Resistance Training Increases the Expression of AMPK, mTOR, and GLUT4 in Previously Sedentary Subjects and Subjects with the Metabolic Syndrome.

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Resistance Training Increases the Expression of AMPK, mTOR, and GLUT4 in Previously Sedentary Subjects and Subjects with the Metabolic Syndrome

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the faculty of the Department of Kinesiology, Leisure, and Sport Sciences

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Master of Arts in Exercise Physiology and Performance

by

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ABSTRACT

Resistance Training Increases the Expression of AMPK, mTOR, and GLUT4 in Previously Sedentary Subjects and Subjects with the Metabolic Syndrome

by

Andrew Steven Layne

Exercise has been considered a cornerstone of diabetes prevention and treatment for decades, but the benefits of resistance training are less clear. Nineteen non-diabetic subjects (10 metabolic syndrome, 9 sedentary controls) underwent 8 weeks of supervised resistance training. After training, strength and \( \dot{V} O_2_{\text{max}} \) increased by 10% in both groups. Percent body fat decreased in subjects with the metabolic syndrome. Additionally, lean body mass increased in both groups (p<0.05). Expression of glucose transporter protein-4 (GLUT4), the principle insulin-responsive glucose transporter, increased significantly in both groups. 5'-adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) expression increased in both groups, indicating increased protein synthesis and mitochondrial biogenesis. Markers of insulin resistance measured by a euglycemic hyperinsulinemic clamp did not improve in subjects with the metabolic syndrome but increased significantly in control subjects (13%). Resistance training upregulates intracellular signaling pathways that may be beneficial for ameliorating the metabolic syndrome.
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CHAPTER 1
INTRODUCTION

Problem Statement

The prevalence of the metabolic syndrome is increasing rapidly worldwide. Exercise causes an array of physiological adaptations that may ameliorate many aspects of the metabolic syndrome. The purpose of this study was to identify the role of several molecular mediators that are potentially responsible for the beneficial adaptations to 8 weeks of resistance training in subjects with the metabolic syndrome.

Review of the Literature

The Metabolic Syndrome

Advancements in technology over the past century have greatly improved the quality of life for humans across the globe. Consequently, many humans have become less physically active and have relatively unimpeded access to excessively rich nutrition, particularly in developed and developing countries (1-3). These and other lifestyle and behavioral changes have led to a marked increase in the prevalence of obesity and other metabolic disorders (4). Over the past 20 years, the number of people diagnosed with metabolic abnormalities has increased dramatically (5;6). Several studies indicate that over half of all U.S. adults are overweight (BMI > 25kg/m^2) (1;7;8), and as many as 1 in 4 U.S. adults meet the criteria for having metabolic syndrome (9;10). The average life expectancy in the U.S. may soon decrease for the first time due to obesity-related illness (11). Individuals with the metabolic syndrome are at an increased risk for developing cardiovascular disease (CVD), type 2 diabetes (9), and for mortality from CVD (10). The prevalence of the metabolic syndrome is continuing to rise (12), highlighting the need for the development of effective prevention and treatment strategies.
The metabolic syndrome has existed conceptually since at least the 1920s (5;13), when a Swedish physician named Kylin described the association of hypertension and hyperglycemia (13). Later, obesity (particularly visceral obesity) was observed to be associated with type 2 diabetes and CVD (13;14). The term “metabolic syndrome,” the most commonly used term for the syndrome, was coined in the 1970s by German researchers (15). In 1988, Reaven described what he termed Syndrome X: insulin resistance, hyperglycemia, hypertension, and low HDL-cholesterol (16). Because these risk factors tend to occur in the same individual, Reaven suggested that the body’s attempt to compensate for these risk factors, particularly insulin resistance, leads to the development of coronary artery disease (16). Around the same time, Ferrannini et al. coined the term “insulin resistance syndrome” and concluded that insulin resistance is the underlying cause of risk factors for coronary artery disease (17). Based on these observations, it became apparent that a criterion for diagnosing patients at risk of developing type 2 diabetes and CVD was essential for detection and treatment of the metabolic syndrome.

Definitions of the Metabolic Syndrome

Since the late 1990s, several groups have established guidelines for clinical diagnosis of the metabolic syndrome. According to guidelines published by the World Health Organization in 1999, a patient is considered to have the metabolic syndrome if he or she has diabetes, impaired glucose tolerance, impaired fasting glucose, or insulin resistance as well as 2 or more of the following: high resting blood pressure (140/90 mmHg); hyperlipidemia (triglyceride concentration ≥150 mg/dL) and/or HDL cholesterol <35 mg/dL in men and <39 mg/dL in women; waist-to-hip ratio of >0.90 in men or >0.85 in women and/or BMI >30kg/m²; microalbuminuria (urinary albumin excretion rate ≥20 µg/min or albumin:creatinine ratio ≥20 mg/g (18;19). It was soon realized that the WHO definition was inadequate for the clinical
setting because of the need for a euglycemic insulin clamp procedure to identify insulin resistance (20).

Since the original guidelines published by the WHO, other groups have published various definitions of the metabolic syndrome. The European Group for the Study of Insulin Resistance released an alternative definition in 1999. In 2002, the National Cholesterol Education Program’s Adult Treatment Panel III report (ATP III) released slightly different guidelines. A diagnosis under ATP III guidelines requires abdominal obesity (defined as a waist circumference >102 cm in men and >88 cm in women); triglycerides ≥150 mg/dL; HDL cholesterol <40mg/dL in men and <50mg/dL in women; resting blood pressure ≥130/85 mmHg; and fasting glucose ≥110 mg/dL (9;21). The ATP III definition does not require a euglycemic clamp for diagnosis, making it more applicable for the clinical setting than the WHO definition.

Following the release of the ATP III definition of the metabolic syndrome, the American Association of Clinical Endocrinologists (AACE) developed a modified version of the ATP III definition; however, it has received criticism due to its exclusion of obesity as a key factor in the metabolic syndrome (3;20).

The growing number of definitions of the metabolic syndrome along with the discrepancies in diagnosis criteria highlighted the need for a unifying definition of the syndrome (10). In 2005, the International Diabetes Federation (IDF) proposed a new set of guidelines with the hope of accomplishing such a goal (20). The IDF guidelines focused on central adiposity as a requirement for diagnosis of the metabolic syndrome. In addition to central adiposity, diagnosis of the metabolic syndrome under the IDF definition requires 2 or more of the following: triglycerides ≥150 mg/dL; reduced HDL cholesterol <40mg/dL in males and <50mg/dL in females or specific treatment for this lipid abnormality; raised resting blood pressure ≥130/85
mmHg; family history of diabetes (first-degree relative); and pre-existing CVD (6;22).

Additionally, the IDF published ethnic-specific cut off values for waist circumference, a measure of central obesity (6).

Prevalence of the Metabolic Syndrome

Several studies and meta-analyses have been conducted to determine the actual prevalence of the metabolic syndrome using differing definitions of the syndrome (3). A 2006 study compared the prevalence of the metabolic syndrome in Japanese men and women when using the ATP III and IDF definitions. Of the 2228 people in the study, 37.3% of men and 4.4% of women were diagnosed with metabolic syndrome according to the IDF definition, and 26.9% of men and 14.7% of women were diagnosed using the ATP III definition (23). A low number of women with a waist circumference greater than 90 cm could account for much of the discrepancy in the rate of diagnosis between men and women in this study. A similar study was conducted with Asian Indians in Chennai, India. 2350 individuals aged ≥20 years were screened for metabolic syndrome using the WHO, ATP III, and IDF definitions. The percentage of men diagnosed with metabolic syndrome was similar to the rates found Japanese males. Approximately 23.3%, 28.3%, and 25.8% of males were identified as having metabolic syndrome according to the WHO, ATP III, and IDF definitions in Asian Indians, while 19.7%, 19.4%, and 28.2% of females were diagnosed with metabolic syndrome using the same definitions. Interestingly, only 224 of the subjects were diagnosed by all three criteria (24). Another study in Jaipur, India found that out of 1091 participants, 9.8% of men and 20.4% of women were diagnosed with metabolic syndrome according to the ATP III definition (25).

Additional studies have been conducted in countries throughout the world. According to the ATP III definition of the metabolic syndrome, the prevalence in European countries ranges
from 10% in French men and 7% in French women (26) to 26.2% in Scottish men (27).

According to the WHO definition, the prevalence of the metabolic syndrome in Europe ranges from 44% in British men and 33.9% in British women to 12.2% in Italian men and 5.1% in Italian women (28). The prevalence in the Mexican population was found to be 26.6% and 13.6% according to the ATP III definition and the WHO definition, respectively (29). A meta-analysis of studies using the ATP III and WHO definitions of the metabolic syndrome indicated that the prevalence of the metabolic syndrome ranges from 8.8% to 92.3% in the United States and Europe; however, the studies that reported the highest rates of metabolic syndrome used participants who had already been diagnosed with type 2 diabetes. The meta-analysis indicated that the metabolic syndrome is an important risk factor for CVD, and that the association of the metabolic syndrome with risk of cardiovascular disease was higher in studies that used the WHO definition of the metabolic syndrome compared to the ATP III definition (30).

Several studies have been conducted in the United States in an attempt to determine the prevalence of the metabolic syndrome across different age and ethnicity groups. A study of 439 U.S. children between 4 and 20 years old used an age-adjusted version of the ATP III definition of the metabolic syndrome and found that the prevalence of the metabolic syndrome was 38.7% in moderately obese participants and 49.7% in severely obese subjects. In this particular study, no overweight or normal participants met the criteria for metabolic syndrome (31). The study found that the percentage of subjects with glucose intolerance increased with the severity of the obesity regardless of ethnicity, and biomarkers of increased risk of cardiovascular complications (decreased adiponectin, increased C-reactive protein) were already present in these children.

In U.S. adults, the prevalence of the metabolic syndrome varies depending on the criteria used for diagnosis and the age and ethnicity of the population measured (3). Meigs and others
(32) conducted a study of the prevalence of the metabolic syndrome according to the WHO and ATP III definitions in non-Hispanic white and Hispanic subjects. The study found the prevalence of the metabolic syndrome among white U.S. adults to be 26.7% by ATP III criteria and 27.3% by WHO criteria. The prevalence among Hispanic subjects was 41.4% (ATP III) and 41.1% (WHO). A 2005 study by Ford sought to compare the prevalence of the metabolic syndrome using the IDF and ATP III definitions. The study concluded that the prevalence of the metabolic syndrome among all U.S. adults is 40.5% according to the IDF definition and 34.5% according to the ATP III definition. Ford (6) also noted increased prevalence of the metabolic syndrome across all ethnic groups as age increased. Similarly, Alexander et al. (15) found the prevalence of the metabolic syndrome among U.S. adults ≥50 years old to be 43.5% according to the ATP III definition. Earlier studies by Ford (10;33) indicate the unadjusted and age-adjusted prevalence of the metabolic in the United States is 21.8% and 23.7, respectively. Park and others (34) found similar prevalence rates (22.8% in men and 22.6% in women).

Studies indicate a high prevalence of the metabolic syndrome in the U.S. and worldwide, and the prevalence is expected to rise in the future. The presence of the metabolic syndrome in children and the increased prevalence among older adults indicates that the metabolic syndrome could be a lifelong struggle for a relatively large portion of the population; therefore, practical and cost-effective measures for ameliorating the risk of developing the metabolic syndrome are essential.

Clinical Outcomes of the Metabolic Syndrome

CVD and type 2 diabetes are recognized as the main clinical outcomes of untreated metabolic syndrome (9;13;33;35;36). Isomaa et al. (37) found a threefold increase in the risk for coronary heart disease and stroke in subjects with metabolic syndrome versus those without, and
cardiovascular mortality rate was 12% in subjects with metabolic syndrome and only 2.2% in those without. A review by Ford (38) indicates that the population-attributable fraction for the metabolic syndrome is 12-17% for CVD and 30-52% for diabetes. Ford also notes a relative risk of 1.65 for CVD and 2.99 for diabetes in studies using the ATP III definition of the metabolic syndrome. A meta-analysis by Gami et al. (39) concluded that subjects with the metabolic syndrome had a relative risk of 1.78 of cardiovascular events and death in studies between 1971 and 1997. A more recent meta-analysis found that individuals with the metabolic syndrome have relative risk factor of 1.17 for mortality from CVD, as well as a relative risk of 1.53 for incidence of CVD, 1.52 for coronary heart disease, and 1.76 for stroke (30). These and numerous other studies point to the metabolic syndrome as an important risk factor for CVD related events and type 2 diabetes. The complications from CVD and type 2 diabetes cost individuals thousands of dollars each year (40), and the enormous human and monetary toll on society explains much of the recent research interest in the underlying causes and risk factors involved in the development of these diseases.

**Molecular Basis of the Metabolic Syndrome**

**Blood Glucose**

Glucose is the primary fuel source most tissues in the body, including skeletal muscle and the brain (41). Many cells rely on a small amount of glucose that is constantly circulating in the blood. Blood glucose is maintained at a fasting level of about 80 to 90mg/100ml of blood. A sharp decrease in circulating blood glucose (20 to 50 mg/100ml of blood) can lead to neuroglycopenia, seizure, and even death; however, chronically elevated blood glucose levels can lead to β-cell dysfunction and other complications associated with diabetes (42). Due to the
importance of blood glucose, an extensive physiological monitoring and control system has been developed for maintaining blood glucose at a normal level.

In 1921, Banting and Best (43) discovered pancreatic secretions that led to temporary increases in blood glucose, and insulin was soon isolated from the secretions. In 1923, Murlin and others proposed the possibility of a second pancreatic hormone that they termed glucagon (44). Insulin and glucagon are secreted directly into the blood via islets of Langerhans. The islets contain alpha, beta, and delta cells of which 60% are beta cells. Beta cells produce insulin while alpha cells, which comprise about 25% of the cells in the islets, produce glucagon (45).

**Role of Glucagon and Insulin in Blood Glucose Regulation**

The development of an immunoassay for glucagon by Unger and colleagues (46) allowed researchers to determine if glucagon can be identified in circulation and how its secretion influences blood glucose (47). Glucagon exerts a hyperglycemic effect when released into the bloodstream. As little as 1µg/kg of glucagon can raise blood glucose levels by 25% in 20 minutes (45). Glucagon primarily increases blood glucose levels by initiating glycogenolysis in the liver, a process by which glycogen is converted to glucose-1-phosphate (48). Once dephosphorylated, glucose is released from liver cells into the bloodstream, thus raising blood glucose levels. Glucagon is also responsible for gluconeogenesis in the liver, a process by which glucose is created from amino acids (49). Gluconeogenesis can provide about 180g of glucose during a 24-hour fast (50).

Insulin primarily affects the metabolic machinery within skeletal muscle cells. Insulin binds to receptors on the outside of the cell membrane that causes phosphorylation of intracellular enzymes. Phosphorylation of these enzymes causes increases in growth and gene expression as well as increases in fat, protein, and glucose synthesis. Insulin also causes the cell
membrane to become more permeable to glucose, particularly in skeletal and adipose cells (45). The increased permeability of the cell membrane to glucose is due to translocation of vesicles contained within the cell which release glucose transport proteins at the cell membrane. These glucose transport proteins bind with the cell membrane and facilitate glucose uptake into the cell (51). During periods of time when insulin secretion is high such as after a meal, muscle cells use glucose as the preferred fuel source over fatty acids. When glucose is not needed for energy in muscle cell, it can be stored as glycogen for later use (45).

Insulin exerts slightly different effects on other tissues in the body. In the liver, insulin acts primarily to increase storage of glucose. Insulin accomplishes this by increasing hepatic cell permeability to glucose and by inactivating liver phosphorylase. Insulin also activates glycogen synthase, an enzyme involved in glycogen formation (52). The liver generally stores about 100 grams of glucose which can be released between meals to maintain blood glucose (53).

Insulin also affects fat metabolism. The continuous availability of food is a recent development for humans, and humans have yet to adapt; therefore, the body attempts to store nutrients when they are plenty. As previously noted, insulin release can be associated with the increased use of glucose as a fuel source, which effectively spares fat. Additionally, insulin increases fatty acid synthesis and storage and inhibits phosphorylation of hormone-sensitive lipase (HSL), a hormone responsible for fatty acid mobilization (54). Insulin also increases adipose cell membrane permeability to glucose, which is used to form α-glycerol phosphate, a substance that provides the glycerol backbone of triglycerides (55). In the liver, glucose that is not stored as glycogen can be split into pyruvate through the glycolytic pathway. Pyruvate is then converted into acetyl-coenzyme A (acetyl CoA) (56), a necessary substrate for fatty acid synthesis. When glucose is being used for energy, citrate and isocitrate ions activate acetyl-CoA
carboxylase (ACC), an enzyme required for the reductive synthesis of fatty acids from acetyl-CoA and malonyl-CoA (57). Triglycerides formed through these pathways are then released into the blood stream in lipoproteins for uptake and storage in adipose tissue.

**Physiological Regulation of Glucagon and Insulin Release**

Several feedback mechanisms are in place to influence secretion and clearance of insulin and glucagon. The main stimulus that initiates both insulin and glucagon secretion is changes in the blood glucose level. As blood glucose levels fall from a normal level, glucagon is secreted to correct the problem. As described earlier, glucagon causes the liver to increase its output of glucose that raises blood glucose levels. Blood glucose is also a potent stimulator of insulin release; however, its effects on insulin secretion are the opposite of those for glucagon. As blood glucose levels rise above 100mg/100ml of blood, plasma insulin levels rise quickly. Plasma insulin levels tend to “overshoot” initially, but insulin secretion is not maintained and plasma levels decrease. After a short time, plasma insulin levels begin to rise again and reach a plateau or steady state (58;59). As blood glucose levels return to normal, insulin secretion is rapidly decreased.

While blood glucose is a potent stimulator of insulin release, other stimuli can cause insulin secretion. Gastrointestinal hormones such as gastrin and secretin can initiate an anticipatory increase in insulin secretion in response to feeding (60). Parasympathetic activation of the pancreas can also cause insulin secretion and may contribute to postprandial insulin secretory response (61).
Glucose Uptake and Use in Skeletal Muscle

Skeletal muscle makes up a large percentage of an individual’s body mass, and is the primary site for insulin-stimulated glucose uptake and use (62). As early as 1926, Best demonstrated that insulin stimulates glucose uptake by skeletal muscle (63). A subsequent series of studies by Gemmill demonstrated that insulin promotes glucose uptake in isolated rat diaphragm muscle (64;65). In 1944, Gammeltoft noted that fructose and glucose uptake occurs through separate mechanisms in perfused cat hind limbs (66). However, it was not until 1949 that Levine proposed the existence of a facilitated transport system for transporting sugars across the membrane of skeletal muscle cells (67). Decades later, work in adipocytes elucidated the possibility of glucose transport protein (GLUT) translocation from an intracellular pool to the plasma membrane in the presence of insulin (68). Subsequent work led to the characterization and cloning of glucose transporter-4 (GLUT4) (69;70). The development of GLUT4 knockout mice led to the realization that the absence of GLUT4 does not necessarily preclude the development of diabetes, suggesting the presence of other GLUTs involved in glucose transport (71-73).

Today, as many as 14 members of the GLUT hexose transport family have been identified (74;75). Due to differences in tissue expression, structure, and function, the GLUT family has been subdivided into three categories (74). Of these classes, class one GLUTs, including GLUT1 through GLUT4 are the most extensively researched to date. GLUT4 is the predominant transport protein in skeletal muscle cells; however, the mRNA for at least seven other GLUT isoforms are present in skeletal muscle (75). Of these GLUTs, GLUT4, GLUT5, and GLUT12 account for the vast majority of the GLUT isoform mRNA found in skeletal muscle, and along with GLUT1, contribute most of the facilitated transport of hexoses through
the plasma membrane. Furthermore, Stuart et al. (75) demonstrated that the fructose transporter GLUT5 was predominantly expressed in type II muscle fiber, while GLUT12 was predominantly expressed in type I muscle fibers. A subsequent study in humans demonstrated that GLUT12 accounts for as much as 12% of insulin-translocatable GLUTs and works together with GLUT4 to facilitate glucose uptake into muscle cells in response to insulin (76).

While it appears there is some redundancy to the GLUT system, GLUT4 accounts for the majority of facilitated glucose transport through the sarcolemma in response to insulin and exercise. Insulin stimulation can cause a 2- to 3- fold increase in GLUT4 translocation from intracellular storage vesicles to the plasma membrane (77); however, the mechanisms behind GLUT4 translocation have only recently been elucidated. Studies indicate that GLUT4 has unique sequences in its N- and C- termini which contribute to its recycling and transport characteristics (78). Piper et al (79) showed that the N-terminus contains a phenylalanine targeting motif that when deleted or replaced with alanine at position 5 resulted in the accumulation of GLUT4 in the plasma membrane. However, other groups have suggested that the C- terminus is the most important targeting motif of GLUT4. In particular, the dileucine motif in the C- terminus has been suggested to be important in the intracellular trafficking of GLUT4 (80;81).

Insulin and exercise mediate endocytosis and exocytosis of GLUT4 through complex signaling pathways that are not yet clearly understood; however, evidence suggests that insulin-dependent and exercise-induced glucose uptake occur through separate mechanisms and rely on different intracellular GLUT4 pools (82). The plasma membrane of insulin-sensitive tissues contain transmembrane tyrosine kinase insulin receptors (IR) which target IRS proteins when bound with insulin (83). In particular, conformational change of the IR primarily phosphorylates
IRS-1 and IRS-2 in skeletal muscle cells that attract phosphatidylinositol PI 3-kinase (PI3-K) to their location (84). The signal is then transmitted through PDK1 and mammalian target of rapamycin (mTOR) to other downstream targets, including two serine/threonine protein kinases, Akt/protein kinase B (PKB) and atypical PKC (77). Activation of the Akt isoform Akt2 appears to control GLUT4 trafficking in skeletal muscle cells (85), although the mechanisms are unclear. Other substrates including platelet-derived growth factor (PDGF) and 5’ AMP-activated protein kinase (AMPK) increase GLUT4 translocation to the plasma membrane. Currently, research is being conducted to elucidate downstream cellular machinery responsible for GLUT4 translocation, including Rab GTPase Activating Protein (AS160) (86).

Exercise does not appear to cause phosphorylation of IRS-1, suggesting that exercise and insulin signaling occur through separate pathways (87). Additionally, PI3-K inhibition by wortmannin eliminates insulin-stimulated glucose uptake but not exercise-stimulated glucose transport in contracting skeletal muscle (88). Exercise-induced glucose uptake occurs through at least 2 possible pathways. Exercise causes a decrease in the ATP:AMP ratio, which is a powerful stimulator of AMPK activity (89). AMPK is a heterotrimer with two catalytic α-subunits and two regulatory subunits, β and γ. Of the two α-subunits, α2 has the highest expression in skeletal muscle (90) and has been suggested as the primary isoform responsible for metabolic adaptations in human skeletal muscle (91). Phosphorylation at threonine residue 172 (Thr172) of AMPK by the upstream serine/threonine protein kinase 11 (LKB1) stimulates AS160 and atypical PKC which leads to GLUT4 translocation (92). The interaction of AMPK and AS160 in response to exercise may explain the additive effect of the insulin and exercise stimulated GLUT4 translocation signaling pathways (93;94). Interestingly, impaired AS160 phosphorylation has
been observed in individuals with type II diabetes; however, it is unclear if this defect significantly contributes to insulin resistance (95).

Muscle contraction also causes the release of calcium from the sarcoplasmic reticulum, which increases intracellular calcium concentration. Caffeine has been used to stimulate calcium release independent of muscle contraction to determine the effects of calcium concentration on glucose uptake (96). While the mechanisms are still unknown, conventional protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase (CaMK) are potential intracellular proteins responsible for contraction-stimulated glucose uptake (93). Inhibition of LKB1 results in a decrease in contraction-stimulated glucose uptake; however, inhibition of AMPK phosphorylation has little to no effect on contraction-stimulated glucose uptake, suggesting an AMPK-independent signaling pathway (97). CaMK-Kinases (CaMKK) have recently been identified as upstream regulators of AMPK activity in skeletal muscle cells, and studies have demonstrated that CaMKKα may be implicated in glucose uptake signaling (97-99). More research is needed to elucidate the signaling pathway responsible for the increase in glucose uptake following muscle contraction.

The importance of GLUT4 in regulating blood glucose in response to insulin and exercise has made GLUT4 a major target for the treatment and prevention of the metabolic syndrome and diabetes. It has been demonstrated that a single bout of exercise is enough to increase GLUT4 gene expression in skeletal muscle (100). Several short- and long-term training studies have also demonstrated increases in GLUT4 expression in response to exercise (101-105). More recent human studies have shown increases in skeletal muscle (106;107) and whole body (108;109) glucose uptake, possibly due to exercise-induced increases in GLUT4 expression. Furthermore, epidemiological studies indicate that physical inactivity increases the risk of developing type 2
diabetes in humans (110), which underscores the important role of GLUT4 expression and exercise in the prevention of insulin resistance.

**Insulin Resistance and the Metabolic Syndrome**

In 1939, Himsworth observed that the decreased ability of tissues to take up glucose is the major underlying defect of diabetes mellitus (111). Twenty years later Yalow and Berson (112) developed an immunoassay of endogenous plasma insulin that was sufficiently specific and sensitive to measure plasma insulin levels. Using this immunoassay, Yalow and Berson demonstrated that subjects with type II diabetes had higher fasting plasma insulin levels than non-diabetic subjects, and diabetic subjects had a delayed insulin response when given an oral glucose load. These findings were later confirmed by other studies (113-115). Interestingly, these studies indicate that normal and obese non-diabetic subjects reach a higher plasma insulin concentration in response to a glucose load when compared to diabetic subjects.

Longitudinal studies indicate that the development of insulin resistance occurs prior to the development of hyperglycemia (116-119). Accordingly, recent research has begun to link obesity to insulin resistance, as opposed to the traditional “glucocentric” view of the metabolic syndrome (5). As discussed previously, most widely accepted definitions of the metabolic syndrome include some measure of abdominal obesity in the diagnostic criteria. Indeed, high plasma triglycerides and reduced HDL cholesterol are typically seen in individuals with abdominal obesity (120). Moreover, increased plasma free fatty acid (FFA) concentration is elevated in obese subjects (121), possibly due to increased fat mass (122). In 1963, Randle and coworkers (123) found that FFA interfere with the oxidation of glucose in rat heart and diaphragm muscle in vitro. This finding remained controversial until recent studies demonstrated similar findings in human skeletal muscle. In 1996, Roden et al. (124) observed a decrease in
carbohydrate oxidation following lipid infusion under euglycemic insulin clamp conditions in humans. Roden concluded that elevated plasma FFA content “causes insulin resistance by inhibition of glucose transport and/or phosphorylation with a subsequent reduction in rates of glucose oxidation and muscle glycogen synthesis.” A subsequent study by Santomauro et al. (125) showed that treatment with an antilipolytic drug, Acipimox, reduced fasting plasma FFA and insulin concentrations in obese subjects. Furthermore, euglycemic-hyperinsulinemic clamps and glucose tolerance tests revealed improved glucose tolerance after plasma FFA reduction in obese non-diabetic patients and obese patients with type 2 diabetes.

Although link exists between obesity and insulin resistance, the mechanisms behind this relationship are less clear. Recent investigations attempting to explain this relationship have focused on the endocrine functions of adipose tissue. Tumor necrosis factor-alpha (TNF-α) is a cytokine produced primarily in macrophages which has been shown to be elevated in obese rodent and human models. In rodents, over-expression of TNF-α has been shown to induce peripheral insulin resistance, which is restored with neutralization of TNF-α (126). In humans, it has been demonstrated that obese subjects express significantly more TNF-α mRNA in fat tissue than in lean controls, and a strong positive correlation exists between TNF-α mRNA expression and hyperinsulinemia (127-129). Furthermore, TNF-α mRNA expression and insulin insensitivity decreased with a corresponding reduction in body mass (127). Other studies failed to find a relationship between TNF-α secretion and insulin sensitivity in vivo in humans (130;131), possibly due to differences in sample collection and analysis used in these studies. Nonetheless, Feinstein and others examined the effect of TNF-α on rat Fao hepatoma cells to determine the mechanism behind TNF-α induced insulin resistance. As discussed previously, insulin exerts its effects at the cellular level by binding to insulin receptors on the cell surface.
The binding of insulin to the α-subunit of the IR activates the tyrosine kinase in the β-subunit, which is necessary for insulin action in the cell. Feinstein and coworkers (132) demonstrated that TNF-α exposure inhibits tyrosine phosphorylation of the IR β-subunit and IRS-1 in Fao cells, which inhibits insulin receptor signaling. This finding suggests a possible mechanism for obesity-induced insulin resistance.

Adipose tissue also secretes interleukin-6 (IL-6), another cytokine associated with inflammation. Similar to TNF-α, high plasma concentrations of IL-6 correspond to increased obesity and insulin resistance (128;133;134). Kern et al. (128) demonstrated that IL-6 may affect insulin sensitivity in mouse hepatocytes and human HepG2 cells via similar mechanisms to TNF-α. Specifically, IL-6 may inhibit IR activity due to decreased tyrosine phosphorylation of IRS-1, which inhibits insulin-dependent activation of Akt. However, a study in IL-6-deficient (IL-6−/−) mice indicates that hypothalamic IL-6 may exert anti-obesity effects (135). IL-6−/− mice developed type 2 diabetes, which was partially ameliorated by IL-6 treatment. Furthermore, substrate metabolism was impaired in IL-6−/− mice. Similarly, studies in human skeletal muscle indicate that IL-6 exposure improves glucose metabolism but has no effect on insulin-dependent glucose uptake (136). It has been demonstrated that contracting skeletal muscle produces IL-6, which leads to an increase in plasma IL-6 levels (137). Recent evidence suggests that the effects of IL-6 vary depending on the target tissue. Chronic elevation of circulating IL-6 levels are an indicator of insulin resistance, while the acute presence of IL-6 may be beneficial for certain tissues such as skeletal muscle (138).

Other adipocyte secretions include plasminogen activator inhibitor 1 (PAI-1) and apidonectin. PAI-1 has recently been linked to the development of thrombosis, fibrosis, and insulin resistance (139). Using PAI-1 deficient mice Ma and colleagues demonstrated that PAI-1
deficiency protects against diet-induced obesity and insulin resistance, suggesting that PAI-1 may be important in the development of obesity. Others have hypothesized that PAI-1 contributes to a “hypercoagulable” state, which may result in increased platelet formation and associated cardiovascular risks (140). Adiponectin is a protein secreted by adipocytes. Circulating levels of adiponectin are inversely related to obesity and insulin resistance, while up-regulation of adiponectin protects against obesity-induced insulin resistance (141). Adiponectin also acts as an insulin-sensitizing agent, possibly through phosphorylation and activation of the AMPK signaling pathway (142).

While recent work has elucidated many potential mechanisms by which insulin resistance develops, the underlying cellular processes are poorly understood. The concept of obesity as a chronic state of inflammation is supported by evidence that adipocytes release inflammatory mediators, and subsequent work has shown that obese individuals generally express abnormal levels of these inflammatory markers. As discussed earlier, CVD is the primary clinical outcome of the metabolic syndrome. Obesity has been linked to proven mechanisms contributing to the development of CVD and an increased risk of cardiovascular events (140) including atherosclerosis, hypercholesterolemia, increased blood viscosity, and platelet dysfunction (143). Interestingly, weight loss has been shown to reduce inflammatory markers and improve insulin resistance in humans (144-146), which suggests that weight reduction by dietary alterations or increased physical activity may be an effective means of ameliorating the effects of the metabolic syndrome.
Exercise and the Metabolic Syndrome

The health benefits of regular physical exercise have been widely reported. Regular physical exercise is beneficial in alleviating the independent risk factors for the metabolic syndrome, including reduced systolic and diastolic blood pressure (147), improved body composition (148), modestly increased HDL cholesterol (149-151), and slight lowering of serum triglycerides (possibly due to weight loss) (151;152). Moreover, exercise can improve insulin sensitivity acutely and chronically (153). In a study by Katzmarzyk et al. (154), 30.5% of the subjects originally diagnosed with the metabolic syndrome no longer met the ATP III criteria for the metabolic syndrome after 20 weeks of endurance training, illustrating the effectiveness of exercise as a means to treat and prevent the metabolic syndrome. In fact, cardiovascular fitness level has been shown to be associated with the incidence of the metabolic syndrome (155-157).

Exercise has been recognized as an essential component in the management of the metabolic syndrome and diabetes for decades (110). Until recently, much of the available research focused on endurance training as a means to alleviate the metabolic syndrome; however, individuals at risk for developing the metabolic syndrome and type 2 diabetes tend to have a greater preponderance of type II muscle fibers (158) which may make endurance training poorly tolerated. A growing body of literature indicates that resistance training is a safe and effective alternative for reducing the risk of the metabolic syndrome (159-163).

Endurance and resistance training have been shown to improve glycemic control (159;164;165) and insulin resistance (165-167). However, the physiological adaptations that contribute to these improvements differ depending upon the type of exercise performed. For example, endurance training causes an increase in mitochondrial biogenesis, oxidative enzymes, and fatty acid oxidation in type I muscle fibers (168-171), while resistance training does not
Additionally, endurance training increases capillary density (168), while capillary changes in response to resistance training are less clear (173;175); however, both forms of training seem to increase blood flow to skeletal muscle (173;176). Recent work has begun to elucidate the molecular basis for the diverse adaptations to different training modalities.

Muscle Fiber Composition

Skeletal muscle shows remarkable plasticity in response to training. Even acute exercise can cause an increase in protein synthesis (177) and mitochondrial oxidative enzymes (178;179); however, endurance and resistance exercise seem to exert a number of their effects on type I and type II muscle fibers, respectively (180). Muscle fiber type is largely determined by the motor neuron that innervates it, as demonstrated by cross-innervation studies in cat hindlimb muscles (181). The α-motor neuron and all of the muscle fibers it innervates make up a motor unit (182). Burke and coworkers (183) identified three types of motor units based on certain contractile properties. Slow twitch (type S) motor units develop relatively low force, speed, and power and have the longest time to peak force (182); however, they are fatigue resistant, have high oxidative and mitochondrial enzyme content and have extensive capillarization. Another motor unit type, fast-twitch fatigue sensitive (type FF), produce the greatest speed and force of contraction, as well as the highest peak force. Unlike type S motor units, type FF have low mitochondrial enzyme content and capillarization, which contributes to their high susceptibility to fatigue; however, type FF have high glycogen content. A third, intermediate motor unit termed fast-twitch fatigue resistant (type FR) expresses properties of type S and type FF motor units. Type FR produce moderate force and speed of contraction, and contain moderate levels of glycogen and mitochondrial enzymes, which makes them somewhat fatigue resistant (184).
The muscle fibers that make up a motor unit have traditionally been identified based on the myosin ATPase activity of the fibers. Myosin is a contractile protein that, along with actin, makes up the majority of myofibril protein content. Myosin consists of two subunits – heavy and light meromyosin (HMM and LMM, respectively). HMM-\(S_1\) is a globular fragment of HMM that contains two myosin heads. Within HMM-\(S_1\) are myosin heavy chains (MHC), the primary determinants of the ATPase activity and the speed of contraction of a muscle fiber (182;185). Myosin light chains (MLC) are present in the neck region of the myosin molecule, and the isoforms of MHC and MLC are the primary determinants of muscle fiber type classification (182). Peter et al. (186) identified three types of muscle fibers in rabbit and guinea pig hindlimb muscles using myosin ATPase and metabolic enzyme activity. Based on metabolic activity, the authors proposed a new classification system consisting of fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG), and slow-twitch oxidative (SO) motor units. Another classification system is based on the observation that fast and slow myosin ATPase enzymes have different alkaline and acidic stability (187). Brooke and Kaiser (188) found that type IIx fibers (fast twitch) are intensely stained for ATPase activity after preincubation at pH 10.4, while preincubation at pH 4.6 results in strong staining of type I (slow-twitch) fibers. Additionally, the intermediate type IIa fibers are moderately stained at pH 4.6. More recently, Behan and colleagues (189) demonstrated that MHC type 1 and MHC type 2 can be labeled using differently tagged monoclonal antibodies. Type IIx muscle fibers contain little or no MHC type 1, while type IIa fibers contain a mixture of MHC type 1 and 2, which allows for differentiation of type IIa and IIx muscle fibers.
Bioenergetics of Exercise

Normal human skeletal muscle contains a mixture of type I, IIa, and IIx muscle fibers. The metabolic and enzymatic properties of these fibers make them suited for different types of activity. As discussed briefly, type II muscle fibers fatigue quickly but have rich glycogen stores. Resistance exercise is generally short term, high intensity work. Energy for resistance exercise is primarily supplied through the phosphagen and glycolytic systems. At the onset of exercise, adenosine triphosphate (ATP) is hydrolyzed during muscle contraction in a reaction catalyzed by myosin ATPase. Hydrolysis of ATP produces ADP and inorganic phosphate (P$_i$). In order to rapidly produce ATP, creatine kinase catalyzes a reaction in which the phosphate group of phosphocreatine (PCr) is donated to ADP, which results in ATP formation (182). ATP can also be rapidly produced via the myokinase reaction, whereby two ADP are used to form ATP and AMP. Type II muscle fibers generally contain higher concentrations of phosphagens than type I muscle fibers (190); however, phosphagens can only supply energy for about 10-15 seconds of high intensity work. Fast glycolysis is the primary energy system used during high and moderately high intensity exercise lasting between approximately 30 seconds and 2 minutes. Fast glycolysis involves the breakdown of carbohydrates, particularly blood glucose or glycogen, into pyruvate. Fast glycolysis nets 2 molecules of ATP for each molecule of glucose consumed. Each of the reactions involved in ATP production via fast glycolysis occurs without the presence of molecular oxygen. In the continued absence of oxygen, pyruvate formed through fast glycolysis is converted to lactate (50), which can act as an energy substrate during long-term exercise (182;191). Slow glycolysis occurs in the presence of oxygen, when pyruvate produced through fast glycolysis enters the matrix of the mitochondria and is converted to acetyl CoA. Formation of citrate from oxaloacetate and the acetyl group of acetyl CoA marks the beginning of the Krebs
cycle. The Krebs cycle produces 2 molecules of GTP that can be converted into ATP. More importantly, however, the Krebs cycle produces 6 molecules of nicotinamide adenine dinucleotide (NADH) and 2 molecules of FADH$_2$. These molecules are used in the inner membrane of the mitochondria to produce ATP via oxidative phosphorylation (45;50). Overall, anaerobic metabolism can produce 2 molecules of ATP from one molecule of glucose, while aerobic mechanisms can produce 38 molecules of ATP from one molecule of glucose. Although slow glycolysis and oxidative phosphorylation are considerably more efficient than fast glycolysis, the relatively slow rate of ATP production dictates that aerobic mechanisms can only supply energy for low intensity exercise; however, aerobic mechanisms can oxidize carbohydrate, fat, and --to a lesser extent-- protein sources to provide ATP during long periods of physical activity.

As discussed previously, endurance training results in an increase in oxidative enzymes and mitochondrial density in type I and type II muscle fibers. Strength training may result in increases of ATP and PCr concentration within muscle cells (192;193), although some controversy exists as to whether the concentration increases or whether there is simply an increase in proportion to muscle fiber hypertrophy. Regardless, endurance and resistance training result in an increase in the corresponding muscle fiber cross sectional area (173;194;195). Type II fibers tend to undergo greater hypertrophy than type I fibers in response to training, and training may cause a shift in the proportion of type II fibers from type IIb toward type IIa (196). Training also influences glycogen storage in skeletal muscle, which may play a role in whole body glucose disposal (169;197;198). In the context of these somewhat divergent metabolic and functional adaptations, it is not surprising that the molecular mediators of skeletal muscle adaptations to endurance and resistance training are quite different (199).
**Molecular Mediators of Skeletal Muscle Adaptations to Exercise**

Endurance training primarily causes an increase in AMPK concentration and activity. AMPK activates downstream targets resulting in greater energy production capabilities of the cell (200). In addition to its role in contraction-mediated GLUT4 translocation and glucose metabolism, AMPK plays a role in fatty acid synthesis and oxidation. As early as 1973, kinase-dependent phosphorylation of acetyl-CoA carboxylase (ACC) was demonstrated in rat hepatic cells (201). 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) has been used to activate AMPK in skeletal muscle to determine the role of AMPK in fat oxidation. Merrill et al. (202) found that AICAR activated AMPK, inactivated ACC, and decreased malonyl-CoA content in perfused rat hindlimbs that led to 2.8 fold increase in fat oxidation. Similar results have been demonstrated in isolated rat skeletal muscle (203). Other groups have suggested a link between AMPK activity and increased fatty acid oxidation during muscle contraction in rats (204-206) and humans (207;208).

In humans, type 2 diabetes is often treated with thiazolidinediones (TZDs), which are ligands for peroxisome proliferator-activated receptor gamma (PPARγ). Activation of PPARγ and subsequent gene activation appears to enhance insulin sensitivity (209); however, TZDs may also exert their effects independently of PPARγ-regulated gene transcription by activating the AMPK signaling pathway (210). Coletta et al. found that treatment with pioglitazone significantly increased plasma adiponectin and decreased plasma FFA levels in patients with type 2 diabetes. Furthermore, AMPK and ACC phosphorylation increased with a concomitant increase in insulin stimulated glucose disposal (211). A similar study found that 3 months of treatment with rosiglitazone normalized basal AMPK activity and partially restored basal rates of fatty acid oxidation in patients with type 2 diabetes. (212). Metformin, another drug used for the
treatment of type 2 diabetes, has also been shown to increase AMPK activation in skeletal muscle (213;214) and decrease insulin resistance through similar mechanisms (215;216).

Activation of PPARγ co-activator 1 alpha (PGC-1α) by AMPK is a key step whereby AMPK increases the oxidative capabilities of skeletal muscle. PGC-1α coactivates nuclear respiratory factors-1 and -2 (NRF-1 and NRF-2), which regulate mitochondrial transcription factor A (Tfam) (217). Tfam lies upstream of several mitochondrial DNA (mtDNA) gene products and is responsible for mtDNA transcription. AMPK activation by AICAR (218;219) and exercise (220-222) have resulted in a concomitant increase in PGC-1α activation and mitochondrial biogenesis in multiple models. Mitochondria provide most of the energy consumed by a cell (50) and increased mitochondrial density is related to improved endurance performance (223). Furthermore, PGC-1α has been shown to increase in mitochondrial oxidative enzymes in type I and type II skeletal muscle fibers of transgenic mice, suggesting that PGC-1α causes a phenotypic shift in muscle fibers towards having higher oxidative capabilities (224). It has been demonstrated that individuals with type 2 diabetes have a low proportion of type 1 muscle fibers (225), smaller mitochondria, and reduced mitochondrial enzyme activity (226-228), leading to the hypothesis that mitochondrial dysfunction may contribute to insulin resistance; however, some groups contest this finding (229;230). Nonetheless, multiple studies indicate that decreased PGC-1α expression is related to insulin resistance in humans (231-234).

**AMPK**

Activation of the AMPK signaling pathway and subsequent changes in skeletal muscle by endurance exercise have been extensively studied in animals and humans. Acute endurance exercise has been shown to cause increased GLUT4 translocation (235), increased AMPK α2 expression, and decreased blood glucose concentrations in type 2 diabetics (236). AMPK
activation by endurance exercise may depend on several factors including the intensity of the exercise and prior training. Chen and coworkers (237) obtained muscle biopsies from the vastus lateralis of 8 men at rest and after three 20-minute exercise sessions at low, medium, and high intensity. AMPK α2 activity increased 5-fold from low intensity (~40% VO_{2peak}) to medium intensity (~59% VO_{2peak}), and continued to increase at high intensity (~79% VO_{2peak}). Additionally, glucose clearance increased as the intensity of exercise increased, and fat oxidation increased from rest to medium intensity. Similarly, it has been suggested that obese and diabetic individuals may need to exercise at higher intensities to elicit the same AMPK signaling response present in lean individuals after an acute exercise bout. Sriwijitkamol et al (238) found that low- and moderate-intensity exercise significantly lowered blood glucose concentration in subjects with type 2 diabetes but not lean and obese subjects. Furthermore, low- and moderate-intensity exercise increased AMPK phosphorylation in lean subjects, while only moderate-intensity exercise increased AMPK phosphorylation in obese subjects and type 2 diabetics. To determine the effect of previous training on AMPK activation, Coffey and colleagues (239) observed the AMPK and mTOR signaling response to endurance and resistance exercise in trained individuals. Cycling for 1 hour at 70% VO_{2peak} caused a significant increase in AMPK phosphorylation in resistance-trained individuals, but not endurance-trained individuals. Additionally, resistance exercise caused a significant increase in AMPK phosphorylation in endurance-trained individuals, indicating that prior training may affect the signaling response to acute exercise.

It has been demonstrated that long-term endurance training can restore abnormalities in the AMPK signaling pathway observed in obese Zucker rats (240). Additionally, endurance training improved whole-body insulin sensitivity in high-fat fed rats (241). Lessard and
colleagues (242) demonstrated that rats bred for high endurance running capacity had improved insulin sensitivity and lower fasting plasma glucose and triglyceride levels than low endurance capacity rats. Endurance training also increases GLUT4 protein content in rats (104;243) and obese Zucker rats (244). Furthermore, endurance training increases muscle PGC-1α and rescues high fat feeding-induced mitochondrial dysfunction in mice (245).

Copious studies have been published demonstrating the beneficial effects of endurance training for humans with insulin resistance and type 2 diabetes. Training periods typically range from 2 to 9 months and employ various training modalities and intensities, although as little as 1 week of training has been shown to be beneficial (246). Eight weeks of cycle ergometer training at approximately 65-70% VO2peak significantly increased mitochondrial FA oxidation and glucose tolerance in obese humans (247). Similarly, Venables and Jeukendrup (248) demonstrated that 4 weeks of endurance training increased FA oxidation rates by 44% and increased whole-body insulin sensitivity index by 27%. Other groups have demonstrated that the increase in FA oxidation in obese subjects is due to endurance training and not weight loss (249;250). In subjects with type 2 diabetes, 8 weeks of endurance training increased VO2peak approximately 20% and insulin sensitivity approximately 30% (251). Houmard and colleagues (103) demonstrated that 14 weeks of training caused a nearly twofold increase in GLUT4 content in vastus lateralis muscle. Seven weeks of endurance exercise on a cycle ergometer significantly increased citrate synthase activity, FA oxidation and mitochondrial area in untrained subjects (252). Coggan et al. (253) demonstrated that 9-12 months of walking or jogging caused a 23% increase in VO2max and increased the activity of several mitochondrial enzymes. The literature reviewed in this section highlights the importance of AMPK in mediating the molecular adaptations to exercise and the subsequent impact on risk factors for the metabolic syndrome.
As discussed previously, resistance exercise is short duration, high intensity exercise that primarily causes skeletal muscle hypertrophy by increasing protein synthesis and decreasing protein degradation. The Akt/mTOR signaling pathway is up regulated during skeletal muscle hypertrophy and has been identified as a major regulator of cell growth (254). mTOR is thought to be a sensor of substrate availability, particularly amino acids (255;256). The Akt/mTOR pathway regulates the cellular metabolic response to the availability of substrates and the presence of insulin and other growth factors (257). Additionally, the Akt/mTor pathway may be activated by changes in cellular ATP levels (258). Recently, possible roles of mTOR and its downstream targets in the development and treatment of insulin resistance and the metabolic syndrome have been elucidated. Decreased β-cell size and a concomitant decrease in glucose-stimulated insulin secretion has been observed in S6 kinase 1 (S6K1) deficient mice (259); however, Um and colleagues (260) demonstrated that S6K1<sup>−/−</sup> mice are hypersensitive to insulin and are protected from diet-induced obesity. Furthermore, it has been demonstrated that inhibition of the mTOR signaling pathway by the immunosuppressive drug rapamycin results in increased insulin-stimulated glucose transport (261), suggesting that mTOR plays a role in glucose disposal and insulin resistance.

mTOR is a 289 kDa protein that belongs to the phosphoinositide-3 kinase (PI3K)-related kinase family (262). mTOR nucleates 2 distinct protein complexes, termed mTORC1 and mTORC2. mTORC1, which is sensitive to rapamycin and nutrient signaling, contains regulatory associated protein of mTOR (RAPTOR), G-protein beta-like protein (GßL), protein rich Akt substrate 40 kDa (PRAS40), and DEP-domain-containing mTOR-interacting protein (Deptor). mTORC2, sometimes referred to as the rapamycin-insensitive complex, contains mammalian
stress-activated protein kinase interacting protein (mSIN1) and rapamycin-insensitive companion of mTOR (RICTOR) instead of RAPTOR (263). The activity of mTOR and its downstream targets are regulated by a complex signaling pathway that can be activated through several mechanisms, primarily via insulin signaling. Phosphorylation of IRS proteins by insulin and insulin-like growth factors (IGFs) causes association of IRS-1 with PI3K. This association results in the production of phosphatidylinositol (3,4,5)-triphosphate (PIP3) which activates PI3K-dependent kinase 1 (PDK1) (264). PDK1 in turn activates Akt, which inactivates tuberous sclerosis complex 2 (TSC2), an inhibitor of cell growth. Consequent activation of Rheb activates mTORC1 that phosphorylates downstream targets, including S6K1 and eukaryotic initiation factor 4E (eIF3E)-binding protein 1 (4E-BP1) (265). Phosphorylation of 4E-BP1 by mTORC1 inhibits its binding to eIF4E allowing eIF4E to promote cap-dependent translation (266). Similarly, S6K1 activation results in increased cap-dependent translation and elongation, mRNA biogenesis, and ribosomal protein translation (262).

Considering the central role of mTOR in the energy regulation of mammalian cells, it is no surprise that mTOR is an important mediator of the metabolic responses to exercise. A single bout of resistance exercise can cause an transient increase in protein synthesis of up to 115% (177). This increase in protein synthesis in response to acute resistance exercise appears to occur in an mTOR-dependent manner (267). Baar and Esser (268) demonstrated that electrical stimulation mimicking resistance training resulted in increased activity of S6K1, which correlated with hypertrophy of extensor digitorum longus and tibialis anterior in rats. Nader and Esser (269) performed a similar investigation employing high and low frequency electrical stimulation of tibialis anterior and soleus muscles. High frequency stimulation produced a transient increase in Akt phosphorylation and a long-term increase in S6K1 activation. A later
investigation by Atherton and coworkers (270) demonstrated that high-frequency electrical stimulation of isolated rat muscle caused an immediate increase in mTOR and Akt phosphorylation. In trained and untrained humans, acute resistance exercise causes increased myofibrillar protein synthesis with a concomitant increase in mTOR and S6K1 activation (271).

Considerable evidence suggests that resistance training is an effective method for ameliorating insulin resistance in humans (159;164-167); however, little is known about the potential role of mTOR in mediating the positive effects of resistance training in insulin resistant individuals. Recently, Izumiya and colleagues (272) generated a transgenic mouse expressing a constitutively activated form of Akt1. Two weeks of Akt1 activation led to a significant increase in type IIb muscle fiber cross-sectional area in the gastrocnemius. In addition, Akt1 activation in high fat/high sucrose fed mice led to a 44% reduction in subcutaneous fat pad mass as well as normalization of fasting blood glucose levels, insulin levels, and glucose uptake. These results suggest a possible mechanism by which resistance training and the consequent activation of the Akt/mTOR signaling pathway may ameliorate the metabolic syndrome.

Summary

The prevalence of metabolic disorders such as obesity, diabetes, and the metabolic syndrome is on the rise. Left untreated, these conditions can lead to cardiovascular disease and other complications. Exercise has been considered a cornerstone of diabetes prevention and treatment for decades. Increased activity levels can positively alter body composition, blood lipids, blood pressure, and other risk factors for the metabolic syndrome. At the molecular level, exercise activates intracellular signaling pathways that mediate the skeletal muscle adaptations to exercise. Activation of the AMPK pathway leads to mitochondrial biogenesis and increases GLUT4 protein expression and translocation, while mTOR increases protein synthesis. Several
studies have demonstrated that activation of the AMPK signaling pathway in response to endurance and resistance training has a positive impact on insulin sensitivity in humans; however, the exact mechanisms leading to increased insulin sensitivity in response to mTOR activation have yet to be elucidated in humans.
CHAPTER 2

RESISTANCE TRAINING INCREASES THE EXPRESSION OF AMPK, MTOR, AND GLUT4 IN PREVIOUSLY SEDENTARY SUBJECTS AND SUBJECTS WITH THE METABOLIC SYNDROME

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ABSTRACT

The prevalence of the metabolic syndrome is increasing at an alarming rate. Endurance exercise has been considered a cornerstone of diabetes treatment and prevention for decades, but the benefits of resistance training are less clear. Ten subjects with the metabolic syndrome (MS subjects; 5 male, 5 female) and 9 sedentary controls (4 male, 5 female) were recruited to undergo 8 weeks of resistance training. Euglycemic hyperinsulinemic clamps were performed before and after training to quantify insulin sensitivity. Muscle biopsies were taken from the vastus lateralis and changes in muscle fiber composition, muscle adenosine monophosphate-activated protein kinase (AMPK), mammalian target of rapamycin (mTOR), glucose transporter protein 4 and 5 (GLUT4 and GLUT5), and ATP synthase were quantified. Additionally, functional capabilities (isometric strength and VO$_{2\text{max}}$) were measured before and after training. After training, strength and stamina increased by 13% and 35% in both groups, respectively. Insulin sensitivity reflected by glucose infusion rate did not improve in MS subjects after training (3.3 mg/kg.min ± 1.3 before training vs.3.1 ± 1.0 post); however, the increase in insulin sensitivity was statistically significant in controls (p<0.01, 7.0 ± 2.0 pre vs. 8.7 ± 3.1 post). Controls increased muscle expression of p-AMPK (+50.0%) and ATP synthase (+63.0%) to a greater extent than MS Subjects (+13.0% and 25.0%, respectively); however, MS subjects increased expression of p-mTOR to a greater extent (57.0% vs. 32.0%, respectively). These data indicate that while resistance training improved functional capabilities in MS subjects, insulin sensitivity was not improved, possibly due to inhibition of the AMPK signaling pathway.

Key Words: METABOLIC SYNDROME, RESISTANCE TRAINING, ADENOSINE MONOPHOSPHATE ACTIVATED PROTEIN KINASE, MAMMALIAN TARGET OF RAPAMYCIN, GLUCOSE TRANSPORT PROTEINS
INTRODUCTION

The prevalence of metabolic disorders such as obesity, the metabolic syndrome and diabetes is widespread and is continuing to rise. Exercise is considered one of the cornerstones of diabetes prevention and treatment. Both endurance and strength training are effective for ameliorating insulin resistance and improving blood sugar control (164); however, the skeletal muscle adaptations and the signaling pathways through which these adaptations occur appear to be dependent upon the type of exercise performed (199).

An array of physiological adaptations occur in response to endurance training. Endurance training can cause an increase in the oxidative capacity of both Type I (slow twitch, red) and Type II (fast twitch, white) muscle fibers primarily by increasing oxidative enzyme content and upregulating mitochondrial biogenesis (168;273). These adaptations enhance the efficiency of energy production from substrate use (fatty acids and glucose). 5-adenosine monophosphate-activated protein kinase (AMPK) is a key energy sensor in most cells and is activated during endurance exercise due to an increase in the adenosine monophosphate-adenosine triphosphate ratio (89). Activation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), a downstream target of AMPK, coactivates nuclear respiratory factors-1 and -2 (NRF-1 and NRF-2), which regulate mitochondrial transcription factor A (Tfam). In turn, Tfam is responsible for regulating mitochondrial DNA gene products (217). Endurance exercise also increases glucose transport protein-4 (GLUT4) expression in skeletal muscle (198). Activation of the AMPK signaling pathway may contribute to GLUT4 translocation to the plasma membrane (83).

Resistance training results in skeletal muscle hypertrophy, particularly in Type II fibers, with a concomitant increase in muscular strength (274). Muscle fiber hypertrophy occurs via an increase in protein synthesis and a decrease in protein degradation, which may occur in a fiber
type-specific manner (275). Increased protein synthesis occurs through a cell signaling pathway involving mammalian target of rapamycin (mTOR). mTOR integrates intracellular and extracellular signals from growth factors (insulin, insulin-like growth factors), substrate availability and cellular energy levels to regulate metabolic responses within the cell (262). Upstream of mTOR, insulin receptor proteins interact with phosphoinositide 3 kinase (PI3K) in response to insulin resulting in the production of phosphatidylinositol (3,4,5)-triphosphate (PIP3). PIP3 activates PDK1 that in turn activates Akt. Akt activation eventually phosphorylates mTOR, a protein that nucleates two mult-protein complexes, mTORC1 and mTORC2. mTORC1 phosphorylates 70-kDa S6 protein kinase (S6K1) and dephosphorylates 4E binding proteins resulting in increased protein synthesis (262). Animal and human studies indicate that acute resistance exercise and training result in increased mTOR activation and increased protein synthesis (269;271). Recently, a transgenic mouse model expressing a constitutively activated form of Akt1 has demonstrated a potential link between mTOR activation and improved metabolic parameters. In this model, Akt1 activation decreased fat pad mass and normalized fasting blood glucose levels, insulin levels, and glucose uptake with a concomitant increase in type IIb muscle fiber size in high fat/high sucrose fed mice (272).

The aim of the current study was to quantify the effectiveness of a resistance training program for increasing the skeletal muscle expression of molecular mediators that may be beneficial for individuals with the metabolic syndrome. Sedentary subjects (9 control, 10 metabolic syndrome) volunteered for 8 weeks of supervised resistance training. Muscle biopsies were obtained from the vastus lateralis prior to and after completion of the resistance training program. Changes in molecular mediators of skeletal muscle adaptations including AMPK and mTOR expression were measured. Additionally, glucose transport protein expression was
measured, and muscle fiber-type specific changes were quantified. Furthermore, insulin sensitivity was assessed using the euglycemic-hyperinsulinemic clamp technique and related to the skeletal muscle adaptations to training.

MATERIALS AND METHODS

Subject Selection. Nineteen subjects were recruited to undergo 8 weeks of supervised resistance exercise. The research protocol and the consent documents were approved by the East Tennessee State University Institutional Review Board. Each subject gave signed informed consent. The exercise program was performed at the ETSU Exercise and Sports Sciences Laboratory with students from the Department of Kinesiology. Subjects were divided into 2 groups based on the inclusion and exclusion criteria presented in Table 1. Ten MS subjects and 9 controls were able to complete the training and planned measurements.
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<td>3 Family history of type 2 diabetes</td>
<td>Current diabetes: Fasting blood glucose ≥126 mg/dL</td>
<td>Sedentary – less than 1.5 hours exercise/week</td>
<td>Family history of type 2 diabetes</td>
<td></td>
</tr>
<tr>
<td>4 Visceral obesity waist ≥40 inches for males, ≥35 inches for females</td>
<td>Acute or chronic illness that would impair participation in training</td>
<td>Acute or chronic illness that would impair participation in training</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Clinical coronary disease</td>
<td></td>
<td>Current diabetes or impaired fasting blood glucose ≥110 mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Uncontrolled hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1**
Inclusion and exclusion criteria for MS subjects and controls.

*Exercise Protocol.* Training consisted of large muscle mass free weight exercises (Table 2). A light familiarization and baseline measurement period was followed by 4 weeks of high volume, low intensity training (Phase I). The intensity of the exercise (based on an estimate of relative repetition maximum) increased approximately 10% each week for the first 3 weeks, followed by a 10% drop in intensity from week 3 to week 4 to allow for recovery. During Phase II (weeks 5-8), volume decreased and intensity increased to allow for greater strength gains. Similar to Phase I, intensity increased approximately 10% week to week and was decreased by 10% during week 8 to allow for recovery. All sets (warm up and target sets) were recorded and calculated as total volume load (sets x reps x weight). Subjects were instructed to maintain weight during the study period. Blood pressure, blood lipids, body composition, and strength and endurance measurements were taken before and after training.
Table 2
List of exercises performed during the eight weeks of resistance training. Weeks 1-4 were high volume training designed to facilitate gains in fitness and strength-endurance. Weeks 5-8 were high intensity and low volume training emphasizing strength.

Subject Assessments. Several assessments related to body composition were measured at baseline and after 8 weeks of resistance training (Table 3). Subject height was measured to the nearest 0.1 cm using a stadiometer and body mass was measured to the nearest 0.1 kg using an electronic scale. Body composition was measured by air displacement plethysmography.
(BodPod, Concord, CA). Additionally, a 7-site skinfold measurement was performed on each subject (Lange Skinfold Caliper, Beta Technology Inc, Cambridge, MD). Waist circumference was measured just above the iliac crest to the nearest 0.1 cm.

<table>
<thead>
<tr>
<th></th>
<th>MS Subjects</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(5 male, 5 female)</td>
<td>(4 male, 5 female)</td>
</tr>
<tr>
<td>Age</td>
<td>45.0 ± 8.6</td>
<td>36.4 ± 12.2</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>99.5 ± 13.8</td>
<td>69.2 ± 16.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.7 ± 3.0</td>
<td>24.3 ± 3.6</td>
</tr>
<tr>
<td>Resting Systolic</td>
<td>131 ± 20</td>
<td>113 ± 10</td>
</tr>
</tbody>
</table>
| Blood Pressure (mmHg)| Still thigh pulls (20kg) and 3 sets of 5 repetitions with either 40kg (women) or 60kg (men). The lifting rack was set so that the subjects pulled from the mid-thigh pull position used in training (knee angle 120° – 135°, hip angle 170° – 175°). The subjects performed 2 warm up isometric mid-thigh pulls (50% and 75% effort), proceeded by 2 maximum effort pulls. Additional pulls were performed if the pull was preceded by a countermovement, or if the peak force of each pull differed by ≥250N as measured on a force plate (Rice Lake

**Strength Testing.** Strength was assessed isometrically using a custom built lifting rack.
Weighing Systems, Rice Lake, WI, USA) with a sampling rate of 1000Hz. Data were collected and analyzed for peak force (PF) and rate of force development (RFD) using custom Labview 8.6 software (National Instruments, Upper Saddle River, NJ).

**Endurance Testing.** Endurance was measured using a Monark Ergomedic 874E cycle ergometer (Monark Exercise AB, Vansbro, Sweden). After a brief warm up period with no resistance, a graded exercise test was performed. Stage 1 began with 0.5 kg of resistance, and the resistance was increased 0.5 kg every 2 minutes until a resistance of 1.5 kg was reached. The resistance was then increased 0.2 kg every 2 minutes until the subject could no longer continue. Expired air was analyzed using a TrueOne 2400 Metabolic Measurement System (ParvoMedics, Sandy, Utah, USA). Heart rate, VO$_{2\text{max}}$ (expressed as ml/kg/min), respiratory exchange ratio (RER) and time to exhaustion were recorded.

**Muscle Biopsies.** Percutaneous needle biopsies of vastus lateralis were performed after an overnight fast and 2 hours of quiet recumbency as previously described (75:276). Briefly, after local lidocaine anesthesia, a 7-10 mm skin incision was made and a Bergstrom-Stille 5 mm muscle biopsy was introduced through the fascia, and under suction a 50-100 mg specimen was obtained. After quickly blotting, the sample was divided in half, with one piece frozen immediately in liquid nitrogen for later analysis, and the second piece mounted on cork and quickly frozen in a slurry of isopentane cooled in liquid nitrogen (277). The cork-mounted piece is later sectioned on a cryostat (Leica, Wetzlet, Germany) for evaluation of fiber type composition, fiber cross sectional areas, and immunohistochemistry.

**Euglycemic Hyperinsulinemic Clamp.** Immediately following the muscle biopsy, a euglycemic hyperinsulinemic clamp was performed. After a 2-hour baseline period, a single infusion of regular insulin was performed at 15 mU/m2 for 3 hours in order to achieve a
physiological insulin concentration of about 40 μU/mL and a stable glucose infusion rate to quantify insulin sensitivity (58;278;279).

**Quantification of Muscle Fiber Type Composition and Fiber Size.** Fiber composition was determined using methods described by Behan et al. (280). Muscle sections were stained for light microscopy in a 2-step method using commercial monoclonal antibodies to fast and slow isoforms of myosin. After acetone fixation and incubation with 20% normal rabbit serum, the slow myosin antibody (Sigma clone NOQ7.5.4D) was applied, followed by a peroxidase-conjugated rabbit anti-mouse IgG antibody. The fast myosin antibody (Sigma clone MY-32 alkaline phosphatase conjugate) was then applied after another incubation with normal rabbit serum as described by Behan (281). Slides are finally alcohol dehydrated, cleared with xylene, and preserved in synthetic medium. This technique allows discrimination of type 1, type IIα, and type IIx. All sections were coded and then quantified independently by 3 observers who were unaware of which subject or treatment the image represented. Fiber size was quantified by numbering at least 100 identifiable fibers on color images from a camera-equipped microscope. Muscle specimens were cut perpendicular to the fiber direction using a Leica CM3050 S cryostat and pre-training and post-training sections were placed on the same slide in the same order for each subject. The digital images obtained included a key of a known dimension and were coded and submitted to a technician for quantification. In each image, type 1 fibers, type IIα, and type IIx fibers had their 2 major diameters measured ($d_1$ and $d_2$) and the area was estimated by using the smaller of the 2 diameters as described by Dubowitz (277).

**Preparation of Muscle Homogenates.** Briefly, a small piece of muscle was removed from the -80°C freezer and slowly thawed on ice. Muscle homogenate was prepared by placing 25-50 mg muscle in 500 μL 0.25 M sucrose, 20 mM HEPES, pH 7.4, containing protease inhibitors.
(Halt Protease Inhibitor Cocktail Kit from Pierce), and homogenized with two 30 second bursts of a hand-help homogenizer (Pellet Pestle Motor from Kontes).

**Mitochondrial Markers.** Confocal microscopic assessment of specific fluorescent labeling of several proteins in normal human muscle sections were performed using methods previously described (75). All evaluations of changes in expression of these mitochondrial markers were performed on slides containing both the pre- and post-training muscle sample transverse sections as described recently (76). A mixture of antibodies to 5 different mitochondrial components were purchased from MitoSciences (No. MS604). These antibodies are directed against Complexes I, II, III, and IV, and to ATP synthase subunit alpha. The principle component used for these analyses was ATP synthase. In general, 4-20% gradient gels (Thermo Scientific, Rockford, IL) were loaded with 7.5 µg protein from muscle homogenates. Blots were blocked with 0.25% non-fat dry milk.

**Key regulatory Protein Kinases (AMPK, mTOR).** Comparison of expression of these proteins were also quantified in immunoblots as previously described (282). Antibodies for AMPK, phospho-AMPKα1, mTOR, and phospho-mTOR were purchased from Cell Signaling Technology (#2531, #2531, #2972, #2971). For AMPK and phospho-AMPK, samples containing 5 µg protein from muscle homogenates were applied to 10% polyacrylamide gels. Immunoblots were blocked with 5% non-fat dry milk. Gradient gels 3-8% (Invirogen) were used for mTOR and phospho-mTOR immunoblots. These samples were 10 µg per lane and blocking was 0.25% non-fat dry milk.

**Fiber-Specific Expression of Principle Muscle Hexose Transporters (GLUT4 and GLUT5).** The techniques for quantifying these glucose transporters were described previously (75;76). Affinity-purified rabbit antibodies against hGLUT5 (GT52-A) were purchased from
Alpha Diagnostics (San Antonio, TX). GLUT4 antibodies (AB1049, goat anti-human) were purchased from Chemicon. Determining the fiber type that stains more intensely requires the use of fluorescent tagged antibodies that can be mixed together and evaluated with either the confocal microscope or a fluorescent microscope with multiple filters. We used both types of microscopes, but the confocal microscope was the primary device for these studies.

Statistics. All data are displayed as mean ± SE. Paired t-test was used for comparisons before and after training. Effect size correlations were calculated using Cohen’s d (Table 4) (283). Relationships between select variables were assessed using a Pearson correlation coefficient. Statistical procedures were performed using SigmaStat version 3.11 from Systat Software (San Jose, California).

<table>
<thead>
<tr>
<th></th>
<th>Cohen's d Effect Sizes</th>
</tr>
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<tbody>
<tr>
<td>Trivial</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Small</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.3-0.5</td>
</tr>
<tr>
<td>Large</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>

Table 4

RESULTS

Anthropometrics, Functional Capabilities and Volume Load. Eight weeks of resistance training had a positive impact on body composition (Table 5). Overall, training had little effect on body mass or fat mass; however, the percentage change in body mass was strongly correlated with the percent gain in lean body mass (LBM) in both groups (r=0.532). Although the increase in LBM was relatively small (d=0.122), the change was statistically significant in both groups. The decrease in waist circumference was statistically significant (p = 0.022); however, the effect size was small in metabolic syndrome and controls (-0.375 and -0.319, respectively). Training
tended to decrease body fat percentage in both groups, but the change was only statistically significant in metabolic syndrome (p=0.010). The sum of the skinfold thickness measured at 7 sites also tended to decrease in both groups (-1.5% in metabolic syndrome and -2.4% in controls), but this decrease was not statistically significant.

<table>
<thead>
<tr>
<th></th>
<th>Body Mass (kg)</th>
<th>BMI (kg/m²)</th>
<th>Waist Circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>d</td>
</tr>
<tr>
<td>All (n=19)</td>
<td>85.2 ± 21.3</td>
<td>85.1 ± 21.3</td>
<td>-0.006</td>
</tr>
<tr>
<td>MS Subjects</td>
<td>99.5 ± 13.8</td>
<td>99.3 ± 14.3</td>
<td>-0.02</td>
</tr>
<tr>
<td>Controls (n=9)</td>
<td>69.2 ± 16.2</td>
<td>69.2 ± 16.1</td>
<td>0.004</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fat Mass (kg)</th>
<th>LBM (kg)</th>
<th>% Body Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>d</td>
</tr>
<tr>
<td>All</td>
<td>31.1 ± 13.9</td>
<td>31.0 ± 14.0</td>
<td>-0.009</td>
</tr>
<tr>
<td>MS Subjects</td>
<td>41.4 ± 8.1</td>
<td>41.3 ± 8.4</td>
<td>-0.006</td>
</tr>
<tr>
<td>Controls</td>
<td>19.8 ± 9.3</td>
<td>19.5 ± 9.1</td>
<td>-0.024</td>
</tr>
</tbody>
</table>

**Table 5**

Body mass, BMI, waist circumference, fat mass, lean body mass, and percent body fat (mean±SD) for all subjects before and after eight weeks of resistance training. *d=Cohen's d effect size. *p≤0.05 **p≤0.01

Overall, VO₂max increased 10.3% and time to exhaustion increased approximately 35% in both groups (Table 6). The increase in VO₂max was statistically significant in both groups, and both groups achieved relatively large effect sizes (d=0.819 in MS subjects and 0.535 in controls). The increase in time to exhaustion was also statistically significant (p<0.001). MS subjects improved in isometric peak force (IPF) and rate of force development (RFD) after training. Controls tended to increase in IPF and RFD, but the increase was not statistically significant.
<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>d</th>
<th>Pre</th>
<th>Post</th>
<th>d</th>
<th>Pre</th>
<th>Post</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isometric Peak Force (N/kg^{2/3})</td>
<td>134.5 ± 37.1</td>
<td>148.8** ± 41.5</td>
<td>0.374</td>
<td>3061 ± 1745</td>
<td>3716* ± 2180</td>
<td>0.341</td>
<td>27.0 ± 5.4</td>
<td>29.6** ± 5.7</td>
<td>0.482</td>
</tr>
<tr>
<td>Rate of Force Development (N/s)</td>
<td>135.0 ± 39.5</td>
<td>153.1** ± 36.3</td>
<td>0.503</td>
<td>2937 ± 2166</td>
<td>3763* ± 2480</td>
<td>0.374</td>
<td>23.3 ± 3.1</td>
<td>26.2** ± 4.1</td>
<td>0.819</td>
</tr>
<tr>
<td>VO_{2max} (mL/kg/min)</td>
<td>133.9 ± 36.7</td>
<td>143.5 ± 49.3</td>
<td>0.234</td>
<td>3184 ± 1321</td>
<td>3664 ± 1957</td>
<td>0.305</td>
<td>31.0 ± 4.5</td>
<td>33.4* ± 4.9</td>
<td>0.535</td>
</tr>
</tbody>
</table>

Table 6

Functional capabilities. Isometric peak force and rate of force development were measured using a custom built force rack and a force plate (Rice Lake Weighing Systems, Rice Lake, WI, USA) sampling at 1000Hz. VO_{2max} was measured on a Monark Ergomedic 874E cycle ergometer (Monark Exercise AB, Vansbro, Sweden).

Volume load calculated as sets*reps*weight was tracked for each subject. Average weekly volume load is shown in Figure 1 below. Total volume load was 16% higher in MS subjects than in controls (93796 kg ± 32153 and 80737 kg ± 27022, respectively); however, this difference was not statistically significant. Isometric peak force measured prior to training and total volume load were strongly correlated (r=0.681).
Blood Lipids, Glucose, Insulin, and Euglycemic Hyperinsulinemic Clamp. Circulating levels of triglycerides, HDL, and LDL cholesterol were measured at baseline and after training. Triglyceride levels were 114.2 ± 52.3 in controls and 198.4 ± 173.4 in MS subjects at baseline. Controls had HDL and LDL cholesterol concentrations of 48.0 ± 11.0 and 87.3 ± 16.4 at baseline, while MS subjects had low HDL cholesterol (39.8 ± 9.0) and elevated LDL cholesterol (112.4 ± 41.3). Triglycerides and total cholesterol tended to be lower in both groups after resistance training, but the differences were not statistically significant and the effect sizes were small (d=-0.158 and -0.148, respectively).

Insulin sensitivity was assessed using the euglycemic hyperinsulinemic clamp technique described in the methods. Fasting insulin was higher in MS subjects both at baseline and after training (Table 7). Fasting insulin decreased 22% in controls after training (p < 0.05). Fasting blood glucose was higher before and after training in MS subjects. Although fasting blood
glucose tended to be lower after training, the effect size was small ($d = -0.123$). As shown in Table 7 and Figure 2, MS subjects showed marked insulin resistance at baseline (glucose infusion rate $3.3 \pm 1.3$), and training had little effect on glucose infusion rate. In contrast, controls demonstrated normal insulin sensitivity prior to training (GIR $7.0 \pm 2.0$), and glucose infusion rate increased 25% after resistance training. The increment in insulin concentration achieved by the insulin infusion was similar in both groups before and after training. The insulin concentration increment averaged 52 and 52 µU/ml for MS subjects and controls at baseline and 52 and 49 µU/ml post-training, respectively.

<table>
<thead>
<tr>
<th>Fasting Insulin (µU/mL)</th>
<th>Achieved Insulin Concentration (µU/mL)</th>
<th>Glucose Infusion Rate (mg/kg.min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>All</td>
<td>11.3 ± 8.0</td>
<td>96.3 ± 10.0</td>
</tr>
<tr>
<td>MS Subjects</td>
<td>14.7 ± 13.6</td>
<td>103.3 ± 10.5</td>
</tr>
<tr>
<td>Controls</td>
<td>7.6 ± 6.0</td>
<td>88.6 ± 9.0</td>
</tr>
</tbody>
</table>

Table 7

Markers of insulin resistance. Fasting insulin and fasting blood glucose were measured after an overnight fast. A single infusion of regular insulin was performed at 15 mU/m²/min for three hours in order to achieve a physiological insulin concentration of about 40 µU/mL. Glucose was infused at a variable rate to maintain euglycemia. After training, fasting insulin concentration tended to decrease in both groups. The decrease was only statistically significant in controls (p<0.01). Glucose infusion rate (GIR) increased significantly in controls after training (p=0.03), indicating increased insulin sensitivity; however, GIR was unchanged in MS subjects after training.
Glucose infusion rate (GIR). After a 2-hour baseline period, a single infusion of regular insulin was performed at 15 mU/m²/min for three hours in order to achieve a physiological insulin concentration of about 40 μU/mL. Glucose was infused at a variable rate to maintain euglycemia. The rate of steady state glucose uptake in the last 30 minutes reflects insulin sensitivity. MS subjects demonstrated lower GIR than controls before and after eight weeks of resistance training. GIR tended to be lower in MS subjects after training; however, the decrease was not statistically significant. Controls demonstrated a statistically significant increase in GIR due to training (p<0.05).

Skeletal Muscle Fiber Composition and Size. Percutaneous muscle biopsies of the vastus lateralis were performed at baseline and after training, and muscle fiber composition was determined using monoclonal antibodies for fast and slow myosin. As shown in Figure 3, MS subjects had a lower percentage of type I muscle fibers than controls at baseline (36.3% ± 10.2% vs. 50.0% ± 17.7%, p = 0.03). Percentage of type 1 fibers was unchanged after training. Training tended to cause a shift in fiber composition from type IIx to type IIa; however, the effect was small (d =0.119) and not statistically significant. Fiber cross sectional area did not change in either group.
Figure 3

Muscle fiber type portion. Percutaneous muscle biopsies were taken from the vastus lateralis and muscle sections were stained for light microscopy using monoclonal antibodies of fast and slow myosin. (A) Fiber type portion for each group at baseline. MS subjects had fewer type I muscle fibers and tended to have a greater proportion of type IIa/x muscle fibers. (B) Muscle fiber composition for controls before and after training. Fiber composition tended to shift away from type I fibers and toward type IIx; however, the effect sizes were relatively small ($d = -0.295$ for type I fibers and $d = 0.293$ for type IIx fibers). (C) Muscle fiber composition in MS subjects. Fiber type tended to shift towards type I and type IIa muscle fibers. The increased oxidative capacity of these muscle fibers may be reflective of the skeletal muscle adaptations elicited by the increased energy demands of training.

Glucose Transport Proteins. Expression of glucose transport proteins GLUT4 and GLUT5 were also quantified. As shown in Figure 4, MS subjects had slightly higher GLUT4 expression than controls prior to training ($2.80 \pm 1.41$ and $2.38 \pm 1.11$, respectively). GLUT4 expression increased significantly after training in both groups ($p \leq 0.05$). The percent increase in muscle GLUT4 content was greater in controls than MS subjects ($67\%, d = 1.634$ vs. $36\%, d = 0.843$, respectively). GLUT5 expression increased significantly in MS subjects ($p \leq .05, d = 1.0512$) but was unchanged in controls. The increase in muscle GLUT5 in MS subjects may be reflective of the higher proportion of type II muscle fibers in these subjects.
Figure 4

GLUT4 and GLUT5 expression before and after training. (A and B) Representative immunoblots of GLUT4 and GLUT5 expression. 5 µg of muscle homogenate was applied to polyacrylamide gel and probed with antibodies against GLUT4 and GLUT5. (C and D) Changes in GLUT4 and GLUT5 expressed as femtomoles per 5 µg of muscle homogenate. The increase in GLUT4 due to training was statistically significant in both groups (p<0.05); however, controls increased to a greater extent than MS subjects (67.0% and 36.0%, respectively). GLUT5, a fructose transporter protein, was unchanged after training in controls. GLUT5 increased 21.2% in MS subjects.

AMPK. Total and phospho-AMPK expression were quantified by immunoblot analysis as shown in Figure 5. Total AMPK expression increased significantly in both groups, with very strong effect sizes (d = 1.338 in MS subjects and d = 1.77 in controls). Phospho-AMPK expression increased 50% in controls (p < 0.001, d = 1.924). Phospho-AMPK expression
increased 13% in MS subjects; however, the increase was not statistically significant and the effect size was relatively small ($d = 0.368$).

*ATP synthase* ATP synthase expression, a marker of mitochondrial enzyme activity, increased significantly in both groups in response to training. MS subjects had higher ATP synthase expression at baseline than controls ($p=0.112$), but training increased ATP synthase expression by 63% in controls compared to 25% in MS subjects.
Figure 5

Immunoblot analysis of total AMPK, phospho-AMPK, and ATP synthase expression. (A and B) Representative immunoblots of total AMPK and phospho-AMPK before and after training. Each lane of total AMPK and phospho-AMPK contained 5 µg of protein from muscle homogenates and were applied to 10% gels. The pretraining sample is indicated by "A" and the posttraining sample is indicated by "B." Both groups showed a statistically significant increase in total AMPK expression after eight weeks of resistance training as indicated in D and E; however, only controls showed a significant increase in phospho-AMPK by paired t-test (p<0.01). (C) Representative immunoblots of ATP synthase expression. ATP synthase, a marker of mitochondrial biogenesis, was quantified before and after training. Generally, each lane was loaded with 7.5 µg of protein homogenate and blocked with 0.25% non-fat dry milk. Both groups showed a statistically significant increase in ATP synthase expression, as indicated in F. Controls tended to increase to a greater extent than MS subjects (63.6% and 24.6%, respectively).

mTOR. Total and phospho-mTOR, a molecular mediator of protein synthesis, increased significantly in MS subjects in response to training (Figure 6). While the increase in total mTOR expression was not statistically significant in controls, total mTOR expression increased 32% and the effect size was strong (d = 0.772). Total mTOR expression increased 57% in MS subjects in response to resistance training. Phospho-mTOR expression had a similar response to
resistance training. Phospho-mTOR increased 55% in MS subjects ($d = 1.49$, $p < 0.01$) and 39% in controls. Again, the effect size was large in controls ($d = 0.754$), but the increase was not statistically significant.

**Figure 6**

Total and phospho-mTOR expression. (A and B) Representative immunoblots of total mTOR and phospho-mTOR expression before and after training. Each lane contains 10 µg of muscle homogenate and was applied to 3-8% gradient gels. (C and D) Percent changes in expression of total and phospho-mTOR in both groups before and after training. Only MS subjects demonstrated statistically significant changes in total and phospho-mTOR.

**DISCUSSION**

In the present study, 8 weeks of resistance training improved several metabolic and physiological parameters in healthy, previously sedentary subjects as well as previously
sedentary subjects with the metabolic syndrome. Training had a positive impact on body composition in both groups (n=19). While body mass did not change, both groups increased LBM (p≤0.001) and decreased waist circumference (p≤0.05). Additionally, percent body fat tended to decrease in both groups; however, the decrease was only statistically significant in MS subjects. The sum of the thickness of 7 skinfolds also tended to decrease in both groups, further strengthening the evidence that training positively affected body composition.

Several groups have established criteria for diagnosing the metabolic syndrome, and most include a measurement of visceral adiposity (5). Recent work has demonstrated that obesity may play a key role in the development of insulin resistance. Adipose tissue acts as an endocrine tissue, excreting several cytokines that may be involved in the development of insulin resistance. Tumor necrosis factor-alpha (TNF-α) is a cytokine produced primarily in macrophages which has been shown to be elevated in obese rodent and human models of obesity. In rodents, over-expression of TNF-α has been shown to induce peripheral insulin resistance, which is restored with neutralization of TNF-α (126). In humans, it has been demonstrated that obese subjects express significantly more TNF-α mRNA in fat tissue than in lean controls, and a strong positive correlation exists between TNF-α mRNA expression and hyperinsulinemia (127-129). Furthermore, TNF-α mRNA expression and insulin insensitivity decreased with a corresponding reduction in body mass (127). TNF-α may inhibit tyrosine phosphorylation of the β-subunit of insulin receptors and insulin receptor substrate-1 (IRS-1) by insulin, which inhibits insulin signaling (132).

Adipose tissue also secretes interleukin-6 (IL-6), another cytokine associated with inflammation. Similar to TNF-α, high plasma concentrations of IL-6 correspond to increased obesity and insulin resistance (128;133;134). Kern et al. (128) demonstrated that IL-6 may affect
insulin sensitivity in mouse hepatocytes and human HepG2 cells via mechanisms similar to TNF-α. Specifically, IL-6 may inhibit insulin receptor activity due to decreased tyrosine phosphorylation of IRS-1 that inhibits insulin-dependent activation of Akt. However, a study in IL-6-deficient (IL-6<sup>−/−</sup>) mice indicates that hypothalamic IL-6 may exert anti-obesity effects (135). IL-6<sup>−/−</sup> mice developed type 2 diabetes that was partially ameliorated by IL-6 treatment. Furthermore, substrate metabolism was impaired in IL-6<sup>−/−</sup> mice. Similarly, studies in human skeletal muscle indicate that IL-6 exposure improves glucose metabolism but has no effect on insulin-dependent glucose uptake (136). It has been demonstrated that contracting skeletal muscle produces IL-6 that leads to an increase in plasma IL-6 levels (137). Recent evidence suggests that the effects of IL-6 vary depending on the target tissue. Chronic elevation of circulating IL-6 levels are an indicator of insulin resistance, while the acute presence of IL-6 may be beneficial for certain tissues such as skeletal muscle (138).

Other adipocyte secretions include plasminogen activator inhibitor 1 (PAI-1) and adiponectin. PAI-1 has recently been linked to the development of thrombosis, fibrosis, and insulin resistance (139). Using PAI-1 deficient mice, Ma and colleagues demonstrated that PAI-1 deficiency protects against diet-induced obesity and insulin resistance, suggesting that PAI-1 may be important in the development of obesity. Others have hypothesized that PAI-1 contributes to a “hypercoagulable” state that may result in increased platelet formation and associated cardiovascular risks (140).

Adiponectin is a protein secreted by adipocytes. Circulating levels of adiponectin are inversely related to obesity and insulin resistance, while up-regulation of adiponectin protects against obesity-induced insulin resistance (141). Adiponectin also acts as an insulin-sensitizing agent, possibly through phosphorylation and activation of the AMPK signaling pathway (142).
Excessive adiposity may also adversely affect blood lipids. Indeed, high plasma triglycerides and reduced HDL cholesterol are typically seen in individuals with abdominal obesity (120). Moreover, plasma free fatty acid (FFA) concentration is elevated in obese subjects (121), possibly due to increased fat mass (122). Santomauro and colleagues (125) showed that treatment with an antilipolytic drug called Acipimox reduced fasting plasma FFA and insulin concentrations in obese subjects. Furthermore, euglycemic-hyperinsulinemic clamps and glucose tolerance tests revealed improved glucose tolerance after plasma FFA reduction in obese non-diabetic patients and obese patients with type 2 diabetes.

In this context, we expected that improvements in body composition due to training would lead to improved insulin sensitivity and other markers of the metabolic syndrome. Both groups tended to decrease total cholesterol, serum triglycerides, and LDL cholesterol after training. Despite these improvements, only controls showed a statistically significant improvement in fasting insulin levels. Fasting insulin and fasting blood glucose tended to decrease in MS subjects, but the effects were small (d=-0.087 and -0.123, respectively). Fasting blood glucose was still above 100 mg/dL after training. Glucose infusion rate during the euglycemic hyperinsulinemic clamp increased 25% in controls (p≤0.05) but was unchanged in MS subjects.

Skeletal muscle makes up a large percentage of an individual’s body mass and is the primary site for insulin-stimulated glucose uptake and use (62). It has been widely reported that muscle contraction increases skeletal muscle glucose uptake (93). Not surprisingly, low physical activity levels (284;285) and low cardiovascular fitness (286) are associated with an increased prevalence of the metabolic syndrome. Interestingly, muscular strength may also be associated with metabolic syndrome prevalence and insulin resistance (287;288). Endurance and resistance
training have been shown to improve glycemic control (159;164;165) and insulin resistance (165-167). Many of these benefits can be achieved without any accompanying weight loss.

The lack of improvement in insulin resistance and glycemic control in the present study may be explained several ways. First, while not statistically significant, the average age of the controls was approximately 9 years younger than subjects with the metabolic syndrome. Previous studies have suggested that the quantitative adaptations to resistance exercise are reduced with age (289;290). In the present study, however, older subjects (both controls and metabolic syndrome) showed comparable improvements to younger subjects. It is possible that this observation is due to the extremely sedentary lifestyle of many of the older subjects prior to participation in the study. It does not appear that this difference in age affected the outcome of the current study. Second, many studies that suggest resistance training may be effective at improving insulin resistance demonstrate a decrease in fat mass as a result of training (159;164). In the present study, fat mass did not change in either group. One possible explanation is that many of the subjects simply were not strong enough at the beginning of the training program to cause significant adaptations (figure 1). Volume load is typically higher during periods of high volume training (weeks 1-4) than during periods of lower volume training (weeks 5-8); however, volume load was higher during weeks 5-8 for every subject in the present study. Higher volume work increases general fitness and is associated with greater improvements in body composition compared to low volume training (291). It is possible that the subjects needed to achieve a certain threshold of strength before they can lift with enough intensity to force adaptation to the high volume phase of training.

Normal human skeletal muscle contains a mixture of type I, IIa, and IIx muscle fibers. Each fiber type is suited for different types of physical activity. Type I (red, slow) fibers contain
many mitochondria and are well suited for oxidative energy production. While type I fibers can provide energy for long periods of time, their relatively slow contraction speed and low force production make them best suited for long-term, low intensity activity (182;184). Conversely, Type IIx (white, fast) fibers are best suited for energy production via phosphagens and fast glycolysis. Type IIx fibers have the fastest speed of contraction and highest force production capabilities, making them well suited for short term, high intensity activity such as resistance training (35;36). Type IIA fibers are an intermediate fiber type with properties of both type I and type IIx fibers (184). Training typically causes a shift from type IIx muscle fibers toward type IIA fibers, indicative of the altered energy need of the trained muscles (182). In view of the divergent functional capabilities of each skeletal muscle fiber type, it is not surprising that many of the adaptations to training are at least to some extent fiber type dependent (180) and may occur through separate cell signaling pathways (199).

Endurance training is associated with improved efficiency of substrate uptake and use by skeletal muscle. These improvements are brought about by increases in mitochondrial biogenesis, oxidative enzymes, and fatty acid oxidation (168;169;171;288;292). In addition, endurance training appears to increase glucose uptake into the cell by increasing the expression of GLUT4 in skeletal muscle (103). Many of these adaptations are mediated by AMPK and its downstream targets (293). The adaptations brought about by AMPK activation are similar to the type I fiber phenotype. Interestingly, type I muscle fiber content (158) and AMPK activation are positively associated with whole body insulin sensitivity (294).

Resistance training results in increased protein synthesis (177) and may increase GLUT4 expression in skeletal muscle (295;296). The mTOR signaling pathway appears to be crucial in mediating the adaptations to resistance exercise; however, mTOR’s role in improving insulin
sensitivity following resistance training has remained elusive. It has been demonstrated in transgenic mice that knockout of S6-kinase 1 (S6K1), a downstream target of mTOR, results in decreased pancreatic β-cell size and a concomitant decrease in glucose-stimulated insulin secretion (259). Izumiya and colleagues (272) generated a transgenic mouse expressing a constitutively activated form of Akt1, another protein kinase upstream of mTOR in this signaling pathway. Activation of Akt1 resulted in type II fiber hypertrophy and reduced fat pad mass. Additionally, Akt1 activation normalized fasting blood glucose and insulin levels in high fat/high fructose fed mice. The authors concluded that increased type II muscle mass positively affected obesity and other metabolic parameters via altered fatty acid oxidation.

Impaired glucose uptake is a major defect in individuals with the metabolic syndrome (16). Glucose is transported across the plasma membrane via specialized glucose transporter proteins (GLUTs), of which GLUT4 is the predominant GLUT in human skeletal muscle (75). Insulin signaling and exercise cause GLUT4 translocation from intracellular pools to the plasma membrane (87). Increased GLUT4 protein expression after exercise training has been shown to increase whole body glucose disposal in type 2 diabetics (109). As many as 14 members of the GLUT family have been identified, including the fructose transporter GLUT5 (75). Stuart and colleagues (296) demonstrated in a previous study of sedentary, non-metabolic syndrome subjects that GLUT5 protein expression decreases in skeletal muscle after cycle ergometer training.

In the present study, resistance training caused a statistically significant increase in the expression of AMPK, mTOR, ATP synthase, and GLUT4 for the entire group. Disappointingly, these increases were only weakly correlated with changes in markers for insulin resistance. It has been demonstrated that AMPK activation may attenuate the mTOR signaling response to
exercise (297). Moreover, recent work suggests that AMPK signaling may be partially inhibited by activation of S6K1 through the mTOR signaling pathway (298). The lack of improvement in insulin responsiveness in the metabolic syndrome subjects in this study may be due to diminished activation of AMPK and thus decreased mitochondrial biogenesis. While insulin sensitivity did not improve, resistance training appears to have produced several positive benefits in both healthy subjects as well as those with the metabolic syndrome. Body composition improved, along with increases in strength and endurance, which has been shown to improve functional capabilities and quality of life (299). Additionally, intracellular pathways leading to increased protein synthesis and mitochondrial biogenesis were activated. Based upon numerous studies, it appears that long-term activation of these pathways through training will ameliorate many of the negative aspects of the metabolic syndrome.
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APPENDIX A

Informed Consent Document

PRINCIPAL INVESTIGATOR: Charles A. Stuart, M.D.

TITLE OF PROJECT: Molecular Mechanisms By Which Strength Training Ameliorates The Metabolic Syndrome

INFORMED CONSENT DOCUMENT

This Informed Consent will explain about being a participant in a research study. It is important that you read this material carefully and then decide if you wish to be a volunteer.

PURPOSE: In this protocol, we will carefully measure the changes in several muscle proteins and their genes that eight weeks of strength training brings about. To do this we will take two very small pieces of muscle from your thigh muscle using a needle under local anesthesia. One biopsy will be done before and the other at the end of eight weeks of supervised exercise training. Before and after the training, you will also have strength testing, aerobic fitness test, body fat and muscle measurement, and a test of how well insulin works in your body. You will be one of twenty people who will participate in this study.

DURATION: Your participation will last about eleven weeks and the entire project will last about six months. Before scheduling the study, you will be asked a few questions about your health status. In the week before your first muscle biopsy, your strength will be tested, you will have your body fat content measured using a DEXA scanner and/or a BodPod and you will have a test measuring how much oxygen you consume during a brief maximal exercise bout. A blood sugar concentration will be measured after pricking your finger. On the day of your first scheduled muscle biopsy, you will also have a test to measure how well insulin works in your body. The combined time needed on this day will be about six hours. The next week you will begin supervised exercise training in the ETSU Exercise and Sports Science Laboratory of the ETSU Sports complex in the Minidome. This will be continued for eight weeks, beginning with lighter weights and eventually progressing to heavier weights. The training will be for about one hour on five days each week. The exercise will be conducted under the supervision of qualified exercise physiologists and sports science students. Body fat measurement, oxygen consumption, and strength testing will be done again at the end of the last week of training. Two or three days after the last strength training session, a second muscle biopsy will be performed and insulin response tests will be repeated.

PROCEDURES: In the week before your first muscle biopsy, body fat will be measured using a DEXA scanner or equivalent instrument. The body fat will be measured in the research laboratory of the Extended Care Facility on the VA campus or in the Exercise and Sports Science lab. You will also have an aerobic fitness test that measures how much oxygen you use during a brief bout of intense exercise. This test will be done in the ETSU Exercise and Sports Science Laboratory in the Minidome. Muscle biopsy and insulin infusion test will be done in the ETSU Physicians and Associates clinic building or in the Exercise and Sports Science Laboratory. The night before your first muscle biopsy, you should not eat or drink anything except water after 10:00 PM. After an overnight fast, at about 8:00 a cup of blood by finger prick will be taken to measure your blood sugar. You will then lie quietly for about two hours. During this time, two intravenous lines will be started to be used in the study that follows the muscle biopsy. At about 10:00 AM, the muscle biopsy will be done. The muscle biopsy is done with you lying on an exam table. The skin and muscle surface will be numbed with

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Subject initials
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TITLE OF PROJECT: Molecular Mechanisms By Which Strength Training Ameliorates The Metabolic Syndrome

Lidocaine. Any adverse reactions to lidocaine or other anesthetic agents should be reported to the investigator before beginning this study. A small cut (7-10 mm) in the skin will be made and a 5 mm diameter muscle biopsy needle will be introduced. A small snip of muscle (about 100 mg - the size of an apple seed) will be taken and quickly frozen in a special container. Usually one stitch is placed in the skin and an elastic bandage is applied. Normal physical activity may be resumed when you leave, but strenuous exercise should be delayed until the next day.

After the biopsy is done, an insulin infusion study will begin. This procedure is called a euglycemic insulin clamp study. During the two hours before the muscle biopsy, blood will be drawn for cholesterol, potassium, and insulin measurements (a total of three 5 ml tubes). Following the biopsy, an intravenous insulin infusion will be started and a glucose infusion will be begun. Your blood sugar will be monitored about every five minutes (about forty 1 ml samples from the IV line) and adjustments will be made to keep your blood sugar close to 85 mg/dl. Finger prick glucose measurements may be done occasionally during the infusion to corroborate venous blood glucose determinations. Three more 5 ml blood samples will be drawn at 5-10 minute intervals after two and one half hours of insulin infusion. At the end of three hours, the insulin infusion will be stopped and you will eat some food. The infusion of glucose will be gradually stopped while you are monitored over the next half hour. The total amount of blood taken will be about 60 ml (the equivalent of about one third cup). Normal physical activity may be resumed when you leave.

Orientation to the exercise equipment and the training facility will take place over the first two weeks of this program. An exercise training program will begin the week after your first muscle biopsy and the euglycemic insulin clamp study. This program will involve about one hour of supervised exercise five days each week for eight weeks. Over the time of the protocol, training will begin with lighter weights and intensity and will increase to heavier weights to gradually increase your strength. During the last week of your training, we will repeat the body fat measurement and the aerobic fitness test of oxygen consumption. Two or three days after your last exercise session, another muscle biopsy will be performed just like the first, except it will be done on the opposite side. The insulin infusion test will be repeated on the same day as the second muscle biopsy.

Failure to adhere to the exercise program is sufficient reason to terminate your participation with prorated compensation as described below.

ALTERNATIVE PROCEDURES/TREATMENTS: This is a voluntary research project to assess strength training effectiveness in improving obesity and insulin resistance. Subjects who choose not to participate may withdraw from our study and may pursue other exercise training on their own.

POSSIBLE RISKS/DISCOMFORTS: You might experience mild temporary pain when the needle is inserted into the vein in your arm. Occasionally a bruise may form resulting from slight bleeding under the skin. It is possible to become light-headed or dizzy for a few minutes,
rarely leading to fainting. Please notify the person drawing your blood if you know that you tend to have these reactions, so that precautions can be taken to minimize the response. Sterile disposable supplies are used in your blood draw, but as with any break in the skin, there may be a slight chance of infection at the puncture site. Each muscle biopsy is done using sterile techniques. Pain can occur, but the use of lidocaine minimizes the discomfort and the elastic bandage minimizes bruising. Reaction to lidocaine can rarely occur. There may be a slight chance of infection or bruising at the biopsy site. The skin should be kept clean and dry for about a week after the biopsy. There may be some discomfort at the muscle biopsy site for 5-7 days.

Intravenous insulin can cause very low blood sugar, but this will be prevented by close monitoring of your blood sugar throughout the insulin infusion by experienced investigators.

Exercising can make subclinical lung or heart disease apparent. Subjects with a history of lung or heart disease will be excluded. Exercising on a treadmill or stationary bicycle can result in injury if proper safety precautions are not followed. Direct supervision by experienced trainers will be done to decrease the risk of injury.

POSSIBLE BENEFITS: Volunteers who participate in this study may learn new forms of beneficial exercise. They may improve their strength and their general feeling of well being.

COMPENSATION FOR MEDICAL TREATMENT: East Tennessee State University (ETSU) will pay the cost of emergency first aid for any injury that may happen as a result of your being in this study. ETSU makes no commitment to pay for any other medical treatment. Claims against ETSU or any of its agents or employees may be submitted to the Tennessee Claims Commission. These claims will be settled to the extent allowable as provided under TCA Section 9-8-307. For more information about claims call the Chairman of the Institutional Review Board of ETSU at 423/439-6055.

FINANCIAL COSTS: There is no cost to you for your participation in this study.

COMPENSATION IN THE FORM OF PAYMENTS TO RESEARCH PARTICIPANTS: The investigators will provide $500 at the end of the study to help offset your time commitment and inconvenience. For those who do not complete the study, $150 will be given for the first biopsy and insulin infusion study, $200 for completing the exercise program (about $25 per week), and $150 for the second biopsy and insulin infusion.

VOLUNTARY PARTICIPATION: Participation in this research experiment is voluntary. You may refuse to participate. You can quit at any time. If you quit or refuse to participate, the benefits or treatment to which you are otherwise entitled will not be affected. You may quit by calling Dr. Stuart, whose phone number is 439-6282. You will be told immediately if any of the results
APPENDIX B

HIPPA Form

AUTHORIZATION TO USE AND DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES

The privacy law, Health Insurance Portability & Accountability Act (HIPAA), protects my individually identifiable health information (protected health information). The privacy law requires me to sign an authorization (or agreement) in order for researchers to be able to use or disclose my protected health information for research purposes in the study entitled "Glucose Transporter Expression in Human Skeletal Muscle."

I authorize Dr. Charles Stuart and his/her research staff to use and disclose my protected health information for the purposes described below. I also permit my doctors and other health care providers to disclose my protected health information for the purposes described below.

My protected health information that may be used and disclosed includes:

- demographic information
- family history
- health history
- height and weight

The Investigator, Dr. Charles Stuart, may use and share my health information with:

- The East Tennessee State University Human Research Protections Program (HRPP) Institutional Review Board Administration when the researcher or the research site is undergoing Quality Improvement Program (QIP) auditing.
- The James H. Quillen Veterans Affairs Medical Center Office of Research & Development when the researcher or the research site is undergoing Quality Improvement Program (QIP) auditing.
- Government representatives, when required by law

Once my health information has been disclosed to anyone outside of this study, the information may no longer be protected under this authorization. Dr. Stuart agrees to protect my health information by using and disclosing it only as permitted by me in this Authorization and as directed by state and federal law.

I do not have to sign this Authorization. If I decide not to sign the Authorization:

- It will not affect my treatment, payment or enrollment in any health plans nor effect my eligibility for benefits.
- I may not be allowed to participate in this research study.
After signing the Authorization, I can change my mind and:

- Not let the researcher disclose or use my protected health information (revoke the Authorization).
- If I revoke the Authorization, I will send a written letter to Dr. Stuart to inform him of my decision.
- If I revoke this Authorization, researchers may only use and disclose the protected health information already collected for this research study.
- If I revoke this Authorization my protected health information may still be used and disclosed should I have an adverse event (a bad effect, or experience something unanticipated).
- If I change my mind and withdraw the authorization, I may not be allowed to continue to participate in the study.

This Authorization does not have an expiration date.

If I have not already received a copy of the Privacy Notice, I may request one by contacting the Privacy Officer. If I have any questions or concerns about my privacy rights, I should contact the East Tennessee State University, James H. Quillen College of Medicine Privacy Officer, Paula Wright, at 423/433-6074 or the Compliance Manager at Phone: (423)439-5651.

I am the subject or am authorized to act on behalf of the subject. I have read this information, and I will receive a copy of this form after it is signed.

Signature of research subject or *research subject's legal representative ____________________________ Date ________________

Printed name of research subject or *research subject's legal representative ____________________________ Representative's relationship to research subject ____________________________

*Please explain Representative's relationship to patient/subject and include a description of Representative's Authority to act on behalf of Patient:

______________________________

ETSU/VArv93005
VITA

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