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DISSERTATION

Behavioral, Metabolic and Molecular Correlates of Insulin Sensitivity in Humans

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY RICHARD C. HO ENTITLED BEHAVIORAL, METABOLIC AND MOLECULAR CORRELATES OF INSULIN SENSITIVITY IN HUMANS BE ACCEPTED AS FULFILLING IN PART THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

Behavioral, Metabolic and Molecular Correlates of Insulin Sensitivity in Humans

Whole body insulin resistance appears to precede many of the metabolic abnormalities that are involved in the progression toward type 2 diabetes mellitus (T2DM), obesity, dyslipidemia, hypertension, cardiovascular disease and some cancers. The overall objective of this project was to characterize modifiable correlates and sequelae that are associated with insulin sensitivity in humans.

In **Study 1**, we determined whether differences in insulin sensitivity persist between nonobese, nondiabetic Mexican American (MA) (n=13; 27.0 ± 2.0 yrs; BMI=23.0 ± 0.7) and Non-Hispanic White (NHW) (n=13; 24.8 ± 1.5 yrs; BMI=22.8 ± 0.6) males and females after accounting for effects of exercise, adiposity, dietary intake and skeletal muscle insulin signaling protein abundance. Significant differences in insulin sensitivity, estimated by the homeostatic model assessment of insulin resistance, between MA and NHW persisted (1.53 ± 0.22 vs. 0.87 ± 0.16, $p<0.05$, respectively) after accounting for effects of acute and chronic exercise, and adiposity. Protein levels of IRβ, PI3K p85, Akt1, Akt2 and GLUT4 were not different between the two groups. Differences in HOMA-IR scores lost significance after accounting for percent intake of palmitic acid, palmitoleic acid and skeletal muscle protein abundance of IRβ. Our results suggest that differences in insulin sensitivity between nonobese, nondiabetic MA and NHW are not due to differences in level of cardiorespiratory fitness or adiposity, however dietary intake and key insulin signaling protein levels could contribute to these ethnic differences.

In **Study 2**, we determined whether the TNF-α system accounted for differences in insulin sensitivity between MA (n=13; 27.0 ± 2.0 yrs; BMI=23.0 ± 0.7) and NHW (n=13; 24.8 ± 1.5 yrs; BMI=22.8 ± 0.6) subjects. MA were less insulin sensitive compared to NHW, while circulating levels of TNF-α were higher (3.11 ± 0.38 vs. 2.10 ± 0.24 pg/ml, $p<0.05$) and sTNFR2 (1323.52 ± 84.73 vs. 1924.65 ± 127.36 pg/ml, $p<0.05$) were significantly lower among MA subjects. TNFα, sTNFR1 and sTNFR2 were not related to estimates of insulin sensitivity or abdominal fat patterning when the two groups were analyzed in aggregate. These data indicate that although circulating levels of TNFα and sTNFR2 are different between nonobese, nondiabetic MA and NHW, they do not account for the observed differences in insulin sensitivity.

In **Study 3**, we determined the relationship between various estimates of insulin sensitivity, LDL size and oxidized LDL in a group of overweight, nondiabetic males (N=34, BMI 25-35 kg/m^2, 50-75y). Estimates of insulin sensitivity were inversely related to LDL size (r=.41, $P<0.05$), although these relationships were largely mediated by VLDL triglyceride and HDL cholesterol concentrations. Estimates of insulin sensitivity were not related to plasma oxidized LDL concentrations. Furthermore, LDL size was not significantly associated with oxidized LDL. In this homogeneous group of overweight, nondiabetic men, estimates of insulin sensitivity are not independent markers of atherogenic small, dense and oxidized LDL.

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CHAPTER 1

INTRODUCTION AND SPECIFIC AIMS

Whole body insulin resistance (subnormal cellular response to insulin) is now recognized as one of the primary defects in the development of type 2 diabetes mellitus, and is associated with other metabolic abnormalities (dyslipidemia, hypertension, cardiovascular disease, abdominal obesity, and even some cancers), which cluster together as part of the Metabolic Syndrome. It appears that one factor central to the induction of cellular insulin resistance is the excessive accumulation of lipids in nonadipocytes, such as skeletal muscle. This phenomenon of 'lipotoxicity' is predicated on positive intracellular lipid balance, characterized by excessive uptake and/or reduced oxidation of lipid.

Genetic factors undoubtedly play a role in an individual's propensity for lipotoxic phenotypes (insulin resistance, hypertension, dyslipidemia, etc.). However, lifestyle factors, including diet and exercise, also contribute to the expression of the insulin resistant phenotype in humans, possibly acting via intracellular lipid accumulation and increased activity of cytokines such as tumor necrosis factors-alpha, an inhibitor of cellular insulin signaling. Thus, these lifestyle factors (adiposity, dietary intake, physical activity, etc.) can influence risk for the untoward sequelae of insulin resistance, including dyslipidemia and cardiovascular disease. However, as yet, the relationships between insulin sensitivity and markers of cardiovascular dyslipidemia, including a decrease in low-density lipoprotein (LDL) size, elevations of oxidized LDL, and increased plasma triglyceride concentrations have yet to be fully elucidated. Furthermore, there is a paucity of data regarding the extent to

which the aforementioned lifestyle factors mediate differences in insulin sensitivity in populations at high and low risk for whole-body insulin resistance, type 2 diabetes mellitus, and ultimately, the Metabolic Syndrome. For example, Mexican Americans exhibit higher risk for insulin resistance and type 2 diabetes compared to their non-Hispanic White counterparts, but the contributions of diet, exercise, and adiposity to explaining their greater risk is not entirely clear.

To address these issues, three studies were conducted with the following specific aims and hypotheses:

Study 1 Specific Aims: To determine whether differences in insulin sensitivity exist between nonobese, nondiabetic Mexican American and Non-Hispanic White males and females after accounting for levels of cardiorespiratory fitness, regional fat distribution and dietary intake. A corollary aim was to determine whether difference between the two groups exist in the content of proteins involved in insulin-stimulated glucose transport (insulin receptor, phosphatidylinositol 3-kinase, Akt, GLUT4).

Hypotheses:

1.) After accounting for level of cardiorespiratory fitness, regional fat distribution, and dietary intake, no differences in insulin sensitivity between the two groups will be evident.

2.) There will be no group differences in skeletal muscle insulin signaling protein concentrations.

Study 2 Specific Aims: To determine whether, or not, differences in the tumor necrosis (TNF)-alpha system (TNFα, soluble TNF receptor 1 and 2) exist between Mexican American

and Non-Hispanic White males and females. Furthermore, we sought to determine the strength of the relationships between the TNF system, estimates of insulin sensitivity and abdominal fat patterning within these groups.

Hypotheses:

1.) Nonobese, nondiabetic Mexican American and Non-Hispanic White subjects will exhibit no differences in concentrations of circulating TNFα, soluble TNF receptor 1, and soluble TNF receptor 2.

2.) TNFα and its two soluble receptors will be associated with estimates of insulin sensitivity and abdominal visceral fat.

Study 3 Specific Aims: To determine whether estimates of whole-body insulin sensitivity are associated with LDL size and oxidized LDL in a group of overweight, nondiabetic men. Furthermore, we sought to determine whether, or not, insulin sensitivity is an independent predictor of LDL size and after accounting for very-low density lipoprotein-triglyceride. Finally, we examined the relationship between LDL size and oxidized LDL in this population at an increased risk for cardiovascular disease.

Hypotheses:

1.) Estimates of insulin sensitivity will significantly correlate with both LDL size and oxidized LDL.

2.) After accounting for VLDL-TG, insulin sensitivity will not be an independent predictor of LDL size.

3.) LDL size will be inversely related to oxidized LDL.

CHAPTER 2

REVIEW OF LITERATURE

The prevalence of *type 2* diabetes mellitus (T2DM) has steadily increased over the past few decades, and has been projected to increase by 35% on a worldwide basis in the next 25 years (1). When considering health care expenditures in addition to losses of productivity and earnings due to disability, T2DM carries a significant economic burden, estimated in excess of $19 billion annually (2). In the United States, type 2 diabetes is associated with close to 150,000 deaths annually, and the total disability of 950,000 persons (2). Complications associated with T2DM include retinopathy, nephropathy, peripheral neuropathy, amputation, autonomic neuropathy, sexual dysfunction, dyslipidemia, hypertension, and obesity (3).

The pathogenesis of T2DM stems from a combination of both environmental and genetic factors. The major risk factors associated with T2DM are a family history of diabetes, hypertension, dyslipidemia (low HDL cholesterol and high triglyceride), race/ethnicity, and impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) (Table 1). Furthermore, the risk of developing type 2 diabetes increases with age, obesity, and lack of physical activity (4-6).

The progression from prediabetes to diagnosed T2DM typically occurs in four stages: 1.) basal, 2.) compensated peripheral insulin resistance, 3.) uncompensated insulin resistance, and 4.) reduced insulin clearance. In the basal stage, individuals at an elevated risk for

developing T2DM often exhibit normoglycemia. As these individuals progress toward T2DM, they exhibit impaired fasting glucose (110 ≤ fasting blood glucose <126 mg/dL)

TABLE 1. Established and potential risk factors for type 2 diabetes

Family history of diabetes
Obesity
Abdominal obesity
Habitual physical inactivity
Race/ethnicity (Native Americans, African-Americans, Hispanic Americans, Asians/South Pacific Islanders)
Previously identified IFG or IGT
Peripheral insulin resistance
Elevated plasma non-esterified fatty acids
Dietary fat
Preponderance of type IIb skeletal muscle fibers

Adapted from American Diabetes Association (4)

and/or impaired glucose tolerance (2-h plasma glucose ≥ 140 mg/dL during an oral glucose tolerance test). This stage appears to reflect losses in peripheral insulin sensitivity and uncompensated insulin secretion. As peripheral insulin sensitivity continues to decline, the degree of hyperglycemia worsens. Commonly, pancreatic β cell compensate for the relative hyperglycemia and elevated insulin requirements by increasing the production and secretion of insulin (compensatory hyperinsulinemia). Individuals in this stage are typically diagnosed with T2DM, exhibiting both hyperglycemia (FBG ≥ 126 mg/dL) and hyperinsulinemia. In an attempt to spare β cell insulin production, the liver may become insulin resistant, decreasing insulin clearance and further increasing circulating insulin concentrations. Some individuals with T2DM also progress further to exhibit pancreatic β cell exhaustion in the latter stages of the disease, where insulin production fails to match peripheral insulin

requirements. Collectively, recent data indicate that insulin resistance and hyperinsulinemia may exist years before the development of the disease (7).

Insulin Resistance. Prospective studies have shown that insulin resistance is a major predictor of subsequent development of diabetes (8, 9). Insulin resistance is considered to exist when normal insulin concentrations produce a less than normal cellular biologic response (10). In adipocytes, insulin resistance impairs the storage of lipids and fails to suppress lipid mobilization (anti-lipolysis). In the liver, insulin resistance results in increased hepatic glucose production (via gluconeogenesis and glycogenolysis), yet insulin continues to stimulate lipoprotein synthesis. While insulin resistance has been observed in various tissues (liver, adipose, pancreas, endothelium, etc.), it is skeletal muscle that accounts for over 85% of insulin-mediated glucose disposal during a hyperinsulinemic-euglycemic clamp (11). Recently, it has been suggested that skeletal muscle is the primary site of insulin resistance, and impaired muscle glucose uptake represents the earliest detectible metabolic abnormality in normoglycemic individuals at risk for T2DM (12-15), and possibly other conditions (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome) (4, 16).

Insulin is a pleiotropic hormone, activating numerous intracellular pathways (17). Upon binding to the membrane bound insulin receptor, insulin activates signaling cascades that stimulate cell proliferation, differentiation, protein synthesis, glycogen synthesis and glucose transport (18). The insulin-dependent glucose transporter (GLUT4) is translocated from intracellular vesicles to the cell membrane by signaling through the phosphatidylinositol 3-kinase (PI3K) pathway (19, 20). Insulin-stimulated cell proliferation and differentiation signals utilize the pathways belonging to the family of mitogen-activated

protein kinases (MAPK), which include jun-amino terminal kinase (JNK), p38, extracellular-regulated kinase (ERK) and mitogen-activated protein kinase kinase (MEK) cascades (21, 22).

Skeletal muscle insulin resistance appears to selectively affect the PI3K pathway (23, 24). In an elegant study utilizing the euglycemic clamp technique, Cusi and colleagues (24) showed that while activity of the PI3K cascade is impaired in diabetic vs. nondiabetic humans, no such differences exist in the MAPK pathways (ERK phosphorylation, ERK activity, MEK1 activity). In the past few years, studies have suggested that one of the key impairments in insulin-stimulated glucose transport results from the serine phosphorylation of insulin receptor substrate-1 (IRS-1) (24). Under normal conditions, the β-subunit of the insulin receptor phosphorylates IRS-1 on tyrosine residues. The active adaptor/scaffolding protein, IRS-1, recruits the docking of PI3K, which through a series of poorly understood downstream steps stimulates GLUT4 translocation and glucose transport. When phosphorylated on serine residues, IRS-1 has been shown to inhibit intrinsic tyrosine kinase activity of the insulin receptor β subunit and decrease IRS-1-associated PI3K activity. Consequently, serine phosphorylated IRS-1 has been associated with impaired insulin-stimulated glucose uptake (25, 26). Furthermore, this specific defect in the PI3K pathway has been widely associated with elevations in circulating non-esterified fatty acid (NEFA) concentrations (27). In fact, the impact that obesity and regional fat distribution have on inducing peripheral insulin resistance are apparently mediated, at least in part, by increases in plasma NEFA (28).

Elevated fasting plasma NEFA are commonly associated with insulin resistant states and have been shown to be a risk factor for T2DM (29-32). Plasma NEFA can impact both

insulin secretion, by impairing β-cell function (33-35), and insulin action by inducing insulin resistance (36, 37). Various experimental models (diabetes, obesity, lipodystrophy, and glucocorticoid excess) have been studied whereby plasma NEFA are elevated, inducing insulin resistance in skeletal muscle (38-40). Evidence suggests that elevations in plasma NEFA increase lipid availability in nonadipocytes (steatosis), which in turn contributes to the development of skeletal muscle insulin resistance (41). Lowering plasma NEFA below basal values results in increases in insulin-stimulated glucose uptake (42, 43).

The excessive accumulation of lipid, particularly long-chain acyl CoA (LCACoA), in nonadipocytes results from imbalance between uptake and disposal. It has recently been demonstrated that plasma fatty acids require plasma membrane fatty acid binding proteins (FABP) for transport into skeletal muscle cells (44, 45). Studies have found that plasma membrane FABP and NEFA uptake were significantly higher among obese, insulin resistant vs. control rats (46-48). Obese individuals have also exhibited higher contents of cytosolic FABP(C) and plasma membrane FABP(PM) (49). Blaak and colleagues found that skeletal muscle FABP-PM, FABP-C and oxidative capacity of plasma NEFA were lower in T2DM (50). Furthermore, dietary-induced obese mice with a targeted mutation in the gene (aP2) that encodes for FABP did not develop insulin resistance (51), presumably due to less accumulation of lipid within cells. FABP-PM content is positively associated with NEFA uptake and negatively associated with insulin sensitivity and glucose uptake (47, 49).

Upon entry into the cytosol of skeletal muscle, NEFA are converted to LCACoA by the catalytic activity of long-chain acyl-CoA synthetase (ACS1). The majority of LCACoA are stored as triglyceride (TG), while limited amounts are incorporated into lipid second messengers, phospholipids (PL) or oxidized directly.

Plasma-derived fatty acid oxidation is significantly lower among obese and T2DM subjects compared to their nonobese and nondiabetic counterparts (52, 53). Carnitine palmitoyltransferase (CPT)-1 is the rate-limiting enzyme in skeletal muscle fat oxidation. Covalent inhibition of CPT-1 (malonyl CoA, etomoxir) impairs fatty acid oxidation and results in the accumulation of intramyocellular lipid (54). CPT-1 inhibition is also strongly associated with impaired insulin-stimulated glucose disposal. In skeletal muscle of obese subjects CPT-1 and citrate synthase activities are lower than in lean individuals (49). Muscle CPT-1 activity is strongly related to insulin sensitivity in humans (49). Consequently, it has been shown that fat oxidation is impaired in T2DM compared with nondiabetic individuals (50, 53). Skeletal muscle fiber type has been shown to impact the propensity for intracellular lipid accumulation. Interestingly, insulin-stimulated glucose disposal occurs in a skeletal muscle fiber specific manner, with higher responsiveness in type I (oxidative) compared to type IIa and IIb (glycolytic) fibers (55, 56). Studies have documented a higher proportion of type IIb muscle fibers in obesity and T2DM (57, 58). Presumably, the lower oxidative capacity of type IIb fibers make them more susceptible to lipid accretion and impaired insulin responsiveness. Collectively, these studies suggest that the excessive accumulation of lipid in skeletal muscle of insulin resistant individuals is due to increases in fat uptake and decreases in fat oxidation.

The mechanisms by which intramuscular lipid accretion impairs insulin-stimulated glucose uptake are obscure. Studies have revealed a strong correlation between the degree of insulin resistance in skeletal muscle and the local accumulation of TG (59-62). However, some individuals, such as athletes, exhibit high TG levels and therefore triglyceride appears to represent a relatively inert marker of excess lipid accretion in muscle cells. Elevations in

intramuscular LCACoA concentrations have been demonstrated in numerous models of insulin-resistance (63-66). A significant relationship was observed between whole body insulin action and muscle LCACoA (63). Randle et al. (67) suggested that substrate competition exists between lipid and glucose availability. Thus, when lipid is readily available, increases in citrate production allosterically inhibit pyruvate dehydrogenase (PDH) and phosphofructokinase (PFK) activity (68, 69). This would result in increases in glucose 6-phosphate (G6P) levels. The same increase in G6P would result from decreases in glycogen synthase activity. However, studies have provided substantial evidence showing that fat-induced decreases in glucose uptake are associated with depressed, rather than elevated, levels of G6P (70-72). Fat induced reductions in glucose uptake may also result from decreases in glucose transport and phosphorylation, leading to diminished glycogen synthesis/glycolysis. Dresner and colleagues reported lower intracellular glucose concentrations during lipid infusion (41). Insulin typically increases hexokinase II (HKII) transcription via the PI3K pathway (73). Studies have shown that insulin fails to increase HKII expression and activity in skeletal muscle of T2DM subjects (74, 75). Furthermore, LCACoA have been shown to inhibit HKII activity in skeletal muscle (76). Subsequently, the rates of glucose transport and phosphorylation inversely correlate with the content of intramuscular LCACoA (59). Hence, lipid-induced impairments in glucose disposal occur at the level of transport into, and phosphorylation within, the cell. Therefore, partitioning of excess intracellular LCACoA into TG, PL, lipid second messengers or into the mitochondria for β-oxidation is critical in determining both its own cytosolic pool and impairments in glucose uptake.

Excessive accumulation of lipid in skeletal muscle is associated with defects in the PI3K pathway (lipotoxicity). Various serine kinases have been implicated in the deactivation of IRS-1 (PKCθ, IkappaB kinase β, etc.). Evidence suggests that these serine kinases are activated by various cytokine signaling cascades (TNFα), as well as lipid and lipid-derived products.

Tumor Necrosis Factor Alpha. TNFα possesses diverse bioregulatory functions, including inflammation, cell proliferation, differentiation, and apoptosis (77). Binding of TNFα to its two receptors, TNFR1 and TNFR2, leads to the recruitment of various adaptor proteins (TRADD, RIP1, FADD, TRAF2), which activate distinct downstream signaling cascades (78). These cascades stimulate apoptosis by the activation of caspases, or stimulate cell proliferation by activation of two transcription factors, activating protein-1 (AP-1) and nuclear factor kappaB (NFκB) (79).

Several lines of evidence suggest that TNFα is a mediator of lipotoxicity. TNFα is associated with the degree of insulin resistance and obesity in both animals and humans (discussed in Chapter 4). Injection of TNFα results in decreases in insulin-stimulated glucose disposal. Infusion of TNFα into humans results in peripheral insulin resistance, particularly skeletal muscle (80, 81). Using adenovirus-mediated gene transfer, Cheung and colleagues expressed a TNF-α inhibitor gene (TNFi) in rats and reported a significant reduction in plasma insulin and free fatty acids levels in obese rats (82). These changes were associated with a 50% increase in peripheral insulin sensitivity. Targeted null mutations in the gene encoding TNFα or either of its two receptor are associated with increased insulin sensitivity in obese mice (83, 84). Insulin resistance has also been reversed by infusion of a TNFα neutralizing antibody containing soluble TNFR domains in obese *fa/fa* rats (85, 86).

Expression of TNFα in skeletal muscle is increased in obesity, insulin resistance and T2DM (87, 88). Exposure to TNFα has been associated with impairments in the insulin signaling pathway (89-91) and downregulation of GLUT4 (92). Numerous studies have confirmed the association between TNFα and serine phosphoryation of IRS-1. TNFα neutralization is associated with a 2.5-fold increase in tyrosine phosphorylation of IR in skeletal muscle (82). The mechanism by which TNFα induces serine phosphorylation of IRS-1 is best elucidated by the recent work involving a specific serine kinase.

Inactive NFκB is typically sequestered by IκB in the cytosol. In response to TNFα, IκB is phosphorylated by the serine kinase IkappaB kinase β (IKKβ), releasing NFκB for nuclear translocation (93). However, recent data have shown that IKKβ is also intimately involved in the serine phosphorylation of IRS-1 (27). Kim and colleagues reported that IKKβ knockout mice were protected from lipid-induced insulin resistance (27). Furthermore, the beneficial effects of salicylates on improving glucose tolerance are apparently mediated through the inhibition of IKKβ (27). Some evidence suggests that the activation of IKKβ may be further mediated by crosstalk between TNFα and atypical PKCs (94-99).

While TNFα negatively modulates insulin sensitivity in skeletal muscle, it may also contribute to lipotoxic diabetes in other ways. TNFα stimulates hepatic lipogenesis (100) and has also has been shown to increase hepatic gluconeogenesis from alanine and produce a three-fold increase in plasma glucagon (101). Injection of recombinant TNFα has been shown to increase the activity of 6-phosphogluconate dehydrogenase (6PGD), the key enzyme of the hexose monophosphate shunt (102).

Ceramide. Ceramide, a sphingolipid, has been implicated in the lipotoxicity observed in T2DM. Two distinct pathways generate intracellular ceramide: sphingomyelinase catalyzed hydrolysis of sphingomyelin to ceramide and choline (103) and *de novo* synthesis from the fatty acid, palmitate. High ceramide content observed in islets of obese Zucker Diabetic Fatty (ZDF) rats (*fa/fa*) resulted from *de novo* synthesis of ceramide, rather than a product of sphingomyelin hydrolysis (104). Numerous studies have shown that increased availability of palmitate results in elevations in intracellular ceramide levels (105-111). Overproduction of [^{3}H]ceramide from [^{3}H]palmitate is blocked by fumonisin-B_1, an inhibitor of dihydroceramide synthase (112). Ceramide synthesis is also inhibited by Triacsin-C blockade of fatty acyl-CoA synthetase. In cells preincubated in the absence or presence of NEFA, ceramide was found to be elevated 2.1-fold by palmitate, while oleate and linoleate were without effect (106). Furthermore, a recent study found decreased fat oxidation contributed to a diversion of palmitate into the synthesis of ceramide (113, 114).

Ceramide has been found to significantly inhibit insulin signaling, GLUT4 translocation (50-60%) and glucose uptake (115, 116). Studies have reported that ceramide impairs signaling through the PI3K pathway by inhibiting IR, IRS-1-associated PI3K , and/or Akt activity (90, 103, 115, 117, 118). Ceramide also mediates the action of TNFα (119). Studies have documented the activation of IKKβ with ceramide, although to a lesser extent compared to TNFα.

Long Chain Acyl CoA. Long chain acyl-CoA are strongly correlated with skeletal muscle insulin resistance (59). In particular, several lines of evidence suggest that palmitate (C16) plays a primary role in skeletal muscle insulin resistance. Palmitate is the most abundant NEFA in plasma and skeletal muscle cytosol. Thus, changes in plasma NEFA

directly correlate with changes in cytosolic C16. CPT-1 has a higher affinity for fatty acids exhibiting a greater degree of unsaturation and shorter chain length (120). Thus, C16 and stearate (C18) are the least preferred substrates for β-oxidation. Palmitate uptake and oxidation are decreased in skeletal muscle of insulin resistant individuals (50, 53, 121). However, the rate of C16 uptake saturates with an increase in the concentration of unbound C16 in skeletal muscle (44). Individuals with T2DM exhibit high plasma NEFA (C16) and low C16 oxidation, which together produce the increase in the cytosolic palmitoyl CoA pool.

As mentioned previously, nonoxidative pathways involved in LCACoA partitioning include esterification to DAG and TAG, synthesis of lipid second messengers, and incorporation into membrane phospholipids. Palmitate content in skeletal muscle triglyceride (122) and plasma membrane phospholipids (123) is higher in insulin resistant vs. insulin sensitive individuals.

Palmitate was found to be a potent inhibitor of insulin-induced glucose transport, whereas all other fatty acids (C18, C14, C12) and analogues tested did not prevent insulin action (124). Palmitate inhibits glucose disposal and Akt activation in myocytes (125) and myotubes (106, 124). Palmitate inhibited tyrosine phosphorylation of IRβ and IRS-1 by about 50% and also reduced Akt serine phosphorylation by 50% (124). Incubation with C16 did not affect insulin binding, insulin receptor autophosphorylation, or PI3K activity (126). Because elevated C16 availability appears to drive its incorporation into non-oxidative metabolic pathways, an interesting mechanism for C16's role in impaired insulin signaling involves the synthesis of ceramide. A study by Schmitz-Peiffer et al. (106) strongly suggests that the effects of C16 in the reduction of Akt phosphorylation are mediated by ceramide. Palmitoyl-CoA is rate limiting in *de novo* ceramide synthesis (114, 127). These data indicate

that palmitate may exert its effects on various insulin signaling proteins (Akt, in particular) via *de novo* ceramide synthesis.

Fat-induced insulin resistance has typically been observed only after three or more hours of fat infusion. This delayed onset of insulin resistance may be that NEFA need to first accumulate as TG (or DAG) in muscle fibers (128). As TG and DAG levels rise, the flux of LCACoA through nonoxidative pathways (de novo ceramide synthesis) presumably increases. Unlike the delayed effect of lipid infusion on inducing insulin resistance, TNFα has been shown to exhibit rapid effects on insulin-stimulated glucose disposal (81, 129). Lipid overload in skeletal muscle, pancreatic islets, myocardium, and liver are common in all of the foregoing clinical states, causing insulin resistance, T2DM, cardiac dysfunction and hepatic steatosis (130, 131).

Metabolic Syndrome. Conditions which contribute to lipid accumulation in nonadipocytes and potentially impair insulin-stimulated glucose transport, are also related to the clustering of metabolic abnormalities (T2DM, cardiovascular disease, hypertension, dyslipidemia, endothelial dysfunction, dysfibrinolysis, abdominal obesity) commonly referred to as the Metabolic Syndrome.

Abdominal obesity is intimately related to other metabolic anomalies associated with the Metabolic Syndrome. Studies have shown that central adiposity is more strongly related to insulin resistance compared to peripheral adiposity (132). Furthermore, abdominal visceral fat is a better predictor of insulin resistance (discussed in Chapter 3) and cardiovascular complications compared to abdominal subcutaneous fat (133-136). Larger visceral adipocytes tend to be resistant to the anti-lipolytic actions of insulin, exhibit reduced α2-adrenergic receptor (antilipolytic), and increased β3-adrenergic receptor (lipolytic) (137).

Consequently, visceral fat is associated with an increased portal NEFA flux, which contributes to unfavorable hepatic and peripheral sequelae.

Dyslipidemia is associated with elevated plasma NEFA, hypertriglyceridemia, hyperapoB, elevated LDL cholesterol and depressed HDL cholesterol concentrations (138-140). The preponderance of small, dense LDL particles is now recognized as part of the dyslipidemic profile (141, 142). Larger visceral adipocytes (as seen in abdominal obesity) appear to be particularly resistant to the anti-lipolytic effects of insulin. Increases in the efflux from central adipocytes drive the circulating NEFA concentrations up, contributing to the enhanced lipid availability for peripheral tissues (skeletal muscle). Furthermore, elevated plasma NEFA are cleared by the liver, and packaged into lipoprotein triglyceride (143). Hepatic steatosis is associated with the increased synthesis of triglyceride-rich lipoproteins, and subsequently, hepatic insulin resistance (144). Cholesterol ester transfer protein (CETP) catalyzes the exchange of VLDL-TG for LDL and HDL cholesterol ester (145). Hepatic lipase catalyzes the hydrolysis of triglyceride-rich LDL and HDL, resulting in small, dense particles. Small, dense LDL particles are less readily cleared from the plasma compartment and are more susceptible to oxidation compared to larger LDL particles (146-148). Interestingly, oxidized LDL particles have been shown to increase the production of both TNFα (149) and ceramide (150), and have been associated with an increased risk for T2DM (150, 151). Hence, dyslipidemia characterized by small, dense LDL particles may both contribute to and result from central and peripheral insulin resistance (discussed in Chapter 5).

Ethnicity. Numerous epidemiological studies have provided evidence suggesting that the clustering of the aforementioned risk factors and metabolic abnormalities are more

prevalent in certain populations. Among these groups, the problems of T2DM and abdominal obesity are particularly acute among the Mexican American (MA) population (152). Compared to non-Hispanic white (NHW) adults, MA tend to be more obese, exhibit a more centralized distribution of body fat (although the latter finding is based on surrogate measures such as waist circumference, rather than actual measures of abdominal visceral fat) and are less physically active (152-154). Although, the prevalence of hypertension appears to be similar between these two groups (155), Aguirre et al. reported that MA women had higher plasma triglyceride concentrations and lower HDL-cholesterol concentrations compared to NHW women (155, 156).

Consequently, the prevalence of T2DM is two to five times higher among MA compared to NHW individuals (152, 157-160). It is estimated that 1 in 10 individuals over the age of 20 years and at least 1 in 3 of those over the age of 65 years have diabetes (155, 161, 162). Mexican American diabetics exhibit more severe hyperglycemia (163), an increased prevalence of peripheral vascular disease (164, 165), an increased incidence of end-stage renal disease (166), and an increased prevalence of clinical proteinuria (166) and retinopathy (167). Accordingly, diabetes-related mortality rates are substantially higher for MA compared to their NHW counterparts (168).

Several studies have documented an earlier age of onset of T2DM among the MA population (165, 169, 170). While MA exhibit a higher prevalence of many risk factors associated with T2DM, recent studies have suggested that insulin resistance may be one of the primary defects in the etiology of the disease (8, 171-174). MA adults carry a greater burden of hyperinsulinemia and insulin resistance compared to NHW, independent of obesity, blood pressure or age (152, 156, 159, 175-177). Haffner and colleagues found that

young, nonobese, normoglycemic MA were less insulin sensitive compared to NHW (178). In large pediatric centers, diagnosed T2DM was recently reported to account for 8-45% of the new cases of diabetes (179). Additionally, recent studies have shown that third-grade MA children exhibit a greater degree of insulin resistance compared to their NHW counterparts (7). MA children and adults have higher levels of many cardiovascular risk factors, which appear to be significantly related to their higher insulin levels (180). The well-known correlates of insulin resistance, total and abdominal body fat, do not entirely account for their greater prevalence of insulin resistance in MA compared to NHW (152, 154). Given this scenario, it has been suggested that genetic factors could explain the higher prevalence of insulin resistance in Hispanic Americans, possibly owing to the fact that up to 30% of the genetic make-up of Hispanics is attributable to Native American ancestry (181). The latter is strongly correlated with insulin resistance. However, to date, the possible contributions of low physical fitness and elevated abdominal visceral body fat to the disproportionately higher prevalence of insulin resistance in MA compared to NHW have not been addressed (discussed in Chapter 3).

Many of the risk factors associated with insulin resistance and T2DM have also been implicated in the development of lipotoxicity phenotypes and the Metabolic Syndrome. Lifestyle factors account for approximately 50% of the variability in insulin-mediated glucose disposal between apparently healthy individuals (25% to differences in weight and 25% to differences in physical activity) (182). Obesity (visceral adiposity) and dietary fat can contribute to elevations in circulating NEFA and increase the availability of lipid for nonadipocytes. Furthermore, body fat mass and dietary fat are correlated with the production of various cytokines (183, 184). Both weight loss and decreases in dietary fat intake are

associated with reductions in plasma NEFA levels and improvements in insulin sensitivity (185). Oxidative capacity of skeletal muscle is decreased with advancing age, physical inactivity and a skeletal muscle fiber type distribution favoring a preponderance of type IIb glycolytic fibers (55-56). Subsequently, exercise and physical activity are associated with increases in fat oxidation and improvements in insulin-stimulated glucose disposal (186). The main thrust of the research documented herein, was to determine the role that various modifiable acquired factors play in the phenotypes associated with lipotoxic diabetes.

REFERENCES

1. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 1998;21(9):1414-31.
2. Huse DM, Oster G, Killen AR, Lacey MJ, Colditz GA. The economic costs of non-insulin-dependent diabetes mellitus. Jama 1989;262(19):2708-13.
3. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, et al. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation 1999;100(10):1134-46.
4. Association AD. Screening for diabetes. Diabetes Care 2001;24(Supplement 1):S21-S24.
5. Paolisso G, Rizzo MR, Mazziotti G, Tagliamonte MR, Gambardella A, Rotondi M, et al. Advancing age and insulin resistance: role of plasma tumor necrosis factor-alpha. Am J Physiol 1998;275(2 Pt 1):E294-9.
6. Paolisso G, Tagliamonte MR, Rizzo MR, Giugliano D. Advancing age and insulin resistance: new facts about an ancient history. Eur J Clin Invest 1999;29(9):758-69.
7. Batey LS, Goff DC, Jr., Tortolero SR, Nichaman MZ, Chan W, Chan FA, 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):4319-25.
8. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR. 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):925-9.
9. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 1993;329(27):1988-92.
10. Kahn CR. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 1978;27(12 Suppl 2):1893-902.

11. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 1981;30(12):1000-7.
12. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995;75(3):473-86.
13. Gulli G, Ferrannini E, Stern M, Haffner S, DeFronzo RA. The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. Diabetes 1992;41(12):1575-86.
14. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 1985;76(1):149-55.
15. Baron AD, Laakso M, Brechtel G, Edelman SV. 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):1186-94.
16. Dunaif A, Wu X, Lee A, Diamanti-Kandarakis E. Defects in insulin receptor signaling in vivo in the polycystic ovary syndrome (PCOS). Am J Physiol Endocrinol Metab 2001;281(2):E392-9.
17. Smith RM, Harada S, Jarett L. Insulin internalization and other signaling pathways in the pleiotropic effects of insulin. Int Rev Cytol 1997;173:243-80.
18. Shepherd PR, Withers DJ, Siddle K. Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. Biochem J 1998;333(Pt 3):471-90.
19. Gould GW, Holman GD. The glucose transporter family: structure, function and tissue-specific expression. Biochem J 1993;295(Pt 2):329-41.
20. Shepherd PR, Nave BT, Rincon J, Nolte LA, Bevan AP, Siddle K, et al. Differential regulation of phosphoinositide 3-kinase adapter subunit variants by insulin in human skeletal muscle. J Biol Chem 1997;272(30):19000-7.
21. Sale EM, Atkinson PG, Sale GJ. Requirement of MAP kinase for differentiation of fibroblasts to adipocytes, for insulin activation of p90 S6 kinase and for insulin or serum stimulation of DNA synthesis. Embo J 1995;14(4):674-84.
22. Kim SJ, Kahn CR. Insulin regulation of mitogen-activated protein kinase kinase (MEK), mitogen-activated protein kinase and casein kinase in the cell nucleus: a possible role in the regulation of gene expression. Biochem J 1997;323(Pt 3):621-7.
23. Folli F, Saad MJ, Backer JM, Kahn CR. Regulation of phosphatidylinositol 3-kinase activity in liver and muscle of animal models of insulin-resistant and insulin-deficient diabetes mellitus. J Clin Invest 1993;92(4):1787-94.
24. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 2000;105(3):311-20.
25. Bjornholm M, Kawano Y, Lehtihet M, Zierath JR. 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):524-7.
26. Shulman GI. Cellular mechanisms of insulin resistance. The Journal of Clinical Investigation 2000;106(2):171-176.
27. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, et al. Prevention of fat-induced insulin resistance by salicylate. J Clin Invest 2001;108(3):437-46.

28. Bergman RN. Non-esterified fatty acids and the liver: why is insulin secreted into the portal vein? Diabetologia 2000;43(7):946-52.
29. Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. Diabetes 1988;37(8):1020-4.
30. Steiner G, Morita S, Vranic M. Resistance to insulin but not to glucagon in lean human hypertriglyceridemics. Diabetes 1980;29(11):899-905.
31. Paolisso G, Tataranni PA, Foley JE, Bogardus C, Howard BV, Ravussin E. A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. Diabetologia 1995;38(10):1213-7.
32. Charles MA, Eschwege E, Thibult N, Claude JR, Warnet JM, Rosselin GE, et al. The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study. Diabetologia 1997;40(9):1101-6.
33. Bollheimer LC, Skelly RH, Chester MW, McGarry JD, Rhodes CJ. Chronic exposure to free fatty acid reduces pancreatic beta cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. J Clin Invest 1998;101(5):1094-101.
34. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. Proc Natl Acad Sci U S A 1994;91(23):10878-82.
35. Lee Y, Hirose H, Zhou YT, Esser V, McGarry JD, Unger RH. Increased lipogenic capacity of the islets of obese rats: a role in the pathogenesis of NIDDM. Diabetes 1997;46(3):408-13.
36. Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA. Effect of fatty acids on glucose production and utilization in man. J Clin Invest 1983;72(5):1737-47.
37. Lillioja S, Bogardus C, Mott DM, Kennedy AL, Knowler WC, Howard BV. Relationship between insulin-mediated glucose disposal and lipid metabolism in man. J Clin Invest 1985;75(4):1106-15.
38. Fraze E, Donner CC, Swislocki AL, Chiou YA, Chen YD, Reaven GM. Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance. J Clin Endocrinol Metab 1985;61(5):807-11.
39. Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI. Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. J Biol Chem 2000;275(12):8456-60.
40. Mokuda O, Sakamoto Y. Peripheral insulin sensitivity is decreased by elevated non-esterified fatty acid level in dexamethasone-treated rats. Diabetes Nutr Metab 1999;12(4):252-5.
41. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest 1999;103(2):253-9.
42. Gazdag AC, Tucker MZ, Turcotte LP, Dean DJ, Cartee GD. Effect of extracellular palmitate on 2-deoxy-d-glucose uptake in muscle from Ad libitum fed and calorie restricted rats. Biochem Biophys Res Commun 1998;252(3):733-7.
43. Boden G. Free fatty acids, insulin resistance, and type 2 diabetes mellitus. Proc Assoc Am Physicians 1999;111(3):241-8.

44. Turcotte LP, Petry C, Kiens B, Richter EA. Contraction-induced increase in Vmax of palmitate uptake and oxidation in perfused skeletal muscle. J Appl Physiol 1998;84(5):1788-94.
45. Turcotte LP, Swenberger JR, Tucker MZ, Yee AJ, Trump G, Luiken JJ, et al. Muscle palmitate uptake and binding are saturable and inhibited by antibodies to FABP(PM) [In Process Citation]. Mol Cell Biochem 2000;210(1-2):53-63.
46. Carey JO, Neufer PD, Farrar RP, Veerkamp JH, Dohm GL. Transcriptional regulation of muscle fatty acid-binding protein. Biochem J 1994;298 Pt 3:613-7.
47. Turcotte LP, Swenberger JR, Zavitz Tucker M, Yee AJ. Increased fatty acid uptake and altered fatty acid metabolism in insulin-resistant muscle of obese Zucker rats. Diabetes 2001;50(6):1389-96.
48. Memon RA, Fuller J, Moser AH, Smith PJ, Grunfeld C, Feingold KR. Regulation of putative fatty acid transporters and Acyl-CoA synthetase in liver and adipose tissue in ob/ob mice. Diabetes 1999;48(1):121-7.
49. Simoneau JA, Veerkamp JH, Turcotte LP, Kelley DE. 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):2051-60.
50. Blaak EE, Wagenmakers AJ, Glatz JF, Wolffenbuttel BH, Kemerink GJ, Langenberg CJ, 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):E146-54.
51. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science 1996;274(5291):1377-9.
52. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol Endocrinol Metab 2000;279(5):E1039-44.
53. Blaak EE, van Aggel-Leijssen DP, Wagenmakers AJ, Saris WH, van Baak MA. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise.[In Process Citation]. Diabetes 2000;49(12):2102-7.
54. Dobbins RL, Szczepaniak LS, Bentley B, Esser V, Myhill J, McGarry JD. Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. Diabetes 2001;50(1):123-30.
55. Song XM, Kawano Y, Krook A, Ryder JW, Efendic S, Roth RA, et al. Muscle fiber type-specific defects in insulin signal transduction to glucose transport in diabetic GK rats. Diabetes 1999;48(3):664-70.
56. Song XM, Ryder JW, Kawano Y, Chibalin AV, Krook A, Zierath JR. Muscle fiber type specificity in insulin signal transduction. Am J Physiol 1999;277(6 Pt 2):R1690-6.
57. Hickey MS, Weidner MD, Gavigan KE, Zheng D, Tyndall GL, Houmard JA. The insulin action-fiber type relationship in humans is muscle group specific. Am J Physiol 1995;269(1 Pt 1):E150-4.
58. Nyholm B, Qu Z, Kaal A, Pedersen SB, Gravholt CH, Andersen JL, et al. Evidence of an increased number of type IIb muscle fibers in insulin- resistant first-degree relatives of patients with NIDDM. Diabetes 1997;46(11):1822-8.
59. Thompson AL, Lim-Fraser MY, Kraegen EW, Cooney GJ. Effects of individual fatty acids on glucose uptake and glycogen synthesis in soleus muscle in vitro. Am J Physiol Endocrinol Metab 2000;279(3):E577-84.

60. Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 1997;46(6):983-8.
61. Storlien LH, Pan DA, Kriketos AD, Baur LA. High fat diet-induced insulin resistance. Lessons and implications from animal studies. Ann N Y Acad Sci 1993;683:82-90.
62. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. Diabetes 1991;40(2):280-9.
63. Ellis BA, Poynten A, Lowy AJ, Furler SM, Chisholm DJ, Kraegen EW, 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):E554-60.
64. Laybutt DR, Schmitz-Peiffer C, Saha AK, Ruderman NB, Biden TJ, Kraegen EW. Muscle lipid accumulation and protein kinase C activation in the insulin-resistant chronically glucose-infused rat. Am J Physiol 1999;277(6 Pt 1):E1070-6.
65. Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ, Kraegen EW. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. Diabetes 1997;46(11):1768-74.
66. Oakes ND, Bell KS, Furler SM, Camilleri S, Saha AK, Ruderman NB, et al. Diet-induced muscle insulin resistance in rats is ameliorated by acute dietary lipid withdrawal or a single bout of exercise: parallel relationship between insulin stimulation of glucose uptake and suppression of long-chain fatty acyl-CoA. Diabetes 1997;46(12):2022-8.
67. Randle PJ, Garland PB, Newsholme EA, Hales CN. The glucose fatty acid cycle in obesity and maturity onset diabetes mellitus. Ann N Y Acad Sci 1965;131(1):324-33.
68. Randle PJ, Priestman DA, Mistry S, Halsall A. Mechanisms modifying glucose oxidation in diabetes mellitus. Diabetologia 1994;37 Suppl 2:S155-61.
69. Randle PJ, Newsholme EA, Garland PB. Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies and pyruvate, and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscles. Biochem J 1964;93(3):652-65.
70. Hawkins M, Barzilai N, Liu R, Hu M, Chen W, Rossetti L. Role of the glucosamine pathway in fat-induced insulin resistance. J Clin Invest 1997;99(9):2173-82.
71. Rothman DL, Shulman RG, Shulman GI. 31P nuclear magnetic resonance measurements of muscle glucose-6- phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. J Clin Invest 1992;89(4):1069-75.
72. Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, et al. Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest 1996;97(12):2859-65.
73. Osawa H, Sutherland C, Robey RB, Printz RL, Granner DK. Analysis of the signaling pathway involved in the regulation of hexokinase II gene transcription by insulin. J Biol Chem 1996;271(28):16690-4.
74. Pendergrass M, Koval J, Vogt C, Yki-Jarvinen H, Iozzo P, Pipek R, et al. Insulin-induced hexokinase II expression is reduced in obesity and NIDDM. Diabetes 1998;47(3):387-94.

75. Vestergaard H, Bjorbaek C, Hansen T, Larsen FS, Granner DK, Pedersen O. Impaired activity and gene expression of hexokinase II in muscle from non-insulin-dependent diabetes mellitus patients. J Clin Invest 1995;96(6):2639-45.
76. Thompson AL, Cooney GJ. Acyl-CoA inhibition of hexokinase in rat and human skeletal muscle is a potential mechanism of lipid-induced insulin resistance. Diabetes 2000;49(11):1761-5.
77. Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. Annu Rev Cell Biol 1993;9:317-43.
78. Wajant H, Scheurich P. Tumor necrosis factor receptor-associated factor (TRAF) 2 and its role in TNF signaling. Int J Biochem Cell Biol 2001;33(1):19-32.
79. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol 2001;11(9):372-7.
80. Miles PD, Romeo OM, Higo K, Cohen A, Rafaat K, Olefsky JM. TNF-alpha-induced insulin resistance in vivo and its prevention by troglitazone. Diabetes 1997;46(11):1678-83.
81. Ling PR, Bistrian BR, Mendez B, Istfan NW. Effects of systemic infusions of endotoxin, tumor necrosis factor, and interleukin-1 on glucose metabolism in the rat: relationship to endogenous glucose production and peripheral tissue glucose uptake. Metabolism 1994;43(3):279-84.
82. Cheung AT, Ree D, Kolls JK, Fuselier J, Coy DH, Bryer-Ash M. An in vivo model for elucidation of the mechanism of tumor necrosis factor-alpha (TNF-alpha)-induced insulin resistance: evidence for differential regulation of insulin signaling by TNF-alpha. Endocrinology 1998;139(12):4928-35.
83. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF- alpha function. Nature 1997;389(6651):610-4.
84. Ventre J, Doebber T, Wu M, MacNaul K, Stevens K, Pasparakis M, et al. Targeted disruption of the tumor necrosis factor-alpha gene: metabolic consequences in obese and nonobese mice. Diabetes 1997;46(9):1526-31.
85. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity- diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 1994;94(4):1543-9.
86. Qi C, Pekala PH. Tumor necrosis factor-alpha-induced insulin resistance in adipocytes. Proc Soc Exp Biol Med 2000;223(2):128-35.
87. Hrebicek A, Rypka M, Chmela Z, Vesely J, Kantorova M, Golda V. Tumor necrosis factor alpha in various tissues of insulin-resistant obese Koletsky rats: relations to insulin receptor characteristics. Physiol Res 1999;48(1):83-6.
88. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. The expression of TNF alpha by human muscle. Relationship to insulin resistance. J Clin Invest 1996;97(4):1111-6.
89. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996;271(5249):665-8.
90. Kanety H, Hemi R, Papa MZ, Karasik A. Sphingomyelinase and ceramide suppress insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1. J Biol Chem 1996;271(17):9895-7.

91. Miura A, Ishizuka T, Kanoh Y, Ishizawa M, Itaya S, Kimura M. et al. Effect of tumor necrosis factor-alpha on insulin signal transduction in rat adipocytes: relation to PKCbeta and zeta translocation. Biochim Biophys Acta 1999;1449(3):227-38.
92. Szalkowski D, White-Carrington S, Berger J, Zhang B. Antidiabetic thiazolidinediones block the inhibitory effect of tumor necrosis factor-alpha on differentiation, insulin-stimulated glucose uptake, and gene expression in 3T3-L1 cells. Endocrinology 1995;136(4):1474-81.
93. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol 2000;18:621-63.
94. Vertegaal AC, Kuiperij HB, Yamaoka S, Courtois G, van der Eb AJ, Zantema A. Protein kinase C-alpha is an upstream activator of the IkappaB kinase complex in the TPA signal transduction pathway to NF-kappaB in U2OS cells. Cell Signal 2000;12(11-12):759-68.
95. Khoshnan A, Bae D, Tindell CA, Nel AE. The physical association of protein kinase C theta with a lipid raft- associated inhibitor of kappa B factor kinase (IKK) complex plays a role in the activation of the NF-kappa B cascade by TCR and CD28. J Immunol 2000;165(12):6933-40.
96. Chen CC, Sun YT, Chen JJ, Chiu KT. TNF-alpha-induced cyclooxygenase-2 expression in human lung epithelial cells: involvement of the phospholipase C-gamma 2, protein kinase C- alpha, tyrosine kinase, NF-kappa B-inducing kinase, and I-kappa B kinase 1/2 pathway. J Immunol 2000;165(5):2719-28.
97. Trushin SA, Pennington KN, Algeciras-Schimnich A, Paya CV. Protein kinase C and calcineurin synergize to activate IkappaB kinase and NF-kappaB in T lymphocytes. J Biol Chem 1999;274(33):22923-31.
98. Sanz L, Diaz-Meco MT, Nakano H, Moscat J. The atypical PKC-interacting protein p62 channels NF-kappaB activation by the IL-1-TRAF6 pathway. Embo J 2000;19(7):1576-86.
99. Sanz L, Sanchez P, Lallena MJ, Diaz-Meco MT, Moscat J. The interaction of p62 with RIP links the atypical PKCs to NF-kappaB activation. Embo J 1999;18(11):3044-53.
100. Beylot M, Vidal H, Mithieux G, Odeon M, Martin C. Inhibition of hepatic ketogenesis by tumor necrosis factor-alpha in rats. Am J Physiol 1992;263(5 Pt 1):E897-902.
101. Blumberg D, Tsuburaya A, Burt M, Donner DB, Brennan MF. Acute metabolic effects of human recombinant tumor necrosis factor beta in the rat. Ann Surg Oncol 1994;1(5):373-7.
102. Spolarics Z, Wu JX. Tumor necrosis factor alpha augments the expression of glucose-6- phosphate dehydrogenase in rat hepatic endothelial and Kupffer cells. Life Sci 1997;60(8):565-71.
103. Peraldi P, Hotamisligil GS, Buurman WA, White MF, Spiegelman BM. Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. J Biol Chem 1996;271(22):13018-22.
104. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proc Natl Acad Sci U S A 1998;95(5):2498-502.
105. Schmitz-Peiffer C. Signalling aspects of insulin resistance in skeletal muscle. mechanisms induced by lipid oversupply. Cell Signal 2000;12(9-10):583-594.

106. Schmitz-Peiffer C, Craig DL, Biden TJ. 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):24202-10.
107. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proc Natl Acad Sci U S A 1998;95(5):2498-502.
108. Bose R, Verheij M, Haimovitz-Friedman A, Scotto K, Fuks Z, Kolesnick R. Ceramide synthase mediates daunorubicin-induced apoptosis: an alternative mechanism for generating death signals. Cell 1995;82(3):405-14.
109. Cabot MC, Han TY, Giuliano AE. The multidrug resistance modulator SDZ PSC 833 is a potent activator of cellular ceramide formation. FEBS Lett 1998;431(2):185-8.
110. Garzotto M, White-Jones M, Jiang Y, Ehleiter D, Liao WC, Haimovitz-Friedman A, et al. 12-O-tetradecanoylphorbol-13-acetate-induced apoptosis in LNCaP cells is mediated through ceramide synthase. Cancer Res 1998;58(10):2260-4.
111. Xu J, Yeh CH, Chen S, He L, Sensi SL, Canzoniero LM, et al. Involvement of de novo ceramide biosynthesis in tumor necrosis factor- alpha/cycloheximide-induced cerebral endothelial cell death. J Biol Chem 1998;273(26):16521-6.
112. Wang E, Norred WP, Bacon CW, Riley RT, Merrill AH, Jr. Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with Fusarium moniliforme. J Biol Chem 1991;266(22):14486-90.
113. Hickson-Bick DL, Buja ML, McMillin JB. Palmitate-mediated alterations in the fatty acid metabolism of rat neonatal cardiac myocytes. J Mol Cell Cardiol 2000;32(3):511-9.
114. Paumen MB, Ishida Y, Muramatsu M, Yamamoto M, Honjo T. Inhibition of carnitine palmitoyltransferase I augments sphingolipid synthesis and palmitate-induced apoptosis. J Biol Chem 1997;272(6):3324-9.
115. Wang CN, O'Brien L, Brindley DN. Effects of cell-permeable ceramides and tumor necrosis factor-alpha on insulin signaling and glucose uptake in 3T3-L1 adipocytes. Diabetes 1998;47(1):24-31.
116. Summers SA, Garza LA, Zhou H, Birnbaum MJ. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. Mol Cell Biol 1998;18(9):5457-64.
117. Schubert KM, Scheid MP, Duronio V. Ceramide inhibits protein kinase B/Akt by promoting dephosphorylation of serine 473. J Biol Chem 2000;275(18):13330-5.
118. Zundel W, Swiersz LM, Giaccia A. Caveolin 1-mediated regulation of receptor tyrosine kinase-associated phosphatidylinositol 3-kinase activity by ceramide. Mol Cell Biol 2000;20(5):1507-14.
119. Doornbos RP, Theelen M, van der Hoeven PC, van Blitterswijk WJ, Verkleij AJ, van Bergen en Henegouwen PM. Protein kinase Czeta is a negative regulator of protein kinase B activity. J Biol Chem 1999;274(13):8589-96.
120. DeLany JP, Windhauser MM, Champagne CM, Bray GA. Differential oxidation of individual dietary fatty acids in humans. Am J Clin Nutr 2000;72(4):905-11.
121. Kelley DE, Simoneau JA. Impaired free fatty acid utilization by skeletal muscle in non-insulin- dependent diabetes mellitus. J Clin Invest 1994;94(6):2349-56.
122. Manco M, Mingrone G, Greco AV, Capristo E, Gniuli D, De Gaetano A, et al. Insulin resistance directly correlates with increased saturated fatty acids in skeletal muscle triglycerides. Metabolism 2000;49(2):220-4.

123. Clore JN, Harris PA, Li J, Azzam A, Gill R, Zuelzer W, et al. Changes in phosphatidylcholine fatty acid composition are associated with altered skeletal muscle insulin responsiveness in normal man. Metabolism 2000;49(2):232-8.
124. Storz P, Doppler H, Wernig A, Pfizenmaier K, Muller G. Cross-talk mechanisms in the development of insulin resistance of skeletal muscle cells palmitate rather than tumour necrosis factor inhibits insulin-dependent protein kinase B (PKB)/Akt stimulation and glucose uptake. Eur J Biochem 1999;266(1):17-25.
125. Eckel J, Asskamp B, Reinauer H. Induction of insulin resistance in primary cultured adult cardiac myocytes. Endocrinology 1991;129(1):345-52.
126. Usui I, Takata Y, Imamura T, Morioka H, Sasaoka T, Sawa T, et al. Fatty acid induced insulin resistance in rat-1 fibroblasts overexpressing human insulin receptors: impaired insulin-stimulated mitogen-activated protein kinase activity. Diabetologia 1997;40(8):894-901.
127. Merrill AH, Jr., Jones DD. An update of the enzymology and regulation of sphingomyelin metabolism. Biochim Biophys Acta 1990;1044(1):1-12.
128. Boden G, Chen X, Ruiz J, White JV, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest 1994;93(6):2438-46.
129. Evans DA, Jacobs DO, Wilmore DW. Tumor necrosis factor enhances glucose uptake by peripheral tissues. Am J Physiol 1989;257(5 Pt 2):R1182-9.
130. Unger RH, Zhou YT. Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. Diabetes 2001;50 Suppl 1:S118-21.
131. Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. Faseb J 2001;15(2):312-21.
132. Bjorntorp P. Abdominal obesity and the development of noninsulin-dependent diabetes mellitus. Diabetes Metab Rev 1988;4(6):615-22.
133. Despres JP. Dyslipidaemia and obesity. Baillieres Clin Endocrinol Metab 1994;8(3):629-60.
134. Despres JP, Nadeau A, Tremblay A, Ferland M, Moorjani S, Lupien PJ, et al. Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. Diabetes 1989;38(3):304-9.
135. Pouliot MC, Despres JP, Nadeau A, Moorjani S, Prud'Homme D, Lupien PJ, et al. Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. Diabetes 1992;41(7):826-34.
136. Despres JP, Lemieux S, Lamarche B, Prud'homme D, Moorjani S, Brun LD, et al. The insulin resistance-dyslipidemic syndrome: contribution of visceral obesity and therapeutic implications. Int J Obes Relat Metab Disord 1995;19 Suppl 1:S76-86.
137. Lonnqvist F, Thome A, Nilsell K, Hoffstedt J, Arner P. A pathogenic role of visceral fat beta 3-adrenoceptors in obesity. J Clin Invest 1995;95(3):1109-16.
138. Ginsberg HN. Lipoprotein physiology in nondiabetic and diabetic states. Relationship to atherogenesis. Diabetes Care 1991;14(9):839-55.
139. Laakso M, Sarlund H, Mykkanen L. Insulin resistance is associated with lipid and lipoprotein abnormalities in subjects with varying degrees of glucose tolerance. Arteriosclerosis 1990;10(2):223-31.
140. Laws A, Reaven GM. Evidence for an independent relationship between insulin resistance and fasting plasma HDL-cholesterol, triglyceride and insulin concentrations. J Intern Med 1992;231(1):25-30.

141. Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J Clin Invest 1993;92(1):141-6.
142. Haffner SM, Mykkanen L, Robbins D, Valdez R, Miettinen H, Howard BV, et al. A preponderance of small dense LDL is associated with specific insulin, proinsulin and the components of the insulin resistance syndrome in non- diabetic subjects. Diabetologia 1995;38(11):1328-36.
143. Havel RJ, Kane JP, Balasse EO, Segel N, Basso LV. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. J Clin Invest 1970;49(11):2017-35.
144. Byrne CD, Brindle NP, Wang TW, Hales CN. Interaction of non-esterified fatty acid and insulin in control of triacylglycerol secretion by Hep G2 cells. Biochem J 1991;280(Pt 1):99-104.
145. Bruce C, Chouinard RA, Jr., Tall AR. Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. Annu Rev Nutr 1998;18:297-330.
146. Austin MA. Genetic epidemiology of dyslipidaemia and atherosclerosis. Ann Med 1996;28(5):459-63.
147. Hurt-Camejo E, Olsson U, Wiklund O, Bondjers G, Camejo G. Cellular consequences of the association of apoB lipoproteins with proteoglycans. Potential contribution to atherogenesis. Arterioscler Thromb Vasc Biol 1997;17(6):1011-7.
148. Deckelbaum RJ, Galeano NF. Small dense low density lipoprotein: formation and potential mechanisms for atherogenicity. Isr J Med Sci 1996;32(6):464-8.
149. Hamilton-Wessler M, Ader M, Dea M, Moore D, Jorgensen PN, Markussen J, et al. Mechanism of protracted metabolic effects of fatty acid acylated insulin, NN304, in dogs: retention of NN304 by albumin. Diabetologia 1999;42(10):1254-63.
150. Deigner HP, Claus R, Bonaterra GA, Gehrke C, Bibak N, Blaess M, et al. Ceramide induces aSMase expression: implications for oxLDL-induced apoptosis. Faseb J 2001;15(3):807-14.
151. Barakat HA, Carpenter JW, McLendon VD, Khazanie P, Leggett N, Heath J, et al. Influence of obesity, impaired glucose tolerance, and NIDDM on LDL structure and composition. Possible link between hyperinsulinemia and atherosclerosis. Diabetes 1990;39(12):1527-33.
152. Stern MP, Gaskill SP, Hazuda HP, Gardner LI, Haffner SM. Does obesity explain excess prevalence of diabetes among Mexican Americans? Results of the San Antonio Heart Study. Diabetologia 1983;24(4):272-7.
153. Mueller WH, Joos SK, Hanis CL, Zavaleta AN, Eichner J, Schull WJ. The Diabetes Alert study: growth, fatness, and fat patterning, adolescence through adulthood in Mexican Americans. Am J Phys Anthropol 1984;64(4):389-99.
154. Haffner SM, Stern MP, Hazuda HP, Rosenthal M, Knapp JA, Malina RM. Role of obesity and fat distribution in non-insulin-dependent diabetes mellitus in Mexican Americans and non-Hispanic whites. Diabetes Care 1986;9(2):153-61.
155. Lindeman RD, Romero LJ, Hundley R, Allen AS, Liang HC, Baumgartner RN, et al. Prevalences of type 2 diabetes, the insulin resistance syndrome, and coronary heart disease in an elderly, biethnic population. Diabetes Care 1998;21(6):959-66.

156. Aguirre MA, Jones CN, Pei D, Villa ML, Reaven GM. 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):M56-60.
157. Stern MP, Rosenthal M, Haffner SM, Hazuda HP, Franco LJ. Sex difference in the effects of sociocultural status on diabetes and cardiovascular risk factors in Mexican Americans. The San Antonio Heart Study. Am J Epidemiol 1984;120(6):834-51.
158. Gardner LI, Jr., Stern MP, Haffner SM, Gaskill SP, Hazuda HP, Relethford JH, et al. Prevalence of diabetes in Mexican Americans. Relationship to percent of gene pool derived from native American sources. Diabetes 1984;33(1):86-92.
159. Hamman RF, Marshall JA, Baxter J, Kahn LB, Mayer EJ, Orleans M, et al. Methods and prevalence of non-insulin-dependent diabetes mellitus in a biethnic Colorado population. The San Luis Valley Diabetes Study. Am J Epidemiol 1989;129(2):295-311.
160. Diehl AK, Stern MP. Special health problems of Mexican-Americans: obesity, gallbladder disease, diabetes mellitus, and cardiovascular disease. Adv Intern Med 1989;34:73-96.
161. Ramachandran A, Snehalatha C, Viswanathan V, Viswanathan M, Haffner SM. Risk of noninsulin dependent diabetes mellitus conferred by obesity and central adiposity in different ethnic groups: a comparative analysis between Asian Indians, Mexican Americans and Whites. Diabetes Res Clin Pract 1997;36(2):121-5.
162. Carter JS, Pugh JA, Monterrosa A. Non-insulin-dependent diabetes mellitus in minorities in the United States. Ann Intern Med 1996;125(3):221-32.
163. Haffner SM, Hazuda HP, Stern MP, Patterson JK, Van Heuven WA, Fong D. Effects of socioeconomic status on hyperglycemia and retinopathy levels in Mexican Americans with NIDDM. Diabetes Care 1989;12(2):128-34.
164. Haffner SM, Mitchell BD, Stern MP, Hazuda HP. Macrovascular complications in Mexican Americans with type II diabetes. Diabetes Care 1991;14(7):665-71.
165. Beach KW, Brunzell JD, Strandness DE, Jr. Prevalence of severe arteriosclerosis obliterans in patients with diabetes mellitus. Relation to smoking and form of therapy. Arteriosclerosis 1982;2(4):275-80.
166. Pugh JA, Stern MP, Haffner SM, Eifler CW, Zapata M. Excess incidence of treatment of end-stage renal disease in Mexican Americans. Am J Epidemiol 1988;127(1):135-44.
167. Harris MI, Klein R, Cowie CC, Rowland M, Byrd-Holt DD. Is the risk of diabetic retinopathy greater in non-Hispanic blacks and Mexican Americans than in non-Hispanic whites with type 2 diabetes? A U.S. population study. Diabetes Care 1998;21(8):1230-5.
168. Carter JS, Wiggins CL, Becker TM, Key CR, Samet JM. Diabetes mortality among New Mexico's American Indian, Hispanic, and non-Hispanic white populations, 1958-1987. Diabetes Care 1993;16(1):306-9.
169. Stern MP, Haffner SM. Type II diabetes and its complications in Mexican Americans. Diabetes Metab Rev 1990;6(1):29-45.
170. Neufeld ND, Raffel LJ, Landon C, Chen YD, Vadheim CM. Early presentation of type 2 diabetes in Mexican-American youth. Diabetes Care 1998;21(1):80-6.
171. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988;37(12):1595-607.
172. DeFronzo RA. Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: a balanced overview. Diabetologia 1992;35(4):389-97.

173. McCance DR, Pettitt DJ, Hanson RL, Jacobsson LT, Bennett PH, Knowler WC. Glucose, insulin concentrations and obesity in childhood and adolescence as predictors of NIDDM. Diabetologia 1994;37(6):617-23.
174. Pyorala K, Savolainen E, Kaukola S, Haapakoski J. Plasma insulin as coronary heart disease risk factor: relationship to other risk factors and predictive value during 9 1/2-year follow-up of the Helsinki Policemen Study population. Acta Med Scand Suppl 1985;701:38-52.
175. Hanis CL, Ferrell RE, Barton SA, Aguilar L, Garza-Ibarra A, Tulloch BR, et al. Diabetes among Mexican Americans in Starr County, Texas. Am J Epidemiol 1983;118(5):659-72.
176. Haffner SM, Hazuda HP, Mitchell BD, Patterson JK, Stern MP. Increased incidence of type II diabetes mellitus in Mexican Americans. Diabetes Care 1991;14(2):102-8.
177. Haffner SM. Hyperinsulinemia as a possible etiology for the high prevalence of non insulin dependent diabetes in Mexican Americans. Diabete Metab 1987;13(3 Pt 2):337-44.
178. Haffner SM, Stern MP, Dunn J, Mobley M, Blackwell J, Bergman RN. Diminished insulin sensitivity and increased insulin response in nonobese, nondiabetic Mexican Americans. Metabolism 1990;39(8):842-7.
179. Fagot-Campagna A. Emergence of type 2 diabetes mellitus in children: epidemiological evidence. J Pediatr Endocrinol Metab 2000;13(Suppl 6):1395-402.
180. Reaven P, Nader, P.R., Berry, C., Hoy, T. Cardiovascular disease insulin risk in Mexican American and Anglo American children and mothers. Pediatrics. 1998;101:E12.
181. Hanis CL, Hewett-Emmett D, Bertin TK, Schull WJ. Origins of U.S. Hispanics. Implications for diabetes. Diabetes Care 1991;14(7):618-27.
182. Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G. Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol 1985;248(3 Pt 1):E286-91.
183. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 2001:280(5):E745-51.
184. Wallace FA, Neely SJ, Miles EA, Calder PC. Dietary fats affect macrophage-mediated cytotoxicity towards tumour cells. Immunol Cell Biol 2000;78(1):40-8.
185. Blaak EE, Wolffenbuttel BH, Saris WH, Pelsers MM, Wagenmakers AJ. Weight reduction and the impaired plasma-derived free fatty acid oxidation in type 2 diabetic subjects. J Clin Endocrinol Metab 2001;86(4):1638-44.
186. Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. Annu Rev Med 1998;49:235-61.

CHAPTER 3

Differences in Insulin Sensitivity Between Mexican American and Non-Hispanic White Men and Women Persist After Accounting for Cardiorespiratory Fitness, Abdominal Fat, and Skeletal Muscle Insulin Signaling Proteins

ABSTRACT

Non-diabetic Mexican Americans (MA) tend to be less insulin sensitive compared to non-Hispanic White (NHW) Americans, although reasons for this discrepancy are not readily apparent. We determined whether differences in insulin sensitivity persist between MA and NHW after accounting for acute and chronic effects of exercise, abdominal fat distribution, dietary intake and skeletal muscle insulin signaling proteins. Cardiorespiratory fitness (VO_2max), abdominal fat distribution (by computerized tomography), and dietary intake were determined in young, nonobese, nondiabetic MA (n=13; 27.0 ± 2.0 yrs; BMI=23.0 ± 0.7) and NHW (n=13; 24.8 ± 1.5 yrs; BMI=22.8 ± 0.6) males and females. Whole body insulin sensitivity was estimated by fasting insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and the Quantitative Insulin Sensitivity Check Index (QUICKI). Muscle biopsies were obtained for measurement of the insulin receptor β subunit (IRβ), phosphotidylinositol 3-kinase (PI3K) p85 subunit, Akt1, Akt2 and GLUT4 protein levels. There were no differences between the two groups in regards to VO_2max, total adiposity, abdominal visceral fat (AVF) or abdominal subcutaneous fat (ASF). MA exhibited a tendency toward higher intakes of dietary fat. MA were significantly less insulin sensitive

compared to their NHW counterparts when estimated by HOMA-IR (MA: 1.53 ± 0.22 vs. NHW: 0.87 ± 0.16, $p<0.05$), with a trend for lower insulin sensitive based on QUICKI (0.37 ± 0.01 vs. 0.41 ± 0.02, $p=0.06$) and fasting insulin (44.84 ± 6.1 vs. 29.86 ± 4.8 pmol/L, $p=0.07$) as well. Skeletal muscle protein abundance of IRβ, PI3K p85, Akt1, Akt2 and GLUT4 were not significantly different between the two groups. Differences in HOMA-IR scores lost significance after accounting for percent intake of palmitic acid, palmitoleic acid and skeletal muscle protein abundance of IRβ. Our results suggest that differences in insulin sensitivity between nonobese, nondiabetic MA and NHW persist after accounting for effects of chronic and acute exercise, total and abdominal fat distribution. However, these differences may be mediated, in part, by dietary fat intake and skeletal muscle IRβ protein levels.

INTRODUCTION

Mexican Americans (MA) exhibit a three- to fivefold excess prevalence of type 2 diabetes mellitus (T2DM) compared to non-Hispanic white (NHW) Americans (1). Insulin resistance plays a central role in the pathophysiology of (T2DM) (2). Studies have shown that across a wide range of ages, both MA males and females demonstrate greater levels of insulin resistance compared to NHW (3-6). Reasons for this discrepancy are not clear. It has been suggested that genetic factors could explain the higher prevalence of insulin resistance in MA, possibly owing to the fact that up to 35% of their genetic make-up is attributable to Native American ancestry (7, 8).

It is also possible that lifestyle factors, including diet and exercise, contribute to the ethnic differences in insulin resistance. Visceral adiposity (9, 10), exercise (11-13) and dietary fat (14-18) have all been shown to impact peripheral insulin resistance.

In several previous studies, Mexican-Americans have been shown to exhibit lower insulin sensitivity independent of body fat and body fat patterning (1, 4-6). However, this issue is not entirely resolved given that studies which have documented diminished insulin sensitivity in nonobese, nondiabetic MA compared to NHW (3, 6, 19) utilized less sensitive methods (skinfolds, body mass index, waist-to-hip ratio) to estimate body fat and central adiposity. Furthermore, the possible contribution of lower physical activity and physical fitness in the MA, as well as differences in dietary intake were not accounted for in these studies. Because MA compared to NHW tend to exhibit greater central adiposity (19, 20), are less physically active (21) and consume a more atherogenic diet (22) it is important to more carefully examine these factors as possible contributors to the lower insulin sensitivity in MA.

Numerous studies have provided evidence that skeletal muscle insulin resistance seen in T2DM is primarily isolated to the insulin-stimulated phosphatidylinositol 3-kinase (PI3K) signaling cascade (23, 24). Under normal physiologic conditions insulin binds to the α subunit of the insulin receptor (IR). This binding activates tyrosine kinase activity in the β subunit and causes tyrosine phosphorylation of various IR substrates (IRS). Once activated, IRS-1 docks with the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) and activates its p110 catalytic subunit. Catalytic activity of PI3K phosphorylates $PI(4,5)P_2$ to $PI(3,4,5)P_3$. PIP_3 is necessary to activate 3-phosphoinositide-dependent protein kinase-1 (PDK1), which phosphorylates Akt (protein kinase B or related to A and C) on threonine

308. Subsequent phosphorylation of Akt on serine 473 by PDK2 further activates the enzyme. Unknown downstream steps enhance the translocation of GLUT4 from intracellular vesicles to the plasma membrane resulting in glucose entry into the cell. Some studies have documented diminished abundance of some of these proteins in association with insulin resistance, while others have not (25-28). Despite the well-recognized differences in insulin sensitivity between MA and NHW, there are no data available regarding possible differences in the expression of these skeletal muscle-signaling proteins, which could account for the lower insulin sensitivity in MA.

We undertook the present study to accomplish two specific aims. First, we sought to determine whether or not differences in insulin sensitivity persist between these two groups after controlling for the effects of acute and chronic exercise, abdominal fat distribution and dietary intake. Second, we sought to determine whether MA exhibit lower skeletal muscle protein concentrations of IRβ, PI3K p85, Akt1, Akt2 and GLUT4 compared to NHW, after controlling for these same potential confounders.

RESEARCH DESIGN AND METHODS

Subjects. A total of 13 nonobese MA (7 female, 6 male) were matched to 13 nonobese NHW (7 female, 6 male) aged 18-40 years, based on gender, age, and aerobic fitness (VO_2 max within 5 $ml*kg^{-1}*min^{-1}$) as measured by a graded exercise test. Subjects were eligible for participation based on the following characteristics: nonobese (BMI<30), non-smoking, apparently healthy individuals with no overt signs or symptoms of disease based on a medical history, normal fasting blood glucose (< 110 mg/dl), absence of past or present history of endocrine disorders, and resting blood pressure < 140/90 mmHg. Individuals were

excluded from participation for the following reasons: pregnancy, oral contraceptive use, tobacco use, diabetes mellitus, history of any eating disorders, history of menstrual cycle irregularities, a history of hypo- or hyperthyroidism, and use of any medications that could influence insulin sensitivity. In addition, subjects had no orthopedic problems that prohibited them from engaging in the maximal exercise bout. Subjects were weight stable (± 2.5 kg) for the previous six months. To be appropriately identified as a Mexican American, each participant traced his/her ethnicity to the four grandparents. The study protocol was approved by the Colorado State University Human Research Committee. Verbal and written informed consent was obtained from each volunteer.

Body Mass, Height, and Composition: Body weight was measured on a balance scale to the nearest 100 g. Body height was measured with a wall-mounted stadiometer to the nearest 0.1 cm. The percentage of body fat, absolute fat mass and fat-free mass were measured in all subjects using dual-energy x-ray absorptiometry (DEXA) using software version 4.5c (Lunar model DPX-IQ Lunar Corp., Madison, WI).

Abdominal Visceral Fat: The measurement of total, visceral, and subcutaneous fat in the abdominal region was performed using a General Electric High Speed CT scanner (Milwaukee, WI) with helical capability (29). A cross-sectional scan 10 mm thick, centered at the L4-L5 intervertebral space, was obtained using 170 mA with a scanning time of 2 s and a 512 X 512 matrix. Abdominal visceral fat (AVF) was measured by sectioning the intra-abdominal area by pixel density. Abdominal subcutaneous fat (ASF) was calculated by determining the area of adipose tissue within the abdominal wall. The areas of deep (DSF)

and superficial subcutaneous (SSF) adipose tissue were determined by dividing the two compartments based on the fascial delineation (30). Superficial subcutaneous adipose tissue was calculated by subtracting deep subcutaneous from total subcutaneous adipose tissue. Within each of the three compartments, the cross-sectional area of AT was measured in pixels (0.6 mm) in the attenuation range of –190 to –30 HU, using commercially available software (sliceOmatic, Tomovision Inc., Montreal, Canada).

Dietary Intake: Subjects were instructed to accurately record food intake (e.g., portion sizes, food preparation methods, brand names of products) over a four-day period using two-dimensional food models. Records were checked for completion and sufficiency of detail. Subjects were asked to provide food labels for products used to determine appropriate substitutions when actual items consumed were not in the software database. Food intake records were analyzed using the Food Intake Analysis System (FIAS 3.98 nutrient analysis program, University of Texas School of Public Health, 1998). The FIAS database consists of the Primary Data Set (PDS) and the Survey Nutrient Data Base (Survey NDB) of the National Nutrient Data Bank, developed and maintained by the United States Department of Agriculture (USDA).

Cardiorespiratory Fitness: Acute and chronic exercise are related to insulin sensitivity, and MA have been reported to exhibit lower levels of physical activity compared to NHW. Thus, we sought to control for this potential confounder by recruiting individuals from both groups who were similar in maximal oxygen consumption. Maximal oxygen consumption was measured during incremental treadmill exercise to volitional exhaustion as previously

described by Balke (31). Measurements of oxygen consumption, carbon dioxide production, pulmonary ventilation, and the respiratory exchange ratio were determined by on-line computer assisted open circuit spirometry (CPX Express, MedGraphics, Minnesota, MN). To ensure that maximal oxygen consumption was obtained, at least three out of the four following criteria were satisfied: (a) a plateau in oxygen consumption with increasing workload; (b) a respiratory exchange ratio of at least 1.15; (c) achievement of 100% of the age-predicted maximal heart rate; and (d) a maximal rating of perceived exertion of at least 18 on the Borg scale.

Insulin Sensitivity: For the determination of insulin sensitivity, subjects were instructed to remain fasted for 12 hours prior to blood collection. Furthermore, subjects refrained from participation in any form of exercise for 48 hours prior to the study. Female subjects were tested during the early follicular phase of their menstrual cycles (days 3-10). Three estimates of insulin sensitivity were used: fasting plasma insulin, homeostatic model assessment of insulin resistance (HOMA-IR) and the Quantitative Insulin Sensitivity Check Index (QUICKI) (32, 33). These estimates have been shown to correlate well with the more direct measures of insulin sensitivity, such as the frequently sampled intravenous glucose tolerance test (FSIVGT) and hyperinsulinemic-euglycemic clamp (33-36). We have previously found that QUICKI and insulin sensitivity estimated by the FSIVGT and MINMOD analysis were strongly related (see Chapter 5).

$$\text{HOMA-IR} = (\text{Fasting insulin, } \mu\text{U/mL} \times \text{Fasting glucose, mmol/L})/22.5$$

$$\text{QUICKI} = 1/[\log(\text{Fasting insulin, } \mu\text{U/mL}) + \log(\text{Fasting glucose, mg/dL})]$$

Muscle Biopsies: Muscle biopsies were obtained from all subjects to examine insulin signaling pathway intermediates and glucose transporters in skeletal muscle. Subjects reported to the laboratory following an overnight fast and 48 hours following any exercise. Percurtaneous muscle biopsies (≈75 mg) were obtained from the belly of the vastus lateralis by using a 5 mm Bergstrom needle with suction applied as described previously (37). Muscle obtained from the subjects was immediately frozen in liquid nitrogen and subsequently analyzed for the determination of IRβ, PI 3-kinase p85α/β subunit, Akt1, Akt2 and GLUT4 protein abundance.

Immunoblotting: Approximately 50 mg of frozen muscle was homogenized in ice-cold buffer (50 mM HEPES, pH 7.4, 100 mM NaF, 10 mM $Na_4P_2O_7$, 2.5 mM EDTA, 2 mM Na_3VO_4, 2 mM phenylmethylsulfonyl fluoride, 1 μM leupeptin, 1 μM pepstatin, and 0.2 μM aprotinin). Tissue lysates were solubilized with gentle mixing in 1% Triton X-100 for 1.5 h at 4° C and centrifuged 14,000 g for 15 minutes at 4° C. Total protein concentration was determined by the BCA assay (Pierce, Rockford, IL). Positive controls (fat cell lysate, Jurkat cell lysate) were used as internal standards for IRβ, PI3K p85α, Akt1, Akt2 and GLUT4. Data are expressed as protein abundance in arbitrary units, comparing one group relative to the other. Solubilized proteins (50 μg) were resolved by 7.5% SDS-PAGE and transferred to PVDF membrane. The membrane was blocked with 5% nonfat dry milk containing Tris-buffered saline (TBS) containing 0.05% Tween-20 for 1 h at room temperature. The membrane was then incubated with 3% milk for 1 h in TBS containing 0.05% Tween-20 at room temperature with antibodies to IRβ, PI3K p85α, Akt1, Akt2 or GLUT4. Membranes

were incubated with the horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature, then washed with TBS containing 0.05% Tween-20. The bands were visualized using the Enhanced Chemiluminescence System (Amersham, Arlingtom Heights, IL) and quantified by phosphorimager.

Assays: Blood glucose was measured by the glucose oxidase method using an autoanalyzer (YSI2300 Stat Plus, Yellow Springs Incorporated, Yellow Springs, OH). Plasma insulin was measured using a two-step sandwich ELISA (DSL, Webster, TX). This assay demonstrates a 2.6% intra-assay and 6.2% interassay variation. Total plasma non-esterified fatty acid (NEFA) concentrations were analyzed by colorimetric assay (Waco Chemicals, Richmond, VA).

Statistical Analysis: Data were analyzed for normality and homogeneity of variance. Dependent variables were initially subjected to normally testing using both qualitative (data plots) and quantitative (Kolmogorov-Smirnov test) approaches. Group differences were analyzed using a 2 X 2 analysis of variance (ANOVA) (ethnicity x gender). When gender differences failed to reach significance, males and females were analyzed together within ethnic groups by independent t-tests. Pearson product moment correlations and partial correlations were used to determine associations between independent and dependent variables. Analysis of covariance (ANCOVA) was performed when potentially confounding variables exhibited a significant relationship to the dependent variables. The significance level was set a priori at $P<0.05$.

RESULTS

Subject characteristics are given in Table 1. On average, both groups were composed of young, normal weight men and women of moderate cardiorespiratory fitness. There were expected gender differences in several of the physical characteristics such as height, weight, body composition, fat patterning, and VO_2 max. However, there were no ethnic differences in any of these physical characteristics, except for the lower stature of MA compared with NHW. Computerized tomography revealed that total AVF, total ASF, SSF, and DSF were not different between the two groups (Table 1).

Dietary intake of energy and macronutrients are shown in Table 2. Percent of total energy intake from palmitoleic acid was significantly higher among MA, with a trend for higher percent of total energy intake from palmitic acid, and oleic acid, and lower fiber intake among MA (Table 2). After co-varying for gender, total intake of palmitic acid was significantly higher among MA (11.39 ± 1.53 vs. 15.74 ± 2.07 g/d, $p<0.05$). Total fasting plasma NEFA were not significantly different between the two groups.

HOMA-IR was significantly higher among MA compared with NHW subjects (1.53 ± 0.22 vs. 0.87 ± 0.16, $p<0.05$). Insulin sensitivity estimated by QUICKI tended to be lower among MA compared with NHW subjects (0.37 ± 0.01 vs. 0.41 ± 0.02, $=<0.058$). There was a tendency for MA subjects to exhibit higher fasting insulin (44.84 ± 6.14 vs. 29.86 ± 4.85 pmol/l, $p=0.068$) and glucose (4.70 ± 0.19 vs. 4.22 ± 0.22 mmol/l, $p=0.11$) concentrations compared with NHW subjects, however these differences were not statistically significant.

Despite accounting for possible confounding factors shown in Table 1, differences in insulin sensitivity between MA and NHW subjects persisted (Table 3). Differences in HOMA-IR between the groups remained significant after accounting for percent intakes of

total fat, saturated fat, and all fatty acids except for palmitate and palmitoleate. Differences in HOMA-IR between the groups lost significance when percent energy intake from palmitic acid, percent energy intake from palmitoleic acid, or protein abundance of IRβ were accounted for. Correlations between HOMA-IR and these covariates are shown in Figure 1.

Representative immunoblots are shown in Figure 2. There were no significant differences between the two groups in regard to skeletal muscle protein abundance of IRβ subunit, PI3K p85, Akt1, Akt2 or GLUT4 (Figure 3). Skeletal muscle protein abundance of IRβ was significantly associated with fasting plasma insulin ($r = -0.46$, $p<0.05$), HOMA-IR ($r = -0.43$, $p<0.05$) and QUICKI ($r=0.41$, $p< 0.05$). Skeletal muscle Akt2 protein levels were associated with HOMA-IR after accounting for gender, although this relationship did not reach statistical significance ($r = -0.37$, $p=0.07$). Abundance of the other signaling proteins studied were not related to estimates of insulin sensitivity.

Pearson product-moment correlations were used to analyze relationships between estimates of insulin sensitivity and dietary variables (Table 4). When the groups were analyzed in aggregate, fasting insulin was associated with percent of energy intake from fat ($r=0.43$, $p<0.05$), percent of energy intake from monounsaturated fat and fiber intake. The degree of insulin resistance estimated by HOMA-IR and QUICKI were associated with percent energy intake from fat, percent energy intake from monounsaturated fat, and percent energy intake from saturated fat. HOMA-IR and QUICKI were significantly associated with the intake of individual fatty acids, palmitate, stearate, palmitoleate and oleate. The degree of insulin resistance estimated by HOMA-IR and QUICKI were also associated with percent energy intake from carbohydrate and fiber intake.

Pearson product-moment correlations were used to analyze relationships between among insulin sensitivity, adiposity measures, plasma NEFA, and cardiorespiratory fitness. Indices of insulin sensitivity (fasting insulin, HOMA-IR, QUICKI) were not related to BMI, percent body fat, abdominal visceral fat, abdominal subcutaneous fat, superficial subcutaneous fat or deep subcutaneous fat (Table 5). Fasting plasma NEFA concentrations were not related to fasting insulin, HOMA-IR, or QUICKI. Fasting plasma NEFA was significantly associated with both fasting glucose ($r=0.49$, $p<0.05$) and percent of total calories from fat ($r=0.44$, $p<0.05$). Among the entire sample, cardiorespiratory fitness expressed as maximal metabolic equivalents (maximal exercise VO_2 relative to resting VO_2) was associated with indices of insulin sensitivity (HOMA-IR: $r=0.48$, $p<0.02$, QUICKI: $r=0.47$, $p<0.02$, fasting insulin: $r=0.49$, $p<0.02$) and also IR-β ($r=0.43$, $p<0.05$).

DISCUSSION

There a several important findings from this study that warrant discussion. First, nonobese, nondiabetic MA adults were less insulin sensitive compared to NHW adults, even when accounting for the potential role of cardiorespiratory fitness, acute exercise, and total and regional adiposity. Second, skeletal muscle protein abundance of IRβ, PI3K p85, Akt1, Akt2 and GLUT4 were not significantly different between the two groups, and therefore do not account for the group differences in insulin sensitivity. Finally, group differences in insulin sensitivity failed to reach statistical significance after accounting for dietary intake of palmitic acid, palmitoleic acid or skeletal muscle IRβ protein content.

The results of our study extend previous findings in several important areas. Previous studies have used crude anthropometric methods (body mass index, skinfolds) to measure

body adiposity (1, 3), while the present data suggest that differences in insulin sensitivity between nonobese, nondiabetic MA and NHW persist after accounting for both total and abdominal adiposity determined by computerized tomography. It has recently been suggested that fat stored in various abdominal compartments (visceral vs. subcutaneous) confers different degrees of association with insulin resistance (38). Abdominal obesity is widely recognized as a strong correlate of insulin resistance. Visceral adiposity, independent of total adiposity, has been implicated in the etiology of skeletal muscle insulin resistance and T2DM (39, 40). Recently, it has reported that both abdominal visceral and deep subcutaneous adiposity are strongly associated with peripheral insulin resistance, while superficial abdominal fat is not (38). The use of CT scans in the present study allowed us to characterize these specific aspects of fat patterning, while other studies have used less sophisticated approaches such as measurement of skinfold thickness, waist circumference, or waist-to-hip ratios. Why then, given the use of the gold standard, was the magnitude of visceral adiposity not associated with the measures of insulin sensitivity in our study? One possible explanation is that because all subjects were non-obese, the lack of adequate heterogeneity in both abdominal fat and insulin sensitivity may have masked a relationship. Nevertheless, it is evident that even within a homogeneous sample, non-obese MA are less insulin sensitive than NHW, with this difference unrelated to visceral adiposity.

Dietary fat has been considered to be a risk factor in the development of insulin resistance and T2DM (18), but many studies have failed to account for the higher intake of dietary fat commonly reported among MA. Here, we have shown that MA exhibit a tendency to consume higher amounts of dietary fat, which partially mediate differences in insulin sensitivity when compared with NHW. Our findings are in agreement with numerous

studies showing that high dietary fat and saturated fat intake is associated with reduced insulin sensitivity (41-43). MA children exhibiting a greater degree of insulin resistance compared to NHW have been reported to consume higher than recommended percent energy from fat and saturated fat (22, 44). There was a trend for MA in our study to ingest more total and saturated fatty acids than NHW, with differences reaching statistical significance for palmitate and palmitoleate. Previous reports have shown an inverse relationship between insulin sensitivity and the dietary intake of palmitate and palmitoleate (45). Together these data suggest the possibility that the higher intake of specific dietary fatty acids among MA may contribute to their lower insulin sensitivity.

While it has been clearly established that exercise improves insulin action in skeletal muscle, some previous studies have neglected to account for the possible influences of acute and chronic exercise on estimates of insulin sensitivity (3, 6). Our data suggest that differences in insulin sensitivity between these two groups persist after accounting for the effects of acute exercise and cardiorespiratory fitness. No data are available regarding the effect of cardiorespiratory fitness on insulin sensitivity in MA. The effects of exercise on skeletal muscle insulin action have been clearly demonstrated, although not in MA (12, 46, 47). These improvements are typically observed within the first 24 hours following an exercise bout (48-50). Some studies have suggested that chronic exercise training is also associated with improved insulin responsiveness (51-53). A recent study found that cardiorespiratory fitness levels (VO_2max) were associated with fasting insulin concentrations after controlling for age, percent body fat and waist circumference (54). We also found a significant relationship between cardiorespiratory fitness and measures of insulin sensitivity in our entire sample. We hypothesized that when controlling for any confounding effects of

acute exercise (by eliminating all exercise for 48 h prior to blood sampling), and matching MA and NHW for cardiorespiratory fitness there would be no decrement in insulin sensitivity in MA. However, our data indicate the lower insulin sensitivity in MA persists even when rigorously controlling for fitness and acute exercise.

We were also interested in examining whether or not nonobese, nondiabetic MA displayed differences in several of the main proteins involved in the PI3K insulin-signaling pathway. While insulin receptor number have been shown to be significantly decreased in isolated adipocytes from obese T2DM patients (25), the number has been shown to be only slightly reduced in skeletal muscle of these individuals (55). One study found that protein levels of PI3K were lower in diabetic vs. nondiabetic mice (26), while Andreelli and colleagues (56) found that mRNA levels of IRS-1, PI3K p85α and GLUT4 were not different in skeletal muscle of controls and type 2 diabetic patients. Another study reported the amount of Akt2 in skeletal muscle of obese rats was 56% lower compared to lean rats, but there were no differences in the amount of IR or the p85 regulatory subunit of PI3K in insulin resistant vs. control skeletal muscle (57). Kim et al. (27) found no differences in the amount of Akt1 between insulin resistant obese and lean rats. While diabetic individuals exhibit impaired Akt activity, no differences in Akt protein levels between healthy volunteers and type 2 diabetics have been documented (58, 59). Several studies have documented a reduction in GLUT4 expression in isolated adipocytes (28,67) and skeletal muscle (71) of patients with T2DM. However, other studies have failed to show reduced GLUT4 expression in skeletal muscle of type 2 diabetic patients (60-62). One study has reported that GLUT4 density is lower in slow-twitch skeletal muscle fibers of T2DM individuals (63). In the present study, we found that in the entire sample, estimates of insulin sensitivity were

related to IR-β and Akt2 expression. However, the lack of group differences in signaling proteins indicates that the lower insulin sensitivity in MA is not the result of reduced skeletal muscle expression of IRβ, PI3K p85, Akt1, Akt2 or GLUT4.

We can only speculate on the mechanisms responsible for the lower insulin sensitivity in MA compared with NHW in the present investigation. Palmitate is one of the least preferred substrates for skeletal muscle β oxidation (64), and has been implicated in the *de novo* synthesis of ceramide, a known inhibitor of insulin-stimulated glucose uptake (65). The possible relation of fatty acid intake, ceramide production, and insulin sensitivity should be addressed in future studies of MA.

There are some potential limitations of the present study that should be addressed. First, we used only fasting samples of glucose and insulin to determine insulin sensitivity via HOMA-IR and QUICKI, rather than the more clinically sophisticated frequently sampled glucose tolerance test or the hyperinsulinemic-euglycemic clamp. The reasons we used this approach are as follows. Our laboratory has previously established strong associations between the FSIGT and the indices of insulin sensitivity used in the current study: fasting insulin ($r= -0.53$, $p=0.001$), HOMA-IR ($r= -0.53$, $p=0.001$), QUICKI ($r=0.70$, $p<0.001$, unpublished observations). Other studies have also reported strong associations between these estimates and the hyperinsulinemic-euglycemic clamp technique (33-35). Haffner and colleagues have previously shown the HOMA-IR method able to readily characterize the lower insulin sensitivity in MA compared to NHW (66). Furthermore, it has been suggested that estimates of insulin sensitivity based on fasting insulin and glucose may be the best markers of early insulin resistance in nondiabetic individuals (67, 68). Thus this approach seemed especially well suited for the study of young, non-obese, non-diabetic individuals, all

of whom exhibited fasting blood glucose concentrations less than 110 mg/dl. Note that although the MA subjects were less insulin sensitive based on HOMA-IR, they are clearly not to be considered 'insulin resistant' based on fasting insulin, HOMA-IR or QUICKI values. However, previous studies have implicated diminished insulin sensitivity among nondiabetic MA as a risk factor for future insulin resistance and development of T2DM (3, 4).

The lack of group differences in skeletal muscle cytosolic insulin signaling proteins is a novel finding. However, we did not evaluate functional activity or insulin-stimulated phosphorylation of these intermediates. Clinical states of insulin resistance have been associated with impairments in insulin-stimulated glucose uptake, and are associated with decreases in IRS-1 tyrosine phosphorylation, PI3K activity and Akt serine/threonine phosphorylation. Kim et al. (69) and others (23, 70, 71) have reported that insulin-stimulated IRS-1-associated PI3K activity is decreased in skeletal muscle of diabetic subjects. While impaired activities of these proteins appear to be primary defects insulin-stimulated GLUT4 translocation, reduced protein content may contribute to some models of human insulin resistance.

A third caveat to this study is that, while we found that total plasma NEFA was associated with percent fat intake, we did not determine the distribution of individual plasma NEFA. Therefore, it is possible that while total plasma NEFA are similar between the two groups, the preponderance of individual fatty acid species (e.g. palmitate) may differ and contribute to the observed insulin resistance. Future research should examine the distribution of individual fatty acids (i.e. saturated vs. unsaturated) in plasma NEFA.

In summary, the present study demonstrates that lower insulin sensitivity persists in nonobese, nondiabetic Mexican Americans compared to their nonHispanic White counterparts, even after accounting for acute and chronic effects of exercise and abdominal fat distribution. Furthermore, these differences are not explained by the protein abundance of skeletal muscle IRβ, PI3K p85, Akt1, Akt2 or GLUT4. Differences in insulin sensitivity are lost when skeletal muscle IRβ protein abundance and dietary intakes of palmitate and palmitoleate are accounted for, suggesting the possible role of these factors in contributing to the lower insulin sensitivity seen in Mexican Americans.

TABLE 1. Subject Characteristics.

	NHW males and females	MA males and females	NHW females	NHW males	MA females	MA males
n	13	13	7	6	7	6
Age, y	24.84 ± 1.49	27.00 ± 2.00	23.6 ± 1.8	26.3 ± 2.5	28.14 ± 2.88	25.67 ± 2.94
Height, m	1.76 ± 0.02[1]	1.68 ± 0.02	1.70 ± 0.02[2]	1.83 ± 0.02[134]	1.64 ± 0.02[2]	1.74 ± 0.01[23]
Weight, kg	71.23 ± 3.30	65.48 ± 2.34	63.25 ± 3.04[2]	80.55 ± 3.37[13]	60.81 ± 2.90[2]	70.92 ± 2.41
BMI, kg/m^2	22.80 ± 0.58	23.04 ± 0.69	21.72 ± 0.72	24.05 ± 0.65	22.63 ± 1.09	23.53 ± 0.86
% Body fat	20.10 ± 2.14	21.67 ± 2.67	24.13 ± 2.46	15.40 ± 2.67[3]	26.83 ± 3.96[2]	15.65 ± 1.27
AVF, cm^2	43.74 ± 7.26	38.29 ± 4.90	29.41 ± 7.55[2]	58.07 ± 7.55[13]	29.97 ± 6.99[2]	47.99 ± 7.55
ASF, cm^2	145.38 ± 17.81	174.72 ± 24.96	134 ± 31.84	156.52 ± 31.84	200.23 ± 29.48	144.96 ± 31.84
SSF, cm^2	76.53 ± 9.78	86.22 ± 12.50	74.57 ± 16.72	78.51 ± 16.72	96.87 ± 15.48	73.81 ± 16.72
DSF, cm^2	68.85 ± 8.86	85.72 ± 12.35	59.69 ± 15.62	78.01 ± 15.63	98.22 ± 14.47	71.15 ± 15.63
V/S	0.33 ± 0.06	0.29 ± 0.05	0.24 ± 0.03	0.43 ± 0.13	0.19 ± 0.04	0.39 ± 0.09
VO_2max						
mL/kg/min	44.57 ± 1.43	44.84 ± 2.33	41.84 ± 1.58	47.75 ± 1.84	40.23 ± 3.07[4]	50.22 ± 2.11[3]
mL/kg FFM/min	55.77 ± 0.88	56.86 ± 1.42	55.21 ± 1.37	56.42 ± 1.09	54.64 ± 1.76	59.45 ± 1.90
L/min	3.18 ±0.19	2.94 ± 0.19	2.64 ± 0.13[24]	3.82 ± 0.09[13]	2.42 ± 0.15[24]	3.54 ± 0.12[13]

Mean ± SEM; AVF = abdominal visceral fat; ASF = abdominal subcutaneous fat; SSF = superficial subcutaneous fat; DSF = deep subcutaneous fat; [1] $p<0.05$ vs. MA males and females; [2] $p<0.05$ vs. NHW males; [3] $p<0.05$ vs. MA females; [4] $p<0.05$ vs. MA males

Table 2. Dietary intake for MA and NHW subjects determined by 4-day diet record.

	NHW n = 13	MA n = 13
Total energy intake, kcal/day	2047.64 ± 225.93	2326.20 ± 240.32
Carbohydrate, g/d	289.55 ± 34.45	302.35 ± 32.09
Carbohydrate, %	56.8 ± 2.5	52.6 ± 2.5
Protein, g/d	84.19 ± 5.41	93.51 ± 10.02
Protein, % of total kcal	17.3 ± 0.8	16.2 ± 0.6
Fat, g/d	65.65 ± 9.56	85.58 ± 10.53
Fat, % of total kcal	28.0 ± 2.0	32.7 ± 2.1
Saturated fat, g/d	21.36 ± 3.08	28.61 ± 3.98
Saturated fat, % of total kcal	9.2 ±0.8	10.8 ± 0.7
Palmitic acid, g/d	11.39 ± 1.53	15.74 ± 2.07
Palmitic acid, %	4.9 ± 0.4	6.0 ± 0.4
Stearic acid, g/d	5.60 ± 0.95	7.18 ± 0.99
Stearic acid, %	2.3 ± 0.2	2.7 ± 0.1
MUFA, g/d	24.58 ± 3.89	32.74 ± 4.07
MUFA, % of total kcal	10.4 ± 0.9	12.5 ± 0.7
Palmitoleic acid, g/d	0.88 ± 0.09	1.53 ± 0.25[1]
Palmitoleic acid, %	0.4 ± 0.04	0.6 ± 0.06[1]
Oleic acid, g/d	22.90 ± 3.49	30.54 ± 3.77
Oleic acid, %	9.7 ± 0.8	11.7 ± 0.7
PUFA, g/d	15.22 ± 2.55	17.45 ± 2.43
PUFA, % of total kcal	6.5 ± 0.7	6.9 ± 0.8
Linoleic acid, g/d	12.61 ± 1.88	14.88 ± 2.53
Linoleic acid, %	5.4 ± 0.5	5.7 ± 0.9
Linolenic acid, g/d	1.39 ± 0.24	1.37 ± 0.91
Linolenic acid, %	0.6 ± 0.06	0.5 ± 0.09
P/S	0.79 ± 0.11	0.65 ± 0.06
Fiber, g/1000 kcal	12.93 ± 1.59	9.09 ± 1.10

Mean ± SEM; MUFA, monounsaturated fat; PUFA, polyunsaturated fat; P/S, polyunsaturated-to-saturated fat ratio; [1] $p<0.05$ vs. NHW

TABLE 3. Estimates of insulin sensitivity for MA and NHW subjects.

	NHW n = 13	MA n = 13
Fasting glucose, mmol·L	4.22 ± 0.22	4.70 ± 0.19
Fasting insulin, pmol·L	29.86 ± 4.86	44.84 ± 6.14
HOMA-IR	0.91 ± 0.15	1.53 ± 0.22[1]
QUICKI	0.41 ± 0.02	0.37 ± 0.01

Mean ± SEM; [1] $p<0.05$

Table 4. Relationship between dietary intake and estimates of insulin sensitivity.

	Fasting insulin	HOMA-IR	QUICKI
Total energy intake, kcal/day	0.13	0.21	-0.21
Carbohydrate, % of total kcal	-0.39	-0.43[1]	0.50[1]
Protein, % of total kcal	-.017	-0.23	0.28
Fat, % of total kcal	0.43[1]	0.52[1]	-0.57[2]
Saturated fat	0.35	0.42[1]	-0.46[1]
Palmitic acid	0.38	0.47[1]	-0.51[1]
Stearic acid	0.35	0.42[1]	-0.48[1]
Monounsaturated fat	0.48[1]	0.58[2]	-0.64[2]
Palmitoleic acid	0.39	0.54[2]	-0.49[1]
Oleic acid	0.46[1]	0.56[2]	-0.63[2]
Polyunsaturated fat	0.15	0.22	-0.23
Linoleic acid	0.30	0.34	-0.31
Linolenic acid	0.16	0.20	-0.13
P/S	-0.26	-0.23	0.34
Fiber, g/1000 kcal	-0.52[1]	-0.51[1]	0.64[2]

Values are expressed in terms of Pearson's r-coefficients. [1] $p<0.05$; [2] $p<0.01$

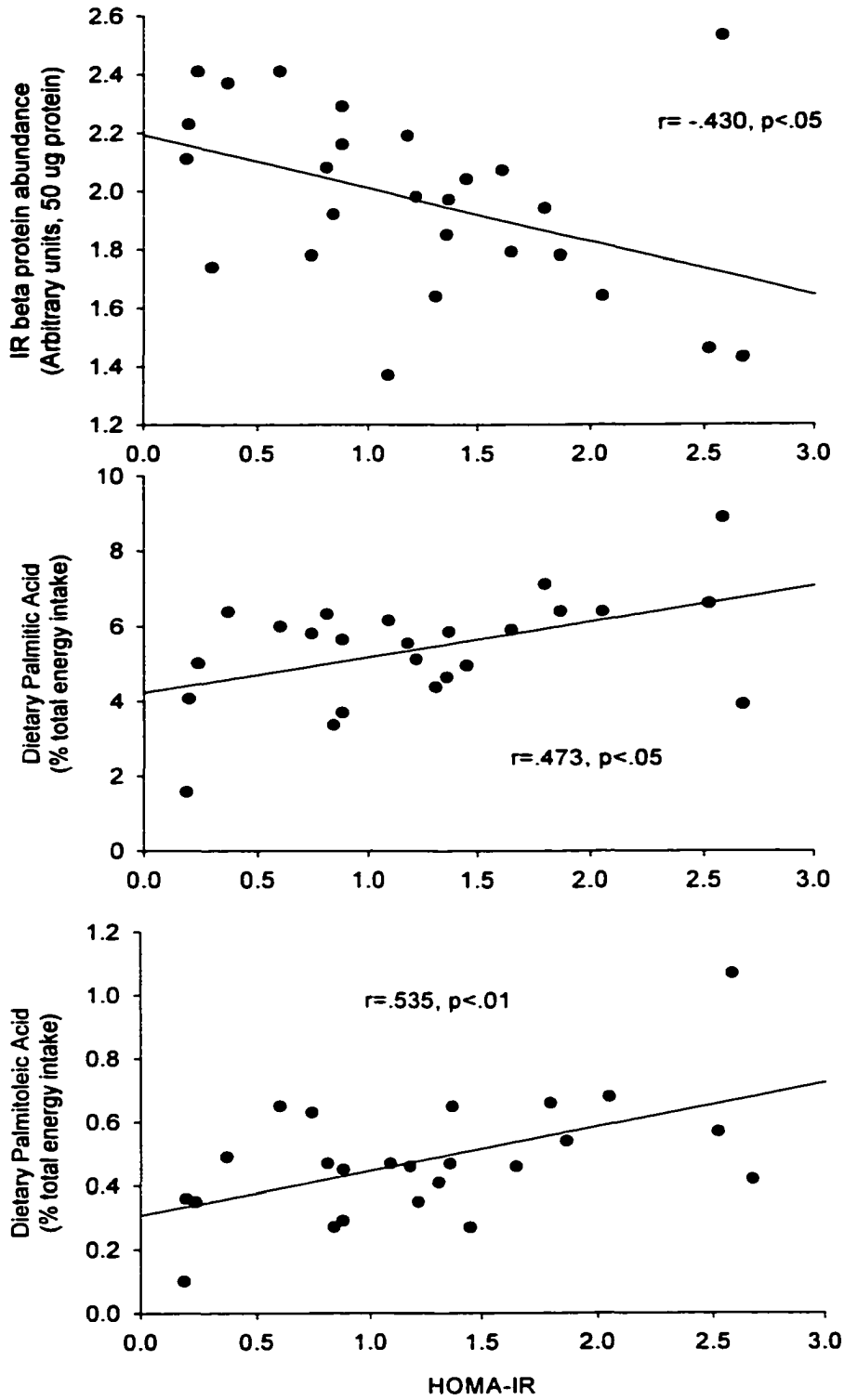

Figure 1. Relationships between HOMA-IR and IRβ, dietary palmitate and dietary palmitoleic acid intake.

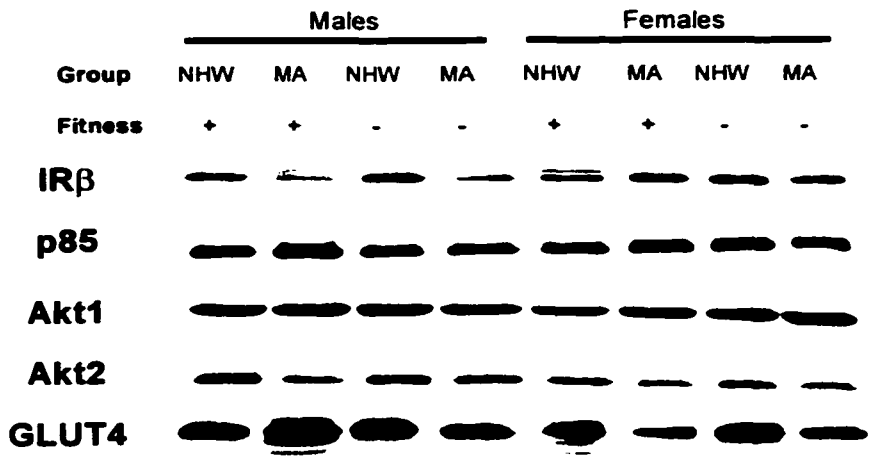

Figure 2. Representative immunoblots of IRβ, p85, Akt1, Akt2 and GLUT4 from more fit (+) and less fit (-) Mexican American and Non-Hispanic White men and women.

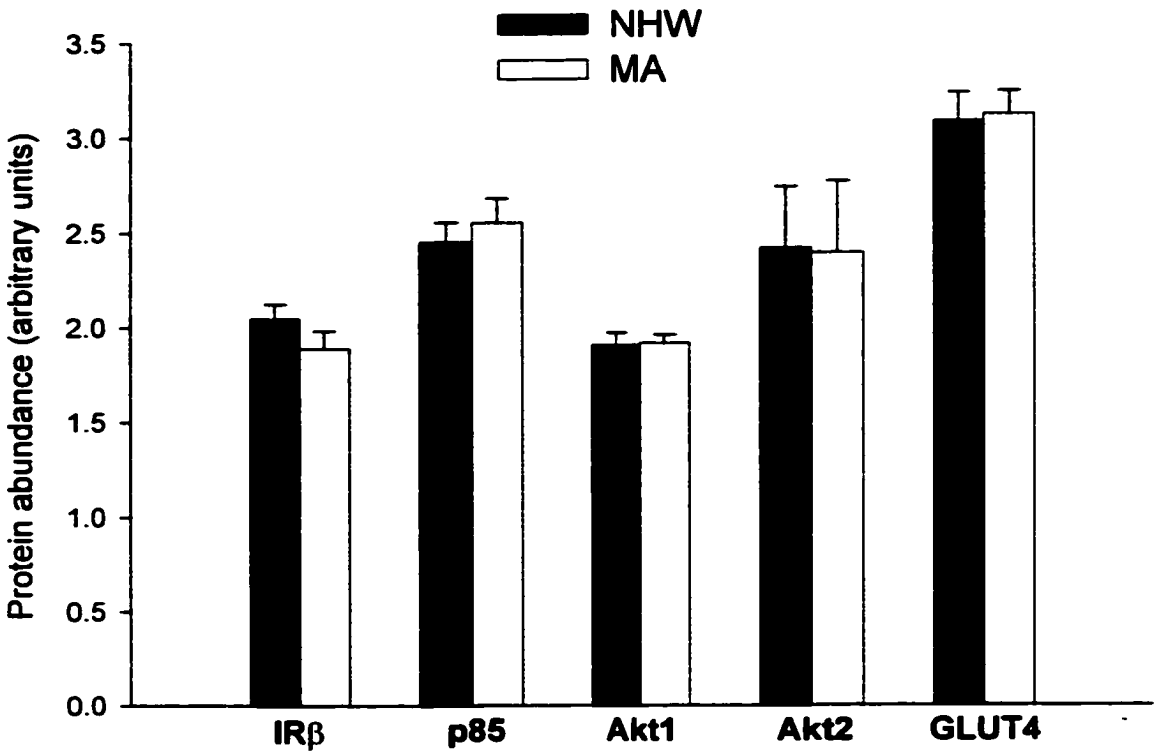

Figure 3. Protein abundance of IRβ. PI3K p85, Akt1, Akt2 and GLUT4 in Mexican American and Non-Hispanic White Men and Women.

REFERENCES

1. Stern MP, Gaskill SP, Hazuda HP, Gardner LI, Haffner SM. Does obesity explain excess prevalence of diabetes among Mexican Americans? Results of the San Antonio Heart Study. Diabetologia 1983;24(4):272-7.
2. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995;75(3):473-86.
3. Haffner SM, Stern MP, Dunn J, Mobley M, Blackwell J, Bergman RN. Diminished insulin sensitivity and increased insulin response in nonobese, nondiabetic Mexican Americans. Metabolism 1990;39(8):842-7.
4. Batey LS, Goff DC, Jr., Tortolero SR, Nichaman MZ, Chan W, Chan FA, 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):4319-25.
5. Tortolero SR, Goff DC, Jr., Nichaman MZ, Labarthe DR, Grunbaum JA, Hanis CL. Cardiovascular risk factors in Mexican-American and non-Hispanic white children: The Corpus Christi Child Heart Study. Circulation 1997;96(2):418-23.
6. Aguirre MA, Jones CN, Pei D, Villa ML, Reaven GM. 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):M56-60.
7. Chakraborty R, Ferrell RE, Stern MP, Haffner SM, Hazuda HP, Rosenthal M. Relationship of prevalence of non-insulin-dependent diabetes mellitus to Amerindian admixture in the Mexican Americans of San Antonio, Texas. Genet Epidemiol 1986;3(6):435-54.
8. Hanis CL, Hewett-Emmett D, Bertin TK, Schull WJ. Origins of U.S. Hispanics. Implications for diabetes. Diabetes Care 1991;14(7):618-27.
9. Kissebah AH. Intra-abdominal fat: is it a major factor in developing diabetes and coronary artery disease? Diabetes Res Clin Pract 1996;30 Suppl:25-30.
10. Kim JY, Nolte LA, Hansen PA, Han DH, Ferguson K, Thompson PA, et al. High-fat diet-induced muscle insulin resistance: relationship to visceral fat mass [In Process Citation]. Am J Physiol Regul Integr Comp Physiol 2000;279(6):R2057-65.
11. LeBlanc J, Nadeau A, Richard D, Tremblay A. Studies on the sparing effect of exercise on insulin requirements in human subjects. Metabolism 1981;30(11):1119-24.
12. Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. Annu Rev Med 1998;49:235-61.
13. Goodyear LJ, Hirshman MF, Horton ED, Knutson SM, Wardzala LJ, Horton ES. Exercise training normalizes glucose metabolism in a rat model of impaired glucose tolerance. Metabolism 1991;40(5):455-64.
14. Pedersen O, Kahn CR, Flier JS, Kahn BB. 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):771-7.
15. Hansen PA, Han DH, Marshall BA, Nolte LA, Chen MM, Mueckler M, et al. A high fat diet impairs stimulation of glucose transport in muscle. Functional evaluation of potential mechanisms. J Biol Chem 1998;273(40):26157-63.

16. Swinburn BA, Boyce VL, Bergman RN, Howard BV, Bogardus C. Deterioration in carbohydrate metabolism and lipoprotein changes induced by modern, high fat diet in Pima Indians and Caucasians. J Clin Endocrinol Metab 1991;73(1):156-65.
17. Zierath JR, Houseknecht KL, Gnudi L, Kahn BB. High-fat feeding impairs insulin-stimulated GLUT4 recruitment via an early insulin-signaling defect. Diabetes 1997;46(2):215-23.
18. Mayer-Davis EJ, Monaco JH, Hoen HM, Carmichael S, Vitolins MZ, Rewers MJ, 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):79-87.
19. Haffner SM, Stern MP, Hazuda HP, Rosenthal M, Knapp JA, Malina RM. Role of obesity and fat distribution in non-insulin-dependent diabetes mellitus in Mexican Americans and non-Hispanic whites. Diabetes Care 1986;9(2):153-61.
20. Stern MP, Patterson JK, Mitchell BD, Haffner SM, Hazuda HP. Overweight and mortality in Mexican Americans. Int J Obes 1990;14(7):623-9.
21. Crespo CJ, Keteyian SJ, Heath GW, Sempos CT. Leisure-time physical activity among US adults. Results from the Third National Health and Nutrition Examination Survey. Arch Intern Med 1996;156(1):93-8.
22. Trevino RP, Marshall RM, Jr., Hale DE, Rodriguez R, Baker G, Gomez J. Diabetes risk factors in low-income Mexican-American children. Diabetes Care 1999;22(2):202-7.
23. Bjornholm M, Kawano Y, Lehtihet M, Zierath JR. 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):524-7.
24. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 2000;105(3):311-20.
25. Sinha MK, Pories WJ, Flickinger EG, Meelheim D, Caro JF. Insulin-receptor kinase activity of adipose tissue from morbidly obese humans with and without NIDDM. Diabetes 1987;36(5):620-5.
26. Bonini JA, Colca JR, Dailey C, White M, Hofmann C. Compensatory alterations for insulin signal transduction and glucose transport in insulin-resistant diabetes. Am J Physiol 1995;269(4 Pt 1):E759-65.
27. Kim YB, Peroni OD, Franke TF, Kahn BB. Divergent regulation of Akt1 and Akt2 isoforms in insulin target tissues of obese Zucker rats. Diabetes 2000;49(5):847-56.
28. Sinha MK, Raineri-Maldonado C, Buchanan C, Pories WJ, Carter-Su C, Pilch PF, et al. Adipose tissue glucose transporters in NIDDM. Decreased levels of muscle/fat isoform. Diabetes 1991;40(4):472-7.
29. Ferland M, Despres JP, Tremblay A, Pinault S, Nadeau A, Moorjani S, et al. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr 1989;61(2):139-48.
30. Johnson D, Dixon AK, Abrahams PH. The abdominal subcutaneous tissue: computed tomographic, magnetic resonance, and anatomical observations. Clin Anat 1996;9(1):19-24.
31. Balke B, Ware, R.W. An experimental study of physical fitness in Air Force personnel. US Armed Forces Medicine. 1959;10:675-688.
32. Haffner SM, Kennedy E, Gonzalez C, Stern MP, Miettinen H. A prospective analysis of the HOMA model. The Mexico City Diabetes Study. Diabetes Care 1996;19(10):1138-41.

33. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85(7):2402-10.
34. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23(1):57-63.
35. Anderson RL, Hamman RF, Savage PJ, Saad MF, Laws A, Kades WW, et al. Exploration of simple insulin sensitivity measures derived from frequently sampled intravenous glucose tolerance (FSIGT) tests. The Insulin Resistance Atherosclerosis Study. Am J Epidemiol 1995;142(7):724-32.
36. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.
37. Hickey MS, Weidner MD, Gavigan KE, Zheng D, Tyndall GL, Houmard JA. The insulin action-fiber type relationship in humans is muscle group specific. Am J Physiol 1995;269(1 Pt 1):E150-4.
38. Kelley DE, Thaete FL, Troost F, Huwe T, Goodpaster BH. Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. Am J Physiol Endocrinol Metab 2000;278(5):E941-8.
39. Despres JP, Lemieux S, Lamarche B, Prud'homme D, Moorjani S, Brun LD, et al. The insulin resistance-dyslipidemic syndrome: contribution of visceral obesity and therapeutic implications. Int J Obes Relat Metab Disord 1995;19 Suppl 1:S76-86.
40. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. 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):2893-8.
41. Lovejoy J, DiGirolamo M. Habitual dietary intake and insulin sensitivity in lean and obese adults. Am J Clin Nutr 1992;55(6):1174-9.
42. Marshall JA, Hamman RF, Baxter J. High-fat, low-carbohydrate diet and the etiology of non-insulin- dependent diabetes mellitus: the San Luis Valley Diabetes Study. Am J Epidemiol 1991;134(6):590-603.
43. Budohoski L, Panczenko-Kresowska B, Langfort J, Zernicka E, Dubaniewicz A, Ziemlanski S, et al. Effects of saturated and polyunsaturated fat enriched diet on the skeletal muscle insulin sensitivity in young rats. J Physiol Pharmacol 1993;44(4):391-8.
44. Webber LS, Osganian V, Luepker RV, Feldman HA, Stone EJ, Elder JP, et al. Cardiovascular risk factors among third grade children in four regions of the United States. The CATCH Study. Child and Adolescent Trial for Cardiovascular Health. Am J Epidemiol 1995;141(5):428-39.
45. Vessby B, Tengblad S, Lithell H. Insulin sensitivity is related to the fatty acid composition of serum lipids and skeletal muscle phospholipids in 70-year-old men. Diabetologia 1994;37(10):1044-50.
46. Devlin JT. Effects of exercise on insulin sensitivity in humans. Diabetes Care 1992;15(11):1690-3.
47. Henriksson J. Influence of exercise on insulin sensitivity. J Cardiovasc Risk 1995;2(4):303-9.

48. Devlin JT, Hirshman M, Horton ED, Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. Diabetes 1987;36(4):434-9.
49. Youngren JF, Keen S, Kulp JL, Tanner CJ, Houmard JA, Goldfine ID. Enhanced muscle insulin receptor autophosphorylation with short-term aerobic exercise training. Am J Physiol Endocrinol Metab 2001;280(3):E528-33.
50. Kirwan JP, del Aguila LF, Hernandez JM, Williamson DL, O'Gorman DJ, Lewis R, et al. Regular exercise enhances insulin activation of IRS-1-associated PI3- kinase in human skeletal muscle. J Appl Physiol 2000;88(2):797-803.
51. Stallknecht B, Larsen JJ, Mikines KJ, Simonsen L, Bulow J, Galbo H. Effect of training on insulin sensitivity of glucose uptake and lipolysis in human adipose tissue. Am J Physiol Endocrinol Metab 2000;279(2):E376-85.
52. Mikines KJ, Sonne B, Farrell PA, Tronier B, Galbo H. Effect of physical exercise on sensitivity and responsiveness to insulin in humans. Am J Physiol 1988;254(3 Pt 1):E248-59.
53. Mikines KJ, Sonne B, Farrell PA, Tronier B, Galbo H. Effect of training on the dose-response relationship for insulin action in men. J Appl Physiol 1989;66(2):695-703.
54. Kriska AM, Hanley AJ, Harris SB, Zinman B. Physical Activity, Physical Fitness, and Insulin and Glucose Concentrations in an Isolated Native Canadian Population Experiencing Rapid Lifestyle Change. Diabetes Care 2001;24(10):1787-1792.
55. Caro JF, Sinha MK, Raju SM, Ittoop O, Pories WJ, Flickinger EG, et al. Insulin receptor kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes. J Clin Invest 1987;79(5):1330-7.
56. Andreelli F, Laville M, Vega N, Riou JP, Vidal H. Regulation of gene expression during severe caloric restriction: lack of induction of p85 alpha phosphatidylinositol 3-kinase mRNA in skeletal muscle of patients with type II (non-insulin-dependent) diabetes mellitus. Diabetologia 2000;43(3):356-63.
57. Dunaif A, Wu X, Lee A, Diamanti-Kandarakis E. Defects in insulin receptor signaling in vivo in the polycystic ovary syndrome (PCOS). Am J Physiol Endocrinol Metab 2001;281(2):E392-9.
58. Krook A, Roth RA, Jiang XJ, Zierath JR, Wallberg-Henriksson H. Insulin-stimulated Akt kinase activity is reduced in skeletal muscle from NIDDM subjects. Diabetes 1998;47(8):1281-6.
59. Carvalho E, Eliasson B, Wesslau C, Smith U. Impaired phosphorylation and insulin-stimulated translocation to the plasma membrane of protein kinase B/Akt in adipocytes from Type II diabetic subjects [In Process Citation]. Diabetologia 2000;43(9):1107-15.
60. Ciaraldi TP, Carter L, Mudaliar S, Kern PA, Henry RR. Effects of tumor necrosis factor-alpha on glucose metabolism in cultured human muscle cells from nondiabetic and type 2 diabetic subjects. Endocrinology 1998;139(12):4793-800.
61. Pedersen O, Bak JF, Andersen PH, Lund S, Moller DE, Flier JS, et al. Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity or NIDDM. Diabetes 1990;39(7):865-70.
62. Garvey WT, Maianu L, Hancock JA, Golichowski AM, Baron A. Gene expression of GLUT4 in skeletal muscle from insulin-resistant patients with obesity, IGT, GDM, and NIDDM. Diabetes 1992;41(4):465-75.
63. Gaster M, Staehr P, Beck-Nielsen H, Schroder HD, Handberg A. GLUT4 is reduced in slow muscle fibers of type 2 diabetic patients: is insulin resistance in type 2 diabetes a slow, type 1 fiber disease? Diabetes 2001;50(6):1324-9.

64. DeLany JP, Windhauser MM, Champagne CM, Bray GA. Differential oxidation of individual dietary fatty acids in humans. Am J Clin Nutr 2000;72(4):905-11.
65. Summers SA, Garza LA, Zhou H, Birnbaum MJ. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. Mol Cell Biol 1998;18(9):5457-64.
66. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. Diabetes Care 1997;20(7):1087-92.
67. Laakso M. How good a marker is insulin level for insulin resistance? Am J Epidemiol 1993;137(9):959-65.
68. Ferrannini E, Haffner SM, Mitchell BD, Stern MP. Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia 1991;34(6):416-22.
69. Kim YB, Nikoulina SE, Ciaraldi TP, Henry RR, Kahn BB. Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes [see comments]. J Clin Invest 1999;104(6):733-41.
70. Krook A, Bjornholm M, Galuska D, Jiang XJ, Fahlman R, Myers MG, Jr., et al. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. Diabetes 2000;49(2):284-92.
71. Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ, Dohm GL. 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):2195-204.

CHAPTER 4

Circulating Tumor Necrosis Factor Alpha is Higher and Soluble Tumor Necrosis Factor Receptor 2 is Lower in Non-Obese, Non-diabetic Mexican Americans compared to Non-Hispanic White Men and Women

ABSTRACT

Mexican Americans (MA) exhibit lower insulin sensitivity and greater risk for type 2 diabetes mellitus than non-Hispanic white (NHW) adults. The reasons for this phenomenon remain obscure. Because tumor necrosis factor-alpha (TNFα) is associated with insulin resistance in various models of obesity and diabetes, we sought to determine whether this cytokine and its soluble receptors could accouint, at least in part, for any differences in insulin sensitivity between these groups. Fasting blood samples were used to determine concentrations of TNFα, soluble TNF receptor-1 (sTNFR1), and soluble TNF receptor-2 (sTNFR2) in thirteen MA (7 women, 6 men, age=27.0 ± 2.0 y, BMI=23.0 ± 0.7) and 13 NHW (7 women, 6 men, age=24.8 ± 1.5 y, BMI=22.8±0.6). Estimates of insulin sensitivity were made using the Quantitative Insulin Sensitivity Check Index (QUICKI) and the Homeostatic Model of Insulin Resistance (HOMA-IR) using fasting insulin and glucose concentrations. MA were significantly less insulin sensitive compared to their NHW counterparts when estimated by HOMA-IR (1.53 ± 0.22 vs. 0.87 ± 0.16, $p<0.05$). MA tended to be less insulin sensitive compared to NHW based on QUICKI (0.37 ± 0.01 vs. 0.41 ± 0.02, $p=0.06$) and fasting insulin (44.84 ± 6.1 vs. 29.86 ± 4.8, $p=0.07$) as well. Circulating

levels of TNFα were significantly higher (3.11 ± 0.38 vs. 2.10 ± 0.24 pg/ml, $p<0.05$) and sTNFR2 was significantly lower (1323.52 ± 84.73 vs. 1924.65 ± 127.36 pg/ml, $p<0.05$) among MA compared to NHW subjects, respectively. Soluble TNFR1 tended to be higher among NHW subjects (1217.94 ± 73.21 vs. 969.78 ± 111.11 pg/ml, $p=0.07$). TNFα was inversely correlated with both sTNFR1 ($r= -0.50$, $p<0.05$) and sTNFR2 ($r= -0.40$, $p<0.05$). Among the NHW subjects, sTNFR1 was significantly associated with both fasting insulin ($r=0.60$, $p<0.05$) and the QUICKI estimate of insulin sensitivity ($r= -0.57$, $p<0.05$). However, TNFα, sTNFR1 and sTNFR2 were not related to estimates of insulin sensitivity in the MA, and when the two groups were analyzed in aggregate. These data indicate that although circulating levels of TNFα and sTNFR2 are different between nonobese, nondiabetic Mexican Americans and NHW, they do not appear to account for the observed differences in insulin sensitivity.

INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is 2-3 times higher among Mexican Americans (MA) compared with non-Hispanic whites (NHW) (1). Across a wide range of age groups non-diabetic MA tend to be more insulin resistant compared to their NHW counterparts (2-5). While some studies indicate that MA exhibit greater levels of total and abdominal obesity vs. NHW individuals, we, and others have demonstrated that differences in insulin sensitivity between these two groups persist after adjusting for various measures of obesity (1, 4). Thus, the specific contributors to the lower insulin sensitivity in the MA population remain obscure.

Numerous studies have suggested that the cytokine tumor necrosis factor alpha (TNFα) may play an important role in skeletal muscle insulin resistance (6-8). Various models of obesity and insulin resistance are associated with elevated circulating levels of TNFα (9-12). TNFα concentrations are lower in individuals with normal glucose tolerance compared with individuals with impaired glucose tolerance or T2DM (13). Circulating TNFα also tends to correlate with the degree of insulin resistance in humans (14).

TNFα concentrations appear to be related to body fatness. The expression of this cytokine is higher among obese individuals with T2DM, compared with nonobese, nondiabetic and nonobese individuals with T2DM (15). TNFα is expressed in adipocytes and is positively related to the degree of adiposity in humans (12). TNFα is elevated to a greater extent with android-type compared to gynoid-type obesity and decreased by weight loss (12, 16, 17). Surgical removal of visceral fat has been associated with a 72% decrease in the expression of TNFα (18). Infusion of TNFα into humans results in peripheral insulin resistance, particularly skeletal muscle (19, 20). Evidence suggests that the mechanism by which TNFα impairs insulin-stimulated glucose uptake involves the serine phosphorylation of IRS-1 and subsequent inhibition of both insulin receptor tyrosine kinase and IRS-1-associated phosphatidylinositol 3-kinase activity (21). Targeted null mutations in the gene encoding TNFα or either of its two receptor are associated with increased insulin sensitivity in obese mice (22-24).

Recent evidence suggests that the circulating soluble form of the two TNF receptors (sTNFR1 and sTNFR2) may function to neutralize circulating TNFα and negatively modulate its interorgan effects. Insulin resistance has been reversed by infusion of a TNFα neutralizing antibody containing soluble TNFR domains in obese *fa/fa* rats (25, 26).

However, others have suggested that the soluble receptors may actually enhance TNFα activity.

To date, no information is available regarding potential ethnic difference in TNF system. Therefore, in the present study we tested the hypothesis that non-diabetic MA exhibit greater levels of TNFα, sTNFR1 and sTNFR2 compared with NHW. We further hypothesized that elevated levels of the soluble TNF system would be associated with lower insulin sensitivity.

RESEARCH DESIGN AND METHODS

Subjects. A total of 13 nonobese (BMI<30) MAs (7 female, 6 male) and 13 nonobese NHWs (7 female, 6 male) aged 18-40 years were. All subjects were non-smoking, apparently healthy individuals with no overt signs or symptoms of disease based on a medical history. Subjects inclusion criteria were: fasting blood glucose levels < 110 mg/dl, no past or present history of endocrine disorders, and demonstrated blood pressure under resting conditions < 140/90 mmHg. Individuals were excluded from participation for the following reasons: pregnancy, oral contraceptive use, tobacco use, diabetes mellitus, history of any eating disorders, history of menstrual cycle irregularities, a history of hypo- or hyperthyroidism, and use of any medications that could influence insulin sensitivity. In addition, subjects had no orthopedic problems that prohibited them from engaging in a maximal graded exercise test. Subjects were weight stable (± 2.5 kg) for the previous six months. To be appropriately identified as a Mexican American, each participant traced his/her ethnicity to the four grandparents. The protocol of the study was approved by the

Colorado State University Human Research Committee, and both written and verbal informed consent was obtained from each volunteer.

Body Mass, Height, and Composition: Body weight was measured on a physicians balance scale to the nearest 100 g. Body height was measured to the nearest 0.1 cm with a wall-mounted stadiometer. The percentage of body fat, absolute fat mass and fat-free mass were measured in all subjects using dual-energy x-ray absorptiometry (DEXA) using software version 4.5c (Lunar model DPX-IQ Lunar Corp., Madison, WI).

Abdominal Visceral Fat: The measurement of total, visceral, and subcutaneous fat in the abdominal region was performed using a General Electric High Speed CT scanner (Milwaukee, WI) with helical capability (27). A cross-sectional scan 10 mm thick, centered at the L4-L5 intervertebral space, was obtained using 170 mA with a scanning time of 2 s and a 512 X 512 matrix. The visceral fat was measured by sectioning the intra-abdominal area by pixel density. The subcutaneous fat was calculated by determining the area of adipose tissue within the abdominal wall. The areas of deep and superficial subcutaneous adipose tissue were determined by dividing the two compartments based on the fascial delineation (28). Superficial subcutaneous adipose tissue was calculated by subtracting deep subcutaneous from total subcutaneous adipose tissue. Within each of the three compartments, the cross-sectional area of AT was measured in pixels (0.6 mm) in the attenuation range of –190 to –30 HU, using commercially available software (sliceOmatic, Tomovision Inc., Montreal, Canada).

Cardiorespiratory Fitness: Acute and chronic exercise are related to insulin sensitivity, and MA have been reported to exhibit lower levels of physical activity compared to NHW. Thus, we sought to control for this potential confounder by recruiting individual from both groups who were similar in regarding to maximal oxygen consumption. Maximal oxygen consumption was measured during incremental treadmill exercise to volitional exhaustion as previously described by Balke (29). Measurements of oxygen consumption, carbon dioxide production, pulmonary ventilation, and the respiratory exchange ratio was determined by on-line computer assisted open circuit spirometry (CPX Express, MedGraphics, Minnesota, MN). To ensure that maximal oxygen consumption has been obtained, at least three out of the four following criteria were satisfied: (a) a plateau in oxygen consumption with increasing workload; (b) a respiratory exchange ratio of at least 1.15; (c) achievement of age-predicted maximal heart rate; and (d) a maximal rating of perceived exertion of at least 18 on the Borg scale.

Insulin Sensitivity: For the determination of insulin sensitivity, subjects were instructed to remain fasted for 12 hours prior to blood collection. Furthermore, subjects refrained from participation in any form of exercise for 48 hours prior to the study. Female subjects were tested during the early follicular phase of their menstrual cycles (days 3-10). Three surrogate measures of insulin sensitivity were used: fasting plasma insulin, homeostatic model assessment of insulin resistance (HOMA-IR) and the Quantitative Insulin Sensitivity Check Index (QUICKI) (30, 31). These estimates have been shown to correlate well with the more direct measures of insulin sensitivity, such as the frequently sampled intravenous glucose tolerance test (FSIVGT) and hyperinsulinemic-euglycemic clamp (31-34). Our laboratory

has previously established strong associations between the FSIGT and the indices of insulin sensitivity used in the current study: fasting insulin ($r = -0.53$, $p=0.001$), HOMA-IR ($r = -0.53$, $p=0.001$), QUICKI ($r=0.70$, $p<0.001$, unpublished observations).

Assays: Blood glucose was measured by the glucose oxidase method using an autoanalyzer (YSI2300 Stat Plus, Yellow Springs Inc., Yellow Springs, OH). Plasma insulin was measured using a two-step sandwich ELISA (DSL, Webster, TX). This assay demonstrates a 2.6% intra-assay and 6.2% interassay variation. Plasma TNFα, sTNFR1 and sTNFR2 were measured using ELISA (R&D Systems, Minneapolis, MN). The high sensitivity TNFα assay demonstrates an 8% intra-assay and 15% inter-assay variation, while the sTNFR assays demonstrate 7% intra-assay and 9% inter-assay variations.

Statistical Analysis: Dependent variables were initially subjected to normality testing using both qualitative (data plots) and quantitative (Kolmogorov-Smirnov test) approaches. Group differences were analyzed using a 2 X 2 analysis of variance (ANOVA) (ethnicity x gender). When gender differences failed to reach significance, males and females were analyzed together within ethnic groups by independent t-tests. Pearson product moment correlations and partial correlations were used to determine associations between independent and dependent variables. Analysis of covariance (ANCOVA) was performed when potentially confounding variables exhibited a significant relationship to the dependent variables. The significance level was set a priori at $P<0.05$.

RESULTS

Subject characteristics are given in Table 1. On average, both groups were composed of young, normal weight men and women of moderate cardiorespiratory fitness. There were expected gender differences in several of the physical characteristics such as height, weight, body composition, fat patterning, and VO_2 max. However, there were no ethnic differences in any of these physical characteristics, except for the lower stature of MA compared with NHW.

Table 2 shows that there was a trend for MA subjects to exhibit higher fasting insulin and glucose concentrations compared with NHW subjects (insulin: 44.84 ± 6.14 vs. 29.86 ± 4.86 pmol/l, p=0.06; glucose: 4.70 ± 0.19 vs. 4.22 ± 0.22 mmol/l, p=0.11, respectively). Insulin sensitivity was lower in MA compared with NHW based on HOMA-IR (1.53 ± 0.22 vs. 0.91 ± 0.15, $p<0.05$), with a strong trend for lower insulin sensitivity estimated by QUICKI in the MA compared with NHW subjects (0.37 ± 0.01 vs. 0.41 ± 0.02, p=0.06).

As shown in Figure 1, circulating TNFα was significantly higher among MA vs. NHW subjects (3.11 ± 0.38 vs. 2.10 ± 0.24 pg/ml, $p<0.05$). Plasma sTNFR1 was not statistically different between the two groups; however, there was a tendency for this soluble receptor to be higher among NHW subjects (1217.93 ± 73.21 vs. 969.78 ± 111.11 pg/ml, p=0.07). Plasma sTNFR2 was significantly higher among NHW subjects compared with MA subjects (1924.65 ± 127.36 vs. 1323.52 ± 84.73 pg/ml, $p<0.05$).

Pearson product-moment correlations were used to analyze relationships between estimates of insulin sensitivity and the TNFα system. There were no significant associations between estimates of insulin sensitivity and TNFα, sTNFR1 or TNFR2 among MA subjects. Fasting glucose was inversely related to sTNFR2 in the MA study subjects (r= -0.58,

$p<0.05$). Insulin sensitivity estimated by QUICKI was significantly associated with circulating sTNFR1 in NHW subjects ($r= -0.57$, $p<0.05$). Additionally, fasting insulin was associated with sTNFR1 in the NHW subjects ($r=0.59$, $p<0.05$). When the study subjects were analyzed in aggregate, fasting insulin, HOMA-IR and QUICKI were not related to any components of the TNF system (Table 3). Fasting glucose was significantly associated with sTNFR2 ($r=-0.51$, $p<0.01$). There were no gender differences in estimates of insulin sensitivity, TNFα, sTNFR1 or sTNFR2 within or between groups.

Pearson product-moment correlations were used to analyze relationships between the TNF-α system and various compartments of abdominal adipose tissue, however, abdominal fat patterning was not associated with TNFα, sTNFR1 or sTNFR2.

DISCUSSION

There were three important findings from the present study. First, TNFα was higher and sTNFR2 was significantly lower in the MA compared with NHW. Second, non-obese and nondiabetic MA demonstrated lower indices of insulin sensitivity compared with NHW. This difference was unaltered after adjusting for cardiorespiratory fitness, total and abdominal adiposity. Finally, we did not observe any significant relation between the TNF system and the three indices of insulin sensitivity in the pooled sample in the present study.

The results of our study significantly extend previous findings in at least two important aspects. To the best of our knowledge, this is the first study to document higher circulating TNFα and lower sTNFR2 levels in nonobese, nondiabetic MA compared with NHW individuals. There is significant controversy regarding the role of this cytokine in human insulin resistance. Several lines of evidence suggest that TNFα is involved in various

models of insulin resistance in experimental animals, possibly by way of increased phosphorylation of serine rather than tyrosine residues on insulin receptor substrates involved in the PI-3 kinase signaling cascade. Studies have shown that TNF-α is expressed in skeletal muscle, and its expression is increased in obesity, insulin resistance and T2DM (35-37). Exposure to TNFα has been associated with impairments in the insulin signaling pathway (9. 38, 39) and downregulation of GLUT4 (40). TNFα neutralization is also associated with a 50% improvement in insulin sensitivity index and a 2.5-fold increase in tyrosine phosphorylation of IR in skeletal muscle (10). Therefore. skeletal muscle derived TNFα may be more closely related to insulin resistance. TNFα expression and secretion are both increased in human obesity (12. 41). However, similar to our findings. others have not found a relation between TNFα and estimates of obesity and insulin sensitivity (42). Patiag et al. showed that TNFα did not impair insulin-stimulated glucose uptake in skeletal muscle (43). Nolte and colleagues also reported that skeletal muscle does not become insulin resistant in response to short-term exposure to TNFα (44).

Controversy exists regarding the role of sTNFRs in modulating TNFα activity. Some studies suggest that sTNFRs function to neutralize and decrease circulating TNFα activity (45). Other studies have shown that TNFα bioactivity is increased in the presence of sTNFR (46, 47). If sTNFRs neutralize TNFα bioactivity, our data would suggest that MA (higher TNFα, lower sTNFR2) exhibit a stronger predisposition for TNFα cytotoxicity compared to NHW (lower TNFα, higher sTNFR2). The lack of association between circulating TNFα and estimates of insulin sensitivity among the study participants in our study provides no

support for this theory. Even the ratio of TNFα/sTNFR, calculated as an index of circulating bioactive TNFα, was unrelated to insulin sensitivity in our subjects.

Second, while our observation of lower indices of insulin sensitivity in MA compared with NHW adults is consistent with numerous other studies, there has previously been inadequate control for two potential confounders, cardiorespiratory fitness and fat patterning. In our study, even after matching MA and NHW subjects for fitness and body fatness using the best available techniques to measure these characteristics, insulin sensitivity still remained lower in the MA. Abdominal obesity is widely recognized as a strong correlate of insulin resistance. Visceral fat (omental, mesenteric adipose tissue) has been implicated in the etiology of skeletal muscle insulin resistance and T2DM. TNFα expression in adipose tissue is elevated in human obesity, and is strongly associated with hyperinsulinemia (41). Studies have shown that abdominal visceral fat secretes TNFα to a greater extent than abdominal subcutaneous fat (48). The present findings do not support the data of Mohamed-Ali et al. who reported that levels of both sTNFR1 and sTNFR2 correlated significantly with BMI and %body fat (49-51). Hotamisligil and colleagues (52) demonstrated that sTNFR2 was elevated sixfold in obese vs. lean women. Although TNFα was higher among our group of MA subjects, this was not associated with differences in abdominal fat patterning. Our data suggest that circulating TNFα, sTNFR1 and sTNFR2 are not associated with regional or abdominal fat distribution in nonobese, nondiabetic MA and NHW. This study suggests then, that ethnic differences in insulin sensitivity are not solely due to higher levels of total and central adiposity, and lower levels of physical activity and physical fitness as we hypothesized.

Finally, the higher plasma TNFα and lower sTNFR2 concentrations in the MA subjects is a new and interesting finding, but this phenomenon appears to be disassociated from the lower insulin sensitivity in the MA compared to NHW subjects. The lack of relationship between TNF-α and insulin sensitivity could be interpreted in various ways. If sTNFRs function to stabilize and increase circulating TNFα activity, as has been suggested (46) this would assist in explaining the lack of association between insulin sensitivity and TNFα. That is, higher TNFα coupled with lower sTNFR2 among MA could attenuate the ethnic differences in insulin resistance. Also, several studies (53, 54) have shown that exposure to TNFα increases basal glucose transport by increasing the translocation of GLUT1 glucose transporters. Thus, TNFα appears to selectively inhibit insulin-stimulated GLUT4 translocation. The estimates of insulin sensitivity used in the current study all involved fasting blood samples. Thus, it is entirely plausible that the strength of the relationship between TNFα and insulin sensitivity was masked by the cytokine's effect on basal glucose transport. While the estimates of insulin sensitivity reported in the current investigation correlate well with more direct measures of insulin sensitivity, further work is warranted examining the relationship between the TNFα system and insulin sensitivity under insulin-stimulated conditions. Further work is required to examine the association between circulating TNFα and basal vs. insulin-stimulated glucose disposal in groups at high risk for developing T2DM.

We can only speculate on the mechanisms responsible for the higher TNFα and lower sTNFR2 in MA compared with NHW in the present investigation. Dietary fat intake has been shown to increase the production of TNFα from activated macrophages and hepatocytes (55-57). We have previously shown that nonobese, nondiabetic MA tend to consume higher

amounts of dietary fat when compared with NHW (Chapter 3). It is possible that the higher TNFα levels observed in the current study are related to differences in dietary fat intake and macrophage production of the cytokine. This hypothesis seems plausible in light of our results showing a lack of association between circulating TNFα and visceral adipose tissue.

There are some potential limitations of the present study that should be addressed. Differences in the circulating TNF system were observed between these two groups, however, the TNF system was not associated with either estimates of insulin sensitivity or regional fat distribution. It should be noted that while we did not use the FSIGT or hyperinsulinemic-euglycemic clamp to measure whole-body insulin sensitivity, our laboratory has previously established a strong association between HOMA-IR, QUICKI and the FSIGT. Furthermore, it has been suggested that estimates of insulin sensitivity based on fasting insulin and glucose may be the best markers of early insulin resistance in nondiabetic individuals (58, 59). Additionally, all of the subjects tested in the current study exhibited normal fasting blood glucose levels (<110 mg/dl). Thus, MA subjects were not considered to be 'insulin resistant', but rather less insulin sensitive compared to the group of NHW subjects. However, previous studies have implicated this diminished insulin sensitivity among nondiabetic MA in the propensity for the development of T2DM in this ethnic group (2, 4).

In summary, the present study demonstrates that insulin sensitivity is lower in young, nonobese, nondiabetic MA compared to NHW, even after controlling for potential confounders of cardiorespiratory fitness and body fatness and fat patterning. Circulating TNFα and sTNFR2 are lower in nondiabetic, nonobese MA compared to NHW individuals. However, neither TNFα nor its two soluble receptors were associated with estimates of

insulin sensitivity. Further work is warranted in order to examine the effects of local production of TNFα on explaining differences in insulin sensitivity between NHW and MA.

TABLE 1. Subject Characteristics.

	NHW males and females	MA males and females	NHW females	NHW males	MA females	MA males
n	13	13	7	6	7	6
Age, y	24.84 ± 1.49	27.00 ± 2.00	23.6 ± 1.8	26.3 ± 2.5	28.14 ± 2.88	25.67 ± 2.94
Height, m	1.76 ± 0.02	1.68 ± 0.02[1]	1.70 ± 0.02[2]	1.83 ± 0.02[34]	1.64 ± 0.02[2]	1.74 ± 0.01[23]
Weight, kg	71.23 ± 3.30	65.48 ± 2.34	63.25 ± 3.04[2]	80.55 ± 3.37[3]	60.81 ± 2.90[2]	70.92 ± 2.41
BMI, kg/m^2	22.80 ± 0.58	23.04 ± 0.69	21.72 ± 0.72	24.05 ± 0.65	22.63 ± 1.09	23.53 ± 0.86
% Body fat	20.10 ± 2.14	21.67 ± 2.67	24.13 ± 2.46	15.40 ± 2.67[3]	26.83 ± 3.96[2]	15.65 ± 1.27
AVF, cm^2	43.74 ± 7.26	38.29 ± 4.90	29.41 ± 7.55[2]	58.07 ± 7.55[3]	29.97 ± 6.99[2]	47.99 ± 7.55
ASF, cm^2	145.38 ± 17.81	174.72 ± 24.96	134 ± 31.84	156.52 ± 31.84	200.23 ± 29.48	144.96 ± 31.84
SSF, cm^2	76.53 ± 9.78	86.22 ± 12.50	74.57 ± 16.72	78.51 ± 16.72	96.87 ± 15.48	73.81 ± 16.72
DSF, cm^2	68.85 ± 8.86	85.72 ± 12.35	59.69 ± 15.62	78.01 ± 15.63	98.22 ± 14.47	71.15 ± 15.63
VO_2max						
mL/kg/min	44.57 ± 1.43	44.84 ± 2.33	41.84 ± 1.58	47.75 ± 1.84	40.23 ± 3.07[4]	50.22 ± 2.11[3]
mL/kg FFM/min	55.77 ± 0.88	56.86 ± 1.42	55.21 ± 1.37	56.42 ± 1.09	54.64 ± 1.76	59.45 ± 1.90
L/min	3.18 ±0.19	2.94 ± 0.19	2.64 ± 0.13[24]	3.82 ± 0.09[3]	2.42 ± 0.15[24]	3.54 ± 0.12[3]

Mean ± SEM; AVF = abdominal visceral fat; ASF = abdominal subcutaneous fat; SSF = superficial subcutaneous fat; DSF = deep subcutaneous fat; [1] $p<0.05$ vs. MA males and females; [2] $p<0.05$ vs. NHW males; [3] $p<0.05$ vs. MA females; [4] $p<0.05$ vs. MA males

TABLE 2. Estimates of insulin sensitivity.

	NHW n = 13	MA n = 13
Fasting glucose, mmol/L	4.22 ± 0.22	4.70 ± 0.19
Fasting insulin, pmol/L	29.86 ± 4.86	44.84 ± 6.14
HOMA-IR	0.91 ± 0.15	1.53 ± 0.22[1]
QUICKI	0.41 ± 0.02	0.37 ± 0.01

Mean ± SEM; [1] $p<0.05$ vs. NHW

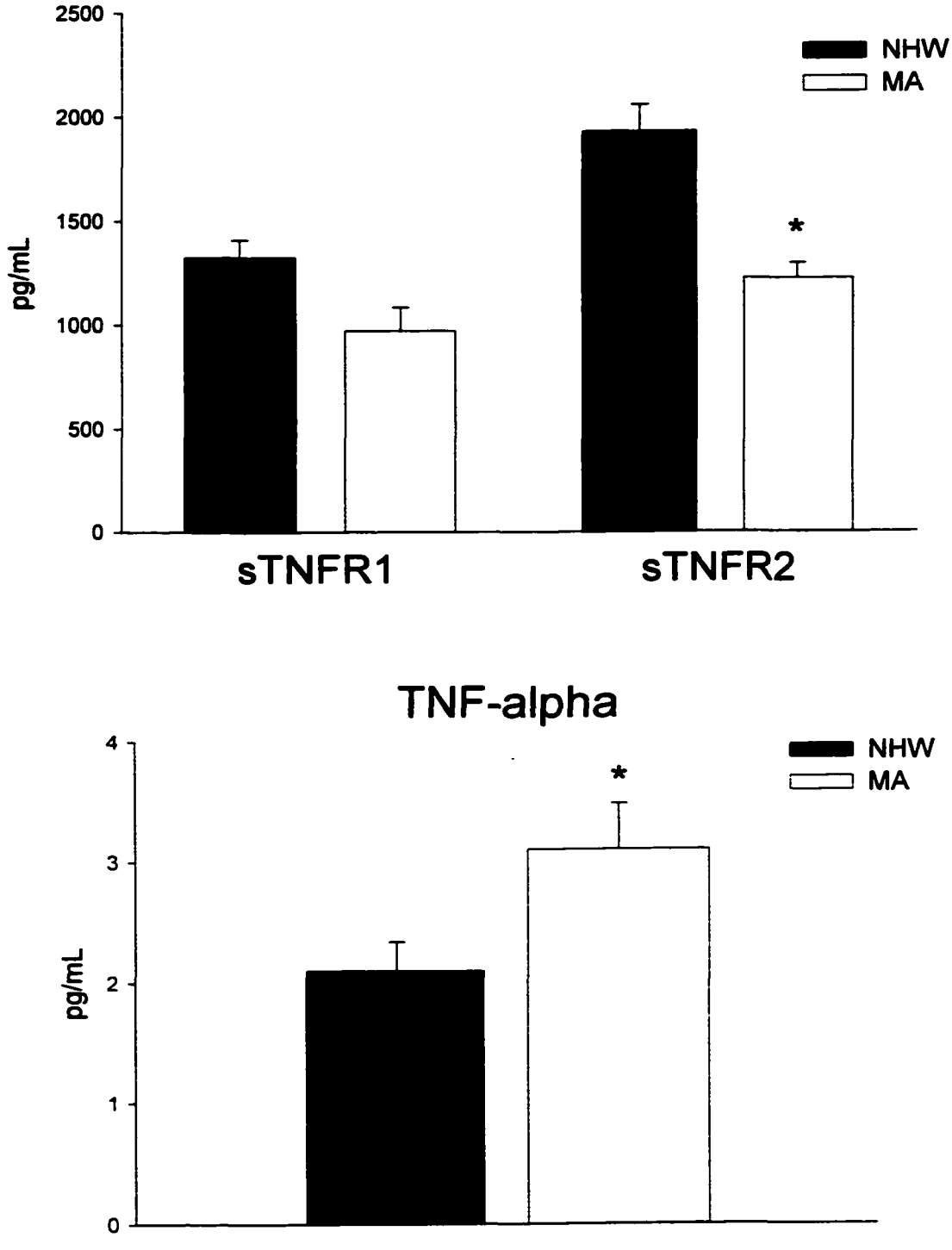

Figure 1. Circulating TNFα, sTNFR1 and sTNFR2 among Mexican American and Non-Hispanic White Men and Women.

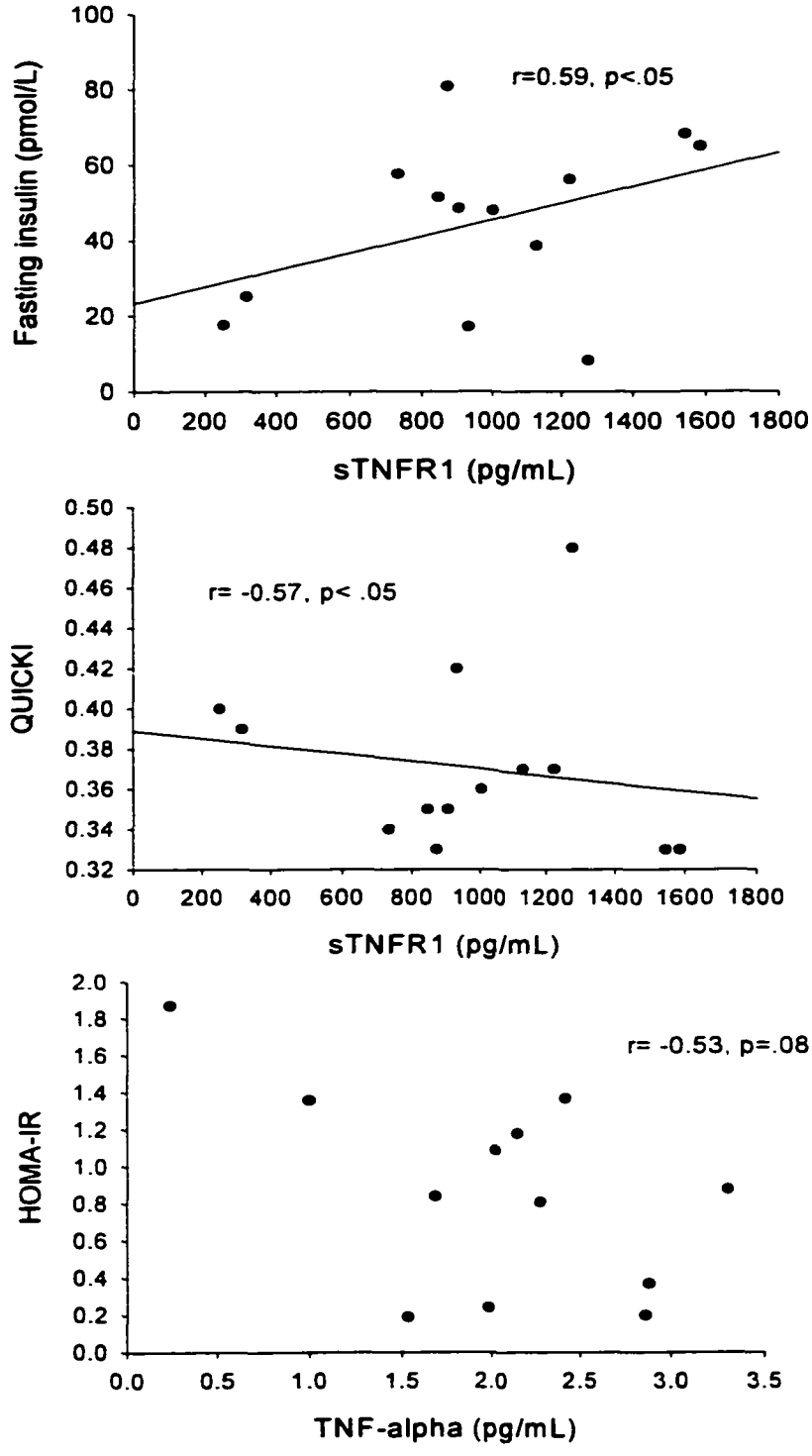

Figure 2. Relationship between sTNFR1 and estimates of insulin sensitivity in NHW subjects.

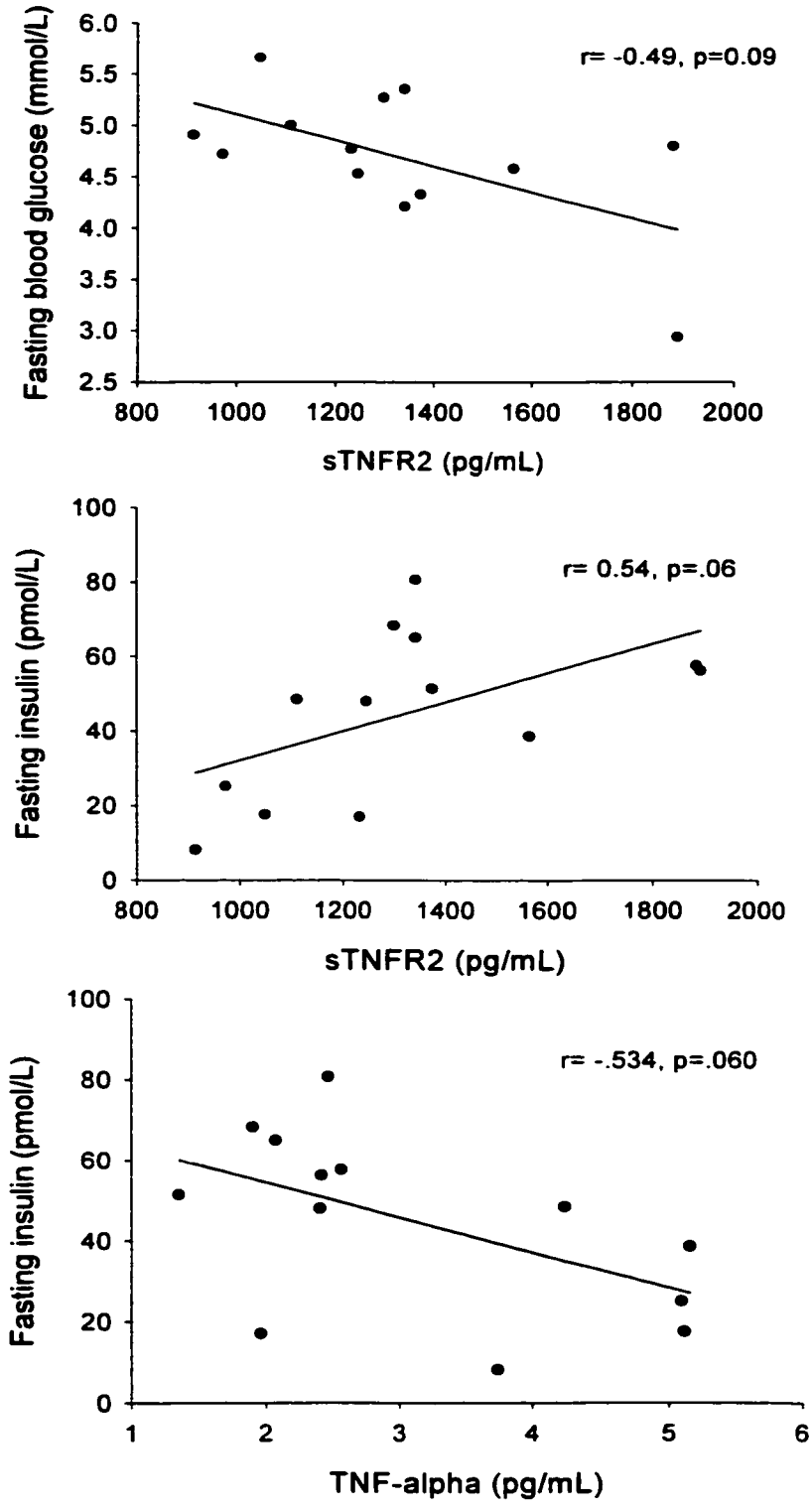

Figure 3. Relationship between the TNF-α system and surrogate measures of insulin sensitivity in Mexican American males and females.

REFERENCES

1. Stern MP, Gaskill SP, Hazuda HP, Gardner LI, Haffner SM. Does obesity explain excess prevalence of diabetes among Mexican Americans? Results of the San Antonio Heart Study. Diabetologia 1983;24(4):272-7.
2. Batey LS, Goff DC, Jr., Tortolero SR, Nichaman MZ, Chan W, Chan FA, 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):4319-25.
3. Tortolero SR, Goff DC, Jr., Nichaman MZ, Labarthe DR, Grunbaum JA, Hanis CL. Cardiovascular risk factors in Mexican-American and non-Hispanic white children: The Corpus Christi Child Heart Study. Circulation 1997;96(2):418-23.
4. Haffner SM, Stern MP, Dunn J, Mobley M, Blackwell J, Bergman RN. Diminished insulin sensitivity and increased insulin response in nonobese, nondiabetic Mexican Americans. Metabolism 1990;39(8):842-7.
5. Aguirre MA, Jones CN, Pei D, Villa ML, Reaven GM. 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):M56-60.
6. Brindley DN, Wang CN, Mei J, Xu J, Hanna AN. Tumor necrosis factor-alpha and ceramides in insulin resistance. Lipids 1999;34(Suppl):S85-8.
7. del Aguila LF, Claffey KP, Kirwan JP. TNF-alpha impairs insulin signaling and insulin stimulation of glucose uptake in C2C12 muscle cells. Am J Physiol 1999;276(5 Pt 1):E849-55.
8. Peraldi P, Hotamisligil GS, Buurman WA, White MF, Spiegelman BM. Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. J Biol Chem 1996;271(22):13018-22.
9. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996;271(5249):665-8.
10. Cheung AT, Ree D, Kolls JK, Fuselier J, Coy DH, Bryer-Ash M. An in vivo model for elucidation of the mechanism of tumor necrosis factor-alpha (TNF-alpha)-induced insulin resistance: evidence for differential regulation of insulin signaling by TNF-alpha. Endocrinology 1998;139(12):4928-35.
11. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 2001;280(5):E745-51.
12. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95(5):2111-9.
13. Zinman B, Hanley AJ, Harris SB, Kwan J, Fantus IG. Circulating tumor necrosis factor-alpha concentrations in a native Canadian population with high rates of type 2 diabetes mellitus. J Clin Endocrinol Metab 1999;84(1):272-8.
14. Nilsson J, Jovinge S, Niemann A, Reneland R, Lithell H. Relation between plasma tumor necrosis factor-alpha and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1998;18(8):1199-202.

15. Katsuki A, Sumida Y, Murashima S, Murata K, Takarada Y, Ito K, et al. Serum levels of tumor necrosis factor-alpha are increased in obese patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1998;83(3):859-62.
16. Winkler G, Lakatos P, Salamon F, Nagy Z, Speer G, Kovacs M, et al. Elevated serum TNF-alpha level as a link between endothelial dysfunction and insulin resistance in normotensive obese patients. Diabet Med 1999;16(3):207-11.
17. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. J Clin Endocrinol Metab 1998;83(8):2907-10.
18. Barzilai N, She L, Liu BQ, Vuguin P, Cohen P, Wang J, et al. Surgical removal of visceral fat reverses hepatic insulin resistance. Diabetes 1999;48(1):94-8.
19. Miles PD, Romeo OM, Higo K, Cohen A, Rafaat K, Olefsky JM. TNF-alpha-induced insulin resistance in vivo and its prevention by troglitazone. Diabetes 1997;46(11):1678-83.
20. Ling PR, Bistrian BR, Mendez B, Istfan NW. Effects of systemic infusions of endotoxin, tumor necrosis factor, and interleukin-1 on glucose metabolism in the rat: relationship to endogenous glucose production and peripheral tissue glucose uptake. Metabolism 1994;43(3):279-84.
21. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci U S A 1994;91(11):4854-8.
22. Hotamisligil GS. Mechanisms of TNF-alpha-induced insulin resistance. Exp Clin Endocrinol Diabetes 1999;107(2):119-25.
23. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF- alpha function. Nature 1997;389(6651):610-4.
24. Ventre J, Doebber T, Wu M, MacNaul K, Stevens K, Pasparakis M, et al. Targeted disruption of the tumor necrosis factor-alpha gene: metabolic consequences in obese and nonobese mice. Diabetes 1997;46(9):1526-31.
25. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity- diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 1994;94(4):1543-9.
26. Qi C, Pekala PH. Tumor necrosis factor-alpha-induced insulin resistance in adipocytes. Proc Soc Exp Biol Med 2000;223(2):128-35.
27. Ferland M, Despres JP, Tremblay A, Pinault S, Nadeau A, Moorjani S, et al. Assessment of adipose tissue distribution by computed axial tomography in obese women: association with body density and anthropometric measurements. Br J Nutr 1989;61(2):139-48.
28. Johnson D, Dixon AK, Abrahams PH. The abdominal subcutaneous tissue: computed tomographic, magnetic resonance, and anatomical observations. Clin Anat 1996;9(1):19-24.
29. Balke B, Ware, R.W. An experimental study of physical fitness in Air Force personnel. US Armed Forces Medicine. 1959;10:675-688.
30. Haffner SM, Kennedy E, Gonzalez C, Stern MP, Miettinen H. A prospective analysis of the HOMA model. The Mexico City Diabetes Study. Diabetes Care 1996;19(10):1138-41.
31. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85(7):2402-10.

32. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23(1):57-63.
33. Anderson RL, Hamman RF, Savage PJ, Saad MF, Laws A, Kades WW, et al. Exploration of simple insulin sensitivity measures derived from frequently sampled intravenous glucose tolerance (FSIGT) tests. The Insulin Resistance Atherosclerosis Study. Am J Epidemiol 1995;142(7):724-32.
34. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.
35. Noguchi Y, Yoshikawa T, Marat D, Doi C, Makino T, Fukuzawa K, et al. Insulin resistance in cancer patients is associated with enhanced tumor necrosis factor-alpha expression in skeletal muscle. Biochem Biophys Res Commun 1998;253(3):887-92.
36. Hrebicek A, Rypka M, Chmela Z, Vesely J, Kantorova M, Golda V. Tumor necrosis factor alpha in various tissues of insulin-resistant obese Koletsky rats: relations to insulin receptor characteristics. Physiol Res 1999;48(1):83-6.
37. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. The expression of TNF alpha by human muscle. Relationship to insulin resistance. J Clin Invest 1996;97(4):1111-6.
38. Kanety H, Hemi R, Papa MZ, Karasik A. Sphingomyelinase and ceramide suppress insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1. J Biol Chem 1996;271(17):9895-7.
39. Miura A, Ishizuka T, Kanoh Y, Ishizawa M, Itaya S, Kimura M, et al. Effect of tumor necrosis factor-alpha on insulin signal transduction in rat adipocytes: relation to PKCbeta and zeta translocation. Biochim Biophys Acta 1999;1449(3):227-38.
40. Szalkowski D, White-Carrington S, Berger J, Zhang B. Antidiabetic thiazolidinediones block the inhibitory effect of tumor necrosis factor-alpha on differentiation, insulin-stimulated glucose uptake, and gene expression in 3T3-L1 cells. Endocrinology 1995;136(4):1474-81.
41. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995;95(5):2409-15.
42. Kellerer M, Rett K, Renn W, Groop L, Haring HU. Circulating TNF-alpha and leptin levels in offspring of NIDDM patients do not correlate to individual insulin sensitivity. Horm Metab Res 1996;28(12):737-43.
43. Patiag D, Gray S, Idris I, Donnelly R. Effects of tumour necrosis factor-alpha and inhibition of protein kinase C on glucose uptake in L6 myoblasts. Clin Sci (Colch) 2000;99(4):303-7.
44. Nolte LA, Hansen PA, Chen MM, Schluter JM, Gulve EA, Holloszy JO. Short-term exposure to tumor necrosis factor-alpha does not affect insulin-stimulated glucose uptake in skeletal muscle. Diabetes 1998;47(5):721-6.
45. Kern P, Hemmer CJ, Gallati H, Neifer S, Kremsner P, Dietrich M, et al. Soluble tumor necrosis factor receptors correlate with parasitemia and disease severity in human malaria. J Infect Dis 1992;166(4):930-4.
46. Aderka D, Engelmann H, Maor Y, Brakebusch C, Wallach D. Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med 1992;175(2):323-9.

47. Smith RA, Baglioni C. The active form of tumor necrosis factor is a trimer. J Biol Chem 1987;262(15):6951-4.
48. Bertin E, Nguyen P, Guenounou M, Durlach V, Potron G, Leutenegger M. Plasma levels of tumor necrosis factor-alpha (TNF-alpha) are essentially dependent on visceral fat amount in type 2 diabetic patients. Diabetes Metab 2000;26(3):178-82.
49. Mohamed-Ali V, Goodrick S, Bulmer K, Holly JM, Yudkin JS, Coppack SW. Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo. Am J Physiol 1999;277(6 Pt 1):E971-5.
50. Hauner H, Bender M, Haastert B, Hube F. Plasma concentrations of soluble TNF-alpha receptors in obese subjects. Int J Obes Relat Metab Disord 1998;22(12):1239-43.
51. Fernandez-Real JM, Broch M, Ricart W, Casamitjana R, Gutierrez C, Vendrell J, et al. Plasma levels of the soluble fraction of tumor necrosis factor receptor 2 and insulin resistance. Diabetes 1998;47(11):1757-62.
52. Hotamisligil GS, Arner P, Atkinson RL, Spiegelman BM. Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance. Diabetes 1997;46(3):451-5.
53. Ciaraldi TP, Carter L, Mudaliar S, Kern PA, Henry RR. Effects of tumor necrosis factor-alpha on glucose metabolism in cultured human muscle cells from nondiabetic and type 2 diabetic subjects. Endocrinology 1998;139(12):4793-800.
54. Hauner H, Petruschke T, Russ M, Rohrig K, Eckel J. Effects of tumour necrosis factor alpha (TNF alpha) on glucose transport and lipid metabolism of newly-differentiated human fat cells in cell culture. Diabetologia 1995;38(7):764-71.
55. Wallace FA, Neely SJ, Miles EA, Calder PC. Dietary fats affect macrophage-mediated cytotoxicity towards tumour cells. Immunol Cell Biol 2000;78(1):40-8.
56. Holm T, Berge RK, Andreassen AK, Ueland T, Kjekshus J, Simonsen S, et al. Omega-3 fatty acids enhance tumor necrosis factor-alpha levels in heart transplant recipients. Transplantation 2001;72(4):706-11.
57. Rusyn I, Bradham CA, Cohn L, Schoonhoven R, Swenberg JA, Brenner DA, et al. Corn oil rapidly activates nuclear factor-kappaB in hepatic Kupffer cells by oxidant-dependent mechanisms. Carcinogenesis 1999;20(11):2095-100.
58. Laakso M. How good a marker is insulin level for insulin resistance? Am J Epidemiol 1993;137(9):959-65.
59. Ferrannini E, Haffner SM, Mitchell BD, Stern MP. Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia 1991;34(6):416-22.

CHAPTER 5

Whole Body Insulin Sensitivity, LDL Particle Size and Oxidized LDL in Overweight, Nondiabetic Men

ABSTRACT

Insulin resistance is often accompanied by elevated plasma triglyceride (TG) and a preponderance of small, dense low-density lipoprotein (LDL) particles. However, it remains unclear whether or not insulin resistance is related to LDL particle size, independent of plasma TG. Therefore, we sought to determine the strength of the relationships among these variables in a group of overweight, nondiabetic men (N=34, BMI 25-35 kg/m^2, 50-75y), and to also examine the possible relation between insulin sensitivity and oxidized LDL. Insulin sensitivity (Si) was assessed by the frequently sampled insulin-augmented glucose tolerance test (FSIGTT), LDL size by nuclear magnetic resonance spectroscopy (NMR) and oxidized LDL (oxLDL) by ELISA. We also examined the strength of the relationships between these lipid variables and estimates of insulin sensitivity using calculated indices based on fasting insulin and glucose concentrations. Si was significantly associated with total TG (r= -0.61, P<0.001), VLDL-TG (r= -0.60, P<0.001), total cholesterol (r= -0.39, P<0.05) and LDL size (r=0.41, P<0.05). LDL size was also significantly associated with TG (r= -0.73, P<0.001), VLDL-TG (r= -0.73, P<0.001), total cholesterol (r= -0.37, P<0.05) and HDL cholesterol (r=0.65, P<0.001). Si was a significant predictor of LDL size when analyzed by stepwise regression analysis with age and BMI (R^2= 0.17). However, when TG and HDL-C were

added to the model, Si was no longer a significant predictor of LDL size. After controlling for age and body fatness, Si was significantly associated with oxLDL (r= -0.38, P<0.05). These data suggest that the relation between insulin sensitivity and LDL size is largely mediated by plasma TG, and that Si is weakly related to oxLDL in overweight, nondiabetic men.

INTRODUCTION

Several lines of evidence suggest that insulin resistance may be the primary defect in the clustering of risk factors (obesity, hypertension, dyslipidemia) associated with the Metabolic Syndrome [Reaven, 1995 #2262; DeFronzo, 1991 #2848]. These metabolic abnormalities are associated with an increased risk for atherogenesis, although the mechanisms explaining these co-morbitities are not entirely clear. Whole-body insulin resistance is associated with increases in plasma non-esterified fatty acids (NEFA). Elevations in plasma NEFA increase hepatic synthesis of triglyceride-rich particles, primarily very low density lipoprotein (VLDL). Initially, via exchange of cholesterol ester for VLDL triglyceride, LDL and HDL particles become triglyceride enriched, but later have substantial amounts of TG removed by the action of hepatic triglyceride lipase, resulting in higher circulating concentrations of small dense LDL and HDL particles.

Small, dense LDL particles appear to be more atherogenic than larger, less dense LDL particles (1, 2), and individuals with a predominance of plasma small, dense LDL (pattern B lipoprotein phenotype) carry a three-fold higher risk for coronary heart disease (CHD) as compared to those with a predominance of large LDL particles (pattern A phenotype)(1). An elevated LDL particle number, often recognized by high apoprotein B

concentrations, has also been linked to an increased CHD risk (3, 4). Small, dense LDL particles are cleared from plasma at a slower rate compared to larger LDL particles (5), and appear more susceptible to oxidative damage and accumulation in the artery wall. It has recently been suggested that circulating oxidized LDL may be a marker for coronary heart disease risk (6, 7). Individuals with the Pattern B phenotype are typically more insulin resistant and hyperinsulinemic compared to those with the pattern A phenotype (8, 9).

An association between whole-body insulin sensitivity and LDL particle size has been shown in some studies (8, 10-13) but not all (14-17). Howard et al. (18) found a significant relation between insulin sensitivity and LDL size among nondiabetic men and women. Mykkanen et al. (11) found an association between insulin resistance and the preponderance of small, dense LDL particles in normoglycemic middle-aged men. However, others have reported that neither fasting insulin concentrations nor insulin sensitivity were related to LDL size independently of circulating plasma TG and HDL cholesterol levels (12, 17). It remains unclear, then, whether or not insulin resistance results in higher circulating concentrations of small, dense LDL particles, independent of elevated plasma TG. If plasma TG rather than insulin sensitivity were found to be more predictive of small dense LDL concentrations, this finding would be of substantial clinical relevance given the convenience of TG measurement compared to the difficulty in obtaining measures of insulin sensitivity in clinical practice.

The possibility also exists that insulin resistance directly contributes to the oxidative modification of LDL. After adjusting for age, gender, BMI and WHR, Carantoni M et al. (19) found that the steady-state plasma glucose (SSPG) concentration, and plasma glucose and insulin responses to oral glucose remained significantly correlated with oxLDL.

Carantoni M et al. (19) reported a significant and independent correlation between insulin resistance and compensatory hyperinsulinemia and the amount of poxLDL in nondiabetic patients. Previous studies have suggested that a preponderance of small, dense LDL particles is related to increased oxidized LDL in obesity and individuals with T2DM (9, 20, 21).

Obesity and insulin resistance are strong risk factors in the development of both T2DM and atherosclerosis, yet there is a paucity of data regarding the relationship between insulin sensitivity, LDL size, and oxLDL in overweight individuals. Therefore, the purpose of this study was to determine whether, or not, insulin sensitivity, as determined by the FSIGTT, is related to LDL particle size and circulating oxidized LDL, independent of total plasma triglycerides in overweight, nondiabetic males. Given the difficulty in measuring insulin sensitivity in clinical practice, we further sought to determine the strength of the relations among LDL particle size, oxLDL and several different surrogate estimates of insulin sensitivity that depend on only a single fasting measurement of plasma glucose and insulin.

METHODS

Subjects. Male subjects aged 50-75 y with a body mass index (BMI) between 25-35 kg/m^2 and elevated BP (systolic BP 130-160 mmHg and/or diastolic BP 85-99 mmHg) and fasting blood glucose less than 110 mg/dl were recruited for this study from the surrounding community. Only subjects who were sedentary or minimally physically active (< two 30-minute aerobic exercise sessions per week) were included. Individuals were excluded if they reported or were observed to have any of the following: overt cardiovascular, metabolic, or pulmonary disease or use of any medications known to affect any of the dependent variables

in this study. This study was approved by the Human Research Committee at Colorado State University. All subjects provided written informed consent prior to participation.

Anthropometric Measurements. Body weight was determined to the nearest 0.1 kg using a balance scale (Detecto, Webb City, MO) and height (cm) (without shoes) was measured using a wall-mounted stadiometer. Skinfold thickness measurements (mm) were obtained at eight sites (chest, abdomen, triceps, suprailiac, midaxillary, subscapular, thigh, and medial calf) using calipers (Lange, Cambridge Scientific Industries, Cambridge, MD). Waist circumference was measured (cm) at the level of the umbilicus using a Gulick II measuring tape (Country Technology, Gays Mills, WI). The percentage of body fat, absolute fat mass and fat-free mass was measured in all subjects using dual-energy x-ray absorptiometry (DEXA) using software version 4.5c (Model DPX-IQ Lunar Corp., Madison, WI). Medium length scans (20 min) were used for all subjects except for those with an anteriorposterior thickness >27 cm for whom the slow (40 min) speed was used.

Insulin sensitivity. An insulin-augmented frequently sampled intravenous glucose tolerance test (FSIGT) was administered with the subjects in the supine position following an overnight, 12-hour fast, and after a 30-minute relaxation period. An intravenous (IV) catheter was placed in each antecubital vein, one for the administration of insulin and glucose, and one for collecting blood samples. Two blood samples were obtained at time (t) = -10, -5 minutes, with these two samples used for determination of the mean baseline insulin and glucose concentrations. A bolus of glucose (0.3g/kg in a of 50% dextrose solution) was infused over a 90 second period at t=0. At t = 20 minutes, a bolus of insulin (0.03 U/kg) was

injected. Blood samples were obtained at t = 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes and immediately centrifuged at 4°C and analyzed for glucose concentrations by the glucose oxidase method using a glucose autoanalyzer (Yellow Springs Instruments, Yellow Springs, Ohio). A sample of plasma was stored at –20°C for later determination of insulin concentrations by the ELISA method (Diagnostic Systems Laboratories, Inc., Webster, TX). The MINMOD program (version 3.0, R. Bergman, University of Southern California) was used for determination of insulin sensitivity (Si). This model uses measurements of plasma glucose and insulin concentrations over the 3-h period to deduce in vivo whole-body insulin sensitivity (22). Whole-body insulin sensitivity was also estimated with fasting insulin, homeostatic model assessment of insulin resistance (HOMA-IR), the quantitative insulin sensitivity index (QUICKI) (23-25).

Biochemical assays. Following an overnight fast, blood was obtained from an indwelling IV catheter into tubes containing EDTA. Samples were inverted, and then centrifuged to obtain plasma, which was then stored at –70° C until analysis. Plasma lipid and lipoprotein profiles and characteristics were determined from plasma samples using nuclear magnetic resonance (NMR) spectroscopy (LipoMed, Inc., Raleigh, NC). This method is based on the principle that when plasma is exposed to the NMR magnet, the methyl group protons of each of the four types of lipids (phospholipid, cholesterol, cholesterol ester, and TG) in the lipoproteins together emit a bulk NMR signal. The unique signal produced by the various lipoprotein particles of differing diameters (based on the phospholipid shell) contribute to this bulk signal, analgous to the sound produced by simultaneously ringing numerous bells of differing size and shape. This composite signal then undergoes deconvolution using linear least–

squares regression analysis to separate the lipoprotein subclasses based on prior knowledge of the methyl signal frequency and shape emitted by individual lipoprotein subclasses. The plasma concentration of each lipoprotein subclass is then calculated based on knowledge about the known relationship between signal amplitude and subclass concentration (26, 27). To perform these calculations, software is used which contains a reference library of subclass spectra obtained by isolating lipoprotein particle size ranges from fasting plasma of normolipidemic and dyslipidemic subjects using ultracentrifugation and agarose gel filtration chromatography (27). This process allows the simultaneous measurement of 15 lipoprotein subclasses including VLDL-TG (V1-V6), LDL-C (IDL, L1-L3), HDL-C (H1-H5) and chylomicrons. HDL_2 corresponds to the sum of H4 and H5, HDL_3 is the sum of H1, H2, and H3. Based on comparisons with the lipoproteins separated by flotation ultracentrifugation, "small" LDL corresponds to L1 and "large" LDL refers to the sum of L2 and L3 (26, 27). Individual lipoprotein lipid concentrations are then calculated based on the average measured lipid values for each of the lipoproteins, based on values previously determined from spectrophotometric analysis of lipids from reference samples (26). Total plasma cholesterol, triacylglycerol, LDL-cholesterol, and HDL-cholesterol concentrations are then determined by summing the lipid concentrations of the various subclasses. Values were expressed in mmol/l of cholesterol (LDL-C and HDL-C) and triacylglycerol (VLDL-TG). Strong correlations between NMR and chemical analysis for VLDL-TG, LDL-C, and HDL-C have been reported ($r = 0.98$, 0.91, and 0.93 respectively) (26). LDL and HDL subclass distributions between NMR and gradient gel electrophoresis (GGE) also correlate well (26). Average particle size (diameter, nm) of VLDL, LDL, and HDL, and concentration (particle number) of the LDL particles (nmol/L) were also determined. Reference standards for VLDL and LDL subclass

diameters for the NMR method are based upon electron microscopy and for HDL by polyacrylamide GGE (27). Correlations between NMR and GGE for LDL and HDL particle size have been reported to be 0.7-0.9 and 0.88 (26), respectively. LDL subclass diameters obtained with the NMR method are ~5nm smaller than those obtained using GGE, but are in agreement with electron microscopy data and calculations based upon LDL chemical composition (27). Coefficients of variation using the NMR procedure have been reported to be 1.5-2.9% for standard lipid panel variables, 1.8% for LDL particle concentration, and about 0.5% for average LDL and HDL particle size (27). A more detailed treatment of the use of NMR for measuring plasma lipid and lipoprotein concentrations and lipoprotein particle size has been described previously (26-28).

Oxidized LDL was measured by ELISA (Mercodia, Uppsala, Sweden). This method is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants raised against copper-oxidized human LDL. The antibodies are directed against a conformational epitope in the apoB-100 moiety of LDL that is generated as a consequence of the condensation of lysine residues of apoB-100 with aldehydes (29).

Statistical analysis. Pearson product-moment correlations and partial correlation coefficients were utilized to determine relationships between variables. Multiple stepwise regression analyses were also performed to determine how well metabolic variables predict LDL size and oxidized LDL. Data were analyzed with SPSS for Windows 10.0 statistical software (SPSS, Inc.) and are expressed as the mean ± SEM.

RESULTS

Subject characteristics for the 36 men are shown in Table 1. Simple and partial correlations were used to determine the relationship between insulin sensitivity (Si as determined from the FSIGTT), LDL size and oxLDL (Table 2). As shown in Figure 1, LDL size was significantly associated with Si ($r=0.41$, $p=0.01$), total triglyceride ($r=-0.73$, $p<0.001$), VLDL triglyceride ($r=-0.73$, $p<0.001$), total cholesterol ($r=-0.37$, $p=0.026$), and HDL cholesterol ($r=0.65$, $p<0.001$). After adjusting for age, % body fat and abdominal skinfold, these associations remained statistically significant. Oxidized LDL was significantly associated with LDL cholesterol ($r=0.34$, $p=0.04$), and with Si ($r=-0.38$, $p=0.039$) after adjusting for age, % body fat and abdominal skinfold, and approached significance with fasting insulin ($r=0.33$, $p=0.079$). LDL size was not significantly associated with oxLDL (Figure 1).

Fasting glucose and insulin concentrations were used to calculate two different estimates of insulin sensitivity. Insulin sensitivity determined by HOMA was significantly associated with Si ($r= -0.53$, $p< 0.005$) as was QUICKI ($r= 0.70$, $p<0.001$). Table 3 shows that QUICKI was significantly associated with LDL size ($r=0.42$, $p=0.011$), with the correlation coefficient for this relationship similar to that of Si and LDL size. QUICKI was also related to serum TG ($r= -0.55$, $p<0.001$). Fasting insulin ($r=-0.30$, $p=0.079$) and HOMA-IR ($r=-0.28$, $p=0.10$) were not significantly associated with LDL size. Neither HOMA or QUICKI were associated with oxLDL.

Stepwise regression was performed to determine whether or not estimates of insulin resistance could predict LDL size independently of other covariates (total TG, VLDL-TG, HDL-C, age, BMI). Table 4 shows that Si alone accounted for 22% of the variance in LDL

size, while total triglyceride alone accounted for almost 60% of the variance in LDL size. Together, total triglyceride and HDL cholesterol accounted for more than 65% of the variance in LDL size. Neither Si nor QUICKI remained in the model explaining the variance in LDL size when plasma TG was included in the regression analysis.

DISCUSSION

The major findings in this study are: 1.) insulin sensitivity and LDL particle size are significantly correlated in this group of overweight/obese, nondiabetic men, but the ability of this particular index of insulin sensitivity to explain the variance in LDL particle size is apparently mediated, to a large extent, by total plasma TG; 2.) the Quick Index, an estimate of insulin sensitivity based on fasting glucose and insulin concentrations obtained from a single sample of blood, is as strongly correlated with LDL particle size as is the much more labor intensive and expensive frequently sampled glucose tolerance test; 3.) insulin sensitivity is only weakly correlated with plasma oxidized LDL concentrations in middle-aged and older overweight, nondiabetic men.

Insulin resistance is associated with elevations in plasma NEFAs, which stimulate the hepatic synthesis of triglyceride-rich VLDL particles. Circulating VLDL exchanges triglyceride for LDL/HDL cholesterol esters via the action of cholesterol ester transfer protein. Hepatic lipase cleaves triglyceride-rich LDL and HDL particles, producing small, dense lipoproteins. Additionally, insulin promotes the degradation of LDL through interaction with the LDL receptor. Some have suggested that larger LDL particles are preferentially cleared by insulin-stimulated LDL receptor endocytosis (34), while small, dense LDL particles remain longer in circulation (35-38). Our results show that there was,

indeed, a strong relationship between Si and both total triglyceride and VLDL-triglyceride. As one might expect, we also found a significant relationship between LDL size and both total triglyceride and VLDL-triglyceride. These data support those reported by Rainwater et al. (35) and Ambrosch et al. (13) who showed that LDL size was strongly associated with plasma TG and VLDL-TG concentrations. However, total plasma triglyceride concentration was the strongest determinant of LDL particle size, explaining 58% of the variance in LDL particle size. VLDL triglyceride and HDL cholesterol were slightly weaker, explaining 50 and 39% of the variance in LDL size, respectively. When analyzed by stepwise regression with age and BMI, Si accounted for 22% of the variance in LDL particle size. However, only TG concentration emerged as an independent predictor when added to the multivariate model (17). These findings are in agreement with those reported by Mykkanen et al. (11) and Festa et al. (12) who found that whole-body glucose uptake and Si were not independently associated with LDL size after controlling for VLDL-TG and HDL cholesterol. Ambrosch et al. (13) reported that although both TG and insulin sensitivity were associated with LDL size in young, nondiabetic subjects, only TG was a significant determinant of LDL size. Slyper AH et al. (17) did not find a significant correlation between LDL size and Si among healthy, nondiabetic males.

The fact that Si did not remain an independent predictor of LDL size when fasting plasma TGs were included in the multivariate model, should not detract from the role of insulin resistance in contributing to smaller, denser LDL particles. As discussed above, insulin resistance is strongly related to elevations in plasma TGs, which in turn contributes to the formation of small, dense LDL particles. However, these findings do suggest that in

clinical practice, the measurement of plasma TG is more important in predicting LDL size than the measurement of insulin sensitivity.

The FSIGTT is relatively cost and time prohibitive for estimating insulin sensitivity in large-scale studies and in the clinical setting. Therefore, we sought to determine whether various estimates of insulin sensitivity that rely solely on fasting blood samples were associated with LDL size and oxidized LDL. Of the additional surrogate measures of insulin sensitivity analyzed, the QUICKI was significantly associated with LDL particle size, with the strength of the association similar to that of Si and LDL size. As was true of the latter relationship, when TG were entered into the model, QUICKI was no longer a significant independent predictor of LDL size.

Several lines of evidence also suggest that insulin resistance may directly affect the production of oxLDL. Physiological hyperinsulinemia is associated with decreases in plasma vitamin E as well as increases in H_2O_2 production (36, 37). Quinones-Galvan et al. (31) reported that acute in vivo insulin administration increased the susceptibility of LDL cholesterol to both copper-induced and cell-mediated oxidation. Carantoni and colleagues (19) reported that insulin resistance was associated with increased serum levels of partially oxidized LDL in nondiabetic men and women. Individuals with type 2 diabetes mellitus exhibit increased free radical production, which is also associated with hyperglycemia (38). Also, the susceptibility of LDL to oxidation may be higher with increasing levels of total and abdominal adiposity (39), well known concomitants of insulin resistance.

While insulin resistance may contribute to increased levels of oxLDL, the reverse may also be true. Chavakis et al. (40) recently showed that oxLDL induced dephosphorylation and subsequent inactivation of Akt, a key signaling intermediate involved

in insulin-stimulated glucose transport (Cho et al., 2001). Previous studies have reported that defects in Akt activity are associated with increases in the production of the sphingolipid, ceramide. Interestingly, oxLDL has been shown to increase the cellular ceramide content through the activation of acid sphingomyelinase (45, 46).

In our study sample, we found a weak, but significant inverse relation between Si and oxLDL, after partialling out the effects of age and body fatness. There was only a trend for plasma insulin concentrations to be related to oxLDL. The lack of a stronger relationship should not discount the possible importance of the relation between insulin sensitivity and oxLDL. A stronger relation may have been masked by the lack of substantial heterogeneity in our study sample—all men were overweight or obese, and none exhibited type 2 diabetes. It is possible that had our sample included lean individuals as well as those with diabetes, a wider range of Si values and average LDL sizes would have resulted in a stronger relation. Note that previous studies have utilized NMR and alanine methods to quantify oxidized LDL in circulation, while we utilized an ELISA technique which recognizes antigenic determinants on oxidized apoprotein B. While total LDL cholesterol was positively associated with oxLDL in our study sample, it is unclear why we did not find oxLDL to be related to LDL size, as has been previously reported by Carantoni et al. (21).

In summary, the results of the current study suggest that among middle-aged and older, overweight, nondiabetic men, the relationship between insulin sensitivity and LDL particle size is highly dependent on plasma triglyceride and HDL cholesterol concentrations. Furthermore, an estimate of insulin sensitivity, the QUICKI, which has more clinical utility because it relies a single fasting blood sample, is as strongly correlated to LDL size as the

more burdensome FSIGTT. Finally, insulin sensitivity is modestly associated with oxidized LDL.

TABLE 1. Clinical and Metabolic Characteristics

Variable	N = 36
Age, y	59.2 ± 1.1 (50-75)
Weight, kg	91.8 ± 2.1 (70.7-123.9)
BMI, $kg \times m^2$	29.4 ± 0.5 (24.8-39.1)
% Fat	28.1 ± 0.7 (14.3-37.8)
WHR	0.985 ± 0.005 (0.87-1.07)
Si x 10^4 ($mU \times ml^{-1} \times min^{-1}$)	2.07 ± 0.23 (0.21-5.63)
Sg x 10^2 (min^{-1})	0.0193 ± 0.013 (0.0037-0.400)
AIR ($pmol \times ml^{-1} \times min^{-1}$)	472.5 ± 33.9 (97.2-951.1)
Fasting insulin, $pmol \times l^{-1}$	62.33 ± 9.38 (16.20-62.33)
Fasting glucose, $mmol \times l^{-1}$	5.33 ± 0.07 (4.51-6.19)
Total triglycerides, $mmol \times l^{-1}$	1.66 ± 0.12 (0.58-3.35)
Total cholesterol, $mmol \times l^{-1}$	5.08 ± 0.12 (3.52-6.38)
LDL cholesterol, $mmol \times l^{-1}$	3.42 ± 0.11 (1.84-4.62)
HDL cholesterol, $mmol \times l^{-1}$	0.88 ± 0.03 (0.60-1.51)
LDL size, nm	20.26 ± 0.13 (19.0-21.7)
Oxidized LDL, U/L	51.15 ± 2.31 (10.14-83.46)
VLDL triglyceride, $mmol \times l^{-1}$	1.28 ± 0.12 (0.21-3.00)

Mean ± SEM (range)

TABLE 2. Relationship between LDL size, oxidized LDL and anthropometric and metabolic variables.

	LDL size		Oxidized LDL	
Variable	**r**	**Partial r***	**r**	**Partial r***
Si x 10^4 (mU× ml^{-1}× min^{-1})	0.414[1]	0.446[1]	-0.304	-0.379[1]
Sg x 10^2 (min^{-1})	0.026	0.069	-0.293	-0.310
AIRg (pmol×ml^{-1} ×min^{-1})	-0.047	0.007	-0.169	-0.130
Fasting insulin, pmol× l^{-1}	-0.296	-0.270	0.157	0.326
Fasting glucose, mmol× l^{-1}	-0.212	-0.268	0.001	0.065
Total triglycerides, mmol× l^{-1}	-0.728[1]	-0.721[1]	0.125	0.226
Total cholesterol, mmol× l^{-1}	-0.371[1]	-0.428[1]	0.247	0.190
LDL cholesterol, mmol× l^{-1}	-0.216	-0.298	0.343[1]	0.269
HDL cholesterol, mmol× l^{-1}	0.648[1]	0.690[1]	-0.003	0.052
VLDL triglyceride, mmol× l^{-1}	-0.731[1]	-0.727[1]	0.099	0.207

Values are expressed in terms of Pearson's r-coefficients. [1] $p<0.05$; *Adjusting for age (y), % body fat, abdominal skinfold (mm).

TABLE 3. Correlations between LDL size/oxidized LDL and surrogate measures of insulin sensitivity

	Fasting insulin	HOMA-IR	QUICKI
LDL size			
Pearson's r	-0.296	-0.279	0.421
P-value	0.790	0.100	0.011
Oxidized LDL			
Pearson's r	0.157	0.146	-0.204
P-value	0.369	0.404	0.240

TABLE 4. Stepwise regression: contributions to the variance of LDL particle size

Variable	B	SE(B)	P	R2
Model 1				
Si	0.232	0.062	.001	0.223
Model 2				
Total TG	-0.009	0.001	.001	0.584
Model 3				
Total TG	-0.008	0.002	.001	
HDL cholesterol	0.046	0.013	.001	0.656

Independent variables that were tested for entry in the regression models were: Model 1: age, BMI, Si; Model 2: age, BMI, Si, total plasma triglyceride; Model 3: age, BMI, Si, total plasma triglyceride, HDL cholesterol.

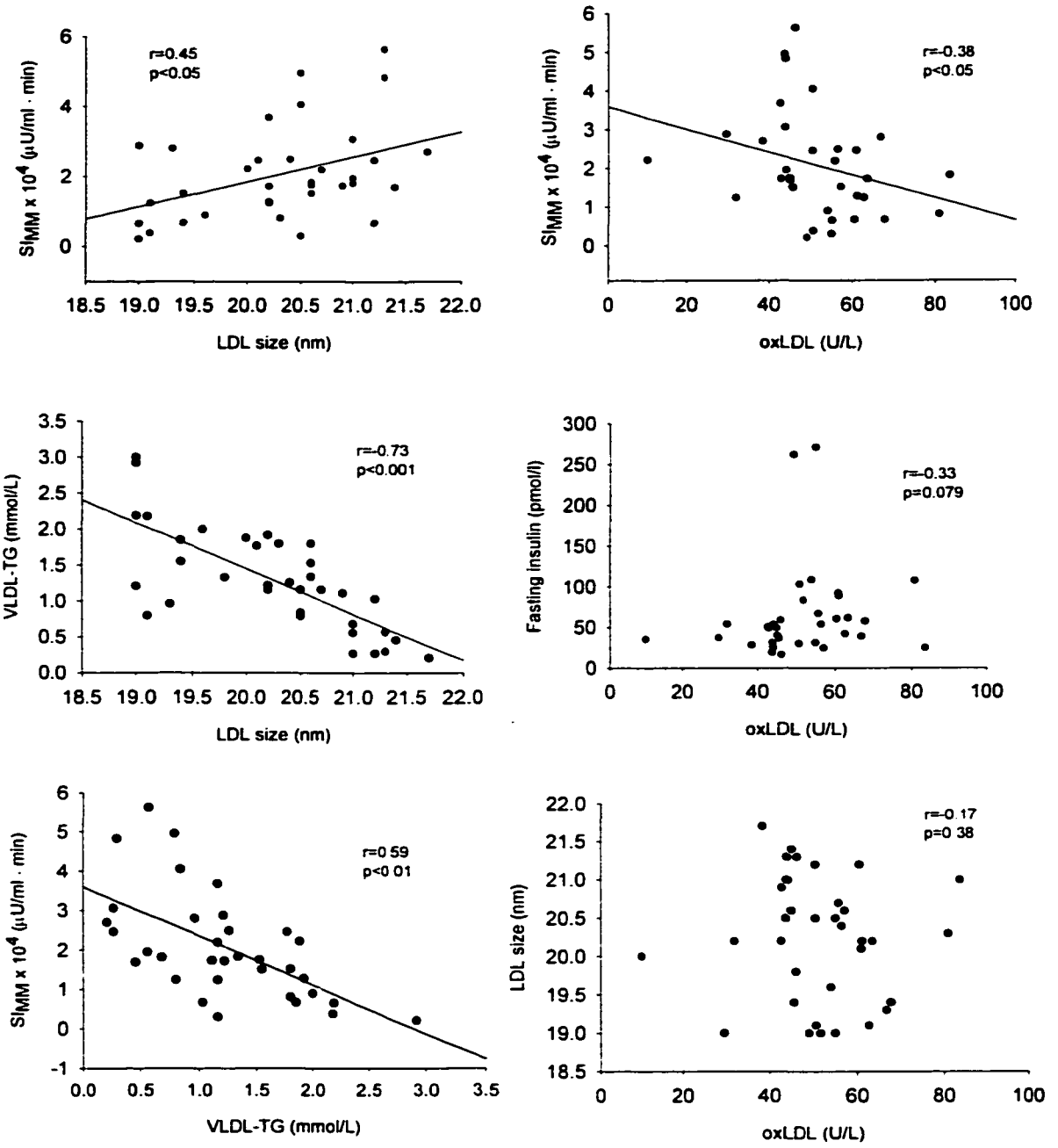

Figure 1. Correlation coefficients for variables associated with LDL size and oxidized LDL.

REFERENCES

1. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995;75(3):473-86.
2. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991;14(3):173-94.
3. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. Jama 1988;260(13):1917-21.
4. Campos H, Genest JJ, Jr., Blijlevens E, McNamara JR, Jenner JL, Ordovas JM, et al. Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 1992;12(2):187-95.
5. Austin MA, Rodriguez BL, McKnight B, McNeely MJ, Edwards KL, Curb JD, et al. Low-density lipoprotein particle size, triglycerides, and high-density lipoprotein cholesterol as risk factors for coronary heart disease in older Japanese-American men. Am J Cardiol 2000;86(4):412-6.
6. Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997;95(1):69-75.
7. Nigon F, Lesnik P, Rouis M, Chapman MJ. Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. J Lipid Res 1991;32(11):1741-53.
8. Toshima S, Hasegawa A, Kurabayashi M, Itabe H, Takano T, Sugano J, et al. Circulating oxidized low density lipoprotein levels. A biochemical risk marker for coronary heart disease. Arterioscler Thromb Vasc Biol 2000;20(10):2243-7.
9. Holvoet P, Donck J, Landeloos M, Brouwers E, Luijtens K, Arnout J, et al. Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure. Thromb Haemost 1996;76(5):663-9.
10. Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J Clin Invest 1993;92(1):141-6.
11. Selby JV, Austin MA, Newman B, Zhang D, Quesenberry CP, Jr., Mayer EJ, et al. LDL subclass phenotypes and the insulin resistance syndrome in women. Circulation 1993;88(2):381-7.
12. Haffner SM, Mykkanen L, Robbins D, Valdez R, Miettinen H, Howard BV, et al. A preponderance of small dense LDL is associated with specific insulin, proinsulin and the components of the insulin resistance syndrome in non- diabetic subjects. Diabetologia 1995;38(11):1328-36.
13. Mykkanen L, Haffner SM, Rainwater DL, Karhapaa P, Miettinen H, Laakso M. Relationship of LDL size to insulin sensitivity in normoglycemic men. Arterioscler Thromb Vasc Biol 1997;17(7):1447-53.
14. Festa A, D'Agostino R, Jr., Mykkanen L, Tracy RP, Hales CN, Howard BV, et al. LDL particle size in relation to insulin, proinsulin, and insulin sensitivity. The Insulin Resistance Atherosclerosis Study. Diabetes Care 1999;22(10):1688-93.

15. Ambrosch A, Muhlen I, Kopf D, Augustin W, Dierkes J, Konig W, et al. LDL size distribution in relation to insulin sensitivity and lipoprotein pattern in young and healthy subjects. Diabetes Care 1998;21(12):2077-84.
16. Lahdenpera S, Sane T, Vuorinen-Markkola H, Knudsen P, Taskinen MR. LDL particle size in mildly hypertriglyceridemic subjects: no relation to insulin resistance or diabetes. Atherosclerosis 1995;113(2):227-36.
17. Stewart MW, Laker MF, Dyer RG, Game F, Mitcheson J, Winocour PH, et al. Lipoprotein compositional abnormalities and insulin resistance in type II diabetic patients with mild hyperlipidemia. Arterioscler Thromb 1993;13(7):1046-52.
18. Friedlander Y, Kidron M, Caslake M, Lamb T, McConnell M, Bar-On H. Low density lipoprotein particle size and risk factors of insulin resistance syndrome. Atherosclerosis 2000;148(1):141-9.
19. Slyper AH, Zvereva S, Schectman G, Hoffmann RG, Mueller RA, Walker JA. Insulin resistance is not a major determinant of low-density lipoprotein particle size. Metabolism 1997;46(11):1275-80.
20. Howard BV, Mayer-Davis EJ, Goff D, Zaccaro DJ, Laws A, Robbins DC, et al. Relationships between insulin resistance and lipoproteins in nondiabetic African Americans, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Metabolism 1998;47(10):1174-9.
21. Carantoni M, Abbasi F, Warmerdam F, Klebanov M, Wang PW, Chen YD, et al. Relationship between insulin resistance and partially oxidized LDL particles in healthy, nondiabetic volunteers. Arterioscler Thromb Vasc Biol 1998;18(5):762-7.
22. Barakat HA, Carpenter JW, McLendon VD, Khazanie P, Leggett N, Heath J, et al. Influence of obesity, impaired glucose tolerance, and NIDDM on LDL structure and composition. Possible link between hyperinsulinemia and atherosclerosis. Diabetes 1990;39(12):1527-33.
23. Feingold KR, Grunfeld C, Pang M, Doerrler W, Krauss RM. LDL subclass phenotypes and triglyceride metabolism in non-insulin- dependent diabetes. Arterioscler Thromb 1992;12(12):1496-502.
24. Bergman RN, Phillips LS, Cobelli C. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 1981;68(6):1456-67.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.
26. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85(7):2402-10.
27. Anderson RL, Hamman RF, Savage PJ, Saad MF, Laws A, Kades WW, et al. Exploration of simple insulin sensitivity measures derived from frequently sampled intravenous glucose tolerance (FSIGT) tests. The Insulin Resistance Atherosclerosis Study. Am J Epidemiol 1995;142(7):724-32.
28. Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. Clin Chem 1992;38(9):1632-8.

29. Otvos J. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. In: Rifai N. WG, Dominiczk M., editor. Handbook of Lipoprotein Testing. 2nd Edition ed. Washington D.C.: American Association of Clinical Chemistry; 2000. p. 609-623.
30. Otvos J. Measurement of triglyceride-rich lipoproteins by nuclear magnetic resonance spectroscopy. Clin Cardiol 1999;22(6 Suppl):II21-7.
31. Holvoet P. Oxidative modification of low-density lipoproteins in atherothrombosis. Acta Cardiol 1998;53(5):253-60.
32. Holvoet P, Collen D. Oxidation of low density lipoproteins in the pathogenesis of atherosclerosis. Atherosclerosis 1998;137 Suppl:S33-8.
33. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 1998;98(15):1487-94.
34. Mazzone T, Foster D, Chait A. In vivo stimulation of low-density lipoprotein degradation by insulin. Diabetes 1984;33(4):333-8.
35. Quinones-Galvan A, Sironi AM, Baldi S, Galetta F, Garbin U, Fratta-Pasini A, et al. Evidence that acute insulin administration enhances LDL cholesterol susceptibility to oxidation in healthy humans. Arterioscler Thromb Vasc Biol 1999;19(12):2928-32.
36. Beard CM, Barnard RJ, Robbins DC, Ordovas JM, Schaefer EJ. Effects of diet and exercise on qualitative and quantitative measures of LDL and its susceptibility to oxidation. Arterioscler Thromb Vasc Biol 1996;16(2):201-7.
37. Chait A, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Am J Med 1993;94(4):350-6.
38. Okumura K, Matsui H, Kawakami K, Morishima I, Numaguchi Y, Murase K, et al. Modulation of LDL particle size after an oral glucose load is associated with insulin levels. Clin Chim Acta 1998;276(2):143-55.
39. Rainwater DL. Lipoprotein correlates of LDL particle size. Atherosclerosis 2000;148(1):151-8.
40. Galvan AQ, Muscelli E, Catalano C, Natali A, Sanna G, Masoni A, et al. Insulin decreases circulating vitamin E levels in humans. Metabolism 1996;45(8):998-1003.
41. Rifici VA, Schneider SH, Khachadurian AK. Stimulation of low-density lipoprotein oxidation by insulin and insulin like growth factor I. Atherosclerosis 1994;107(1):99-108.
42. Matteucci E, Giampietro O. Oxidative stress in families of type 1 diabetic patients. Diabetes Care 2000;23(8):1182-6.
43. Van Gaal LF, Zhang A, Steijaert MM, De Leeuw IH. Human obesity: from lipid abnormalities to lipid oxidation. Int J Obes Relat Metab Disord 1995;19 Suppl 3:S21-6.
44. Chavakis E, Dernbach E, Hermann C, Mondorf UF, Zeiher AM, Dimmeler S. Oxidized LDL Inhibits Vascular Endothelial Growth Factor-Induced Endothelial Cell Migration by an Inhibitory Effect on the Akt/Endothelial Nitric Oxide Synthase Pathway. Circulation 2001;103(16):2102-2107.
45. Harada-Shiba M, Kinoshita M, Kamido H, Shimokado K. Oxidized low density lipoprotein induces apoptosis in cultured human umbilical vein endothelial cells by common and unique mechanisms. J Biol Chem 1998;273(16):9681-7.

46. Deigner HP, Claus R, Bonaterra GA, Gehrke C, Bibak N, Blaess M, et al. Ceramide induces aSMase expression: implications for oxLDL-induced apoptosis. Faseb J 2001;15(3):807-14.

CHAPTER 6

Overall Conclusions

The development of T2DM and other conditions associated with the Metabolic Syndrome stems from a combination of acquired, environmental and genetic factors. Peripheral insulin resistance is an early defect that appears to explain many of the untoward sequelae associated with these diseases. Several lines of evidence suggest that excessive lipid accumulation in nonadipocytes results in impairments in specific insulin signaling pathways (glucose transport, glycogen synthesis). Lipotoxicity stemming from high lipid availability (obesity, dyslipidemia, dietary fat) and low lipid oxidation (physical inactivity, skeletal muscle fiber type) may be a unifying hypothesis incorporating established and potential risk factors in the etiology of insulin resistance, T2DM and the Metabolic Syndrome. Inflammatory cytokines are also associated with the many risk factors related to the Metabolic Syndrome, and have been implicated in the progression of these diseases. Hence, the dysregulation of homeorhetic mechanisms determining the balance between lipid availability and lipid disposal in nonadipocytes is a salient feature in the appearance of peripheral insulin resistance in humans. The studies provided herein were designed to evaluate the relationships between some of the major modifiable risk factors and insulin sensitivity in humans.

Study 1 Specific Aims: To determine whether differences in insulin sensitivity exist between nonobese, nondiabetic Mexican American and Non-Hispanic White males and

females after accounting for level of cardiorespiratory fitness, regional fat distribution and dietary intake. A corollary aim was to determine whether differences between the two groups exist in the content of proteins involved in insulin-stimulated glucose transport (insulin receptor, phosphatidylinositol 3-kinase, Akt, GLUT4).

Hypotheses: After accounting for level of cardiorespiratory fitness, regional fat distribution, and dietary intake, no differences in insulin sensitivity between the two groups will be evident. Furthermore, no differences in insulin signaling proteins will be apparent between the two groups.

Primary findings: Our findings suggest that differences in insulin sensitivity between nonobese, nondiabetic MA and NHW persist after accounting for effects of acute and chronic exercise, abdominal fat patterning, and skeletal muscle protein levels of key insulin signaling intermediates. However, after adjusting for differences in intake of the fatty acids palmitate and palmitoleate, the ethnic differences in insulin sensitivity were attenuated and no longer significant. These data suggest that greater dietary intake of these fatty acids among MA may contribute to their lower insulin sensitivity.

Future directions: While we have accounted for the potential effects of various lifestyle factors in determining differences in insulin sensitivity, it would be of particular interest to examine whether or not the present findings would be similar among a more heterogeneous group (in terms of body fat distribution, cardiorespiratory fitness, etc.). We found that dietary intake of certain fatty acids was related to insulin sensitivity in this group of individuals; however, we did not determine the proportions of individual fatty acids in plasma. Furthermore, since our data suggest that skeletal muscle protein levels do not differ

between groups, it would be advisable to examine possible differences in the insulin-stimulated activity of these signaling intermediates.

Study 2 Specific Aims: To determine whether, or not, differences in the tumor necrosis (TNF)-alpha system (TNFα, soluble TNF receptor 1 and 2) exist between Mexican American and Non-Hispanic White males and females. Furthermore, we sought to determine the strength of the relationships between the TNF-α system, estimates of insulin sensitivity and abdominal fat patterning within these groups.

Hypotheses: Nonobese, nondiabetic Mexican American and Non-Hispanic White subjects will exhibit similar levels of circulating TNFα, soluble TNF receptor 1, and soluble TNF receptor 2. TNFα and its two soluble receptors will be associated with estimates of insulin sensitivity and abdominal visceral fat.

Primary findings: The data indicate that although circulating levels of TNFα and sTNFR2 are different between nonobese, nondiabetic Mexican Americans and NHW, they do not appear to account for the observed differences in insulin sensitivity.

Future directions: While our data suggest that the TNF system does not function as an endocrine hormone, we did not evaluate the potential local effect of this cytokine. It has yet to be determined whether or not skeletal muscle expression of TNFα differs between these two groups. Furthermore, the role of the soluble TNF receptors in various models of insulin resistance has yet to be firmly elucidated.

Study 3 Specific Aims: To determine whether estimates of whole-body insulin sensitivity are associated with LDL size and oxidized LDL in a group of overweight, nondiabetic men.

Furthermore, we sought to determine whether, or not, insulin sensitivity is an independent predictor of LDL size after accounting for very-low density lipoprotein-triglyceride. Finally, we examined the relationship between LDL size and oxidized LDL in this population at an increased risk for cardiovascular disease.

Hypotheses: Estimates of insulin sensitivity will significantly correlate with both LDL size and oxidized LDL. After accounting for VLDL-TG, insulin sensitivity will not be an independent predictor of LDL size. LDL size will be inversely related to oxidized LDL.

Primary findings: Our data suggest that the relationship between insulin sensitivity and LDL size is largely mediated by plasma VLDL-TG. Furthermore, insulin sensitivity estimated by the FSIGT is weakly related to oxLDL in overweight, nondiabetic men.

Future directions: There remains a paucity of data describing the relationship between insulin sensitivity and modified LDL in humans. While small, dense LDL particles are more susceptible to oxidation, a clear association between LDL size and oxidized LDL has yet to be firmly established. Circulating oxidized LDL has been associated with total oxidized LDL; however, the subendothelial accumulation of oxidized LDL presumably confers a greater risk for CVD. This association may be clouded by data that have suggested that oxidized LDL activates ceramide and TNF-α production. Furthermore, oxidized LDL can function as a ligand for PPARγ. The antidiabetic thiazoledinedions (TZD), synthetic PPARγ ligands, have been shown to decrease the oxidation of LDL, an effect that is associated with the TZD tocopherol moiety allowing it to function as an antioxidant. Whether elevated oxidized LDL occurs secondary to insulin resistance, or induces insulin resistance remains to be elucidated.

APPENDIX A:

INFORMED CONSENT

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Exercise and Insulin Sensitivity in Mexican Americans

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

NAME OF CO-INVESTIGATORS: Richard Ho, M.S., Kevin Davy, Ph.D., Matt Hickey, Ph.D.

CONTACT NAME AND PHONE NUMBER FOR QUESTIONS/PROBLEMS: Chris Melby; 970-491-6736

SPONSOR OF PROJECT: National Institutes of Health, Colorado Agricultural Experiment Station

This consent form may contain words that you do not understand. Please ask Chris Melby or the study staff to explain any words or information that you do not clearly understand.

PURPOSE OF THE RESEARCH: The research described in this form is to see how physical fitness is related to insulin sensitivity. Insulin sensitivity is a measure of how well the hormone insulin causes sugar to go from your blood into your cells. We also want to look at how specific skeletal muscle proteins are related to physical fitness. We want to learn how these molecules may link fitness to insulin sensitivity.

PROCEDURES/METHODS TO BE USED: If you agree to take part in this study, we will ask you to fill out a form about your health. We will look at the form. If you don't have any health problems like the ones listed below, we will ask you to start the next part of the research. You must be between the ages of 18-40. You cannot participate in the study for any of the following reasons: heart problems, high blood pressure, asthma, bleeding disorder, pregnancy, oral contraceptive use, tobacco use, diabetes mellitus, history of any eating disorders, history of menstrual cycle irregularities, a history of hypo- or hyperthyroidism, and use of any medications that could influence insulin sensitivity. In addition, you must have no physical problems that would keep you from doing an exercise test. You must be weight stable (± 2.5 kg) for the previous six months. If you meet the qualifications for participation based on the questions we ask you, you will be involved in a series of up to five visits, which will take place in Room 216 of the Gifford Building, the Human Peformance/Clinical Research Lab of the Moby Complex, Hartshorn Student Health Center and Poudre Valley Hospital.

Screening Questionnaires- We will ask you questions about your medical history and personal health habits. Time and Schedule: 30 minutes total.

Pregnancy test- All women in the study will be asked to take a pregnancy test. We want to make sure that you are not pregnant. If the pregnancy test is positive, you will not be able to participate in this study. It is important that you do not become pregnant during the course of the study. This will disqualify you from being in the study. Time and Schedule: 10 minutes prior to your entry into the study.

Screening tests for high blood pressure and diabetes- Following a 12-h fast (no food or beverages except water) 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. Time: 20 minutes.

Diet, Physical Activity and Depression Questionnaires- In order to see what kinds of food you eat (fat, protein, carbohydrate, fiber, fruits and vegetables), you will be asked to write down everything you eat and drink (with the exception of water) over a 4-day period of time. You will also be asked questions about your participation in specific physical activities. Additionally, you will be asked to choose specific statements that best describe the way you have been feeling in the past week. Time: 45 minutes

Ethnicity Questionnaire- You will be asked to complete a questionnaire about your ethnic background. 5 min.

Resting and Exercise Test- Following a 12-h fast (no food or beverages except water), the amount of calories you burn at rest will be determined. You will lay quietly in a bed while you breathe into a respiratory canopy or a mouthpiece for 30 minutes. You will then take a treadmill exercise test while you breathe into a hose so that samples of your breath will be collected. You will have a brief warm-up period walking on the treadmill, and then will have to jog at the same speed throughout the test. Every minute, the treadmill incline will become more difficult (steep) until you can no longer jog at that speed. Your heart rate will be watched using a heart rate monitor, and your blood pressure will be taken at least twice during the exercise test. Time: 1 hour.

The previously described procedures will all take place in either Room 216 of the Gifford Building or in the Human Performance Clinical/Research Laboratory in the Moby Complex

Insulin Sensitivity- In order to estimate the ability of insulin to cause sugar to move from your blood into your cells, you will have a sample of blood drawn from your arm following a 12-h fast (no food or drink except water). For this test, a small sterile needle will be placed in your arm and blood will be collected into two tubes. This blood will later be analyzed for insulin and sugar levels. If these levels are not normal, you will be able to contact a physician if you want. During this test, an

additional tube (approximately 2 teaspoonfuls) of your blood will be drawn to analyze the types of fat in your blood, as well as some hormones that may influence insulin sensitivity. The total amount of blood obtained from this test will be about 4 teaspoons. Time: 20 minutes.

Body weight and body fat- Your height will be measured without 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. Your body composition (% body fat) will be measured using a machine called a dual energy X-ray absorptiometer (DEXA) following at least a 4-h fast (no food or drink except water). This unit uses 2 low energy X-rays to measure the amount of body fat you have. You should know that the amount of X-ray exposure in this procedure is very low. This amount is about 1/1000 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. This is about 1/40th the exposure from a normal stomach X-ray you might receive at a hospital. 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: 45 min.

Muscle Biopsy- A small sample of your leg muscle will be obtained to analyze for molecules that affect the ability of your muscle to take in and use sugar. The muscle sample will be obtained from the vastus lateralis, which is a large muscle in your thigh. The procedure involves numbing the skin using lidocaine. This is an anesthetic similar to novocaine, which you may have received at the dentist. If you are allergic to novocaine (or any local anesthetic) or have had any reaction to novocaine from your dentist, you should notify Professor Hickey immediately and should not participate in the biopsy procedure. After numbing the skin, a small cut (about 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 usually ~ ½ the size of an eraser on the end of a pencil. It is common to experience some mild soreness in the muscle that lasts for about a day. You should NOT stop exercising, although you should also not perform any unusual or intense activity for a few days. You will be given written instructions about proper care of the cut, and a telephone contact should you have any questions. A 12-h fast (no food or drink except water) before the biopsy procedure is required. Time :1 hour.

The body composition test (DEXA) will be performed in the Human Performance Clinical/Research Laboratory at the Moby Complex. The insulin sensitivity test and muscle biopsy procedures will take place in this same facility or at the Hartshorn Student Health Center. Because the three procedures above require you to be fasted, it is possible that for your convenience, more than one procedure may be conducted during the same day.

Computed Tomography (CT) Scan- The amount of total fat, fat around your internal organs, and fat under the skin in the abdominal area will be measured by computed tomography (CT scan). For this procedure, you will be asked to lie very still on a table. An x-ray machine (the CT scanner) will rotate around you, and the table will moved back and forth slightly making it possible to take x-rays from several angles. Time: 1 hour. *The computed tomography scan will take place at Poudre Valley Hospital, Fort Collins, CO. We will give you specific directions.*

Time Involvement: Once it has been determined that you are eligible to participate in the study, you will be scheduled for testing sessions, with the order depending on your schedule and the availability of the laboratory and investigators. You will attend a minimum of 5 testing sessions (some possibly on the same day if it convenient for you) in several locations including the Nutrition and Fitness Lab in 216 Gifford and the Human Performance/Clinical Research Lab in Moby. You will also make a visit to Poudre Valley Hospital. These visits will require a total of approximately 10 hours of your time, covering a span of approximately 4-6 weeks.

Participation: The investigators have a lot experience with the above listed tests, and take special precautions to make sure you are safe. As the study participant, you have the option of deciding to stop participating at any point during a particular test, or at any time in the study.

RISKS INHERENT IN THE PROCEDURES:

Blood Draw: Drawing a small sample of blood (no more than 2 teaspoons) may cause a hematoma (bruise), slight risk of infection, local soreness, and fainting.

Blood Samples: Any time a blood needle is inserted, there is a small risk that you may faint, experience local soreness, bruising or infection, and there is a possibility of the vein becoming inflamed and/or painful in the hours or days after the needle

Graded Exercise Test: In people with heart problems, the maximal exercise testing may cause fatigue, muscle strains, an irregular heartbeat (arrhythmias), and a change in blood pressure. There is a 0.01% chance of death, and a 0.02% risk of cardiac arrhythmias requiring hospitalization. These risks are much less in healthy individuals.

Body composition DEXA Scan: The amount of radiation that you will receive in the DEXA exam is less than the amount allowed by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to less than 1/20 of a chest x-ray. The more radiation received over the course of your life, the more is the risk of developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risk is not known.

CT Scan: The amount of radiation that you will receive in the CT scan (combined with the DEXA exam) is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount you will receive is equal to that produced by a dental x-ray. The more radiation received over the course of your life, the more is the risk of developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risk is not known.

Muscle Biopsies: May cause discomfort, localized soreness, bruising, infection, minor scarring, and occasionally a person may faint from the procedure. An allergic reaction to the numbing medicine is rare (less than 1 in 10,000).

It is important for you to recognize that several of the tests including the body composition DEXA and CT scans should not be performed if you are pregnant. It is important that you inform the principal investigator immediately should you become pregnant during the study.

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

BENEFITS: You will benefit from being informed of your individual values for dietary intake, fitness level, energy expenditure, and insulin sensitivity. Referrals to physicians will be made if your blood glucose levels are outside the normal range, and if you have an abnormal graded exercise test. You will also be paid $100 for your participation in the study. Failure to complete all aspects of the study will result in less than full payment. You will not be paid for completion of the questionnaires but will be paid according to the following:

Resting and Exercise Test:	$25
Muscle Biopsy:	$25
Blood Sampling	$25
DEXA and CT Scan	$25

CONFIDENTIALITY:
All the data obtained for you will be kept in a separate file in a locked cabinet. Data will be published as group data—no publication will include any data that can be linked to you as an individual.

LIABILITY:
Because Colorado State University is a publicly-funded institution, it may have only limited legal responsibility for injuries incurred as a result of participation in this study under a Colorado law known as the Colorado Governmental 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 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 your own 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.

Questions about subjects' rights may be directed to Celia S. Walker at (970) 491-1563.

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 4 pages.

_____

Participant name (printed)

_____ _____

Participant signature Date

_____ _____

Investigator or co-investigator signature Date

APPENDIX B:

HEALTH HISTORY QUESTIONNAIRE

COLORADO STATE UNIVERSITY
CONFIDENTIAL HEALTH HISTORY QUESTIONNAIRE

STUDY_____ **DATE**_____ **SUBJECT ID #**_____

Reviewed by (must be PI or MD):_____

Current Age_____ **Height**_____ **Weight**_____

PLEASE PRINT

GENERAL MEDICAL HISTORY

	YES	NO	
Do you have any current medical conditions?	☐	☐	If Yes, please explain:
Have you had any major illnesses in the past?	☐	☐	If Yes, please explain:
Have you ever been hospitalized or had surgery?	☐	☐	If Yes, please explain: (include date and type of surgery, if possible)
Have you ever had an electrocardiogram (EKG)? (a test that measures your heart's activity using an electrical tracing)	☐	☐	If Yes, please explain:

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?

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, including any medications taken:

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	NO	Relation	Age at Diagnosis
a. High blood pressure	☐	☐		
b. Heart Attack	☐	☐		
c. Coronary bypass surgery	☐	☐		
d. Angioplasty	☐	☐		
e. Stroke	☐	☐		
f. Diabetes	☐	☐		
g. Obesity	☐	☐		
g. Other (Please list)	☐	☐		

TOBACCO HISTORY (check any that apply)

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

Total years of tobacco use_____

CURRENT TOBACCO USE
(if applicable)

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

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 by an investigator so we can be sure to safely determine your ability to participate.

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 or 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?	☐	☐

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):_____

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

d). Duration of diet_____

What was your weight 12 months ago? _____

What is your current weight?_____

	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?	☐	☐

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. Other Spanish
G. Caucasian
H. Black
I. Asian/Pacific Islander

2.What are your parent's surnames?

Father:_____
Mother:_____

3.What are your parent's country 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?