To the Graduate Council:

I am submitting herewith a dissertation written by William Joseph Banz entitled "The Relationship Between Insulin Resistance and Glucose Transport and Transporter (GLUT4) Levels in a Rodent Model of Syndrome X." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Human Ecology.

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The Relationship Between Insulin Resistance and Glucose Transport and Transporter (GLUT4) Levels in a Rodent Model of Syndrome X

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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William Joseph Banz
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DEDICATION

This thesis is dedicated to my parents

Mrs. Geraldine Banz

&

Mr. Steve Banz

who have given me the opportunity to reach this moment.

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Abstract

(Part I.) The purpose of this study was to measure insulin regulated glucose transport and GLUT4 protein in aortic tissue from insulin-resistant vs. sensitive rats. We assessed basal and insulin-stimulated [^{3}H]-2-deoxyglucose transport in aorta from lean vs. obese Zucker rats. In a muscle-bath system, insulin significantly stimulated aortic 2-deoxyglucose transport by 30% in lean and obese animals. Further, in the perfusion system with only the luminal vessel exposed, insulin significantly stimulated 2-deoxyglucose transport in aorta of lean animals. Western blot analysis demonstrated that aorta contained substantial concentrations of GLUT4 protein. These data demonstrate that the aorta is insulin-sensitive and partially impaired in the insulin resistant animals. **(Part II.)** The purpose of this study was to determine if there is a *fa* allele effect in *BN/fa* rats subjected to a high fat diet. *BN/BN* and *BN/fa* male/female rats were fed 12% or 48% fat diets for 7 weeks. Food intake and weight change were recorded weekly to obtain an index of energy efficiency ratio (EER) and blood and tissue were harvested for subsequent analysis. Plasma insulin, glucose and cholesterol concentrations, perirenal and epididymal fat pad weights, EER, weight gain and food intake were all significantly higher in male rats on the 48% vs. 12% fat diet. *BN/fa* animals had heavier fat pads than *BN/BN* animals. The animals on the 48% fat diet had a significantly higher concentration of soleus GLUT4 protein vs. 12% fat diet. We have demonstrated that a high fat diet exacerbates insulin resistance and the aberrations associated with insulin resistance.

The effects of the high fat diet are amplified in animals carrying one copy of the *fa* allele and female animals appear to be somewhat resistant to this diet-gene interaction. Additionally, we have demonstrated that a high fat diet increases the insulin responsive glucose transporter (GLUT4) protein in skeletal muscle. These data support the concept that syndrome X is a multifactorial syndrome that predominates in males, which is genetically determined and modulated by environmental influences.

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PART I.

INTRODUCTION

INTRODUCTION

Cardiovascular disease is one of the major killers in industrialized nations today (Morbidity and Mortality Weekly Report 1992). Hyperinsulinemia, hypertension, dyslipidemia and android obesity are all considered risk factors for cardiovascular disease. These risk factors have a tendency to aggregate and this combination has been coined "syndrome X" or "the deadly quartet" (Barnard et al. 1992, DeFronzo and Ferrannini 1991, Kaplan 1989, Reaven 1988). Insulin resistance appears to be the key factor that links this "syndrome X", especially in genetically susceptible persons (DeFronzo and Ferrannini 1991, Reaven 1988). It has been shown that impaired cellular responses to insulin result in increased vascular smooth muscle tone, decreased expression and/or translocation of the insulin-responsive glucose transporter (GLUT4) protein, and altered transport of glucose in skeletal muscle and adipose cells (Dohm et al. 1991, Ivy et al. 1986, Kahn and Pedersen 1993, Sherman et al. 1988, Zemel et al. 1992).

Both clinical data and studies in animal models demonstrate that environmental manipulations may affect some of the symptoms associated with syndrome X. For example, physical training can improve the impaired cellular responses to insulin in skeletal muscle and adipose cells associated with syndrome X (Douen et al. 1990, Friedman et al. 1990, Fushiki et al. 1989, Hirshman et al. 1993, Houmard et al. 1991, Ploug et al. 1990, Rodnick et al. 1990, Talmadge and Silverman 1991, Young et al. 1989). Physical training has been shown to induce recruitment

and/or translocation of GLUT4 (Douen et al. 1990, Friedman et al. 1990, Fushiki et al. 1989, Hirshman et al. 1993, Houmard et al. 1991, Young et al. 1989). Physical training has also been shown to alleviate the insulin resistance associated with android-shaped obesity (Krotkiewski and Bjorntorp 1986). Moreover, dietary manipulation has the potential to modify peripheral insulin sensitivity (Kahn and Pedersen 1993, Lillioja et al. 1987, Saltin and Gollnick 1986). Rats that are obese from high fat feeding have suppressed GLUT4 expression in skeletal muscle (Kahn and Pedersen 1993). It has been demonstrated that a high sodium intake has the potential to exacerbate insulin resistance (Donovan et al. 1993). Recent research demonstrates that manipulating fat, complex carbohydrate, and sucrose in the diet has a profound effect on peripheral insulin sensitivity (Barnard et al. 1993). Further, rats fed diets high in saturated fat became more obese, had elevated fasting insulin levels and exhibited hypertension when compared with control animals and animals on high polyunsaturated fat diets (Kaufman et al. 1994). Indeed, both activity and diet appear to have profound effects on risk factors associated with syndrome X.

Genetics also play an important role in the etiology of syndrome X. The obese Zucker rat is a genetically extreme animal model which exhibits some of the symptoms analogous to human syndrome X. Insulin-resistant skeletal muscle is a chronic defect of the obese Zucker rat *(fa/fa)*, which is the best-known and most widely used rat model of genetic obesity (Cortez et al. 1991, Crettaz et al.1980, Czech et al. 1978, Ivy et al. 1989, Ivy et al. 1986, Sherman et al. 1988). The obese Zucker rat has also been established as a model of hypertension and insulin resistance. The

obese Zucker rat exhibits obesity and hyperinsulinemia by 4-5 weeks of age (Bray 1977, Fletcher et al. 1986, Ionescu et al. 1985, Kasiske et al. 1985, Martin and Gahagan 1977, Stern et al. 1975, Zucker and Antoniades 1972), and insulin resistance is evident by 11-13 weeks of age (Ionescu et al. 1985). The aberrations of syndrome X progress in this model relatively independent of environmental influences (Cleary et al. 1980). Alternatively, the heterozygous *(Fa/fa)* lean Zucker rat may exhibit a gene dosage effect which is more sensitive to environmental manipulations (Abel et al. 1995, Figlewicz et al. 1985, Phillips and Cleary 1994, Truett et al. 1995, York et al. 1984). Therefore, it may be a more appropriate animal model for the study of syndrome X.

Traditionally insulin resistance associated with syndrome X has been considered somewhat pathway selective (primarily involving glucose transport and metabolism) and tissue specific (primarily affecting skeletal muscle, adipose and liver) (Castillo et al. 1991, DeFronzo et al. 1993, Ferrannini et al. 1987, Rocchini 1991, Zemel et al. 1992). However, recent evidence from this laboratory and others suggests that insulin may, in fact, exert a local vasodilatory effect on the vasculature, which is impaired in insulin resistant states (Abel and Zemel 1993, Kim and Zemel 1993, Shehin et al. 1989, Zemel et al. 1992, Zemel et al. 1991, Zemel et al. 1990a and 1990b). Altered glucose metabolism and associated calcium regulation appear to be the link between hypertension and insulin resistance in this syndrome (Abel and Zemel 1993, Kim and Zemel 1993, Shehin et al. 1989, Zemel et al. 1992, Zemel et al. 1991, Zemel et al. 1990a and 1990b).

Accordingly, the focus of this dissertation research was to: (a) determine whether aortic tissue exhibits "classical" insulin-stimulated glucose transport and if so, is this action impaired in an insulin resistant model; and (b) determine if there is a gene-diet interaction associated with the *fa* (fatty) allele, potentially modulating GLUT4 protein and classical risk factors for syndrome X. These studies are intended to provide insight into the interaction between genetics and environment and how this impacts insulin sensitivity in an animal model representative of syndrome X.

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PART II.

LITERATURE REVIEW

SYNDROME X

Introduction

Cardiovascular disease is one of the major killers in industrialized nations today (Morbidity and Mortality Weekly Report 1992). The incidence of cardiovascular disease appears to be much more prevalent in specific groups, which possess a clustering of anthropometric, hemodynamic and metabolic risk factors. For example, hyperinsulinemia, hypertension, dyslipidemia and android obesity are all considered fundamental risk factors for coronary artery disease (Barnard et al. 1992, Basset 1994, Bjorntorp 1991, Bjorntorp 1987, DeFronzo and Ferrannini 1991, Hall 1994, Hall et al. 1993, Julius et al. 1992, Julius et al. 1991, Kaplan 1989, Larsson 1991, Mykkanen et al. 1992, Reaven 1988, Salvetti et al. 1993, Zemel 1995a and 1995b).

In the Banting Lecture, Reaven (1988) suggested "there is a series of related variables - syndrome X - that tend to occur in the same individual and may be of enormous importance in the genesis of coronary artery disease". The common feature of syndrome X appeared to be insulin resistance and all other changes were secondary to this basic abnormality. Moreover, Reaven (1988) described the related variables (resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased very-low-density lipoprotein triglyceride, decreased high-

density lipoprotein cholesterol and hypertension) and proposed that a significant portion of the variance in insulin resistance observed from person to person is genetically determined and that insulin's action can also be modulated by environmental influences. Hence, he proposed that life-style modifications, in particular avoiding obesity and remaining physically active, provide an approach to minimize the risk factors for coronary artery disease associated with resistance to insulin-stimulated glucose uptake.

Kaplan (1989) described an analogous clustering of aberrations and coined it the "deadly quartet". His proposed syndrome, like Reaven, included glucose intolerance, hypertriglyceridemia and hypertension. However, he also incorporated upper-body obesity into the clustering of risk factors. He proposed that caloric excess in the presence of androgens, mediates the aberrations by way of hyperinsulinemia. Consequently, Kaplan (1989) suggested there was a need to identify and prevent upper-body obesity or, provide therapies that will control the associated problems without aggravating hyperinsulinemia.

More recently, hyperinsulinemia, hypertension, dyslipidemia and android obesity have been considered the fundamental risk factors converging in "syndrome X" or the "deadly quartet" (Barnard et al. 1992, Basset 1994, Bjorntorp 1991, Bjorntorp 1987, DeFronzo and Ferrannini 1991, Kaplan 1989, Larsson 1991, Mykkanen et al. 1992, Reaven 1988). Apparently, insulin resistance is the key factor that links syndrome X, especially in genetically susceptible persons (Ferrannini et al. 1990a and 1990b, Mykkanen et al. 1992, Pollare et al. 1990, Rocchini 1991, Slater

1991, Zemel 1995a and 1995b). Reaven (1988) suggested that all of the risk factors may not occur in every individual with insulin resistance but it is likely that the majority of risk factors occur in most individuals. Consequently, the incidence and/or role of insulin resistance in the etiology of the aberrations accompanying syndrome X has been the focus of extensive investigation.

Syndrome X and Insulin Resistance

Several researchers have examined and confirmed the relationship between insulin resistance and the aggregate of aberrations associated with syndrome X. Individuals with increased fasting plasma insulin and/or increased plasma insulin in response to a glucose challenge appear to have an aggregation of the aberrations associated with syndrome X. (Ferrannini et al. 1990a and 1990b, Ferrannini et al. 1987). Swan et al. (1994) compared healthy controls to patients presenting with clinical syndrome X and confirmed the relationship between insulin resistance and the aberrations associated with this syndrome. Insulin sensitivity was 31% lower in men with syndrome X and fasting insulin concentration was 30% higher. The patient group also had a 64% higher mean triglyceride concentrations and a 20% lower mean high density lipoprotein cholesterol concentration. Systolic blood pressure was 10% higher in the syndrome X group. Upon administration of an oral glucose load Chauhan et al. (1994) found an increase in immunoreactive insulin levels in patients with syndrome X when compared to an age matched control group. A similar study by Zavaroni et al. (1994) divided volunteers into four equal groups based on the degree of obesity and plasma insulin response to a 74 g oral glucose challenge. The mean integrated plasma glucose response area for 2 hours following a 75 g oral glucose load was significantly higher in the hyperinsulinemic group, as were the fasting triglyceride levels and uric acid concentrations. In contrast, high density lipoprotein cholesterol concentrations were lower in the hyperinsulinemic group.

Furthermore, systolic and diastolic blood pressures were higher in the hyperinsulinemic group. Division of the two groups into obese and non-obese subjects did not alter the differences outlined above. In a subsequent study by Zavaroni et al. (1993), 20 males with asymptomatic hyperuricemia and 20 males with normal serum uric acid concentrations received a 75 g oral glucose challenge. The plasma glucose and insulin responses to the oral glucose was increased in the hyperuricemia group, as were systolic and diastolic blood pressures. In addition, subjects with hyperuricemia had higher plasma triglycerides and cholesterol and lower high density lipoprotein cholesterol concentrations. The authors provided these results as a possible explanation for the well known association of hyperuricemia with coronary heart disease. Furthermore, they suggested that hyperuricemia be added to the cluster of metabolic and hemodynamic abnormalities associated with insulin resistance, hyperinsulinemia and syndrome X.

Several researchers (Bjorntorp 1992, Bjorntorp 1991, Bjorntorp 1987, Haffner et al. 1990, Kaplan 1989, Mbanya et al. 1988, Mykkanen et al. 1992) have examined and postulated on the relationship between obesity and syndrome X. It appears android obesity, indicated by waist/hip circumference ratio, is a risk factor for cardiovascular disease, in parallel with other previously established risk factors. The important component of waist/hip ratio appears to be the mass of visceral fat, relatively independent of total fat mass. Bjorntorp (1992,1991 and 1987) postulated that visceral fat mass is probably increased by multiple endocrine aberrations, with steroid hormones involved in the basic etiology of these aberrations. Moreover, this

appears to cause insulin resistance by direct effects on the periphery, which may be amplified by the metabolism of the enlarged visceral adipose tissues. Hence, this association of insulin resistance and android obesity is consistent with Kaplan's (1989) initial hypothesis and may therefore be considered one of the primary aberrations likely to occur in syndrome X.

The relationship between syndrome X and insulin resistance has been assessed in several diverse populations. Chaiken et al. (1993) assessed the relationship between insulin resistance and the other aberrations associated with syndrome X in 37 black men and 53 black women with NIDDM. In the total group 30% were insulin sensitive and 70% were insulin resistant. No relationship existed between insulin sensitivity and blood pressure in either sex. However, fasting triglyceride and high density lipoprotein cholesterol levels were highly correlated in an inverse relationship within men, but not women. In summary, blood pressure did not correlate with insulin resistance in blacks with NIDDM.

Stern et al. (1992) examined the San Antonio Heart Study cohort, a population-based cohort of 3,301 Mexican Americans and 1,857 non-Hispanic whites. The researchers defined individuals who were hyperdynamic (pulse pressure and heart rate in the upper quartile of their respective distributions), intermediate, and hypodynamic (pulse pressure and heart rate in the bottom quartile). The characteristics of syndrome X were examined according to the previously defined hemodynamic categories, as was the 8-year incidence of hypertension and NIDDM. A hyperdynamic circulation was significantly associated with an increase in body mass

index, subscapular-to-tricep skinfolds ratio, triglyceride, 2-hour glucose, and insulin. When the groups were broken down into obese and non-obese individuals the above effects persisted. The odds ratio for the hyperdynamic state as a predictor of future NIDDM was significant, but this was not true for hypertension. Haffner et al. (1990 and 1992) also used the population-based San Antonio Heart study to examine the relationship of fasting insulin concentration to the incidence of multiple metabolic abnormalities associated with syndrome X. In multivariate analyses, after adjustment for obesity and body fat distribution, elevated fasting insulin continued to be significantly related to decreased high density lipoprotein cholesterol, increased triglycerides and the increased incidence of NIDDM. Baseline insulin concentrations were higher in subjects who subsequently developed multiple metabolic disorders. The results could not be attributed to differences in baseline obesity and were similar in Mexican Americans and non-Hispanic whites. The previous results support the existence of a syndrome related to multiple metabolic disorders, which reveal that elevations in insulin concentration precede the development of numerous metabolic disorders.

Additionally, Saad et al. (1991) examined racial differences in the relation between blood pressure and insulin resistance. Pima Indians, whites, and blacks who were normotensive and did not have diabetes were screened for insulin resistance using the euglycemic-hyperinsulinemic clamp technique. The Pima Indians had higher fasting plasma insulin concentrations and lower rates of whole-body glucose disposal than whites or blacks. After adjustment for age, sex, body weight, and

percentage of body fat, mean arterial blood pressure was significantly correlated with fasting plasma insulin concentration and rate of glucose disposal in whites, but not Pima Indians or blacks. The authors concluded that the relationships among insulinemia, insulin resistance and blood pressure differ among racial groups and may be mediated by mechanisms active in whites, but not in Pima Indians or blacks. Falkner et al. (1990) investigated blood pressure in young black men to determine if insulin resistance was an antecedent to hypertension. The results revealed a relationship between insulin-mediated glucose uptake and blood pressure. Furthermore, in this young black population insulin resistance appears to precede the onset of established essential hypertension. In conclusion, insulin resistance appears to be under genetic control, although genetic predisposition alone can not account for the prevalence of hypertension against a divergent ethnic background (Ferrannini et al. 1990a and 1990b, Ferrannini et al. 1987).

Insulin Signalling and Insulin Resistance

Insulin acts on several tissues to elicit several metabolic effects. The insulin molecule consists of two polypeptide chains connected by two disulfide bridges. Most mammalian insulin molecules have similar sequences; in fact, the primary structure of insulin has been relatively stable throughout evolution. Insulin is formed from a biosynthetic precursor of a high molecular weight, proinsulin. Recently, it has been demonstrated that insulin binds to the α subunit of its receptor, which results in the autophosphorylation of the β subunit and activation of the β subunit tyrosine kinase. This elicits a cascade of phosphorylation and dephosphorylation. For example, ribosomal protein S6 and acetyl-CoA-carboxylase are phosphorylated, whereas pyruvate dehydrogenase and glycogen synthase are dephosphorylated. Moreover, after insulin binds to its receptor and activates tyrosine kinase, it stimulates glucose transport by promoting the translocation and/or activation of transport proteins. Further, it is believed that following activation of insulin receptor tyrosine kinase, there is a site-specific phosphorylation and activation of phosphoserine phosphatase-1. Hence, phosphoserine phosphatase-1 dephosphorylates a number of intracellular substrates of insulin action, including GLUT4, which leads to activation of this glucose transporter. Insulin also exerts an effect on the transcription of specific mRNAs. Furthermore, subsequent to receptor kinase activity insulin may generate second messengers (insulin receptor substrate 1, IRS-1) that would modulate some

of the effects of insulin within the cell (Baulieu and Kelly 1990, Begum et al. 1993, Quon et al. 1994, Saltiel 1990).

Initially, insulin's action was thought to primarily be the enhancement of glucose disposal in skeletal muscle via insulin sensitive transporters. Hence, this launched the investigation of glucose transport in classical insulin sensitive tissues. This led to the recognition of GLUT4 as the primary insulin-stimulated glucose transporter, which is present in adipose tissue, cardiac and skeletal muscle. More recently, GLUT4 has been found in A-10 vascular smooth muscle cells, renal vascular cells and glomerular cells (Cooper et al. 1993, Marcus et al. 1994). Subsequent studies revealed that GLUT4 is activated and/or translocated by an insulin induced second messenger possibly IRS-1 (Quon et al. 1994). Moreover, there may be a direct interaction between the actin network and glucose transporter vesicles which may be mediated through a spectrin-containing skeleton attached to glucose transporter-containing vesicles (Tsakiridis et al. 1994). In addition, GLUT4 appears to be the transporter responsible for exercise-induced, insulin independent glucose uptake.

The two primary glucose transporters found in skeletal muscle are GLUT1 and GLUT4. It appears that GLUT1 is not insulin responsive and functions to maintain the basal uptake of glucose into skeletal muscle (Barnard and Youngren 1992). In contrast, the insulin-responsive GLUT4 is recruited when elevated glucose levels elicit an increase in circulating insulin. Furthermore, non-insulin contractile mediated glucose uptake associated with exercise may utilize a separate pool of

GLUT4 transporters, since the combination of insulin-induced and exercise-induced glucose uptake is additive (Barnard and Youngren 1992). The proposed mechanism of GLUT4 activation suggests that GLUT4 is present in tubulovesicular structures clustered in the transgolgi reticulum and that these transporters are translocated to the sarcolemma with either insulin stimulation and/or exercise (Barnard and Youngren 1992, Galante et al 1994, Holman et al. 1994). It also has been demonstrated (Kainulainen et al. 1993) that there is a marked difference in the response of glucose transport to hypoinsulinemia between oxidative and glycolytic fibers. Furthermore, in contrast to GLUT1, GLUT4 levels are regulated similarly in several tissues (heart and skeletal muscle) in response to hypoinsulinemia.

Additionally, it has been demonstrated that increases in cytoplasmic Ca^{2+} concentration too low to elicit muscle contraction induce an increase in glucose transport activity in skeletal muscle (Youn et al. 1991). On the other hand, Begum et al. (1993) demonstrated in adipocytes that elevated levels of cytosolic calcium interfere with insulin's ability to dephosphorylate GLUT4, thus reducing its intrinsic activity. Moreover, it has been demonstrated in adipocytes that there is an optimal range of cytosolic free calcium for insulin-stimulated glucose transport (Draznin et al. 1987, Levy et al. 1989). Thus, cellular calcium homeostasis appears to be linked to classical actions of insulin in skeletal muscle and adipocytes. It has been proposed that sustained levels of intracellular Ca^{2+} inhibit phosphoserine phosphatase-1 via the phosphorylation and activation of its inhibitor. This effect appears to be mediated by cAMP-dependent pathways. Thus, inhibition of phosphoserine phosphatase-1

activity results in impaired dephosphorylation of several insulin-sensitive enzymes and proteins such as glycogen synthase and GLUT4 (Begum et al. 1992).

Traditionally insulin resistance has been considered pathway specific (primarily involving glucose transport and metabolism) and tissue specific (primarily affecting skeletal muscle, adipose, and liver tissues) (Castillo et al. 1991, Defronzo et al. 1993, Ferrannini et al. 1987, Rocchini 1991, Zemel et al. 1992). However, recent data suggest there are other "non-classical" insulin sensitive pathways (calcium metabolism) and tissues (vascular smooth muscle) which are affected in insulin resistance (Abel and Zemel 1993, Kim and Zemel 1993, Marcus et al. 1994, Quon et al. 1994, Shehin et al. 1989, Zemel et al. 1992, Zemel et al. 1991, Zemel et al. 1990a and 1990b).

The demonstrated elevations in blood pressure accompanying insulin resistant states have been attributed to a lack of feedback inhibition for insulin release, resulting in hyperinsulinemia and consequent increases in sympathetic output (Hall et al. 1989). However, recent evidence from several laboratories, indicates that insulin exerts a direct local vasodilatory effect on the vasculature, and this effect is impaired in insulin resistant states (Kim and Zemel 1993, Shehin et al. 1989, Zemel et al. 1991, Zemel et al. 1990a and 1990b). Insulin accelerates the rate of vascular smooth muscle recovery from pressor agonist-induced intracellular free calcium transients, while these recovery rates are blunted in both insulin resistant and insulinopenic rats (Abel and Zemel 1993a and 1993b, Kim and Zemel 1993). Similarly, insulin attenuates vascular reactivity responses to pressor agonists, as both

insulin resistant and insulinopenic animals exhibit exaggerated vascular reactivity (Reddy at al. 1990, Zemel et al. 1990a). Further, it has been recently demonstrated that some of these effects are dependent, in part, upon glucose transport and/or metabolism (Kim and Zemel 1995).

In conclusion, there appears to be an accumulating body of evidence that insulin may have "non-classical" actions on a tissues that are not traditionally considered insulin sensitive. Moreover, it appears that insulin acts as a vasodilator in vascular smooth muscle (via regulation of calcium transport and cellular calcium levels) and this action is blunted or lost in insulin resistant states (Zemel 1995a and 1995b).

Insulin Resistance and Environmental Influences

As previously mentioned, environmental manipulations may alleviate some of the symptoms associated with syndrome X. For example, physical training can improve the impaired cellular responses to insulin in skeletal muscle and adipose cells associated with syndrome X (Douen et al. 1990, Friedman et al. 1990, Fushiki et al. 1989, Hirshman et al. 1993, Houmard et al. 1991, Ploug et al. 1990, Rodnick et al. 1990, Talmadge and Silverman 1991, Young et al. 1989). Physical training has been shown to induce recruitment and/or translocation of GLUT4 (Douen et al. 1990, Friedman et al. 1990, Fushiki et al. 1989, Hirshman et al. 1993, Houmard et al. 1991, Young et al. 1989). Physical training has also been shown to alleviate the insulin resistance associated with android-shaped obesity (Krotkiewski and Bjorntorp 1986). Moreover, dietary manipulation has the potential to modify peripheral insulin sensitivity (Kahn and Pedersen 1993, Lillioja et al. 1987, Saltin and Gollnick 1986). Rats that are obese from high fat feedings have a suppression of GLUT-4 expression in skeletal muscle (Kahn and Pedersen 1993). It has been demonstrated that a high sodium intake has the potential to exacerbate insulin resistance (Donovan et al. 1993). Recent research demonstrates that manipulating fat, complex carbohydrate, and sucrose in the diet has a profound effect on peripheral insulin sensitivity (Barnard et al. 1993). Further, rats fed diets high in saturated fat became more obese, had elevated fasting insulin levels and exhibited hypertension when compared with control animals and animals on high polyunsaturated fat diets (Kaufman et al.

1994). Therefore it appears that insulin resistance is the locus of a multifactorial syndrome that is genetically based, but modifiable by environmental manipulation (Henriksen et al. 1994, Martin et al. 1991, Serjeantson and Zimmet 1991). This gene-environment interaction will be addressed in greater detail in subsequent sections.

ANIMAL MODELS OF SYNDROME X

Introduction

There are several animal models exhibiting genetic mutations which result in obesity and/or insulin resistance. In the mouse the *ob/ob*, *db/db* and *Avy/-*, whereas, in the rat the *fa/fa*, ZDF/Drt-*fa*, JCR:LA-*ca* and SHR/N-*cp* are commonly used models of genetic obesity and insulin resistance. Analogous to the human syndrome X, these rodent models of obesity possess a clustering of risk factors revolving around insulin resistance. For example, hyperinsulinemia, hypertension, dyslipidemia and obesity appear together in several of these models (Apweiler et al. 1993, Johnson et al. 1991, Kasiske et al. 1992, Kava et al. 1990, Paulson et al. 1992, Pederson et al. 1991, Truett et al. 1991, Zemel et al. 1995c, Zhang et al. 1994).

The main focus of this section will be on the obese Zucker rat (*fa/fa*), a genetically extreme animal model which exhibits some of the symptoms analogous to human syndrome X (Apweiler et al. 1993, Kasiske et al. 1992, Kava et al. 1990, Paulson et al. 1992). The obese Zucker rat model was discovered in 1961 by Zucker and Zucker. This has been the most extensively studied model of obesity in terms of metabolic and cardiovascular alterations (Paulson et al. 1992). Obese Zucker rats exhibit many of the classical cardiovascular risk factors: hyperlipidemia,

hyperinsulinemia, insulin resistance, impaired glucose tolerance and debatable hyperglycemia and hypertension (Paulson et al. 1992).

The Zucker Rat as a Genetic Model of Syndrome X

It has been speculated that the obesity gene fatty (*fa*) of the Zucker rat has homology with the mouse gene diabetes (*db*) and that a homolog of the *fa* and *db* gene is likely to occur in humans (Truett et al. 1991). Moreover, with the recent cloning of the mouse obese (*ob*) gene and its human homologue, the putative role of the *fa* gene becomes much more apparent. It has been speculated (Zhang et al. 1994) that the *ob* gene product is a signal molecule that regulates body weight, potentially interacting with central nervous system and/or other receptors to modulate food intake and the activity of the autonomic nervous system. Furthermore, experiments suggest that the *ob* receptor is encoded by the mouse *db* gene (Zhang et al. 1994). Hence, the *fa* gene may code for the obese (the unidentified rat homologue of *ob*?) receptor in the Zucker rat (Truett et al. 1991).

Insulin-resistant skeletal muscle is a chronic defect of the obese Zucker rat *(fa/fa)*, (Apweiler et al. 1993, Cortez et al. 1991, Crettaz et al. 1980, Czech et al. 1978, Ivy et al. 1989, Ivy et al. 1986, Kasiske et al. 1992, Kava et al. 1990, Paulson et al. 1992, Sherman et al. 1988, Zucker and Antoniades 1972). The obese Zucker rat has also been established as a model of hypertension and insulin resistance (Ionescu et al. 1985, Kurtz et al. 1989, Shehin et al. 1989, Yoshioka et al. 1993, Zemel et al. 1992, Zemel et al. 1990a and 1990b). The obese Zucker rat exhibits obesity (present in obese Zucker rats when compared to lean *(Fa/?)* siblings) and hyperinsulinemia by 4-5 weeks of age (Bray 1977, Fletcher et al. 1986, Ionescu et al. 1985, Kasiske et

al. 1985, Martin et al. 1977, Stern et al. 1995, Zucker and Antoniades 1972), and insulin resistance is evident by 11-13 weeks of age (Ionescu et al. 1985). Hyperglycemia appears to be dependent on the specific colony of obese Zucker rats, as some colonies exhibit hyperglycemia and others do not (Bray 1977, Ionescu et al. 1985). Nonetheless, skeletal muscle glucose transport is lower in obese animals (Cortez et al. 1991, Ivy et al. 1989, Sherman et. al. 1988). Obese Zucker rats also exhibit hyperphagia which plateaus with maturation. The relative hyperphagia is influenced by dietary components, as animals on a high fat diet reach this plateau earlier (Vasseli and Maggio 1990). Although the obese Zucker rat has been extensively studied as a model of hypertension, findings of obese versus lean Zucker rats are conflicting consistent with hypertension in human syndrome X. On the other hand, direct arterial measurements in conscious, unrestrained animals demonstrate that obese Zucker rats are hypertensive by six weeks of age (Kurtz et al. 1989, Shehin et al. 1989, Yoshioka et al. 1993, Zemel et al. 1992, Zemel et al. 1990a and 1990b). Moreover, vascular smooth muscle from obese animals exhibits increased in vitro vascular reactivity (Zemel et al. 1990a) and exaggerated intracellular calcium responses to vasoactive agonists compared to lean animals (Abel and Zemel 1993).

Distinguishing between genotypes of lean and obese Zucker rats has been relatively impossible until later in maturation, when phenotypic markers (rectal temperature, body weight and fat pad size) are discernible. Furthermore, distinguishing between homozygous (*Fa/Fa*) and heterozygous (*Fa/fa*) lean animals has been dependent on the known breeding history of parent animals, which appears

problematic in regard to controlling for the litter effect (Truett et al. 1995) and the unequivocal identification of pure *Fa/Fa* and *Fa/fa* populations. Consequently, little research has been done to examine gene dosage effects of the *fa* allele. Nonetheless, a few studies have shown lean heterozygote animals to be phenotypically intermediate on several physical and metabolic parameters (Baskin et al. 1985, Blonz et al. 1985, Figlewicz et al. 1985, Phillips and Cleary 1994, York et al. 1984, Truett et al. 1995, Abel et al. 1995). Phillips and Cleary (1994) examined lean (*Fa/Fa, Fa/fa*) and obese Zucker (*fa/fa*) rats at 17 days of age. They determined inguinal fat pad weight, inguinal fat pad weight/body weight ratio and inguinal fat pad cell size and number. It was demonstrated that lean Zucker heterozygotes were intermediate between obese and homozygote lean Zucker pups. Furthermore, lipid metabolic enzymes such as lipoprotein lipase and 6-phosphogluconate dehydrogenase were also intermediately produced in adipose and liver tissue. Baskin et al. (1985) demonstrated that insulin binding in brain and liver is reduced in both lean heterozygote and obese animals when compared to lean homozygote animals. Figlewicz et al. (1985) also demonstrated that brain and liver insulin binding is decreased in Zucker rats carrying the *fa* gene. Moreover, dynamic pancreas release of insulin is increased in lean heterozygote and obese rats compared to homozygote lean Zucker rats (Blonz et al. 1977). Intermediate effects have also been demonstrated in brown adipose tissue thermogenesis when lean Zucker homozygous and obese Zucker rats were compared with lean Zucker heterozygous rats (York et al. 1984). Truett et al. (1995) demonstrated a codominant effect of the *fa* gene

during early development of obesity. At 14-days-of-age, *fa* copy number had linear effects on body, inguinal adipose pads and interscapular brown adipose tissue weights. The recessive effects of the *fa* on growth appear during the second week of life. Furthermore, these effects became much more dramatic when the litter effect was statistically controlled. Recent findings from our laboratory (Abel et al. 1995) indicate that feeding lean animals a high fat diet unmasked heterogeneity in mean arterial blood pressure, plasma insulin and glucose, and food efficiency. The variability in mean arterial blood pressure was correlated with blood glucose and food efficiency in high fat fed rats. Lean animals fed a high fat diet with the highest mean arterial blood pressures also had higher circulating insulin and glucose as well as a higher food efficiency ratio. The ability of a high fat diet to raise mean arterial blood pressure was related to the ability of a high fat diet to exert a diabetogenic effect and possibly promote fat storage. Thus, we observed two populations of lean animals, which suggests a *fa* gene dosage effect being environmentally altered in heterozygous (*Fa/fa*) animals.

Orosco et al. (1991) investigated the effect of insulin on brain monoamine metabolism in the Zucker rat. It has been postulated that disturbances of insulin or brain monoamine metabolism may play a role in the impaired regulation of food intake and body weight in the obese Zucker rat. Accordingly, the researchers investigated a possible insulin-monoamine interaction by measuring monoamine levels in the hypothalamus and striatum of obese (*fa/fa*) and lean (*Fa/Fa,Fa/fa*) Zucker rats after peripheral insulin administration. The classical effect of insulin

(increased monoamines) was observed in the hypothalamus of lean (*Fa/Fa,Fa/fa*) rats, but not obese rats. Furthermore, insulin administration increased monoamine levels in the striatum of the lean (*Fa/Fa*) rats only, suggesting a central insulin-related disturbance related to the presence of the "*fa*" gene. In addition, certain effects of insulin on striatal dopamine release were observed in only the *Fa/Fa* and *fa/fa* rats, suggesting a particular disturbance related to the heterozygous character. In conclusion, there appears to be an accumulating body of evidence that suggests a gene dosage effect related to the *fa* allele. Moreover, it appears that this effect may be manipulated by environmental factors (Abel et al. 1995).

The Locus of Insulin Resistance in The Zucker Rat

As previously mentioned insulin's action is elicited by the molecule binding to the α subunit of its receptor, which results in the autophosphorylation of the β subunit and activation of the β subunit tyrosine kinase. This elicits a cascade of phosphorylations and dephosphorylations. Moreover, after insulin binds to its receptor and activates tyrosine kinase, it stimulates glucose transport by promoting the translocation and/or activation of transport proteins. Insulin also exerts an effect at the gene level, on the transcription of specific mRNAs. One example of this is the expression of Ca^{2+} ATPase in vascular smooth muscle (Zemel 1995a). Furthermore, subsequent to receptor kinase activity insulin generates second messengers that modulate some of the effects of insulin within the cell (Baulieu and Kelly 1990, Saltiel 1990). Insulin-resistant skeletal muscle is a chronic defect of the obese Zucker rat *(fa/fa)* (Cortez et al. 1991, Crettaz et al.1980, Czech et al. 1978, Ivy et al. 1989, Ivy et al. 1986, Kasiske et al. 1992, Sherman et al. 1988). The obese Zucker rat is a well established model of hypertension and insulin resistance. The obese Zucker rat exhibits obesity and hyperinsulinemia (Bray 1977, Fletcher et al. 1986, Ionescu et al. 1985, Kasiske et al. 1985, Martin and Gahagan 1977, Stern et al. 1975, Zucker and Antoniades 1972), and insulin resistance early in life (Ionescu et al. 1985). The metabolic and hemodynamic alterations progress in the obese Zucker rat relatively independent of environmental manipulation (Cleary et al. 1980).

Skeletal muscle is one of the traditional sites for insulin's action and is the primary insulin-induced disposal site for glucose. Approximately 75-80% of glucose removed from the blood under maximal insulin stimulation goes into skeletal muscle (Barnard and Youngren 1992). Furthermore, most of the diseases associated with syndrome X exhibit insulin resistance in skeletal muscle as one of their primary characteristics (Barnard and Youngren 1992, Dohm 1991, Elsas and Longo 1992, Kaplan 1989, Pessin and Bell 1992). Both glucose transport and GLUT4 protein function in skeletal muscle of the obese Zucker rat appear to be defective relative to lean littermates (Brozinick et al. 1994). Thus, many researchers have examined the mechanisms of altered insulin-induced glucose disposal in skeletal muscle of the obese Zucker rat. A primary focus of this laboratory has been the role of insulin in regulating vascular smooth muscle reactivity and cell calcium regulation (Abel et al. 1993b, Kim and Zemel 1993, Zemel et al. 1992, Zemel et al. 1991, Zemel et al. 1990a and 1990b). This regulation is blunted or lost in insulin resistant states, as these "non-classical" actions of insulin are not well preserved in vascular smooth muscle of insulin resistant animals (Abel et al. 1993b, Zemel et al. 1991, Zemel et al. 1990a and 1990b). In conclusion, it appears that insulin resistance is inherent in the obese Zucker rat. Moreover, this resistance to insulin is manifested in traditional skeletal muscle carbohydrate metabolism and in additional pathways of other tissues such as vascular smooth muscle. The locus of altered insulin-stimulated glucose disposal in the obese Zucker rat will be addressed in subsequent sections.

Environmental Influences on Insulin Resistance in Syndrome X and the Zucker Rat

There is limited research addressing environmental modulation of insulin resistance in syndrome X and the obese Zucker rat. Further, most of the research has focused on manipulating activity, while limited studies have addressed dietary manipulation. For example, physical training can improve the impaired cellular responses to insulin in skeletal muscle and adipose cells associated with syndrome X (Douen et al. 1990, Friedman et al. 1990, Fushiki et al. 1989, Hirshman et al. 1993, Houmard et al. 1991, Ploug et al. 1990, Rodnick et al. 1990, Talmadge and Silverman 1991, Young et al. 1989). Physical training has been shown to induce recruitment and/or translocation of GLUT4 (Douen et al. 1990, Friedman et al. 1990, Fushiki et al. 1989, Hirshman et al. 1993, Houmard et al. 1991, Young et al. 1989). Physical training has also been shown to alleviate the insulin resistance associated with android-shaped obesity (Krotkiewski and Bjorntorp 1986). Moreover, dietary manipulation has the potential to modify peripheral insulin sensitivity (Kahn and Pedersen 1993, Lillioja et al. 1987). Rats that are obese from high fat feedings have a suppression of GLUT-4 expression in skeletal muscle (Kahn and Pedersen 1993). In addition, a high sodium intake has the potential to exacerbate insulin resistance (Donovan et al. 1993). Likewise, manipulating fat, complex carbohydrate, and sucrose in the diet has a profound effect on peripheral insulin sensitivity (Barnard et al. 1993). Further, rats fed diets high in saturated fat became more obese, had elevated fasting insulin levels and exhibited hypertension when compared with

control animals and animals on high polyunsaturated fat diets (Kaufman et al. 1994). The details of environmental manipulation of glucose transport and GLUT4 will be addressed in subsequent sections.

As previously mentioned the Zucker rat is a genetic rodent model which exhibits insulin-resistance in skeletal muscle as a chronic defect of the obese rat *(fa/fa)* (Cortez et al. 1991, Crettaz et al.1980, Czech et al. 1978, Ivy et al. 1989, Ivy et al. 1986, Sherman et al. 1988). The obese Zucker rat has also been established as a model of hypertension and insulin resistance. The obese Zucker rat exhibits obesity and hyperinsulinemia by 4-5 weeks of age (Bray 1977, Fletcher et al. 1986, Ionescu et al. 1985, Kasiske et al. 1985, Martin and Gahagan 1977, Stern et al. 1975, Zucker and Antoniades 1972), and insulin resistance is evident by 11-13 weeks of age (Ionescu et al. 1985). The aberrations of syndrome X progress in this model relatively independent of dietary influences (Cleary et al. 1980), although, it has been demonstrated that specific aberrations can be blunted with exercise (Brozinick et al. 1993).

Alternatively, Smoller et al. (1993) has recently discovered a restriction fragment length polymorphism corresponding to a cDNA library clone (VC85) in Brown Norway/Zucker offspring. Therefore, distinguishing between obese (*fa/fa*), lean homozygous (*BN/BN*) and lean heterozygous (*BN/fa*) animals is possible. Accordingly, it is now possible to determine if *fa* gene dosage alters the severity and developmental progress of obesity, insulin resistance, hyperglycemia, dyslipidemia and hypertension.

In conclusion, the aberrations of syndrome X progress in the Zucker model *(fa/fa)* relatively independent of environmental influences (Cleary et al. 1980). Alternatively the heterozygous *(Fa/fa)* lean Zucker rat may exhibit a gene dosage effect which may be more sensitive to environmental manipulations (Figlewicz et al. 1985, Phillips and Cleary 1994, York et al. 1984). Therefore, the heterozygous lean Zucker rat may be a more appropriate animal model for the study of gene-environment interaction in syndrome X than the genetic extreme obese Zucker rat.

GLUCOSE TRANSPORTERS AND TRANSPORT IN INSULIN RESISTANT STATES

Introduction

Glucose transport appears to be the rate-limiting step for glucose disposal in skeletal muscle. Glucose uptake is maximized by the presence of insulin and/or muscle contraction, which are additive and appear to be independent of each other and tissue specific (Brozinick et al. 1994, Brozinick et al. 1992, King et al.1993, King et al. 1992). Furthermore, in muscle and adipose tissue, glucose transport is regulated by intrinsic and extrinsic factors (Rodnick et al. 1992). Several glucose transporter proteins work together to maintain glucose homeostasis in the body by balancing glucose absorption with glucose utilization (Elsas and Longo 1992, Pessin and Bell 1992). The glucose transporter proteins can be divided into two main classes which include the sodium-dependent glucose cotransporters (SGTP1) and the facilitative glucose transporters (GLUT1-GLUT5). The sodium-dependent glucose cotransporters transport glucose against a concentration gradient inside intestinal and renal cells using the electrochemical potential of sodium as their energy source (Elsas and Longo 1992). Since glucose is a hydrophilic molecule and cannot freely cross the plasma membrane, a series of facilitative glucose transporters are required to promote entry of glucose into cells. The facilitative glucose transporters transport

glucose down a concentration gradient and do not require energy (Barnard et al. 1992a and 1992b, Elsas and Longo 1992).

The first glucose transporter (GLUT1) is located in brain, erythrocyte, and fibroblast cells and is minimally stimulated by insulin. The primary purpose of GLUT1 is to maintain basal glucose transport in the cell. On the other hand, GLUT2 is a low affinity glucose transporter found primarily in liver, kidney, intestine, and β cells. GLUT2 activity increases when blood glucose levels are elevated beyond the basal state. GLUT3 has a function (basal glucose transport) similar to GLUT1 and is also found in brain and fibroblast cells. GLUT4 is the primary insulin stimulated glucose transporter and is found in adipose tissue, cardiac muscle, and skeletal muscle (Barnard and Youngren 1992, Elsas and Longo 1992, Pessin and Bell 1992).

More recently, GLUT4 has been found in A-10 vascular smooth muscle cells, renal vascular cells and glomerular cells (Cooper et al. 1993, Marcus et al. 1994). GLUT4 is activated and/or translocated by an insulin induced second messenger. Subsequent demonstration revealed that GLUT4 is activated and/or translocated by an insulin induced second messenger possibly IRS-1 (Quon et al. 1994). Moreover, there may be a direct interaction between the actin network and glucose transporter vesicles which may be mediated through a spectrin-containing skeleton attached to glucose transporter-containing vesicles (Tsakiridis et al. 1994). Furthermore, it has been demonstrated that increases in cytoplasmic Ca^{2+} concentration too low to cause muscle contraction can induce an increase in glucose transport activity in skeletal

muscle (Youn et al. 1991). On the other hand, Begum et al. (1993) demonstrated in adipocytes that elevated levels of cytosolic calcium interfere with insulin's ability to dephosphorylate GLUT4, thus reducing its intrinsic activity. Moreover, it appears there is an optimal calcium range for some of insulin's actions. It has been demonstrated in adipocytes that there is an optimal range of cytosolic free calcium for insulin-stimulated glucose transport (Draznin et al. 1987, Levy et al. 1989), thus, linking cellular calcium homeostasis to classical actions of insulin in skeletal muscle and adipocytes. It has also been proposed that sustained levels of intracellular Ca^{2+} inhibit phosphoserine phosphatase-1 via the phosphorylation and activation of its inhibitor. This effect appears to be mediated by cAMP-dependent pathways. Thus, inhibition of phosphoserine phosphatase-1 activity elicits impaired dephosphorylation of several insulin-sensitive enzymes and proteins such as, glycogen synthase and GLUT4 (Begum et al. 1992). GLUT4 also appears to be the transporter responsible for exercise-induced insulin independent glucose uptake.

The final glucose transporter (GLUT5) is located in the small intestine and facilitates the absorption of glucose from the lumen. Although much progress has been made in understanding the specific glucose transporters, there are still many important unanswered questions (Barnard and Youngren 1992, Elsas and Longo 1992, Pessin and Bell 1992).

The two primary glucose transporters found in skeletal muscle and adipocytes consist of GLUT1 and GLUT4. It appears that GLUT1 functions to maintain the basal uptake of glucose into skeletal muscle (Barnard and Youngren 1992), whereas

GLUT4 is recruited when elevated glucose levels elicit an increase in circulating insulin. GLUT4 also appears to be responsible for non-insulin mediated glucose (contractile) uptake associated with exercise. This phenomena may utilize a separate pool of GLUT4 transporters since the combination of insulin-induced and exercise-induced glucose uptake is additive (Barnard and Youngren 1992). The proposed mechanism on how GLUT4 is activated suggests that GLUT4 is present in tubulovesicular structures clustered in the transgolgi reticulum and that these transporters are translocated to the sarcolemma with either insulin stimulation and/or exercise (Barnard and Youngren 1992, Galante et al 1994, Holman et al. 1994). It also has been demonstrated (Kainulainen et al. 1993) that there is a marked difference in the response of glucose transport between oxidative and glycolytic fibers. Furthermore, GLUT1 and GLUT4 levels are regulated differently in various tissue types.

Skeletal muscle is the primary insulin-induced disposal site for glucose. Approximately 75% of glucose removed from the blood under maximal insulin stimulation goes into skeletal muscle (Barnard and Youngren 1992). Furthermore, most of the diseases associated with altered insulin-induced glucose disposal have insulin resistance in skeletal muscle as one of their primary characteristics (Barnard and Youngren 1992, Dohm 1991, Elsas and Longo 1992, Kaplan 1989, Pessin and Bell 1992). Both glucose uptake and GLUT4 protein distribution in skeletal muscle of NIDDM and the obese Zucker rat appear to be defected (Brozinick et al. 1994, Wallberg et al. 1992). Thus, many investigators have focused their research on

impaired insulin-induced glucose disposal in skeletal muscle. Moreover, with GLUT4 being one of the primary regulators of insulin and contractile-induced glucose disposal, it has become the locus of extensive research. Therefore, the emphasis of this section will be to examine the effects of environmental manipulations such as exercise training and diet on insulin-sensitivity and GLUT4 regulation in skeletal muscle and adipocytes. Furthermore, the interaction of exercise and the glucose transport system has been investigated much more extensively then the interaction of diet and the glucose transport system, thus, it is logical to examine both interactions in this section.

Environmental Influences on Glucose Transport and GLUT4

Exercise

It been shown that increasing the expression and/or insulin-induced translocation of GLUT4 is beneficial for individuals that possess any disease that encompasses a glucose disposal abnormality. Moreover, it appears that skeletal muscle GLUT4 aberrations result from a defect in GLUT4 compartmentalization, translocation and/or functional activity, and there appears to be a divergence between GLUT4 mRNA levels and translation (Garvey et al. 1991, Garvey et al. 1992a and 1992b, Hager et al. 1991, Hainault et al. 1991, Kahn et al. 1992a and 1992b, King et al. 1992, Knott 1992). Hence, several researchers have investigated the potential benefit of exercise training in increasing glucose transport, GLUT4 activity and GLUT4 translocation in insulin resistant models (Garvey et al. 1991, Garvey et al. 1992a and 1992b, Hager et al. 1991, Hainault et al. 1991, Kahn et al. 1992a and 1992b, King et al. 1992, Knott 1992).

Several investigators have demonstrated that exercise has the potential to ameliorate the defects in glucose transport and/or GLUT4 associated with syndrome X and a sedentary lifestyle. For instance, Dela et al. (1994) demonstrated that physical training increased muscle GLUT4 protein and mRNA in patients with NIDDM. Seven men with NIDDM and eight healthy controls were cycle trained and

biopsies were obtained from the vastus lateralis leg muscle before and after the training program. Prior to training, GLUT4 protein content was similar in individuals with NIDDM and controls. Nonetheless, GLUT4 content increased in all individuals after training. The authors concluded that the antidiabetogenic effects associated with training were partially due to the increase in muscle GLUT4 protein content. Similarly, Houmard et al. (1991) investigated the effect of exercise training on skeletal muscle glucose transporter (GLUT4) levels. Muscle GLUT4 and oral glucose tolerance test (OGTT) responses were obtained in trained and sedentary middle-aged men. Plasma insulin levels during the OGTT were significantly lower in the trained men, whereas no differences were seen in the plasma glucose responses. GLUT4 protein content was approximately twofold higher in the trained men. Accordingly, GLUT4 levels appear to be increased in conjunction with insulin sensitivity in chronically exercise-trained middle-aged men. In a subsequent study Houmard et al. (1993) investigated the effect of exercise training on GLUT4 protein concentration in previously sedentary middle-aged men. In this study GLUT4 concentration was assessed before and after 14 weeks of exercise training. The investigators also examined muscle fiber-type composition. As speculated, the GLUT4 concentration increased by approximately 1.8-fold. Moreover, as the GLUT4 protein increased, the percentage of GLUT4 rich type IIa muscle fibers increased by 10%. Therefore, it appears that the concentration of GLUT4 is, in part, inherent to the muscle fiber-type and as the fiber-type is modified, so is the concentration of GLUT4.

On the other hand, in a group of athletes, Andersen et al. (1993) examined the importance of GLUT4 mRNA, GLUT4 protein and fiber type composition of skeletal muscle. A biopsy of the vastus lateralis muscle was used to determine fiber type and GLUT4 concentrations, while the euglycemic hyperinsulinemic clamp was used to assess insulin-stimulated glucose uptake. The authors concluded that the abundance of GLUT4 protein and mRNA is increased in skeletal muscle from endurance trained subjects compared to sedentary subjects. However, it appeared that GLUT4 protein concentration was not the determinant for the increased insulin-stimulated whole body glucose uptake in endurance trained subjects.

The interaction of exercise training and glucose transport and/or GLUT4 have been investigated extensively in animal models. For example, Hirshman et al. (1993) examined the abundance and subcellular distribution of GLUT1 and GLUT4 in basal and insulin-stimulated adipose cells from wheel cage exercise-trained rats. Exercise training increased total GLUT4 by 31% compared with controls. In conclusion exercise training increased the total amount of GLUT4 protein in adipose cells. Further, the increased rate of insulin-stimulated glucose transport in adipose cells from exercise training is due to the translocation of GLUT4 to the plasma membrane from an enlarged intracellular pool.

The relationship between insulin induced and exercise induced glucose transport has been investigated. Nesher et al. (1985) examined the effects of insulin and contraction on in vitro glucose transport and metabolism in rat epitrochlearis muscle. It was demonstrated that insulin and contraction activated glucose transport

and metabolism via independent mechanisms. In addition, the insulin dose-response curves showed a threshold and saturation-type kinetics, whereas isometric contraction activated glucose transport and metabolism in a linear fashion with no evidence of a threshold. Furthermore, the relationships among fiber type, GLUT4 protein content, and glucose transport activity stimulated maximally with insulin and/or contractile activity has also been examined (Henriksen et al. 1992). Insulin-stimulated 2-deoxyglucose (2-DG) uptake was greatest in predominantly type I and least in predominantly type IIb muscle fibers. On the other hand, contractile activity induced the greatest uptake of 2-DG in predominantly type IIa and least in predominantly type IIb muscle fibers. The effects of insulin and contractile activity on 2-DG uptake were additive in all muscle preparations and were greatest in predominantly type IIa and least in predominantly type IIb muscle fibers. In parallel, GLUT4 protein concentrations were greatest in predominantly type IIa and least in predominantly type IIb muscle fibers. Moreover, linear regression analysis revealed low if any correlations between GLUT4 protein content and insulin-stimulated 2-DG uptake, but there were significant correlations between GLUT4 protein content and contractile-stimulated 2-DG uptake. The relationship between GLUT4 protein content and insulin + contractile stimulated 2-DG uptake had the strongest ($r = 0.992$) correlation. It was concluded that the differences in maximally (insulin + contractile) stimulated glucose transport activity among the three fiber types are highly related to their GLUT4 protein content.

Ploug et al. (1990) examined the effect of endurance training on glucose transport capacity and glucose transporter expression in rat skeletal muscle. The effect of 10 weeks of endurance swim training on 3-O-methylglucose uptake in skeletal muscle was studied. Training resulted in a 33% increase in maximum insulin-stimulated glucose uptake in fast-twitch red fibers, and a 33% increase for contraction-stimulated transport in slow-twitch red fibers compared with non-exercised sedentary muscle. A fully additive effect of insulin and contraction was observed both in trained and untrained muscle. The mRNA for GLUT1 and GLUT4 increased approximately twofold by training in fast-twitch red muscle fibers. Parallel to this, Western blot analysis demonstrated an increase in GLUT1 and GLUT4 protein. The authors concluded that the increase in maximum glucose transport in trained muscle is due to an increased number of glucose transporters.

To elucidate the chronic effect of exercise on GLUT4, Goodyear et al. (1992) examined glucose transporter number, function, and subcellular distribution in rat skeletal muscle after exercise training. The authors examined the mechanism of insulin-stimulated glucose uptake in skeletal muscle by comparing female rats exercise-trained in a wheelcage for 6 weeks with untrained female rats. The total number of skeletal muscle plasma membrane glucose transporters, total muscle homogenate and plasma membrane GLUT4 proteins, and rates of plasma membrane glucose transport were higher in exercise-trained rats immediately after exercise training and did not decrease significantly during the five days after cessation of training. On the other hand, exercise training did not alter microsomal membrane

total glucose transporter number or GLUT4 protein. GLUT1 protein in total muscle was unaffected by exercise. Consequently, the increased rates of glucose uptake in endurance-trained skeletal muscle appeared to result primarily from an increased number and not an increase in the average functional activity of glucose transporters present in the plasma membrane. Furthermore, these increases persisted for several days after cessation of exercise training. The specific increase in the GLUT4, but not the GLUT1 glucose transporter isoform, in response to training demonstrates that a common, chronic physiological stimulus can regulate the expression of the two glucose transporter isoforms present in skeletal muscle tissue differentially.

To determine the latent effect of exercise on insulin's action, Douen et al. (1990) investigated the effect of exercise on the recruitment of the insulin-responsive glucose transporter (GLUT4). The investigators examined the distribution of the muscle glucose transporters GLUT1 and GLUT4 in plasma membrane and intracellular membrane fractions of skeletal muscle preparations from control, exercised, and acutely insulin-treated rats. Acute insulin treatment increased GLUT4 transporters in the plasma membrane fraction and decreased GLUT4 transporters in the intracellular membrane fraction. Exercise also increased the GLUT4 transporters in the plasma membrane fraction, but in contrast to insulin, did not significantly decrease GLUT4 transporters in the intracellular fraction. Neither insulin nor exercise increased GLUT1 transporters in the plasma membrane fraction. The investigators concluded that exercise-sensitive GLUT4 transporters do not originate from the insulin-sensitive intracellular membrane fraction, suggesting the

existence of distinct intracellular insulin and exercise-recruitable GLUT4 transporter pools.

Marette et al. (1992) investigated the abundance, localization, and insulin-induced translocation of glucose transporters in red and white muscle. Glucose transporter number was twofold higher in isolated plasma membranes of red than of white muscle. In contrast, the number of transporters in an isolated insulin-sensitive intracellular membrane organelle was similar in the two muscle groups. The Slow twitch oxidative (ST) and Fast twitch oxidative-glycolytic (FT_a) fibers demonstrated an increased number of GLUT4 protein compared with the fast twitch glycolytic (FT_b) fibers. GLUT1 activity was restricted to the surface of the muscle cell. Insulin increased the membrane distribution of GLUT4 protein and glucose transport activity in red and white muscle. In contrast, insulin did not change the membrane distribution of GLUT1 in red and white skeletal muscle. The molar ratio of GLUT4 to GLUT1 in red and white muscle plasma membranes was found to be 4:1 in the basal state and 7:1 in the insulin-stimulated state. The researchers concluded that red muscle contains a higher amount of GLUT1 and GLUT4 transporters at the plasma membrane than white muscle in the basal and insulin-stimulated states but that GLUT4 translocation does not differ between muscle types. In addition, GLUT4 expression correlates with the metabolic nature (oxidative vs. glycolytic) of skeletal muscle fibers rather than with their contractile properties (slow twitch vs. fast twitch). Similarly, Megeney et al. (1993) examined glucose uptake and GLUT4 in oxidative and glycolytic rat muscle. In the perfused rat hindlimb muscles the

proportion of oxidative fibers was highly correlated with the muscle's insulin-stimulated 3-O-MG uptake and GLUT4 content. Insulin-stimulated 3-O-MG uptake and GLUT4 were also highly correlated. In 3-day denervated muscles, insulin-stimulated 3-O-MG uptake and GLUT4 content was reduced in almost all muscles. A very high correlation was observed between the decrements in GLUT4 and the decrements in 3-O-MG uptake. In conclusion, it was demonstrated that glucose uptake and GLUT4 are regulated by insulin-independent means, namely the oxidative capacity of the muscle and the normal activity level of the muscle.

On the other hand, Neufer et al. (1992) investigated the effects of training and detraining on skeletal muscle glucose transporter GLUT4 content in rats. After 1 day or 1 week there was no change in muscle GLUT4 content. Although 6 weeks of training increased GLUT4 protein content approximately 1.5 fold over controls in the soleus and red vastus lateralis, whereas no significant change was evident in the white vastus lateralis muscle. GLUT4 protein content returned to near control values after 1 week of detraining. In parallel, citrate synthase activity showed similar responses to training and detraining. The authors suggested that expression of GLUT4 protein is coordinated with the well-documented adaptations in oxidative enzyme activity with endurance training and detraining.

To determine if a decrease in GLUT4 was an aging phenomena, Kern et al. (1992) investigated the effect of aging and exercise on GLUT4 protein in skeletal muscle. This study focused on the relationship between muscle GLUT4 protein and aging induced deterioration in glucose tolerance. Furthermore, it investigated

whether exercise training would increase GLUT4 protein levels in older animals. Exercise training produced a significant increase in GLUT4 protein in the soleus and gastrocnemius muscle of young and middle-aged rats compared with sedentary controls. In the oldest rats, GLUT4 was not significantly increased with training, but a trend toward an increase was apparent. The main effect of aging was primarily due to a statistically significant difference between the old trained and young trained rats. The training program increased the old trained GLUT4 content to equal that of the middle-aged untrained animals.

The effects of exercise on insulin resistance in the obese Zucker rat have been examined. Friedman et al. (1990) studied the effect of exercise training on glucose transporter protein GLUT4 in skeletal muscle of obese Zucker rats. The authors examined the level of GLUT4 protein in the gastrocnemius muscle of 36 week old genetically obese Zucker rats and their lean littermates, and in obese Zucker rats following 18 or 30 weeks of treadmill exercise training. The level of GLUT4 protein was similar in the lean and insulin-resistant obese Zucker rats. Exercise training the obese Zucker rats increased GLUT4 protein levels 2.3 fold above sedentary obese Zucker rats. The authors suggest that endurance training stimulates expression of skeletal muscle GLUT4 protein which may be responsible for the previously observed increase in insulin sensitivity with training. Consequently, Brozinick et al. (1992) examined the rates of muscle glucose uptake of lean and obese Zucker rats under basal conditions, maximally stimulating concentrations of insulin and muscle contraction elicited by electrical stimulation of the sciatic nerve. Basal glucose

uptake was similar for lean and obese rats, although plasma membranes from lean rats contained 82% more GLUT4 protein than obese rats. Insulin stimulation resulted in a significant increase in plasma membrane GLUT4 protein concentration in lean but not obese rats. Glucose uptake of lean rats in the presence of insulin was approximately fourfold greater than that of obese rats. Moreover, this difference in glucose uptake could not be completely accounted for by the difference in plasma membrane GLUT4 concentrations. Stimulation by contraction resulted in similar increases in plasma membrane GLUT4 concentration and glucose uptake rates in both lean and obese rats. It was concluded that muscle insulin resistance in the obese Zucker rat is due to a decrease in GLUT4 translocation and activity. However, contraction stimulated glucose uptake and GLUT4 protein translocation and activation are normal in the obese Zucker rat. In a subsequent study Brozinick et al. (1993) examined the rate of skeletal muscle glucose uptake in trained and untrained obese Zucker rats under basal, insulin stimulated and contractile conditions. In this study it was demonstrated that exercise training did not alter basal, insulin-, or contraction-stimulated GLUT4 functional activity. Furthermore, aerobic exercise increased insulin- and contraction-stimulated muscle glucose uptake via increased plasma membrane GLUT4 protein concentration and not an increased GLUT4 protein translocation or functional activity.

Subsequently, the effects of muscle activity and fiber composition on glucose transport and GLUT4 were examined. To elucidate the effects of exercise training intensity on insulin-stimulated 3-O-MG transport and GLUT4 protein, Banks et al.

(1992) examined red and white quadriceps of high and low intensity exercised trained obese Zucker rat. Sedentary obese and lean Zucker rats were used as controls. Although, no differences existed between red quadriceps of high and low intensity trained rats, training in general increased both 3-O-MG transport and GLUT4 protein concentrations over sedentary obese rats. In the white quadriceps 3-O-MG transport between high and low intensity trained rats approached significance ($p<0.07$). GLUT4 protein concentrations and citrate synthase activity of high intensity trained rats were significantly greater than sedentary obese rats. The authors concluded that the improvement in muscle insulin resistance of the obese Zucker rat after exercise training is due in part to an increased GLUT4 concentration, which is related with the degree to which the muscle is trained.

In conclusion, the decrease in insulin-stimulated glucose transport associated with insulin resistant states is in part due to a reduction in the compartmentalization, activity and/or translocation of GLUT4 protein. It appears that not all models of insulin resistance exhibit a decrease in total GLUT4 protein (NIDDM and the Zucker obese rat). Moreover, compartmentalization, activation and translocation appears to be aberrant in the Zucker obese rat. Nonetheless, it is apparent that physical activity can have a significant affect on the glucose transport system. It has been demonstrated that training, detraining and age influence the capacity of the glucose transport system and GLUT4 protein levels. Furthermore, exercise can increase glucose transport to the same level as a maximum dose of insulin. Although, it appears that insulin resistance is associated with the insulin-induced

glucose transport system and not the contractile-induced glucose transport system. The mechanisms that elicit the insulin and contractile responses appear to have an additive effect and tissue GLUT4 protein has it's highest correlation with insulin+contractile-stimulated glucose transport. Further, increasing the plasma membrane GLUT4 protein via exercise training also enhances insulin-stimulated glucose transport. Regular exercise training also increases insulin sensitivity for several days after the final bout of exercise. Thus, exercise appears to increase the number (especially in the plasma membrane) and/or the translocation/activation of the insulin-responsive glucose transporter protein GLUT4. In addition, physical inactivity has been shown to elicit an insulin resistant state and a decrease in GLUT4. Therefore, it appears that exercise training has a positive and predictable effect on glucose transport and GLUT4 protein.

Diet

Recently, it has been demonstrated that dietary manipulation has the potential to modify peripheral insulin sensitivity (Kahn and Pedersen 1993). Rats that are obese from high fat feedings have a suppression of GLUT4 expression in skeletal muscle and fat cells (Kahn and Pedersen 1993 and Pedersen et al. 1991). Similarly, it has been demonstrated that a high sodium intake has the potential to exacerbate insulin resistance (Donovan et al. 1993). Hence, a growing body of

research has focused on manipulating fat, complex carbohydrate, sucrose, and other nutrients in the diet to elucidate their effects on peripheral insulin sensitivity.

For example, Barnard et al. (1993) examined the effects of a high-fat-sucrose diet on serum insulin and related syndrome X associated atherosclerotic risk factors in rats. Fischer 344 rats were fed a high-fat-sucrose diet or low-fat, high-complex-carbohydrate diet for 2 years. The high-fat-sucrose diet rats were obese, hypertensive, hyperinsulinemic, and hypertriglyceridemic. Furthermore, they exhibited enhanced clotting and impaired fibrinolytic response to streptokinase. Insulin was positively correlated with body weight, triglycerides, and systolic blood pressure. Total cholesterol and HDL-cholesterol were unchanged. Hence, the authors concluded that many syndrome X associated risk factors can be induced with high-fat-sucrose diets. The aggregation of risk factors for atherosclerosis was found in the high-fat-sucrose diet rats and not the low-fat, high-complex-carbohydrate diet animals. In fact, most of the rats on the low-fat, high-complex-carbohydrate diet developed no risk factors after 2 years, indicating that the development of risk factors is not an aging phenomenon. Consequently, Barnard et al. (1994) examined the reversibility of the diet-induced skeletal muscle insulin resistance. They demonstrated that 8 weeks on a high-fat-sucrose diet did not change the number of insulin receptors assessed by ^{125}I-insulin binding. However, maximum insulin-stimulated glucose transport and maximum insulin-stimulated receptor tyrosine kinase activity were significantly decreased but could be restored with 4 weeks of low-fat, high-complex-carbohydrate diet.

It appears that obesity alone has a profound effect on glucose transport. Elton et al. (1994) investigated the relationship between glucose transport and body mass index using muscle biopsies from 30 females (BMI from 16-40). There was a significant negative relationship between stimulation of glucose transport and BMI. There was a continuous decline in glucose transport at BMI's greater then 30 kg/m^2. However, the decrease in glucose transport associated with obesity was ameliorated with weight loss. Friedman et al. (1992) demonstrated with the euglycemic hyperinsulinemic clamp that weight loss via gastric bypass in obese patients could partially restore blunted glucose transport. Although, impaired in vivo glucose transport was partially restored in these patients with weight loss, weight loss elicited no change in vastus lateralis GLUT4 protein concentration. The authors suggested that skeletal muscle insulin resistance in morbid obesity is reversible with weight loss and is associated with enhanced transporter translocation and/or activation but not an increase in GLUT4 protein.

Peripheral insulin resistance can also be modified by dietary manipulation. Grimditch et al. (1988) demonstrated in Sprague-Dawley rats that the presence of sucrose and/or the absence of complex carbohydrates, not high fat, was responsible for the insulin resistance and it was not improved by adding fiber to the diet or by exercise training.

A high-fat diet with or without exercise can have a significant impact on glucose transport and GLUT4 protein. Rosholt et al. (1994) examined the effect of insulin and exercise on male Sprague-Dawley rats fed a high-fat or high-carbohydrate

diet. It was demonstrated that maximal glucose transport increased three-fold with insulin treatment or acute exercise training compared to the treatment free controls in the high-carbohydrate diet rats. In the high-fat animals the glucose transport increases were less than twofold. In contrast to findings from Grimditch et al. (1988), the high-fat diet animals exhibited resistance to both exercise and insulin stimulation of muscle glucose transport. Similarly, Pedersen et al. (1991) examined the effect of high-fat feeding, overfeeding, or energy-restriction on insulin resistance and the expression of GLUT4 in fat cells of Sprague-Dawley rats. High-fat feeding was associated with relative postprandial hypoglycemia and hypoinsulinemia. The high-fat fed animals had decreased body weight but increased adiposity as demonstrated by epididymal fat pad weight. Fat feeding caused a 78% reduction in insulin-stimulated glucose transport per adipocyte. In addition there was a 92-94% reduction in GLUT4 mRNA and protein per adipocyte in fat-fed rats. Even though energy restriction and overfeeding elicited opposite changes in adiposity, no changes occurred in glucose transport or GLUT4. Thus, the impaired insulin-stimulated glucose transport in adipose cells from high fat-fed rats occurs in the presence of a dramatic decrease in the expression of GLUT4. Moreover, it was speculated that the reduced gene expression may be caused by chronic hypoinsulinemia and may contribute to the insulin resistance observed in this state. Consequently, Kahn et al. (1993) demonstrated that obesity due to high fat feeding, but not due to high calorie/carbohydrate feeding or genetics, is associated with pretranslational suppression of GLUT4 expression in skeletal muscle. The authors went on to

conclude that in at least some forms of obesity, the level of GLUT4 expression in muscle appears to be only one factor in, or may even be unrelated to, the degree of insulin-responsive glucose transport in vivo. In contrast, Okamoto et al. (1992) demonstrated that insulin resistance in rats fed a high-fat diet exhibit a post-receptor defect in muscle, although tyrosine-phosphorylation of both insulin receptor and its endogenous substrate are decreased in liver. Moreover, levels of both GLUT4 from muscle and GLUT2 from liver in the crude membrane were comparable to those of the controls. Penicaud et al. (1991) demonstrated an enhanced expression of GLUT4 in white adipose tissue of young obese *fa/fa* rats subjected to a high fat diet upon weaning. These data indicated the existence of an enhanced expression of GLUT4 in white adipose tissue of young obese rats which could be related to an increased plasma insulin level.

Specific fatty acids have unique effects on insulin sensitivity. Hunnicutt et al. (1994) demonstrated that saturated fatty acids induce insulin resistance in rat adipocytes. These investigators demonstrated that saturated fatty acids stimulate glucose transport acutely, but on prolonged exposure induce insulin resistance via a post-insulin binding defect. It has also been demonstrated that dietary fish oil decreases the sensitivity of glucose uptake to insulin in isolated rat adipocytes (Macho et al. 1993). Furthermore, arachidonic acid has been shown to down-regulate the insulin-dependent glucose transporter gene (GLUT4) in adipocytes by inhibiting transcription and enhancing mRNA turnover (Tebbey et al. 1994). Kaufman et al. (1994) fed male Sprague-Dawley rats a diet high in either saturated

fat or polyunsaturated fat beginning at 10 weeks of age. After 10 weeks of diet treatment, blood pressure was 17% higher in rats fed saturated fat and 8% higher in rats fed polyunsaturated fat than in rats fed a low-fat control diet. Moreover, rats fed the saturated fat diet became obese, and their fasting insulin levels were elevated (38% above control). The authors concluded that both saturated and polyunsaturated dietary fats induce hypertension. Findings from our work (Abel et al. 1995) indicate that feeding lean animals a high fat diet unmasked heterogeneity in mean arterial blood pressure, plasma insulin and glucose, and energy efficiency. The variability in mean arterial blood pressure was correlated with blood glucose and energy efficiency in high fat fed rats. Lean animals fed a high fat diet with the highest mean arterial blood pressures also had higher circulating insulin and glucose as well as a higher energy efficiency ratio. The ability of a high fat diet to raise mean arterial blood pressure was related to the ability of a high fat diet to exert a diabetogenic effect and possibly promote fat storage.

Further, Ivy et al. (1986) demonstrated that a high carbohydrate diet and exercise training increased the rate of glucose uptake in the obese Zucker rat. More importantly, the combined effect of diet and exercise training was greater than the sum of their individual effects. It appears that the effect of diet and exercise is synergistic in reducing the muscle insulin resistance in the obese Zucker rat. Moreover, Sherman et al. (1993) found that hyperglycemia associated with pancreatectomized lean and obese Zucker rats may be an important factor in the reduction of muscle GLUT4 levels in lean and obese rats. The reduced GLUT4 was

accompanied by reduced maximal glucose transport in lean but not obese rats. Notably, exercise training reduced hyperglycemia, normalized glucose transport, and increased muscle GLUT4 in obese animals. Thus, the beneficial effects of exercise training resulted in a reduction in hyperglycemia and chronic adaptions in skeletal muscle glucose transport capacity including increased GLUT4. It also appears that carbohydrate metabolism may alter insulin resistance in the obese Zucker rat. Ivy et al. (1994) demonstrated that pair-feeding for 3 weeks with 6 energy % calcium-pyruvate or pyruvylglycine resulted in: decreased weight gain; lower food conversion efficiency; and higher resting oxygen consumption than control rats. The experimental diets also resulted in increased lipid oxidation and decreased carbohydrate oxidation. Moreover, the pyruvylglycine rats had a lower insulin response despite their elevated plasma triglyceride concentrations. Thus, it appears that both experimental diets favorably alter the metabolism of the obese Zucker rats. In addition, pyruvylglycine reduces the insulin resistance that develops spontaneously in the obese rats.

The mechanism of fatty acid-induced inhibition of glucose uptake has been investigated. It has been demonstrated that glucocorticoid-induced muscle insulin resistance is due to excessive non-esterified fatty acid oxidation. The proposed mechanisms of this insulin resistance was that increased fatty-acid oxidation ultimately inhibited glucose transport, or decreased glycogen synthesis, or directly affected glucose transport translocation and/or activity (Guillaume et al. 1993). In parallel, Boden et al. (1994) examined the mechanisms of fatty acid-induced inhibition of glucose uptake. The authors investigated the effects of three steady

state plasma free fatty acid levels on rates of glucose uptake, glycolysis, glycogen synthesis, carbohydrate oxidation, hepatic glucose output, and nonoxidative glycolysis during euglycemic-hyperinsulinemic clamp. Increased free fatty acid concentrations decreased glucose uptake in a dose-dependent fashion. The decrease was caused mainly by a reduction in glycogen synthesis and to a lesser extent by a reduction in carbohydrate oxidation. At high free fatty acid infusion there was an impaired glycogen synthesis activity in muscle, associated with an increase in glucose-6-phosphate concentration, which developed after 4-6 hours of fat infusion. At medium free fatty acid concentrations there was a decrease in muscle glucose-6-phosphate concentration, which may have been the consequence of reduced glucose transport/phosphorylation. In addition, free fatty acids and/or glycerol increased insulin-suppressed hepatic glucose output. It was concluded that free fatty acids cause a dose-dependent inhibition of insulin-stimulated glucose uptake and that free fatty acids and/or glycerol increase insulin-suppressed hepatic glucose output and thus caused insulin resistance at the peripheral and the hepatic level.

In summary, skeletal muscle insulin resistance appears to be the hallmark of syndrome X. The decrease in insulin-stimulated glucose transport associated with insulin resistant states is in part due to a reduction in the activity, translocation and/or compartmentalization of GLUT4 protein. It is apparent that physical activity and dietary modification can have a significant effect on the glucose transport system. Furthermore, environmental manipulation of insulin sensitivity has been documented in several models of insulin-resistance. Exercise can increase glucose transport to the

same level as a maximum dose of insulin. The mechanism that elicits this response has an additive effect when coupled with insulin, but it also enhances the activity of the insulin-stimulated response. Regular exercise training also increases insulin sensitivity for several days after the final bout of exercise. Chronic exercise appears to increase the number and/or the translocation/activation of the insulin-responsive glucose transporter protein GLUT4. In addition, physical inactivity has been shown to elicit an insulin resistant state and decrease GLUT4 protein. Thus, exercise training has a positive and predictable effect on glucose transport and GLUT4 protein. On the other hand, the effect of various dietary constituents on insulin sensitivity are not as predictable as manipulating activity. Moreover, it appears that there may be diet-gene interactions adding to the unpredictability of dietary modification in various models of insulin resistance.

In conclusion, there has been tremendous progress made in understanding the complex relationship between insulin resistance, glucose transport, and environmental influences, but there are still many important unanswered questions. There are numerous studies examining the effect of exercise training on the glucose transport system, but few studies have addressed dietary influences on this system. Moreover, there appears to be relatively few studies that investigate the possibility of gene-diet interactions influencing the aberrations associated with syndrome X. Indeed, insulin resistance is the hallmark of many chronic diseases that plague our society today. Therefore, it is important to understanding the interaction of genetics and environment in insulin resistance.

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PART III.

INSULIN REGULATION OF VASCULAR SMOOTH MUSCLE GLUCOSE TRANSPORT IN INSULIN-SENSITIVE AND RESISTANT RATS

ABSTRACT

Previous studies from this laboratory have demonstrated insulin regulation of vascular smooth muscle intracellular calcium transport and of vascular tone. Accordingly, we measured insulin regulated glucose transport and GLUT4 protein in aortic tissue from insulin-resistant and sensitive rats in order to document classical insulin action in this tissue. We assessed, via *in vitro* muscle-bath and perfusion; and via *in vivo* infusion, basal and insulin-stimulated [^{3}H]-2-deoxyglucose transport in aorta from lean and obese Zucker rats. In the muscle-bath system, insulin significantly stimulated aortic 2-deoxyglucose transport by 30%, but there was no difference in either baseline or insulin-stimulated glucose transport in lean versus obese rats. However, in the perfusion system, in which only the luminal surface of the vessel was e1xosed, insulin significantly ($p < 0.04$) stimulated 2-deoxyglucose transport in the aorta of only the lean animals. Western blot analysis demonstrated that aort1xfrom lean and obese Zucker rats contained substantial concentrations of GLUT4 protein. Surprisingly, in the muscle-bath system 2-deoxyglucose transport was markedly higher in the aorta than in the soleus ($p < 0.0002$). These data demonstrate that vascular smooth muscle is an insulin-sensitive tissue which appears to be partially impaired in the insulin-resistant animals.

INTRODUCTION

The obese Zucker rat (*fa/fa*), a well characterized and widely used rat model of genetic obesity (Cortez et al. 1991, Ivy et al. 1989, Sherman et al.1988), exhibits chronic skeletal muscle insulin resistance. These animals exhibit obesity and hyperinsulinemia by 4-5 weeks of age (Bray 1977, Fletcher et al.1986, Ionescu et al. 1985, Kasiske et al. 1985, Martin and Gahagan 1977, Stern et al. 1975, Zucker and Antoniades 1972), and insulin resistance is evident by 11-13 weeks of age (Ionescu et al. 1985). The obese Zucker rat is also an established model of hypertension, with significant elevations in systolic and diastolic blood pressure evident as early as six weeks of age (Kurtz et al. 1989, Shehin et al. 1989, Yoshioka et al. 1993, Zemel et al. 1990a and 1990b, Zemel et al. 1992).

Traditionally, insulin resistance has been considered pathway specific (primarily involving glucose transport and metabolism) and tissue specific (primarily affecting skeletal muscle, adipose, and liver) (Castillo et al., DeFronzo et al. 1983, Ferrannini et al. 1987, Rocchini 1991, Zemel et al. 1992). However, recent data suggest there are other "non-classical" insulin sensitive pathways such as calcium metabolism and tissues including vascular smooth muscle which are affected in insulin resistance (Abel and Zemel 1993b, Kim and Zemel 1993, Zemel et al. 1992, Zemel et al. 1991, Zemel et al. 1990a and 1990b).

The demonstrated elevations in blood pressure accompanying insulin resistant states have been attributed to a lack of feedback inhibition for insulin release,

resulting in hyperinsulinemia and consequent increases in sympathetic output (Hall et al. 1989). However, recent evidence, from our laboratory and others, indicates that insulin exerts a direct vasodilatory effect on the vasculature, and this effect is impaired in insulin resistant states (Abel and Zemel 1993b, Shehin et al. 1989, Zemel et al. 1991, Zemel et al. 1990a and 1990b). Insulin accelerates the rate of vascular smooth muscle recovery from pressor agonist-induced intracellular free calcium transients, while these recovery rates are blunted in both insulin resistant and insulinopenic rats (Abel and Zemel 1993a and 1993b, Kim and Zemel 1993). Similarly, insulin attenuates vascular reactivity responses to pressor agonists, as both insulin resistant and insulinopenic animals exhibit exaggerated vascular reactivity (Reddy et al. 1990, Zemel et al. 1990a). Further, we have recently found these effects to be dependent, in part, upon glucose transport and metabolism (unpublished data). Accordingly, the purpose of this study was to determine if insulin stimulates glucose transport in aortic tissue and if so, determine whether aortic tissue glucose transport is impaired in insulin resistant states.

MATERIALS AND METHODS

Housing and care of animals

Lean (*Fa/?*) and obese (*fa/fa*) Zucker rats were purchased from Charles River Inc. (Wilmington, MA) or generated from our breeding colony (parent animals from an established vendor). Upon arrival the rats were housed 2/cage and provided food (Agway Prolab Rat Mouse Hamster 3000, Syracuse, NY) and water *ad libitum*. A temperature of 21°C and an artificial 12-h light-dark cycle were maintained in the animal room. This study was approved by the Institutional Animal Care and Use Committee of the University of Tennessee.

Muscle-bath system with 2-deoxyglucose

Glucose transport activity was measured by using the nonmetabolizable glucose analogue [^{3}H]-2-deoxyglucose and a modified *in vitro* muscle-bath procedure previously used in rat skeletal muscle (Karlstad and Sayeed 1986, Youn et al. 1991, Young et al. 1986). Food was withheld overnight, 4 lean and 4 obese animals (20 weeks of age) were then anesthetized with 50 mg/kg sodium pentobarbital and soleus and thoracic aorta were carefully removed and cleaned in ice-cold Krebs-Henseleit buffer (Karlstad and Sayeed 1986). The rat hindlimb contains tissue that exhibits the three types of skeletal muscle fibers (Armstrong and Phelps 1984): slow twitch, fast twitch $_a$, and fast twitch $_b$. The soleus muscle contains predominantly (89%) slow twitch muscle fibers, which have been demonstrated to be extremely insulin sensitive (Cortez et al. 1991, Ivy et al. 1989). Thus, the soleus muscle has been established as an insulin sensitive tissue, which exhibits insulin resistance in the obese Zucker rat (Sherman et al. 1988, Zucker and Antoniades 1972). The aorta was sectioned into 4 similar pieces and the soleus was gently split and mounted on small wires to resting length with each animal serving as its own control. After a 30 minute incubation in oxygenated Krebs-Henseleit buffer, muscles were transferred to a flask containing 10 ml Krebs-Henseleit buffer (pH 7.4), 32 nmol/L [^{3}H]-2-deoxyglucose (NEN, Boston, MA) (3.7 kBq), 1.5 umol/L [^{14}C]mannitol (NEN, Boston, MA) (185 Bq) (for extracellular volume correction), 0.1% albumin, and 140 nmol/L insulin or acetic acid control (Karlstad and Sayeed 1986). Incubations 10 minutes aorta and 20

minutes soleus were carried out in a Dubnoff metabolic shaker (Chicago, IL) at 37° C in an atmosphere of 95% O_2 and 5% CO_2. Following incubation, the muscles were gently blotted on filter paper and frozen in liquid nitrogen. The samples were then weighed, dried overnight at 68° C, reweighed and solubilized in 1 ml of Solvable (NEN, Boston, MA). Ten ml of liquid scintillation fluid (Formula 989 NEN, Boston, MA) was added to the solubilized samples. Simultaneous determination of ^{3}H and ^{14}C was used to calculate the amount of 2-Deoxyglucose incorporated into tissues in the presence or absence of insulin.

Perfusion system with 2-deoxyglucose

Glucose transport activity was measured using an *in vitro* perfusion procedure in isolated, intact aorta. Food was withheld overnight, 6 lean and 6 obese animals (25 weeks of age) were then anesthetized with sodium pentobarbital and aorta were carefully removed, sectioned (each animal served as its own control), and cleaned in ice-cold Krebs-Henseleit buffer. Sections of aorta were attached to a syringe mounted on an infusion pump (Harvard Apparatus Pump 22, Southnatick, MA) and perfused with 5 ml (0.5 ml/min) of oxygenated Krebs-Henseleit buffer (pH 7.4) containing 32 nmol/L [^{3}H]-2-deoxyglucose, (3.7 kBq), 1.5 umol/L [^{14}C]mannitol (185 Bq) and 0.1% albumin, with or without 140 nmol/L insulin. Following perfusion, the aorta were gently blotted on filter paper and weighed. The samples were then dried in a vacuum oven at 90° C, reweighed and solubilized in 1 ml of Solvable. Ten ml of liquid scintillation fluid was added to the solubilized samples. Simultaneous determination of ^{3}H and ^{14}C was used to calculate the amount of 2-deoxyglucose incorporated into tissues in the presence or absence of insulin.

Infusion system with 2-deoxyglucose

Glucose transport activity was measured using a modification of an *in vivo* infusion procedure previously used in rat skeletal muscle (Turinsky 1987). Food was withheld overnight, 8 lean and 8 obese animals (25-30 weeks of age) were then anesthetized with sodium pentobarbital (50 mg/kg body wt) and injected intravenously (penile vein) with a bolus dose of 32 nmol/L [^{3}H]-2-deoxyglucose, (3.7 kBq), 1.5 umol/L [^{14}C]mannitol (185 Bq) and 0.1% albumin, with or without 140 nmol/L insulin. Following a 10 minute infusion, the aorta and soleus were removed and weighed. The samples were then dried in a vacuum oven at 90° C, reweighed and solubilized in 1 ml of Solvable. Ten ml of liquid scintillation fluid was added to the solubilized samples. Simultaneous determination of ^{3}H and ^{14}C was used to calculate the amount of 2-deoxyglucose incorporated into tissues in the presence or absence of insulin.

Determination of GLUT4 Protein

Thoracic aorta and soleus were removed from 4 lean and 4 obese Zucker rats (17-20 weeks of age), washed in ice-cold phosphate-buffered saline, minced and dissolved in suspension and gel loading buffers (Sambrook et al. 1989). Following ten minutes of boiling, the preparation was sonicated, centrifuged and the supernatant was removed. Proteins (200 μg) were separated by 10% SDS-PAGE Tris-glycine and electroblotted and/or immobilized on PVDF membrane for immunoblot analysis. ^{125}I-Protein A (NEN, Boston, MA) and anti-rabbit polyclonal antibody to GLUT4 protein (gift from Dr. Cushman) were used to determine GLUT4 concentrations. Aortic tissue GLUT4 protein was also demonstrated via BioRad (Hercules, CA) Immuno-lite kit for chemiluminescent determination of proteins in conjunction with Genzyme (Cambridge, MA) mouse-anti-rat monoclonal antibody to GLUT4 protein. Blots were exposed to several films for 2-4 days and densitometry was performed to determine relative concentrations of tissue GLUT4 protein.

Statistical Considerations

These studies used a completely randomized design with two treatments (basal and maximal insulin-stimulated glucose transport) and two levels (lean and obese Zucker rats). Two-way analysis of variance was used to determine statistical significance of the main effects. Comparisons between aorta and soleus in each transport assay system were evaluated via t-tests and significance was confirmed at ($p < 0.05$). All values are reported as means $\pm$ standard error.

RESULTS

In the muscle-bath system, insulin stimulated aortic 2-deoxyglucose transport by 30% (Figure III.1a, Table III.1), but there was no difference in either baseline or insulin-stimulated glucose transport in lean versus obese Zucker rats. Moreover, in the perfusion system insulin significantly ($p < 0.04$) stimulated 2-deoxyglucose transport in the aorta of the lean, but not in the aorta of the obese Zucker rats (Figure 1b). Not surprisingly, there appeared to be a trend toward reduced baseline transport in the aorta of the obese versus lean Zucker rats (Figure III.1b), although this difference was not statistically significant ($p < 0.07$). In contrast, in the *in vivo* infusion system, insulin failed to elicit stimulation of 2-deoxyglucose transport in the aorta of lean Zucker rats. The lack of insulin stimulation in the aortic infusion system resulted from several limitations of the technique. Nonetheless, western blot analysis demonstrated that aorta contained substantial concentrations of GLUT4 protein, with no significant difference between lean and obese animals (Figure III.2).

As expected in the muscle-bath system, both baseline ($p < 0.03$) and insulin-stimulated ($p < 0.004$) 2-deoxyglucose transport in soleus were lower in obese than in lean rats. Although these findings do not parallel the findings in the aorta, they are consistent with the previous literature on this topic (Cortez et al. 1991, Ivy et al. 1989, Sherman et al. 1988). In the infusion system, insulin significantly ($p < 0.02$) stimulated 2-deoxyglucose transport in the soleus of all animals. Paradoxically, western blot analysis demonstrated that obese rat soleus contained substantial

($p < 0.01$) higher concentrations of GLUT4 protein then there lean littermates (Figure III.2).

Surprisingly, in the muscle-bath system, basal and insulin stimulated 2-deoxyglucose transport were markedly higher in the aorta than in the soleus (123%, $p < 0.0002$) of lean and obese animals (Figure III.3). Similarly, in the *in vivo* infusion system the rate of basal and insulin stimulated 2-deoxyglucose transport in aorta was 265% ($p < 0.02$) greater then basal 2-deoxyglucose transport in soleus.

Table III.1: 2-deoxyglucose transport in soleus and/or aorta of lean and/or obese rats.

group	basal (nM/min/g)	insulin-stimulated	
MUSCLE-BATH			
lean soleus	1.8 ± 0.2	2.2 ± 0.1	* #
lean aorta	14.1 ± 0.7	18.3 ± 1.0	* $
obese soleus	1.1 ± 0.3	1.4 ± 0.3	*
obese aorta	14.2 ± 0.8	18.2 ± 0.9	* $
PERFUSION			
lean aorta	8.41 ± 2.06	13.5 ± 1.87	*
obese aorta	5.3 ± 0.65	8.2 ± 3.64	
INFUSION			
lean soleus	13.0 ± 2.2	23.5 ± 5.0	*
lean aorta	48.9 ± 11.2	38.0 ± 12.2	* $

* $p<0.05$ versus basal

# $p<0.05$ versus obese

$ $p<0.05$ versus soleus

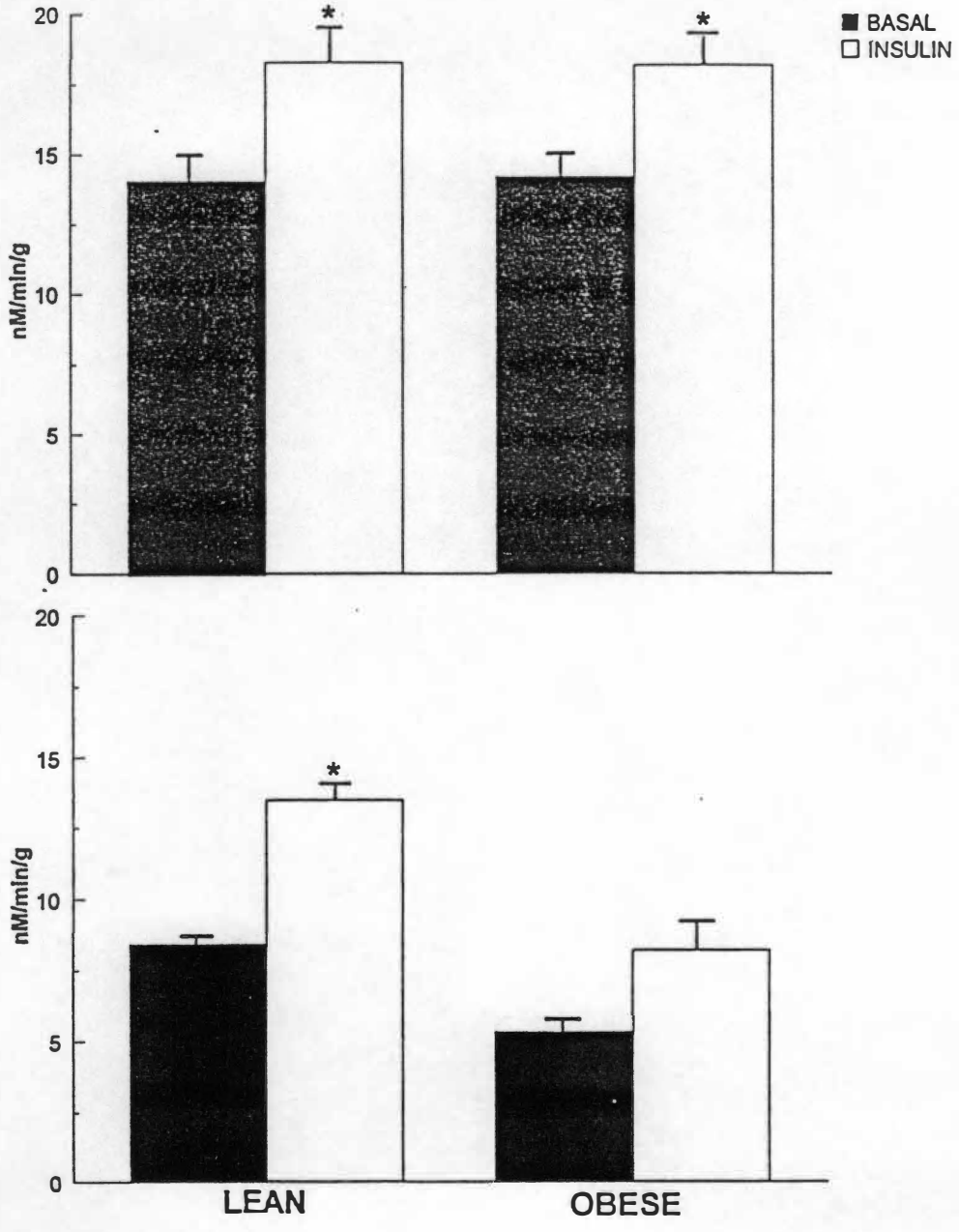

Figure III.1 (a. upper panel) Aortic muscle-bath measurements of basal and insulin stimulated 2-deoxyglucose transport in lean and obese Zucker rats. Lean and obese animals exhibited a significant insulin stimulated increase in 2-deoxyglucose uptake (* indicates $p < 0.03$, $n = 4$ lean and 4 obese). (b. lower panel) Aortic perfusion measurements of basal and insulin stimulated 2-deoxyglucose transport in lean and obese Zucker rats. Only lean animals exhibited a significant insulin stimulated increase in 2-deoxyglucose uptake (* indicates $p < 0.04$, $n = 6$ lean and 6 obese) (values are means $\pm$ SEM).

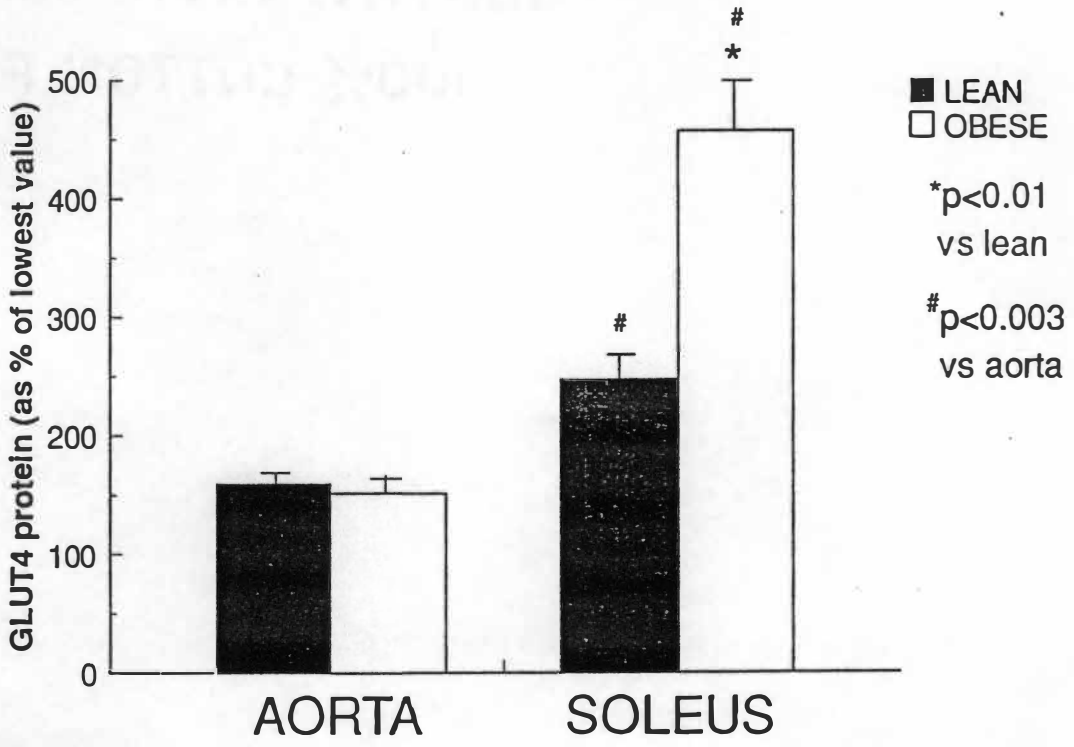

Figure III.2 Relative GLUT4 protein concentrations (% of lowest densotometric value) from soleus and aorta of 4 lean vs. 4 obese Zucker rats.(values are means ± SEM).

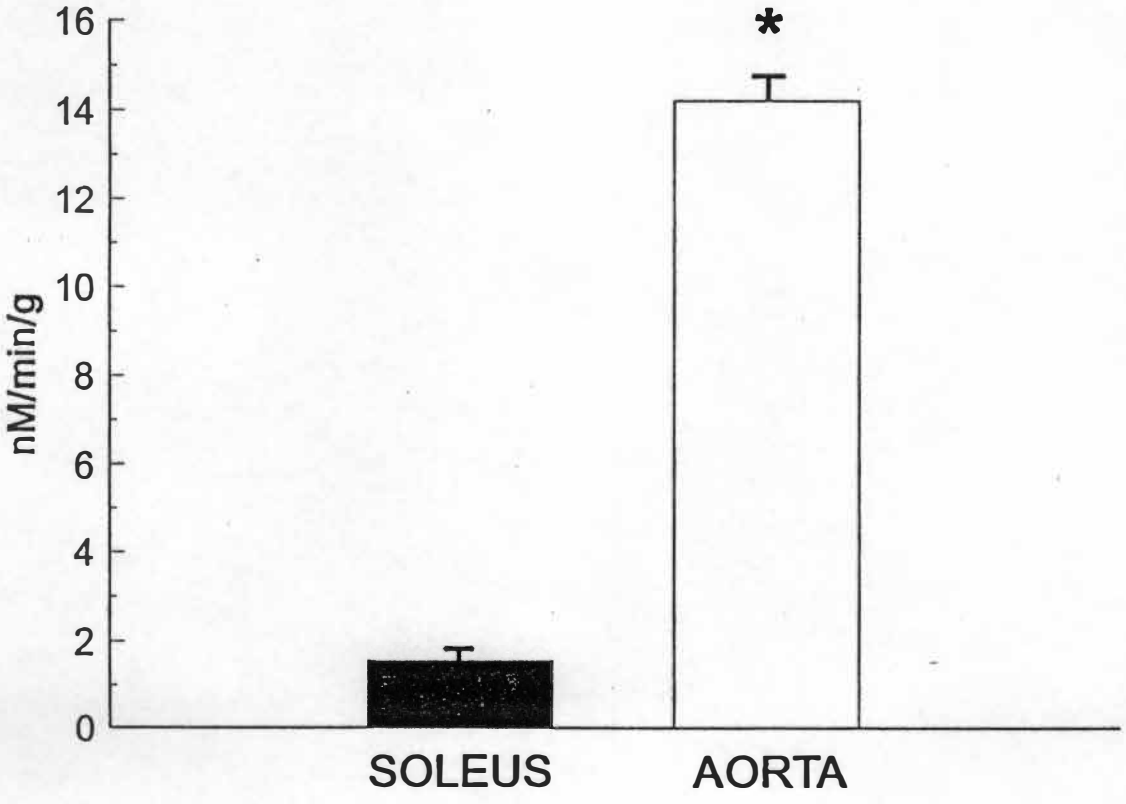

Figure III.3 Insulin stimulated 2-deoxyglucose transport in soleus versus aorta of all (lean and obese) Zucker rats using the muscle-bath system (* indicates $p < 0.03$, $n = 4$ lean and 4 obese) (values are means $\pm$ SEM).

DISCUSSION

We have demonstrated that aortic tissue contains a substantial concentration of the insulin-stimulated glucose transporter (GLUT4) and exhibits insulin dependent glucose transport. This is consistent with Cooper et al. (1993), who demonstrated the presence of GLUT4 protein, GLUT4 mRNA and glucose transport in A-10 vascular smooth muscle cells. In previous studies, this laboratory has demonstrated the role of insulin in regulating vascular smooth muscle reactivity and cell calcium regulation (Abel et al. 1993b, Kim and Zemel 1993, Zemel et al. 1992, Zemel et al. 1991, Zemel et al. 1990a and 1990b). This regulation is blunted or lost in insulin resistant states, as these "non-classical" actions of insulin are not well preserved in vascular smooth muscle of insulin resistant animals (Abel et al. 1993b, Zemel et al. 1991, Zemel et al. 1990a and 1990b). Further, we have recently shown these effects to be glucose dependent, as inhibition of glucose transport and/or metabolism (with appropriate replacement of energy substrate) eliminates these effects (unpublished data). Accordingly, we sought to determine if aortic tissue transports glucose in an insulin dependent fashion and if so, determine if aortic tissue exhibits resistance to this action in an insulin-resistant state. If so, this could explain, in part, the loss of the "non-classical" actions of insulin on vascular smooth muscle function. Data from the present study supports this concept, as insulin perfusion stimulated 2-deoxyglucose transport in lean, but not obese Zucker rats.

The muscle-bath and perfusion methods elicited significant insulin stimulated 2-deoxyglucose transport in aorta from lean Zucker rats. However, in the muscle-bath system we demonstrated similar basal and insulin stimulated 2-deoxyglucose transport in aorta from lean and obese Zucker rats (Figure 1a). In contrast, in the *in vitro* perfusion system we demonstrated insulin stimulated 2-deoxyglucose transport in aorta from only the lean animals. In retrospect, the muscle-bath technique presents potential limitations. For example, we question the consequence of allowing insulin and 2-deoxyglucose exposure to all surfaces of the aorta and the possible removal of the endothelial layer from the aorta during dissection. Consequently, the perfusion technique appears to be a more accurate portrayal of glucose transport across the luminal surface of the vessel. Therefore, the perfusion technique may be the most physiologically pertinent procedure.

The infusion method elicited significant insulin stimulated 2-deoxyglucose transport in the soleus but not in the aorta. Clearly, 2-deoxyglucose transport is dependent upon the concentration of labeled 2-deoxyglucose substrate in the media. However, unlike the *in vitro* systems, muscle-bath and perfusion, we could not control the 2-deoxyglucose concentration in the *in vivo* infusion system. Consequently, in the infusion system there was a dramatic decrease in blood 2-deoxyglucose and glucose concentration of the insulin-stimulated group compared to the control group, which created a distinct discrepancy in the concentration of 2-deoxyglucose that the aortic tissue was exposed to for the 10 minutes of infusion. This rapid decline in 2-deoxyglucose concentration in the insulin stimulated group created an artifact

rendering the results of the procedure invalid. Several attempts at compensating for this rapid decline of 2-deoxyglucose in the insulin stimulated animal by simultaneous infusion of glucose and/or 2-deoxyglucose did not resolve this problem, as skeletal muscle served as a substantial sink for insulin-stimulated 2-deoxyglucose uptake. Accordingly, the lack of aortic 2-deoxyglucose transport *in vivo* may be attributed to limitations of the infusion technique, hence, the *in vivo* infusion system does not appear to be as suitable for assessment of aortic 2-deoxyglucose transport as it is for skeletal muscle.

Paradoxically, the aorta contained substantially ($p < 0.0003$) less GLUT4 protein, but transported significantly ($p < 0.0002$) greater amounts of 2-deoxyglucose than the soleus. Most likely, this discrepancy is due to the direct relationship between tissue weight and glucose transport (Crettaz et al. 1980). Alternatively, this discrepancy could result from a different distribution of glucose transporters (GLUT4/GLUT1 ratio) present in the aorta relative to soleus transporter distribution.

Surprisingly, the soleus of the obese animals contained substantially ($p < 0.01$) higher concentrations of GLUT4 protein than soleus from lean littermates, but transported significantly ($p < 0.03$) less 2-deoxyglucose than the lean animals. There is an accumulating body of evidence suggesting there is no significant difference of GLUT4 protein content in normal vs insulin resistant states (Bader et al. 1992, Brozinick et al. 1991 and 1992, Davidson 1993, Eriksson et al. 1991, Friedman et al. 1992 and 1990, Garvey et al. 1992, Handberg et al. 1990, Pedersen et al. 1990). It

has also been demonstrated that plasma insulin is positively correlated with glucose transport and GLUT4 protein concentration (Barnard et al. 1990, Kahn et al. 1987). Thus, as plasma insulin increases (ie. obese vs. lean Zucker rats) one would expect a concomitant increase in GLUT4 protein concentration. Moreover, there appears to be little or no correlation between insulin-stimulated 2-deoxyglucose uptake and total tissue GLUT4 protein content (Brozinick et al. 1993, Henriksen et al. 1992). In part, this lack of correlation is due to the possible existence of two distinct pools of GLUT4, one stimulated by insulin and the other stimulated by contraction. Further, the two pools are fully additive (Brozinick et al. 1993) with respect to 2-deoxyglucose transport and together they correlate highly with 2-deoxyglucose uptake (Henriksen et al. 1992). It has also been demonstrated that this impairment in 2-deoxyglucose transport is related to aberrations in GLUT4 protein activation, translocation and compartmentalization, not total tissue GLUT4 protein content (Brozinick et al. 1992 and 1993, Friedman et al. 1990, Henriksen et al. 1992). Consequently, the total tissue concentration of GLUT4 protein may be of limmited value in predicting transporter capacity. In fact, activation, translocation and compartmentalization of GLUT4 is more instrumental in the aberrant transport of glucose associated with the obese Zucker rat (Brozinick et al. 1992 and 1993, Friedman et al. 1990, Henriksen et al. 1992).

In summary, we have demonstrated that aortic tissue contains a substantial concentration of the insulin-stimulated glucose transporter (GLUT4) and exhibits insulin dependent glucose transport, which appears to be impaired in obese Zucker

rats. These data support the concept that vascular smooth muscle is an insulin-sensitive tissue which appears to be partially impaired in obese Zucker rats. Further, this impairment of "classical" insulin action on vascular smooth muscle may result from a loss of regulation of "non-classical" insulin actions ie. cell Ca^{2+} regulation of vascular relaxation in the insulin resistant state.

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PART IV.

A NOVEL GENE-DIET-GENDER RELATIONSHIP ASSOCIATED WITH THE *fa* ALLELE

ABSTRACT

The purpose of this study was to determine if there is a *fa* allele effect in lean heterozygous (*BN/fa*) rats subjected to a high fat diet. Lean (*BN/BN*) and lean (*BN/fa*) male/female rats were fed 12% or 48% fat diets for 7 weeks. Food intake and weight change were recorded weekly to obtain an index of energy efficiency. After seven weeks, direct intra-arterial blood pressure was measured in conscious, unrestrained animals. Blood and tissue were collected for subsequent analyses. Plasma insulin, plasma glucose, plasma cholesterol, perirenal and epididymal fat pad weights, energy efficiency ratio, weight gain and food intake were all significantly higher in male rats on the 48% fat diet than male rats on the 12% fat diet. Animals on the 48% fat diet demonstrated higher fat pad weights than animals on the 12% fat diet ($p<0.00001$) and *BN/fa* animals had heavier fat pads than *BN/BN* animals ($p<0.0018$). Not surprisingly, males had higher perirenal fat pad weights than females ($p<0.00001$). Paradoxically, the animals on the 48% fat diet had a significantly higher ($p<0.009$) concentration of soleus GLUT4 protein than animals on the 12% fat diet. In summary, we have demonstrated that a high fat diet exacerbates insulin resistance and the aberrations associated with insulin resistance. Furthermore, the effects of the high fat diet are amplified in animals carrying one copy of the *fa* allele and female animals appeared to be somewhat resistant to this diet-gene interaction. Additionally, we have demonstrated that a high fat diet increases the insulin responsive glucose transporter (GLUT4) protein in skeletal

muscle regardless of genotype or gender. These data support the concept that syndrome X is predominantly a male syndrome that is genetically determined and modulated by environmental influences.

INTRODUCTION

Hyperinsulinemia, hypertension, dyslipidemia and android obesity are all considered risk factors for cardiovascular disease. Further, these risk factors have a tendency to aggregate and this combination has been termed "syndrome X" or "the deadly quartet" (Barnard et al. 1992, DeFronzo and Ferrannini 1991, Kaplan 1989, Reaven 1988). Insulin resistance appears to be the key factor that links this "syndrome X", especially in genetically susceptible persons (DeFronzo and Ferrannini 1991, Reaven 1988). It has been shown that impaired cellular responses to insulin results in increased vascular smooth muscle tone, decreased expression and/or translocation of the insulin-responsive glucose transporter (GLUT4) protein, and altered transport of glucose in skeletal muscle and adipose cells (Dohm et al. 1991, Ivy et al. 1986, Kahn and Pedersen 1993, Sherman et al. 1988, Zemel et al. 1992).

Additionally, it has been demonstrated that manipulating activity and diet can alleviate or exacerbate some of the symptoms associated with syndrome X. For example, physical training can improve the impaired cellular responses to insulin in skeletal muscle and adipose cells associated with syndrome X (Douen et al. 1990, Friedman et al. 1990, Fushiki et al. 1989, Hirshman et al. 1993, Houmard et al. 1991, Ploug et al. 1990, Rodnick et al. 1990, Talmadge and Silverman 1991, Young et al. 1989). Physical training has also been shown to induce recruitment and/or translocation of GLUT4 (Douen et al. 1990, Friedman et al. 1990, Fushiki et al. 1989, Hirshman et al. 1993, Houmard et al. 1991, Young et al. 1989) and alleviate

the insulin resistance associated with android-shaped obesity (Krotkiewski and Bjorntorp 1986).

Dietary manipulation also has the potential to modify peripheral insulin sensitivity and GLUT4 protein functional activity (Kahn and Pedersen 1993, Lillioja et al. 1987, Penicaud et al. 1991, Saltin and Gollnick 1986). Rats with diet induced obesity exhibit suppresed GLUT-4 expression in skeletal muscle (Kahn and Pedersen 1993). Manipulating fat, complex carbohydrate, and sucrose in the diet has a profound effect on peripheral insulin sensitivity (Barnard et al. 1993). Further, rats fed diets high in saturated fat became more obese, had elevated fasting insulin levels and exhibited hypertension when compared with control animals and animals on high polyunsaturated fat diets (Kaufman et al. 1994).

The obese *(fa/fa)* Zucker rat is a genetic animal model which exhibits many of the symptoms of syndrome X. Insulin-resistant skeletal muscle is a chronic defect of the obese Zucker rat (Cortez et al. 1991, Crettaz et al.1980, Czech et al. 1978, Ivy et al. 1989, Ivy et al. 1986, Sherman et al. 1988). The obese Zucker rat has also been established as a model of hypertension and insulin resistance. The obese Zucker rat exhibits obesity and hyperinsulinemia by 4-5 weeks of age (Bray 1977, Fletcher et al. 1986, Ionescu et al. 1985, Kasiske et al. 1985, Martin and Gahagan 1977, Stern et al. 1975, Zucker and Antoniades 1972), and insulin resistance is evident by 11-13 weeks of age (Ionescu et al. 1985). The aberrations of syndrome X progress in this model relatively independent of dietary influences (Cleary et al. 1980), although, it has been demonstrated that specific aberrations can be blunted with exercise (Brozinick et al.

1993). In contrast, limited evidence suggests that the heterozygous *(Fa/fa)* lean Zucker rat may exhibit a gene dosage effect which may be more sensitive to environmental manipulations (Figlewicz et al. 1985, Phillips and Cleary 1994, York et al. 1984). Therefore, the heterozygous lean Zucker rat may be a more appropriate animal model for the study of gene-environment interaction in syndrome X than the obese Zucker rat.

Unfortunately, distinguishing between genotypes of lean and obese Zucker rats has not been possible until later in maturation, when phenotypic markers such as rectal temperature, body weight and fat pad size are discernible. Furthermore, distinguishing between homozygous (*Fa/Fa*) and heterozygous (*Fa/fa*) lean animals has been dependent on the known breeding history of parent animals, which appears problematic in regard to controlling for the litter effect (it is impossible to compare littermates) and the unequivocal identification of pure *Fa/Fa* and *Fa/fa* populations (Truett et al. 1995). Consequently, little research has been done to examine gene dosage effects of the *fa* allele. Nonetheless, a few studies have shown lean heterozygote animals to be phenotypically intermediate on several physical and metabolic parameters (Abel et al. 1995, Baskin et al. 1985, Blonz et al. 1985, Figlewicz et al. 1985, Orosco et al. 1991, Phillips and Cleary 1994, York et al. 1984, Truett et al. 1995).

However, with the recent discovery of a restriction fragment length polymorphism (RFLP) corresponding to a cDNA library clone (VC85) in Brown Norway/Zucker offspring (Smoller et al. 1993), distinguishing between obese (*fa/fa*),

lean homozygous (*BN/BN*) and lean heterozygous (*BN/fa*) animals is possible. Thus, it is now possible to determine if *fa* gene dosage alters the severity and developmental progress of obesity, insulin resistance, hyperglycemia, dyslipidemia and hypertension. Accordingly, the focus of this research was to determine if there is a gene-diet interaction associated with the *fa* allele, potentially modulating GLUT4 protein and classical risk factors associated with syndrome X.

MATERIALS AND METHODS

Breeding and genotyping of animals

In Brown Norway/Zucker offspring, the restriction fragment length polymorphism corresponding to a cDNA library clone (VC85) (Smoller et al. 1993) was used to distinguish between lean homozygous (*BN/BN*) and lean heterozygous (*BN/fa*) animals. The animals were bred and genotyped at Pennington Biomedical Research Center. Tail segments were used for extraction of high molecular weight DNA utilizing phenol-chloroform (Autoextract Kit, Isogene Biotechnology, Overland Park, KS). Restriction endonuclease Taq-1 (Boehringer Mannheim, Indianapolis, IN) was used to digest DNA, followed by electrophoresis through 1% LE agarose gel (Sigma, St. Louis, MO) in 40 mM tris(hydroxymethyl)aminomethane acetate and 1 mM EDTA and transferred to nylon membranes (Hybond N+, Amersham, UK). Blots were then hybridized with a 2.1 kb subclone VC85 (American Type Culture Collection 61067), labelled with ^{32}P-alpha-dATP random priming kit (DECAprime, Ambion, Austin, TX) in 10% dextran sulfate, 1M NaCl, 1% sodium dodecyl sulfate and 10 μg/ml sheared salmon sperm DNA at 65°C for 16-24 hours (Truett et al. 1995). Subsequently, the animals were weaned, randomized by sex and litter (Table IV.1), and placed on a standard rodent chow diet (Agway Prolab Rat Mouse

Hamster 3000, Syracuse, NY) until seven weeks of age. At seven weeks of age the animals were placed on their respective diets (Table IV.2).

Housing and care of animals

Lean (*BN/BN*) and lean (*BN/fa*) rats were housed in hanging cages and provided food and water *ad libitum*. A temperature of 21°C and an artificial 12-h light-dark cycle were maintained in the animal room. This study was approved by the Institutional Animal Care and Use Committee of the University of Tennessee.

Diet

At seven weeks of age, 16 (*BN/BN*) and 10 (*BN/fa*) male and 8 (*BN/BN*) and 11 (*BN/fa*) female rats were randomly (within litter) assigned to a 12% or 48% fat diet (Table IV.2). Weekly food intake and body weight were recorded for the duration of the seven week study. Coconut oil replaced corn starch in the high fat diet, and the essential micronutrient content was adjusted to be equivalent on an energy basis. Over the seven week period animals were allowed free access to their assigned diet and water. Energy efficiency ratio was calculated as weight gain divided by energy intake over the seven week feeding period. Perirenal and epididymal fat pads were removed and weighed for subsequent analysis.

Determination of blood pressure and plasma measures

After approximately seven weeks on the diet treatment each animal (14-15 weeks of age) was anesthetized with sodium pentobarbital and a femoral arterial catheter was implanted, exteriorized at the nape of the neck and stored in a tissue culture cap. Approximatily, seventy two hours post-surgery blood pressure was measured directly (Micromed Inc. Lexington, KY) in the conscious unrestrained state. The animals were then anesthetized and exsanguinated by cardiac puncture. Plasma was collected and cholesterol, triglyceride, glucose and insulin concentrations were determined using the standard cholesterol, triglyceride and glucose methods (Sigma, St. Louis, MO) and insulin via radioimmunoassay (INCSTAR, Stillwater, MN) method, respectively.

Determination of GLUT4 Protein

Thoracic aorta, perirenal fat and soleus were removed from all animals, washed in ice-cold phosphate-buffered saline, minced and dissolved in suspension and gel loading buffers (Sambrook et al. 1989). Following ten minutes of boiling, the preparation was sonicated, centrifuged and the supernatant was removed. Proteins (200 μg) were separated by 10% SDS-PAGE Tris-glycine and electroblotted and/or immobilized on PVDF membrane for immunoblot analysis. ^{125}I-Protein A (NEN, Boston, MA) and anti-rabbit polyclonal antibody to GLUT4 protein (a gift from Dr. Samuel Cushman, NIDDK, National Institutes of Health, Bethesda, MD) were used to determine GLUT4 protein concentrations. Blots were exposed to several films for 2-4 days and densitometry was performed to determine relative concentrations of tissue GLUT4 protein.

Statistical Considerations

These studies used a randomized design with two treatments (low and high fat diets) and four levels (male *BN/BN*, male *BN/fa*, female *BN/BN*, and female *BN/fa* rats)(Table IV.1). The analysis of data had to accommodate unbalanced groups as previously described by Truett et al. (1995). Therefore, the data were analyzed under a mixed linear model employing the software PROC MIXED of SAS (Cary, NC). The dependent variables (weight, weight change, fat pads weight, GLUT4 protein concentrations, food intake, energy efficiency ratio, mean arterial pressure, plasma glucose, plasma insulin, glucose/insulin ratio, plasma cholesterol, and plasma triglycerides) were regressed on the independent variables litter (random factor), diet, sex, genotype and interactions (fixed factors). Type III sum of squares for unbalanced data was used to compute the F statistic for analysis of variance of the fixed factors. Significance was confirmed at ($p < 0.05$) and all data are reported as means $\pm$ standard error.

RESULTS

The male and female animals responded differently to the high fat diet (Figure IV.1). For example, the female rats did not show a significant difference in the amount of weight gained between 48% and 12% fat diets. In contrast, the male rats on the 48% fat diet gained significantly ($p < 0.0027$) more weight than the male rats on the 12% fat diet and the male rats gained significantly ($p < 0.00001$) more weight than the female rats. Furthermore, there was a trend ($p < 0.055$) toward increased weight gain (97.6 ± 5.9 g vs. 107.1 ± 5.9 g) in *BN/BN* vs. *BN/fa* animals. In parallel, food intake (kcal/week) was higher in male rats on the 48% fat diet than male rats on the 12% fat diet ($p < 0.046$) and no difference existed in females on the respective diets. Similarly, the *BN/fa* rats consumed significantly ($p < 0.036$) more food than the *BN/BN* rats. Energy efficiency ratio [weight change(g)/food intake(kcal)] exhibited trends similar to weight change and food intake (Figure IV.2). Furthermore, energy efficiency ratio was higher in the 48% fat compared to the 12% fat diet rats ($p < 0.026$) and this diet effect was only evident in the male animals. In addition, the male animals had higher energy efficiency ratios than female animals ($p < 0.00001$). Figure IV.1 depicts the growth curves of all eight groups over a seven week period.

Animals on a 48% fat diet demonstrated higher fat pad weights than animals on a 12% fat diet ($p < 0.00001$) and *BN/fa* animals had heavier fat pads than *BN/BN* animals ($p < 0.0018$). Not surprisingly, males had higher perirenal fat pad weights

than females ($p<0.00001$). Moreover, when fat pad weights were adjusted on an animal weight basis [pads weight(g)/animal weight(g)] these relationships persisted (Figures IV.3,IV.4). There also appeared to be differential distribution of adiposity in the two fat pads of the male animals. When comparing body fat partitioning (perirenal/epididymal fat pad ratio) by diet, the 48% fat diet animals put on more adiposity in the perirenal fat pads. Moreover, the male *BN/fa* animals on the 48% fat diet exhibited a significant ($p<0.03$) diet-genotype interaction, as they had the greatest change in fat pad weights.

Fasting plasma insulin values exhibited a significant increase in all rats on the high fat diet ($p<0.05$). The males had higher fasting plasma insulin than the female rats ($p<0.002$) and there was a significant ($p<0.04$) diet-sex-genotype interaction. Similarly, fasting plasma glucose was higher in the 48% fat diet ($p<0.02$), higher in males ($p<0.04$) and higher in male *BN/BN* vs. male *BN/fa* animals ($p<0.026$). Glucose to insulin ratios were compared as indices of insulin sensitivity. This revealed decreased insulin sensitivity in animals on a 48% fat diet ($p<0.003$) and a decreased in insulin sensitivity in male vs. female animals ($p<0.004$). There was also a significant sex-genotype and diet-sex-genotype interaction ($p<0.0008$) regarding insulin sensitivity, with male *BN/fa* and female *BN/BN* rats exhibiting the most dramatic decline in insulin sensitivity in response to the 48% fat diet. Not surprisingly, total cholesterol was significantly ($p<0.0002$) higher in male animals when compared to female animals. Moreover there was a diet-sex interaction ($p<0.023$) revealing an increase in total cholesterol of male animals on a 48% fat

diet, while females demonstrated no diet effects. Plasma triglycerides were not significantly different among all eight groups. Mean arterial blood pressures were similar in most groups, with the exception of *BN/fa* animals on 48% fat diet, which exhibited the highest mean arterial blood pressures ($p < 0.03$).

Animals on the 48% fat diet had a significantly higher ($p < 0.009$) concentration of soleus GLUT4 protein than animals on the 12% fat diet (Figure IV.5). Comparisons in aorta and perirenal fat GLUT4 protein concentrations revealed no significant relationships, although, there was a trend ($p < 0.06$) toward an increase in aorta GLUT4 protein of *BN/BN* animals on a 48% fat diet vs. *BN/BN* animals on a 12% fat diet. This trend was not seen in the *BN/fa* animals on their respective diets. There was also a trend ($p < 0.06$) in perirenal fat GLUT4 protein concentration, which suggested a higher amount of GLUT4 protein in female than male rats.

TABLE IV.1: Distribution of Brown Norway/Zucker offspring by diet, sex and genotype.

DIET	SEX	COPIES *fa*	n
12% fat diet	female	0	4
		1	6
	male	0	8
		1	5
48% fat diet	female	0	4
		1	5
	male	0	8
		1	5
TOTAL			45

TABLE IV.2: Composition of 12% and 48% fat diets.

Component	12% fat diet[1]	48% fat diet[1]
Cornstarch[2]	35.1 (g/100g)	0.0 (g/100g)
Coconut oil[2]	0.0	19.4
Corn oil	5.0	6.2
Casein	20.0	24.8
Cellulose	5.0	6.2
Sucrose	29.9	37.2
AIN Mineral mix	3.5	4.3
AIN Vitamin mix	1.0	1.2
D-l-methionine	0.3	0.5
Choline bitartrate	0.2	0.3
Energy	3.86 (kcal/g)	4.80 (kcal/g)

[1]12% and 48% of energy derived from fat

[2]coconut oil was substituted for cornstarch in the high fat diet.

TABLE IV.3: Measurements of Brown Norway/Zucker offspring fed 12% or 48% fat diets.

GENDER	FEMALE		MALE	
DIET	12%	48%	12%	48%
N	10	9	13	13
EER	0.030±0.002	0.030±0.002	0.046±0.002[1]	0.053±0.002[1,2]
PFP % BW	0.90±0.14	1.33±0.15[2]	0.91±0.14	1.77±0.13[1,2]
TC (mg/dL)	79.4±4.1	75.5±4.3	86.8±4.0	98.8±4.0[1,2]
TG (mg/dL)	46.4±6.0	48.4±5.8	50.7±5.6	53.7±5.5
Glucose (mg/dL)	143.9±6.1	161.9±6.3[2]	158.8±5.8	168.0±5.5
Insulin (ng/mL)	2.43±1.01	2.43±0.98	4.01±1.11	6.70±0.96[1,2]
MAP (mmHg)[3]	126±4	128±5	126±4	131±4
HR	365±18	355±21	366±18	359±18

[1] significant difference versus females ($p<0.05$)

[2] significant difference versus 12% fat diet ($p<0.05$)

[3] The sample sizes for mean arterial pressure were 8, 6, 10, and 8 respectively.

KEY: N = sample size, EER = energy efficiency ratio (weight change (g)/energy intake (kcal)) over 7 weeks, PFP % BW = perirenal fat pads expressed as percent body weight, TC = total cholesterol, TG = triglyceride, MAP = mean arterial blood pressure, HR = heart rate

TABLE IV.4: Measurements of male Brown Norway/Zucker offspring fed 12% or 48% fat diets.

DIET	12%		48%	
GENOTYPE	*BN/BN*	*BN/fa*	*BN/BN*	*BN/fa*
N	8	8	5	5
EER	0.047±0.002	0.046±0.003	0.053±0.003[1]	0.053±0.003[1]
PFP % BW	0.73±0.16	1.11±0.22	1.41±0.16[1]	2.12±0.19[1,2]
EFP % BW	1.17±0.13	1.34±0.17	1.66±0.13[1]	2.30±0.15[1,2]
TC (mg/dL)	81.8±4.5	91.7±6.3	98.9±4.6[1]	98.8±6.3
TG (mg/dL)	49.0±6.5	52.3±7.7	51.5±6.3	55.8±7.7
Glucose (mg/dL)	149.4±6.7	168.3±9.5	164.3±7.2	171.8±8.5
Insulin (ng/mL)	5.10±1.19	2.93±1.74	5.65±1.19	7.73±1.27[1]
MAP (mmHg)[3]	127±5	125±6	128±5	134±6
HR	375±19	355±25	366±21	352±25

[1]significant difference versus 12% fat diet ($p<0.05$)

[2]significant difference versus BN/BN ($p<0.05$)

[3]The sample sizes for mean arterial pressure were 8, 6, 10, and 8 respectively.

KEY: N = sample size, EER = energy efficiency ratio (weight change (g)/energy intake (kcal)) over 7 weeks, PFP % BW = perirenal fat pads expressed as percent body weight, EFP % BW = epididymal fat pads expressed as percent body weight, TC = total cholesterol, TG = triglyceride, MAP = mean arterial blood pressure, HR = heart rate

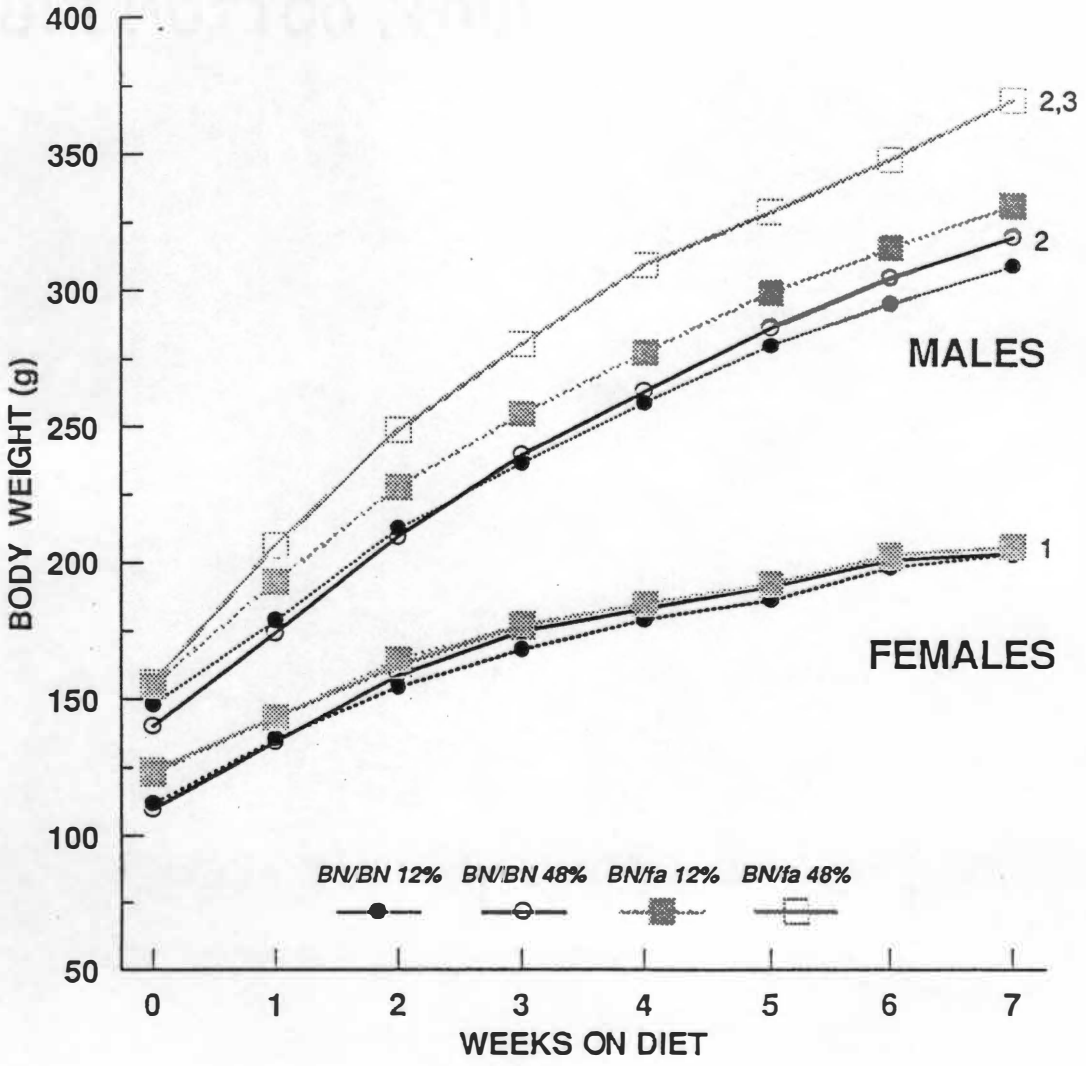

Figure IV.1 Growth curves of all Brown Norway/Zucker offspring by diet and gender (mean). [1] $p<0.05$ versus males, [2] $p<0.05$ versus 12% fat diet, [3] $p<0.05$ versus BN/BN

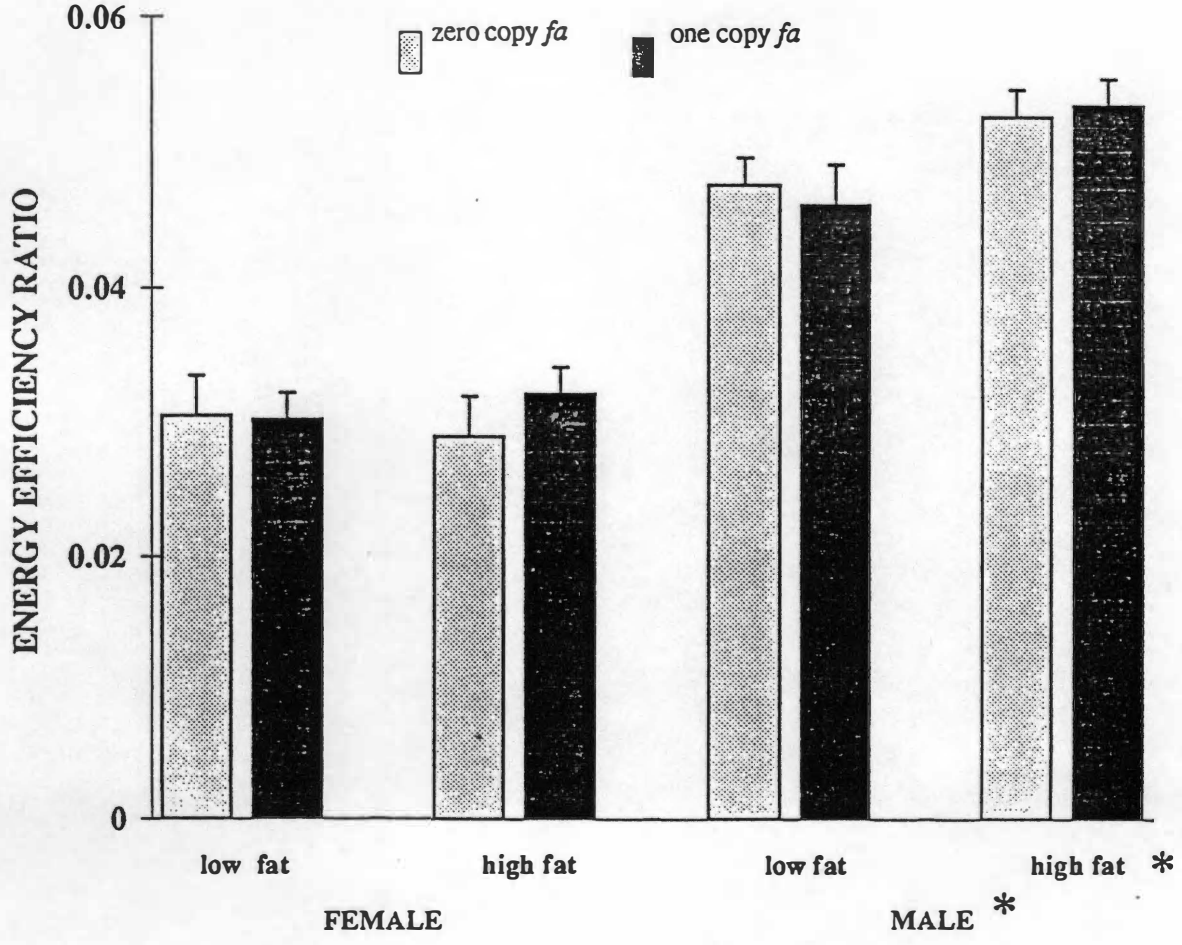

Figure IV.2 Energy efficiency ratios of all Brown Norway/Zucker offspring by gender and diet. (means ± SEM, * indicates significance at $p<0.05$)

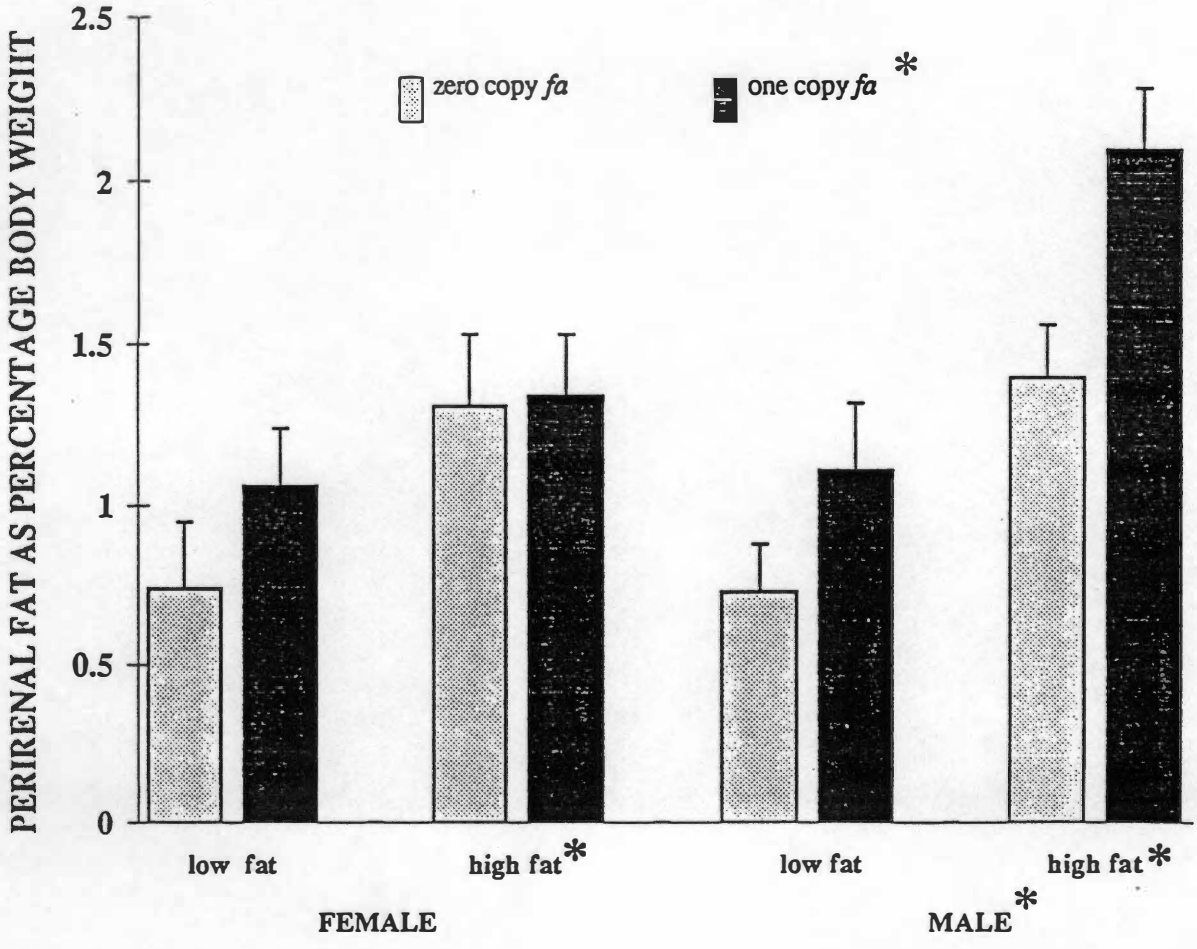

Figure IV.3 Perirenal fat pads as % of body weight by gender and diet (mean ± SEM, * indicates significance at $p<0.05$)

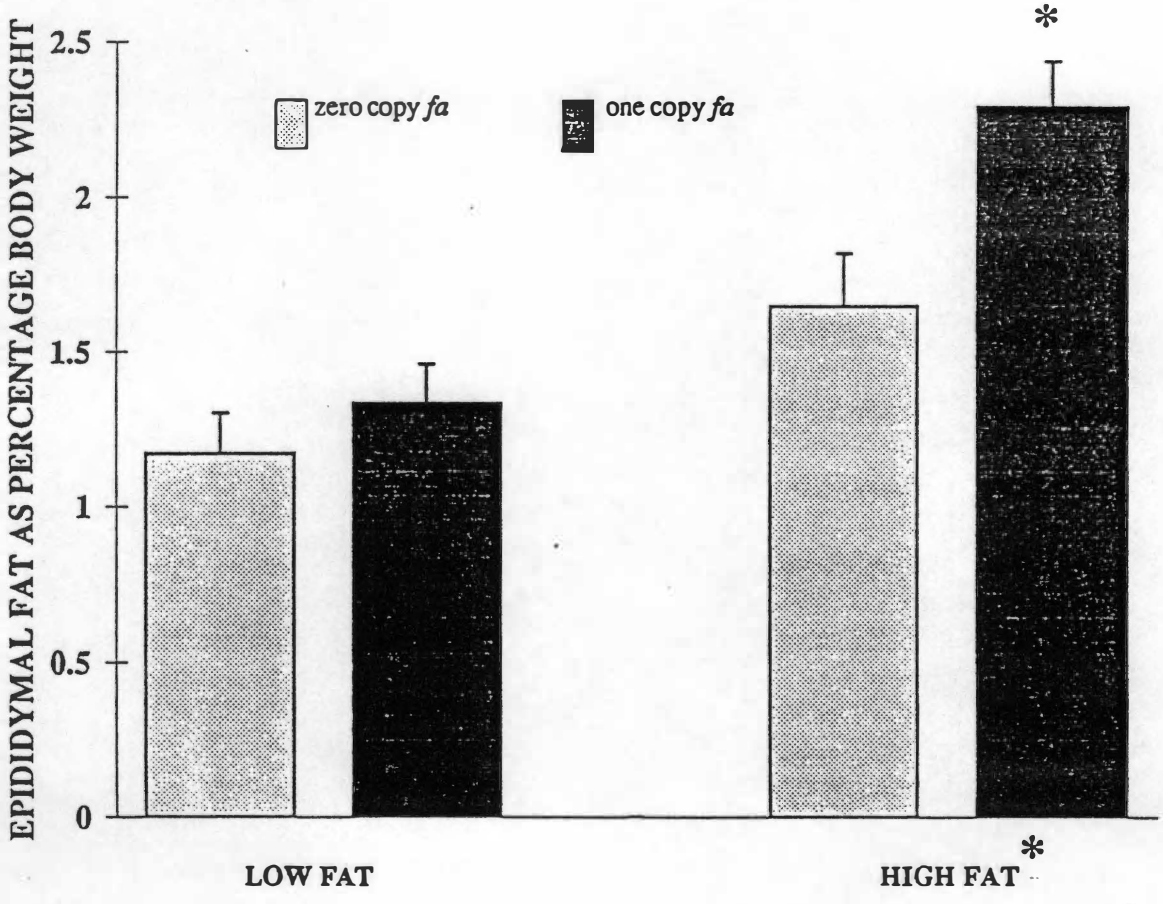

Figure IV.4 Epididymal fat pads as % of body weight by diet (mean ± SEM, * indicates significance at $P<0.05$).

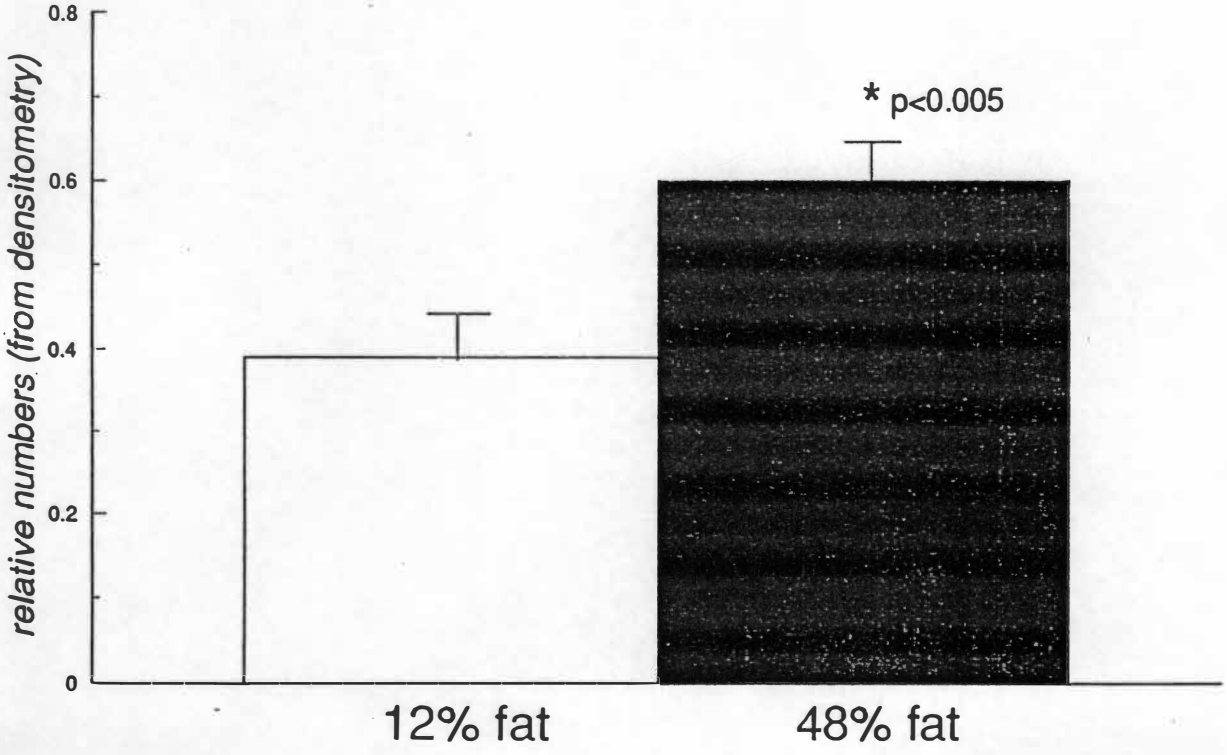

Figure IV.5 Densitometry evaluations of GLUT4 protein concentrations by diet (means ± SEM, * indicates significance at $p<0.05$).

DISCUSSION

We have demonstrated that a high fat diet exacerbates insulin resistance and the aberrations associated with insulin resistance. Moreover, many of the deleterious effects related to high fat feeding are amplified in animals carrying one copy of the *fa* allele and female animals appear to be somewhat resistant to these diet and gene effects. We have also demonstrated that a high fat diet increases the insulin responsive glucose transporter (GLUT4) protein in skeletal muscle.

There were several genotype effects related to the *fa* allele (Table IV.4). For instance, both epididymal and perirenal fat pad weights were significantly higher in *BN/fa* animals when compared to *BN/BN* littermates. *BN/fa* animals consumed significantly more food than *BN/BN* animals and exhibited a trend ($p < 0.055$) toward increased weight gain when compared to *BN/BN* littermates. On the 48% fat diet *BN/fa* animals had higher mean arterial blood pressures than representative *BN/BN* animals. Again, many of the genotype effects were blunted or nonexistent when female *BN/BN* were compared to female *BN/fa* animals.

As expected, animals on the high fat diet exhibited an exacerbation of cardiovascular disease risk factors (Table IV.3). Plasma insulin, plasma glucose, plasma cholesterol, perirenal and epididymal fat pad weights, energy efficiency ratio, weight gain and food intake were all significantly higher in rats on a 48% fat diet than rats on a 12% fat diet. Furthermore, male rats exhibited an increase in cardiovascular disease risk factors relative to female rats. For instance, plasma

insulin, plasma glucose, plasma cholesterol, perirenal fat pad weight, energy efficiency ratio, weight gain and food intake were all significantly higher in male rats vs. female rats.

Additionally, there were several diet-genotype interactions revealing the most profound risk factor aberrations occurring in the *BN/fa* males on the high fat diet. For example, when comparing epididymal fat pads in male animals on 12% vs 48% fat diets, there was a diet-genotype interaction revealing a *fa* effect on adiposity exacerbated by a high fat diet. Similar to human syndrome X, the male animals with a genetic predisposition (one *fa* allele) for insulin resistance appear to exhibit an exaggerated response to a high fat diet with regard to several syndrome X risk factors. Moreover, these data support Reaven's (1988) original hypothesis of syndrome X, stating insulin resistance observed from person to person is genetically determined and insulin's action can be modulated by environment. Furthermore, these data support the speculation of Kaplan (1989) and Bjorntorp (1991) that androgens appear to be involved in the basic etiology of syndrome X. Android obesity, as indicated by waist/hip circumference ratio, is a risk factor for cardiovascular disease. The important component of waist/hip ratio appears to be the mass of visceral fat, relatively independent of total fat mass. Bjorntorp (1992,1991 and 1987) postulated that visceral fat mass is probably increased by multiple endocrine aberrations, with steroid hormones involved in the basic etiology of these aberrations. In this study both epididymal and perirenal fat pad weights were significantly higher in *BN/fa* animals when compared to *BN/BN* littermates, but

BN/fa animals only exhibited a trend toward increased weight gain when compared to *BN/BN* littermates. In addition, there was a diet-genotype interaction revealing a *fa* effect on this visceral adiposity exacerbated by a high fat diet. This suggests that in *BN/fa* male rats on high fat diet visceral fat mass increases in greater proportion then body weight relative to all other groups. Consequently, these data support the notion that syndrome X develops in individuals who inherited a genetic predisposition, which is triggered by the combination of androgens and a high fat diet.

Surprisingly, the soleus of the animals on a high fat diet contained substantially ($p < 0.009$) higher concentrations of GLUT4 protein than soleus from littermates on a low fat diet. In contrast, other researchers (Kahn and Pedersen 1993, Pedersen et al. 1991) have demonstrated that high fat feeding resulted in suppression of GLUT4 protein and mRNA in rat skeletal muscle and adipose tissue. Differences between these investigations and results of the present study may be, in part, due to differences in experimental design. In the previous studies, the high fat groups consumed 80% of their energy as fat and 15% as protein, while the two comparison groups consumed 26% of their energy as protein and 12% and 14% of their energy as fat, respectively. The lack of appropriate dietary controls in these studies may have confounded the results. For instance, the high fat group had lower body weights, plasma glucose levels and plasma insulin levels than control rats. Further, the high fat group exhibited a decrease in expression of the cytoskeletal protein β-actin, reflecting one or several dietary inadequacies leading to growth

failure. It is likely that animals subjected to an artificially high fat-low protein/carbohydrate diet may exhibit ketosis and alterations in satiety, thus resulting in growth failure. In addition, it was not apparent that the high fat diets in these studies were adequately adjusted for micronutrient content, thus creating the potential for key nutrient deficiencies.

Insulin stimulates glucose transport by promoting the translocation and/or activation of GLUT4 transporter proteins. Further, it is believed that following activation of insulin receptor tyrosine kinase, there is a site-specific phosphorylation and activation of phosphoserine phosphatase-1, which dephosphorylates the GLUT4 transporter protein leading to it's activation. Insulin also exerts an effect on the transcription of specific mRNAs (ie. GLUT4 mRNA) (Baulieu and Kelly 1990, Begum et al. 1993, Quon et al. 1994, Saltiel 1990). Consequently, it has been demonstrated that plasma insulin is positively correlated with glucose transport and GLUT4 protein concentrations (Barnard et al. 1990, Kahn et al. 1987). Clearly, in a hyperinsulinemic model of insulin resistance there would be an expected increase in GLUT4 protein.

There is an accumulating body of evidence suggesting there is no significant difference of GLUT4 protein content in normal vs insulin resistant states (Bader et al. 1992, Brozinick et al. 1991 and 1992, Davidson 1993, Eriksson et al. 1991, Friedman et al. 1992 and 1990, Garvey et al. 1992, Handberg et al. 1990, Pedersen et al. 1990). Moreover, there appears to be little or no correlation between insulin-stimulated 2-deoxyglucose uptake and tissue GLUT4 protein content (Brozinick et

al. 1993, Henriksen et al. 1992). In part, this lack of correlation is due to the existence of two distinct pools of GLUT4, one stimulated by insulin and the other stimulated by contraction. Further, the two pools are fully additive (Brozinick et al. 1993) with respect to 2-deoxyglucose transport and together they correlate highly with 2-deoxyglucose uptake (Henriksen et al. 1992). It has also been demonstrated that this impairment in 2-deoxyglucose transport is related to aberrations in GLUT4 protein activation, translocation and compartmentalization, not total tissue GLUT4 protein content (Brozinick et al. 1992 and 1993, Friedman et al. 1990, Henriksen et al. 1992). Consequently, the total tissue concentration of GLUT4 protein may be of limited value in predicting transporter capacity. In fact, activation, translocation and compartmentalization of GLUT4 appears to be more instrumental in the aberrant transport of glucose associated with insulin resistant states (Brozinick et al. 1992 and 1993, Friedman et al. 1990, Henriksen et al. 1992). Theoretically, a decrease in the function of GLUT4 protein may elicit an upregulation of the transporter in an attempt to compensate for the aberrations in GLUT4 activation, translocation and/or compartmentalization.

In summary, we have demonstrated that a high fat diet exacerbates insulin resistance and the aberrations associated with insulin resistance. Furthermore, the effects of the high fat diet are amplified in animals carrying one copy of the *fa* allele and female animals appear to be somewhat resistant to this diet-gene interaction. Additionally, we have demonstrated that a high fat diet increases the insulin responsive glucose transporter (GLUT4) protein in skeletal muscle regardless of

genotype or gender. These data support the concept that syndrome X is genetically determined and modulated by endocrine and environmental influences (ie. androgens and diet). Future research should focus on the effect of a high fat diet on the glucose transport system and examine gene-environment interactions in genetic models of syndrome X.

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PART V.

SUMMARY AND CONCLUSION

The focus of this dissertation research was to: (a) determine whether aortic tissue exhibits classical insulin-stimulated glucose transport and if so, is this action impaired in an insulin resistant model; and (b) determine if there is a gene-diet interaction associated with the *fa* (fatty) allele, potentially modulating GLUT4 protein and classical risk factors for syndrome X. These studies were intended to provide insight into the interaction between genetics and environment and how this impacts insulin sensitivity in an animal model representative of syndrome X.

Data presented demonstrates that aortic tissue contains a substantial concentration of the insulin-stimulated glucose transporter (GLUT4) and exhibits insulin dependent glucose transport. Further, insulin-stimulated aortic glucose transport appeared impaired in obese Zucker rats. These data support our hypothesis that vascular smooth muscle is an insulin-sensitive tissue which appears to be partially impaired in obese Zucker rats. Further, we demonstrated that a high fat diet exacerbates insulin resistance and the aberrations associated with insulin resistance. Moreover, the effects of the high fat diet are amplified in animals carrying one copy of the *fa* allele and female animals seem some what resistant to this diet-gene interaction. Additionally, we have demonstrated that a high fat diet increases the insulin responsive glucose transporter (GLUT4) protein in skeletal muscle regardless of genotype or gender. Consequently, the total tissue concentration of GLUT4 protein may be of limited value in predicting transporter capacity. In fact, activation, translocation and compartmentalization of GLUT4

appears to be more instrumental in the aberrant transport of glucose associated with insulin resistant states. These data support the concept that syndrome X is a multifactorial syndrome that is genetically determined and predominates in males but, has the potential to be modulated by environmental manipulation. Future research should focus on elucidating the effect of a high fat diet on the glucose transport system and examine gene-environment interactions in genetic models of syndrome X.

VITA

William Joseph Banz was born in Mosinee, Wisconsin on September 8, 1963. He attended school (K-12) in the public system of Mosinee, Wisconsin, where he graduated from Mosinee High School in June, 1982. He entered The University of Wisconsin-Stevens Point during August of 1983 where in December, 1988 he received the Bachelor of Science Degree in Dietetics with a Minor in Health Education. He entered the Master's program in Nutritional Sciences at The University of Wisconsin-Stevens Point in June of 1989, officially receiving the Master's degree in August, 1990. In August of 1990, he entered The University of Tennessee to pursue the Doctorate of Philosophy Degree in Nutrition with a Minor in Exercise Physiology and concomitantly entering the Approved Pre-Professional Practice Program to obtain eligibility to sit for the Registered Dietitian exam. The doctoral degree was received May, 1995.