CHANGES IN GLUT4 AND GLUT1 IN FELINE OBESITY

by

CHRISTINA LEIGH BRENNAN

(Under the direction of Margarethe Hoenig)

ABSTRACT

As in man, obesity is a risk factor for many diseases including diabetes mellitus. The cat is an important and unique model for the study of obesity and its progression to diabetes. Recently, tissue specific regulation of glucose metabolism in fat and muscle has been identified as an important factor for insulin sensitivity and it has been hypothesized that glucose uptake into tissues is altered in obesity causing insulin resistance. Seventeen cats were tested in the lean state and again after a 6 month period of ad libitum food intake which led to a significant increase in weight ($p < 0.0001$). The Glut4 expression was significantly decreased in both muscle ($p< 0.01$) and adipose tissue ($p<0.001$), respectively, in the obese cats. Glut1 expression showed no significant change in the obese cats in either muscle or adipose tissue.

INDEX WORDS: Obesity, Diabetes Mellitus, Glut1, Glut4, Muscle, Adipose tissue

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DEDICATION

To my parents, Jim and Velma Brennan, who have always given me so much love and support, allowing me to pursue my goals. I am so fortunate to have you as my parents. You have always been there for me whenever I needed you. I will be eternally grateful for all that you both have given me.

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CHAPTER 1

INTRODUCTION

The increase in obesity in cats and other species, including man, has been phenomenal. Currently, the rate of obesity in humans is almost 50%, while in the cat over 25% are considered overweight. There are several devastating diseases associated with obesity including type 2 diabetes mellitus. Obesity increases the risk of developing diabetes 3-5 fold in cats and is also considered a major risk factor for the diseases in people. The cat is an important and unique model for the study of obesity and its progression to diabetes because the cat shows very similar alterations in glucose metabolism and insulin sensitivity as seen in people. Most importantly, the pre-diabetic and diabetic cat develops amyloid, which has been called the hallmark of type 2 diabetes in people. Recently, tissue specific regulation of glucose metabolism in fat and muscle has been identified as an important factor for insulin sensitivity and it has been hypothesized that glucose uptake into tissues is altered in obesity causing insulin resistance.

The mammalian cell membrane is predominately impermeable to glucose. There is a family of glucose transporters with the protein symbol GLUT, responsible for the transport of glucose across the cell membrane. The transporter GLUT1 is responsible for passive diffusion of glucose during basal states. GLUT1 has been shown to be

predominately located at the plasma membrane and is considered predominately insulin insensitive.

GLUT4 is considered the major glucose transporter. In skeletal muscle and fat, 90% of the glucose transporters expressed are GLUT4. It is considered the insulin mediated transporter. In the presence of insulin, GLUT4 can rapidly augment glucose transport rate into the cell by 10-30 fold. In basal states, unlike GLUT1, GLUT4 is located in intracellular compartments. It is then translocated to the plasma membrane in response to insulin.

Numerous studies have shown that obese rats and mice have depressed levels of GLUT4, while glucose transport via GLUT1 remains the same. The study of the changes in GLUT4 during the progression from the normal state to the obese state may help us to understand why cats, like humans, develop Type 2 diabetes.

The purpose of this research was to:

(1) determine the expression of GLUT4 in muscle and fat tissue from cats in lean and obese states

(2) determine the expression of GLUT1 in muscle and fat tissue from cats in lean and obese states

CHAPTER 2

MATERIALS AND METHODS

Materials

3T3-L1 murine fibroblast cell lines were obtained from American Type Tissue Culture Collection (Manassas, VA). Complete protease inhibitor cocktail tablets were obtained from Roche (Mannheim, Germany). Horseradish peroxidase-labeled donkey anti rabbit IgG were from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polycolonal to GLUT 1 antibodies (ab652) were from Abcam Limited (Cambridge, UK). Rabbit polyclonal GLUT 4 antibodies (GT41-S) were from Alpha Diagnostics (San Antonio, TX). West Dura chemiluminescence substrate was from Pierce (Rockford, IL). Fetal bovine serum was obtained from Gibco (Rockville, MD). PVD membranes were from Millipore (Bedford, MA). All other reagents for western blot, and cell culture were obtained from Sigma (St. Louis, MO), unless otherwise noted.

Animals

Seventeen healthy neutered adult female cats Sinclair Research Center, Columbia, MO, USA) were used in this study. The cats were maintained at the University of Georgia College of Veterinary Medicine Animal Care Facility under standard colony conditions. They were housed in individual cages and were given free access to water.

Animal studies were approved by the University of Georgia Animal Care and Use Committee and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The animals were determined to be healthy based on results of physical examination and clinical laboratory data. All cats were used to daily handling. They were fed a commercially available dry ration (Ocean Fish and Rice; Iams, Dayton OH) twice daily. Each cat was tested in the lean state (L) and again after a 6 month period of ad libitum food intake (OB).

To allow blood sampling, catheters were placed in the jugular and cephalic vein 15-17 hours before any tests were performed. Catheter patency was maintained by administration of 0.5 ml 0.38% sterile citrate flush (citric acid, trisodium salt dihydrate, Sigma Co., MO, USA) every 6 hours. Blood was taken through the jugular catheter and was allowed to clot for serum collection. After centrifugation, the serum was harvested and frozen at -20^{o} C until the assays were performed. Blood was also taken for routine complete blood count and biochemical profile. Intravenous glucose tolerance tests (IVGTT) were performed using 1 g/kg 50% dextrose as described in detail for measurement of serum glucose, insulin, and nonesterified fatty acid (NEFA) concentrations (Hoenig et al. 2002; Hoenig et al. 2003).

Measurement of weight, percent body fat by dual-energy X-ray absorptiometry (DEXA), and body mass index (BMI; expressed in kg/m^2) was performed as described (Hoenig et al. 2003).

Biopsy of muscle and fat tissue

Biopsies were taken after injection of (11 mg/kg intramuscularly) according to standard procedures (Dickinson and LeCouteur 2002). Approximately 100 mg of muscle tissue was excised from the cranial tibialis muscle and approximately 1 g of fat was excised from subcutaneous adipose tissue (flank area). The tissue was snap frozen in liquid nitrogen and stored at - 80C until assayed.

Cell culture of 3T3-L1 adipocytes

As a positive control, 3T3-L1 murine fibroblast cells were used. This cell line has been shown to positively express the GLUT1 protein (Schroer et al. 1986; Calderhead et al. 1990) as well as GLUT4 protein (de Herreros and Birnbaum 1989; Calderhead et al. 1990). Using quantitative immunoblotting with antibodies directed against the carboxyl-terminal peptides of GLUT1 and GLUT4, there are approximately 950,000 and 280,000 copies of GLUT1 and GLUT4 respectively per 3T3-L1 cell (Calderhead et al. 1990).

The cells were differentiated using the protocol as previously described (Haruta, T. et al, 1995) with the exception that cells were kept in an 8% CO2 environment.

Muscle preparation

On average, approximately 50 mg of muscle tissue was used per experiment. Tissue was prepared by a modification of Hocquette et al 1997. Briefly, a buffer containing 0.1% Triton/5M Tris-base/ 62.5mM urea and complete protease inhibitor cocktail according to manufacturer's instructions was used to homogenize the tissue using a glass-Teflon homogonizer. The muscle sample was then centrifuged at 42,000 X *g* for 2 hours to separate cytosolic and particulate fractions. The pellet (particulate) was solubilized

again in Triton/Tris/urea buffer and sonicated on ice (4 x 10 seconds at an amplitude of 60). The supernatant (cytosolic) fraction was collected.

Adipose tissue preparation

On average, approximately 0.5 g of adipose tissue was used per experiment. Adipose tissue was pulverized in frozen condition with a mortar and pestel. Tissue was then sonicated on ice as described above in a buffer containing Triton 10% and 500 mM Tris-HCL and complete protease inhibitor cocktail according to manufacturer's instructions. The protein was allowed to solubilize on ice for 1 hour. Next, the adipose tissue was centrifuged for 20 minutes at 12000 x g at 4˚C. The supernatant was collected and precipitate buffer (1 g trichloroacetic acid (TCA), 0.3085 g dithiothreitol (DTT), in 100 ml acetone) was added. The protein was allowed to precipitate overnight at -20˚C. The precipitate was sedimented the following day by centrifugation at 7500 x g for 15 minutes. The supernatant was discarded. One ml of cold acetone was added to the pellet and centrifuged for 3 minutes at 7500 x g. This step was repeated 2-3 times to remove all TCA. The acetone was then removed and the pellet was allowed to air dry for 5 minutes. The pellet was sonicated in a buffer containing triton 10%, 5 M urea, and 62.5 mM tris-base and centrifuged for 42,000 X *g* for 2 hours to separate cytosolic and particulate fractions. The supernatant (cytosolic) fraction was collected. The pellet (particulate) was sonicated again as described above.

Western blotting

Blotting was essentially done as described before (Knutson KL and Hoenig M 1994). Protein samples of 60-100 ug (fat) and 50-80 ug (muscle) were applied to a 10% sodium dodecyl sulfate-polyacrylamide gel. The amount of protein in L was always

identical to that in Ob. Four ug of 3T3-L1 served as positive control for GLUT4 and 1 ug for GLUT1. The protein was transferred onto PVDF membranes. Overnight incubation occurred with either a 1:1000 dilution of a polyclonal GLUT4 antiserum or a 1:750 dilution of a polyclonal GLUT1 antiserum. Following 4 washings, membranes were incubated at room temperature with a 1: 8000 dilution (for GLUT1) of a horseradish peroxidase-labeled donkey anti rabbit IgG or a 1: 10,000 dilution (for GLUT4) in phosphate buffered saline (PBS), pH 7.2, with 1% bovine serum albumin (BSA) and 0.05% Tween-20. Membranes were again washed 4 times with PBS pH 8.1. West Dura Chemiluminescence substrate was then added to the membranes and bands were detected using the Bio Rad Fluor-S (Hercules, CA) multiimager system.

Quantification of bands

Bands were quantified using Bio Rad's Quantity One program on the Fluor-S Max Multiimager system BioRad, City, State). Background subtractions were taken from each reading.

Protein analysis

Protein content of the fractions was determined by the method of Bradford (Bradford MM 1976) using BSA as standard.

Statistical analysis

The data were analyzed using Data Desk software (Ithaca, NY) and Prism software (Graph Pad Software, Inc., San Diego, Ca) for Macintosh computers. The data are expressed as means ± SD, unless stated otherwise. The significance of differences of means between groups was evaluated by (Student's t-test for paired samples). A p value < 0.05 was considered statistically significant.

CHAPTER 3

LITERATURE REVIEW

A. OBESITY. Obesity is defined as the excessive storage of energy as fat. Currently, over half of the population in the United States is inflicted with obesity. It has become the most common nutritional disease in the U.S, with 54.9% of the population considered obese (Reda et al. 2002). This nutritional disease has also become a problem in many of our domestic animals, including cats. Currently, approximately 25% of domestic cats are considered overweight (Scarlett et al. 1994). Obesity in cats and in people leads to an increased risk of other diseases. In obese cats, diabetes mellitus is seen at a 3-5 fold increased incidence, whereas in obese people, type 2 diabetes mellitus as well as hypertension, hypertriglyceridemia, hyperinsulinemia, hyperglycemia, artherosclerosis, and premature death are frequently seen .

The body mass index (BMI) and dual-energy X-ray absorptiometry (DEXA) are two methods to measure obesity in humans and animals. The BMI is calculated using the formula: weight (kg)/ height (m^2) (Taylor et al. 1996). The DEXA scan is a newer method to determine not only fat mass of an individual, but also bone mineral mass and soft lean tissue (Salamone et al. 2000). This method uses minimal radiation with a fan-beam x-ray to estimate % body fat (Salamone et al. 2000).In animals, body condition scores guided by model silhouettes are often used to determine when an animal is obese (Donoghue and Scarlett 1998). Obesity is characterized by both a change in insulin secretion and in insulin action

B. INSULIN SECRETION. Insulin is one of the most important hormones involved in metabolism. It is secreted by the beta cells of the pancreas. Secretion is stimulated by nutrients, neurotransmitters and other hormones, among others substances. However, glucose is considered the major regulator of insulin release. The stimulation of insulin secretion by glucose occurs through two pathways, a triggering and an amplification pathway (Henquin 2000). The pancreatic β-cell is considered electrically excitable (Gilon et al. 1992). The triggering pathway involves metabolism of glucose by oxidative glycolysis, an increase in ATP/ADP ratios, membrane depolarization via closure of ATP-sensitive K+ channels, opening of voltage-gated Ca^{2+} channels resulting in Ca^{2+} influx, a rise in cytoplasmic free Ca^{2+} concentration and activation of the exocytotic mechanisms (Henquin et al. 2002; Gilon et al. 2002). The amplification pathway's mechanisms are not yet completely defined. This pathway also depends on glucose metabolism, but does not involve further increase in Ca^{2+} and serves to amplify the effect of Ca^{2+} on exocytosis of the granules (Henquin et al. 2002; Gilon et al. 2002). There are several hallmark responses of the β-cell in response to increased intracellular glucose. They include: rapidity of insulin release, a high sensitivity to the stimulus, a large amplitude range of the response, and an oscillatory nature of the insulin secretion (Nesher and Cerasi 2002).

A common measurement of the kinetic characteristics of glucose-induced insulin secretion is the hyperglycemic clamp. This clamp uses primed-continuous infusion of glucose with blood samples taken at intervals to measure insulin concentrations (Nesher and Cerasi 2002). In the 1960's it was first reported that insulin secretion showed a biphasic release during glucose-induced secretion. The first phase of insulin release

normally accelerates dramatically before slowing down and lasts approximately minutes (Cerasi et al. 1995; Henquin et al. 2002). The second phase is delayed from the first and is longer in duration and occurs at a slower rate than the first (Cerasi et al. 1995; Henquin et al. 2002). The first phase accounts for only 50-100 of more than 10,000 insulin containing granules in a pancreatic β-cell (Henquin et al. 2002). The reasons for the biphasic insulin secretion are still not completely clear. The oldest hypothesis for this phenomenon is that the β-cell contains 2 distinct pools (relating to geographically or functionally distinct insulin granules) of granules. One of these pools being a small, labile pool which is available for immediate release, and a larger pool that feeds slowly into the smaller pool (Grodsky 1972). The delay between the two phases of insulin release is the consequence of the exhaustion of the smaller insulin pool early during glucose stimulation. After a time, the pool is refilled by transfer of granules from the larger pool, which then allows for the second phase of insulin release (Grodsky 1972). There is much debate as to whether this simple model is adequate in fully explaining the phasicity of insulin release and different hypotheses are being proposed (Henquin et al. 2002; Nesher and Cerasi 2002; Henquin et al. 2002). There is some agreement that the first phase involves a rapid depolarization-mediated rise in cytosolic Ca^{2+}, while the second phase is regulated by other mechanisms, mostly metabolic factors and activity of protein kinase C (PKC) and protein kinase A (PKA) families (Henquin et al. 2002).

The rapid rise in insulin during the first phase has a great impact on the liver, which is normally very sensitive to insulin. The liver is able to take up glucose from the bloodstream and either stores it as glycogen, or discharges it into the bloodstream (Ganong 2001). The liver's ability to detect the rise in insulin causes inhibition of

hepatic glucose output thereby avoiding a state of hyperglycemia (Henquin et al. 2002). Later work found that insulin was secreted in an oscillatory manner like many other hormones.(Henquin and Boitard et al. 2002; Gilon et al. 2002; Sha et al. 2001 Gilon et al. 1992). Evidence points towards cytosolic Ca^{2+} oscillations being a cause of the insulin oscillations (Henquin and Boitard et al. 2002; Gilon et al. 2002; Gilon et al. 1992). However, recent data suggest that Ca^{2+} is not acting alone to cause the oscillations. Current studies indicate that the metabolism of glucose, via changes in the ATP/ADP ratio, determines the level of Ca^{2+} maintained in the cell and drives exocytosis of insulin (Deeney et al. 2001). The reason for these oscillations is not yet clear. Possibilities include that they ensure the release of insulin in a time course that will promote optimal response of target tissues (Henquin and Boitard et al. 2002) and that the oscillations are an important factor in preventing insulin receptor downregulation (Gilon et al. 2002;Goodner et al. 1988).

B1. The change of insulin secretion in obesity. During the IVGTT a bolus of glucose is directly infused into the bloodstream. Blood samples are then taken over a time period. In healthy people and animals the glucose concentrations during the test should not exceed certain values which are determined from results of a large population and insulin secretion should follow the biphasic pattern described above. In obese individuals, first phase of secretion is significantly lower while the second phase of insulin secretion is much higher compared to lean controls (Cerasi and Luft 1972). These same changes are seen in the obese cat (Hoenig 2002). In human subjects during the hyperglycemic clamp, the insulin rate is significantly less in obese compared to lean individuals (Polonsky 1988). Other changes seen in obesity are increased basal insulin

secretion rates which are maintained in the fasting state (Letiexhe et al.; Polonsky et al. 1988). Furthermore, insulin clearance is markedly reduced during the clamp and IVGTT in obese subjects (Letiexhe et al. 1995; Polonsky et al. 1988).

C. INSULIN ACTION

C1. Normal insulin signaling: Insulin-stimulated translocation of glucose transporters is a distinct cascade of events. The insulin receptor spans the lipid bilayer of the plasma membrane. The receptor consists of two alpha subunits which are located extracellularly and are connected to two beta subunits which traverse the membrane. Once insulin binds to its receptor, the alpha subunits come in contact which in turn causes the beta subunits to come together. The tyrosine kinase domains become active and cause auto-phosphorylation of the β subunits which then attracts the insulin receptor substrate protein (IRS). The IRS approaches the intracellular domain of the insulin receptor (an SH2 domain), where tyrosine residues on the IRS are phosphorylated.

Phosphoinositide 3-kinase (PI-3 kinase) then approaches the phosphorylated IRS. Two phospho-tyrosine residues on the IRS act as docking sites for PI-3 kinase. (Matthaei et al. 2000; Czech and Corvera 1999; Laybutt et al. 1999; Dresner et al. 1998). Three phosphatidylinositol 4,5-diphosphate (PIP2) molecules then attach to the PI-3 kinase removing a phosphate so that 3 molecules of phosphatidylinositol 3,4,5-triphosphate (PIP3) are formed. These 3 molecules then recruit 3 kinase molecules, most likely: atypical protein kinase C (aPKC), phosphoinositide-dependent kinase (PDK), and protein kinase B (PKB) (Matthaei et al. 2000). The activated aPKC and PKB move toward a vesicle containing glucose transporter moleclules The exact events that cause these two kinases to translocate the vesicle to the plasma membrane are not yet clear.

C2. Normal glucose uptake: One of the major effects of insulin is in the cellular uptake of glucose. In muscle, fat and some other tissues, insulin facilitates glucose entry via an increase of glucose transporters to the cell surface. There are a number of tissues in which insulin is not required for the uptake of glucose, such as the brain and red blood cells. Glucose is able to enter the cells through either facilitated diffusion or by secondary active transport with Na+ in the kidneys and intestine (Ganong 2001).

A family of transporters exists which have the function of transporting glucose across the membrane. This family is characterized by the gene symbol SLC 2A and the protein symbol GLUT (Joost et al. 2002). Four members of this gene family have been found to function as authentic glucose transporters: GLUT1, GLUT2, GLUT3 and GLUT4 (Pessin et al. 1999; Ganong 2001). However, other members of this family are being discovered (Mann et al. 2003). The members of the GLUT family have several common characteristics. They all have a 12 membrane spanning α-helices domain that is highly hydrophobic and which lacks catalytic activity except when present in membrane bilayers (Stephens and Pilch 1995; Zuniga et al. 2001). The members are similar in their primary sequences and are all considered facilitative transporters working in the direction of the glucose gradient (Joost et al. 2002, Stephens and Pilch 1995). This review will discuss the importance of 2 of the glucose transporters: GLUT 1 and GLUT 4.

C3a. Insulin dependent glucose transporter-GLUT4. GLUT4 is the major insulin sensitive transporter found in insulin sensitive muscle and fat tissues. In these tissues, 90% of the glucose transporters expressed are GLUT4. (Santalucia et al. 1999). GLUT4 is considered the major glucose transporter in the mouse, rat, and human and is not only insulin-sensitive but also is stimulated by exercise (see below) (James and Piper

1993). This transporter is able to rapidly augment glucose transport rate in response to insulin by 10-40 fold (Hashiramoto and James 2000). It has a high Km value of 5 mM (Ganong 2001). The half life of the GLUT4 protein has been found to be 50 hours in 3T3-L1 cells (Sargeant and Paquet 1993), and similarly 48 hours in rat adipose tissue (Sivitz et al. 1992). Muscle represents the major disposal site for insulin-stimulated glucose metabolism during the post prandial phase (Ploug et al. 1998).

In basal states, GLUT4 is not located at the plasma membrane, but rather is found in intracellular compartments (Stephens and Pilch 1995; Pessin et al. 1999). The major intracellular storage sites of GLUT4 include the trans-Golgi network (TGN) region and tubulovesicular elements in the cytoplasm just beneath the cell surface (Slot et al. 1997; Hashiramoto and James 2000). Unlike other insulin-insensitive glucose transporters, GLUT4 contains an internal N-terminal sequence, which increases the ability of GLUT4 to be retrieved from the plasma membrane, as well as C-terminal sequence, which limits GLUT4 to the intracellular compartments if insulin is absent (Dauterive 1996). The docking of the GLUT4 vesicle with the plasma membrane occurs through interactions of soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins. SNAREs are membrane proteins shown to be associated with intracellular membrane fusion events. V-SNARE proteins are vesicle membrane SNAREs, while t-SNAREs are target membrane proteins. Interactions between the plasma membrane t-SNARE proteins syntaxin 4 and SNAP 23 with the GLUT4 vesicle's v-SNARE protein VAMP 2 appear to be required for GLUT4 vesicle docking (Pessin et al. 1999; Foster and Klip 2000; Olson et al. 1997; Yang et al. 2001).

Following the insulin-stimulated translocation of GLUT4 to the plasma membrane,

the GLUT4 protein accumulates in clathrin-coated invaginations of the plasma membrane (Pessin et al. 1999). The exact mechanisms for this are not definite. Dynamin, a GTP binding protein involved in coated endocytic vesicles, is sequestered to these structures and is required for the formation of the intracellular GLUT4 clathrin-coated vesicles. These vesicles have the ability to rapidly lose their clathrin coat and are then able to either undergo another cycle of translocation and/or to repopulate the intracellular GLUT4 storage sites (Pessin et al. 1999).

In addition to insulin, exercise has also been shown to translocate GLUT4 to the plasma membrane (Hirshman et al. 1988; Douen et al. 1989; King et al. 1988) from different pools than that of insulin (Douen et al. 1989; Fushiki et al. 1989; Coderre et al. 1995). It has been documented that a single bout of exercise can increase whole body glucose disposal and increase the sensitivity of skeletal muscle glucose uptake to insulin. Furthermore, these effects can last for a number of hours after exercise is finished (Ivy and Holloszy 1981; Richter et al. 1982). This increase in insulin sensitivity for glucose uptake is a local phenomenon which has been shown to be limited to the exercised muscle.

The exact mechanisms for GLUT4 translocation via exercise are not yet clear. There are several pieces of evidence that the increase in intracellular calcium during muscle contraction is a crucial step of exercise-stimulated glucose transport (King et al 1988; Cleland 1989). This rise in cytosolic calcium may initiate the activation of signaling molecules, such as protein kinase C (PKC), which might lead to an immediate and prolonged effect of exercise on glucose transport. There has also been evidence of an

autocrine or paracrine contribution, possibly nitric oxide or adenosine, to this translocation (Goodyear and Kahn 1998).

C3b. Insulin independent glucose transporter-GLUT1 The glucose transporter GLUT1is found in most tissues throughout the body including the brain, red blood cells, blood-brain barrier and numerous other tissues. It accounts for a small amount of total glucose carriers in skeletal muscle and adipose tissue. GLUT1 is localized mainly in the plasma membrane of tissue (Ramos et al. 2001). This glucose transporter is considered insulin insensitive and acts at basal states to transport glucose across the cell membrane (Santalucia et al. 1999; Ganong 2001). GLUT1 has a lower affinity for glucose transport (Km 1-2 mM) than GLUT4 (Ganong et al. 2001). There have been contradictory reports of the half-life for the GLUT1 protein in the 3T3-L1 cell line. Haspel et al. (1985) found the half life to be 90 minutes. However other studies have found a half-life of 19 hours (Sargeant and Paquet 1993) and 14 hours (McMahon RJ and Frost SC 1995).

During fetal life, GLUT1 is expressed at much higher rates than GLUT4 which is the major glucose transporter. During the perinatal phase, a continuous increase of GLUT4 mRNA and protein takes place (Ramos et al. 2001). These high GLUT1 levels are important because during fetal stages insulin receptors are low. Therefore, GLUT1 is essential for maintaining glucose regulation because it acts in the absence of insulin. Also, during the late fetal stage, in the fetal heart and other muscles, glucose consumption is very high. Because glucose transport is the rate limiting step for glycolysis (Ren et al. 1993), the high levels of GLUT1 would be crucial for the fetus to overcome any hypoxic events that could possibly occur during birth (Santalucia et al. 1999). Thyroid hormones

have been shown to play an essential role in regulating these changes in GLUT1 and GLUT4 content after birth (Castello et al. 1994).

Studies have found 2 channels in GLUT1, one of which travels the transporter completely and is lined by solvent-accessible residues (Zuniga et al. 2001). It is thought that it is through this channel that glucose passes. GLUT1 exhibits a unique kinetic property of "accelerated exchange" in which Vmax (the frequency of the interconversion of the outward and inward facing forms) for transport of glucose into a cell is much higher when measured under equilibrium exchange compared to conditions where little or no intracellular sugar is present (Dauterive et al. 1996; Whitesell et al. 1989). The stimulation of glucose transport via GLUT1 during high intracellular concentration of glucose occurs through an increase in rate constant for conversion of this transporter from an inward to an outward facing binding conformation when the inward facing site becomes bound by sugar (Dauterive et al. 1996; Walmsley 1988; Bell et al. 1993). Evidence for a structural separation between these two binding sites has been presented (Bell et al. 1993).

C4. Changes in insulin action in obesity. Insulin resistance is defined as the impaired ability of insulin to suppress glucose output from the liver and to promote peripheral glucose disposal (McGarry 2002; Castillo et al. 1995; DeFronzo and Ferrannini 1991; Bonadonna et al. 1991). The presence of insulin resistance is observed years before the onset of hyperglycemia and the diagnosis of type 2 diabetes and is considered a common feature of human obesity (Groop et al. 1991; Paquot et al. 2002; Solini et al. 1997) The gold standard of determining insulin resistance in people and

animals is the hyperinsulinemic-euglycemic clamp (see above) (Matthaie et al. 2000; Castillo et al. 1995**;** Scheen et al. 1995).

In obese human subjects, there is an impairment of total body glucose uptake and suppression of hepatic glucose production (Bonadonna et al. 1990; Paquot et al. 2002; Groop et al. 1991). Furthermore, the insulin sensitivity index which measures the effect of plasma insulin to enhance glucose clearance, is markedly reduced in obese subjects (Letiexhe et al. 1995). Obese subjects in clamped conditions show a significant increase of total glucose output, net endogenous glucose production, and glucose cycling and a decrease in glucose rate of disappearance, indicating insulin resistance (Paquot et al. 2002).

Studies in the 1970's and 1980's *in vivo* to assess glucose disposal, showed that insulin resistance is caused by impaired insulin action in insulin-sensitive tissue, such as fat and muscle (Cefalu, WT 2001).

C4a. Cellular changes in insulin resistance- Lipid and glucose metabolism in fat and muscle. The synthesis, storage and uptake of adipose tissue triglycerides (TG) and mobilization of this energy source as free fatty acids (FFA) are processes that are highly regulated (Lewis et al. 2002). There is much evidence that the breakdown in lipid dynamics, seen as disordered fat storage and mobilization, are contributors to the pathogenesis of insulin resistance (Lewis et al. 2002; McGarry 2002; Reaven 1995; Schalch and Kipnis 1965; Shulman 2000; Kelley et al. 1999). In an obese individual, glucose accounts for ~ 75% of the energy production, whereas lipids account for ~25% which is a greater amount than that of lean individuals. (Solini et al. 1997). Lipolysis results in the release of FFA and glycerol from stored TG, while uptake of FFA occurs

mainly through re-esterification in adipose tissue and liver (Lewis et al. 2002). The concentration of free FFA in the plasma is due to a balance between these events.

Plasma FFA levels are increased during the basal state as well as during the insulin clamp in obese subjects and correlate with increased rate of FFA turnover (Groop et al. 1992). This leads to the conclusion that in human obesity, the insulin resistant state involves not only glucose but also lipid metabolism (Solini et al. 1997). The elevated fasting plasma FFA concentrations are probably secondary to an increase in the supply of FFA from lipolysis. Hormone sensitive lipase (HSL) is the enzyme that is the principal regulator of lipolysis from adipocyte (Lewis et al. 2002). Under normal states, insulin inhibits HSL, thereby reducing intracellular lipolysis of TG and causing adipocyte TG storage (Lewis et al. 2002). Studies have found resistance to insulin's suppressive effect on HSL appear to be present postprandially in insulin resistant states leading to increased lipolysis in adipocyte tissue and an increase in FFA (Coppack et al. 1992). The reasons for this decrease in HSL sensitivity to insulin are not yet clear. However, it is speculated that it may be largely due to a mass effect of overall expansion of body fat depots (Lewis et al. 2002).

The enzyme lipoprotein lipase (LPL) is stimulated by insulin to increase the uptake of fatty acids derived from circulating lipoproteins in adipose tissue (Kahn and Flier 2000; Lewis et al. 2002). In normal individuals, LPL is stimulated by insulin to decrease its effect in muscle and to stimulate its activity in adipose tissue. This results in partitioning lipoprotein derived fatty acids toward adipose and away from muscle (Farese et al. 1991). However, in obese individuals, LPL activity in adipose tissue is delayed and LPL activity in muscle is increased instead of decreased by hyperinsulinemia (Yost et al.

1995; Sadur et al. 1984). This leads to a further decrease of esterification of FFA in adipose tissue. This is amplified by the fact that obesity also causes a defect in insulin-mediated glucose uptake. This leads to decreased glycerol-3-phosphate concentrations which are needed for esterification of FFA (Lewis et al. 2002).

The outcome of increased FFA lipolysis and diminished FFA esterification in adipose tissue during insulin resistant obesity tends to be a diversion of FFAs toward nonadipose tissue such as muscle, liver, heart and in some species pancreatic β-cells (Lewis et al. 2002; Groop et al. 1992). Furthermore, there appears to be a reciprocal channeling of energy between fat and muscle when one or the other becomes insulin resistant (Lewis et al. 2002). Evidence for this is the fact that down regulation of GLUT4 and glucose transport in adipose tissue causes insulin resistance in muscle. The converse of this has also been shown to occur (Kim et al 2000; Abel et al. 2001). This phenomenon may occur through the diversion of FFAs and other energy fuels from adipose to nonadipose tissues**.**

Studies suggest that impaired fatty acid oxidation in muscle is the primary defect that causes intramyocellular triglyceride accumulation in this tissue thereby leading to muscle insulin resistance in obesity (Bell et al. 2000; McGarry 2001). It has been shown in both rodents and humans that the TG content of muscle bears a negative correlation to whole body insulin sensitivity (Kelley et al.1999). The cellular mechanisms that cause the increase in skeletal muscle TG deposition are uncertain. The changes could possibly be caused to defects of fatty acid oxidation at the carnitine palmitoyl-transferase-1 (CPT-1) and post-CPT-1 levels (Kim et al. 2000). Impaired muscle FFA oxidation could also be a result of chronic exposure to FFA (Lewis et al. 2002). Another possibility for the

defect in muscle oxidation is through defects in binding and transport proteins. Studies have shown that muscle fatty acid binding and transport proteins may be altered in obesity (Blaak et al. 2000). Experimental evidence suggests that excessive delivery of FFA to muscle via the circulation might be a source of muscle TG accumulation (Oakes et al. 1997; Schmitz-Peiffer et al. 1997; Boden et al. 2001).

Numerous studies suggest that there is a link between the effects of FFAs on insulin resistance with an increase in long chain acyl-CoA (LCA-CoA) (Dobbins 2001; Ye 2001; Ellis 2000). There is a negative correlation between whole body insulin sensitivity during euglycemic clamps and content of LCACoA in muscle (Ellis et al 2000). Also, a decrease in insulin mediated glucose uptake into muscle is accompanied by a marked increase in plasma TG and muscle LCACoA levels (Ye et al. 2001). These findings suggest that LCACoA might be the cause of the interference with glucose transport.

LCA-CoAs accumulate in the cytosol when increased FFA influx is associated with malonly-CoA inhibition of carnitine palmitoyltransferase I (CPT-1) (the enzyme that transports fatty acid into the mitochondria for oxidation) (Lewis et al. 2002). During an increase in glucose, followed by a rise in insulin and citrate, acetyl CoA carboxylase (ACC) activity occurs (Wititsuwannakul and Kim 1977). ACC causes synthesis of malonyl-CoA. The increase in malonyl-CoA is paralleled by an increase in cytosolic LCA-CoA (Wititsuwannakul and Kim 1977).

There has been evidence that glucose may signal via altering lipid metabolism (Antinozzi et al. 1998; Corkey et al. 1989; Prentki et al. 1992). In studies by Antinozzi et al (1998), glucose administration to insulinoma cells resulted in an increase in malonyl-CoA levels which preceded the rise in insulin. The increase in malonyl CoA, which

inhibited CPT-1, resulted in inhibition of fatty acid oxidation, increased *de novo* lipid synthesis, and an increase in DAG content leading to an increase in cytosolic LCACoA esters This led to the hypothesis that increases in cytosolic LCACoA acts as signal transduction intermediate in glucose-stimulated insulin secretion (Prentki et al. 1992; Corkey et al. 1989).

Studies have been able to pinpoint the site of defect caused by increased LCACoA to the glucose transport step (Dresner et al. 1999; Cline et al. 1999; Kelley et al. 1996). It is the insulin-mediated translocation of the GLUT4 vesicles in insulin resistant states such as obesity that is believed to be affected by the rise in LCACoA. The proposed mechanism for this defect is that the LCA-CoAs are esterified to diacylgylcerol (DAG) in muscle cells and possibly other insulin sensitive tissue. This pool of DAG causes increased PKC activity (Lewis et al. 2002). PKC is inhibitory towards insulin action, due to serine-threonine phosphorylation of the insulin receptor substrate. The phosphorylation of this serine residue reduces the ability of the insulin receptor substrate to activate the PI-3 kinase. Glucose transport activity and any other events downstream of insulin receptor signaling are reduced (Shulman 2000; Laybutt et al. 1999; Dresner et al. 1999; McGarry 2002). It has been shown that this defect in muscle glucose transport/phosphorylation is an early event in the development of type 2 DM because it is also present in normoglycemic first-degree relatives of such individuals who also exhibit elevated intramyocellular lipid values (McGarry 2002, Rothman et al. 1995, Jacob et al. 1999).

D. THE OBESE CAT AS A MODEL FOR TYPE 2 DIABETES. One of the major diseases which has high association with obesity is type 2 diabetes mellitus (DM).

Today, about 5% of the human population is considered to be type 2 diabetic (McGarry 2002). Approximately 0.6% of felines have been diagnosed as type 2 diabetic.

There are several pathophysiologic features of type 2 diabetes including insulin resistance, pancreatic β cell dysfunction and an increase in endogenous glucose production seen in humans (Matthaei et al. 2000), as well as cats (Hoenig et al. 2002). The cat has several features that make it an ideal model for type 2 diabetes in man. The obese cat can spontaneously develop a form of type 2 diabetes very similar to humans. In both normal and diabetic humans and cats, obesity is associated with impaired glucose tolerance and reversible insulin resistance (Hoenig et al. 2002). The feline model shows very similar changes in insulin secretion during progression from a lean to a diabetic state as seen in humans (Cerasi et al. 1995), cats also have a decrease or complete absence of the first phase of insulin secretion. Furthermore, the second phase may be both delayed and exaggerated (Hoenig et al. 2002). Eventually, the secretion becomes erratic (Hoenig et al., 2000). The most common and consistent morphological features of the pancreatic islets of prediabetic and diabetic humans and cats include the formation of amyloid deposits which originate from islet amyloid polypeptide (IAPP) (Hoenig et al. 2000). It has been shown that insulin resistance not only increases the secretion of insulin but also that of IAPP. This may lead to the depletion of factors that are necessary for normal IAPP processing and cause amyloid deposition and beta cell death. (Hoenig et al. 2000). However, it is unknown at this time if the insulin resistance of obesity leads to increased amyloid deposits and a progression of beta cell failure.

CHAPTER 4

RESULTS

The weight, BMI, girth and % fat of the 17 cats in the lean state (L) and after a 6 months period of ad libitum food intake (OB) are shown in Table 1.

Obese cats showed a significantly higher area under the curve (AUC) for glucose mol/L) (1.78 ± 0.46L; 1.98 ± 0.41 OB; p = 0.013) and AUC for insulin (nmol/L) (34.8 ± 12.9 L; 42.0 ± 11.2OB; p=0.018). Obese cats also had significantly higher baseline insulin concentrations (pmol/L) (91.4 ± 53.2 L; 139.6 ± 48.1 OB; p=0.003 than lean cats, as well as significantly higher insulin at 120 minutes concentrations (pmol/L) (151.87 ± 87.4L; 305.3 ± 167.2 OB; p=0.001). There was also a significant difference in % disappearance/min (k value) during the IVGTT (1.73 ±0 .60 L; 1.41 ± .59 OB; p = 0.017).However, no significant change was seen in baseline glucose concentrations or in glucose at 120 minutes. They ranged from 85.1 ± 11.97 to 88.4 ± 40.9 mg/dL and 115.98 ± 66.9 to 124.3 ±54.0 mg/dL respectively (See figure 1). There was also no significant change in NEFA baseline, although there was a trend in the obese cats for higher concentrations (0.43 ± 0.18 mEq/L in lean cats and 0.64 ± 0.53 in obese cats).

TABLE 1. Measurements of obesity.

		% FAT	WEIGHT (kg)	BMI (kg/m²)	GIRTH (m)
LEAN STATE	MEAN ±SD	25.0±3.1	4.2±0.38	47.1±5.3	0.37±.04
OBESE STATE	MEAN ±SD	31.8±5.6	5.1±0.7	53.0±7.4	0.43±.04
p VALUE		≤0.0001	≤0.0001	0.0003	≤0.0001

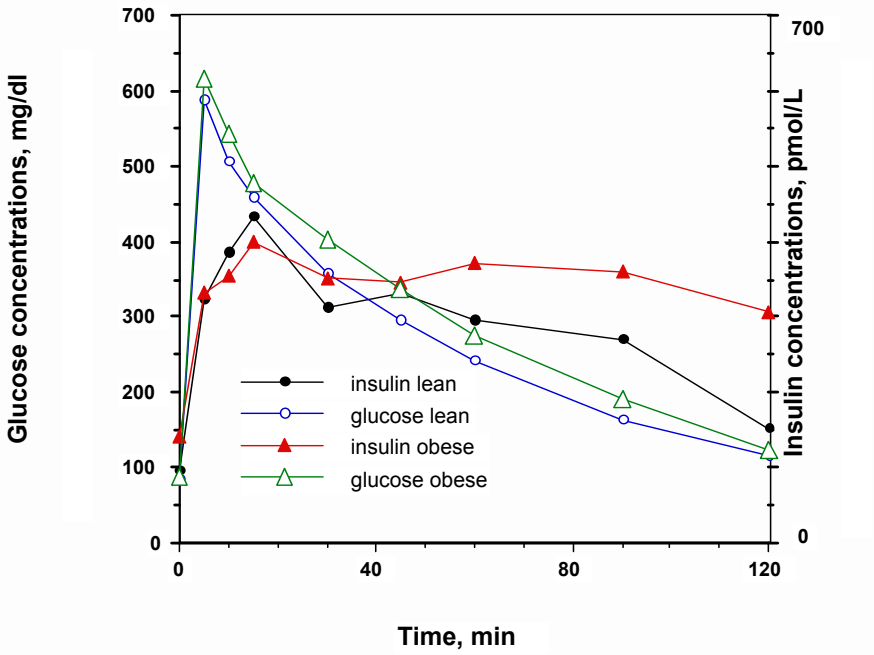

FIGURE 1. Glucose and insulin concentrations.

WESTERN BLOTS

Abbreviations used: MP= muscle particulate; MC= muscle ctyosolic; MPMC= combined MP & MC; FP= fat particulate; FC= fat cytosolic; FPFC= combined FP & FC. Glut1 expression showed no significant change in the obese cats in either total muscle or adipose tissue compared to lean cats. Comparing each fraction between L and OB, there was also no significant difference in MP, MC, FP or FC. (See table 2; figure 2).

TABLE 2. GLUT1 expression.

	LEAN	OBESE	p Value
tissue fractions	(counts•mm²)•10³	(counts•mm²)•10³	lean vs obese
MP	25.7±12.6	28.9±9.6	0.394
MC	24.4±10.3	21.8±10.1	0.21
MPMC	50.1±21.6	50.7±17.2	0.9
FP	23.0±7.4	22.4±10.2	0.828
FC	20.2±8.0	17.7±9.8	0.43
FPFC	43.2±13.2	38.5±19.4	0.411

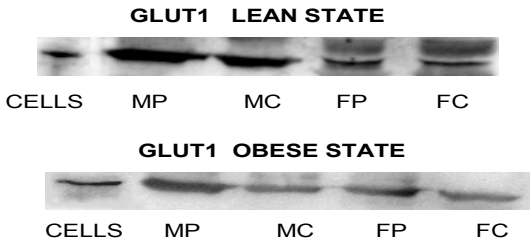

FIGURE 2. GLUT1 western blot images.

The Glut4 expression was significantly decreased in both muscle (p= 0.002) and adipose tissue (p=0.001), respectively, in the obese. Glut 4 in muscle and fat significantly and negatively correlated with the insulin AUC ($p < 0.004$ and $p < 0.040$, respectively) and Glut 4 in muscle but not fat also correlated significantly and negatively with baseline insulin concentrations ($p < 0.021$). There was no significant correlation with any of the glucose parameters that we measured and Glut 4 expression.

Glut4 expression showed a significant decrease in muscle cytosol, fat particulate and fat cytosol (p=0.006, p=0.032, and p=0.004 respectively). However there was no significant change in the muscle particulate fraction of the lean versus obese cats (p=0.086 respectively). (See table 3; figure 2).

TABLE 3. GLUT4 expression.

	LEAN	OBESE	p Value
tissue fractions	(counts•mm²)•10³	(counts•mm²)•10³	lean vs obese
MP	12.2±8.5	16.4±2.2	0.086
MC	14.3±8.6	3.0±4.5	0.006
MPMC	26.5±16.2	3.9±4.7	0.002
FP	12.7±6.2	5.3±6.5	0.032
FC	10.9±7.0	4.0±4.4	0.004
FPFC	23.8±11.5	8.1±7.6	0.001

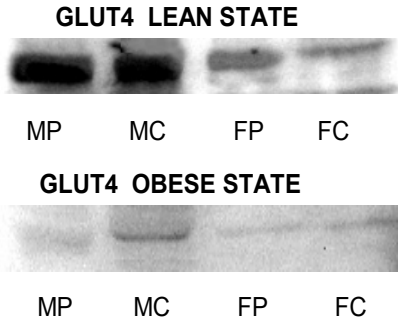

FIGURE 3. GLUT4 western blot images.

CHAPTER 5

DISCUSSION

The changes in the insulin secretion patterns that were seen in obese cats in this study closely replicated the findings of Hoenig (2002) with feline models and are similar to those seen in prediabetic, obese human subjects (Cerasi and Luft 1972). Consistent with these changes were an increased baseline insulin secretion and increased insulin concentrations at 120 min of the glucose tolerance test This study confirms our previous findings that obesity leads to abnormal insulin secretion (Hoenig 2000; 2002) and that this insulin secretory defects occurs before clinically obvious changes in fasting glucose concentrations and glucose tolerance are seen.. However, it was not only insulin secretion that was altered in the obese state. Obese cats also had a significant decrease in K value during the IVGTT indicative of glucose intolerance. The increase in the AUC for glucose is also suuportive of a the decrease in glucose clearance However, no significant change in baseline glucose concentrations was seen in the obese cats.

The increase in the AUC for insulin and the decreased glucose clearance is strongly suggestive but not conclusive of a decrease in insulin sensitivity, i.e. insulin resistance because glucose tolerance was not tested at steady state using the euglycemic hyperinsulinemic clamp. .

The finding of a glucose intolerance and a decrease in GLUT 4 expression in obese cats supports the hypothesis that the state of insulin resistance occurs at least in part, due to a defect in this major insulin sensitive glucose transporter. Some discrepancies exist

regarding differences of Glut 4 expression in muscle and fat of obese subjects. Impaired glucose transport in response to insulin in the adipose tissue of obese models has been attributed to decreased GLUT4 content; however, whether obesity alters GLUT4 expression in skeletal muscle is less clear.

Obese cats showed a reduction in GLUT4 content in both whole adipose tissue and skeletal muscle. This is similar to the findings by Sevilla et al. (1997) in rats; these investigators documented a significant decrease in basal 2-deoxyglucoes uptake as well as insulin-stimulated 2-deoxyglucoes uptake. Furthermore, glucose transport in the incubated soleus muscle and isolated adipocytes was greatly reduced. These combined results suggest, as our findings do, that obesity leads to a decrease in insulin sensitivity and a glucose intolerant state, which are related to a decrease in GLUT4 expression in adipose tissue and muscle.

Tremblay et al. (2001) also found a decrease in GLUT4 in muscle of obese rats. The GLUT4 content in type IIa enriched muscles (tibialis and soleus), was significantly lower in obese rats, similar to our findings of a decrease in GLUT4 in the tibialis muscle. They showed loss of insulin sensitivity evident by the decrease in glucose infusion rate during the euglycemic-hyperinsulinemic clamp. In addition, similar to our results, they also saw significantly higher fasting insulin concentrations, indicating abnormal insulin secretion. In support of the data by Tremblay et al. (2001) and the data presented in this study, Kahn and Pedersen (1992) found reduced levels of GLUT4 in muscles from rats displaying obesity by 3-fold increases in epididymal fat pad weight and 5-fold increases in adipocyte volume compared to lean litter mates. After 20 weeks on a high fat diet, the rats displayed basal hyperinsulinemia but normal basal blood glucose levels, similar to

our findings in obese cats of increased baseline and 120 minute insulin concentrations, with no change in baseline or 120 minute glucose concentrations.

In studies of Kahn (1994) and Pedersen et al. (1991), Glut 4 expression was not only altered in muscle but also in adipose tissue which was also seen in the obese cats of our study. They documented decreased glucose transport in response to insulin and decreased GLUT4 content, as well as a reduction in insulin stimulated glucose transport.

Numerous studies have also documented a decrease in GLUT4 mRNA in the obese state associated with a decrease in insulin sensitivity in these animals. Pedersen O et al. (1991) and Hamann A et al. (1996) found a decrease in GLUT4 mRNA in adipocyte tissue from obese rats and mice. Kahn and Pedersen (1993) found a decrease in GLUT4 mRNA in skeletal muscle from obese rats.

The fact that we saw a difference in the GLUT4 content in the cytosolic and particulate fractions of adipose tissue and muscle deserves further consideration. Studies have demonstrated that constitutive synthesis and turnover of transporters occurs (Holmon et al. 1990; Yang et al. 1992). Therefore, the cytosolic fraction of muscle and fat will contain some of the newly synthesized GLUT1 and GLUT4 protein. Also, as far as GLUT4 is concerned, the cytosolic fraction will also contain transporters in the process of being translocated to the plasma membrane. For these reasons, we measured immunoreactivity for both glucose transporters in the cytosolic fractions. As far as we are aware, other studies have not examined both cytosolic as well as particulate fractions. It is therefore, difficult to compare our results of decreased GLUT4 in both cytosolic and particulate fractions in fat and a decrease in cytosolic not particulate GLUT4 from muscle of these obese cats. Other studies have either examined whole tissue homogenate, or

crude membrane fractions. Studies by Tremblay et al. (2001) and Sevilla et al. 1997), used crude membrane fractions of muscle and adipose. The study by Tremblay had decrease GLUT4 content in crude membrane and Sevilla had decreased GLUT4 in muscle and adipose tissue in the obese state. If we compare these findings to our muscle particulate which is most similar to crude membrane, we too had a decrease in adipose tissue particulate GLUT4 however, we had no change in muscle particulate GLUT4.

Other studies have found normal GLUT4 expression, but abnormal GLUT4 translocation. Although we did not look at translocation, but only expression of GLUT4, this is an important point to address. Zierath et al. (1997) found that obese mice fed high fat-diets had decreased insulin-stimulated glucose transport in muscle. The GLUT4 expression in soleus muscle was normal, but insulin stimulated cell surface recruitment of GLUT4 was reduced. Furthermore, they found a reduction in insulin-receptor substrate 1 (IRS-1) associated PI3-kinase activity stimulated by insulin. Hansen et al. (1998) and Han et al. (1997) showed a decrease in insulin-stimulated 3-O methylglucose uptake in muscle of obese rats indicative of decreased insulin sensitivity, similar to our findings.

While the GLUT4 content was normal, the rats had a reduction in insulin-stimulated increase in GLUT4 at the cell surface, suggesting an impairment of one or more of the steps involved in GLUT4 translocation mechanisms. Tremblay et al. (2001) also found a complete absence of GLUT4 translocation in response to insulin in the plasma membrane of muscle as well as T-tubules, the major component of muscle cell surface. Furthermore, they identified PI-3 kinase as the first step of the insulin signaling pathway to be altered by high fat-feeding. Further studies are needed in obese cats to identify possible defects in Glut 4 translocation and signaling pathways.

GLUT1 expression level in obese animals has not been examined extensively and conflicting data have been presented. Although GLUT1 and GLUT4 are similar in structure, GLUT1 does not translocate to the plasma membrane in response to insulin like GLUT4, but is localized in the plasma membrane of tissue (Ramos et al. 2001) and is responsible for basal transport of glucose (Santalucia et al. 1999; Ganong 2001). It accounts for a small amount of total glucose carriers in skeletal muscle and adipose tissue. GLUT1 has a lower affinity for glucose transport (Km 1-2 mM) than GLUT4 (Km 5) (Ganong et al. 2001).

Hansen et al. (1998) and Han et al. (1995) had similar findings to ours in obese rats. They found no change in GLUT1 expression in muscle. However, all of these studies had a decrease in insulin-stimulated glucose uptake. Because GLUT1 was not decreased, this is evidence that GLUT1 is not involved in the decreased glucose uptake that occurred in the obese animals they studied.

However, other studies have had conflicting data from ours. Tremblay et al. (2001), showed a decrease of GLUT1 content in muscle from obese rats, while in the studies of Kahn (1994) a decrease in GLUT1 content in adipose tissue from obese rats was found. However, both of these studies had decreased glucose transport in response to insulin.

If a defect in the transporter GLUT4 is involved in the development of insulin resistance, as our recent findings as well as others have suggested, it is probably a defect in the translocation of this transporter which causes the defect. The fact that we found no decrease in GLUT1 content, but a significant decrease in GLUT4, lends evidence to this hypothesis. Because GLUT1 is found in the plasma membrane, and does not have to be

translocated as does GLUT4, this would suggest that it is the translocation of the GLUT4 vesicle that is defective. Two models have primarily been proposed to explain the defect in insulin-induced activation of glucose transport in obesity (Tremblay et al. 2001). In the first model, an alteration in insulin signaling (i.e.activation of PI-3 kinase) is an early event in the development of impaired glucose transport in skeletal muscle (Zierath et al. 1997), whereas in the second model, defects in insulin signaling of GLUT4 are a late occurrence and are not the primary defect causing muscle insulin resistance in obese animals (Hansen et al. 1998).

There is much evidence that the breakdown in lipid dynamics, seen as disordered fat storage and mobilization, are contributors to the pathogenesis of insulin resistance (Lewis et al. 2002; McGarry 2002; Reaven 1995; Schalch and Kipnis 1965; Shulman 2000; Kelley et al. 1999). Elevated plasma FFA concentrations have been found in obese individuals (Groop et al. 1992) similar to the trend we saw in the obese cats. These elevated levels are probably secondary to an increase in the supply of FFA from lipolysis due to a decrease in HSL sensitivity to insulin in insulin resistant states such as obesity (Lewis et al. 2002). The fact that the increase in NFA concentrations in the obese cats was not significant might be explained by the fact that the study was comprised of all female cats. Hoenig et al. (2003), found a significant increase in NEFA concentrations in male obese cats compared to male lean cats, with no significant change in female obese cats.

One mechanism that has been suggested to explain insulin resistance of muscle glucose transport that develops with obesity is a change in lipid composition of the plasma membrane as influx of FFA into the muscle bed occurs (Hansen et al. 1998). As

membrane lipid composition changes, these changes can affect the functioning of membrane-associated proteins. This could help to explain the defects that occur with GLUT4 because 2 important proteins involved in regulation of glucose transport stimulation by insulin include the glucose transporter itself, as well as the insulin receptor, both being part of the plasma membrane. It is possible that as lipid composition changes in adipose tissue with the progression of obesity (i.e. increased lipolysis due to a decrease in insulin action on HSL, possibly due to a mass effect of overall expansion of body fat depots), changes occur in the plasma membrane which effect GLUT4 translocation, causing insulin resistance in adipose tissue.

We can conclude from our findings that the changes in glucose transporter activity in muscle and fat are early derangements in obesity and occur before glucose intolerance is clinically evident. Because the cat is such a unique model for the development of Type 2 diabetes, further studies with this model will be very beneficial in determining the progression of this disease.

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