were collected from n=6 participants. The resistance exercise session resulted in a significant 1.4-fold increase in FOXO1 expression (p=0.034), a 1.4-fold increase in PDK4 expression (p=0.028), a 1.3-fold increase in LPL expression (p=0.041), a 1.27-fold increase in HSL expression (p=0.039), and a 1.28-fold increase in ADIPOQ expression (p=0.038) (Figure 5). There was, likewise, a trend toward increased CD36 expression (1.2-fold increase, p=0.070), and ATGL expression (1.23-fold increase p=0.100).

Discussion

In this study, we found that a single bout of moderate intensity resistance exercise increased skeletal muscle maximal lipid respiration, as well as coupled and uncoupled respiration in in obese men with prediabetes. To our knowledge, this is the first study to demonstrate that
acute resistance exercise increases the contribution of lipid oxidation to overall energy production (evidenced by increased lipid oxidation relative to maximal oxidative phosphorylation and the lipid to glucose oxidation ratio) and that these increases occurred concurrently with increased expression of PGC1alpha, PDK4, and FOXO1, as well as reductions in SREBP1. This pattern of gene expression in skeletal muscle could be expected to promote glucose storage and lipid oxidation in skeletal muscle. Previous studies have found reduced skeletal muscle lipid oxidation capacity (or rate) in individuals with prediabetes, which has been implicated in the development of insulin resistance. Increasing lipid oxidation with resistance exercise could potentially prevent the development of lipotoxicity by clearing lipid and toxic intermediates that are known to interfere with insulin signaling. Therefore, improvements in mitochondrial respiration and oxidative gene expression could be important in preventing disease progression and reducing the risk for cardiovascular disease.

Additionally, we found that a single session of resistance exercise increased the expression of FOXO1, PDK4, HSL, ADIPOQ, and LPL in adipose tissue. These genes work cooperatively to mobilize stored FFA and increase lipoprotein hydrolysis (while storing glucose) in adipose tissue, which supports our previous findings of increased FFA rate of appearance (i.e. adipose tissue lipolytic rate) after resistance exercise. Increases in FFA availability complements the increase in FFA oxidation we observed in skeletal muscle mitochondria.

Compared with healthy people, individuals with prediabetes demonstrate diminished responses to exercise, even after high-intensity interventions. Likewise, in mouse models of diabetes, chronic resistance exercise failed to improve state 3 (coupled) respiration in cardiac mitochondria, suggesting that resistance exercise may not be enough of a stimulus to improve mitochondrial function in striated muscle. In contrast to these results, we were able to find
significant improvements in skeletal muscle mitochondrial respiration in obese men with prediabetes after just a single session of moderate intensity resistance exercise. Of note, the greatest improvements in respiration occurred in complex I-supported respiration, which coincides with work by Porter et al. (2015) and van Schaardenburgh et al. (2016), who found that resistance exercise tended to increase protein content of complex I only, and that changes in complex I-supported respiration occur in as little as 15 minutes after resistance exercise.\textsuperscript{16,30} Further research is needed to identify the mechanism of this rapid response.

The mechanisms associated with improvements in skeletal muscle mitochondria following acute resistance exercise in individuals with prediabetes are not known. Following acute resistance exercise, we found increased gene expression of PGC1alpha, PDK4, and FOXO1, as well as reduction in SREBP1. PGC1alpha is a transcription co-activator that regulates several transcription factors including those associated with adaptive thermogenesis, mitochondrial biogenesis, glucose and fatty acid metabolism, fiber-type switching to oxidative phenotypes, and cardiac development.\textsuperscript{31,32} Knockout of skeletal muscle PGC1alpha results in declines in state 3 and state 4 respiration in both subsarcolemmal and intramyofibrillar mitochondria.\textsuperscript{33} In contrast, cardiac myocytes overexpressing PGC1alpha demonstrate 2.8-fold greater rate in mitochondrial oxygen consumption secondary to amplification of coupled respiration.\textsuperscript{34} Previous studies have shown that PGC1alpha expression increases mRNA content of genes involved in the electron transport chain (most notably in subunits complexes II, IV, and cytochrome C) as well most genes involved in the tricarboxylic acid cycle, β-oxidation, and fatty acid transport.\textsuperscript{35-37} While PGC1alpha may exert the majority of its influence on coupled respiration, overexpression of PGC1alpha in myotubes has also been shown to increase uncoupled respiration above control cells.\textsuperscript{36} Although PGC1alpha regulates many aspects of oxidative metabolism, previous research
is conflicting with regard to the ability of resistance exercise to increase PGC1alpha expression. Some studies have found increases in PGC1alpha mRNA content (38, 39, 40), while other have not (41, 42) following acute and chronic resistance exercise in mice and healthy humans. In the current study, we found a 1.76-fold increase in PGC1alpha mRNA content, providing support for the capacity for resistance exercise to induce skeletal muscle PGC1alpha expression. The increases in coupled and uncoupled respiration we identified in this study are consistent with the known functions of PGC1alpha in skeletal muscle.

Acute resistance exercise also increased the expression of PDK4 (pyruvate dehydrogenase kinase 4). PDK4 has been shown to stimulate free fatty acid oxidation by: 1) inhibiting pyruvate dehydrogenase kinase, reducing the concentration of acetyl-coA, and limiting malonyl-coA production (inhibits fatty acid entry into the mitochondria); and 2) by shunting absorbed glucose to glycogen storage rather than oxidation. Following aerobic or resistance exercise, the combined signals from glycogen depletion and increased FFA availability may be a strong stimulus for PDK4 expression via activation of forkhead box (FOXO) transcription factors 1 and 3 to the nucleus, where FoxO1 can bind directly to the promoter region of the PDK4 gene. FOXO1 is highly sensitive to changes in FFA and insulin concentrations, and has therefore been implicated in the balance between lipid and carbohydrate oxidation and storage.

Similar to PDK4, we found increased expression of FOXO1 gene expression, indicating an upregulation of this signaling pathway after resistance exercise to promote lipid oxidation.

Indeed, we found a significant increase in the ratio of lipid to carbohydrate oxidation -- suggesting a greater contribution from free fatty acid to oxidation, which closely mirrors the known influence of both PDK4 and PGC1alpha. In healthy men, Gallagher et al. (2013) found that acute moderate-intensity resistance exercise (3 sets of knee extensor resistance exercise at
90% of their 10-repetition maximum) increased PDK4 gene expression in type I and type II muscle fibers (7.4-fold and 8.1-fold respectively). These results, together with the current study, suggest that acute moderate intensity resistance exercise increases skeletal muscle lipid oxidation through activation of PDK4 in both healthy and prediabetic men. Of note, previous studies have shown that overexpression of PGC1alpha increases PDK4 mRNA content and slows glycolytic flux – providing a potential mechanistic link between PGC1alpha and PDK4.

In response to acute resistance exercise, we also found reductions in skeletal muscle Sterol Regulatory Element Binding Protein 1 (SREBP1) expression. SREBP proteins are transcription factors that control lipid metabolism and cholesterol synthesis primarily in lipogenic tissues (liver and adipose tissue). In skeletal muscle, SREBP1 may play a role in the anabolic response to resistance exercise, with previous studies demonstrating that overexpression SREBP1 in myoblasts inhibits the expression of myogenic regulatory factors (e.g. myogenin, MyoD, and MEF2C) needed for myogenic differentiation. Moreover, overexpression of SREBP1 reduced protein synthesis rates and increased protein degradation, providing a mechanistic link between SREBP1 expression and muscle size and hypertrophy. Previous studies have found that SREBP1 expression increases fatty acid synthase activity and intramyocellular triglyceride content and could therefore play a role in skeletal muscle lipid balance. After acute resistance exercise, we found a significant reduction in SREBP1 expression, as well as fatty acid synthase expression (trend toward significant). This pattern of expression is consistent with the changes in PGC1alpha and PDK4 in that it promotes free fatty acid mobilization and oxidation rather than storage. However, we did not measure intramyocellular triglyceride content in the current study is should be further studied.
While SREBP1 has been shown to regulate the expression of myogenic regulatory factors, MURF1 is an E3 ligase expressed in striated skeletal muscle where is involved in the ubiquitination and proteolysis of muscle proteins. A recent review by Murton and Greenhaff found that MuRF1 mRNA expression was upregulated 1-4 hours after acute bouts of resistance exercise in healthy subjects. MuRF1 expression, like PDK4, is stimulated by FOXO1 and is believed to be a central component of protein breakdown needed for muscle remodeling after resistance exercise. Taken together, MuRF1 expression may have been stimulated by resistance exercise as part of skeletal muscle remodeling responses to the exercise session.

Acute resistance exercise also tended to increase skeletal muscle lipoprotein lipase (LPL) expression 4.5 hour following exercise. One of the major contributors to increased FFA availability in the postprandial period is the secretion of triglyceride-rich lipoproteins in the form of chylomicrons (from the small intestine), and very low density lipoproteins (VLDL from the liver). These lipoproteins are hydrolyzed by LPL in the endothelium of the vasculature of peripheral tissues, including skeletal muscle and adipose tissue. Few studies have examined changes in skeletal muscle lipoprotein lipase expression after resistance exercise, and the results have been conflicting. Tsintzas et al. (2017) found that an acute bout of resistance exercise (leg press and knee extension) did not have an effect on skeletal muscle mRNA content of LPL in healthy young men. In contrast, Kiens et al. (1989) reported that single leg knee extensions increased muscle LPL activity 4-hours after exercise, but not after 8 hours in healthy young men. Our data support the findings by Kiens et al. although our data lacked statistical significance. Individuals with prediabetes might have a blunted LPL response to acute resistance exercise; however this requires further study. In individuals with prediabetes and type 2 diabetes, there is a positive correlation between insulin sensitivity and skeletal muscle LPL.
activity, suggesting a role of lipoprotein metabolism in disease progression. Resistance exercise might increase LPL expression and with chronic resistance exercise, increase LPL content, thereby improving lipoprotein and FFA clearance, reducing postprandial triglycerides, thus reducing the risk for cardiovascular disease. However, this requires further investigation.

In addition to changes in skeletal muscle gene expression, we also found differences in genes regulating glucose and lipid metabolism in subcutaneous adipose tissue, including FOXO1, PDK4, LPL, HSL, and adiponectin. Like skeletal muscle, FOXO1 expression plays a central role in lipid and glucose metabolism in adipose tissue. In periods of stress, cold exposure, or nutrient deprivation, FOXO1 is redistributed to the nucleus and is transcriptionally active. Consistent with its role in skeletal muscle, FOXO1 activates a gene network involved in lipid mobilization, inhibition of lipid storage and fat accumulation, and sparing of glucose. This function compliments its role in skeletal muscle by mobilizing fatty acids to support FOXO1-driven increases in lipid oxidation. In contrast, insulin mediates FOXO1 nuclear exclusion through phosphorylation by Akt. We found a trend toward a significant increase in ATGL, which is responsible for the bulk of triglyceride lipolysis in adipose tissue. Previous studies have shown that FOXO1 expression in adipose tissue induces ATGL expression, and that insulin inhibits the expression of ATGL in adipocytes by restraining the nuclear localization of FOXO1. Likewise, we also found a significant increase in hormone sensitive lipase (HSL), another important lipolytic enzyme with function consistent with ATGL.

Upregulation of PDK4 expression in adipose tissue results in an increase in glyceroneogenesis – a pathway that uses pyruvate, alanine, glutamine or any substances from the TCA cycle as precursors to synthesize glycerol-3-phosphate. The increase in glycerol-3-phosphate may promote re-esterification of non-esterified fatty acids from lipolysis, thereby
limiting excessive plasma FFA production while also reducing substrate for glucose oxidation. To the author’s knowledge, no previous studies have explored PDK4 expression in adipose tissue after resistance exercise. This is the first study to show that a single session of resistance exercise may influence glyceroneogenesis in adipose tissue, which could play a role in adipocyte size and body fat accumulation. Likewise, lipoprotein lipase is the rate-limiting enzyme for the uptake of chylomicron and VLDL triglyceride-derived fatty acids in adipose tissue. Increased LPL expression, as we found with resistance exercise, could facilitate increased FFA uptake in the postprandial period, as adipose tissue is a major source of endogenous and exogenous FFA clearance.

Adiponectin is an adipokine that is reduced in obesity and insulin resistance, including type 2 diabetes. Adiponectin has been shown to play a central role in the modulation of glucose and lipid metabolism by promoting glucose uptake and storage, while augmenting lipid oxidation in skeletal muscle. It has also been shown to increase skeletal muscle insulin sensitivity, increase muscle fatty acid transport, and reduce inflammation in endothelial, muscle, and epithelial cells. We found that resistance exercise significantly increased the adiponectin (ADIPOQ) gene expression, which could, together with increased protein translation, improve lipoprotein clearance, free fatty acid uptake in adipose tissue, and further augment lipid oxidation in skeletal muscle. Previous studies on the effects of acute resistance exercise in healthy men and women found that resistance exercise did not increase plasma levels of adiponectin despite lowering plasma glucose when measured 15 hours post-exercise. We found elevated ADIPOQ expression 4.5 hours after resistance exercise. Taking these studies together, it may be that 1) the acute effects of resistance exercise on adiponectin levels may dissipate in less than 15 hours, or 2) that the magnitude of adiponectin mRNA expression after resistance exercise may not be
enough to change adiponectin protein concentrations after just a single session. However, this is unclear as we did not measure plasma adiponectin concentration in the current study.

In conclusion, we found that a single bout of resistance exercise increased mitochondrial respiratory capacity and lipid oxidation in obese men with prediabetes. These adaptations occurred concurrently with changes in skeletal muscle (PGC1alpha, PDK4, SREBP1, FOXO1, and MURF1) and adipose tissue (FOXO1, PDK4, HSL, ADIPOQ, and LPL) gene expression. Together, these genes cooperate to mobilize stored FFA and increase lipoprotein hydrolysis (and storing glucose) in adipose tissue, while increasing lipid uptake and oxidation in skeletal muscle. Improvements in lipid clearance through oxidation could reduce the risk for progression from prediabetes to type 2 diabetes, as well as facilitate weight loss and lower the risk for cardiovascular disease.
Works Cited

29. Ko TH1, Marquez JC1, Kim HK. Resistance exercise improves cardiac function and mitochondrial efficiency in diabetic rat hearts. Pflugers Arch. 2018 Feb;470(2):263-275. doi: 10.1007/s00424-017-2076-x


Table 1. Substrate-uncoupler-inhibitor titration (SUIT) protocol.

<table>
<thead>
<tr>
<th>Definition</th>
<th>Abbreviation</th>
<th>Flux State</th>
<th>Titration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leak</td>
<td>((\text{ETF} + \text{CI})_{\text{LEAK}})</td>
<td>L</td>
<td>PC + Mal</td>
</tr>
<tr>
<td>FFA Oxidation through CI</td>
<td>((\text{ETF} + \text{CI})_{\text{Lip}})</td>
<td>PC + Mal + ADP</td>
<td></td>
</tr>
<tr>
<td>Combined FFA and Pyruvate Oxidation Through CI</td>
<td>((\text{ETF} + \text{CI})_{\text{Lip+Pyr}})</td>
<td>PC + Mal + ADP + Pyr + Glut</td>
<td></td>
</tr>
<tr>
<td>Max OXPHOS</td>
<td>((\text{ETF} + \text{CI} + \text{CII})_{\text{Lip+Pyr}})</td>
<td>P</td>
<td>PC + Mal + ADP + Pyr + Glut + Succ</td>
</tr>
<tr>
<td>Maximal ETS</td>
<td>((\text{ETF} + \text{CI} + \text{CII})_E)</td>
<td>E</td>
<td>PC + Mal + ADP + Pyr + Glut + Succ + FCCP</td>
</tr>
<tr>
<td>CII Supported Respiration</td>
<td>((\text{CII})_E)</td>
<td></td>
<td>PC + Mal + ADP + Pyr + Glut + Succ + FCCP + Rot</td>
</tr>
</tbody>
</table>

### Table 2. Sequence of primers used for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Forward (F) and reverse (R) primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOXO1</td>
<td>NM_002015</td>
<td>F: 5’- TCGTCATAATCTGTCCCTACACA -3’&lt;br&gt;R: 5’- CGGCTTCGGCTCTTAGCAA -3’</td>
</tr>
<tr>
<td>PPARGC1A</td>
<td>NM_013261</td>
<td>F: 5’- TCTGAGTCTGTATGGAGTGACAT -3’&lt;br&gt;R: 5’- CCAAGTCGTTACATCTAGTCA -3’</td>
</tr>
<tr>
<td>PDK4</td>
<td>NM_002612</td>
<td>F: 5’- GGAGCATTCTCGCGCTACA -3’&lt;br&gt;R: 5’- ACAGGCAATTCTTGTGCAA -3’</td>
</tr>
<tr>
<td>LPL</td>
<td>NM_000237</td>
<td>F: 5’- TCATTCCCGGAGTAGCAGAGT -3’&lt;br&gt;R: 5’- GGCACAAGTTTTGGCACCC -3’</td>
</tr>
<tr>
<td>SREBF1</td>
<td>NM_001005291</td>
<td>F: 5’- ACAGTGACTTCCCTGGCCTAT -3’&lt;br&gt;R: 5’- GCAATGGACGGGTACATTCAA -3’</td>
</tr>
<tr>
<td>MURF1</td>
<td>NM_032588</td>
<td>F: 5’- CTTCCAGGCTGCAAATCCCTA -3’&lt;br&gt;R: 5’- ACACTCCGTGACGATCCATGA -3’</td>
</tr>
<tr>
<td>CD36</td>
<td>NM_000072</td>
<td>F: 5’- GTTGGAGCATT TTGATTGAAAT -3’&lt;br&gt;R: 5’- AGGAAATGAAACTGATGACTACAGA -3’</td>
</tr>
<tr>
<td>HSL</td>
<td>NM_005357</td>
<td>F: 5’- ATGTTGGAGCATT TTGATTGAAAT -3’&lt;br&gt;R: 5’- GGTCCAGGTTTGGGCAA -3’</td>
</tr>
<tr>
<td>ATGL</td>
<td>NM_020376</td>
<td>F: 5’- ACCTGGAACATCTCGTTG -3’&lt;br&gt;R: 5’- CCTGGCGCTCTTTAGAC -3’</td>
</tr>
<tr>
<td>ADIPOQ</td>
<td>NM_001177800</td>
<td>F: 5’- GGCTTTCCGGGAATCCCAAGG -3’&lt;br&gt;R: 5’- TGGGGATAGTAACGTAAGTCCTCC -3’</td>
</tr>
<tr>
<td>RPLP0</td>
<td>NM_001002</td>
<td>F: 5’- GTGATGTGCAGCTGATCAGACT -3’&lt;br&gt;R: 5’- GATGACCAGCCAAAGGAGA -3’</td>
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</table>
Table 3. Flux control and substrate control ratios

<table>
<thead>
<tr>
<th>Measure</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flux Control Ratios</strong></td>
<td></td>
</tr>
<tr>
<td>Leak Control</td>
<td>L/E</td>
</tr>
<tr>
<td>OXPHOS Control Ratio</td>
<td>P/E</td>
</tr>
<tr>
<td>Respiratory Acceptor Control Ratio</td>
<td>P/L</td>
</tr>
<tr>
<td>Phosphorylation Control Ratio</td>
<td>L/P</td>
</tr>
<tr>
<td><strong>Substrate Control Ratios</strong></td>
<td></td>
</tr>
<tr>
<td>CI Lipid Normalized to Max OXPHOS</td>
<td>(ETF + Cl)_{Lip}/P</td>
</tr>
<tr>
<td>CI Lipid + Pyruvate Normalized to Max OXPHOS</td>
<td>(ETF + Cl)_{Lip+Pyr}/P</td>
</tr>
<tr>
<td>CI Lipid Normalized to Max ETS Capacity</td>
<td>(ETF + Cl)_{Lip}/E</td>
</tr>
<tr>
<td>CI Lipid + Pyruvate Normalized to Max ETS capacity</td>
<td>(ETF + Cl)_{Lip+Pyr} / E</td>
</tr>
<tr>
<td>CII Respiration normalized to Max OXPHOS</td>
<td>(CII)_E /P</td>
</tr>
<tr>
<td>CII Respiration normalized to Max ETS Capacity</td>
<td>(CII)_{E} / E</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
</tr>
<tr>
<td>Lipid/Glucose Balance</td>
<td>(ETF + Cl)<em>{Lip}/ ((ETF + Cl)</em>{Lip+Pyr} - (ETF + Cl)_{Lip})</td>
</tr>
<tr>
<td>ETS Coupling Efficiency</td>
<td>(E-L)/E</td>
</tr>
<tr>
<td>Excess E-P Capacity (E-P)</td>
<td>E-P</td>
</tr>
<tr>
<td>Excess E-P Capacity Ratio (E-P/E)</td>
<td>(E-P/E)</td>
</tr>
<tr>
<td>Free OXPHOS Capacity (P-L)</td>
<td>(P-L)</td>
</tr>
<tr>
<td>Net OXPHOS Control Ratio (P-L)/E</td>
<td>(P-L)/E</td>
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Table 4. Participant Characteristics (N=9)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SEM)</th>
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<tbody>
<tr>
<td>Age</td>
<td>50 (11)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 (5.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>104.5 (9.9)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>33 (3)</td>
</tr>
<tr>
<td>WB Fat (kg)</td>
<td>32.7 (2.2)</td>
</tr>
<tr>
<td>WB Lean (kg)</td>
<td>66.3 (1.5)</td>
</tr>
<tr>
<td>% Fat</td>
<td>31.8 (1.4)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.7 (.2)</td>
</tr>
<tr>
<td>2-hr OGTT (mmol/L)</td>
<td>9.0 (.5)</td>
</tr>
<tr>
<td>Peak Postprandial TG (mmol/L)</td>
<td>2.5 (.2)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.9 (.4)</td>
</tr>
<tr>
<td>Fasting Insulin (uU/mL)</td>
<td>22 (2)</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>2.6 (.4)</td>
</tr>
<tr>
<td>HOMA2-%B</td>
<td>134.4 (17)</td>
</tr>
<tr>
<td>HOMA2-%S</td>
<td>75.6 (37)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>162.7 (7.5)</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>184.5 (11.4)</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>38.4 (1.7)</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>117.9 (10.3)</td>
</tr>
<tr>
<td>% Time Sedentary</td>
<td>69 (6.1)</td>
</tr>
<tr>
<td>% Time Light Activity</td>
<td>21 (3.6)</td>
</tr>
<tr>
<td>% Time Moderate Activity</td>
<td>10 (3)</td>
</tr>
<tr>
<td>% Time Vigorous Activity</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Values are presented as mean (standard error). % of time spent in each activity were derived from accelerometry data (see methods). WB = whole body. 2-hr OGTT is plasma glucose at 2-hours after a 75 gram oral glucose challenge. TG = triglycerides. HOMA2 calculated using the HOMA2 calculator accessed from (http://www.dtu.oc.ac.uk).
Table 5. Effect of exercise on flux and substrate control ratios (n=8)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Rest</th>
<th>Exercise</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flux Control Ratios</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leak Control</td>
<td>.077 ± .008</td>
<td>.074 ± .003</td>
<td>P=.731</td>
</tr>
<tr>
<td>OXPHOS Control Ratio</td>
<td>.82 ± .07</td>
<td>.92 ± .07</td>
<td>P=.041*</td>
</tr>
<tr>
<td>Respiratory Acceptor Control Ratio</td>
<td>12 ± 1.4</td>
<td>14.8 ± .83</td>
<td>P=.031*</td>
</tr>
<tr>
<td>Phosphorylation Control Ratio</td>
<td>.10 ± .03</td>
<td>.09 ± .02</td>
<td>P=.334</td>
</tr>
<tr>
<td><strong>Substrate Control Ratios</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI Lipid Normalized to Max OXPHOS</td>
<td>.31 ± .03</td>
<td>.36 ± .04</td>
<td>P=.043*</td>
</tr>
<tr>
<td>CI Lipid + Pyruvate Normalized to Max OXPHOS</td>
<td>.54 ± .02</td>
<td>.55 ± .03</td>
<td>P=.459</td>
</tr>
<tr>
<td>CI Lipid Normalized to Max ETS Capacity</td>
<td>.25 ± .02</td>
<td>.31 ± .01</td>
<td>P=.012*</td>
</tr>
<tr>
<td>CI Lipid + Pyruvate Normalized to Max ETS capacity</td>
<td>.45 ± .04</td>
<td>.49 ± .03</td>
<td>P=.107</td>
</tr>
<tr>
<td>CII Respiration normalized to Max OXPHOS</td>
<td>.73 ± .09</td>
<td>.67 ± .09</td>
<td>P=.117</td>
</tr>
<tr>
<td>CII Respiration normalized to Max ETS Capacity</td>
<td>.58 ± .03</td>
<td>.56 ± .02</td>
<td>P=.468</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid/Glucose Balance</td>
<td>.47 ± .08</td>
<td>.67 ± .13</td>
<td>p=.043*</td>
</tr>
<tr>
<td>ETS Coupling Efficiency</td>
<td>.92 ± .01</td>
<td>.93 ± .003</td>
<td>p=.730</td>
</tr>
<tr>
<td>Excess E-P Capacity (E-P) (pmolO₂·mg wt wt⁻¹)</td>
<td>7.9 ± 3</td>
<td>5.9 ± 5.7</td>
<td>p=.616</td>
</tr>
<tr>
<td>Excess E-P Capacity Ratio (E-P/E)</td>
<td>.15 ± .07</td>
<td>.08 ± .08</td>
<td>p=.119</td>
</tr>
<tr>
<td>Free OXPHOS Capacity (P-L) (pmolO₂·mg wt wt⁻¹)</td>
<td>60.7 ± 10.8</td>
<td>80.6 ± 12.8</td>
<td>p=.019*</td>
</tr>
<tr>
<td>Net OXPHOS Control Ratio (P-L)/E</td>
<td>.77 ± .07</td>
<td>.85 ± .08</td>
<td>p=.079</td>
</tr>
</tbody>
</table>

Values are mean ± SE. * denotes significant at p<.05.
Figure 1. Study Protocol

Day Before: Consume standardized breakfast, lunch, and dinner. Fast overnight 10-12 hours

- Exercise Or Rest
- Recovery/Basal Period
- Post-prandial Period

- [6,6-¹³C₁₂]glucose constant infusion (initiated 180 min before meal)
- [¹³C₁₆]palmitate constant infusion (initiated 120 min before meal)
- [¹⁴C₂]glycerol bolus injection

- Indirect Calorimetry
- Adipose and Muscle Biopsy
- Blood Sample

Meal: 35% fat, 49% carbohydrates, 16% protein. Total of 15-16 Kcal/kg FFM up to 800 Kcal separated into 4 aliquots consumed every 4 minutes.
Figure 2. Sample plot from mitochondrial respirometry measurements

**Mitochondrial Respiration**

<table>
<thead>
<tr>
<th>(ETF + Cl)_{LEAK}</th>
<th>(ETF + Cl)_{b}</th>
<th>(ETF + Cl)_{UP+PYR}</th>
<th>(ETF + Cl + CII)_{UP+PYR}</th>
<th>CytoC</th>
<th>(ETF + Cl + CII)_{C}</th>
<th>(CII)_{E}</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-carnitine</td>
<td>Malate</td>
<td>ADP</td>
<td>Pyruvate</td>
<td>Glutamate</td>
<td>Succinate</td>
<td>Cyto-C</td>
</tr>
<tr>
<td>(mmol/ml)</td>
<td>(mmol/ml)</td>
<td>(mmol/ml)</td>
<td>(mmol/ml)</td>
<td>(mmol/ml)</td>
<td>(mmol/ml)</td>
<td>(mmol/ml)</td>
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<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O₂ Flux per mass (pmol O₂ s⁻¹ mg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure shows an example of a respiration curve recorded from permeabilized muscle fibers from the vastus lateralis. Purple lines denote the timing of substrate titrations. The substrate added at each time point is listed to the left of each line. Subsequent substrates were added only after O₂ flux reached a plateau as shown. Respiratory states are listed along the top. For corresponding definitions, see Table 1.
Figure 3. Changes in mitochondrial respiration after resistance exercise (n=8)

Values are mean ± SE. Resistance exercise significantly increases complex I-supported lipid [(ETF + CI)]_{Lip} and lipid + pyruvate respiration [(ETF + CI)_{Lip+pyr}], as well as lipid + pyruvate respiration through complexes I and II [(ETF + CI + CII)_{Lip+pyr}], maximal electron transport capacity [(ETF + CI + CII)_{E}] (uncoupled respiration), and complex II supported respiration [(CII)_{E}] (measured after inhibition of complex I with rotenone). *denotes significantly different than rest at p<.05.
Figure 4. Changes in gene expression in skeletal muscle after resistance exercise (n=7).

Values are mean fold change above rest ± SE. Resistance exercise significantly increases skeletal muscle expression of PGC1alpha (A), PDK4 (B), FOXO1 (C), MURF1 (F), and tended to increase LPL gene expression (E). The exercise session also significantly reduced expression of SREBF1 (also known as SREBP1) relative to rest (D). We also found a trend toward an increase in lipoprotein lipase. * Denotes significant difference between rest and exercise. § Denotes trend toward significant difference between rest and exercise.
Resistance exercise significantly increases adipose tissue expression of FOXO1, PDK4, LPL, HSL, ADIPOQ, and tended to increase CD36 and ATGL expression. * denote significant difference between rest (black) and resistance exercise (gray) at p<.05.
5. Overall Summary

5.1 Significance of Key Findings

The primary purposes of this dissertation were to determine the influence of an acute bout of resistance exercise on: 1) postprandial glucose kinetics (appearance, disappearance), insulin sensitivity, and oxidation; 2) postprandial lipid handling, kinetics, and oxidation; 3) skeletal muscle mitochondrial oxidative capacity; and 4) muscle and adipose tissue gene expression in obese, sedentary men with prediabetes.

5.1.1 Chapter 2

Recent evidence suggests that postprandial hyperglycemia is a major, independent risk factor for cardiovascular disease (CVD) and other comorbidities associated with prediabetes, including hypertension, BMI, intraocular pressure, progression to type 2 diabetes, and all cause mortality.\textsuperscript{1,2} Despite the strong connection between postprandial hyperglycemia and disease severity, no studies have evaluated the influence of pre-meal resistance exercise on postprandial glucose metabolism after consumption of a mixed meal in individuals with prediabetes. Moreover, the mechanisms for the potential effects of resistance exercise on postprandial glucose metabolism including beta cell function, insulin sensitivity, hepatic insulin sensitivity, and skeletal muscle glucose oxidation in individuals with prediabetes, are not known. Therefore, the purpose of this chapter was to determine the effects of a single bout of resistance exercise on
postprandial glucose metabolism following a mixed-meal in obese, sedentary men with prediabetes.

We found that a single bout of resistance exercise performed 4.5 hours before a mixed meal reduces postprandial glycemia and insulin concentrations, and that these changes were associated with improvements in peripheral insulin sensitivity, glucose clearance rates, and whole-body and skeletal muscle mitochondrial carbohydrate oxidation. These results provide preliminary evidence for the potential application of resistance exercise as a non-invasive means of improving postprandial glucose metabolism.

5.1.2 Chapter 3

Resistance exercise is a central component of exercise recommendations for individuals with type 2 diabetes and prediabetes, but the effect of resistance exercise on postprandial lipid metabolism is not known. There is some evidence in healthy lean and healthy obese men and women demonstrating that regular physical activity can improve postprandial lipid metabolism.\textsuperscript{3} For example, in healthy, obese men and women, acute resistance exercise reduced triglyceride concentration, VLDL triglyceride concentrations and mean residence time, improved fatty acid oxidation rate, and increased IMTG turnover in both the fasted and postprandial state; however, not all studies found these improvements.\textsuperscript{4-10} In addition, one study in individuals with type 2 diabetes found no effect of pre-meal and a modest effect of post-meal resistance exercise on postprandial triglyceride area under the curve.\textsuperscript{11} Thus, evidence in healthy and diabetic populations suggests that resistance exercise might improve postprandial lipid metabolism in individuals with prediabetes, however this has not been previously studied. Moreover, the
potential mechanisms of improved postprandial lipid handling after resistance exercise in those with prediabetes (or other populations) remain unknown. Therefore, the overall aim of this chapter was to determine the acute effects of resistance exercise on postprandial lipid metabolism in obese men with prediabetes.

This study demonstrated that a single bout of resistance exercise, performed 210 minutes before consuming a mixed carbohydrate and lipid meal, reduced total plasma TG, chylomicron and TRL-TG concentrations, and tended to reduce VLDL plasma FFA concentrations in men with prediabetes. In addition, our study revealed for the first time, that declines in plasma triglyceride concentration following acute resistance exercise were due to reductions of both exogenous (meal-derived) and endogenous (originating internally) triglycerides and fatty acids. Moreover, acute resistance exercise increased lipoprotein turnover, FFA clearance rate, and postprandial lipid oxidation rate. These data support the use of resistance exercise as a non-invasive treatment approach to reduce postprandial lipemia in men with prediabetes.

5.1.3 Chapter 4

Physical inactivity is known to induce insulin resistance and reduce mitochondrial respiration in skeletal muscle. In contrast, increasing physical activity can increase skeletal muscle oxidative capacity and improve lipid metabolism in healthy and obese populations. Much less is known regarding the benefits of exercise on skeletal muscle oxidative capacity in those with prediabetes and type 2 diabetes. In particular, the mitochondrial response to an acute bout of resistance exercise has received little investigative attention despite being a central component of exercise recommendations for these individuals. Therefore, the purpose of this
chapter was to investigate the impact of a single bout of acute resistance exercise on skeletal muscle mitochondrial oxidative capacity and skeletal muscle and adipose tissue gene expression in the postprandial state in obese, sedentary, pre-diabetic males.

In this study, we found that a single bout of resistance exercise increased mitochondrial respiratory capacity and lipid oxidation in obese men with prediabetes. These adaptations occurred concurrently with changes in skeletal muscle (PGC1alpha, PDK4, SREBP1, FOXO1, and MURF1) and adipose tissue (FOXO1, PDK4, HSL, ADIPOQ, and LPL) gene expression. Together, these genes cooperate to mobilize stored FFA and increase lipoprotein hydrolysis (and storing glucose) in adipose tissue, while increasing lipid uptake and oxidation in skeletal muscle. Improvements in lipid clearance through oxidation could reduce the risk for progression from prediabetes to type 2 diabetes, as well as facilitate weight loss and lower the risk for cardiovascular disease.

5.1.4 Overall Conclusions

This dissertation found that in obese, sedentary men with prediabetes, a single session of resistance exercise: 1) significantly improved postprandial glucose metabolism by increasing glucose clearance, insulin sensitivity, and carbohydrate oxidation; 2) significantly reduced postprandial exogenous and endogenous lipid contributions to chylomicrons, VLDL and free fatty acids, as well as increased lipoprotein clearance, FFA clearance, and lipid oxidation; and 3) increased skeletal muscle oxidative capacity, as well as the expression of genes regulating lipid metabolism in skeletal muscle and adipose tissue. The implications of these findings are summarized in Figure 5.1. In healthy individuals, the uptake of lipid and glucose from the meal
is balanced by the clearance of these substrates in peripheral tissues, such as skeletal muscle, adipose tissue, and the liver through oxidation or storage as glycogen or triglyceride. In obesity and prediabetes, poor diets can contribute to excessive uptake of glucose and lipid, which may be exacerbated by reductions in oxidative capacity or clearance (see discussion section). This imbalance leads to prolonged and excessive postprandial glucose and lipid concentrations, which contribute to CVD and disease progression. Resistance exercise may be beneficial by reducing the uptake of meal-derived nutrients (most notably chylomicron triglycerides), and increasing the clearance of lipoproteins, FFA, and glucose through oxidation. Taken together, these improvements may restore balance between substrate uptake and clearance, thereby reducing the risk for disease progression or life-threatening complications. These support the use of resistance exercise to improve postprandial metabolism in obese men with prediabetes.

Figure 5.1. Summative Diagram

5.2 Future Directions
Future studies are needed to investigate the effects of post-meal resistance exercise on postprandial lipid and glucose metabolism. Previous studies have shown that post-dinner resistance exercise was more effective at reducing postprandial triglyceride and glucose concentrations in obese individuals with type 2 diabetes.\textsuperscript{11} Therefore, while we found beneficial effects for pre-meal resistance exercise in men with prediabetes, post-meal resistance exercise may be a more effective option for these individuals. Additionally, clarifying the timing of resistance exercise relative to the meal that maximizes postprandial responses (eg. pre-meal vs. post-meal, amount of time between exercise and the meal) will be needed to create exercise recommendations for individuals with prediabetes.

Future studies should also investigate the influence of resistance exercise on exogenous and endogenous glucose metabolism. This would be needed to understand if the reductions in post-prandial glucose after resistance exercise were due to an increase in suppression of endogenous glucose production, or a suppression of the rate of appearance of oral glucose from the GI tract, or both. Previous studies have shown that individuals with type 2 diabetes present with elevated endogenous glucose production that is not suppressed appropriately in the post-prandial period.\textsuperscript{20} In contrast, the oral glucose rate of appearance appears normal.\textsuperscript{20} Therefore, understanding if resistance exercise suppresses endogenous glucose production could have a particularly strong impact on disease progression from prediabetes to type 2 diabetes.

Further measurement of VLDL kinetics in isolation will also be needed to understand changes in hepatic function in response to resistance exercise. Since we only measured VLDL kinetics indirectly, additional research is needed to specifically identify changes in postprandial VLDL metabolism after resistance exercise. Future studies that use a compartmental modeling approach would be particularly effective at clarifying the organs and metabolic pathways that are
responding to the resistance exercise session. Previous studies suggest that chylomicrons are the preferred substrate for lipoprotein lipase, and that postprandial triglyceride levels may be due mostly to the accumulation of VLDL that are not hydrolyzed by LPL.\textsuperscript{21} We found trends for most of the outcomes for VLDL, which may be explained by a preference for chylomicrons by LPL in the postprandial period. However, we could not confirm this in this study.

Additionally, experiments employing repeat biopsies would be needed to determine the changes in gene expression throughout the postprandial period, and how these changes correlate with improvements in lipid versus glucose oxidation. Further studies are also needed to determine if these changes in gene expression result in significant changes in protein expression, and if these changes in protein expression correspond to the improvements in mitochondrial respiration and substrate selection. Furthermore, there are multiple different types of exercise that should receive further investigation for their impact on postprandial metabolism. These include eccentric resistance exercise, high-intensity interval training, and low-impact exercises such as yoga. All of these could be beneficial for individuals with prediabetes, but haven’t been investigated with respect to the postprandial period.

Finally, this study should be repeated with female participants. Previous studies have shown that women demonstrate increased lipid oxidation and reduced carbohydrate oxidation, deplete less muscle glycogen, and have lower endogenous glucose production during exercise than men.\textsuperscript{22} There are also differences between men and women on insulin signaling and insulin secretion due to estrogen activity, as well as the rates adipose triglyceride synthesis and lipolysis.\textsuperscript{23} All of these factors could alter the effects of resistance exercise on postprandial metabolism and therefore require further investigation.
5.3 Limitations

First, this study only enrolled adult men. Thus, the results cannot be generalized to women and children. Postprandial responses to resistance exercise may be different between men and women and therefore requires further study. We also had participants consume a liquid test meal. While the nutrient composition of this meal reflects the composition of a typical meal, the liquid composition may change the absorption of carbohydrate and lipid compared to solid meals. Further investigation is needed to examine the influence of liquid versus solid meals on absorption and nutrient handling. Additionally, the study sample size was limited and should be expanded to increase the validity of these findings.


