DISSERTATION

Cardiometabolic Plasticity and Skeletal Muscle Protein Expression in Hispanic and non-Hispanic Whites in Response to a Short-Term Diet and Exercise Intervention

Submitted by

Stacy L. Schmidt

Department of Food Science and Human Nutrition

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY STACY L. SCHMIDT ENTITLED CARDIOMETABOLIC PLASTICITY AND SKELETAL MUSCLE PROTEIN EXPRESSION IN HISPANIC AND NON-HISPANIC WHITE ADULTS IN RESPONSE TO A SHORT TERM DIET AND EXERCISE INTERVENTION BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate work

Co-Advisor

Advisor

Department Head

ABSTRACT OF DISSERTATION

CARDIOMETABOLIC PLASTICITY AND SKELETAL MUSCLE PROTEIN EXPRESSION IN HISPANIC AND NON-HISPANIC WHITES IN RESPONSE TO A SHORT-TERM DIET AND EXERCISE INTERVENTION

The prevalence rates for type 2 diabetes (T2D) and the metabolic syndrome (MetS) have steadily increased to epidemic proportions over the past few decades, with disproportionately high rates of these health problems in Hispanics. The largest minority group in the United States is Hispanics, with Mexican Americans (MA) comprising the largest and fastest growing portion of the US Hispanic population. Insulin resistance is more prevalent in the MA population compared to other ethnic groups, and appears to precede many of the metabolic abnormalities involved in the progression toward T2D and MetS. Insulin resistance and many factors present in the MetS have been shown to improve following an increase in physical activity and consumption of diets low in saturated fatty acids and high in fiber. The overall objective of this project was to determine the combined effects of an increase in exercise combined with dietary lipid and carbohydrate modification on insulin sensitivity and blood lipids, and to determine if differences in expression of skeletal muscle proteins exist in non-obese, non-diabetic sedentary MA and NHW adults.

In **Arm 1** of the study, we determined whether differences in insulin sensitivity persist following a short-term diet and exercise intervention between young, sedentary, non-obese, non-diabetic subjects (20 NHW: 11F, 9M, age=23.0 y, BMI=25.5; 17 MA: 13F, 4M, age=22.7, BMI=25.4). In addition, we determined whether or not differences in insulin sensitivity led to greater dyslipidemia in MA compared to NHW and whether or not these two ethnic groups responded similarly to the same lifestyle intervention. Both MA and NHW had significant

improvements in insulin sensitivity, determined by total insulin area under the curve (AUC) during an intravenous glucose tolerance test, following the intervention, suggesting that MA exhibit similar cardiometabolic plasticity as NHW. However, significant differences in insulin sensitivity were observed both before and following the short-term diet and exercise intervention in MA compared to NHW, and the disparity in insulin sensitivity remained. Despite differences in insulin sensitivity, we observed no differences in blood lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, small dense LDL particles, and non-esterified fatty acids) pre or post intervention between ethnicities. Even though both ethnic groups exhibited normal blood lipids at the outset of the study, both ethnic groups had significant decreases in total cholesterol, LDL-cholesterol, triglycerides, and small dense LDL particles following the short-term intervention. Our results suggest that MA respond to a short-term lifestyle intervention in a similar manner as NHW, although the ethnic disparity in insulin sensitivity is not attenuated.

In **Arm 2** of our study, we utilized a proteomics approach to examine expression of skeletal muscle proteins in a subset of 3 MA and 3 NHW subjects in order to provide potential targets for further analysis on the entire study cohort. We found differences in expression of two mitochondrial proteins, carnitine palmitoyltransferase 1A isoform 2 (CPT-1) and mitochondrial ATP synthase F1 complex beta subunit (ATP synthase). ATP synthase was 3-fold higher in NHW (NHW= 188.4 ± 10.2 and MA = 62.7 ± 28.4), while CPT-1 was 1.7 times higher in NHW compared to MA (NHW= 190.4 ± 1.3 vs MA = 109.3 ± 12.6). Western blot analysis of skeletal muscle ATP synthase in 11 MA (3 males, 8 females) and 10 NHW (3 males, 7 females) revealed no differences in protein abundance between ethnic groups or following the insulin-

sensitizing diet and exercise intervention. Therefore, despite the findings from the proteomic analysis, there is no evidence that ATP synthase differs in skeletal muscle of MA compared to NHW. CPT-1 analysis has not yet been completed due to difficulties in measuring CPT-1 in skeletal muscle. Further work to identify possible differences in skeletal muscle proteins and their activation between MA and NHW is warranted.

Stacy L. Schmidt
Department of Food Science and Human Nutrition
Colorado State University
Fort Collins, Colorado
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TABLE OF CONTENTS

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CHAPTER 1

INTRODUCTION AND SPECIFIC AIMS

The metabolic syndrome (MetS) is estimated to afflict more than 50 million adults in the United States [1]. This condition is characterized by a clustering of metabolic abnormalities that is associated with an increased risk for type 2 diabetes (T2D) and cardiovascular disease (CVD) [2]. These abnormalities include abdominal obesity, hypertension, hyperglycemia, hypertriglyceridemia, and low levels of high density lipoprotein (HDL) cholesterol [2]. A primary factor associated with these metabolic abnormalities is the presence of insulin resistance. Insulin resistance is a condition in which a normal amount of insulin is inadequate to produce a normal response from skeletal muscle, liver, and fat. Factors that contribute to insulin resistance include physical inactivity, a diet high in saturated fat and refined carbohydrates, obesity, visceral adiposity, and a genetic predisposition. Certain ethnic groups within the United States tend to be more insulin resistant than others, despite having similar levels of fitness and fatness, suggesting that genetic differences may contribute to higher risk for metabolic diseases, such as T2D and CVD. Compared to other ethnic groups, Hispanics exhibit the highest overall prevalence of insulin resistance in the US [3] and almost a 2-fold higher prevalence of T2D than non Hispanic whites [4]. Even when factors that are known to contribute to insulin resistance, such as

total and abdominal obesity and physical fitness, are controlled for, Hispanics still exhibit greater insulin resistance compared to non Hispanic whites [5, 6]. In addition, the highest overall prevalence of the MetS is found in Mexican Americans (MA), who represent the largest subgroup of Hispanics and the fastest growing portion of the U.S. population [1]. Despite the clear evidence that these health disparities exist, the specific causes that contribute to MA having higher rates of these adverse health conditions have not been fully elucidated.

Factors that promote insulin resistance such as low levels of physical activity, diets high in saturated fat and refined carbohydrates, abdominal and total obesity are widespread among the MA population, but are also present among many NHW who have lower rates of MetS and T2D. This disconnect between the presence of risk factors and disease appearance in these two ethnic groups points to the possibility that genetic factors, unique to MA, make them more susceptible to the untoward effects of having a sedentary lifestyle and an unhealthy western diet. On the other hand, it is also possible that MA do not respond as favorably to lifestyle changes, that is, physical activity and a diet low in saturated fat and refined carbohydrates might not produce the same magnitude of phenotypic benefits in MA as in NHW, which could partially explain these health disparities. To date, no studies have examined the combined effects of an increase in physical activity and a diet low in saturated fat and refined carbohydrates, without considerable weight loss, on risk factors for T2D, CVD, and specific features of the MetS in MA. The magnitude of reduction of risk factors for T2D, CVD, and MetS might differ between ethnic groups, rendering a "blanketed" or "one-size-fits-all"

approach for prevention and treatment of these conditions to be inadequate. We have therefore set out to determine if a short term diet and exercise intervention affects MA differently from NHW in regard to specific features including insulin sensitivity and circulating blood lipids. We also examined skeletal muscle tissue samples in these two groups. Skeletal muscle is responsible for the majority of glucose uptake in the periphery. Enhanced insulin signaling has been associated with a greater abundance of insulin signaling proteins in skeletal muscle [7-11], and we therefore sought to determine if specific proteins differ between these two ethnic groups which could partially explain differences seen in disease risk. Because the focus of our research group is on the prevention rather than the treatment of T2D and the MetS, we chose to study young, non-obese, non-diabetic sedentary adults.

Specific Aim 1: To determine if there is a difference in insulin sensitivity in response to a one-week diet and exercise intervention between nonobese, nondiabetic Mexican American and non-Hispanic white males and females.

Hypothesis: Mexican Americans will exhibit lower insulin sensitivity at baseline, but will have the same degree of improvement as NHW in insulin sensitivity in response to the diet and exercise intervention

Specific Aim 2: To determine if there is a difference in blood lipids at baseline and in response to a 7-day diet and exercise intervention in MA and NHW

Hypothesis: Mexican Americans will have greater dyslipidemia than NHW at baseline but will have the same degree of improvement as NHW in markers for cardiovascular dyslipidemia in response to the diet and exercise intervention.

Specific Aim 3: To utilize 2D proteomics for a preliminary assessment of possible differences in the baseline expression level of skeletal muscle proteins in MA and NHW.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

The United States and other Westernized countries are facing an epidemic increase in obesity-related health problems, including the metabolic syndrome (MetS) which is estimated to afflict more than 50 million adults in the United States [1, 3]. This condition is characterized by a clustering of metabolic abnormalities that are associated with an increased risk for type 2 diabetes (T2D) and cardiovascular disease (CVD) [2]. These abnormalities include abdominal obesity, hypertension, hyperglycemia, hypertriglyceridemia, and low high density lipoprotein (HDL) cholesterol [2]. A primary factor underlying these metabolic abnormalities is the presence of insulin resistance [12].

Certain ethnic groups within the United States tend to be more insulin resistant than others, suggesting that genetic differences and/or ethnic differences in gene-environment interactions may contribute to the disparity in the prevalence of insulin resistance among population groups. Compared to other ethnic groups, Hispanics exhibit the highest overall prevalence of insulin resistance in the U.S. [3] and almost a 2-fold higher prevalence of T2D than non Hispanic whites [4]. In addition, the highest overall prevalence of the MetS is found in Mexican Americans (MA), who represent the largest subgroup of Hispanics and the fastest growing portion of the U.S. population [1].

Genetic ties to insulin resistance in nonobese individuals are poorly understood from a mechanistic standpoint; however, it is probable that genetic components interact with environmental factors in order for insulin resistance to develop into a pathophysiological state. Mexican Americans often have lifestyle factors that differ from NHWs which may contribute to their higher prevalence of T2D and MetS. Despite the clear evidence that health disparities exist among ethnic groups, the specific causes that contribute to Hispanics having higher rates of these conditions have not been fully elucidated. The higher incidence of T2D and MetS in Hispanics compared to NHW may be explained by the higher degree of insulin resistance in Hispanics, but the physiological explanation for the observed higher insulin resistance in Hispanics is not known. This literature review will primarily focus on insulin resistance and its metabolic consequences in addition to environmental factors that may contribute to the higher prevalence of insulin resistance in the Hispanic population.

Insulin Resistance

Insulin is a classic pleiotropic hormone that elicits effects in multiple cell-signaling pathways, generally involved in activating anabolic processes while simultaneously suppressing catabolic processes. Although insulin has historically been viewed as primarily involved in glucose uptake, it also facilitates the uptake and storage of amino acids and fatty acids, and initiates complex signaling pathways that have multiple functions, including protein synthesis, cell proliferation, cellular differentiation, glycogen synthesis and glucose transport [13-17]. Insulin's effects are mediated through intracellular signaling pathways which are initiated with the binding of insulin to

the membrane-bound insulin receptor in response to an increase in circulating glucose, amino acids, or lipids. Insulin resistance occurs when a normal dose of insulin is incapable of eliciting appropriate responses in target tissues. Given that insulin functions in a variety of tissues, insulin resistance has different effects among its various target tissues. Key characteristics of insulin resistance are a decrease in insulin-stimulated uptake of glucose in skeletal muscle, impaired suppression of glucose output by the liver, and reduced ability of insulin to inhibit lipolysis in adipose tissue [18].

Prospective studies show that insulin resistance is a predictor for subsequent T2D and MetS [19]. In the progression to T2D, the pancreas compensates for subnormal responsiveness to insulin by augmenting the secretion of insulin [20]. This compensatory hyperinsulinemia aids in maintaining normal blood glucose concentrations, but can contribute to the pathophysiological conditions present in the MetS, such as high triglycerides, low HDL cholesterol, and hypertension [21-23]. With time, the β-cells in the pancreas may fail to adequately secrete insulin, in which case, impaired glucose tolerance results. The imbalance between insulin secretion and glucose uptake can lead to hyperglycemia and the clinical diagnosis of T2D [20, 24].

Under normal conditions, insulin binds to the α subunits of the membrane-bound insulin receptor, which causes the β subunits to dimerize and autotransphosphorylate tyrosine residues, activating the receptor [25]. The activated insulin receptor recruits insulin receptor substrate (IRS) proteins to bind to phosphorylated tyrosine residues on the insulin receptor. This binding of IRS to the insulin receptor stimulates the kinase activity of the insulin receptor to catalyze

phosphorylation of tyrosine residues on IRS. There are multiple IRS proteins, with IRS-1 being the most predominate form in skeletal muscle [26]. IRS-1 is a docking protein that contains several potential tyrosine and serine/threonine phosphorylation sites. Phosphorylation specifically on tyrosine residues activates IRS-1, which in turn is recognized by and binds to Src homology 2 (SH2) domains present in various signal transduction proteins [27]. A key signaling molecule downstream of IRS-1 is phosphatidylinositol 3- kinase (PI3K). The PI3K regulatory subunit, p85, contains SH2 domains that bind onto activated IRS-1 [27]. Association of IRS-1 with PI3K leads to downstream signaling through intracellular serine/threonine kinases, such as Akt and AS160, which ultimately stimulates the translocation of the glucose transport protein, GLUT4, from its intracellular pool to the plasma membrane, allowing glucose entry into the cell [28, 29] (Figure 1).

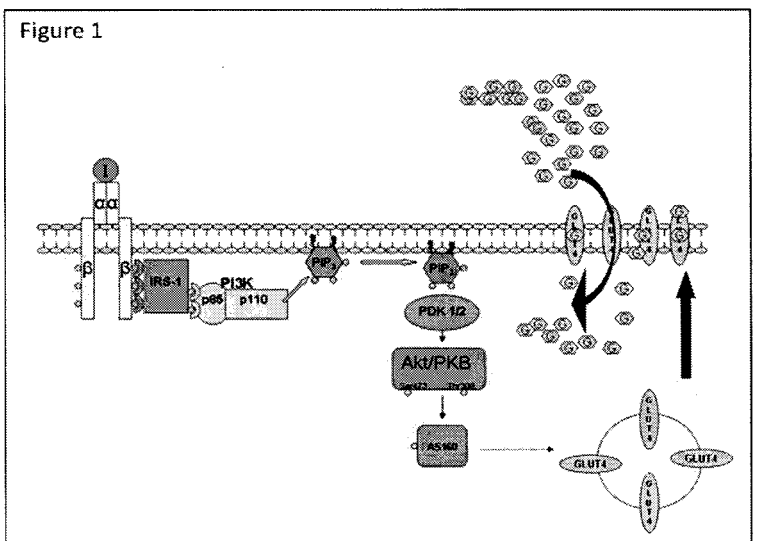


Figure 1. Intracellular Insulin Signaling Cascade

Insulin-mediated glucose uptake requires tyrosine phosphorylation of IRS-1; however, research has demonstrated that skeletal muscle of patients with T2D has a high amount of serine phosphorylation on IRS-1 [30]. Serine phosphorylation impedes IRS-1 tyrosine phosphorylation, which impairs the ability of IRS-1 to associate with PI3K and decreases the activation of this signaling pathway [31]. While serine

phosphorylation of IRS-1 decreases activation of the PI3K pathway, other insulin-stimulated pathways, such as the mitogen-activated kinase (MAPK) pathway, remain fully functional [32]. The MAPK pathway is activated downstream of the insulin receptor through Shc proteins and is responsible for cell-proliferation and differentiation and may even exacerbate the defects in the PI3K pathway [30, 32]. This selective impairment in the PI3K pathway results in a reduction in the ability of insulin to facilitate removal of glucose from the blood.

Skeletal Muscle Lipid Metabolism

Skeletal muscle is accountable for ~80% of insulin-stimulated disposal of intravenous glucose in peripheral tissues and about one-third of postprandial glucose uptake, and it is therefore considered one of the most important sites of insulin action [33, 34]. Impairments in insulin-stimulated glucose uptake in skeletal muscle have been shown to be an early facet to the pathogenesis of T2D and the MetS [35, 36]. While the primary molecular events leading to insulin resistance remain elusive, abnormal lipid metabolism is implicated [37].

Although hyperglycemia is the clinical hallmark of T2D, elevated free or nonesterified fatty acids (NEFA) are also common in insulin resistant states and often precede the onset of hyperglycemia [38, 39]. High plasma NEFA are associated with a number of MetS abnormalities, including hypertension, dyslipidemia, and abdominal obesity [21, 40]. NEFA can become elevated in a number of situations, including overfeeding, high fat diets, physical inactivity and obesity [41]. NEFA tend to be most elevated in obese individuals, especially those with a high amount of visceral adiposity

[42]. In adipose tissue, insulin inhibits the release of fatty acids from adipocytes by decreasing the activity of hormone-sensitive lipase (HSL) [43]. Visceral adipose tissue however is very insulin resistant, more so than subcutaneous adipose tissue, and therefore has a higher rate of lipolysis, which leads to an increase in circulating NEFA with increased visceral adipose tissue [44].

Experimental models elevating NEFA by either a lipid infusion or acutely feeding a high fat diet cause insulin resistance in nonadipose tissue depots such as skeletal muscle, and have been shown to do so in a dose-dependent manner [45-49]. Circulating NEFA enter skeletal muscle cells primarily through specific fatty acid transporter proteins, and this uptake of fatty acids into muscle fibers is associated with an increase in insulin resistance in skeletal muscle [50-52]. Much research has focused on the role of protein-mediated transport in regulating fatty acid entry into skeletal muscle cells [50, 51, 53]. A number of findings have indicated that three groups of long-chain fatty-acid (LCFA) transport proteins facilitate LCFA transport in human skeletal muscle: fatty-acid translocase (FAT/CD36), plasma membrane-associated fatty-acid binding protein (FABPpm), and fatty-acid transport proteins (FATP) 1-6 [54]. Whereas little is known about the regulation of FATP 1–6 in human skeletal muscle, substantial evidence has accumulated to demonstrate that FAT/CD36 and FABPpm are fundamental in regulating the uptake of LCFAs into skeletal muscle [55]. These proteins may, in part, mediate the development of fatty acid-induced insulin resistance.

Bonen et al recently showed that obese Zucker rats had significantly higher FAT/CD36 protein expression in their skeletal muscle compared to lean controls, which

was associated with a higher amount of intramuscular triglyceride (IMTG), the storage form of lipids in skeletal muscle [56]. This group also showed that fatty acid transport into sarcolemmal vesicles in vitro was ~4-fold higher in vesicles prepared from obese and T2D individuals compared to their lean counterparts [57]. This increased uptake was associated with higher CD36 content and IMTG in the obese T2D group of subjects [57]. Skeletal muscle of obese and T2D individuals also has a higher amount of FABPpm compared to healthy individuals [58]. A higher FABP content in the plasma membrane is associated with increased NEFA uptake and reduced insulin sensitivity [59]. Obese Zucker rats have been shown to take up a higher amount of infused palmitate into skeletal muscle than lean control rats, and this increased uptake is associated with higher FABPpm content and incorporation of fat into IMTG [59].

Once fatty acids enter the muscle cell, they are primarily partitioned toward storage as IMTG or entry into the mitochondria for oxidation [60]. The appearance of fatty acids in myocytes can lead to accumulation of lipids as IMTG if there is an imbalance between fatty acid delivery and utilization [61]. The direct relationship reported between the degree of insulin resistance and IMTG content in obese T2D patients, high fat feeding models, and lean offspring of T2D patients, suggests that IMTG could be interfering with insulin signaling [52, 62, 63]. Strong evidence indicates, however, that it is not the IMTG in itself, but the accumulation of lipid intermediates (i.e., long-chain fatty acyl-CoAs, diacylglycerol, and ceramide) in skeletal muscle that may be responsible for impairing insulin signaling, given that healthy physically fit individuals also demonstrate elevations in IMTG [64-66].

Long-chain fatty acyl-CoAs (LCFA-CoA) are the activated form of intracellular fatty acids, produced by the catalytic activity of long-chain acyl-CoA synthetase [37]. Studies using fatty acid infusions and high fat diets to induce insulin resistance have found an increase in LCFA-CoA content that parallels the increase in insulin resistance [67, 68]. Conversely, a decrease in total muscle LCFA-CoA content is observed when circulating NEFA are pharmacologically lowered, resulting in an improvement in insulin sensitivity in T2D patients [69]. These data suggest that LCFA-CoA in skeletal muscle may play a critical role in lipid-mediated insulin resistance. In addition, LCFA-CoAs are a precursor for the synthesis of diacylglycerol (DAG) via the esterification of two LCFA-CoAs to glycerol-3-phosphate [70]. Though DAG can also be generated by the breakdown of phospholipids, synthesis from LCFA-CoAs is the predominant source of DAG [70]. DAG content in skeletal muscle has been shown to be higher in several human and rodent models of lipid-induced insulin resistance [71]. DAG and LCFA-CoA interrupt insulin signaling by activating serine/threonine stress kinases, including protein kinase C-theta (PKC- θ), c-jun N-terminal kinase (JNK), and inhibitor of NF-κB kinase (IKKβ) which are potent stimulators of serine phosphorylation on IRS-1 and antagonize insulin action [65, 72-74]. In humans, PKC-θ, JNK, and IKKβ content and activity are higher in skeletal muscle of obese diabetics compared to nondiabetics [75-78]. Most research supports the notion that DAG promotes insulin resistance through activation of these kinases, and although LCFA-CoA content is also correlated with stress kinase activity, a direct link has not been identified. LCFA-CoA is speculated to act through DAG and another lipid intermediate, ceramide, to interfere with insulin signaling [75].

Ceramide is a lipid metabolite that has received much attention in the literature for its potential role in skeletal muscle insulin resistance [79]. Ceramide can form in skeletal muscle via synthesis from LCFA-CoA, primarily palmitoyl-CoA, or by the hydrolysis of sphingomyelin, which is a phospholipid component of cell membranes [80, 81]. Elevated intracellular ceramide has been found in skeletal muscle of obese, insulin resistant humans, lipid-infused humans, and lean relatives of T2D patients [82-84]. Intracellular ceramide accumulation is associated with a disruption of the insulin signal, specifically with a reduction in the activity of Akt, an insulin signaling protein downstream of PI3K [82, 85]. Akt activity has been shown to be lower in skeletal muscle of obese individuals which was associated with higher ceramide content [82]. It is possible that this reduction in Akt activity reflects the impaired upstream signaling (IRS-1 associated PI3K) that has been shown to be defective in obese individuals [86]. However, *in vitro* studies have indicated that ceramide can impair the ability of Akt to translocate to the plasma membrane, independent of impairments in PI3K signaling, limiting its interaction with membrane-bound protein phosphatidylinositol-3-phosphate (PIP_3), which positions Akt to be activated by PDK [87]. Recent evidence suggests that ceramide might not be a significant player in reduced skeletal muscle insulin sensitivity in humans, given that its levels are no different among T2D patients, glucose intolerant individuals, healthy controls, and endurance athletes [88]. Although the precise mechanisms by which lipids exert their negative effects on insulin signaling in skeletal muscle have not been fully elucidated, current research suggests that intramuscular

lipid accumulation has negative effects on insulin signaling, likely through accumulation of lipid intermediates.

Fatty Acid Oxidation: Evidence that IMTG may not be directly involved in impairing insulin signaling stems from the finding that high IMTG content is seen in endurance athletes who are highly sensitive to insulin, an observation that is commonly referred to as the "athlete's paradox" [89]. A potentially important mechanism by which endurance athletes have higher insulin sensitivity in the face of elevated IMTG content is a higher capacity to oxidize lipids. Endurance-trained individuals typically have a higher oxidative capacity than sedentary individuals and a higher rate of fatty acid turnover (synthesis and oxidation), which limits the exposure time of the muscle cells to the potentially toxic effects of TG accumulation [89]. Therefore oxidative capacity may be an important player in the ability to attenuate the appearance of harmful lipid metabolites that impair insulin signaling in skeletal muscle [90].

Given that the primary metabolic pathways involved in fatty acid metabolism (β-oxidation, the tricarboxylic acid (TCA) cycle, and the electron transport chain (ETC)) are located within the mitochondria, it is possible that deficiencies in this organelle could contribute to a lower ability to oxidize lipids. Cross-sectional studies have revealed a strong direct correlation between insulin sensitivity and skeletal muscle oxidative capacity [91, 92]. A possible link between mitochondrial functionality and insulin resistance is that a reduced oxidative capacity forces lipids to be preferentially partitioned away from mitochondrial oxidation leading to cytosolic accumulation of

IMTG, LCFA-CoA, DAG and ceramide [93]. A reduced capacity to oxidize lipids could explain the higher content of lipid intermediates in insulin resistant individuals.

In order for fatty acid oxidation to occur, LCFA-CoA must cross the inner mitochondrial membrane. This transport requires LCFA-CoA to form an acyl-carnitine complex which is synthesized via carnitine palmitoyltransferase-1 (CPT-1) [94]. The acyl-carnitine complex produced by CPT-1 is able to penetrate the inner mitochondrial membrane, after which, it is converted back to the original LCFA-CoA and carnitine, giving LCFA-CoA access to enzymes of β-oxidation [94] (Figure 2). Skeletal muscle CPT-1 is a key regulatory enzyme in fatty acid oxidation. Overexpression of CPT-1 *in vivo* leads to increases in fatty acid oxidation and reduced storage of lipids, while inhibiting CPT-1 leads to diminished fatty acid oxidation, reduced insulin sensitivity, and increased IMTG content [62, 95]. Kelley et al found obese subjects to have lower CPT-1 activity compared to lean controls; however, CPT-1 activity did not change when these obese subjects significantly improved their insulin sensitivity suggesting that factors other than CPT-1 contribute to lipid-mediated insulin resistance [96]. It has recently been suggested that CPT-1 may work in combination

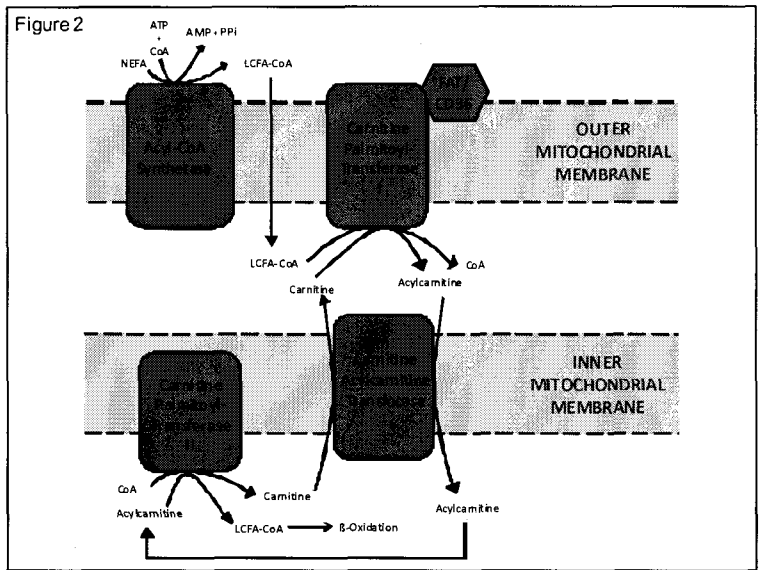


Figure 2. Intramembrane transport of fatty acids via carnitine palmitoyltransferase-1

with FAT/CD36 to promote fatty acid entry into the mitochondria. FAT/CD36 was recently shown to be expressed on the mitochondrial membrane in rat and human muscle [97-99]. Blocking mitochondrial FAT/CD36 significantly decreases fatty acid oxidation, indicating that it is an important player in oxidizing lipids [98, 99]. Although its exact function has not been documented, it appears that FAT/CD36 works in conjunction with CPT-1 to facilitate the transport of fatty acids into the mitochondria for oxidation [97, 100].

As fatty acids undergo β-oxidation and produce acetyl-CoA, additional processes in the mitochondria such as the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) contribute to the production of adenosine triphosphate (ATP) for energy and lead to the complete oxidation of fatty acids to CO_2 and H_2O. The oxidation of fatty acids is regulated at a number of key steps, including β-hydroxy acyl-CoA dehydrogenase (β-HAD) (a key enzyme in β-oxidation), citrate synthase (an enzyme in the TCA cycle), and cytochrome *c* oxidase (an enzyme in the ETC). The activity of these enzymes has been shown to be reduced in skeletal muscle samples of obese sedentary individuals and is associated with reduced insulin sensitivity [91, 101]. These data suggest that impairments in the mitochondria may contribute to insulin resistance. However, recent studies have indicated that fatty acid oxidative capacity in insulin resistant individuals may not be impaired if total mitochondrial content is taken into account [102, 103]. Insulin resistant and T2D individuals typically have ~30% less mitochondria in their skeletal muscle than insulin sensitive control subjects [104, 105]. When fatty acid oxidation rate was measured in *isolated mitochondria* from obese

insulin resistant individuals, there was no difference in the oxidation rate of palmitate compared to lean controls; however, *whole muscle* palmitate oxidation was significantly reduced in obese individuals indicating that mitochondrial number or protein content may be a limiting factor in obese subjects' oxidative capacity [102]. Additionally, the maximal activities (a marker for total protein content) of citrate synthase and β-HAD and cytochrome c oxidase content were lower in whole muscle samples from these obese individuals. Taken together these data indicate that obesity-related reductions in fatty acid oxidation are not necessarily due to inherent impairments within the mitochondria, but perhaps due to lower total protein content of the mitochondrial enzymes and/or lower total mitochondria [102].

Recently Koves et al explored the possibility that skeletal muscle insulin resistance is due to excessive, rather than reduced, flux through mitochondrial β-oxidation [106]. They postulated that inefficient coupling between the metabolic pathways involved in lipid oxidation in skeletal muscle contribute to insulin resistance in obese sedentary individuals. Their explanation for insulin resistance from high fat feeding was that elevated fatty acid oxidation resulted in a buildup of potentially toxic byproducts of incomplete lipid oxidation that may contribute to insulin resistance [106]. They found that there was a higher degree of incomplete fatty acid oxidation as measured *in vitro* by oxidation of labeled oleate to acid-soluble metabolites in rats fed a high fat diet compared to the standard chow-fed rats. In addition there was a lower amount of TCA cycle intermediates in the rats fed the high fat diet. The higher degree of insulin resistance in the high fat diet group may be explained by excess flux through

β-oxidation that exceeded the capacity of the TCA cycle and ETC, resulting in intramitochondrial accumulation of acylcarnitines and insulin resistance. Diabetic patients have also been shown to have reduced maximal mitochondrial oxidative capacity, which was associated with impairments at the level of the ETC and TCA cycle enzymes [107]. More detailed studies are needed however to show precisely how byproducts of incomplete fatty acid oxidation (i.e., acylcarnitines) are related to or cause insulin resistance.

Physical Activity

Individuals with insulin resistance exhibit impaired whole-body insulin action, due in part to impaired insulin action in skeletal muscle [108]. However, a large number of observational studies and exercise interventions have provided evidence demonstrating that regular physical activity can greatly improve insulin sensitivity and reduce risk for developing T2D and MetS, even without weight loss [109-118]. Therefore, current prevention and treatment strategies include regular physical activity as a means to reduce chronic disease risk [119].

It has been known for several years that a single bout of exercise (contraction) significantly increases insulin-stimulated glucose uptake in the exercised skeletal muscle [120-122]. Contraction and insulin stimulate glucose transport into skeletal muscle through independent mechanisms that mediate the translocation of GLUT4 to the plasma membrane, which together cause an additive increase in glucose transport [123, 124]. A single prolonged bout of exercise can lead to a 2-fold increase in both GLUT4 expression and insulin-stimulated glucose uptake in skeletal muscle following the

exercise bout [7]. This acute effect of contraction can persist for up to 48 hours following the exercise bout, depending on the energy expenditure of the exercise [125]. However, if another bout of exercise is not undertaken within 48hr, these enhancements in insulin signaling are reduced [126, 127]. For this reason, it is important for individuals to consistently engage in exercise in order to remain in a constant 'post-exercise' state. Regular physical activity can also lead to chronic training adaptations including changes in gene expression and activity of key proteins involved in insulin signaling [128] enhanced lipid metabolism [129, 130], mitochondrial biogenesis [131], and increased expression and activity of enzymes involved in fatty acid oxidation [129, 130] that contribute to enhanced insulin sensitivity.

An abundance of new evidence indicates that skeletal muscle insulin sensitivity is tightly linked to mitochondrial energy metabolism [123]. Mitochondrial oxidative capacity is increased with exercise training, and this training enhances the muscle's ability to tolerate a heavy lipid load, such as that seen in obesity or high fat feeding [130, 132, 133]. A recent study by Schenk and Horowitz demonstrated that a single bout of aerobic exercise prevented the increase in insulin resistance induced by an overnight lipid infusion [130]. This protective effect of exercise was accompanied by a decrease in DAG and ceramide, with a concomitant increase in IMTG and total fat oxidation measured by indirect calorimetry. Enhanced capacity for fatty acid oxidation and high insulin sensitivity are common in endurance trained athletes, but this was the first study to demonstrate a complete reversal of lipid-induced insulin resistance after a single exercise session, independent of training adaptations such as weight loss, increased

capillary density, or increases in VO_2max [89]. Bruce et al conducted a longer exercise intervention (8 weeks) in obese humans and found that training improved insulin sensitivity, increased mitochondrial fat oxidation, CPT-1, citrate synthase and β-HAD activity, and reduced DAG and ceramide content in skeletal muscle [132]. However, 8 weeks of training did not increase IMTG levels in this study. It is important to note, however, there was no increase in lipid supply, unlike the Schenk and Horowitz study [132]. The protective effect of exercise on insulin sensitivity seems to be associated with a greater ability to shunt lipids into IMTG rather than DAG, as IMTG does not appear to negatively affect insulin signaling [130, 134]. The mechanisms by which exercise reduces intramuscular content of DAG and ceramide are not completely understood, but exercise has been shown to increase diacylglycerol acyltransferase (DGAT) 1, which stimulates conversion of DAG to IMTG, thereby decreasing the DAG content in skeletal muscle. In addition, the lower ceramide content seen with exercise is associated with decreased synthesis and increased degradation of ceramide to sphingosine. Exercise decreases the activity of sphingomyelinase in skeletal muscle, which is responsible for ceramide synthesis from sphingomyelin [135]. Exercise also increases the activity of acid ceramidase, which degrades ceramide to sphingosine [136]. Together, these data show that exercise favorably affects the lipid composition in skeletal muscle, providing another potential mechanism for exercise-induced improvements in insulin action.

Exercise may also improve insulin action through tighter coupling of β-oxidation with the TCA cycle and ETC. Chronic high rates of β-oxidation may outpace metabolic

flux through the TCA cycle and ETC, leading to accumulation of incompletely oxidized fatty acyl-carnitine intermediates and ketones [137]. As mentioned previously, isolated mitochondria from rats fed a high fat diet showed a higher degree of incomplete fatty acid oxidation, as measured *in vitro* by oxidation of labeled oleate to acid-soluble metabolites, compared to the standard chow-fed rats [106]. A short term exercise intervention in mice fed a high fat diet was shown to lower muscle acylcarnitine levels, in association with increased TCA cycle activity [138]. Exercise increases expression of peroxisome proliferator activated receptor (PPAR)γ coactivator 1α (PGC1α) [139]. PGC1α activates transcription factors that have been shown to stimulate mitochondrial biogenesis and increase genes involved in oxidative phosphorylation, including cytochrome c oxidase and ATP synthase [140]. These effects of PGC1 α can potentially lead to better coordination between β-oxidation and the TCA cycle and ETC, which may result in more complete fat oxidation to CO_2 and less accumulation of partially oxidized lipids.

Interventions aimed at augmenting mitochondrial density and/or function, such as aerobic exercise, are likely to improve the balance between fatty acid uptake and complete fat oxidation and should be applied to treat and prevent insulin resistance. Regardless of the precise mechanism, the ability of exercise to enhance insulin sensitivity is clinically significant and emphasizes the importance of incorporating physical activity to prevent and treat T2D and MetS.

Insulin Resistance and Dyslipidemia

Most of the work on insulin resistance has focused on its role in the development of T2D; however, it also represents a major abnormality driving cardiovascular disease. Cardiovascular disease (CVD) is the leading cause of death in the United States, responsible for ~40% of deaths among all ethnic groups [141]. There are many forms of CVD, including hypertension, coronary heart disease, stroke, and heart failure [142]. Coronary heart disease (myocardial infarction or chest pain cause by reduced blood supply to the heart) is caused by atherosclerosis [142]. Atherosclerosis is characterized by the accumulation of cholesterol, fat, calcium and other substances in the inner lining of blood vessels that cause them to be narrowed or blocked, increasing risk for heart attack or stroke [142]. The process of atherosclerosis can begin as early as childhood and is often silent until an acute event (heart attack) occurs later in life [143]. As such, atherosclerosis accounts for ~80% of all diabetes-related deaths [144].

The progression toward atherosclerosis is highly dependent on the trafficking of lipids in the circulation. In healthy individuals, plasma lipids consist of triglycerides, phospholipids, cholesterol, cholesteryl esters, and NEFA [145]. Given that plasma is made up of mostly water, and lipids are insoluble in water, the transport of lipids is accomplished by associating TG and cholesteryl esters (nonpolar lipids) with phospholipids and cholesterol (amphipathic lipids) and proteins to make lipoproteins that readily travel through plasma [145]. There are four major groups of lipoproteins: 1) chylomicrons (CM), derived from intestinal absorption of TG and other lipids; 2) very low density lipoproteins (VLDL), derived from the liver for the export of TG; 3) low-density lipoproteins (LDL), derived from catabolism of VLDL; 4) high-density lipoproteins (HDL),

synthesized by the liver and intestine and involved in chylomicron and VLDL metabolism and cholesterol transport [145]. Chylomicrons and VLDL contain mostly TG, while LDL and HDL contain predominantly cholesterol and phospholipid, respectively. NEFA travel through plasma attached to albumin [145].

The dyslipidemia associated with insulin resistance is a key factor in risk for CVD and is characterized by a high level of circulating triglycerides, lower than average HDL cholesterol, and elevated concentrations of small, dense LDL particles with normal or slightly elevated LDL cholesterol [146]. Insulin is involved in the regulation and interaction of these lipoproteins. Recent evidence suggests that a principal defect in the dyslipidemia seen in insulin resistance is the overproduction of large VLDL particles [147]. The synthesis of VLDL particles involves a process whereby the structural protein apolipoprotein B100 (apoB-100) is lipidated with TG from cytosolic lipid stores within the liver [147]. The synthesis of apoB100 is constitutive under most conditions, with its assembly and secretion regulated posttranslationally by the presence of adequate core lipids (TG and cholesteryl esters) and microsomal triglyceride transfer protein (MTP) activity, which facilitates the lipidation of apoB100 [148]. Under conditions of low lipid or MTP availability, apoB-100 undergoes degradation by the proteasome [149]. This regulation allows for a means to respond to increased lipid supply and readily incorporate lipid into VLDL and secrete it into circulation. In the absence of this need, apoB100 can be degraded as it is synthesized [147].

Insulin can target apoB-100 for degradation; however, studies in cell culture have shown that activation of the PI3K pathway is necessary for the insulin-mediated

degradation [150]. In normal subjects, the effect of hyperinsulinemia (during a euglycemic clamp) inhibits apoB-100 production [151], but the presence of insulin resistance decreases the effectiveness of insulin to suppress apoB-100, possibly due to impairments in hepatic PI3K signaling [152]. Moreover, MTP transcription has been shown to be increased in cells made insulin resistant, which is associated with an increase in apoB100 particle secretion [153]. As mentioned previously, the PI3K pathway can be impaired with elevated fatty acids, and there is evidence that when hepatic lipids are abundant, VLDL particle production is not suppressed by insulin [154]. Adiels et al explored the relationship between liver fat content and the ability of insulin to suppress VLDL production in humans across a range of liver fat contents [154]. They found that in individuals with the lowest liver fat, insulin readily suppressed VLDL secretion, and higher liver fat was associated with a failure of insulin to suppress VLDL secretion. Together these data indicate that insulin resistance in the liver can lead to elevated VLDL secretion through its effects on apoB-100 and MTP.

Insulin resistance also promotes VLDL production by increasing the supply of fatty acids to the liver. Insulin resistance in adipose tissue leads to an impairment in the suppression of lipolysis and subsequent increase in circulating NEFA and uptake by the liver. Increasing NEFA flux stimulates secretion of VLDL in humans by providing lipids for incorporation into VLDL particles [155]. Elevated VLDL particles initiate a cascade of events leading to a preponderance of circulating small dense LDL and HDL particles and higher amounts of remnant particles [147]. An elevation of triglyceride rich VLDL particles in circulation leads to an increase in the uptake of cholesteryl esters from HDL

and LDL into VLDL via the actions of cholesteryl ester transfer protein (CETP). In exchange, VLDLs donate TG to LDL and HDL particles, making them larger and better substrates for hepatic lipase. Hepatic lipase hydrolyzes TG from the core of LDL and HDL, making these particles smaller and denser. Small, dense LDL particles are more atherogenic because they readily cross the endothelial lining and are more prone to oxidation and are less readily cleared [156]. VLDL remnants are also atherogenic because they can cross the vascular intima and remain in the arterial wall [157]. Small, dense HDL particles result in less HDL cholesterol engaged in reverse cholesterol transport, less inhibition of LDL-C oxidation, and less anti-inflammatory actions [148]. Clearly, insulin resistance is not merely a problem of deficient glucose uptake, but a multifaceted condition that significantly increases the risk for CVD.

Lifestyle factors have a significant effect on MetS-associated dyslipidemia. In addition to its effects on insulin signaling, exercise has been shown to have beneficial effects on plasma lipoprotein levels. Specifically, exercise has been shown to increase the amount of circulating HDL cholesterol and lower TG [158, 159]. These changes in lipoprotein levels lead to an overall improvement in MetS conditions and lower risk for an acute cardiac event [159].

Postprandial Lipemia

It is well recognized that alterations in lipid and lipoprotein metabolism increase risk for CVD and are associated with insulin resistance, T2D, and the MetS [160, 161]. A possible explanation for the difference in chronic disease risk in MA compared to NHW could be in the handling of dietary fatty acids in the postprandial state. Most of the

knowledge about the relationship between lipids, lipoprotein metabolism, and CVD is based on measurements taken in the fasting state, however, it is possible for people to have normal fasting blood lipids, but have impairments in postprandial lipid metabolism [162, 163]. Elevated postprandial triglyceride-rich lipoproteins (TRL) such as VLDL and CM, are a prominent finding in several insulin-resistant conditions and are recognized as an independent risk factor for CVD [162-165]. Prolonged elevation of TRLs and remnants leads to an increase in atherosclerotic risk [166]. Much emphasis in the events in the arterial wall leading to atherogenesis such as the formation of macrophage foam cells and monocyte recruitment has been placed on the direct involvement of LDL, particularly oxidized LDL. However, VLDL and CM remnants can cross the vascular intima and may remain in the arterial wall, possibly causing similar detrimental effects to oxidized LDL [157].

It has been suggested that defects in the ability of insulin to coordinate NEFA uptake and release in the postprandial period might underlie many lipid abnormalities associated with insulin resistance [167]. The coordinated regulation by insulin of tissue-specific enzymes and proteins during the postprandial period plays an important role in the partitioning of dietary fat among tissues [168]. Given that MA are typically more insulin resistant than NHW, it seems likely that regulation of fats in the postprandial period could be impaired in this population. In the postprandial period, CM concentration increases substantially, but the majority of the circulating TRL particles are actually VLDL [169]. VLDL compete with CM for hydrolysis, and an increased concentration of VLDL hinders the clearance of CM and prolongs the postprandial

elevation of circulating triacylglycerols [164]. It has recently been recognized that adipose tissue acts as a buffer for postprandial fatty acid flux, in that, uptake of FFA is enhanced while release of FFA from adipose tissue is suppressed [170] in the hours following food ingestion. Insulin stimulates adipose tissue uptake of dietary fatty acids by multiple mechanisms, and if these mechanisms are impaired, a higher amount of substrate is available for the liver to synthesize VLDL particles. The main mechanisms that buffer postprandial lipid flux are: 1) increase in clearance of TRLs, 2) suppression of FFA release, and 3) suppression of hepatic VLDL secretion [170]. The effects of a habitual western diet that is likely to decrease insulin sensitivity and decrease the ability of adipose tissue to buffer lipid flux could contribute to an increased lipid supply to the liver which could result in higher VLDL secretion. MA may be more susceptible to the potentially detrimental effects of a western diet, and it is possible that elevated VLDL in the postprandial period in this group may result in greater overall dyslipidemia.

Diet, Insulin Sensitivity and Features of the Metabolic Syndrome

Saturated Fat

Insulin resistance can be altered not only by total energy intake, but also by diet composition. The role of dietary fat specifically has been of clinical interest for several decades. Epidemiologic studies have found a correlation between a high intake of dietary fat and insulin resistance since the 1930s [171-173]. Saturated fatty acids (SFA), in particular, have a strong association with insulin resistance [174-176]. One of the earliest reports examining fat type and insulin action was by Kinsell et al., who reported in 1959 that intake of saturated fat could influence insulin action in humans [177]. They

reported that a patient with type 1 diabetes required less exogenous insulin after substituting safflower oil, a polyunsaturated fatty acid (PUFA), for fat rich in SFA and oleic acid.

There have since been several cross-sectional or case-control studies comparing dietary fat intake of T2D patients and healthy subjects. The Mediterranean Group for the Study of Diabetes is a multi-center study examining dietary intake with surveys from T2D patients and healthy subjects across six countries. The results from this study have shown that subjects undiagnosed and recently diagnosed with T2D had higher relative intake of total fat and SFA from animal fat sources compared with healthy controls [178]. These data are similar to findings from a Dutch study on patients with newly-diagnosed diabetes, which showed that newly diagnosed subjects had a high intake of total fat (40% of kcals) and saturated fat (15% of kcals) compared to controls [179]. The KANWU study was a large randomized dietary intervention that examined the effects of an isoenergetic substitution of dietary SFA for monounsaturated fatty acids (MUFA) over 90 days. Subjects received edible fats, which differed only in fat quality, to be used as spreads on bread, for cooking, and as dressings. Both the SFA diet (17% SFA, 14% MUFA, 6% PUFA) and the MUFA diet (23% MUFA, 8% SFA, 6% PUFA) had a total fat intake of 37% of calories. The main finding from this study was that decreasing SFA and increasing MUFA improved insulin sensitivity (~10%), as measured by the minimal model method [180].

In a randomized controlled crossover study in healthy subjects, isocaloric diets prepared in a metabolic kitchen were consumed for 4 weeks, with diets differing only in

fatty acid composition [181]. Each diet contained 9% of calories from either palmitic acid, oleic acid or 18:1 trans with total fat comprising ~28% of calories. This intervention caused no differences in insulin sensitivity among the diets, as measured by the minimal model method. However, in a subgroup of overweight subjects, SFA reduced insulin sensitivity by 24% compared to MUFA. This decline was not statistically significant, likely due to the small number of subjects in the subgroup (n = 7), but such a decrease may be clinically relevant nonetheless. Similar results were reported in the Insulin Resistance Atherosclerosis Study (IRAS), in which higher intakes of dietary fat were associated with insulin resistance in obese but not lean individuals [173]. These findings suggest that response to changes in dietary SFA might be more pronounced in overweight subjects who are already prone to insulin resistance. In addition, it is possible that the total amount of fat in the diet must meet a threshold level, regardless of fat type, to negatively affect insulin signaling. The KANWU study showed an improvement in insulin sensitivity with total fat at 37% of calories, whereas, the 4 week controlled crossover study [181] only provided 28% of calories from fat and found no difference in insulin sensitivity in the healthy subjects. To test the effects of different fatty acids on insulin sensitivity in a more controlled scenario, Han et al infused lipid emulsions containing MUFA, PUFA, or SFA into rats for 7 hours. These infusions led to an overall decrease in insulin-stimulated glucose uptake for all fatty acids, but glucose uptake did not differ among the three different fatty acid infusions [182]. Perhaps such a large increase in fatty acid influx among all three groups limited the ability to distinguish any differences in insulin sensitivity from the different fatty acids.

A prospective cohort study examining specific fatty acid composition in plasma showed that circulating SFA were higher and PUFA were lower in men who developed T2D compared to those who remained normoglycemic [183]. A high amount of dietary SFA typically leads to greater SFA in circulation and increased incorporation of SFA into cell membrane phospholipids [71]. Cell membranes contain various proteins and signaling molecules, and a highly saturated fatty acid composition of phospholipids in the membrane may compromise the structural integrity of the cell membrane [71]. Cross-sectional studies evaluating the relationship between insulin sensitivity and the fatty-acid composition of phospholipids within muscle membranes showed that skeletal muscle phospholipids composed of a higher amount of SFA promoted insulin resistance more than unsaturated fatty acids [184, 185]. This is possibly due to more rigid membranes and interference with insulin receptor binding and/or affinity. It has been demonstrated *in vitro* and in the rodent model that more saturated fats in the phospholipid composition of membranes are associated with decreases in the affinity of insulin to the insulin receptor and altered ability to translocate and insert glucose transporters [185, 186].

In addition to membrane phospholipid composition, the type of fat consumed determines the fatty acid composition of IMTG. High SFA in IMTG correlates directly with insulin resistance in humans [63]. IMTG composed of more SFA induces more damaging lipid-derived metabolites, such as DAG and ceramide, than IMTG composed of mostly unsaturated fatty acids [187, 188]. A possible explanation for SFA exerting a more deleterious effect on insulin sensitivity is that SFA are not as readily oxidized as

unsaturated fatty acids in humans, although the overall significance of this effect may be minuscule [189].

Saturated fat has also been known to affect blood cholesterol levels, in addition to its effects on insulin resistance. Epidemiologic data from the Nurses' Health Study revealed a correlation between both higher intake of saturated fat and low PUFA:SFA with increased CVD risk in women with T2D [190]. Saturated fatty acids decrease the activity of acyl-CoA: cholesterol acyltransferase (ACAT), an enzyme responsible for esterifying free cholesterol to cholesterol ester [191]. The free cholesterol pool determines the expression of LDL receptors. When free cholesterol is high, LDL receptor transcription is downregulated, resulting in a decreased uptake of LDL into cells, and higher circulating LDL cholesterol [192]. Conversely, unsaturated fatty acids increase the activity of ACAT, leading to lower LDL cholesterol in circulation [191]. This effect of saturated fat on LDL levels makes it an obvious target for public health dietary recommendations for the prevention/treatment of CVD.

Dietary Carbohydrate

Dietary carbohydrates provide the majority of glucose used for energy production in metabolic pathways. Carbohydrate is the primary nutrient of interest in the context of T2D, as the clinical definition of diabetes is based on fasting or post-challenge blood glucose concentrations. Accordingly, considerable attention has been given to diets in which the type and amount of carbohydrate is varied. Current recommendations by the American Diabetes Association (ADA) do not support restricting carbohydrate intake below 130 g/day [193]. In fact, ADA recommendations

aim for dietary carbohydrate intakes of 45-65% of total energy, or 225-325 g/day on a 2000 kcal/day diet. The main types of carbohydrates found in food can be divided into the following groups: monosaccharides and disaccharides (sugars), oligosaccharides (chains of 3 to 10 glucose or fructose polymers), and polysaccharides (starch and dietary fiber) [194]. About half of dietary carbohydrate is in the form of polysaccharides, derived mostly from cereal grains and vegetables. The remaining half comes from sugars such as glucose, fructose, galactose, sucrose, and maltose [195].

In order for cells to use carbohydrates for energy, they must be absorbed from the gastrointestinal (GI) tract into the bloodstream, normally in the form of monosaccharides, the most nutritionally important being glucose [195]. The influx of glucose from carbohydrates is determined by the rate that carbohydrates become available for absorption at the epithelium of the small intestine. The type of carbohydrate ingested can affect absorption and release into the circulation, which is significant in a nutritional sense because it impacts postprandial blood glucose homeostasis and the associated metabolic responses. Glucose absorbed by the gut enters the portal vein, and 30-40% of glucose is taken up by the liver to replete its glycogen stores, with the remainder of glucose travelling to the periphery [196]. The liver responds to a signal generated by the presence of portal vein glucose concentration in excess of arterial glucose concentration, called the portal signal, which stimulates pancreatic insulin secretion [196]. The liver extracts ~50% of the secreted insulin, with the remainder going into peripheral circulation to stimulate uptake of glucose into peripheral tissues such as skeletal muscle and adipose tissue [197]. The

consumption of sugars is known to rapidly increase blood glucose levels postprandially, but consumption of more complex carbohydrates attenuates postprandial glucose responses [198]. Sugars present a challenge to insulin resistant individuals, as sugars cause a rapid surge in circulating glucose, and, in insulin resistant individuals, a high amount of insulin is required in order to facilitate glucose uptake [199]. A direct link between increased intake of sugars and T2D has not been established, although the increasing prevalence of T2D parallels the increasing consumption of sugar and refined carbohydrates in the U.S. [200].

The presence of dietary fiber is one of the factors in different the types of carbohydrates that can influence postprandial glucose and insulin responses. A high dietary fiber intake (>25g/d in women and >38g/day in men) is recommended by most diabetes and nutritional associations due to a number of epidemiological and experimental studies providing a strong argument for the role of dietary fiber in the prevention of T2D [201, 202]. Pereira et al. found that insulin sensitivity measured with the hyperinsulinemic euglycemic clamp improved after 6 weeks on a whole-grain diet compared with refined grains, independent of changes in body weight [203]. Improvements in insulin sensitivity associated with whole-grains appear to be related to the effect of dietary fiber. Several epidemiologic studies have found significant associations with dietary fiber, with cereal fiber having the strongest association with lower risk for T2D [204, 205].

Dietary fiber consists of a group of complex substances in carbohydrates and lignins that are not digested in the small intestine [206]. There are two classes of

dietary fiber, soluble and insoluble, based on their ability to be dissolved in water. It is important to note, however, that most high-fiber foods contain both soluble and insoluble fiber in varying amounts [207].

Soluble fiber contains substances with a gel-forming ability, such as pectins and gums, mainly found in fruits, vegetables, oats, and beans [201]. The viscous/gel-forming properties of soluble fiber slow gastric emptying and macronutrient absorption from the gut by trapping nutrients inside the viscous gel, shielding them from digestive enzymes, and slowing the movement of nutrients across the surface of the intestines [208, 209]. This property of soluble fiber blunts the increase in glycemia which occurs following ingestion of carbohydrates, therefore, blood glucose and insulin levels rise more slowly when soluble fiber is consumed [201].

Insoluble fiber is made up of components that resist the action of intestinal micro-organisms. These components include cellulose, hemi-cellulose, resistant starch and oligosaccharides, and lignin resin [201]. Wheat, rye, and whole grain cereals are examples of foods containing a high amount of insoluble fiber. Due to its water-holding capacity, insoluble fiber increases fecal mass and is helpful in combating constipation—a condition that often occurs in obesity [208]. Unlike soluble fiber, insoluble fiber has no effect on glucose absorption since it is nonviscous [210]. Nonetheless, when measuring insulin sensitivity with the hyperinsulinemic euglycemic clamp, consumption of insoluble fiber leads to increased glucose disposal in short-term and prolonged studies in humans, independent of changes in body weight [209-211]. Despite a considerable amount of evidence linking insoluble fiber intake to reduced incidence of T2D [212-215], the

metabolic link between chronic ingestion of insoluble fiber and insulin sensitivity has not been determined. A possible explanation may be related to fermentability of fiber by intestinal bacteria.

Fermentation by gut bacteria produces short-chain fatty acids, such as acetate, butyrate, and propionate which are suggested to reduce hepatic glucose output and lower NEFA by inhibition of adipose tissue lipolysis [216, 217]. Resistant starch is an insoluble fiber that is highly fermentable, and Robertson et al showed that supplementation with 30g/d of resistant starch for 4 weeks led to a significant increase in insulin sensitivity [210]. In this study, there was also a decrease in adipose tissue lipolysis and concomitant increase in skeletal muscle glucose uptake and peripheral short-chain fatty acid concentrations [210]. Although these observations potentially explain the decrease in T2D risk commonly seen with increased intake of insoluble fiber, the precise mechanisms by which insoluble fiber and/or short chain fatty acids produce the benefits in adipose and skeletal muscle metabolism require further investigation. Despite clear-cut explanations for the association between fiber and insulin sensitivity, overall, it seems that consumption of high fiber foods could be beneficial in reducing risk for T2D.

In addition to its beneficial effects on insulin sensitivity, dietary fiber is advantageous in the management of blood lipids. Soluble fiber lowers total and LDL-cholesterol levels [218]. Evidence suggests that soluble fiber binds bile acids during formation of micelles in the intestinal lumen [219]. This binding increases excretion of bile acids, in turn, upregulating hepatic synthesis of bile acids from the free cholesterol

pool. As the free cholesterol pool decreases, hepatic LDL cholesterol receptor synthesis is upregulated, which increases the uptake of LDL cholesterol from circulation. In hypercholesterolemic men given 100 g of oat bran for 10 days, a reduction in total and LDL cholesterol levels and an increase in fecal bile acid excretion were observed [220]. These data suggest that incorporating fiber into the diet will beneficially affect cholesterol levels and decrease risk for CVD.

Hispanics and Health Disparities

Hispanic Americans exhibit almost a 2-fold higher prevalence of T2D than non Hispanic whites, with Mexican Americans having the highest percentage of hyperinsulinemia (~48%) compared to other ethnic groups [3, 4]. As of 2002, Mexican Americans also had the highest age-adjusted prevalence of the MetS at 32%, compared to 24% and 22% for non-Hispanic whites and African Americans, respectively [1]. Even at a young age, MA boys and girls have higher median levels of fasting insulin and glucose and are more likely to express MetS risk factors compared to NHW boys and girls [221]. Mexican Americans typically exhibit greater dyslipidemia than NHW, with higher levels of plasma TG concentrations, lower HDL-C, and elevated small, dense LDL particles [222, 223]. The Mexican American population typically also has higher levels of obesity, in particular abdominal obesity, compared to NHW, which could contribute to the higher observed insulin resistance [224, 225]; however, when non-obese MA were compared to NHW using the hyperinsulinemic euglycemic clamp, MA were still significantly more insulin resistant than NHW [226]. Additionally, Ho et al reported higher insulin resistance in MA vs. NHW males and females, even when total body fat,

body fat distribution (subcutaneous vs. visceral) determined via CT scans, and physical fitness (VO_2 max) were similar between groups [227]. In addition, there were no differences in protein abundance of the insulin receptor β-subunit, p85 subunit of PI3K, Akt1/2 or GLUT4. Concentration of tumor necrosis factor (TNF)-α, which is known to impair insulin signaling, is higher in young nonobese Mexican Americans compared to NHW with similar body composition and fitness level [228, 229]. Hispanic ethnicity has also been shown to be a predictor for elevated levels of C-reactive protein (CRP) in young boys [230]. CRP is a nonspecific marker of systemic inflammation, and there is evidence that chronic inflammation plays a role in the pathogenesis of atherosclerosis [231]. Given these data, it is possible that genetic factors could play a role in the higher prevalence of these metabolic abnormalities in MA, owing to the fact that up to 30% of the genetic makeup of most US Hispanics can be traced to Native American ancestry. The latter, seemingly independent of tribe and location within the US, have a severely high prevalence of insulin resistance [232]. In a population study of MA adults from the San Antonio Heart Study, contribution of genes and environmental factors to phenotypic variability in risk factors for CVD were simultaneously estimated in 950 individuals from 42 extended families [233]. From this sample, it was estimated that genes accounted for 13% and 35% of the phenotypic variation in 2-hour and fasting insulin concentrations, respectively [233]. In addition, they reported that environmental factors, such as amount of physical activity and dietary intake, were significantly associated with risk factors for heart disease, but accounted for less of the phenotypic variation in risk factors--about one-third of that attributable to genetic variation.

However, relatively crude instruments were used to assess dietary intake and physical activity, which may have led to an underestimation of the true effects of these variables.

The risk for developing insulin resistance, and subsequent T2D and MetS likely stems from an interaction of genetic and environmental factors. Hispanics living or born in Mexico exhibit a significantly lower prevalence of T2D and MetS risk factors compared to Mexican Americans who have migrated to the US and become acculturated to the US lifestyle [234-236]. As MAs transition from their cultural norms to a more Americanized lifestyle, their leisure time physical activity decreases and there is an increased intake of saturated fats, refined carbohydrates, and total energy [234, 237]. It therefore seems apparent that environmental factors present in the US contribute to the increased disease risk in Mexican Americans. Studies have shown fat, saturated fat and sugar intake in MA to be high, with relatively low intakes of fruits, vegetables, and dietary fiber [238, 239]. Not surprisingly, risk for developing T2D and the MetS increases substantially as MA adopt a Western lifestyle, suggesting that MA have a lower tolerance for the unhealthy environmental factors present in the United States compared to NHW [240, 241]. The presence of these health problems in this growing population presents a need for interventions to help alleviate these health disparities. The interventions aimed at improving risk factors for the MetS and T2D have not been specifically tested in the Hispanic population independent of substantial weight loss. It is possible that MAs experience higher rates of insulin resistance, T2D, and the MetS because they are somewhat resistant to the beneficial effects that a prudent diet and exercise can have on health outcomes. The Diabetes Prevention

Program was a large-scale long term study that demonstrated that lifestyle changes (150 minutes of moderate intensity physical activity per week combined with a low-fat, hypocaloric diet) can favorably affect risk factors for T2D among different ethnic groups [242]. However, this study induced a significant reduction in weight which makes it impossible to ascertain the contributions of the weight loss from the dietary and physical activity changes. It seems important for ethnic groups at higher risk for specific chronic diseases to undergo experimental interventions that will lead to appropriate tailoring of the lifestyle modifications that will have the greatest impact on improving health. Currently the American Diabetes Association recommends engaging in moderate intensity physical activity most days of the week and decreasing intake of saturated fat and refined carbohydrates [243]. To date, no studies have determined how well Mexican Americans, in comparison to NHW, respond to the current public health recommendations on diet and physical activity. Because MAs appear to be more susceptible to the adverse consequences of physical inactivity and a "western" diet, our goal was to determine if MA would show a steeper improvement slope such that the magnitude of the disparity in risk factors for T2D and the MetS among MA and NHW would be attenuated in response to a short diet exercise intervention. In addition, we sought to determine what, if any, baseline differences exist in skeletal muscle proteins of MA and NHW adults that could be related to the ethnic disparity in insulin sensitivity, and whether the magnitude of change in expression of these proteins in response to the intervention is affected by ethnicity.

CHAPTER 3

CARDIOMETABOLIC PLASTICITY IN RESPONSE TO A SHORT-TERM DIET AND EXERCISE INTERVENTION IN YOUNG HISPANIC AND NON HISPANIC WHITE ADULTS

Abstract

Type 2 diabetes (T2D) and the metabolic syndrome (MetS) are more prevalent in Hispanics (HIS) than nonHispanic whites (NHW) in the US, suggesting that HIS may be less responsive to the same lifestyle intervention as NHW. Since Mexican Americans (MA) are the largest subgroup of U.S. Hispanics, we evaluated the effects of a short term exercise and low saturated fat, low sugar, high fiber dietary intervention on T2D and MetS risk factors in MA compared to NHW sedentary adults. Participants (20 NHW: 11F, 9M, age=23.0 y, BMI=25.5; 17 MA: 13F, 4M, age=22.7, BMI=25.4) consumed their habitual diets and remained sedentary for 7 days, after which an intravenous glucose tolerance test (IVGTT) was performed to estimate insulin action by way of determining the 3-h insulin area under the curve (AUC). Subjects then completed a 7 day diet/exercise intervention (6 sessions, 40 min/session at 65% VO2 max) followed by a second IVGTT ~17 h after the final exercise bout. Pre intervention insulin action was lower in MA, improved proportionately with the lifestyle intervention, but remained lower than NHW following the intervention (IAUC μU*min/L pre, post: MA = 2298± 1635; NHW = 1795± 1211). Despite lower insulin action, pre and post test plasma lipids

and lipoproteins were no different in MA compared to NHW, but improved in both groups (total cholesterol: -10%; LDL-C: -10%; TG: -37%; Small dense LDL particles: -13%). These data suggest that despite higher risk for T2D and MetS, young MA adults are as metabolically responsive as NHW to a short term diet-exercise intervention, nevertheless, with this short-term intervention, the ethnic disparity in insulin sensitivity remains.

INTRODUCTION

Type 2 diabetes (T2D) and the metabolic syndrome (MetS) are widespread health problems, with the prevalence increasing among all ethnic groups in the United States [4]. Hispanic Americans, the largest and fastest growing minority group in the U.S., have a disproportionately high prevalence of overweight and obesity, T2D and the MetS compared to non-Hispanic whites (NHW) [1, 244]. Hispanics also exhibit substantially higher levels of insulin resistance compared to NHW, even among nonobese, young adults, foreshadowing their greater future burden of T2D and its complications [3, 5, 245]. Insulin resistance is strongly correlated to high plasma triglycerides (TG), small dense LDL particles, as well as low HDL levels, which could partially explain the greater dyslipidemia that is also prevalent in the Hispanic population [146, 148, 222, 246-249].

The source of this ethnic disparity is unclear, but both genetic predisposition and environmental factors probably play important roles [250]. Lifestyle factors (e.g. lower levels of physical activity and an energy-rich diet) leading to excess adiposity are likely the primary environmental factors related to the greater occurrence of T2D and the MetS among Hispanics. Studies have shown fat, saturated fat and sugar intake in Mexican Americans (MA), the largest subgroup of U.S. Hispanics, to be high, with relatively low intakes of fruits, vegetables, and dietary fiber [238, 239]. As MAs

transition from their cultural norms in Mexico to a more Americanized lifestyle, their leisure time physical activity decreases and there is an increased intake of saturated fats and refined carbohydrates [234, 237]. Not surprisingly, their risk for developing T2D and the MetS increases. Of great concern is that this risk increase is greater than might be predicted based on changes in body weight and composition, suggesting that MA have a low tolerance for the unhealthy environmental factors present in the United States [240, 241].

We have previously shown that after matching for cardiorespiratory fitness, body composition, and fat patterning, young Hispanic adults still exhibit lower insulin sensitivity and greater markers of sub-clinical inflammation than their NHW counterparts, suggesting that factors including dietary composition and ill-defined genetic differences may contribute to the ethnic disparity [251]. Experimental evidence from the Diabetes Primary Prevention Trial (DPPT) suggests that lifestyle changes (diet, regular exercise, and weight loss) can favorably affect risk for T2D among pre-diabetic Hispanic adults despite the apparent genetic predisposition [242]. However, in this landmark study, the contribution of the changes in dietary intake and physical activity on T2D risk cannot be determined independent of the substantial weight loss that also occurred among study participants [252].

Short-term increases in physical activity independent of substantial training adaptations, weight loss, and changes in body composition, alters skeletal muscle protein expression and improves insulin sensitivity and circulating TG, all of which decrease risk for T2D and MetS [253-256]. Seven days of exercise have been shown to

improve insulin sensitivity in a variety of subjects, most of whom have been NHW [114, 253, 257]. We therefore developed a 7-day diet and exercise intervention to compare its effects on cardiometabolic plasticity in young, previously sedentary, non-obese MA and NHW adults. The advantage of examining this question in healthy, young, non-obese, individuals is that they have none of the other confounding factors that are typically associated with obesity and T2D, which have been suggested to play a role in causing the MetS. We hypothesized that MAs compared to NHWs would exhibit lower initial insulin sensitivity and greater dyslipidemia, but would experience intervention-induced improvements in these parameters. Because MAs appear to be more susceptible to the adverse consequences of physical inactivity and a "western" diet, our goal was to determine if MA would show steeper improvement slope such that magnitude of the disparity in risk factors for T2D and the MetS would be attenuated in response to a short diet exercise intervention.

METHODS

Subjects: A total of 17 MA (4 male, 13 female) and 20 NHW (9 male, 11 female) subjects aged 18-39 participated in this study. Subjects were sedentary, non-smoking, with no known diseases. Subject inclusion criteria were: fasting blood glucose <110 mg/dl, blood pressure <140/90 mmHg, and no history of endocrine disorders. Individuals were excluded if they were pregnant, used tobacco, had diabetes, history of eating disorders, were vegetarians, exercised more than once a week for the past 6 months, or used medication that could influence insulin signaling or plasma lipids. Females were studied in the mid-follicular phase of their menstrual cycles (days 3-14), or during the active

phase of hormonal contraceptive use. Subjects were weight stable (±2.5 kg) for the previous six months. Mexican-American subjects verified that at least 3 of their grandparents were of Mexican descent, and NHW subjects traced their ethnicity to at least 3 grandparents having Anglo-European ancestry.

Study Design: Consent was obtained from each volunteer, and the study protocol was approved by the Colorado State University Human Research Committee. Eligible participants spent a baseline period of 7 days consuming their normal diets and refraining from any formal exercise. During this baseline period, subjects recorded their food intake for 3 consecutive days. Immediately following the baseline period, subjects underwent an intravenous glucose tolerance test (IVGTT) and measurement of plasma lipids. For the next 7 days, study participants exercised for 6 of the 7 days and ate only the food that was prepared for them by the study investigators. Following the 7-day intervention, subjects underwent a second IVGTT and measurement of plasma lipids.

Anthropometric measures: Body weight was measured on a physicians balance scale to the nearest 0.1kg. Height was measured with a wall-mounted stadiometer to the nearest 1.0 mm. Waist and hip circumferences were measured to the nearest 0.1 cm. The percentage body fat, absolute fat mass, and fat-free mass were measured using dual-energy x-ray absorptiometry (DEXA) (Model DPX-IQ Lunar Corp., Madison, WI). Medium length scans (20 min) were used for all subjects.

Resting Energy Expenditure (REE): REE was measured to determine each subject's daily energy requirement, which was then used to provide subjects with the appropriate estimated energy intake during the 7 day protocol to keep them in energy balance.

Subjects arrived at the lab after a 12-h overnight fast and before engaging in any type of physical activity. Indirect calorimetry (CPX Express, Med Graphics, St. Paul, MN) was used to establish REE. Subjects rested in a supine position for 20 minutes while resting VO_2 and VCO_2 values were obtained by open circuit spirometry. The final 10 minutes of data were used for determination of REE using the deWeir equation [258].

Cardiorespiratory Fitness: Maximal oxygen consumption (VO_2max) was measured to determine cardiorespiratory fitness. VO_2max was measured via an incremental cycling exercise to volitional exhaustion in each subject. Oxygen consumption, carbon dioxide production, pulmonary ventilation, and the respiratory exchange ratio (RER) were determined with computer assisted open circuit spirometry. For subjects to qualify for the study, females were required to exhibit a VO_2max of <35 ml/kg/min and males < 45 ml/kg/min, which confirmed that subjects were untrained.

Dietary Assessment: To determine the habitual energy and macronutrient intake, a detailed 3-day diet record was obtained from all subjects during the baseline period. Subjects were expected to weigh and measure the volume of foods and beverages consumed, when appropriate, in order to provide the most accurate information possible in regards to their 3-day dietary intake. The Food Intake Analysis Software (FIAS version 3, University of Texas Health Sciences Center, 1998) was used to analyze total energy and macronutrient intake of each subject's 3-day diet.

Insulin Sensitivity: Insulin Sensitivity was measured using a modified intravenous glucose tolerance test (IVGTT) for insulin and glucose area under the curve over 3 hours. Subjects reported to the health center on the campus of Colorado State University

following an overnight fast. An intravenous catheter was placed in an antecubital vein for the collection of blood samples and administration of glucose. Two fasting blood samples were taken 5 minutes apart to determine surrogate measures of insulin sensitivity including homeostasis model assessment of insulin resistance (HOMA-IR), and fasting insulin. The second sample was followed by a 90-second glucose infusion, in the form of a 50% dextrose solution using a relative dose based on body size (0.3g/kg). The catheter was then flushed with 10-15 ml of normal saline solution. Blood samples were then collected in tubes containing EDTA at 2, 4, 8, 19, 22, 30, 40, 50, 58, 63, 70, 100, 140, and 180 min following glucose infusion and placed on wet ice. Blood tubes were centrifuged at 4° C and plasma was stored at -70° C until assayed. The total area under the 3-h response curve (AUC) was calculated for glucose and insulin based on the trapezoidal method [259]. HOMA-IR was determined: HOMA-IR = [insulin (μU/mL) x glucose (mmol/L)/22.5]. Minimal model measurements of Si were not computed due to the inadequacies of the model in calculating insulin sensitivity with the reduced sampling schedule that was employed without insulin augmentation. We chose not to augment insulin due to the risk of inducing hypoglycemia in these non-obese, non-diabetic subjects.

Dietary Intervention: During the experimental phase, subjects were provided with all meals, snacks, and beverages for 7 consecutive days. Meals were prepared and weighed by study investigators to the nearest gram. Meals consisted of a variety of fruits and vegetables, whole grains, dairy products, legumes, lean meats and fish. The diets were designed to be low in both saturated fat and refined carbohydrates and to

keep the participants in energy balance during the 1-week protocol. Energy requirements were established based on a standard multiple of REE for sedentary subjects and calories expended during the exercise were replaced (REE x 1.3 + net cost of exercise). Additionally, because the determination of individual energy requirements is quite difficult, subjects were provided with 200 kcal modules which approximated the macronutrient content of the experimental diet. Prepared foods were sent home with the subjects every 2 days with instructions for consumption. Subjects were asked to consume all their food, but return any uneaten portions. None of the subjects reported having inadequate food available, and few additional food modules were consumed.

Exercise Intervention: Subjects completed 6 exercise sessions in 7 days following the baseline IVGTT. The 6 sessions consisted of stationary cycling for 40 minutes at a heart rate that elicited 65% of VO_2max for the first three exercise bouts, and 45 minutes for the last three bouts over the 7 day period. To determine the heart rate corresponding to each subject's work intensity at 65% of VO_2 max, oxygen consumption and heart rate were measured simultaneously while cycling on a stationary cycle ergometer during the first exercise session. Intensity was gradually increased and oxygen consumption was stabilized at 65% of VO_2max. Steady state heart rate at 65% of VO_2 max was determined, and this became the target heart rate for each of the subsequent exercise bouts for the remainder of the intervention. Study investigators monitored all exercise sessions to ensure that the proper heart rate was maintained for the duration of each bout. The final exercise session was completed ~17 hours prior to the 2nd IVGTT, and

subjects consumed the food that had been provided for them following this last exercise session.

Plasma Assays: Glucose concentrations were measured using the glucose oxidase method on an automated glucose analyzer (YSI 2300, YSI Inc. Yellow Springs, Ohio). Insulin concentrations were measured by the University of Colorado Health Science Center General Clinical Research Center using an enzyme-linked immunosorbent assay (ELISA) kit. HDL-c, TG, and LDL size were measured by NMR spectroscopy (LipoScience). LDL-c and total cholesterol were analyzed by an enzymatic colorimetric kit (Wako, Richmond, VA).

Statistics: SPSS version 16.0 (Cary, NC) was used for data analysis. A repeated measures ANOVA was used to examine pre and post-intervention differences in Si and lipid measures, with P-values identified for interactions and main effects of ethnicity (MA,NHW) and time (pre, post intervention). Because of the differences in the proportion of males and females within each ethnic group, sex was used as a covariate in these analyses. Values are reported as means and standard errors. Statistical significance was set at $p < 0.05$ using a one-tailed test based on the directional research hypothesis established from our previous research studies.

RESULTS

Subject Characteristics: As shown in Table 1, subjects were young, nonobese MA and NHW males and females. The two ethnic groups were similar in age, fitness level, and percent body fat. Mexican Americans were significantly shorter in stature and weighed less than NHW, but BMI values were almost identical. Total energy and macronutrient

intakes based on three-day self-reported diet records were not significantly different between the two ethnicities. In keeping with the goal of the experiment, the intervention diet was significantly lower in total and saturated fat and higher in total calories, protein, and fiber intake compared to subjects' reported dietary intake (Table 2). During the exercise sessions, subjects expended a net average of 293 ± 88 kcals, with no differences between ethnicities. After the intervention, body weight significantly decreased from 77.3±2.9 to 76.5±2.5 kg in NHW and 71.5±2.5 to 71.0±2.5kg in MA.

Table 1. Baseline Characteristics of Study Participants

	non-Hispanic whites		**Mexican Americans**	
Characteristics	***9 males***	***11 females***	***4 males***	***13 females***
Age (years)	21.8±0.8	24.0±1.6	25.5±3.0	21.8±1.5
Weight (kg)	86.2±3.3	71.2±2.9	83.7±1.3	64.7±1.5*
Height (cm)	181.2±2.8	168.9±1.9	177.9±2.2	160.6±1.3*
BMI (m^2/kg)	26.2±0.6	25.0±0.8	26.5±1.0	25.0±0.6
% body fat	20.4±0.9	35.0±1.6	23.1±0.3	36.3±1.5
Waist (cm)	86.5±1.8	76.8±2.6	89.5±0.7	79.1±1.7
VO_2 Max (ml/kg/min)	38.9±1.8	29.4±1.2	37.7±2.0	31.2±1.0

Values are means ± SE. * $p < 0.05$ MA vs. NHW, within sex

Table 2. Dietary Intake before and during the diet-exercise intervention

	Baseline (Self-Reported)		Intervention	
	NHW (n=20)	MA (n=17)	NHW (n=20)	MA (n=17)
Total kcal	2238±130	1889±160	2487±112†	2118±94†*
Carbohydrate (% kcal)	49.3±2.1	46.7±1.7	50.8±0.4	51.9±0.6
Protein (% kcal)	15.2±0.7	15.5±0.9	22.6±0.3†	21.9±0.4†
Fat (% kcal)	32.0±1.3	35.2±1.5	26.6±0.4 †	26.2±0.4†
Sat. Fat (% kcal)	11.6±0.6	11.9±0.6	4.8±0.1 †	4.7±0.1 †
Fiber (gm)	16.6±1.3	13.2±1.4	45.8±2.2†	41.0±2.0†

Values are means ± SE *= MA vs. NHW, within study period ($p < 0.05$)
†= Baseline vs. Intervention Diet, within ethnicity ($p < 0.05$).

Insulin sensitivity: Table 2 shows that at baseline, fasting glucose was not different between MA and NHW. However, fasting insulin and HOMA-IR were significantly higher (~20%) in MA compared to NHW. Based on the baseline IVGTT, there were no significant differences in glucose AUC between ethnic groups (Figure 1). However, despite no difference in glucose AUCs for the two groups, the insulin AUC was ~22% higher in MA compared to NHW (Figure 2), indicating lower insulin sensitivity in the former.

The diet-exercise intervention improved insulin sensitivity in both ethnic groups, as determined by a 33% decrease in insulin AUC in NHW and 29% decrease in MA. While the glucose AUC did not change following the intervention, there were significant main effects of both time and ethnicity for the insulin AUC. However, there was no time by ethnicity interaction, indicating that while both groups improved over time, the ethnic difference remained after the intervention. Specifically, post-intervention insulin

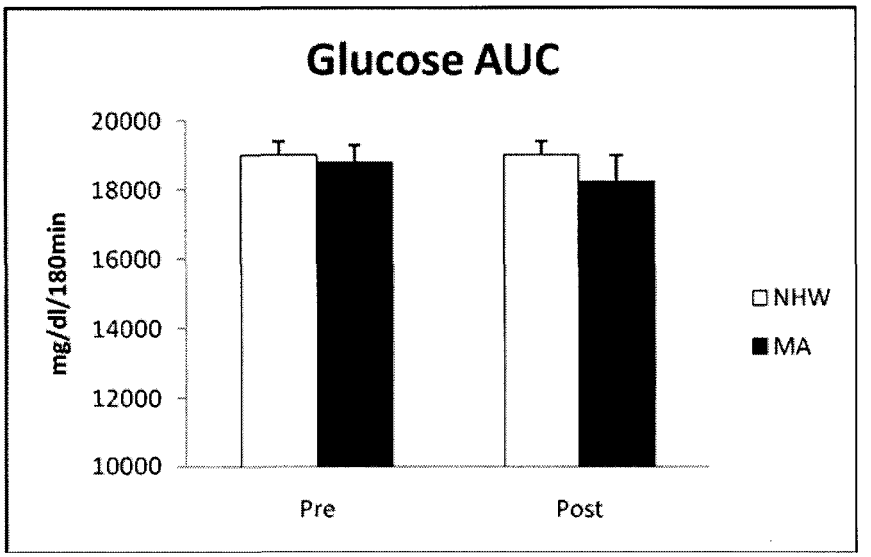
AUC was significantly higher (~26%) for the MA compared to NHW (Figure 2). Fasting indices of insulin resistance followed a similar pattern, although statistical significance was not reached following the intervention (Table 3).

Table 3. Fasting Indices of Insulin Resistance

	Pre-Intervention		Post-Intervention	
	NHW (n=20)	MA (n=17)	NHW (n=20)	MA (n=17)
Fasting Glucose (mg/dl)	86.6±1.6	85.8±1.6	85.0±2.6	81.9±1.4
Fasting Insulin (μU/mL)	4.6±0.4	5.6±0.6*	3.5±0.4†	4.2±0.5†
HOMA-IR	0.99±0.1	1.20±0.3*	0.72±0.1†	0.87±0.1†

Values are means ± SE. * $p < 0.05$ NHW vs. MA, within study period
† $p < 0.05$ Pre vs. Post, within ethnicity

Figure 1. Glucose Area Under the Curve.



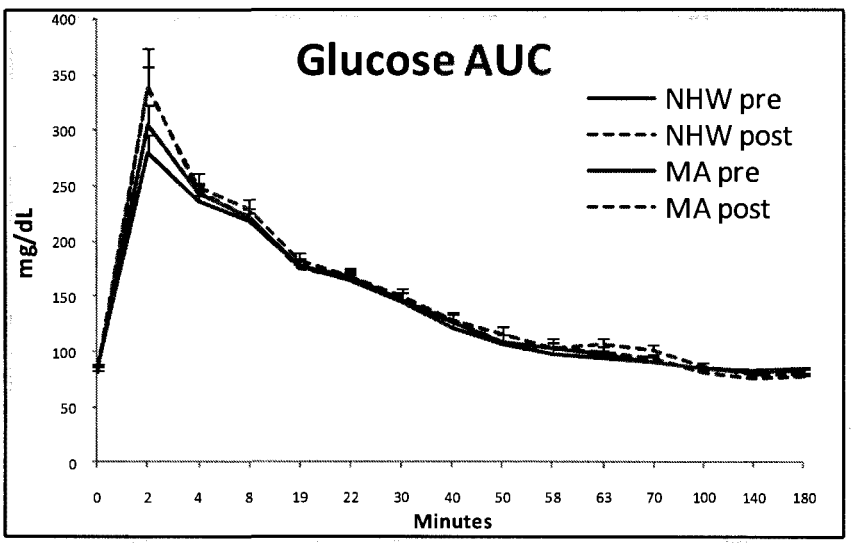
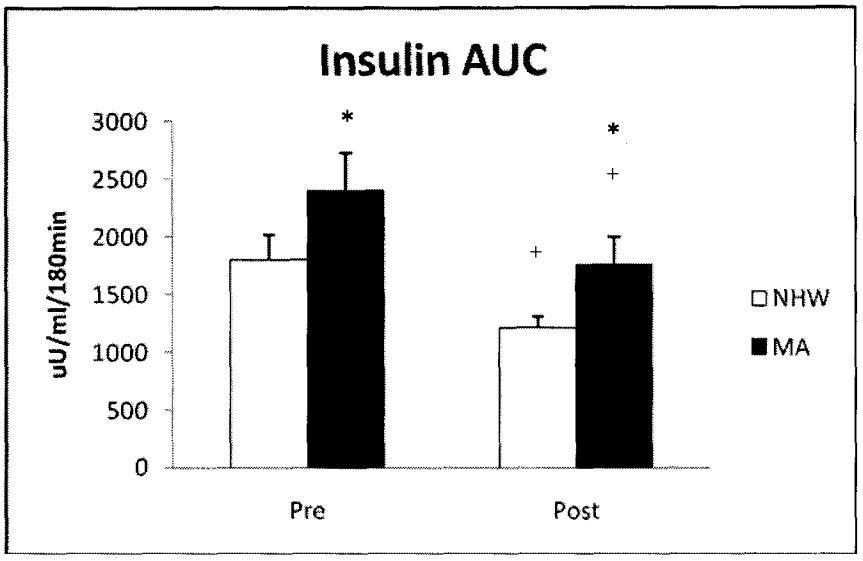


Figure 1 . Plasma glucose area under the curve during the 3h IVGTT for NHW and MA subjects before and after the diet-exercise intervention. Glucose AUC was not different between ethnicities pre or post intervention and did not change after the intervention. Values are means ± SE. AUC values were calculated using the trapezoidal method [259]. NHW n=19, MA n=14.

Figure 2. Insulin Area Under the Curve.



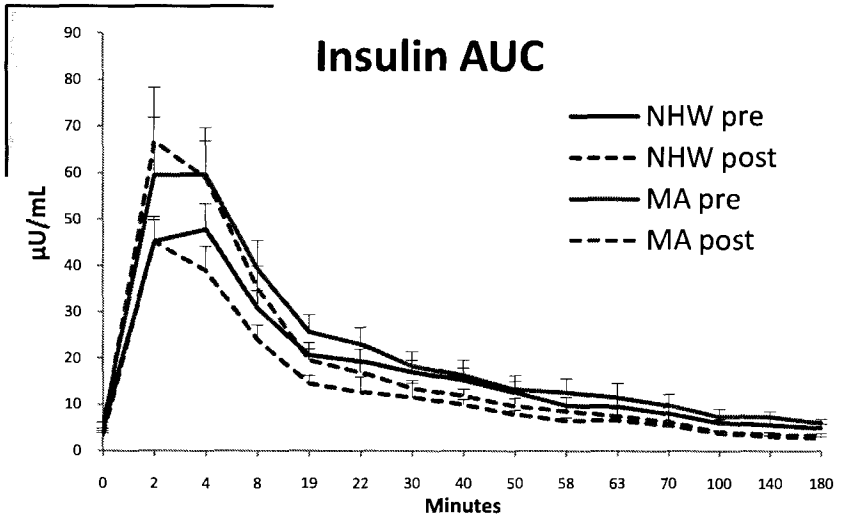


Figure 2. Plasma insulin area under the curve during the 3h IVGTT for NHW and MA subjects before and after the diet-exercise intervention. Insulin AUC was significantly higher in MA subjects before and after the diet-exercise intervention and decreased significantly after the intervention in both groups. Values are means ± SE. AUC values were calculated using the trapezoidal method [259]. NHW n=19, MA n=14.
*= significantly different from NHW, within time period ($p < 0.05$).
+ = significantly different from pre-intervention, within ethnicity ($p < 0.05$).

Lipids: Despite differences in baseline insulin sensitivity, pre-test lipid values did not differ between ethnicities. In response to the diet-exercise intervention, there was a significant time effect indicating that both groups exhibited decreases in total plasma cholesterol: -10%; LDL-cholesterol: -10%; triglycerides: -37% and small dense LDL particles: -13% (Figure 3 A-D). However, there was no main effect of ethnicity, nor was there a time by ethnicity interaction, indicating that improvements in these lipid values were similar across the two groups. HDL-C did not change following the intervention for either ethnic group (Figure 3E). Free fatty acids did not differ between ethnic groups pre or post intervention, however, there was a significant increase following the diet and exercise intervention: +32% for NHW and + 34% for MA (Figure 3F).

Figure 3. Fasting Lipids.

Figure 3A. Total Cholesterol

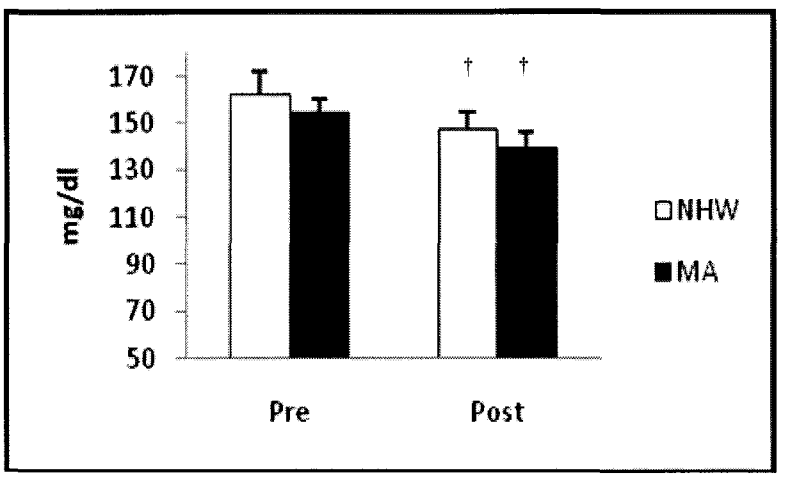


Figure 3B. LDL Cholesterol

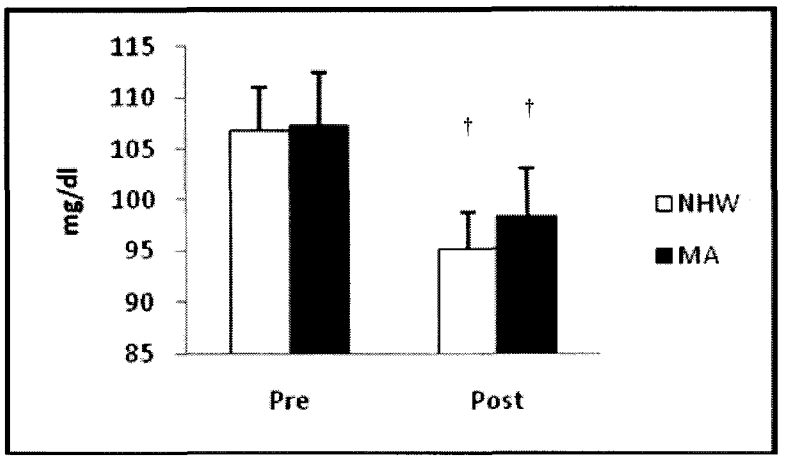


Figure 3C. Triglycerides

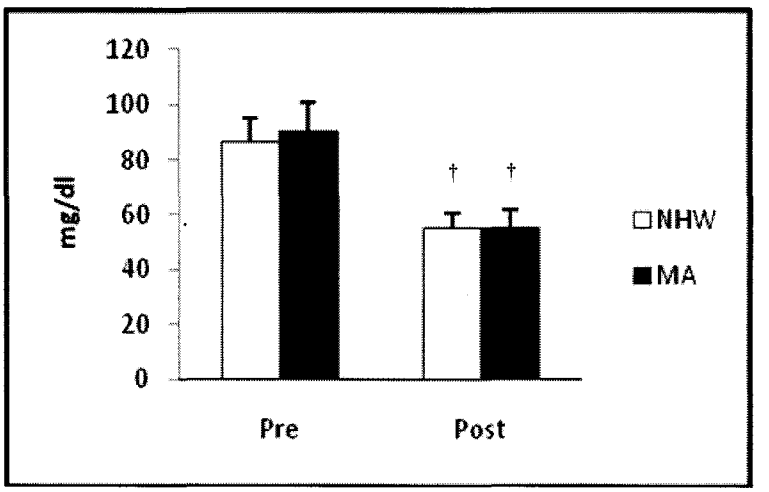


Figure 3D. Small, dense LDL Particles

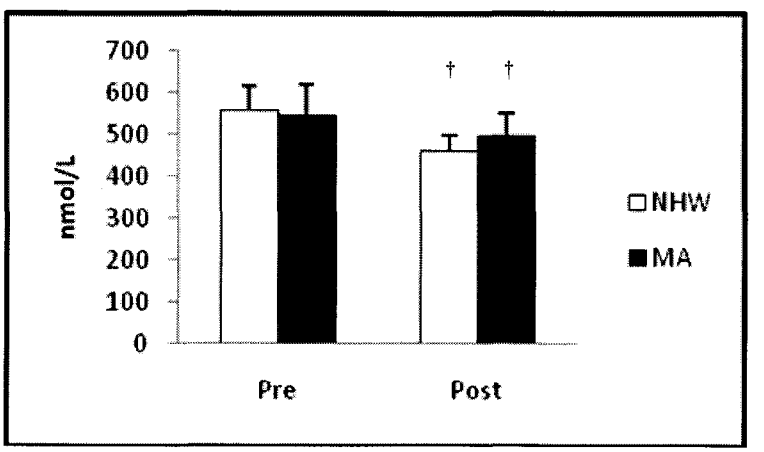


Figure 3E. HDL Cholesterol

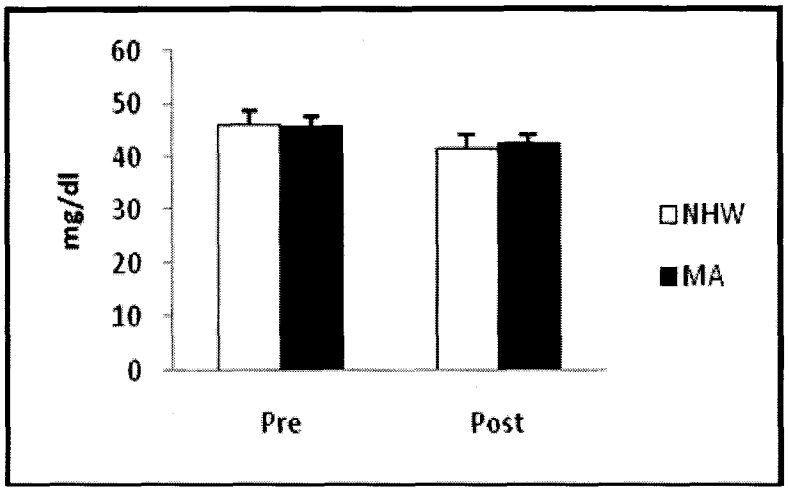


Figure 3F. Free Fatty Acids

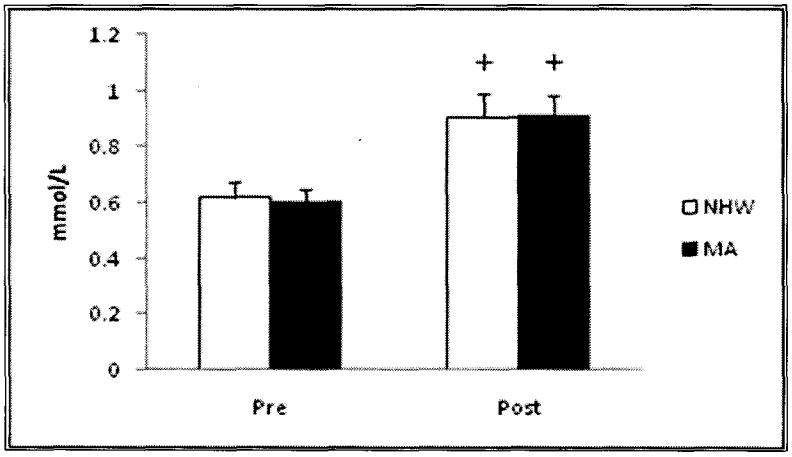


Figure 3 A-F. Fasting plasma lipids for NHW and MA subjects before and after the diet-exercise intervention. 3A.Total cholesterol, 3B. LDL cholesterol, 3C. Triglycerides, 3D. Small, dense LDL particles, 2E. HDL Cholesterol, 3F. Free fatty acids. NHW n=20, MA n=17. † = Pre vs. Post intervention, within ethnicity ($p<0.05$)

DISCUSSION

Insulin Sensitivity

The purpose of this study was to determine whether or not MAs, a high risk population for T2D and the MetS, exhibit the same degree of cardiometabolic plasticity, that is, the same magnitude of change in plasma biomarkers, as that of NHWs (a lower risk population) in response to a short-term diet-exercise intervention. We and others [247, 248] have shown that MAs are at greater risk for obesity, insulin resistance, T2D, and the MetS relative to their NHW counterparts, even when controlling for cardiorespiratory fitness and body fat. Our data clearly show that MA exhibit marked cardiometabolic plasticity such that both insulin sensitivity and plasma lipids improved significantly following the intervention. Note, however, that the ethnic disparity in insulin action remained following the intervention. In fact, the 3-h insulin AUC for MA following the intervention was no different from the insulin AUC for NHW **prior** to the intervention.

This finding differs from that of Jankowski et al [260] who found that MA women had a significant reduction in fasting insulin and insulin AUC following an oral glucose tolerance test (OGTT) in response to 50 min of walking, and were able to "catch up" to the NHW who participated in the same exercise bout. However, this "catch up" occurred without any significant exercise induced improvements in insulin sensitivity in the NHW women, a finding which is at odds with most published exercise studies in this group.

While we have previously reported no differences between MAs and NHWs in skeletal muscle abundance of insulin signaling molecules (insulin receptor-beta, IRS1 and 2, PKB (Akt), and Glut4) [5], possibly there remain ethnic differences in the ability of exercise to activate these molecules by phosphorylation, or differences in dephosphorylation by various protein phosphatases. These possible explanations are speculative, but point to the need to 1.) understand the reasons for the ethnic disparity in the face of similar body composition, age, and diet-exercise changes; and 2.) determine the most effective methods of eliminating this disparity and reducing the excess burden of T2D and the MetS in the MA population.

Lipids

Despite the pre-intervention lower level of Insulin sensitivity, the plasma lipid profiles of the MAs did not differ from the NHWs. This was somewhat unexpected given that insulin resistance is strongly linked to both elevated TG concentrations, VLDL TGs, and concentrations of small dense LDL particles [261]. We have previously shown among overweight and obese men that insulin sensitivity is a strong predictor of total TG, VLDL-TG, and small dense LDL [261]. In addition, Haffner et al in the Insulin Resistance Atherosclerosis Study (IRAS) reported that Hispanics exhibit greater insulin resistance and higher TG, lower HDL-c, and smaller LDL size compared to NHW [222]. However, these subjects were older and had higher BMIs that those participants in our current study. This may suggest that lower insulin sensitivity is not linked to dyslipidemia in young MAs and/or a long time lag is required for the lower insulin

sensitivity to contribute to the dyslipidemic phenotype, a phenomenon which has been previously observed in other ethnicities[262].

The plasticity in plasma lipid responses to the intervention in the MAs was similar to that of the NHWs. In both groups, TGs, total and LDL cholesterol were in the normal range at the outset of the study, and all decreased with only one week of an increase in physical activity and the consumption of a diet low in saturated fat and high in fiber. Decreasing saturated fat and increasing dietary fiber is known to decrease total and LDL cholesterol [263]. However, to our knowledge this is the first study incorporating such a short-term model to favorably improve these plasma lipids.

This short-term intervention also led to a significant decrease in amount of small dense LDL particles with no change in HDL-C. HDL-C typically increases in response to an increase in physical activity [264], however, improvements in HDL-c are quite variable and often depend on baseline levels. Several studies have shown HDL-c to decrease with dietary changes similar to those in our intervention, and 7 days of exercise has previously been shown to induce no changes in HDL-c [114, 257, 264, 265]. Circulating free fatty acids often correlate with degree of insulin sensitivity in obese insulin resistant individuals [38]. Free fatty acids did not correlate with degree of insulin sensitivity in the current study; however, these subjects were young and non- obese, with normal insulin sensitivity at the outset of the study. The increase in FFA following the insulin-sensitizing diet and exercise intervention seems counterintuitive, given that higher concentrations of FFA are associated with increased insulin resistance. However, there was a slight weight loss following the intervention, and this increase in FFA may be

attributed to the weight loss that occurred, as a hypocaloric state is known to stimulate lipolysis and increase circulating FFA. In addition, exercise acutely elevates FFA, and it is possible that FFA were still elevated from the last exercise bout, although it is likely that exercise-induced increases in FFA would have subsided within the ~17hr following exercise.

Strengths and Limitations

There are a number of strengths of this study: 1.) We have developed a short-term model to rapidly test some aspects of cardiometabolic plasticity, independent of any substantial weight loss, that does not require months of exercise and dietary intervention. 2.) All exercise sessions were supervised so that the intensity and duration were well-controlled across all study participants. 3.) Dietary intake was standardized for all subjects over the 7-day period by preparing all foods and providing the same macronutrient composition for all study participants.

There are several caveats which should be noted. 1.) We did not use the hyperinsulinemic, euglycemic clamp, the gold standard for the assessment of insulin sensitivity, and we recognize the shortcomings of our IVGTT approach. However, the dynamic measure of insulin AUC in response to glucose infusion, and the use of fasting insulin and HOMA-IR provide sufficient data to support our contention that the MAs were less insulin sensitive at the commencement of and following the intervention. 2.) The order of the test conditions was not randomized, as the diet-exercise intervention followed the subject's sedentary baseline condition and their typical food intake. Had we randomized conditions, this would have required a lengthy "wash out" period if the

intervention were first. Also, we had concern that the diet-exercise intervention, if provided first, would have potentially biased subjects toward healthier eating and more exercise during their 7 day baseline period. In fact, many of the participants utilized this intervention to begin exercising and consuming healthier foods subsequent to the study. 3.) Despite our efforts to keep subjects in energy balance, there was an average weight loss of 680 g by the end of the intervention—this despite the fact that many subjects found it difficult to consume all of the food that was prepared for them. This decrease in weight, while quite small, may have contributed to the improvements seen in insulin sensitivity and the plasma lipids. However, given that both MA and NHW experienced similar changes in weight, this slight weight change would not confound any differences in the ethnic responses to the intervention. 4.) We chose not to standardize meals leading up to the baseline IVGTT, believing that if there were differences in insulin sensitivity due to differing dietary practices between MA and NHW, these differences should be reflected in the baseline measurement. However, based on the subjects' reported dietary intake, there were no differences between ethnicities in the macronutrient composition of their usual diets and the likelihood that the experimental diet would have a more favorable effect on one group compared to the other seems doubtful. 5.) The short-term pilot study we developed to examine cardiometabolic plasticity incorporated both diet and exercise, and it is therefore impossible to determine the independent contribution of these two lifestyle changes given our study design. While it is likely that the exercise had a greater effect on insulin sensitivity than did diet, we can at least rule out any acute ethnic differences in diet

during the intervention as confounding factors. 6.) The post-intervention measurement of insulin sensitivity was conducted 17 ± 6hr following the previous exercise bout. A single bout of exercise can improve insulin signaling, and it is widely accepted that enhancements in insulin sensitivity are generally attributed to the most recent bout of exercise [118]; therefore, one could surmise that the effect of our intervention was largely due to the effects of the final exercise session. Had we allowed 2-3 days to pass before measuring insulin sensitivity, it is likely that this improvement in insulin sensitivity would have dissipated, and we would not have been able to discern the ability of MA to respond to the diet and exercise intervention. For this reason, we chose to measure insulin sensitivity within 24hr in order to increase the likelihood of capturing any changes in insulin sensitivity. In addition, it is generally believed that a single bout of exercise does not induce significant increases in the expression of insulin signaling proteins in skeletal muscle [266], but seven days of exercise has been shown to increase expression of skeletal muscle proteins associated with insulin signaling without inducing a change in body composition, fitness level or body weight [253, 254, 267]. We therefore chose to employ a 7-day intervention in order to capture any potential changes in skeletal muscle protein expression in MA and NHW (which were analyzed in a separate study), which may help to explain differences in insulin sensitivity in these non-obese individuals.

In summary, our data clearly show that young, sedentary, non-obese MA adults exhibit lower insulin sensitivity compared to their NHW counterparts. Importantly the MA exhibit significant cardiometabolic plasticity such that both insulin sensitivity and

plasma lipids showed marked improvement in response to the short-term diet-exercise intervention. However, it is equally evident that despite their similar plasticity to that of NHW in regard to insulin sensitivity, the MAs fell well short of 'catching up', and the ethnic disparity in insulin sensitivity remains despite exposure to the same lifestyle intervention. In the face of lower insulin sensitivity, the MAs did however exhibit normal plasma lipids that improved in a similar manner to those of the NHWs. Together these data suggest that young, non-obese MA exhibit lower insulin sensitivity than NHWs, show marked improvement from a short-term diet-exercise intervention, but this ethnic disparity is not attenuated. Future research must address the most effective means of eliminating this health disparity in order to lessen the excess burden of T2D and the MetS in this high risk population. Specifically, studies should be conducted to determine the individual effects of diet and exercise in this population to find out which lifestyle factors prove to be the most beneficial to this at-risk population.

CHAPTER 4

TWO-DIMENSIONAL DIFFERENCE GEL ELECTROPHORESIS ANALYSIS OF SKELETAL MUSCLE PROTEINS IN MEXICAN AMERICAN AND NON HISPANIC WHITE ADULTS

ABSTRACT

Skeletal muscle plays an important role in whole-body insulin sensitivity. Since Mexican Americans (MA) typically display lower levels of insulin sensitivity compared to non-Hispanic whites (NHW), independent of differences in percent body fat, abdominal adiposity, and fitness level, we sought to determine if MA exhibit differential expression of skeletal muscle proteins that may help to explain the disparity in insulin sensitivity. We utilized a 2D proteomics approach on skeletal muscle biopsies from 3 MA and 3 NHW subjects in order to identify proteins that may differ between the ethnicities, and planned to confirm these findings in the remaining samples. We found differences in expression of two mitochondrial proteins, carnitine palmitoyltransferase 1A isoform 2 (CPT-1) and mitochondrial ATP synthase F1 complex beta subunit (ATP synthase). ATP synthase was 3-fold higher in NHW (NHW= 188.4 ± 10.2 and MA = 62.7 ± 28.4), while CPT-1 was 1.7 times higher in NHW compared to MA (NHW= 190.4 ± 1.3 vs. MA = 109.3 ± 12.6). However, western blot analysis of ATP synthase in the entire sample revealed no differences in skeletal muscle abundance of this protein between ethnic groups or following an insulin-sensitizing diet and exercise intervention. Therefore, despite the

findings from the proteomic analysis, there is no evidence that ATP synthase differs in skeletal muscle of MA compared to NHW. CPT-1 analysis has not yet been completed due to difficulties in measuring CPT-1 in skeletal muscle. These data provide the potential for differences in expression of mitochondrial proteins in MA to exist, however, no solid data can confirm these findings.

INTRODUCTION

Hispanics exhibit the highest overall prevalence of insulin resistance in the U.S. and almost a 2-fold higher prevalence of type 2 diabetes (T2D) than non Hispanic whites (NHW) [4]. In addition, the highest overall prevalence of the metabolic syndrome (MetS) is found in Mexican Americans (MA), who represent the largest subgroup of Hispanics and the fastest growing portion of the U.S. population [1]. This higher prevalence of T2D and MetS among Mexican Americans is associated with lower insulin sensitivity in MAs even when factors that affect insulin sensitivity, such as physical activity, percent body fat, and abdominal adiposity are accounted for [3, 5] suggesting that at any given level of fitness and fatness, Mexican Americans are at higher risk for T2D than non Hispanic whites.

Skeletal muscle is accountable for the majority of insulin-stimulated disposal of intravenous glucose in peripheral tissues and about one-third of postprandial glucose uptake; it is therefore considered one of the most important sites of insulin-stimulated glucose disposal and thus a primary site of insulin resistance [33, 34]. Given the importance of skeletal muscle in insulin-mediated glucose uptake, it seems possible that differences in skeletal muscle metabolism could, in part, explain differences in insulin sensitivity observed in MA compared to NHW. Skeletal muscle metabolism involves a multitude of proteins that have been found to be associated with insulin sensitivity. However, only a single study [5] has examined possible differences in skeletal muscle protein expression between Mexican Americans and non-Hispanic whites, and this study was limited to only 5 specific proteins related to insulin action. Identifying differences in

skeletal muscle proteins between ethnicities may provide valuable information aimed at understanding the higher risk for T2D in Mexican Americans.

Given the current techniques to quantify differences in protein expression, it is not necessary to choose, a priori, which proteins could be expressed differently. Proteomic methodologies enable characterization of differential expression of proteins among individuals and groups, which are likely attributable to genetic, environmental, and/or physiological influences. Two-dimensional polyacrylamide gel electrophoresis has been the traditional method used for the visualization of thousands of proteins in a single gel. The principle of 2D electrophoresis is based on the separation of proteins by their isoelectric point in the first dimension and by molecular weight in the second dimension. By using a proteomics approach to identify differences in skeletal muscle proteins between these ethnicities, it may be possible to identify differences in proteins that have not been previously explored in this ethnic group which then can be the focus of future research efforts aimed at explaining the increased risk for T2D among Mexican Americans. Lifestyle interventions, such as diet and exercise, are known to decrease risk for T2D, however, it is possible that different ethnic groups respond differently to a given lifestyle intervention aimed at reducing risk for T2D. As such, we sought to determine what, if any, baseline differences exist in skeletal muscle proteins of non-obese, non-diabetic sedentary MA and NHW adults. We also aimed to determine if the magnitude of change in expression of these proteins in response to a diet and exercise intervention is affected by ethnicity.

RESEARCH DESIGN AND METHODS

Subjects: Skeletal muscle samples were obtained from 11 MA (3 males, 8 females) and 10 NHW (3 males, 7 females) subjects prior to and following a 7 day diet and exercise intervention. Subjects arrived following an overnight fast and ~17 hrs following any exercise. Subjects were sedentary, non-smoking, with no known diseases. Subject inclusion criteria were: fasting blood glucose <110 mg/dl, blood pressure <140/90 mmHg, and no history of endocrine disorders. Individuals were excluded if they were pregnant, used tobacco, had diabetes, history of eating disorders, were vegetarians, exercised more than once a week for the past 6 months, or used medication that could influence insulin signaling or plasma lipids. Females were studied in the mid-follicular phase of their menstrual cycles, or during the active phase of hormonal contraceptive use. Subjects were weight stable (±2.5 kg) for the previous six months. Mexican-American subjects verified that at least 3 of their grandparents were of Mexican descent, and NHW subjects traced their ethnicity to at least 3 grandparents having Anglo-European ancestry.

Study Design: Consent was obtained from each volunteer, and the study protocol was approved by the Colorado State University Human Research Committee. Eligible participants spent a baseline period of 7 days consuming their normal diets and refraining from any formal exercise. During this baseline period, subjects recorded their food intake for 3 consecutive days. Immediately following the baseline period, subjects underwent an intravenous glucose tolerance test (IVGTT) and muscle biopsy. For the next 7 days, study participants exercised for 6 of the 7 days and consumed a low

saturated fat, high fiber diet designed to maintain weight that was prepared for them by the study investigators. Following the 7-day intervention, subjects underwent a second IVGTT and biopsy.

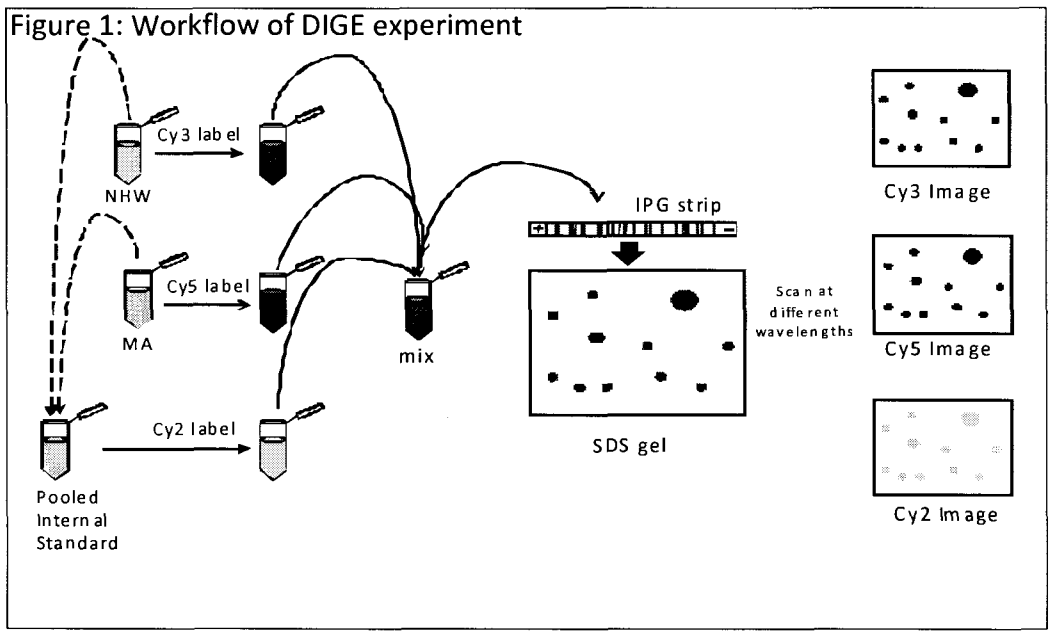
Muscle Biopsies: Percutaneous muscle biopsies (~75mg) were obtained from the belly of the vastus lateralis using 5mm Bergstrom needles with suction applied as previously described [268]. Muscle samples were immediately frozen in liquid nitrogen, stored at -80°C, and subsequently analyzed to determine any protein differences that might exist between ethnic groups at baseline and following the diet and exercise intervention.

Sample Preparation: Ground skeletal muscle samples were homogenized in an ice-cold lysis buffer containing 20 mM HEPES (pH 7.4), 1% Triton X-100, 10% glycerol, 2 mM EGTA, 1 mM sodium vanadate, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 50 mM ß-glycerophosphate, 3 mM benzamidine, 10 µM leupeptin, 5 µM pepstatin, and 10 µg/ml aprotinin. Samples were rotated for 30 minutes at 4°C, sonicated twice for 15 s at 30% power and then centrifuged at 14,000g for 30 minutes at 4° C. Total protein was determined with a 96-well plate reader (Multiskan EX, Waltham, MA).

Proteomics Analysis: Protein lysates from 3 subjects representing each of the two ethnic groups were precipitated with the Bio-Rad 2D clean-up kit (Hercules, CA) following the protocols of the manufacturer in order to remove components found in proteins that are known to interfere with isoelectric focusing, such as lipids, nucleic acids, and salts. These samples were dissolved by sonication in 150 μL rehydration buffer (2M urea, 2% CHAPS, and 30mM Tris, pH 8.5), and 50µg of protein were then

labeled using a lysine labeling protocol (Ettan DIGE, Amersham Biosciences, Piscataway, NJ).

Protein labeling with CyDyes: Each dye (5nmol) was reconstituted in 5µl anhydrous dimethylformamide (DMF) 99.8% to make a 1mM stock dye solution. This stock dye was further diluted to 200pmol by combining 4µl DMF to 1µl stock dye solution which formed the working dye solution for immediate use for labeling proteins. Protein samples from MA and NHW subjects were labeled individually with working Cy3 or Cy5



dye, while equal amounts of protein from all samples were pooled and labeled with working Cy2 dye (Figure 1). A 50µg portion of protein from MA, NHW and the pooled

sample were mixed with 1µl of working Cy2, Cy3, or Cy5 and incubated on ice for 30min in the dark. The labeling reactions were quenched with 1µl of 10mM lysine and incubated for another 10min on ice. Following the labeling reactions, samples were mixed together and adjusted to a total volume of 380 µL with rehydration buffer (8 M urea, 0.3% (w/v) DTT, 2% (w/v) CHAPS at pH 3–10 buffer, a trace amount of bromophenol blue and 4µl BioLyte 3-10).

For the first dimension (isoelectric focusing), the 380 µL sample was pipetted into a rehydrating tray, an immobilized pH gradient (IPG) strip at pH 3–10 (Bio-Rad, Hercules, CA) was placed gel side down onto the sample solution, and mineral oil was pipetted over the IPG strip. The strip was allowed to rehydrate passively for at least 12 h in the dark. Using a Multiphor II system (Pharmacia Biotech, Uppsala, Sweden), the protein mixture was focused using the voltage program of an increase from 0 to 500 V over 1 min, from 500 to 3500 over 5 h, and steady exposure to 3500 V for 17.5 h. For the second dimension (SDS-PAGE) separation, the IPG strip was removed from the focusing tray and reduced in 3 mL of 2% (w/v) dithiothreitol (DTT) in equilibration buffer [6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, and 24 mM Tris-HCl] for 15 min, followed by acetylation in 3 mL of 2.5% (w/v) iodoacetamide (IAA) in equilibration buffer for 5 min. The IPG strip was transferred onto a gel (12% polyacrylamide), overlaid with 1 mL of hot 0.5% agarose, and the agarose was allowed to solidify. Electrophoresis was performed in a Protean II cell (Bio-Rad, Hercules, CA) for 2.5 h at 3000 V, 400 W, and 40 ma. All stages of the protocol were conducted in the dark, using as little exposure to light as possible.

Gels between two low-fluorescence glass plates were scanned with a Typhoon Trio multiwavelength fluorescence scanner (GE Healthcare) and saved in .gel format with ImageQuant Software (GE Healthcare). The excitation wavelength for Cy2 is 488nm, Cy3 is 532 nm and for Cy5 633 nm, and the emission wavelengths are 520, 580 and 670 nm, respectively. Decodon Delta2D version 3.6 gel image analysis software was used to analyze the images. Briefly, after automatic spot detection and background subtraction from each gel image (samples labeled with Cy2, Cy3 and Cy5), spots were edited manually, such as adding spots, splitting spots, and removal of streaks that were detected as protein spots. One gel was chosen as the master gel and the other 2D gel images were warped, i.e., protein spots were matched, so that spots in the Cy2, Cy3 and Cy5 labeled samples were aligned with those in the master gel. After image warping, Cy3 and Cy5 gels were fused together and spots were detected for MA and NHW images. Each spot was assigned a normalized spot volume based on the internal standard, and total spot volume was calculated for each group. The images were then analyzed for protein spot differences between MA and NHW gels, with significantly different spots $p<0.05$.

After spot differences were detected from the MA and NHW gel images, another 2D gel was run without lysine labeling for protein identification. After isoelectric focusing and SDS-PAGE were completed, the gel was fixed for 1 h in an aqueous solution of 10% methanol/7% acetic acid and then stained in 100 mL of Sypro Ruby stain overnight. The gel was destained in the aqueous ethanol-acetic acid solution for 12 h. Images were obtained and protein spots that were determined to be significantly higher

in NHW compared to MA were then excised using ProPic II robotic spot picking. Excised protein spots underwent trypsin digestion without acylation overnight with the ProPrep robotic digestion. The digested proteins were then characterized with the Bruker Ultraflex MALDI-TOF/TOF automated mass spectrometer, and Mascot database search engine was used for protein identification.

Western blot: Western blots were conducted on all subjects in both groups to confirm findings from the subset of samples that were analyzed via proteomics. Equivalent amounts of protein (50 μg) were subjected to SDS-PAGE and transferred to Hybond-P membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Membranes were blocked and incubated with antibodies against ATP synthase (Santa Cruz Biotechnology, sc-16689) and α-tubulin (sc-23948), used as a loading control. Proteins were detected using horseradish peroxidase-conjugated secondary antibodies and a chemiluminescence reagent (Santa Cruz Biotechnology). A UVP Bioimaging system (Upland, CA, USA) was used for detection and analysis of optical density. Due to the difficulties in measuring CPT-1 in human skeletal muscle via Western blot, measurement of this enzyme is pending analysis.

RESULTS

Proteomics: Based on the quantification of protein spots from the Decodon Delta2D analysis program, 1015 spots were detected, and 45 spots differed significantly between MA and NHW at baseline. From these spots, 31 spots had higher expression level in NHW compared to MA. Of these 31 spots, 11 were well-defined protein spots with distinct isoelectric point and molecular weight, and these spots were chosen for excision

and identification. From the analysis of pre vs. post intervention, 46 spots differed from pre to post intervention in gel images from MA and NHW, with all 46 spots having a higher expression level following the intervention. Unfortunately, nearly all of the 46 spots were virtually impossible to visually distinguish in the gel image, and therefore, none of the spots that may have changed due to the diet and exercise intervention were excised for subsequent identification.

Based on the Mascot protein database search results, 5 spots contained only the background peaks of trypsin. Four proteins did not match any proteins in the database, but 2 proteins were positively identified: carnitine palmitoyltransferase 1A isoform 2 (CPT-1) and mitochondrial ATP synthase F1 complex beta subunit (ATP synthase). ATP synthase was 3-fold higher in NHW (NHW= 188.4 ± 10.2 and MA = 62.7 ± 28.4) (Figure 3). CPT-1 was 1.7 times higher in NHW compared to MA (NHW= 190.4 ± 1.3 vs. MA = 109.3 ± 12.6) (Figure 4).

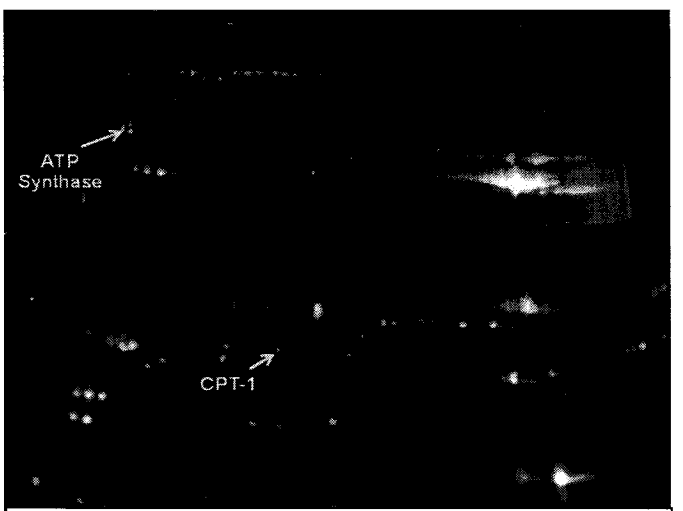


Figure 2. Representative 2D Proteomics gel

Western Blot: Western blot analysis revealed no main effect of time or ethnicity for the expression of ATP synthase F1 β-subunit. There was no difference between ethnic groups either pre or post intervention and no change following the intervention in either group ($p>0.05$). Pre-

intervention: MA = 1.6 ± 0.3, NHW =1.4 ± 0.3. Post intervention: MA = 1.11± 0.2 and NHW = 1.8, 0.5, arbitrary units. Figure 5.

Figure 3. Proteomics analysis of ATP synthase F1 β-subunit

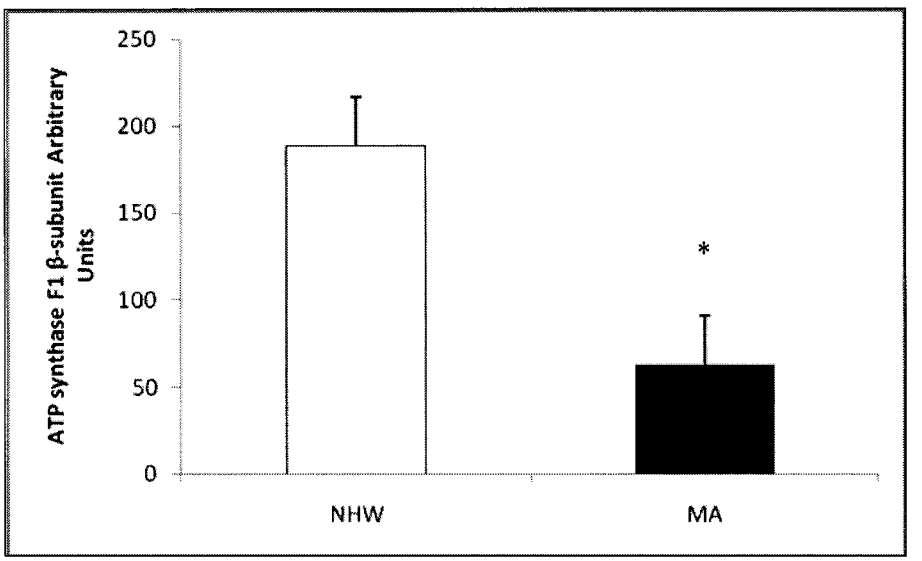


Figure 4. Proteomics analysis of CPT-1

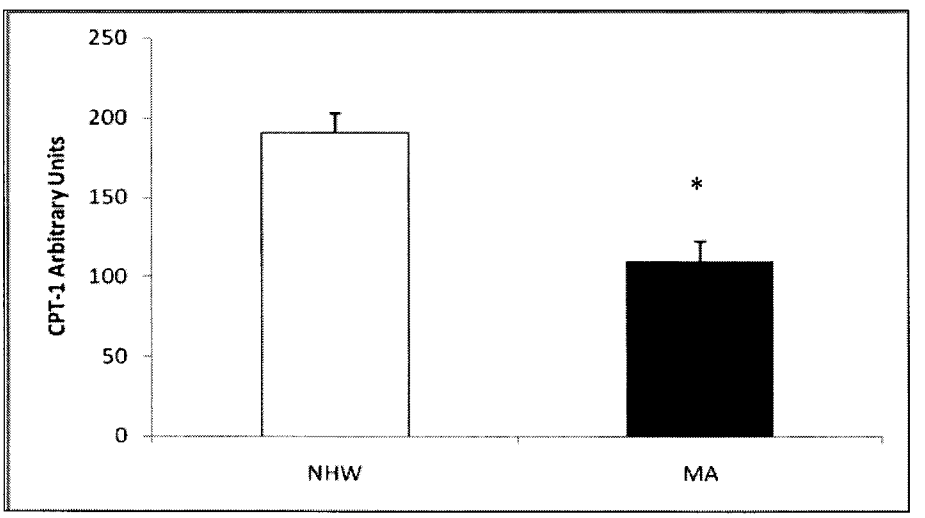
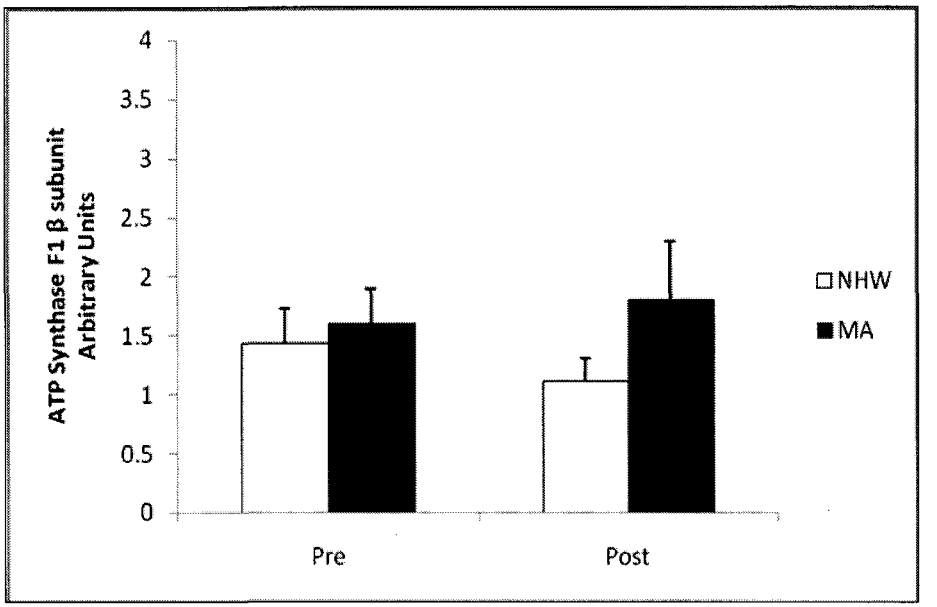


Figure 3 &4. Quantitation of proteomics analysis in NHW (n=3, 2F, 1M) and MA (n=3, 2F, 1M) subjects before the diet-exercise intervention. Values are means ± SE, arbitrary units. * = significantly different from NHW ($p<0.05$)

Figure 5. Western Blot Analysis of ATP Synthase F1 β-subunit



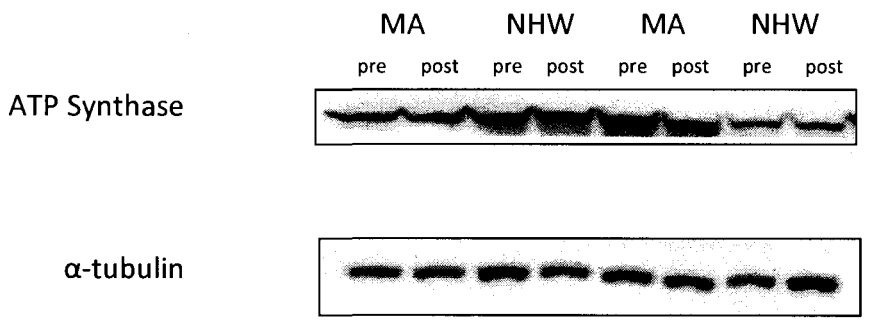


Figure 5.
Representative western blot and quantitation of ATP Synthase F1 β-subunit pre and post intervention in NHW (n=10) and MA (n=11). Values are means ± SE, relative to an internal standard run on all gels, normalized to α-tubulin content.

DISCUSSION

The purpose of this study was to utilize a 2D proteomics approach to determine whether MAs, a high risk population for T2D and the MetS, exhibit differential expression of skeletal muscle proteins compared to NHWs (a lower risk population). While we have previously reported no differences between MAs and NHWs in skeletal muscle abundance of insulin signaling molecules (insulin receptor-β, phosphatidylinositol 3-kinase p85 subunit, Akt1, Akt2, and GLUT4) [5], analysis of skeletal muscle from a subset of MA and NHW subjects from the current study revealed that carnitine palmitoyltransferase 1A isoform 2 (CPT-1) and mitochondrial ATP synthase F1 complex β-subunit (ATP synthase) were expressed significantly higher in NHW compared to MA. While these data are only preliminary, they demonstrate the possibility that differences in mitochondrial metabolism may be playing an important role in risk for insulin resistance in this population, as CPT-1 and ATP synthase are both mitochondrial proteins.

CPT-1 is an enzyme located on the outer mitochondrial membrane that appears to be one of the principal regulators of transport of long chain fatty acyl-CoAs across the inner mitochondrial membrane [269, 270]. The overexpression of CPT-1 leads to a repartitioning of fatty acids away from esterification and toward oxidation, leading to a reduction in IMTG content [95]. A recent study overexpressing CPT-1 at physiologic levels in skeletal muscle of rats showed that this overexpression prevented a high-fat diet induced increase in insulin resistance [271]. Overexpression of CPT-1 also led to

increased fat oxidation, lowered serine phosphorylation of IRS-1, and less membrane: cytosolic DAG, p-JNK, and PKC-θ [271]. In human skeletal muscle, exercise training significantly increases mRNA expression of CPT-1 in skeletal muscle, which is associated with an increase in fat oxidation [272]. Conversely, inhibition of skeletal muscle CPT-1 with etoximir leads to a decrease in insulin-stimulated glucose uptake and concomitant increase in IMTG and hepatic TG in rats fed a high fat diet [62]. These findings demonstrate that CPT-1 is an important protein in regulating fatty acid partitioning in skeletal muscle and may have implications for identifying potential differences in skeletal muscle insulin resistance in MA vs. NHW.

ATP synthase is a membrane-associated complex responsible for catalyzing the phosphorylation of ADP to ATP in exchange for protons generated from the electron transport chain (ETC)[145]. The F1 β-subunit of ATP synthase is considered part of the catalytic subunit of the complex. In a recent study by Rose et al, 3 weeks of one-legged knee extensor exercise for 1-2hr per day, 4-6 days per week led to an increase in ATP synthase F1 β-subunit in the exercised leg of obese humans [273]. This study did not measure insulin sensitivity, however previous studies utilizing knee extensor exercise found an increase in glucose uptake in the exercised leg 3h post exercise [274]. Given these data, it is tempting to speculate that an increase in expression level of F1 β-subunit of ATP synthase could be associated with an improvement in insulin sensitivity. However, western blot analysis of ATP synthase F1 β-subunit in our study sample found no difference in expression between the NHW and MA subjects at baseline or following a diet and exercise intervention that improved insulin sensitivity. It therefore seems

unlikely that differences in expression of ATP synthase in skeletal muscle are contributing to differences in insulin sensitivity in MA vs. NHW adults.

Lean offspring of T2D patients have 30% less mitochondria than lean controls with no family history of T2D [105]. Since Mexican Americans have a higher prevalence of T2D and insulin resistance, it is likely that the lean MA subjects in the current study had less total skeletal muscle mitochondria than the NHW subjects. This potential for a lower amount of total mitochondria may be the reason that we found lower expression level of CPT-1 and ATP synthase in the subset of MA subjects, although this is only speculative, as amount of mitochondria was not measured.

Although the etiology of skeletal muscle insulin resistance has not been fully elucidated, much data in the literature has focused on the impact of functionality and/or total content of mitochondria. The magnitude of reduction in mitochondrial content or functionality necessary to elicit a reduction in insulin sensitivity has not been clarified, however. Clinical strategies that have proven most effective in treating insulin resistance and T2D include increased physical activity, which is known to promote mitochondrial biogenesis and therefore mitochondrial proteins [118]. Although the mechanisms causing MA to be more insulin resistant than NHW remain unknown, an increase in physical activity, which we have shown to improve insulin resistance in MA, may be working through an increased expression of mitochondrial proteins and/or improvement in mitochondrial functionality.

In summary, our data show that a subset of young, sedentary, non-obese MA adults exhibit lower expression level of at least two mitochondrial proteins in skeletal

muscle compared to their NHW counterparts. The possibility for CPT-1 playing a role in the lower degree of insulin resistance in the MA subjects is supported by provocative data in the literature, however, we have no data at this time to confirm a difference in expression level between all subjects in the two ethnic groups. On the other hand, data to support the role of a lower ATP synthase protein content being associated with insulin resistance is less well-documented, and our analysis with western blot did not confirm that MA have lower abundance in skeletal muscle compared to NHW.

CHAPTER 5

OVERALL CONCLUSIONS

Ethnic disparities in the prevalence of type 2 diabetes (T2D) and the Metabolic Syndrome (MetS) are a growing problem in the US, particularly in Mexican Americans (MA). Insulin resistance is an early defect that may explain many of the abnormalities associated with T2D and MetS. Mexican Americans are more insulin resistant than non-Hispanic whites (NHW), but the reasons for the higher prevalence of insulin resistance in MA compared to NHW remain elusive. A potential contribution to the disparity in insulin resistance may be related to expression of skeletal muscle proteins, as insulin action in skeletal muscle has been shown to be important in maintaining whole-body insulin sensitivity. In addition, both diet and exercise have been shown to improve insulin sensitivity and are recommended in the prevention of T2D and MetS, yet no studies have determined how well MA respond to diet and exercise in terms of insulin sensitivity and features of the MetS.

Arm 1: Cardiometabolic Plasticity

Specific Aim 1: To determine if there is a difference in insulin sensitivity in response to a one-week diet and exercise intervention between nonobese, nondiabetic Mexican American and non-Hispanic white males and females.

Hypothesis: Mexican Americans will exhibit lower insulin sensitivity at baseline, but will have the same degree of improvement as NHW in insulin sensitivity in response to the diet and exercise intervention

Specific Aim 2: To determine if there is a difference in blood lipids at baseline and in response to a 7 day diet and exercise intervention in MA and NHW

Hypothesis: Mexican Americans will have greater dyslipidemia than NHW at baseline but will have the same degree of improvement as NHW in markers for cardiovascular dyslipidemia in response to the diet and exercise intervention.

Primary findings: Our findings suggest that a short term exercise and low saturated fat, high fiber diet intervention has similar effects on T2D and MetS risk factors in MA compared to NHW sedentary adults; in that, MA had a similar change in insulin sensitivity and blood lipids as NHW. However, despite an improvement in insulin sensitivity, MA remained less insulin sensitive than NHW both pre and post intervention. The lower insulin sensitivity in MA did not translate into greater dyslipidemia compared to NHW, however. These data suggest that despite higher risk for T2D and MetS, young MA adults are as metabolically responsive as NHW to a short term diet-exercise intervention, but the ethnic disparity in insulin sensitivity remains.

Future directions: While we have shown that MA exhibit a similar cardiometabolic plasticity as NHW in response to diet and exercise, it would be of particular interest to determine the individual effects of diet and exercise in this population to find out which lifestyle factors prove to be the most beneficial to this at-risk ethnic group. In addition, it would also be beneficial for MA to have information regarding particular dietary

factors that may cause a greater degree of insulin resistance in them compared to the general population. Future studies aimed at eliminating this health disparity should focus on the most effective lifestyle strategies in order to prevent the onset of T2D and MetS.

Arm 2: Skeletal Muscle Analysis

Specific Aim 3: To utilize 2D proteomics for a preliminary assessment of possible differences in the baseline expression level of skeletal muscle proteins in MA and NHW and in response to a 7 day diet and exercise intervention

Primary findings: Based on proteomics analysis of a subset of MA and NHW subjects, we found that two mitochondrial proteins were differentially expressed in the MA vs. NHW. We found carnitine palmitoyltransferase-1 and ATP synthase F1 β-subunit to be significantly higher in the NHW compared to MA at baseline. However, upon further investigation in the remaining study cohort, ATP synthase was found not to differ between the two ethnic groups. CPT-1 abundance could not be confirmed due to the difficulties in measurement of this protein in skeletal muscle.

Future directions: We found several proteins to differ between MA and NHW in our preliminary proteomics analysis; however, only 2 proteins were positively identified in the analysis. It would be beneficial to attempt to identify the other protein spots that were deemed to be different between the two ethnic groups in order to determine potential contributions to the ethnic disparity in insulin resistance. In addition, no protein spots were able to be identified between pre and post intervention due to difficulties in visualizing protein spots on the gel. Additional gel analysis should be run

in order to determine if skeletal muscle proteins respond differently to the exercise and diet intervention in MA compared to NHW. It would also be advisable to examine possible differences in activity of skeletal muscle proteins in the insulin stimulated state, since expression levels may not differ between these ethnic groups.

BIBLIOGRAPHY

1. Ford, E.S., W.H. Giles, and W.H. Dietz, *Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey.* Jama, 2002. **287**(3): p. 356-9.
2. NIH, *National Heart Lung and Blood Institute: Diseases and Conditions Index.* 2007.
3. Li, C., et al., *Trends in hyperinsulinemia among nondiabetic adults in the U.S.* Diabetes Care, 2006. **29**(11): p. 2396-402.
4. *Center for Disease Control's Diabetes Program- Publications and Products- National Diabetes Fact Sheet* 2005.
5. Ho, R.C., et al., *Behavioral, metabolic, and molecular correlates of lower insulin sensitivity in Mexican-Americans.* Am J Physiol Endocrinol Metab, 2002. **283**(4): p. E799-808.
6. Haffner, S.M., et al., *Role of obesity and fat distribution in non-insulin-dependent diabetes mellitus in Mexican Americans and non-Hispanic whites.* Diabetes Care, 1986. **9**(2): p. 153-61.
7. Ren, J.M., et al., *Exercise induces rapid increases in GLUT4 expression, glucose transport capacity, and insulin-stimulated glycogen storage in muscle.* J Biol Chem, 1994. **269**(20): p. 14396-401.
8. Bjornholm, M., et al., *Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation.* Diabetes, 1997. **46**(3): p. 524-7.
9. Ryder, J.W., A.V. Chibalin, and J.R. Zierath, *Intracellular mechanisms underlying increases in glucose uptake in response to insulin or exercise in skeletal muscle.* Acta Physiol Scand, 2001. **171**(3): p. 249-57.
10. Yu, M., et al., *Exercise-associated differences in an array of proteins involved in signal transduction and glucose transport.* J Appl Physiol, 2001. **90**(1): p. 29-34.
11. Henriksen, E.J., *Invited review: Effects of acute exercise and exercise training on insulin resistance.* J Appl Physiol, 2002. **93**(2): p. 788-96.
12. DeFronzo, R.A. and E. Ferrannini, *Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease.* Diabetes Care, 1991. **14**(3): p. 173-94.
13. Shepherd, P.R., D.J. Withers, and K. Siddle, *Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling.* Biochem J, 1998. **333 (Pt 3)**: p. 471-90.
14. Wang, D. and H.S. Sul, *Insulin stimulation of the fatty acid synthase promoter is mediated by the phosphatidylinositol 3-kinase pathway. Involvement of protein kinase B/Akt.* J Biol Chem, 1998. **273**(39): p. 25420-6.

15. Hajduch, E., et al., *Constitutive activation of protein kinase B alpha by membrane targeting promotes glucose and system A amino acid transport, protein synthesis, and inactivation of glycogen synthase kinase 3 in L6 muscle cells.* Diabetes, 1998. **47**(7): p. 1006-13.
16. Takata, M., et al., *Requirement for Akt (protein kinase B) in insulin-induced activation of glycogen synthase and phosphorylation of 4E-BP1 (PHAS-1).* J Biol Chem, 1999. **274**(29): p. 20611-8.
17. Chabowski, A., et al., *Insulin stimulates fatty acid transport by regulating expression of FAT/CD36 but not FABPpm.* Am J Physiol Endocrinol Metab, 2004. **287**(4): p. E781-9.
18. DeFronzo, R.A., et al., *Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus.* J Clin Invest, 1985. **76**(1): p. 149-55.
19. Martin, B.C., et al., *Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study.* Lancet, 1992. **340**(8825): p. 925-9.
20. DeFronzo, R.A., R.C. Bonadonna, and E. Ferrannini, *Pathogenesis of NIDDM. A balanced overview.* Diabetes Care, 1992. **15**(3): p. 318-68.
21. Reaven, G.M., *Banting lecture 1988. Role of insulin resistance in human disease.* Diabetes, 1988. **37**(12): p. 1595-607.
22. Ginsberg, H.N., *Risk factors common to insulin resistance and atherosclerosis explain why diabetes is a cardiovascular disease.* Am J Manag Care, 2002. **Suppl**: p. 3, 5.
23. Moller, D.E. and K.D. Kaufman, *Metabolic syndrome: a clinical and molecular perspective.* Annu Rev Med, 2005. **56**: p. 45-62.
24. Reaven, G.M., *Pathophysiology of insulin resistance in human disease.* Physiol Rev, 1995. **75**(3): p. 473-86.
25. De Meyts, P., *The insulin receptor: a prototype for dimeric, allosteric membrane receptors?* Trends Biochem Sci, 2008. **33**(8): p. 376-84.
26. Sesti, G., et al., *Defects of the insulin receptor substrate (IRS) system in human metabolic disorders.* Faseb J, 2001. **15**(12): p. 2099-111.
27. White, M.F. and C.R. Kahn, *The insulin signaling system.* J Biol Chem, 1994. **269**(1): p. 1-4.
28. Ren, J.M., et al., *Evidence from transgenic mice that glucose transport is rate-limiting for glycogen deposition and glycolysis in skeletal muscle.* J Biol Chem, 1993. **268**(22): p. 16113-5.
29. Krook, A., H. Wallberg-Henriksson, and J.R. Zierath, *Sending the signal: molecular mechanisms regulating glucose uptake.* Med Sci Sports Exerc, 2004. **36**(7): p. 1212-7.
30. Bouzakri, K., et al., *Reduced activation of phosphatidylinositol-3 kinase and increased serine 636 phosphorylation of insulin receptor substrate-1 in primary culture of skeletal muscle cells from patients with type 2 diabetes.* Diabetes, 2003. **52**(6): p. 1319-25.

31. Gual, P., Y. Le Marchand-Brustel, and J.F. Tanti, *Positive and negative regulation of insulin signaling through IRS-1 phosphorylation.* Biochimie, 2005. **87**(1): p. 99-109.
32. Cusi, K., et al., *Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle.* J Clin Invest, 2000. **105**(3): p. 311-20.
33. DeFronzo, R.A., et al., *The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization.* Diabetes, 1981. **30**(12): p. 1000-7.
34. Kelley, D., et al., *Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load.* J Clin Invest, 1988. **81**(5): p. 1563-71.
35. Bjornholm, M. and J.R. Zierath, *Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes.* Biochem Soc Trans, 2005. **33**(Pt 2): p. 354-7.
36. Baron, A.D., et al., *Reduced capacity and affinity of skeletal muscle for insulin-mediated glucose uptake in noninsulin-dependent diabetic subjects. Effects of insulin therapy.* J Clin Invest, 1991. **87**(4): p. 1186-94.
37. Hegarty, B.D., et al., *The role of intramuscular lipid in insulin resistance.* Acta Physiol Scand, 2003. **178**(4): p. 373-83.
38. Boden, G. and G.I. Shulman, *Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction.* Eur J Clin Invest, 2002. **32 Suppl 3**: p. 14-23.
39. Fraze, E., et al., *Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance.* J Clin Endocrinol Metab, 1985. **61**(5): p. 807-11.
40. Fagot-Campagna, A., et al., *High free fatty acid concentration: an independent risk factor for hypertension in the Paris Prospective Study.* Int J Epidemiol, 1998. **27**(5): p. 808-13.
41. Cornier, M.A., et al., *The metabolic syndrome.* Endocr Rev, 2008. **29**(7): p. 777-822.
42. Busetto, L., et al., *Omental and epigastric adipose tissue lipolytic activity in human obesity. Effect of abdominal fat distribution and relationship with hyperinsulinemia.* Horm Metab Res, 1993. **25**(7): p. 365-71.
43. Duncan, R.E., et al., *Regulation of lipolysis in adipocytes.* Annu Rev Nutr, 2007. **27**: p. 79-101.
44. Kurioka, S., et al., *Relationship between visceral fat accumulation and anti-lipolytic action of insulin in patients with type 2 diabetes mellitus.* Endocr J, 2002. **49**(4): p. 459-64.
45. Boden, G., et al., *Effects of a 48-h fat infusion on insulin secretion and glucose utilization.* Diabetes, 1995. **44**(10): p. 1239-42.
46. Roden, M., et al., *Mechanism of free fatty acid-induced insulin resistance in humans.* J Clin Invest, 1996. **97**(12): p. 2859-65.

47. Bachmann, O.P., et al., *Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans.* Diabetes, 2001. **50**(11): p. 2579-84.
48. Schrauwen-Hinderling, V.B., et al., *Intramyocellular lipid content and molecular adaptations in response to a 1-week high-fat diet.* Obes Res, 2005. **13**(12): p. 2088-94.
49. Belfort, R., et al., *Dose-response effect of elevated plasma free fatty acid on insulin signaling.* Diabetes, 2005. **54**(6): p. 1640-8.
50. Hamilton, J.A., *Fatty acid transport: difficult or easy?* J Lipid Res, 1998. **39**(3): p. 467-81.
51. Abumrad, N., C. Harmon, and A. Ibrahimi, *Membrane transport of long-chain fatty acids: evidence for a facilitated process.* J Lipid Res, 1998. **39**(12): p. 2309-18.
52. Jacob, S., et al., *Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects.* Diabetes, 1999. **48**(5): p. 1113-9.
53. Bonen, A., et al., *Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase.* J Biol Chem, 2000. **275**(19): p. 14501-8.
54. Bonen, A., et al., *Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence.* Physiology (Bethesda), 2007. **22**: p. 15-29.
55. Holloway, G.P., et al., *Fatty acid binding protein facilitates sarcolemmal fatty acid transport but not mitochondrial oxidation in rat and human skeletal muscle.* J Physiol, 2007. **582**(Pt 1): p. 393-405.
56. Holloway, G.P., et al., *In obese rat muscle transport of palmitate is increased and is channeled to triacylglycerol storage despite an increase in mitochondrial palmitate oxidation.* Am J Physiol Endocrinol Metab, 2009.
57. Bonen, A., et al., *Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36.* Faseb J, 2004. **18**(10): p. 1144-6.
58. Blaak, E.E., et al., *Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle.* Am J Physiol Endocrinol Metab, 2000. **279**(1): p. E146-54.
59. Turcotte, L.P., et al., *Increased fatty acid uptake and altered fatty acid metabolism in insulin-resistant muscle of obese Zucker rats.* Diabetes, 2001. **50**(6): p. 1389-96.
60. Jeukendrup, A.E., *Regulation of fat metabolism in skeletal muscle.* Ann N Y Acad Sci, 2002. **967**: p. 217-35.
61. Olefsky, J.M., *Fat talks, liver and muscle listen.* Cell, 2008. **134**(6): p. 914-6.
62. Dobbins, R.L., et al., *Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats.* Diabetes, 2001. **50**(1): p. 123-30.

63. Manco, M., et al., *Insulin resistance directly correlates with increased saturated fatty acids in skeletal muscle triglycerides.* Metabolism, 2000. **49**(2): p. 220-4.
64. Schmitz-Peiffer, C., et al., *Alterations in the expression and cellular localization of protein kinase C isozymes epsilon and theta are associated with insulin resistance in skeletal muscle of the high-fat-fed rat.* Diabetes, 1997. **46**(2): p. 169-78.
65. Montell, E., et al., *DAG accumulation from saturated fatty acids desensitizes insulin stimulation of glucose uptake in muscle cells.* Am J Physiol Endocrinol Metab, 2001. **280**(2): p. E229-37.
66. Turinsky, J., D.M. O'Sullivan, and B.P. Bayly, *1,2-Diacylglycerol and ceramide levels in insulin-resistant tissues of the rat in vivo.* J Biol Chem, 1990. **265**(28): p. 16880-5.
67. Ellis, B.A., et al., *Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle.* Am J Physiol Endocrinol Metab, 2000. **279**(3): p. E554-60.
68. Chalkley, S.M., et al., *Five-hour fatty acid elevation increases muscle lipids and impairs glycogen synthesis in the rat.* Metabolism, 1998. **47**(9): p. 1121-6.
69. Bajaj, M., et al., *Effect of a sustained reduction in plasma free fatty acid concentration on intramuscular long-chain fatty Acyl-CoAs and insulin action in type 2 diabetic patients.* Diabetes, 2005. **54**(11): p. 3148-53.
70. Timmers, S., P. Schrauwen, and J. de Vogel, *Muscular diacylglycerol metabolism and insulin resistance.* Physiol Behav, 2008. **94**(2): p. 242-51.
71. Corcoran, M.P., S. Lamon-Fava, and R.A. Fielding, *Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise.* Am J Clin Nutr, 2007. **85**(3): p. 662-77.
72. Bronfman, M., M.N. Morales, and A. Orellana, *Diacylglycerol activation of protein kinase C is modulated by long-chain acyl-CoA.* Biochem Biophys Res Commun, 1988. **152**(3): p. 987-92.
73. Griffin, M.E., et al., *Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade.* Diabetes, 1999. **48**(6): p. 1270-4.
74. Schmitz-Peiffer, C., D.L. Craig, and T.J. Biden, *Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate.* J Biol Chem, 1999. **274**(34): p. 24202-10.
75. Itani, S.I., et al., *Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha.* Diabetes, 2002. **51**(7): p. 2005-11.
76. Itani, S.I., et al., *Increased protein kinase C theta in skeletal muscle of diabetic patients.* Metabolism, 2001. **50**(5): p. 553-7.
77. Sell, H., J. Eckel, and D. Dietze-Schroeder, *Pathways leading to muscle insulin resistance--the muscle--fat connection.* Arch Physiol Biochem, 2006. **112**(2): p. 105-13.
78. Bhatt, B.A., et al., *Diet-induced obesity and acute hyperlipidemia reduce IkappaBalpha levels in rat skeletal muscle in a fiber-type dependent manner.* Am J Physiol Regul Integr Comp Physiol, 2006. **290**(1): p. R233-40.

79. Holland, W.L. and S.A. Summers, *Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism.* Endocr Rev, 2008. **29**(4): p. 381-402.
80. Hannun, Y.A. and L.M. Obeid, *The Ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind.* J Biol Chem, 2002. **277**(29): p. 25847-50.
81. Merrill, A.H., Jr. and D.D. Jones, *An update of the enzymology and regulation of sphingomyelin metabolism.* Biochim Biophys Acta, 1990. **1044**(1): p. 1-12.
82. Adams, J.M., 2nd, et al., *Ceramide content is increased in skeletal muscle from obese insulin-resistant humans.* Diabetes, 2004. **53**(1): p. 25-31.
83. Straczkowski, M., et al., *Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle.* Diabetes, 2004. **53**(5): p. 1215-21.
84. Straczkowski, M., et al., *Increased skeletal muscle ceramide level in men at risk of developing type 2 diabetes.* Diabetologia, 2007. **50**(11): p. 2366-73.
85. Hajduch, E., et al., *Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells.* Diabetologia, 2001. **44**(2): p. 173-83.
86. Goodyear, L.J., et al., *Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects.* J Clin Invest, 1995. **95**(5): p. 2195-204.
87. Powell, D.J., et al., *Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism.* Mol Cell Biol, 2003. **23**(21): p. 7794-808.
88. Skovbro, M., et al., *Human skeletal muscle ceramide content is not a major factor in muscle insulin sensitivity.* Diabetologia, 2008. **51**(7): p. 1253-60.
89. Goodpaster, B.H., et al., *Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes.* J Clin Endocrinol Metab, 2001. **86**(12): p. 5755-61.
90. Perdomo, G., et al., *Increased beta-oxidation in muscle cells enhances insulin-stimulated glucose metabolism and protects against fatty acid-induced insulin resistance despite intramyocellular lipid accumulation.* J Biol Chem, 2004. **279**(26): p. 27177-86.
91. Simoneau, J.A., et al., *Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss.* Faseb J, 1999. **13**(14): p. 2051-60.
92. Bruce, C.R., et al., *Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status.* J Clin Endocrinol Metab, 2003. **88**(11): p. 5444-51.
93. Lowell, B.B. and G.I. Shulman, *Mitochondrial dysfunction and type 2 diabetes.* Science, 2005. **307**(5708): p. 384-7.
94. Kelley, D.E. and L.J. Mandarino, *Fuel selection in human skeletal muscle in insulin resistance: a reexamination.* Diabetes, 2000. **49**(5): p. 677-83.

95. Bruce, C.R., et al., *Overexpression of carnitine palmitoyltransferase I in skeletal muscle in vivo increases fatty acid oxidation and reduces triacylglycerol esterification.* Am J Physiol Endocrinol Metab, 2007. **292**(4): p. E1231-7.
96. Kelley, D.E., et al., *Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss.* Am J Physiol, 1999. **277**(6 Pt 1): p. E1130-41.
97. Campbell, S.E., et al., *A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria.* J Biol Chem, 2004. **279**(35): p. 36235-41.
98. Bezaire, V., et al., *Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation.* Am J Physiol Endocrinol Metab, 2006. **290**(3): p. E509-15.
99. Holloway, G.P., et al., *Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise.* J Physiol, 2006. **571**(Pt 1): p. 201-10.
100. Schenk, S. and J.F. Horowitz, *Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training.* Am J Physiol Endocrinol Metab, 2006. **291**(2): p. E254-60.
101. Kim, J.Y., et al., *Lipid oxidation is reduced in obese human skeletal muscle.* Am J Physiol Endocrinol Metab, 2000. **279**(5): p. E1039-44.
102. Holloway, G.P., et al., *Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women.* Am J Physiol Endocrinol Metab, 2007. **292**(6): p. E1782-9.
103. Boushel, R., et al., *Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle.* Diabetologia, 2007. **50**(4): p. 790-6.
104. Mootha, V.K., et al., *PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes.* Nat Genet, 2003. **34**(3): p. 267-73.
105. Asmann, Y.W., et al., *Skeletal muscle mitochondrial functions, mitochondrial DNA copy numbers, and gene transcript profiles in type 2 diabetic and nondiabetic subjects at equal levels of low or high insulin and euglycemia.* Diabetes, 2006. **55**(12): p. 3309-19.
106. Koves, T.R., et al., *Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance.* Cell Metab, 2008. **7**(1): p. 45-56.
107. Phielix, E., et al., *Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients.* Diabetes, 2008. **57**(11): p. 2943-9.
108. Zierath, J.R., A. Krook, and H. Wallberg-Henriksson, *Insulin action in skeletal muscle from patients with NIDDM.* Mol Cell Biochem, 1998. **182**(1-2): p. 153-60.
109. Kriska, A.M., et al., *Association of physical activity and serum insulin concentrations in two populations at high risk for type 2 diabetes but differing by BMI.* Diabetes Care, 2001. **24**(7): p. 1175-80.

110. Kriska, A.M., et al., *Physical activity, physical fitness, and insulin and glucose concentrations in an isolated Native Canadian population experiencing rapid lifestyle change.* Diabetes Care, 2001. **24**(10): p. 1787-92.
111. Schulze, M.B. and F.B. Hu, *Primary prevention of diabetes: what can be done and how much can be prevented?* Annu Rev Public Health, 2005. **26**: p. 445-67.
112. Hu, F.B., et al., *Walking compared with vigorous physical activity and risk of type 2 diabetes in women: a prospective study.* JAMA, 1999. **282**(15): p. 1433-9.
113. Hollenbeck, C.B., et al., *Effect of habitual physical activity on regulation of insulin-stimulated glucose disposal in older males.* J Am Geriatr Soc, 1985. **33**(4): p. 273-7.
114. Rogers, M.A., et al., *Improvement in glucose tolerance after 1 wk of exercise in patients with mild NIDDM.* Diabetes Care, 1988. **11**(8): p. 613-8.
115. Boule, N.G., et al., *Effects of exercise training on glucose homeostasis: the HERITAGE Family Study.* Diabetes Care, 2005. **28**(1): p. 108-14.
116. Mikines, K.J., et al., *Effect of physical exercise on sensitivity and responsiveness to insulin in humans.* Am J Physiol, 1988. **254**(3 Pt 1): p. E248-59.
117. Oshida, Y., et al., *Long-term mild jogging increases insulin action despite no influence on body mass index or VO2 max.* J Appl Physiol, 1989. **66**(5): p. 2206-10.
118. Hawley, J.A. and S.J. Lessard, *Exercise training-induced improvements in insulin action.* Acta Physiol (Oxf), 2008. **192**(1): p. 127-35.
119. Haskell, W.L., et al., *Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association.* Med Sci Sports Exerc, 2007. **39**(8): p. 1423-34.
120. Dolan, P.L., et al., *Contractile activity restores insulin responsiveness in skeletal muscle of obese Zucker rats.* Biochem J, 1993. **289 (Pt 2)**: p. 423-6.
121. Richter, E.A., et al., *Effect of exercise on insulin action in human skeletal muscle.* J Appl Physiol, 1989. **66**(2): p. 876-85.
122. DeFronzo, R.A., et al., *Synergistic interaction between exercise and insulin on peripheral glucose uptake.* J Clin Invest, 1981. **68**(6): p. 1468-74.
123. Thyfault, J.P., *Setting the stage: possible mechanisms by which acute contraction restores insulin sensitivity in muscle.* Am J Physiol Regul Integr Comp Physiol, 2008. **294**(4): p. R1103-10.
124. Goodyear, L.J. and B.B. Kahn, *Exercise, glucose transport, and insulin sensitivity.* Annu Rev Med, 1998. **49**: p. 235-61.
125. Magkos, F., et al., *Improved insulin sensitivity after a single bout of exercise is curvilinearly related to exercise energy expenditure.* Clin Sci (Lond), 2008. **114**(1): p. 59-64.
126. Burstein, R., et al., *Acute reversal of the enhanced insulin action in trained athletes. Association with insulin receptor changes.* Diabetes, 1985. **34**(8): p. 756-60.
127. Kump, D.S. and F.W. Booth, *Alterations in insulin receptor signalling in the rat epitrochlearis muscle upon cessation of voluntary exercise.* J Physiol, 2005. **562**(Pt 3): p. 829-38.

128. Chibalin, A.V., et al., *Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: differential effects on insulin-receptor substrates 1 and 2.* Proc Natl Acad Sci U S A, 2000. **97**(1): p. 38-43.
129. Bonen, A., G.L. Dohm, and L.J. van Loon, *Lipid metabolism, exercise and insulin action.* Essays Biochem, 2006. **42**: p. 47-59.
130. Schenk, S. and J.F. Horowitz, *Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance.* J Clin Invest, 2007. **117**(6): p. 1690-8.
131. Szendroedi, J. and M. Roden, *Mitochondrial fitness and insulin sensitivity in humans.* Diabetologia, 2008. **51**(12): p. 2155-67.
132. Bruce, C.R., et al., *Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content.* Am J Physiol Endocrinol Metab, 2006. **291**(1): p. E99-E107.
133. Yaspelkis, B.B., 3rd, et al., *Exercise reverses high-fat diet-induced impairments on compartmentalization and activation of components of the insulin-signaling cascade in skeletal muscle.* Am J Physiol Endocrinol Metab, 2007. **293**(4): p. E941-9.
134. van Loon, L.J. and B.H. Goodpaster, *Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state.* Pflugers Arch, 2006. **451**(5): p. 606-16.
135. Blachnio-Zabielska, A., et al., *Effect of exercise duration on the key pathways of ceramide metabolism in rat skeletal muscles.* J Cell Biochem, 2008. **105**(3): p. 776-84.
136. Baranowski, M., et al., *Effect of exercise duration on ceramide metabolism in the rat heart.* Acta Physiol (Oxf), 2008. **192**(4): p. 519-29.
137. Watt, M.J. and A.L. Hevener, *Fluxing the mitochondria to insulin resistance.* Cell Metab, 2008. **7**(1): p. 5-6.
138. Koves, T.R., et al., *Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency.* J Biol Chem, 2005. **280**(39): p. 33588-98.
139. Adhihetty, P.J., et al., *Plasticity of skeletal muscle mitochondria in response to contractile activity.* Exp Physiol, 2003. **88**(1): p. 99-107.
140. Wu, Z., et al., *Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1.* Cell, 1999. **98**(1): p. 115-24.
141. Kochanek, K.D., et al., *Deaths: final data for 2002.* Natl Vital Stat Rep, 2004. **53**(5): p. 1-115.
142. *Atherosclerosis.* American Heart Association 2007; Available from: www.americanheart.org
143. Kavey, R.E., et al., *American Heart Association guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood.* Circulation, 2003. **107**(11): p. 1562-6.

144. *Diabetes in America*. 1995, National Diabetes Data Group, National Institutes of Health: Washington, DC.
145. Murray, R.K., et al., *Harper's Illustrated Biochemistry*. 26 ed. 2003, New York, NY: McGraw-Hill Companies.
146. Haffner, S.M., et al., *Cardiovascular risk factors in confirmed prediabetic individuals. Does the clock for coronary heart disease start ticking before the onset of clinical diabetes?* Jama, 1990. **263**(21): p. 2893-8.
147. Adiels, M., et al., *Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome.* Arterioscler Thromb Vasc Biol, 2008. **28**(7): p. 1225-36.
148. Ginsberg, H.N., *Insulin resistance and cardiovascular disease.* J Clin Invest, 2000. **106**(4): p. 453-8.
149. Fisher, E.A., et al., *The degradation of apolipoprotein B100 is mediated by the ubiquitin-proteasome pathway and involves heat shock protein 70.* J Biol Chem, 1997. **272**(33): p. 20427-34.
150. Sparks, J.D., et al., *Insulin-mediated inhibition of apolipoprotein B secretion requires an intracellular trafficking event and phosphatidylinositol 3-kinase activation: studies with brefeldin A and wortmannin in primary cultures of rat hepatocytes.* Biochem J, 1996. **313 (Pt 2)**: p. 567-74.
151. Malmstrom, R., et al., *Metabolic basis of hypotriglyceridemic effects of insulin in normal men.* Arterioscler Thromb Vasc Biol, 1997. **17**(7): p. 1454-64.
152. Lewis, G.F., et al., *Effects of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals.* Diabetes, 1993. **42**(6): p. 833-42.
153. Jin, U.H., et al., *Secretion of atherogenic risk factor apolipoprotein B-100 is increased by a potential mechanism of JNK/PKC-mediated insulin resistance in liver cells.* J Cell Biochem, 2008. **103**(3): p. 908-19.
154. Adiels, M., et al., *Acute suppression of VLDL1 secretion rate by insulin is associated with hepatic fat content and insulin resistance.* Diabetologia, 2007. **50**(11): p. 2356-65.
155. Lewis, G.F., *Fatty acid regulation of very low density lipoprotein production.* Curr Opin Lipidol, 1997. **8**(3): p. 146-53.
156. Nesto, R.W., *Beyond low-density lipoprotein: addressing the atherogenic lipid triad in type 2 diabetes mellitus and the metabolic syndrome.* Am J Cardiovasc Drugs, 2005. **5**(6): p. 379-87.
157. Gianturco, S.H., et al., *Cellular binding site and membrane binding proteins for triglyceride-rich lipoproteins in human monocyte-macrophages and THP-1 monocytic cells.* J Lipid Res, 1994. **35**(9): p. 1674-87.
158. Pronk, N.P., *Short term effects of exercise on plasma lipids and lipoproteins in humans.* Sports Med, 1993. **16**(6): p. 431-48.
159. Johnson, J.L., et al., *Exercise training amount and intensity effects on metabolic syndrome (from Studies of a Targeted Risk Reduction Intervention through Defined Exercise).* Am J Cardiol, 2007. **100**(12): p. 1759-66.

160. Kolovou, G.D., et al., *Clinical relevance of postprandial lipaemia.* Curr Med Chem, 2005. **12**(17): p. 1931-45.
161. Graham, T.E., *Exercise, postprandial triacylglyceridemia, and cardiovascular disease risk.* Can J Appl Physiol, 2004. **29**(6): p. 781-99.
162. Chen, Y.D., et al., *Differences in postprandial lipemia between patients with normal glucose tolerance and noninsulin-dependent diabetes mellitus.* J Clin Endocrinol Metab, 1993. **76**(1): p. 172-7.
163. Paglialunga, S. and K. Cianflone, *Regulation of postprandial lipemia: an update on current trends.* Appl Physiol Nutr Metab, 2007. **32**(1): p. 61-75.
164. Karpe, F., *Postprandial lipoprotein metabolism and atherosclerosis.* J Intern Med, 1999. **246**(4): p. 341-55.
165. Barrows, B.R., M.T. Timlin, and E.J. Parks, *Spillover of dietary fatty acids and use of serum nonesterified fatty acids for the synthesis of VLDL-triacylglycerol under two different feeding regimens.* Diabetes, 2005. **54**(9): p. 2668-73.
166. Zilversmit, D.B., *Atherogenesis: a postprandial phenomenon.* Circulation, 1979. **60**(3): p. 473-85.
167. Frayn, K.N., et al., *Periprandial regulation of lipid metabolism in insulin-treated diabetes mellitus.* Metabolism, 1993. **42**(4): p. 504-10.
168. Lewis, G.F., et al., *Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes.* Endocr Rev, 2002. **23**(2): p. 201-29.
169. Schneeman, B.O., et al., *Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans.* Proc Natl Acad Sci U S A, 1993. **90**(5): p. 2069-73.
170. Frayn, K.N., *Adipose tissue as a buffer for daily lipid flux.* Diabetologia, 2002. **45**(9): p. 1201-10.
171. HP, H., *The dietetic factor determining the glucose tolerance and sensitivity to insulin of healthy men. .* Clin Sci (Lond), 1935. **2**: p. 67-94.
172. Pedersen, O., et al., *High fat feeding causes insulin resistance and a marked decrease in the expression of glucose transporters (Glut 4) in fat cells of rats.* Endocrinology, 1991. **129**(2): p. 771-7.
173. Mayer-Davis, E.J., et al., *Dietary fat and insulin sensitivity in a triethnic population: the role of obesity. The Insulin Resistance Atherosclerosis Study (IRAS).* Am J Clin Nutr, 1997. **65**(1): p. 79-87.
174. Maron, D.J., J.M. Fair, and W.L. Haskell, *Saturated fat intake and insulin resistance in men with coronary artery disease. The Stanford Coronary Risk Intervention Project Investigators and Staff.* Circulation, 1991. **84**(5): p. 2020-7.
175. Parker, D.R., et al., *Relationship of dietary saturated fatty acids and body habitus to serum insulin concentrations: the Normative Aging Study.* Am J Clin Nutr, 1993. **58**(2): p. 129-36.
176. Villegas, R., et al., *Prudent diet and the risk of insulin resistance.* Nutr Metab Cardiovasc Dis, 2004. **14**(6): p. 334-43.
177. Kinsell, L.W., et al., *Dietary fats and the diabetic patient.* N Engl J Med, 1959. **261**: p. 431-4.

178. Thanopoulou, A.C., et al., *Dietary fat intake as risk factor for the development of diabetes: multinational, multicenter study of the Mediterranean Group for the Study of Diabetes (MGSD).* Diabetes Care, 2003. **26**(2): p. 302-7.
179. van de Laar, F.A., et al., *Fat intake in patients newly diagnosed with type 2 diabetes: a 4-year follow-up study in general practice.* Br J Gen Pract, 2004. **54**(500): p. 177-82.
180. Vessby, B., et al., *Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study.* Diabetologia, 2001. **44**(3): p. 312-9.
181. Lovejoy, J.C., et al., *Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults.* Diabetes Care, 2002. **25**(8): p. 1283-8.
182. Han, P., et al., *Effects of different free fatty acids on insulin resistance in rats.* Hepatobiliary Pancreat Dis Int, 2008. **7**(1): p. 91-6.
183. Laaksonen, D.E., et al., *Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men.* Diabet Med, 2002. **19**(6): p. 456-64.
184. Borkman, M., et al., *The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids.* N Engl J Med, 1993. **328**(4): p. 238-44.
185. Storlien, L.H., et al., *Skeletal muscle membrane lipids and insulin resistance.* Lipids, 1996. **31 Suppl**: p. S261-5.
186. Field, C.J., et al., *Diet fat composition alters membrane phospholipid composition, insulin binding, and glucose metabolism in adipocytes from control and diabetic animals.* J Biol Chem, 1990. **265**(19): p. 11143-50.
187. Lee, J.S., et al., *Saturated, but not n-6 polyunsaturated, fatty acids induce insulin resistance: role of intramuscular accumulation of lipid metabolites.* J Appl Physiol, 2006. **100**(5): p. 1467-74.
188. Chavez, J.A. and S.A. Summers, *Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes.* Arch Biochem Biophys, 2003. **419**(2): p. 101-9.
189. DeLany, J.P., et al., *Differential oxidation of individual dietary fatty acids in humans.* Am J Clin Nutr, 2000. **72**(4): p. 905-11.
190. Tanasescu, M., et al., *Dietary fat and cholesterol and the risk of cardiovascular disease among women with type 2 diabetes.* Am J Clin Nutr, 2004. **79**(6): p. 999-1005.
191. Spector, A.A., T.L. Kaduce, and R.W. Dane, *Effect of dietary fat saturation on acylcoenzyme A:cholesterol acyltransferase activity of rat liver microsomes.* J Lipid Res, 1980. **21**(2): p. 169-79.
192. Dietschy, J.M., *Dietary Fatty Acids and the Regulation of Plasma Low Density LipoproteinCholesterol Concentrations*Journal of Nutrition, 1998. **128**: p. 444-448S.
193. *Diagnosis and classification of diabetes mellitus.* Diabetes Care, 2009. **32 Suppl 1**: p. S62-7.

194. Wheeler, M.L. and F.X. Pi-Sunyer, *Carbohydrate issues: type and amount.* J Am Diet Assoc, 2008. **108**(4 Suppl 1): p. S34-9.
195. Groff, J.L. and S.S. Gropper, *Advanced Nutrition and Human Metabolism.* Third Edition ed. 2000, Stamford, CT: Wadsworth.
196. Pagliassotti, M.J. and A.D. Cherrington, *Regulation of net hepatic glucose uptake in vivo.* Annu Rev Physiol, 1992. **54**: p. 847-60.
197. Pagliassotti, M.J.D., S.N.; Cherrington, A. D., ed. *The Role of the Liver in Maintaining Glucose Homeostasis.* 1994, RG Landes Company: Austin, TX.
198. Jenkins, D.J., et al., *Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease.* Curr Opin Lipidol, 2000. **11**(1): p. 49-56.
199. Reaven, G.M., *Banting Lecture 1988. Role of insulin resistance in human disease. 1988.* Nutrition, 1997. **13**(1): p. 65; discussion 64, 66.
200. Gross, L.S., et al., *Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment.* Am J Clin Nutr, 2004. **79**(5): p. 774-9.
201. Kaline, K., et al., *The importance and effect of dietary fiber in diabetes prevention with particular consideration of whole grain products.* Horm Metab Res, 2007. **39**(9): p. 687-93.
202. Howarth, N.C., E. Saltzman, and S.B. Roberts, *Dietary fiber and weight regulation.* Nutr Rev, 2001. **59**(5): p. 129-39.
203. diet Pereira, M.A., et al., *Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults.* Am J Clin Nutr, 2002. **75**(5): p. 848-55.
204. diet Ludwig, D.S., et al., *Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults.* Jama, 1999. **282**(16): p. 1539-46.
205. diet Salmeron, J., et al., *Dietary fiber, glycemic load, and risk of NIDDM in men.* Diabetes Care, 1997. **20**(4): p. 545-50.
206. Topping, D.L. and P.M. Clifton, *Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides.* Physiol Rev, 2001. **81**(3): p. 1031-64.
207. McKee, L.H. and T.A. Latner, *Underutilized sources of dietary fiber: a review.* Plant Foods Hum Nutr, 2000. **55**(4): p. 285-304.
208. Aleixandre, A. and M. Miguel, *Dietary fiber in the prevention and treatment of metabolic syndrome: a review.* Crit Rev Food Sci Nutr, 2008. **48**(10): p. 905-12.
209. Weickert, M.O., et al., *Cereal fiber improves whole-body insulin sensitivity in overweight and obese women.* Diabetes Care, 2006. **29**(4): p. 775-80.
210. Robertson, M.D., et al., *Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism.* Am J Clin Nutr, 2005. **82**(3): p. 559-67.
211. Pereira, M.A., et al., *Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults.* Am J Clin Nutr, 2002. **75**(5): p. 848-55.
212. Salmeron, J., et al., *Dietary fiber, glycemic load, and risk of NIDDM in men.* Diabetes Care, 1997. **20**(4): p. 545-50.

213. Salmeron, J., et al., *Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women.* Jama, 1997. **277**(6): p. 472-7.
214. Schulze, M.B., et al., *Fiber and magnesium intake and incidence of type 2 diabetes: a prospective study and meta-analysis.* Arch Intern Med, 2007. **167**(9): p. 956-65.
215. de Munter, J.S., et al., *Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review.* PLoS Med, 2007. **4**(8): p. e261.
216. Crouse, J.R., et al., *Role of acetate in the reduction of plasma free fatty acids produced by ethanol in man.* J Lipid Res, 1968. **9**(4): p. 509-12.
217. Thorburn, A., J. Muir, and J. Proietto, *Carbohydrate fermentation decreases hepatic glucose output in healthy subjects.* Metabolism, 1993. **42**(6): p. 780-5.
218. Jenkins, D.J., et al., *Dietary fibre, lente carbohydrates and the insulin-resistant diseases.* Br J Nutr, 2000. **83 Suppl 1**: p. S157-63.
219. Eastwood, M.A. and D. Hamilton, *Studies on the adsorption of bile salts to non-absorbed components of diet.* Biochim Biophys Acta, 1968. **152**(1): p. 165-73.
220. Kirby, R.W., et al., *Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations of hypercholesterolemic men.* Am J Clin Nutr, 1981. **34**(5): p. 824-9.
221. ma Batey, L.S., et al., *Summary measures of the insulin resistance syndrome are adverse among Mexican-American versus non-Hispanic white children: the Corpus Christi Child Heart Study.* Circulation, 1997. **96**(12): p. 4319-25.
222. Haffner, S.M., et al., *LDL size in African Americans, Hispanics, and non-Hispanic whites : the insulin resistance atherosclerosis study.* Arterioscler Thromb Vasc Biol, 1999. **19**(9): p. 2234-40.
223. Hertz, R.P., A.N. Unger, and C.M. Ferrario, *Diabetes, hypertension, and dyslipidemia in mexican americans and non-Hispanic whites.* Am J Prev Med, 2006. **30**(2): p. 103-10.
224. Stern, M.P., et al., *Does obesity explain excess prevalence of diabetes among Mexican Americans? Results of the San Antonio Heart Study.* Diabetologia, 1983. **24**(4): p. 272-7.
225. Haffner, S.M., et al., *Upper body and centralized adiposity in Mexican Americans and non-Hispanic whites: relationship to body mass index and other behavioral and demographic variables.* Int J Obes, 1986. **10**(6): p. 493-502.
226. ma Chiu, K.C., et al., *Insulin sensitivity differs among ethnic groups with a compensatory response in beta-cell function.* Diabetes Care, 2000. **23**(9): p. 1353-8.
227. ma Ho, R.C., et al., *Behavioral, metabolic, and molecular correlates of lower insulin sensitivity in Mexican-Americans.* Am J Physiol Endocrinol Metab, 2002. **283**(4): p. E799-808.
228. Hotamisligil, G.S., et al., *Tumor necrosis factor alpha inhibits signaling from the insulin receptor.* Proc Natl Acad Sci U S A, 1994. **91**(11): p. 4854-8.

229. Ho, R.C., et al., *Circulating tumor necrosis factor alpha is higher in non-obese, non-diabetic Mexican Americans compared to non-Hispanic white adults.* Cytokine, 2005. **30**(1): p. 14-21.
230. Lande, M.B., et al., *Elevated blood pressure, race/ethnicity, and C-reactive protein levels in children and adolescents.* Pediatrics, 2008. **122**(6): p. 1252-7.
231. Visser, M., et al., *Low-grade systemic inflammation in overweight children.* Pediatrics, 2001. **107**(1): p. E13.
232. Hanis, C.L., et al., *Origins of U.S. Hispanics. Implications for diabetes.* Diabetes Care, 1991. **14**(7): p. 618-27.
233. Mitchell, B.D., et al., *Genetic and environmental contributions to cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study.* Circulation, 1996. **94**(9): p. 2159-70.
234. Dixon, L.B., J. Sundquist, and M. Winkleby, *Differences in energy, nutrient, and food intakes in a US sample of Mexican-American women and men: findings from the Third National Health and Nutrition Examination Survey, 1988-1994.* Am J Epidemiol, 2000. **152**(6): p. 548-57.
235. Abate, N. and M. Chandalia, *The impact of ethnicity on type 2 diabetes.* J Diabetes Complications, 2003. **17**(1): p. 39-58.
236. Sundquist, J. and M. Winkleby, *Country of birth, acculturation status and abdominal obesity in a national sample of Mexican-American women and men.* Int J Epidemiol, 2000. **29**(3): p. 470-7.
237. Crespo, C.J., et al., *Acculturation and leisure-time physical inactivity in Mexican American adults: results from NHANES III, 1988-1994.* Am J Public Health, 2001. **91**(8): p. 1254-7.
238. Alexander, H., et al., *Risk factors for cardiovascular disease and diabetes in two groups of Hispanic Americans with differing dietary habits.* J Am Coll Nutr, 1999. **18**(2): p. 127-36.
239. Corpeleijn, E., et al., *Direct association of a promoter polymorphism in the CD36/FAT fatty acid transporter gene with Type 2 diabetes mellitus and insulin resistance.* Diabet Med, 2006. **23**(8): p. 907-11.
240. Barcenas, C.H., et al., *Birthplace, years of residence in the United States, and obesity among Mexican-American adults.* Obesity (Silver Spring), 2007. **15**(4): p. 1043-52.
241. Perez-Escamilla, R. and P. Putnik, *The role of acculturation in nutrition, lifestyle, and incidence of type 2 diabetes among Latinos.* J Nutr, 2007. **137**(4): p. 860-70.
242. Knowler, W.C., et al., *Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin.* N Engl J Med, 2002. **346**(6): p. 393-403.
243. *Total Prevalence of Diabetes & Pre-diabetes.* American Diabetes Association, 2008. **www.diabetes.org**.
244. CDC. *National diabetes fact sheet: general information and national estimates on diabetes in the United States, 2003.* 2004; Rev:[
245. Cruz, M.L., et al., *The metabolic syndrome in overweight Hispanic youth and the role of insulin sensitivity.* J Clin Endocrinol Metab, 2004. **89**(1): p. 108-13.

246. Olefsky, J.M., J.W. Farquhar, and G.M. Reaven, *Reappraisal of the role of insulin in hypertriglyceridemia.* Am J Med, 1974. **57**(4): p. 551-60.
247. Garvey, W.T., et al., *Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance.* Diabetes, 2003. **52**(2): p. 453-62.
248. Aguirre, M.A., et al., *Ethnic differences in insulin resistance and its consequences in older Mexican American and non-Hispanic white women.* J Gerontol A Biol Sci Med Sci, 1997. **52**(1): p. M56-60.
249. Hanley, A.J.G., et al., *Homeostasis Model Assessment of Insulin Resistance in Relation to the Incidence of Cardiovascular Disease: The San Antonio Heart Study* Diabetes Care, 2002. **25**(7): p. 1177-1184.
250. Tusie Luna, M.T., *Genes and type 2 diabetes mellitus.* Arch Med Res, 2005. **36**(3): p. 210-22.
251. Ho, R.C., et al., *Circulating tumor necrosis factor alpha is higher in non-obese, non-diabetic Mexican Americans compared to non-Hispanic white adults.* Cytokine. **30**(1): p. 14-21.
252. West, D.S., et al., *Weight loss of black, white, and Hispanic men and women in the Diabetes Prevention Program.* Obesity (Silver Spring), 2008. **16**(6): p. 1413-20.
253. Houmard, J.A., et al., *Effect of short-term exercise training on insulin-stimulated PI 3-kinase activity in human skeletal muscle.* Am J Physiol, 1999. **277**(6 Pt 1): p. E1055-60.
254. Houmard, J.A., et al., *Seven days of exercise increase GLUT-4 protein content in human skeletal muscle.* J Appl Physiol, 1995. **79**(6): p. 1936-8.
255. Kraniou, G.N., D. Cameron-Smith, and M. Hargreaves, *Effect of short-term training on GLUT-4 mRNA and protein expression in human skeletal muscle.* Exp Physiol, 2004. **89**(5): p. 559-63.
256. Kishimoto, H., et al., *Effect of short-term low-intensity exercise on insulin sensitivity, insulin secretion, and glucose and lipid metabolism in non-obese Japanese type 2 diabetic patients.* Horm Metab Res, 2002. **34**(1): p. 27-31.
257. Cononie, C.C., et al., *Seven consecutive days of exercise lowers plasma insulin responses to an oral glucose challenge in sedentary elderly.* J Am Geriatr Soc, 1994. **42**(4): p. 394-8.
258. Weir, J.B., *New methods for calculating metabolic rate with special reference to protein metabolism.* J Physiol, 1949. **109**(1-2): p. 1-9.
259. Le Floch, J.P., et al., *Blood glucose area under the curve. Methodological aspects.* Diabetes Care, 1990. **13**(2): p. 172-175.
260. Jankowski, C., et al., *Effect of exercise on postprandial insulin responses in Mexican American and non-Hispanic women.* Metabolism, 1999. **48**(8): p. 971-7.
261. Ho, R.C., et al., *Whole-body insulin sensitivity, low-density lipoprotein (LDL) particle size, and oxidized LDL in overweight, nondiabetic men.* Metabolism, 2002. **51**(11): p. 1478-83.
262. Srinivasan, S.R., M.G. Frontini, and G.S. Berenson, *Longitudinal changes in risk variables of insulin resistance syndrome from childhood to young adulthood in*

offspring of parents with type 2 diabetes: the Bogalusa Heart Study. Metabolism, 2003. **52**(4): p. 443-50; discussion 451-3.
263. Schaefer, E.J., et al., *Changes in plasma lipoprotein concentrations and composition in response to a low-fat, high-fiber diet are associated with changes in serum estrogen concentrations in premenopausal women.* Metabolism, 1995. **44**(6): p. 749-56.
264. Haskell, W.L., *The influence of exercise on the concentrations of triglyceride and cholesterol in human plasma.* Exerc Sport Sci Rev, 1984. **12**: p. 205-44.
265. Duncan, G.E., et al., *Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults.* Diabetes Care, 2003. **26**(3): p. 557-62.
266. Wojtaszewski, J.F. and E.A. Richter, *Effects of acute exercise and training on insulin action and sensitivity: focus on molecular mechanisms in muscle.* Essays Biochem, 2006. **42**: p. 31-46.
267. Houmard, J.A., et al., *Effect of short-term exercise training on leptin and insulin action.* Metabolism, 2000. **49**(7): p. 858-61.
268. Bergstrom, J., *Percutaneous needle biopsy of skeletal muscle in physiological and clinical research.* Scand J Clin Lab Invest, 1975. **35**(7): p. 609-16.
269. Saggerson, E.D. and C.A. Carpenter, *Carnitine palmitoyltransferase and carnitine octanoyltransferase activities in liver, kidney cortex, adipocyte, lactating mammary gland, skeletal muscle and heart.* FEBS Lett, 1981. **129**(2): p. 229-32.
270. McGarry, J.D., et al., *Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat.* Biochem J, 1983. **214**(1): p. 21-8.
271. Bruce, C.R., et al., *Overexpression of carnitine palmitoyltransferase-1 in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high-fat diet-induced insulin resistance.* Diabetes, 2009. **58**(3): p. 550-8.
272. Tunstall, R.J., et al., *Exercise training increases lipid metabolism gene expression in human skeletal muscle.* Am J Physiol Endocrinol Metab, 2002. **283**(1): p. E66-72.
273. Rose, A.J., et al., *Effect of endurance exercise training on Ca2+ calmodulin-dependent protein kinase II expression and signalling in skeletal muscle of humans.* J Physiol, 2007. **583**(Pt 2): p. 785-95.
274. Thong, F.S., et al., *Prior exercise increases basal and insulin-induced p38 mitogen-activated protein kinase phosphorylation in human skeletal muscle.* J Appl Physiol, 2003. **94**(6): p. 2337-41.

APPENDIX A:

INFORMED CONSENT

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Interaction of Diet and Exercise on Chronic Disease Risk

NAME OF PRINCIPAL INVESTIGATOR: Chris Melby, Dr.P.H.

NAME OF CO-INVESTIGATORS: Matt Hickey, Ph.D., Stacy Schmidt, MS

CONTACT NAME AND PHONE NUMBER FOR QUESTIONS/PROBLEMS: Chris Melby, 491-6736, Matthew Hickey, 491-5727

SPONSOR OF THE PROJECT: Colorado Agricultural Experiment Station

PURPOSE OF THE RESEARCH: The purpose of the present study is to determine if your ethnic background influences how participating in a one-week diet and exercise program affects your risk for diabetes and other chronic diseases.

PROCEDURES/METHODS TO BE USED: After completing initial screening tests, eligible participants will be asked to spend 7 days during a baseline period consuming your usual diet and **not** participating in any formal exercise. You will then be asked to complete a 7-day period in which you exercise for 6 of the 7 days. During this second week, you will also eat food that we provide for you. If you are eligible to be in the study, your participation will require a time commitment of about 20 hours during a 19 day-period. There are a number of "exclusion criteria" (things that will make you ineligible for the study such as having diagnosed diabetes, certain types of medication, etc). Should you meet any of these exclusion criteria during the screening period, we will fully inform you as to the reason you can't be in the study.

Depending on your eligibility for the study, your participation will involve a 19-day period:

DAYS 1-3: INITIAL SCREENING - During one of the initial days of the study, you will complete the following screening tests to help us determine if you are eligible to go the next phases. These tests will require about 90 minutes of your time.

Health and Medical History Questionnaire- You will need to answer questions about your medical history and personal health habits. Time required: 15 minutes

Ethnicity Questionnaire- You will need to identify your ethnic background. Time: 5 minutes

Food Preferences Questionnaire- You will need to answer questions about foods you like and don't like. You will also be asked to list any food allergies you think you might have. Time: 10 minutes

Eating Disorders Questionnaire- You will be asked to complete a form that screens for eating disorders. Time: 5 minutes

Exercise Questionnaire- You will need to answer questions about your exercise habits. Time: 10 minutes

Pregnancy test- All women in the study will be asked to take a pregnancy test. If the pregnancy test is positive, you cannot be in this study. It is important that you do not become pregnant during the study. This will stop you from continuing the study. Time 10 minutes

Body measurements- Your height will be measured without you wearing shoes. Body weight will be measured on a normal scale. This will include the weight of light indoor clothing minus shoes. Your waist and hip circumference will be measured using a measuring tape. Time: 10 minutes.

Blood pressure and blood glucose tests- Following a 12-h fast (no food or beverages except water for 12 hours) you will have your blood pressure taken using normal procedures while you sit quietly in a comfortable chair. You will then have a small amount of blood taken from your fingertip (one drop). From this we will measure your blood sugar levels. If your blood pressure is greater than 140/90 or your blood sugar level higher than 126 mg/dl, you will not be able to participate in the study and you will be told to see your doctor to check for high blood pressure or diabetes. Time: 10 minutes.

DAYS 4-11: BASELINE CONTROL PERIOD- If your screening tests tell us you are eligible for the study, you will then begin a series of additional tests. During this time you will consume your typical diet. We ask that you do not participate in any exercise program during these 8 days. These tests will require a total of about 7 hours of your time.

Body composition (fat and lean tissue) - This will be performed using a machine called a dual energy X-ray absorptiometer (DEXA). This unit uses 2 low energy X-rays to determine the amount of body fat you have. You will be exposed to some radiation. But, the amount of radiation exposure in this procedure is very low, about 1/1,000 of the normal radiation exposure you receive yearly from what is called "background" radiation from the environment. Put another way, the exposure from a DEXA scan is less than the normal exposure in a flight from Denver to Chicago, and about $1/40^{th}$ the exposure from a normal stomach X-ray you might receive at a hospital. This test will be performed in room 124 in the Human Performance Clinical Research Laboratory (HPCRL) located near Moby Gym. You will be asked to lie quietly on a bed in shorts and a T-shirt for about 15 minutes while the scan is performed. Time: 30 minutes.

Resting Metabolic Rate - This test involves reporting to the HPCRL or to room 216 Gifford between 7:00 and 9:30 am after a 12-hour overnight fast. You will be asked to lay on a bed for 30 minutes with a plastic canopy over your head or fitted with a mouthpiece to breathe into. Tubes connected to the canopy or mouthpiece measure how much air you breathe in and out. This measures how many calories you are burning while at rest. Time: 30 minutes

Physical Fitness: This test involves walking and/or jogging on a motorized treadmill or riding on a stationary bicycle. It will be conducted in either Gifford 216 or the HPCRL The grade (steepness) and speed of the treadmill (or pedal tension, if on a bike) will gradually increase until you are no longer able to continue. You will be asked to breathe through a mouthpiece during this test so we can measure the amount of oxygen your body consumes. In addition, we will measure your heart rate (using a heart rate monitor, which is like a small elastic belt you wear around your chest) and your blood pressure (using a small cuff that fits around your upper arm). Time: 40 minutes.

Food Intake Record: You will be asked to record your food intake on 3 consecutive days during this period. Time: 15 minutes.

Step counter: You will be asked to wear a simple step counter and record your step counts for days 4-18.

Fasting Blood Sample and Insulin Sensitivity Test: On day 11 of the study, you will be asked to report to the Hartshorn Health Center following a 12 hour fast for a blood sample. This means you will come to the lab in the morning. You will not have eaten any food or drank any beverages except water during the previous 12 hours. You will lie on a bed A hollow needle/plastic tube will be put in your forearm (or back of your hand if your veins are better there). First, about 2 teaspoons of blood will be taken from the tube in your arm. We will later determine how much glucose, fats (like cholesterol), hormones, and specific proteins are in your blood.

After this blood sample has been taken, you will begin the insulin sensitivity test. This test is to estimate the ability of insulin to cause sugar to move from your blood into your cells. Sugar water will be put into your blood though the tube (catheter). Blood will be collected from the catheter 15 times over a three hour period. After blood is taken from the catheter in your arm each time, a small amount of sterile salt-water will be used to flush the catheter to keep your blood from clotting inside the tubing.

We will later analyze your blood's insulin and sugar levels. The amount of blood taken at each sample is about 1/2 teaspoon. Altogether, we will take about 10 teaspoons of your blood. You will probably not feel any pain when the blood is collected from the catheter in your arm. If the way your body responds to insulin is not normal or if your blood sugar levels are not in the normal range, you will be able to see a physician if you want. Time: 3.5 hours. **This procedure will be performed 2 times: once during the baseline control period at one following the diet/exercise period.**

Muscle Biopsy: During the insulin sensitivity test, you will have one muscle biopsy before you receive the sugar water. The small tissue sample (biopsy) will be obtained from the vastus lateralis, which is a large muscle in your thigh. We will determine your muscle's level of some molecules that allow insulin to work. The procedure involves numbing the skin using lidocaine, an anesthetic similar to novacaine, which you may have received at the dentist If you are allergic to novacaine or have had any reaction to novacaine from your dentist, you should notify Professor Melby or Professor Hickey immediately and should not participate in this study. After numbing the skin, a small incision (less than the width of a pencil) is made in the skin over the muscle. The biopsy is obtained using a sterile needle. The muscle sample obtained is generally ~ ½ the size of an eraser on the end of a pencil. It is not uncommon to experience some mild soreness in the muscle that lasts for about a day. You should NOT restrict your activity, although you should also not perform any unusual or extremely vigorous activity for a few days. You will be provided with written instructions regarding proper care of the incision, and a telephone contact should you have any questions. **This procedure will be performed 2 times: once during the baseline control period at the beginning of the study and once at the end following your 7-day diet and exercise period. This means you will be asked to have a total of TWO muscle biopsies.**

DAYS 12-18: A SEVEN-DAY PERIOD OF DIET AND EXERCISE:
After the screening and baseline tests are done during the first two weeks, you will participate in a diet and exercise program for seven days. Your diet will consist of eating foods (meals, beverages, and snacks) that we give you. The food will be healthy (lower in saturated fat and sugar than the diets of most Americans). You are expected to eat enough so you do not lose weight. You also should not eat so much that you gain weight. You will be expected to participate to exercise 6 of the 7 days during this period. Specifically, you be asked to do the following for the entire 7 days: 1). eat all your meals and snacks from the food we prepare and provide you during the week (breakfast, lunch, dinner, snacks); 2.) come to the Gifford Building at least 3 times during the week to pick up food and be weighed at each visit;. 3). meet with a research dietitian twice when you come to the Gifford Building to determine your satisfaction with the food provided. If you don't like certain foods we give you, we will find other food items to give you that you do like. 4). exercise on a stationery bike or treadmill 6 times during the 7-day period for 35-40 minutes per session. Trained personnel will monitor all exercise sessions. Your exercise program will be in the South College Gym, Moby Gym, or the Gifford Building on the CSU campus. Time: ~ 8 hours total for the week

Day 19: SECOND INSULIN SENSITIVITY TEST AND SET OF BIOPSIES: After the one week of special diet and exercise you will have a second insulin sensitivity test and the last one of two muscle biopsies. Time: 3.5 hours

STUDY TIMELINE:

	Days 1-3	Days 4-11	Days 12-18	Day 19
Screening	X			
BP/ Glucose	X			
Weight	X			
Diet Analysis	X	X		
Fitness test		X		
DEXA		X		
Exercise			X	
Special diet			X	
Muscle Biopsy		X-Day 11		X
Insulin sensitivity		X-Day 11		X
Blood		X-Day 11		X
Time (hours)	1.5	7	8	3.5

TOTAL TIME COMMITMENT: approximately 20 hours

RETENTION OF BLOOD AND MUSCLE SAMPLES

You should understand that we plan to keep any extra muscle and blood samples that are not used in the analysis for this study. In other words, if we have any "extra" blood or muscle we will keep them in a freezer in our lab. It is very possible that we will use all of the blood and muscle obtained in this study and will have none left, but in the event that we do, we would like your permission to keep the samples in the event that they can be used for further research. We will use these samples in the future solely for additional research on obesity and metabolism; specifically, all future research will simply be an extension of what we hope to accomplish with the current study. We may simply analyze your blood for the presence of other hormones or metabolites, or analyze your muscle for other enzymes, etc. We have NO plans to store DNA in this study. Your stored samples will be coded in such a way that your confidentiality will be maintained. Only the Principal Investigators (Professors Melby and Hickey) will have access to the coding system for your samples. There is a possibility that your samples may be shipped to other departments on the CSU campus, or to colleagues at other Universities for assistance with analysis. Under such circumstances, the same coding system will be used, so researchers in other labs will not be able to identify you. We do not anticipate ANY commercial product development from your tissue, the samples will be used solely for research purposes. You should be advised that we do NOT have plans to recontact you in the future regarding any additional analyses, but will seek full approval of the CSU Regulatory Compliance Office prior to initiating any further research on your samples.

By checking "Yes" below and signing on the accompanying line, you are agreeing to allow the investigators retain any muscle and blood samples obtained during this study. If you do not wish the investigators to retain any samples, please check the box marked "No" and also sign on the accompanying line.

The investigators may keep any muscle or blood samples obtained during the course of this study for future research on obesity and metabolism YES ☐ NO ☐

_____

Signature Date

EXERCISE PROGRAM CONTINUATION (PHASE II)

After you have completed the 19-day study detailed above, you will be asked if you would like to continue with the exercise program for an additional 7 weeks. The exercise will consist of 35-40 minutes per session 4 sessions a week on a stationery bike or treadmill. Trained personnel will monitor all exercise sessions. Your exercise program will be in the South College Gym, Moby Gym, or the Gifford Building on the CSU campus. Should you choose to participate, you will *not* be provided with food but you are expected to eat enough so you do not lose weight. To monitor weight stability, you will be weighed the first exercise session of each week. If you choose to participate in the additional 7 weeks of exercise, you will *not* receive additional compensation.

Fasting Blood Sample: On day 68 of phase II (i.e., after completing the 7 additional weeks of exercise training), you will be asked to report to the Hartshorn Health Center following a 12 hour fast for a blood sample. This means you will come to the lab in the morning. You will not have eaten any food or drank any beverages except water during the previous 12 hours. You will lie on a bed. A hollow needle will be put in your forearm (or back of your hand if your veins are better there). Approximately 2 teaspoons of blood will be taken from the tube in your arm. We will later determine how much glucose, fats (like cholesterol), hormones, and specific proteins are in your blood.

Time:~3 hours per week, total time ~21 hours

I would like to participate in an additional 7 weeks of supervised exercise following day 19 of the study.

_____ YES ☐ NO ☐

Signature Date

PHASE II STUDY TIMELINE:

	Days 1-3	Days 4-11	Days 12-18	Day 19	Day 20-68
Screening	X				
BP/ Glucose	X				
Weight	X				X
Diet Analysis	X	X			
Fitness test		X			
DEXA		X			
Exercise			X		X
Special diet			X		
Muscle Biopsy		X-Day 11		X	
Insulin sensitivity		X-Day 11		X	
Blood		X-Day 11		X	X
Time (hours)	1.5	7	8	3.5	21

RISKS INHERENT IN THE PROCEDURES:

1). DEXA: The risks associated with the DEXA are very low. The radiation you will receive in this study is less than 1/3000th of the FDA limit for annual exposure. Put another way, you could receive 3000 DEXA scans in a single year and still not meet the FDA limit for radiation exposure. In this study, you will receive only a single scan. The more radiation you receive over the course of your life, the more is the risk of having cancerous tumors or causing changes in genes. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risks is not known. Women who are pregnant or could be pregnant should receive no unnecessary radiation and should not participate in this study.

2). Blood Samples: The risks associated with blood drawing include bruising, vein inflammation, slight risk of infection, local soreness, and fainting. These are all very minor risks and if present, are generally resolved in less than a day.

3). Resting Metabolic Rate measurement: There is no known risk associated with this procedure. You may experience some minor discomfort associated with this measurement if you have claustrophobia, but this is very unlikely. The canopy used is a large, see-through plastic bubble. There is adequate space and breathing is unrestricted, whether you are in the canopy or you use a mouthpiece.

4). Cardiorespiratory Fitness and Exercise: The exercise test is a standard test for determining the presence of heart and lung problems. 1 in 10,000 individuals with cardiovascular disease may die and 4 in 10,000 may have abnormal heart beats or chest pain. The exercise program will be less intense than your exercise test, so the risks are less. However, as with any exercise, there is the possibility of muscle soreness and muscle, bone, or joint injury.

5). Insulin Sensitivity Test: The risks of obtaining your blood from a tube put in your vein are similar to those that could occur from a blood draw. Any time a blood collection catheter is inserted, there is a small risk that you may faint, experience local soreness, bruising or infection. There is also a small risk of experiencing low blood sugar during the test with symptoms of headache, tremor, and dizziness.

6). Muscle Biopsy: The risks associated with the muscle biopsy include discomfort, localized soreness, bruising, infection, and minor scarring. The discomfort and localized soreness are likely, but generally last only 24-48h. Temporary scarring is also expected, the natural course of wound healing varies substantially from individual to individual, but the scar will be less than ½ inch long, and is generally difficult to distinguish within 8-12 months after the biopsy. The risk of bruising is low, and infections are extremely rare.

7). Research Diets: There is a small risk that you could get a food-borne illness from the research diets. The food will be prepared under the supervision of a trained Chef. Meal preparation will occur in nutrition laboratories in the Department of Nutrition at CSU or in kitchens which supply meals to campus residence halls. All food preparation will be done in accordance with standard procedures designed to minimize the risk of illness. Thus, the likelihood of a food-borne illness is remote.

It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

BENEFITS:

You will receive detailed diet and body composition data, and information on the role of diet and exercise in maintenance of health. You will receive 7 days of food at no cost to you. If you choose to participate in the additional 7 weeks of exercise, you have the potential to benefit from increased physical fitness associated with regular exercise.

COMPENSATION

You will be paid $100 upon completion of this study, and an additional $25 for each of the 2 possible biopsies, for a total of $150 dollars possible. While the biopsies are an important part of the study, the information we obtain from other measures (i.e. insulin sensitivity) is important to us as well. You may still

participate even if you choose not to have the biopsies, but your payment will be less. If you choose at any point not to continue the study, you will be paid $75 upon completion of ALL tests between days 1-11. If you choose to participate in the additional 7 weeks of exercise, you will not receive additional compensation.

REASONS WHY YOU MAY BE REMOVED FROM THE STUDY

As mentioned, we are aware that this study requires a significant time commitment from you as a volunteer. It is very important to the study that you not miss scheduled visits with study personnel. In the event that
something comes up that will make you miss a visit, please call and let us know. Please also note that we may call you if a visit is missed, simply to check and make sure everything is OK. If you have conflicts that
require you to miss more than 10% of your scheduled visits, we will have to remove you from the study. If this happens, we will contact you and let you know the reason why you will not be allowed to continue, and make arrangements to pay you for the portion(s) of the study you have completed. Should our testing reveal information that suggests you need to be referred for medical care, we will put you in contact with Dr. Russell Risma, M.D., at the Hartshorn Health Service at Colorado State University, who is the physician contact for this study.

CONFIDENTIALITY:

Your data will be coded and kept in a locked file cabinet on the CSU campus. A copy of the coded data may be sent to the sponsor of this project. A summary of the findings will be published in a science journal. However, you will not be identified in relation to your data at any point.

LIABILITY:

Because Colorado State University is a publicly-funded, state institution, it may have only limited legal responsibility for injuries as a result of participation in this study under a Colorado law known as the Colorado Government Immunity Act (Colorado Revised Statutes, section 24-10-101, et seq.). In addition, under Colorado law, you must file any claims against the University within 180 days after the date of the injury. In light of these laws, you are encouraged to evaluate your own health and disability insurance to determine whether you are covered for any injuries you might sustain by participating in this research, since it may be necessary for you to rely on individual coverage for any such injuries. If you sustain injuries which you believe were caused by Colorado State University or its employees, we advise you to consult an attorney. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, IRB Administrator, (970) 491-1655 or janell.barker@research.colostate.edu

PARTICIPATION:

Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing __7__ pages.

_____ _____

Participant Name (print) Date Participant Signature

_____ _____

Investigator or co-investigator Signature Date

APPENDIX B:

HEALTH HISTORY QUESTIONNAIRE

Colorado State University
CONFIDENTIAL HEALTH HISTORY QUESTIONNAIRE

Study_____ Date_____ Subject ID_____

Reviewed by PI: _____

PLEASE PRINT Current Age_____ Height_____ Weight_____

GENERAL MEDICAL HISTORY

Do you have any current medical conditions? explain: YES ☐ NO ☐ If Yes, please

Have you had any major illnesses in the past? explain: YES ☐ NO ☐ If Yes, please

Have you ever been hospitalized or had surgery? explain: (include date and type of surgery, if possible) YES ☐ NO ☐ If Yes, please

Have you ever had an electrocardiogram (EKG)? explain: (a test that measures your heart's activity using an electrical tracing) YES ☐ NO ☐ If Yes, please

Are you currently taking any medications, including aspirin, hormone replacement therapy, or other over-the-counter medications? YES ☐ NO ☐ If yes, please explain:

Medication	Reason	Times taken per Day	Taken for how long?

PI Initials_____

Are you currently taking any nutritional supplements, such as Ginko, St. John's Wort, or others?

YES ☐ NO ☐ If Yes, please explain:

Supplement Reason Times taken per Day Taken for how long?

Have you been diagnosed with diabetes? YES ☐ NO ☐ If yes, please explain:

Age at diagnosis_____

Have you been diagnosed with a thyroid disorder? YES ☐ NO ☐ If yes, please explain:

FAMILY HISTORY

Please indicate the current status of your immediate family members.

	Age (if alive)	Age of Death	Cause of Death
Father	_____	_____	_____
Mother	_____	_____	_____
Brothers/Sisters	_____	_____	_____
	_____	_____	_____
	_____	_____	_____

Do you have a family history of any of the following: (Blood relatives only, please give age at diagnosis if possible)

	YES Diagnosis	NO	Relation	Age at
a. High Blood Pressure	☐	☐		
b. Heart Attack	☐	☐		
c. Coronary bypass surgery	☐	☐		
d. Angioplasty	☐	☐		
e. Stroke	☐	☐		
f. Diabetes	☐	☐		
g. Obesity	☐	☐		
h. Other (Please List)	☐	☐		

PI Initials_____

MUSCULOSKELETAL HISTORY

	YES	NO
Any current muscle injury or illness?	☐	☐
Any muscle injuries in the past?	☐	☐
Muscle pain at rest?	☐	☐
Muscle pain on exertion?	☐	☐
Any current bone of joint (including spinal) injuries?	☐	☐
Any previous bone or joint (including spinal) injuries?	☐	☐
Painful joints?	☐	☐
Swollen joints?	☐	☐
Edema (fluid build up)?	☐	☐
Pain in your legs when you walk?	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate

NEUROLOGICAL HISTORY	YES	NO
History of seizures	☐	☐
Diagnosis of epilepsy	☐	☐
History of fainting	☐	☐

GASTROINTESTINAL HISTORY	YES	NO
History of ulcers	☐	☐
History of colitis	☐	☐
History of chronic diarrhea	☐	☐
History of chronic constipation	☐	☐

REPRODUCTIVE HISTORY

	YES	NO
Currently pregnant		
Think you might be pregnant	☐	☐
Planning on becoming pregnant in the near future	☐	☐
Currently using Oral Contraceptives	☐	☐
History of Menstrual cycle irregularities	☐	☐
Hysterectomy	☐	☐
	☐	☐

PI Initials_____

TOBACCO HISTORY (check any that apply)

None	☐	
Quit	☐	(when)_____
Cigarette	☐	
Cigarette	☐	
Pipe	☐	
Chew Tobacco	☐	
Snuff	☐	

CURRENT TOBACCO USE (if applicable)

	# per day
Cigarette	_____
Cigar	_____
Pipe	_____
Chew Tobacco	_____
Snuff	_____

Total Years of tobacco use_____

CARDIORESPIRATORY HISTORY

	YES	NO
Presently diagnosed with heart disease	☐	☐
History of heart disease	☐	☐
Heart murmur	☐	☐
Occasional chest pain or pressure	☐	☐
Chest pain or pressure on exertion	☐	☐
Heart valve problem	☐	☐
Abnormal heart rhythm	☐	☐
Edema (fluid build up)	☐	☐
High Cholesterol	☐	☐
History of rheumatic fever	☐	☐
Episodes of fainting	☐	☐
Daily coughing	☐	☐
High blood pressure	☐	☐
Shortness of breath	☐	☐
At rest	☐	☐
Lying down	☐	☐
After 2 flights of stairs	☐	☐
Asthma	☐	☐
Emphysema	☐	☐
Bronchitis	☐	☐
History of bleeding disorders	☐	☐
History of problems with blood clotting	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response to an investigator so we can be sure to safely determine your ability to participate.

PI Initials_____

DIET HISTORY

	YES	NO
Have you ever dieted?	☐	☐

If YES, have you dieted within the past 12 months or are you currently on a diet?

	YES	NO
	☐	☐

If you have dieted within the past 12 months, please describe the diet:

a). Name (if applicable): _____

b). Prescribed by a Physician/nutritionist	YES ☐	NO ☐
c). Have you lost weight?	YES ☐	NO ☐

d). Duration of the diet?_____

What was your weight 12 months ago?_____

What was your weight 6 months ago?_____

	YES	NO
Have you dieted other than in the past 12 months?	☐	☐

If YES, please answer the following:

a). How many times have you dieted?

b). How old were you?

C). Weight loss (amount)?

	YES	NO
History of eating disorders?	☐	☐

EXERCISE HISTORY

How many times a week do you participate in moderate to high intensity exercise? (examples include jogging, biking, aerobics, basketball, swimming, etc.)_____

How long do these exercise sessions last? _____

You may be asked to complete a more detailed diet survey if you are volunteering for a research study.

APPENDIX C:

ETHNICITY QUESTIONNAIRE

PARTICIPANT ETHNICITY IDENTIFICATION FORM

Nutrition and metabolic Fitness Laboratory, Colorado State University

ID #_____ **Date:** _____

1. Please identify your ethnicity: _____

A. Mexican American
B. Mexican/Mexicano
C. Puerto Rican
D. Cuban
E. Other Latin American
F. Native American
G. Other Spanish
H. Caucasian
I. Black
J. Asian/Pacific Islander

2. What are your parents' surnames?

Father: _____
Mother: _____

3. What are your parents' countries of origin?

Father: _____
Mother: _____

4. Please identify the ethnicity of your 4 grandparents:
(Use the letters from Question #1)

Father's father: _____
Father's mother: _____
Mother's father: _____
Mother's mother: _____

5. What is your primary (first) language spoken?

APPENDIX D

FOOD PREFERENCES QUESTIONNAIRE

Food Preferences and Acceptability Questionnaire

Please circle the number that corresponds to your response.

Have not tried item (check box)	Food Item	Like Extremely	Like Very Much	Like Moderately	Like Slightly	Neither Like Nor Dislike	Dislike Slightly	Dislike Moderately	Dislike Very Much	Dislike Extremely
☐	**1% Milk**	1	2	3	4	5	6	7	8	9
☐	**Almonds**	1	2	3	4	5	6	7	8	9
☐	**Apple**	1	2	3	4	5	6	7	8	9
☐	**Avocado**	1	2	3	4	5	6	7	8	9
☐	**Baked Tortilla Chips**	1	2	3	4	5	6	7	8	9
☐	**Banana**	1	2	3	4	5	6	7	8	9
☐	**Beef tenderloin**	1	2	3	4	5	6	7	8	9
☐	**Bell Peppers**	1	2	3	4	5	6	7	8	9
☐	**Black beans**	1	2	3	4	5	6	7	8	9
☐	**Blueberries**	1	2	3	4	5	6	7	8	9
☐	**Blueberry yogurt**	1	2	3	4	5	6	7	8	9
☐	**Broccoli**	1	2	3	4	5	6	7	8	9
☐	**Brown Rice**	1	2	3	4	5	6	7	8	9
☐	**Cantaloupe**	1	2	3	4	5	6	7	8	9
☐	**Carrots**	1	2	3	4	5	6	7	8	9
☐	**Cheddar Cheese**	1	2	3	4	5	6	7	8	9
☐	**Cheerios Cereal**	1	2	3	4	5	6	7	8	9
☐	**Cherry Yogurt**	1	2	3	4	5	6	7	8	9
☐	**Chicken Breast**	1	2	3	4	5	6	7	8	9
☐	**Corn**	1	2	3	4	5	6	7	8	9
☐	**Corn Flakes Cereal**	1	2	3	4	5	6	7	8	9
☐	**Cottage Cheese**	1	2	3	4	5	6	7	8	9
☐	**Cream Cheese lowfat**	1	2	3	4	5	6	7	8	9
☐	**Cucumber**	1	2	3	4	5	6	7	8	9
☐	**Dried Apricots**	1	2	3	4	5	6	7	8	9
☐	**Fish- Tilapia**	1	2	3	4	5	6	7	8	9
☐	**Graham crackers**	1	2	3	4	5	6	7	8	9
☐	**Granola**	1	2	3	4	5	6	7	8	9
☐	**Granola Bar**	1	2	3	4	5	6	7	8	9

☐	**Grape Jelly**	1	2	3	4	5	6	7	8	9
☐	**Grapes**	1	2	3	4	5	6	7	8	9
☐	**Green beans**	1	2	3	4	5	6	7	8	9
☐	**Green Peas**	1	2	3	4	5	6	7	8	9
☐	**Honey Mustard dressing**	1	2	3	4	5	6	7	8	9
☐	**Italian Dressing**	1	2	3	4	5	6	7	8	9
☐	**Ketchup**	1	2	3	4	5	6	7	8	9
☐	**Lettuce**	1	2	3	4	5	6	7	8	9
☐	**Mayonnaise**	1	2	3	4	5	6	7	8	9
☐	**Mexican rice**	1	2	3	4	5	6	7	8	9
☐	**Mushrooms**	1	2	3	4	5	6	7	8	9
☐	**Mustard**	1	2	3	4	5	6	7	8	9
☐	**Nectarines**	1	2	3	4	5	6	7	8	9
☐	**Nilla wafers**	1	2	3	4	5	6	7	8	9
☐	**Oatmeal**	1	2	3	4	5	6	7	8	9
☐	**Oil/vinegar dressing**	1	2	3	4	5	6	7	8	9
☐	**Onion- sauteed**	1	2	3	4	5	6	7	8	9
☐	**Orange**	1	2	3	4	5	6	7	8	9
☐	**Orange juice**	1	2	3	4	5	6	7	8	9
☐	**Peanut butter**	1	2	3	4	5	6	7	8	9
☐	**Peanuts**	1	2	3	4	5	6	7	8	9
☐	**Pickle relish**	1	2	3	4	5	6	7	8	9
☐	**Pinto Beans**	1	2	3	4	5	6	7	8	9
☐	**Potato- baked**	1	2	3	4	5	6	7	8	9
☐	**Pretzels**	1	2	3	4	5	6	7	8	9
☐	**Ranch dressing**	1	2	3	4	5	6	7	8	9
☐	**Reduced fat cheese**	1	2	3	4	5	6	7	8	9
☐	**Salmon**	1	2	3	4	5	6	7	8	9
☐	**Salsa**	1	2	3	4	5	6	7	8	9
☐	**Shrimp, grilled**	1	2	3	4	5	6	7	8	9
☐	**Skim Milk**	1	2	3	4	5	6	7	8	9
☐	**Spaghetti Sauce**	1	2	3	4	5	6	7	8	9
☐	**Spinach**	1	2	3	4	5	6	7	8	9
☐	**Squash**	1	2	3	4	5	6	7	8	9
☐	**Strawberries**	1	2	3	4	5	6	7	8	9
☐	**Strawberry yogurt**	1	2	3	4	5	6	7	8	9
☐	**Sweet Potato**	1	2	3	4	5	6	7	8	9

☐	**Swiss cheese**	1	2	3	4	5	6	7	8	9
☐	**Tomato**	1	2	3	4	5	6	7	8	9
☐	**Total cereal**	1	2	3	4	5	6	7	8	9
☐	**Tuna-canned in water**	1	2	3	4	5	6	7	8	9
☐	**Turkey Breast**	1	2	3	4	5	6	7	8	9
☐	**Wheat Crackers**	1	2	3	4	5	6	7	8	9
☐	**Wheat Thins**	1	2	3	4	5	6	7	8	9
☐	**Whole wheat bagel**	1	2	3	4	5	6	7	8	9
☐	**Whole wheat bread**	1	2	3	4	5	6	7	8	9
☐	**Whole wheat pasta**	1	2	3	4	5	6	7	8	9
☐	**Whole wheat tortilla**	1	2	3	4	5	6	7	8	9

Please list any food allergies you have:

APPENDIX E

EATING ATTITUDES TEST (EATS-26)

EATS-26

Please place and (X) under the column which applies best to each of the numbered statements. All of the results will be strictly confidential. Most of the questions directly relate to food or eating, although other types of questions have been included. Please answer each question carefully. Thank you.

Always	Very Often	Often	Sometimes	Rarely	Never	
( )	( )	( )	( )	( )	( )	1. Engage in dieting behavior.
( )	( )	( )	( )	( )	( )	2. Have the impulse to vomit after meals.
( )	( )	( )	( )	( )	( )	3. Cut my food into small pieces.
( )	( )	( )	( )	( )	( )	4. Eat diet foods.
( )	( )	( )	( )	( )	( )	5. Feel uncomfortable after eating sweets.
( )	( )	( )	( )	( )	( )	6. Vomit after I have eaten.
( )	( )	( )	( )	( )	( )	7. Take longer than others to eat meals.
( )	( )	( )	( )	( )	( )	8. Enjoy trying new rich foods.
( )	( )	( )	( )	( )	( )	9. Have gone on eating binges and feel that I may not be able to stop.
( )	( )	( )	( )	( )	( )	10. Other people think I am too thin.
( )	( )	( )	( )	( )	( )	11. Avoid foods with sugar in them.
( )	( )	( )	( )	( )	( )	12. Particularly avoid foods with high carbohydrate content.
( )	( )	( )	( )	( )	( )	13. Give too much time and thought to food.
( )	( )	( )	( )	( )	( )	14. Feel that others would prefer if I ate more.
( )	( )	( )	( )	( )	( )	15. Am preoccupied with a desire to be thinner.
( )	( )	( )	( )	( )	( )	16. Like my stomach to be empty.
( )	( )	( )	( )	( )	( )	17. Am preoccupied with the thought of having fat on my body.
( )	( )	( )	( )	( )	( )	18. Find myself preoccupied with food.
( )	( )	( )	( )	( )	( )	19. Feel that others pressure me to eat.
( )	( )	( )	( )	( )	( )	20. Am terrified about being overweight.
( )	( )	( )	( )	( )	( )	21. Avoid eating when I am hungry.
( )	( )	( )	( )	( )	( )	22. Think about burning up calories when I exercise.
( )	( )	( )	( )	( )	( )	23. Feel extremely guilty after eating.
( )	( )	( )	( )	( )	( )	24. Feel that food controls my life.
( )	( )	( )	( )	( )	( )	25. Display self-control around food.
( )	( )	( )	( )	( )	( )	26. Aware of the calorie content of foods.

APPENDIX F

RECRUITMENT FLYER

Research Study Participants Needed

The Departments of Food Science and Human Nutrition and Health and Exercise Science are conducting a research study on the effect of a one-week program of increased physical activity and eating a lower saturated fat, lower sugar diet on risk factors for diabetes and heart disease.

Study participants should be 18-40 years of age, with no history of chronic health problems, and currently exercise no more than one time per week.

Requirements/Compensation:

- Eligible study participants will be expected to participate in a 7-day control period in which you consume your usual diet and refrain from exercise. This control period is then followed immediately by a 7-day period in which you will be expected to eat the food provided you by the study investigators, which will be low in saturated fat and refined sugar. During this second 7-day period, you will also participate in a 40-45 minute exercise program for 6 of the 7 days.

- At the start of the study you will undergo a number of tests including assessment of your dietary intake, physical activity and fitness levels, health and medical history, and body composition. After the control period you will undergo testing to measure insulin sensitivity and a muscle biopsy to measure molecules involved in insulin's action. The insulin sensitivity test and muscle biopsy will be repeated again after the 7-day diet/exercise intervention.

- Upon completion of this study, volunteers will be compensated $150 for their time. For details and additional information, please contact:

Research Assistant Stacy Schmidt, 491-5696, stacy5@cahs.colostate.edu
Professor Chris Melby, 491-6736, melby@cahs.colostate.edu.
Professor Matt Hickey, 491-5727, hickey@cahs.colostate.edu