ASSESSMENT OF INSULIN RESISTANCE IN OBESE CATS

by

JULIANO BRANDAO

(Under the direction of Margarethe Hoenig)

ABSTRACT

Obesity and insulin resistance is a growing problem in people and in cats. Insulin resistance is defined as a condition in which the magnitude of the biological response to insulin is decreased. The major hallmarks of insulin resistance are alterations in glucose and lipid metabolism. Together, they lead to an impairment of insulin secretion and action, and may cause type 2 diabetes. Several methods have been developed in people in order to evaluate early changes in insulin secretion, glucose and lipid metabolism. The goal of this research was to examine the efficacy and sensitivity of different methods, such as the Euglycemic Hyperinsulinemic glucose Clamp (EHC), and the Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT) to detect changes in insulin sensitivity, and to examine glucose and lipid metabolism by using labeled ($^3$H)-glucose and deuterated glycerol and indirect calorimetry. Twenty two cats, 10 lean and 12 obese cats were used in this study. The EHC results of this study indicate that glucose sensitivity in obese cats is approximately 50% of that of lean cats and is negatively correlated with body weight, and that the insulin sensitivity for non-radioactive glucose was double that of radioactive glucose indicating that tracer estimates of insulin sensitivity may reflect only glucose disappearance while the estimate of insulin sensitivity based on total glucose also includes the suppression of hepatic glucose output. Obese cats had higher baseline non esterified fatty acids (NEFA) concentrations than lean cats. Obese males had significantly higher baseline NEFA concentrations than obese females. Obese females had lower RER values than obese males cats during insulin infusion suggesting a higher capacity to oxidize fat than obese males. It is conclude that obesity in cats is characterized by increased lipid oxidation which is maintained in females during insulin infusion but not males. The lower heat production in lean males combined with a decreased ability to oxidize fatty acids in response to insulin may predispose them to obesity. The lower metabolic rate of obese cats of either gender favors an increase in body weight in the face of unaltered food intake.

INDEX WORDS: Obesity, insulin resistance, Euglycemic Hyperinsulinemic Clamp, Frequently Sampled Intravenous Glucose Tolerance Test, Indirect Calorimetry, Respiratory Exchange Ratio, $^3$H-glycerol, $^3$H-glucose
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JULIANO BRANDAO

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JULIANO BRANDAO

Major Professor: Margarethe Hoenig

Committee: Duncan C Ferguson
           James Hargarove

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

This Master thesis is dedicated to a man that I most admire:

A friend whose touches warmed-me;
A mentor whose wisdom guides-me;
An encourager whose words lifted-me;

A leader I love to follow;

My father:

Antonio Carlos Brandao
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TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGEMENTS</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1. INTRODUCTION AND LITERATURE REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>2. MEASUREMENT OF INSULIN SENSITIVITY</td>
<td>25</td>
</tr>
<tr>
<td>OF LEAN AND OBESE CATS WITH THE</td>
<td></td>
</tr>
<tr>
<td>EUGLYCEMIC HYPERINSULINEMIC CLAMP AND</td>
<td></td>
</tr>
<tr>
<td>THE FREQUENTLY-SAMPLED INTRAVENOUS</td>
<td></td>
</tr>
<tr>
<td>GLUCOSE TOLERANCE TEST</td>
<td></td>
</tr>
<tr>
<td>3. LIPID METABOLISM, RESPIRATORY</td>
<td>64</td>
</tr>
<tr>
<td>EXCHANGE RATE AND HEAT PRODUCTION</td>
<td></td>
</tr>
<tr>
<td>DURING THE EUGLYCEMIC HYPERINSULINEMIC</td>
<td></td>
</tr>
<tr>
<td>CLAMP IN LEAN AND OBESE CATS</td>
<td></td>
</tr>
<tr>
<td>4. SUMMARY AND CONCLUSION</td>
<td>87</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW
**Introduction**

Obesity in cats and dogs is the most common veterinary nutritional disorder in the U.S. and its incidence is growing at an alarming rate. Obesity in cats, as in humans, is a risk factor for diabetes mellitus and also many obese cats show glucose intolerance and are thought to be insulin resistant.

Insulin resistance is defined as a condition in which the magnitude of the biological response to insulin is decreased. The major hallmarks of insulin resistance are alterations in glucose and lipid metabolism. Together, they lead to an impairment of insulin secretion and action, and may cause type 2 diabetes.

Several methods have been developed to aid in the early detection of changes in insulin secretion, and glucose and lipid metabolism. The goal of this research is to examine methods that would allow the early detection of changes in insulin sensitivity. These methods have been used in other species but have not been validated for use in cats.

1. The Euglycemic Hyperinsulinemic glucose Clamp (EHC)
2. The Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT)

The goal of these studies was to examine glucose and lipid metabolism by using labeled (³H-glucose and deuterated glycerol) and indirect calorimetry, and to compare these results to the “gold” standard for insulin sensitivity, the EHC.
Literature Review

General Background
The incidence of obesity is increasing at the alarming rate of 6% a year in people, and obesity is also a problem in people but also in pets. One third of the domestic cats are considered obese or overweight. (Scarlet et al., 1994). Obesity is caused by impaired insulin action and insulin secretion in people and in cats (Felber et al 2002; Hoenig et al 2002). It has been shown that the risk of developing insulin resistance and type 2 diabetes increases with the development of obesity in cats (Hoenig et al., 2000).

Evaluation of insulin action in the lean state

A. Glucose Metabolism
Insulin is an important anabolic hormone with many effects on tissue metabolism. Insulin acts by binding to its receptor on the surface of target cells with intracellular signaling followed by tyrosine autophosphorylation and consequently intracellular signaling and cellular response (Cheatam et al., 1995). There are two major sites for insulin control of glucose homeostasis: hepatic glucose production (HGP), and glucose uptake into peripheral tissues, primarily muscle (DeFronzo, 1998). Insulin facilitates entry of glucose into muscle and adipose tissue, as well as other tissues by increasing the number of glucose transporters in the cell (Cheatam et al., 1995). Among the transporters, GLUT 4 is insulin-dependent and is present in skeletal muscle, heart, and white and brown adipose tissue (Brennan et al., 2004; Bouche et al., 2004). Glucose-independent transport is achieved with GLUT1 transporters (Bouche et al., 2004). In liver, insulin is responsible for the suppression of hepatic glucose output by increasing the activity of glycogen synthase and decreasing the activity of glycogen phosphorylase, and also by increasing the activity of hexokinase, which enhance the conversion of glucose into glucose-6-
phosphate. Large amounts of glucose-6-phosphate inside the cell are converted to glycogen. Glycogen is stored in the liver and in skeletal muscle for later use as a nutrient in times of nutrient need, such as exercise or starvation.

In the liver, when insulin and glucose plasma concentration are low, such as during fasting, glycogen is converted to glucose which is released from hepatocytes. In muscle, because the activity of hexokinase is high even when insulin is low, any free glucose available is immediately phosphorylated to enter the glycolytic pathway, where is converted into pyruvate and is consequently used in the Krebs cycle (Bouche et al., 2004).

**B. Fat metabolism**

Lipids are broken down in the intestinal tract into glycerol plus free fatty acids and absorbed by the intestinal mucosa cells. They are re-synthesized and packaged into chylomicrons, which are lipoprotein complexes, in order to be carried through the circulation. The purpose is to transport dietary cholesterol and triglycerides from the intestine to the rest of the body. Chylomicrons from the intestine are released into the blood and are carried to tissues, where the enzyme lipoprotein lipase (LPL) removes the fatty acids of the triacylglycerol. The remaining particle is carried as a chylomicron remnant to the liver where the rest of the triacylglycerols are packaged into very low density lipoproteins (VLDL) and released into the blood directly. The triacylglycerol components of VLDL are hydrolyzed to free fatty acids and glycerol in the capillaries of adipose tissue and skeletal muscle by the action of lipoprotein lipase which is stimulated by insulin. The free fatty acids are then taken up by the cells and the glycerol is returned via the blood to the liver, where it is converted to the glycolytic intermediate dihydroxyacetone phosphate (DHAP) and used in glycolysis for energy needs or converted into glycerol-3-phosphate and used as the back bone of triacylglycerol stored as VLDL. The
conversion of glycerol into glycerol-3-phosphate is mediated by an enzyme called glycerol kinase. However, the adipocytes glucose, uptake by GLUT1 and GLUT4 is important for lipogenesis because the adipose tissue lacks the enzyme glycerol kinase. As a result, glycerol-3-phosphate originates from glucose and is then converted into DHAP and later on into glycerol-3-phosphate to be used as a back bone for triacylglycerol. In adipose tissue and liver, insulin also stimulates fatty acids synthase, which activates de novo lipid synthesis (Stipanuk 2000, Frayn et al., 2003).

Insulin acts on a variety of tissues, most notably adipocytes, skeletal muscle, heart muscle, and pancreatic β-cells by stimulating LPL (Picard et al, 2001) and inhibiting the enzyme hormone sensitive lipase (HSL), which is responsible for the breakdown of intracellular triacylglycerol into free fatty acids plus glycerol (Grosses et al., 1981; Yeaman, 2004). Lipid metabolism is also regulated by the activity of hepatic lipase. In humans, hepatic lipase (HL) is one of the enzymes responsible for the metabolism of chylomicrons and very low density lipoprotein (VLDL) (Grosses et al., 1981; Yeaman, 2004). However in cats, this enzyme is responsible for the hydrolysis of VLDL and intermediate density lipoprotein (IDL) triglycerides (Demacker et al., 1988). In cats, plasma hepatic lipase is present in higher amounts in cats than LPL. FFA released by the action of lipases are taken up into tissues, where they are either oxidized or deposited after esterification or they remain in the circulation bound to albumin as non-esterified fatty acids (NEFAs).

A connection between glucose and lipid metabolism was suggested by Randle et al (1963). He suggested that an increase in free fatty acid (FFA) concentrations increases the rate of FFA oxidation and decreases the rate of glucose oxidation because the increase in FFA oxidation would result in production of acetyl-CoA and consequently increase intramitochondrial levels of
NADH. Higher levels of acetyl-CoA and NADH inhibit glucose oxidation by inhibiting pyruvate dehydrogenase, leading to inhibition of phosphofructokinase-1 (PFK-1) and therefore increasing glucose-6-phosphate levels. When glucose levels are high, there is an increased rate of pyruvate product and conversion to acetyl CoA by the enzyme pyruvate dehydrogenase. The higher levels of acetyl CoA subsequently lead to the production of citrate through the Krebs cycle, which activates acetyl CoA carboxylase (ACC), an enzyme that catalyzes the formation of malonyl CoA. MalonylCoA is a molecule that regulates FFA fluxes inside the mitochondrial matrix by inhibiting the activation of carnitine palmitoyl transferase I (CPT-1) resulting in FFA accumulation in the cytoplasm (Ramussen et al., 2002). The mitochondrial oxidation of fatty acids is controlled by the glucagon to insulin ratio. Under fasting conditions, this ratio is high resulting in the stimulation of lipolysis. Triacylglycerol in fat depots are hydrolyzed to free fatty acids that are released into the circulation and subsequently taken up and oxidized by cells. Glycogenolysis is also stimulated, and, in the liver, glucose is mobilized for extra hepatic utilization (Bartlett et al., 2004). However, when the glucagon insulin ratio is low, glucose is taken up by the cells and stored as glycogen, and mitochondrial fatty acid oxidation is inhibited, with FFA being stored as triacylglycerol.

**Evaluation of insulin secretion in the lean state**

The major stimulus for insulin secretion is glucose. Glucose is transported into β-cells through the glucose transporter GLUT 2 (Bouche et al., 2004) Elevated concentrations of glucose within the β-cells ultimately lead to membrane depolarization and an influx of extracellular calcium, which triggers insulin (Porte Jr, 1991; Kahn, 2001). Insulin secretion occurs in two phases: the first (acute) phase results from the insulin released from secretory granules close to the plasma membrane, while the second (maintenance) phase results from insulin released from granules...
that need to be recruited from the cytoplasm to the plasma membrane (Gerich et al., 1976). The “gold standard” to study the pattern of insulin secretion is the hyperglycemic glucose clamp technique, in which glucose is infused to achieve a high plasma glucose concentration (DeFronzo, 1979).

**Evaluation of insulin action in the obese state**

**A. Glucose Metabolism**

An important action of insulin on glucose metabolism is the lowering of plasma glucose concentration by suppressing hepatic glucose production (HGP) (DeFronzo, 1988) and stimulating tissue glucose uptake. Since skeletal muscle is primarily responsible for stimulating glucose uptake, this tissue is also the major site of insulin resistance (Rosseti et al., 1993). In obesity insulin loses some of its potency to stimulate skeletal muscle glucose uptake (DeFronzo, 1988). Several causes for the impairment of glucose uptake into muscle are possible. In obesity, high plasma levels of FFA are present in the pre-and postprandial period (Balent et al., 2002, Jensen, 1999). High levels of FFA are thought to be responsible for alterations in muscle glucose metabolism (DeFronzo, 1988; Boden et al., 2002). Fatty acids might alter glucose metabolism through changes invoked by the Randle cycle as discussed above. It has also been suggested that in obesity lower expression of GLUT4 exists (Cline et al. 1994). This has been shown in rodents, humans, and cats (Tremblay et al., 2001; Lynis et al., 1991; Brennan et al., 2004). Together with an impairment of glucose transport, a reduction in glucose phosphorylation was proposed (Shulman et al., 1995).

Hepatic glucose production (HGP) contributes to impaired glucose metabolism in obesity. During insulin infusion, HGP is suppressed in lean subjects (Rebrin et al., 1995); however, in obesity, this suppression is impaired (Rebrin et al., 1995). High plasma FFA oxidation leads to
diminished effect of insulin to suppress HGP and stimulate glucose oxidation (Rebrin et al., 1995; Groop et al., 1989). These observations are supported by those of Boden et al. (1995) who demonstrated that insulin was unable to suppress hepatic glucose production after 48 hours of FFA infusion.

Bergman (2000) postulated that insulin action on the liver occurs slowly and in two steps: firstly, insulin acts on the extrahepatic tissues such as adipocytes, inhibiting lipolysis and decreasing NEFA blood concentrations, and secondly, NEFAs act as a signal to the liver, to reduce hepatic glucose production and increase peripheral glucose utilization. Because suppression of lipolysis is impaired in insulin resistant states, (Groop et al., 1989), high NEFA levels are associated with insulin resistance and transmit an impaired signal to the liver and continued hepatic glucose production.

B. Fat metabolism

The major metabolic effects of insulin on lipid metabolism are the suppression of lipolysis and an increase in lipogenesis (Hickener et al., 1999). The plasma concentration of FFA reflects a balance between the hydrolysis of triglycerides and FFA uptake into cells (Lewis et al., 2002). There are several methods to investigate in vivo lipolysis. Frequently, these methods are combined with the EHC technique (Coppack et al., 1994) allowing the investigator to directly examine the effect of insulin on fatty acid turnover. One method measures the appearance rate of glycerol through dilution of an infused isotope, such as D$_5$-glycerol (Beylot et al., 1987). By adding tracer ($^2$H$_5$-glycerol) during the EHC, the true rate of lipolysis can be measured, because glycerol is not an insulin secretagogue (Jensen et al., 2001) and its infusion during the EHC does not influence FFA turnover or lipid oxidation.
During the EHC, the rate of FFA and glycerol production decline after insulin infusion reflects the insulin suppression of lipolysis and the insulin stimulation of FFA reesterification (Zengkui et al., 1999).

Additionally insulin plays a pivotal role in determining plasma FFA concentrations by controlling the flux of FFA from extrahepatic tissues to the liver. (Lewis et al., 2002).

In insulin resistant states, VLDL production is increased (Bioletto et al., 2000; Lewis et al., 2002). High VLDL production is due to high levels of FFA secreted into the portal circulation, especially in abdominal obesity in humans subjects (Bioletto et al., 200; Zenkui et al., 2004).

During the EHC elevation of FFA concentrations reflects a diminished effect of insulin to suppress lipolysis (Zenkui et al., 2004). Insulin promotes FFA reesterification and/ or oxidation. (Jensen et al., 1994; Campbell et al., 1994).

Healthy muscle metabolically switches from lipid oxidation during fasting favoring glucose oxidation in postprandial conditions. (Blaak., 2004). It has been hypothesized that the capacity of muscle to switch between fuels is lost in insulin resistance (Kelly et al., 1999). Boden et al (1997) and Kelly et al. (1999) using euglycemic hyperinsulinemic clamp conditions, detected a decrease in carbohydrate oxidation associated with a decrease in fatty acid oxidation in obese and type 2 diabetic patients. Kelley and coworkers (1999) were unable to detect an increase in fatty acid uptake in obesity and proposed that the diminished capacity for fatty acid oxidation is the major contributing factor to the triglyceride deposition in muscle in obese subjects.

The result of increased FFA lipolysis, decreased oxidation, and increased reesterification in insulin resistant states diverts FFA towards nonadipose tissues such as liver, muscle, and others (Lewis et al., 2002). Shulman et al (1995) has proposed that insulin resistance develops because of an imbalance of fat distribution between tissues where in adipocytes are able to effectively
protect themselves against ongoing caloric overload by developing resistance to insulin’s anabolic effect; consequently, FFA deposition is diverted to other tissues. This notion is also supported by Campbell et al (1994) who found that during insulin infusion, FFA reesterification in muscle and hepatic extraction of FFA is increased in obese volunteers.

**Evaluation of insulin secretion in the obese state**

In obesity, insulin secretion is also abnormal. The first phase of insulin secretion is thought to be primarily responsible for the suppression of hepatic glucose output, whereas the second phase is primarily responsible for glucose disposal by peripheral tissues (DelPrato et al., 2001). In obesity, the area under the curve (AUC) of the first phase of insulin secretion is diminished whereas the second phase is prolonged and increased (Hoenig et al., 2000; Kahn, 2001) primarily because insulin secretion is increased and clearance is reduced. The changes in second phase have been interpreted to be an attempt to compensate for the decreased first phase of insulin secretion in an attempt by β-cells to maintain normal blood glucose concentration. These changes in insulin secretion become more severe with the progression from obesity to type 2 diabetes, eventually leading to a complete disappearance of the first phase and erratic secretion during the second (Hoenig et al., 2000; DelPrato et al., 2002; Kahn, 2001). In a longitudinal study, the amplitude of the first phase of insulin secretion has been found to be directly related to the impairment of glucose tolerance (Bogardus et al., 2002, Haffner et al., 1996).

**Testing insulin sensitivity and metabolism**

In order to measure insulin resistance, several methods have been used, among them the euglycemic hyperinsulinemic glucose clamp (EHC) (DeFronzo, 1979), the frequently sampled intravenous glucose tolerance test (FSIVGTT) (Bergman et al., 1979), the homeostasis model assessment (HOMA) (Matthews et al., 1985), and the quantitative insulin sensitivity check index.
(QUICKI) (Katz et al., 2000). The major goal of the methodology is to quantify insulin stimulated glucose uptake and HGP (Ferrannini, 1998; Raziuk, 2000).

**Euglycemic hyperinsulinemic glucose Clamp**

The EHC technique was first described by Andreas and co-workers in 1970 and is often referred as the “gold-standard” for measure insulin sensitivity in people. This test involves the infusion of a high concentration of insulin at a constant rate with a simultaneous infusion of glucose at a variable rate to keep glycemia at the normal fasting glucose rate (Ferrannini et al., 1998). In this test, the pancreatic insulin production is inhibited and the measurement of tissue insulin sensitivity can be made by measuring the amount of glucose infused, which at steady state is equal at the amount of glucose disposed by the body. (Molina et al., 1999). More information can be obtained by adding a tracer. In one study (Rosseti et al., 1993) tritiated glucose was added to the clamp and by measuring the rate of tritiated water formation, muscle glycogen synthesis and hepatic glucose output could be calculated from the difference of the glucose uptake (glucose infusion) and glycolysis (water plasma formation) (Noel et al., 1997).

During the EHC, hyperinsulinemia decreases HGO and increases in the peripheral glucose uptake into tissues (Raziuk et al., 2000). In insulin resistant states, the opposite occurs resulting in a decreased glucose requirement to maintain euglycemia.

**FSIVGTT**

Using the FSIVGTT, obese individuals have been found to have decreased insulin sensitivity (SI). This low SI has been explained as the result of reduced transport of insulin through the capillary endothelium because obese subjects have a higher lymph insulin concentration (Bergman et al., 1989). This would suggest the time course of insulin action is, in part, determined by the time that is required for insulin to cross the capillary endothelium. Villar et al
(1998) criticized this theory and suggested instead that, obese subjects have a lower SI due to signal transduction defects and insulin binding and associate the accumulation of insulin in lymph with inefficiency of the receptor.

Donner et al (1985) compared the FSIVGTT with the EHC in humans with different metabolic states and found a poor correlation between the two methods. One explanation for the discrepancy is that insulin insensitive states, such as type 2 diabetes, the ability of glucose to lead to its own disposal is impaired. Following this study, several others tried to improve the FSIVGTT method by changing the protocol in order to amplify the insulin signal so that the ability of glucose to lead its own disposal could be truly evaluated. (Beard et al., 1986; Yang et al., 1987; Welch et al., 1990). There is a better correlation between EHC and the modified FSIVGTT when tolbutamide or an insulin bolus was injected after 20 minutes of the glucose infusion. This procedure amplifies the insulin signal in individuals with low insulin secretory capacity. Petrus et al (1998) using the standard FSIVGTT and the EHC in lean cats found a positive correlation between the two tests.

**Indirect Calorimetry**

Indirect calorimetry measures the total oxygen consumption and carbon dioxide production when organic compounds are oxidized. The ratio between O$_2$ consumed and CO$_2$ produced by the metabolism of carbohydrates, fat and protein varies because of the difference in the carbon and oxygen content of each component of nutrients (Klebber, 1975). By measuring the O$_2$/CO$_2$ ratio, the type of substrate oxidized can be identified (Simonson et al., 1990). The ratio of O$_2$/CO$_2$ is called Respiratory Exchange Ratio (RER) or Respiratory Quotient (RQ). Generally, a person in the post-absorptive state would have a RER of 1 for carbohydrates, 0.7 for fat and 0.9 for protein (Simonson et al., 1990). These numbers are calculated based upon the oxidation of one mol of
substrate, i.e. carbohydrates, protein lipids (Simonson et al., 1990). Once the total volume of \( O_2 \) and \( CO_2 \) have been determined in a respiratory chamber, these values can be utilized to calculate the net rate of “disappearance” of substrates from their respective metabolic pools (Simonson et al., 1990) and the heat production based primarily on the fact that energy is produced when organic compounds are oxidized (Simonson et al., 1990).

In the lean and obese state, after an overnight fast, the predominant substrate oxidized by skeletal muscle was lipid (Simonson et al., 1990; Kelley et al., 1999; Groop, 1992). However, lipid oxidation in lean subjects was higher than in obese subjects despite similar rates of fatty acid uptake (Kelley et al., 1999). These differences lead to increased lipid deposition in obese subjects. During the EHC, insulin suppresses lipolysis and free fatty acid release in lean subjects by suppressing fatty acid oxidation (Campbell et al., 1994). However, in obesity, insulin suppression of lipolysis during the EHC is inadequate, and therefore plasma FFA are relatively increased when compared to lean individuals (Campbell et al., 1994, Boden, 1997, Groop, 1992, Perseguin et al., 2001).

When indirect calorimetry was used to measure substrate oxidation, the RER in lean subjects during an EHC was close or equal to 1 indicating that carbohydrates were oxidized. In fact, glucose oxidation accounted for almost 90% and lipid oxidation for only approximately 10% of energy expenditure. However, in obese subjects, the RER was lower compared to lean subjects. Insulin infusion failed to suppress lipid oxidation in obese individuals, and the rates of lipid oxidation were similar to those seen in fasting conditions (approximately 35%; Kelley et al., 1999).

Calorimetry also allows the calculation of heat production. Several studies have shown that low energy expenditure combined with a high RER can be used as a predictor for the development of
obesity. However, during the development of obesity, it was demonstrated that energy expenditure increases and RER decreases indicating a higher fat oxidation rate (Golay et al., 1982). This adaptive response was variable among individuals in studies of lean and obese patients. Lean individuals were found to have a reduced total heat production when measurements were based on total body weight but heat production was similar when normalized for lean body mass (Segal et al., 1987). It has been suggested that obese subjects have increased heat production because both, lean body mass as well as fat mass, are increased (James et al., 1978). In severely obese type 2 diabetics, total heat production was high even after it had been normalized for lean body mass (Huang et al., 2004). These investigators actually found that body weight had a stronger correlation with resting metabolic rate than lean body mass.

During insulin infusion or after a meal, heat production was lower in obese than in lean subjects (Welle et al., 1981). During the EHC, more than one half of the increase in energy expenditure is due to glucose storage, i.e. glycogen synthesis (DeFronzo et al., 1981; Ravussin et al., 1982) which is decreased in insulin resistant obese individuals. (Ravussin et al., 1982). Similarly, in Pima Indians, a reduction in insulin-stimulated thermogenesis is an early event in the development of type 2 diabetes (Weyer et al., 1999).
REFERENCES


CHAPTER 2

MEASUREMENT OF INSULIN SENSITIVITY OF LEAN AND OBESE CATS WITH THE EUGLYCEMIC HYPERINSULINEMIC CLAMP AND THE FREQUENTLY-SAMPLED INTRAVENOUS GLUCOSE TOLERANCE TEST\textsuperscript{1}

\textsuperscript{1}Brandao J, Thomaseth K, Waldron M, Ferguson DC, Hoenig M. To be submitted to American Journal of Comparative Endocrinology.
Introduction

Obesity is becoming a more common problem, not only in people but also in pets. About one third of the cat population is considered obese (1, 2). Cats are excellent models for the disease process because, like humans, many obese cats develop diabetes. The classification is similar to type 2 diabetes in that diabetic cats do not develop antibodies against beta cells (3); however, they develop amyloid, the hallmark of type 2 diabetes in people (4, 5). In order to learn more about the pathogenesis of diabetes, it is important to be able to detect early changes in insulin secretion and action as they become abnormal with the development of obesity. Several methods have been proposed to quantify insulin sensitivity and β-cell response: the most common are the euglycemic hyperglycemic clamp (EHC) (6), the insulin tolerance test (7), the insulin suppression test (8), the frequently sampled intravenous glucose tolerance test with minimal model analysis (FSIVGTT-MINMOD) which is a modification of the intravenous glucose tolerance test (9); the homeostasis model assessment (10), and the quantitative insulin sensitivity check index (11). The gold standard for the measurement of insulin action is the EHC, whereas the hyperglycemic clamp is the preferred method for the measurement of insulin secretion (6, 12). The EHC measures insulin sensitivity, because at steady state the amount of glucose infused equals the amount of glucose disposed by tissues. This test provides even more information when tracer is added to the infusion (13). Addition of D - [3H]-glucose, which is metabolized to [3H]2O through the glycolytic pathway, allows the calculation of whole body glycolysis and total body glycogen synthesis (14, 15). Tracer has also been added to the FSIVGTT with MINMOD analysis to allow the assessment of glucose turnover (16).
The goal of our study is to investigate glucose turnover and insulin sensitivity in lean and obese cats with the EHC, and compare its utility as well as that of the FSIVGTT in the determination of insulin resistance.

**Material and Methods**

Recombinant human regular insulin was supplied by Eli Lilly (Indianapolis, IN); D-[3-H]$^3$ glucose was purchase from Perkin Elmer (Boston MA). Barium hydroxide solution and Zinc sulfate solution were purchased from Sigma Aldrich (St. Louis MO).

**Animals and Diets.** Twenty-two neutered adult Domestic Shorthair cats (Sinclair Research Center, Columbia, MO and Harlan, Indianapolis, IN 46229) were used for these studies. Their gender, weight, body mass index, girth, circumference, and percent body fat are shown in Figure 2.1. Measurement of weight, percent body fat, and body mass index (BMI; expressed in kg/m$^2$) was performed as described previously (17). 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.

All animal studies were approved by the University of Georgia Animal Care and Use Committee and were 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*. Animals were determined healthy based on the results of physical examination and clinical laboratory data. All cats were accustomed to daily handling and were fed the same diet once daily. The composition of the diet is shown in figure 2.2. Food intake (Kcal/kg body weight (BW) was recorded at each feeding (obese male 48.9 ± 7.2, obese female 44.6 ± 5.9, lean male 58 ± 13, lean female 46.3 ± 6.8), and the cats were weighed twice weekly. Food intake was adjusted to maintain body weight within 5% of the weight at the beginning of the study. .
Blood sampling

To allow blood sampling, catheters were placed in the jugular vein of cats 36-40 hours before the study and in the cephalic vein prior to the clamp. Catheter patency was maintained by injection of 0.5 ml of 0.38% sterile citrate flush (citric acid, trisodium salt dihydrate, Sigma Co., MO) every 8 hours. Whole blood was taken through the jugular catheter and allowed to clot for serum collection; for the collection of plasma, blood was placed into tubes containing cold potassium oxalate (1 mg/ml), sodium fluoride (1 mg/ml), and benzamidine (1M) and the samples were centrifuged immediately. Serum and plasma samples were stored at -20°C until assayed. Mixed venous samples were drawn for 3-[3H] glucose, glucose, and insulin determinations. Infusions were administered via cephalic catheter.

Determination of Plasma D-[3-3H]-glucose distribution.

In order to estimate the rate and extent of glucose distribution, a D-[3-3H] glucose bolus of 0.5 µCi/kg was administered intravenously (IV) in 4 adult cats (2 lean neutered cats, 1 female, and 1 male, 3.83 ± 0.12 kg BW and 9.7 ± 2.6 % body fat; 2 obese neutered cats, 1 male and 1 female, 7.48 ± 0.37 kg BW and 37 ± 9.8 % body fat). Blood samples were collected at times 0, 1, 2, 3, 4, 5, 7, 10, 12, 13, 14, 15, 30, 45, 55, 90, 120 minutes.

Euglycemic Hyperinsulinemic clamp

An EHC was performed in 22 cats according to the protocol described in Fig. 2.3 At time 0, a bolus of 3-[3H] glucose (Perkin Elmer, Boston MA) containing 0.12 µCi/kg BW was administered IV followed by an IV infusion of 3-[3H] glucose in saline containing 6nCi/kg/min. The saline infusion was continued for 120 min to insure isotope equilibration. At time 120 min, the clamp was started and lasted for 120 minutes using the protocol described by Petrus and coworkers (18) with the following modifications: At time 120 min, an initial insulin bolus of 26
mU/ml/kg BW recombinant human regular insulin was administered IV and a solution of $[3^3\text{H}]$-glucose (0.4 µCi/ml) in saline with added glucose (20% w/v) was infused for 120 min at a rate necessary to maintain the cats’ fasting blood glucose concentrations. This was achieved by measuring whole blood glucose with a glucometer (Elite XL, Bayer Corporation, Mishawaka, IN) at least every 15 min. At time 125 min a second bolus of insulin (13mU/kg BW) was administered IV followed by the infusion of insulin (1.1mU/min/kg BW) containing $[3^3\text{H}]$-glucose (6nCi/kg/min) for the remainder of the clamp. Plasma radioactivity, glucose, and insulin concentrations were determined at -10, 0, 30, 60, 70, 80, 90, 100, 110, 120, 135, 150, 165, 180, 195, 210, 225, and 240 min. Insulin concentrations were measured as described (19) Glucose measurements were performed using a colorimetric glucose oxidase method (glucose trinder kit; Diagnostic Chemicals limited. Charlottetown, PE, Canada).

**Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT) protocol**

Four months after the euglycemic clamp study, while the cats weight was strictly maintained, a frequently sampled intravenous glucose tolerance test was performed in 8 adult cats (5 lean neutered cats, 3 male and 3 female, 3.8 ± 0.5 kg BW and 15.2 ± 3.3 % body fat; 3 obese neutered cats, 2 male and 1 female, 6.6 ± 1.3 kg BW and 41.7 ± 4.4 % body fat) as follows: At time 0, a glucose bolus (50%, w/v) of 0.8 g/kg BW was administered followed by an IV insulin bolus of 0.05 U/kg/BW at 20 minutes. Thirty-three blood samples were collected for measurement of glucose and insulin concentrations at the following times; -5, 0, 1, 2, 3, 4, 5, 7, 8, 10, 12, 14, 16, 20, 22, 23, 24, 25, 28, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 160, 180, 240 minutes.

**Determination of Plasma D-[3-$^3\text{H}$]-glucose distribution.**

Plasma D-[3-$^3\text{H}$]-glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide-zinc sulfate precipitates (Somogyi procedure) of plasma samples after evaporation to
dryness to eliminate tritiated water as described by Shimoyama et al. (20). The difference between total radioactivity and radioactivity of dried samples was considered to be water production as a result of glucose metabolism.

A three compartmental model (Figure 2.4) was used to describe $^3$H-$\text{H}_2\text{O}$ productions as a proportion of the $^3$H-glucose metabolism, assuming that it takes place in the remote compartment.

**Mathematical model**

The mathematical model equations are:

\[
\frac{dx_1(t)}{dt} = K_c(c_2(t) - c_1(t)) \quad (1)
\]

\[
\frac{dx_2(t)}{dt} = -k_{02}x_2(t) + K_c(c_1(t) - c_2(t)) \quad (2)
\]

\[
\frac{dx_3(t)}{dt} = k_{02}x_2(t) \quad (3)
\]

where $x_1(t)$, $x_2(t)$ are the compartmental masses of $^3$H-Glucose and $x_3(t)$ of $^3$H-$\text{H}_2\text{O}$ expressed in (dpm) with initial conditions $x_1(0) = \text{Dose}$, $x_2(0) = 0$, $x_3(0) = 0$. The concentrations in the compartments are given by $c_1(t) = x_1(t) / V_1$, $c_2(t) = x_2(t) / V_2$, $c_3(t) = x_3(t) / V_3$ (dpm/ml). The model parameters are $K_c$, which represents the inter-compartmental mass transfer (ml/min); $k_{02}$, which represents the fractional glucose clearance rate (1/min); $V_1$ and $V_2$ (ml) are glucose distribution spaces ($V_{\text{tot}} = V_1 + V_2$), and $V_3$ (ml) is the water distribution space.

In addition to the above dynamic model equations, the equations describing the measurements are the following:

\[
y_1(t) = f_Gc_1(t) \quad (4)
\]

\[
y_2(t) = c_1(t) + c_2(t) \quad (5)
\]
Where $0 < f_G < 1$ is the fraction $^3$H-Glucose recovered from plasma samples after drying.

Two volumes of distribution volumes $V_1$ and $V_2$ and the inter-compartmental mass transfer $K_c$ have been expressed as fractions of the total distribution volume for glucose, $V_{tot}$. In particular, $V_1 = f_V^*V_{tot}$ and $V_2 = (1 - f_V) * V_{tot}$, where $0 < f_V < 1$ is the fraction of the volume of the accessible compartment, and $K_c = f_{Kc}V_{tot}$, where $f_{Kc}$ (1/min) is the fraction of the volume exchanged between the two compartments in one time unit. In summary, the model parameters that are estimated from the experimental data are \{$V_{tot}$, $f_{Kc}$, $f_V$, $k_{02}$, $V_3$, $f_G$\}.

For the estimation of these parameters, non-linear transformation was introduced to guarantee the fulfillment of constraints on parameter estimates and to reduce possible problems due to large inter-individual variability in kinetic parameters.

Although only four cats have been studied, the possible effect of body weight on glucose and $^3$H-$H_2O$ was studied using population-modeling approach based on the simultaneous analysis of all data using a statistical model that explicitly accounts for intra- and inter-individual variations of parameters. For this purpose, the $k$-th components of the individual parameter vector $\theta_i$ were described, in general, by a linear model as follows:

$$\theta_{ik} = \theta_{ok} + X_{ik}a_k + \delta_{ik}, \; (6)$$

where $\theta_{ok}$ represents the average population value, $X_{ik}$ are the covariates predicting intra- and inter-individual variability, and $\delta_{ik}$ are so-called random effects that represent “unpredictable” variations. In our case, $X_{ik}$ was simply 0 for lean cats and 1 for obese cats, such that $a_k$ quantifies the difference between lean and obese cats. However, not all kinetic parameters were described using the complete structure of Eq. (6). The need for the covariate $X_{ik}$ or the random effect $\delta_{ik}$ was assessed by statistical criteria to select the minimum number of estimated parameters able to quantify the statistically significant determinants of intra-and inter-individual variability.
Euglycemic hyperinsulinemic clamp

The analysis of the EHC data was performed by using a modified version of the minimal model of glucose disappearance to account for exogenous infusion with a term \( u_g(t)/V_g \), where \( u_g(t) \) represents either the cold or \(^3\)H-Glucose infusion, expressed in mmol/min or dpm/min, respectively, and \( V_G \) is the individual distribution volume, which was determined experimentally to be proportionate to body weight. Given the differences in measurement units for cold (mmol/l) and \(^3\)H-Glucose (dpm/ml), the volume \( V_G \) was either expressed in liter or ml, respectively. The graphical representation of the minimal model for cold glucose is shown in Fig. 2.5 and for \(^3\)H-glucose in Fig. 2.6.

In order to distinguish between cold and tracer glucose and the corresponding kinetic parameters, a star superscript (*) indicates a parameter pertaining to tracer kinetics. Cold glucose is indicated with \( G(t) \) and the corresponding kinetic parameters with a tilde (~). All other variables are the same for both tracer and cold glucose kinetics. To simplify the mathematical notation, the minimal model equations are written in an equivalent notation as follows:

\[
\frac{dG(t)}{dt} = -(S_G + \tilde{S}_I Y(t))G(t) + \tilde{S}_G G_b + \frac{u_g(t)}{V_g} \\
\frac{dY(t)}{dt} = -P2 \left(Y(t) - (I(t) - I_b)\right) \\
\frac{dG^*(t)}{dt} = -(S_G + S_I^* Y(t))G^*(t) + \frac{u^*_g(t)}{V_g \cdot 10^3} \\
\frac{dW^*(t)}{dt} = K_w \left(S_G + S_I^* Y(t)\right) \frac{V_g G^*(t)}{V_{bw}}
\]

with initial conditions (at \( t = -120 \)): \( G_{-120} = G_b, \frac{G^*_{-120}}{D^*/V_g} = D^*/V_g \) (\( D^* \) is the tracer bolus dose).
Equations 9 and 10 represent the $^3$H-Glucose and $^3$H-H$_2$O kinetic equations, respectively. The variable $Y(t)$ in Eq. (8) represents insulin in the remote compartment (interstitial fluid) and is basically a smoothed (and lagged) profile of suprabasal plasma insulin, where $I(t)$ is obtained by linear interpolation of the experimental data of plasma insulin concentrations determined by radioimmunoassay. The parameter that defines the remote insulin dynamics, $P_2$, was fixed to the value estimated from the IVGTT experiments such that $P_2 = e^{-3.426121} = 0.0325$ min$^{-1}$. The glucose effectiveness parameter $S_G$, was initially not assumed to be the same for cold and hot glucose; however, estimates from $^3$H-glucose and $^3$H-H$_2$O kinetics showed that $S^*G$ was not statistically different from the FSIVGTT experiments. The unique values of $S_G$, actually the logarithm of $S_G$, were estimated from the data.

The insulin sensitivity index, represented by $S_I$ (1 min$^{-1}$ x pmol$^{-1}$), was found to be different between cold and hot glucose kinetics. The differences were described using log-transformations as follows: \( \log \hat{S}_I = \log S_I^* + \delta \log S_I \).

The equations of $^3$H-Glucose in Eq. (9) have the basal infusion implicitly contained in $u_g^*(t)$, while basal glucose turnover for cold glucose is expressed by the term $S_G G_b$. The volume for $^3$H-Glucose is expressed in ml. With regard to the production of $^3$H-H$_2$O, Eq. (10) expresses that a fraction $K_w$ of all $^3$H-Glucose disappearance is diluted in total body water $V_{bw}$. In particular, $V_g G^*(t)$ is the amount (mass) of $^3$H-Glucose in the glucose compartment with volume $V_g$, $(S_G + S_I)*Y(t)$ is its elimination rate, and $V_{bw}$ is the new distribution volume.

Non-linear mixed effects models were used for fitting all available data sets simultaneously.

**FSIVGTT MINMOD**

The model in its uniquely identifiable parameterization is described by
\[
\dot{g}(t) = -[p_1 + x(t)] g(t) + p_1 g_b \quad g(0) = g_b + D/V
\]
\[ x(t) = -p_2x(t) + p_3[I(t) - i_b] \quad x(0) = 0 \]  (11)

where \( g \) is plasma glucose concentration (\( g_b \) denotes its basal end test value), \( i \) is plasma insulin concentration (\( i_b \) denotes its basal end test value), \( D \) is the glucose dose in the bolus, \( V \) is the glucose distribution volume, \( x \) is insulin action \( [x = (k_4+k_6)i', \text{ where } i' \text{ is insulin in the remote compartment} ] \), and the \( p_i \) values are parameters related to the \( k_i \) values: \( p_1 = k_1+k_5, p_2 = k_3, p_4 = k_2(k_4+k_6) \).

Parameters \( p_1, p_2, p_3, \) and \( V \) can be estimated from glucose and insulin data by use of nonlinear least squares parameter estimation techniques (21). From them one can calculate the cold indexes of glucose effectiveness, \( S_G \), and insulin sensitivity, \( S_I \), as
\[
S_I = \frac{p_3}{p_2} = \frac{k_2(k_4+k_6)}{k_3} \text{ (min}^{-1} \times \text{U}^{-1} \times \text{ml}) \quad (12)
\]

\( S_G \) and \( S_I \) measure the effects of glucose and insulin, respectively, on both glucose disappearance (\( R_d \)) and endogenous glucose production (EGP). In fact, because \( S_G \) is a function not only of \( k_1 \), but also of \( k_5 \) (see Fig. 2.5), it measures the ability of glucose at basal insulin to stimulate \( R_d \) and to inhibit EGP. Similarly, \( S_I \) is a function not only of \( k_1, k_3, k_4 \), but also of \( k_6 \), and thus measures the ability of insulin to enhance the glucose stimulation of \( R_d \) and inhibition of EGP. Parameter \( p_2 \) is the rate constant of the remote insulin compartment and governs the speed of rise and decay of insulin action.

**Results**

**Determination of Plasma D-[3-3H]-glucose distribution after bolus administration**

The data and best model predictions for “Total” and “Glucose” radioactivity concentrations are shown in figure 2.7. Individual parameter estimates obtained for each cat are reported in figure 2.8 after backtransformation to the original units. The confidence intervals of the actually estimated (log-transformed) parameters are shown in fig. 2.9.
Mixed-effects model parameter estimates

The final nonlinear mixed-effects model deemed to provide the best description of the experimental data is summarized in figure 2.10 (only fixed effects are reported). The information provided in figure 2.10 is related to the fixed effects $\alpha_k$ in Eq. (6) In particular, one can predict the kinetic parameters according to the following equations

\[
\log(k_{02}) = -3.161 - 0.238 \times I_{\text{obese}}
\]  \hspace{1cm} (13)

\[
\log(V_{\text{tot}}) = 6.797
\]  \hspace{1cm} (14)

\[
\log(f_{Kc}) = -2.034 - 0.485 \times I_{\text{obese}}
\]  \hspace{1cm} (15)

\[
\logit(f_{v}) = -1.388 + 0.625 \times I_{\text{obese}}
\]  \hspace{1cm} (16)

\[
\log(V_{3}) = 8.211
\]  \hspace{1cm} (17)

\[
\logit (f_{G}) = 1.697
\]  \hspace{1cm} (18)

where $I_{\text{obese}} = 1$ for obese and 0 for lean cats.

By backtransforming, we obtain the equations to predict individual parameter values depending on the body size (lean vs obese):

\[
k_{02} = 0.0424 \times 0.788 \times I_{\text{obese}}
\]  \hspace{1cm} (19)

\[
V_{\text{tot}} = 895.4
\]  \hspace{1cm} (20)

\[
f_{Kc} = 0.131 \times 0.616 \times I_{\text{obese}}
\]  \hspace{1cm} (21)

\[
f_{v} = 0.199 \times 1.592 \times I_{\text{obese}}
\]  \hspace{1cm} (22)

\[
V_{3} = 3681
\]  \hspace{1cm} (23)

\[
f_{G} = 0.8452
\]  \hspace{1cm} (24)

The above equations indicate that the fractional glucose clearance in obese cats is 78.8% that of lean cats (Eq. 19). The total glucose distribution volume can be assumed constant for all cats (Eq. 20). The fractional mass transfer rate coefficient between accessible and remote
compartments, can be quantified with a reduction of 61.6% in obese compared to lean cats (Eq. 21). Similarly the fraction of the accessible pool increases in obese cats to 159% of that in lean cats (Eq. 22).

The total body water can be assumed constant (Eq. 23), and finally the average efficiency in recovering $^3$H-Glucose with the drying procedure is only 84.5% (Eq. 24).

**Hyperinsulinemic Euglycemic Clamp**

These results have been obtained by using the minimal model approach for both cold and tracer glucose kinetics. The best model fit of the experimental data of cold glucose, $^3$H-Glucose and $^3$H-H$_2$O are shown in figure 2.11, 2.12 and 2.13, respectively. The interpretations of the results presented in the figure 2.14 are the following:

\[
\log S_G = -3.672 - 0.1136 \cdot BW
\]

\[
\log S^* = -10.75 - 0.6969 \cdot I_{obese}
\]

\[
\log \tilde{S} = \log S^* + 0.7395
\]

Where $I_{obese} = 1$ for obese and 0 for lean cats.

By backtransforming the above equations the following relationships were obtained:

\[
S_G = 0.0254 \cdot 0.893BW
\]

\[
S^* = 2.144 \cdot 10^{-5} \cdot 0.50 I_{obese}
\]

\[
\tilde{S} = 2.095 \cdot S^* = 4.492 \cdot 10^{-5} \cdot 0.50 I_{obese}
\]

These model integration solutions predict: (i) a variable glucose effectiveness dependent on body weight, e.g. $S_G = 0.0181$ min$^{-1}$ at 3 kg and 0.0115 at 7 kg; (ii) in obese cats, a 50% reduction of insulin sensitivity of (both tracer and cold) glucose disappearance; and (iii) an insulin sensitivity for cold glucose twice that of tracer glucose, both for lean and obese cats (2.15). The decrease in
insulin sensitivity in obese cats was significant. Insulin sensitivity (1/min)(pmol/L) for obese cats was 0.27 ± 0.002, whereas it was 0.59 ± 0.09 in lean cats (p < 0.002).

**Frequently Sampled Intravenous Glucose Tolerance Test (IVGTT)**

Individual Parameter estimates obtained with the MINMOD, without log-transformation of parameters, and within a traditional non-linear least squares approach are reported in 2.16. It shows that for one data set the procedure failed to give an estimate, and that for one cat the estimated sensitivity was negative.

**Nonlinear mixed-effects model**

The final nonlinear mixed-effects model chosen as the most appropriate description of the experimental data is summarized in 2.17. Results basically confirm the observations made above (2.16) on the basis of individual estimates.

Figure 2.11 indicates that the fixed effects are highly significant, in particular that the size of the cat (lean vs obese) has a significant effect on insulin sensitivity. For the average lean cat in this study, insulin sensitivity of $e^{lnSI (intercept)} = e^{-9.634853} = 6.54 \times 10^{-5}$ can be expected, whereas for the average obese cat, the expected sensitivity will be $e^{lnSI (intercept)} + lnSI_{size obese} = -9.63 - 1.65 = 1.26 \times 10^{-5}$ or 80% less than for the lean cats. There was great inter-individual variability in $SI$. The presence of random effects for $lnSI$ and $V_G$ means that the actual individual parameter estimates will differ from those predicted by the fixed effects alone. In figure 2.16 the mixed effect is clarified by evaluating the contribution of the obese state, by multiplying the individual intercept term by 0.192 when required, we obtain the individual parameter estimates reported in figure 2.17. The model fits are fair in most cases, and the impact of the population modeling approach can be appreciated. In particular, the non-linear mixed effects approach provided estimates for all
cats, including the one in which the algorithm failed with the traditional estimation approach based on individual data.

**DISCUSSION**

Obesity is a disease that is characterized by a change in insulin secretion and action in many species and many obese patients progress to diabetes. It seems therefore important to detect early abnormalities and to be able to identify those patients that are at risk to develop complications from obesity. The IVGTT has been used in cats to measure insulin secretion and action but is not an ideal test for this purpose because the results are actually a reflection of insulin secretion and action. Unless very similar populations are studied, comparisons of insulin sensitivity with IVGTT among subjects with potentially different insulin secretory dynamics are questionable.

The first aim of our study was to determine the inter-animal variability of kinetic parameters and the possible effect of body size. Our results indicate that the average fractional mass transfer (fkc) rate coefficient between accessible (or superficial) and remote (or deep) compartments was reduced in obese cats compared to lean cats and the fraction of the accessible pool (fv) increases in obese cats. The two parameters are negatively correlated. This is in agreement with a “functional” rather than “anatomical” interpenetration of the two compartments in the model similar to what has been observed in human dialysis patients (22). If the paradigm is accepted that inter-compartment mass transfer, fKc, is related to circulatory transport phenomena and peripheral perfusion, then a reduced fKc can be accompanied by an increase of the apparent volume of the accessible pool, which should be in general larger than vascular space if mixing is not a problem.

Except for the glucose distribution volume, V_{tot}, and the water distribution space, V_3, there was a clear separation of values of parameters between lean and obese cats. Obesity led to a reduction
in lnfc, and logitfv, and most importantly, a reduction of fractional glucose clearance, \( k_{02} \). The glucose distribution volume did not change significantly between lean and obese cats suggesting that glucose distribution volume is primarily related to lean body mass. From a technical perspective, it also would mean that the glucose dosage that is used for intravenous glucose tolerance testing in cats should be based on the animal’s ideal, and not actual, body weight. It needs to be investigated if this is also true for other drugs in cats as has been shown in humans (23).

Our second goal was to examine and compare the utility of the EHC, the gold standard method, with the FSIVGTT-MINMOD to evaluate insulin sensitivity and to determine glucose turnover with the analysis of isotopic glucose metabolism to water. To our knowledge, neither test has been performed to evaluate insulin sensitivity in obese cats. The EHC technique was developed in the late 1970’s by DeFronzo and colleagues (6). This method has the advantage that plasma insulin concentrations are kept at high levels and glucose plasma concentrations at basal levels, therefore disrupting the glucose-insulin feedback. With this approach, the response of beta cells to glucose is obliterated, and the tissue response to insulin can then be quantified (6). Since the skeletal muscle is the major organ responsible for insulin mediated glucose disposal (24), the EHC can be used to accurately measure tissue insulin sensitivity (14). Data from EHC have been analyzed with compartmental and non-compartmental models, whose advantages and disadvantages have been discussed in detail (25-30). A minimal model approach was used for both cold and tracer glucose kinetics in this study. Although this may not represent the optimal choice for each measure, the analysis suffers at least from the same modeling bias. The EHC results of this study indicate that glucose sensitivity in obese cats is approximately 50% of that of lean cats and is negatively correlated with body weight. A high negative correlation has also
been found in people between body mass index and insulin sensitivity (31). Interestingly, the insulin sensitivity for non-radioactive glucose was double that of radioactive glucose. It is possible that tracer estimates of insulin sensitivity may reflect only glucose disappearance while the estimate of insulin sensitivity based on total glucose also includes the suppression of hepatic glucose output. Our results also indicate that under the EHC conditions that were used in cats, the majority of insulin-dependent glucose disposal is attributable to glycolysis.

Increasing body weight led to a reduction in insulin sensitivity that was accompanied by a reduction of glucose effectiveness which is defined as the efficiency of insulin-independent glucose removal that is glucose production and utilization at basal insulin concentrations. There is much controversy about the contribution of the different mechanisms involved in glucose effectiveness. While some investigators have found that glucose uptake plays the primary role with equal stimulation of glycolysis and glycogen synthesis (32), others have found that inhibition of hepatic glucose output accounts for the majority of glucose effectiveness (33). The differences are thought to be in part due to different testing methods. In a study in dogs where results from EHC and IVGTT were compared, it was found that >70% of glucose effectiveness was due to glucose’s action on glucose uptake (34). The glucose effectiveness $S_G$ in lean cats was similar to that of lean people; however, it decreased with obesity in the cats of this study and also in monkeys (35), whereas it increased with obesity in horses (36), and did not change significantly in obese humans (30). The reason for the difference is not clear. It suggests that a decrease in insulin sensitivity might be more detrimental in cats and monkeys than horses because it is not met by an increase in glucose-induced glucose removal.

In the present study, FSIVGTT results predicted a much larger reduction in insulin sensitivity than the EHC (80% vs. 50%). This is likely due in part to the larger variability introduced by
technical difficulties associated with the administration of the test. We found it easier to administer the EHC in cats than to administer the FSIVGTT. The rapid sampling, especially in the beginning of the FSIVGTT, proved to be very difficult in conscious cats. Our finding that there was an over-collection of samples during the initial part of the FSIVGTT, which were not necessary for data modeling, will make it easier in the future to perform this test.

In summary, the EHC is a sensitive test to assess insulin sensitivity in lean and obese cats. The FSIVGTT is also a feasible test, and will be more user-friendly for conscious cats when decreasing the number of early samples which are not needed for modeling purposes.
Figure 2.1. Weight (kg), BMI (kg/m$^2$), girth (cm) and percent fat of neutered lean (L) and obese male and female cats (OB).

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>6.6 ± 0.79</td>
<td>3.74 ± 0.36</td>
</tr>
<tr>
<td>BMI</td>
<td>62.95 ± 9.4</td>
<td>40.3 ± 4.49</td>
</tr>
<tr>
<td>Girth</td>
<td>45.29 ± 4.9</td>
<td>31.45 ± 6.25</td>
</tr>
<tr>
<td>% of fat</td>
<td>39.38 ± 5.8</td>
<td>14.02 ± 4.67</td>
</tr>
</tbody>
</table>

p value between lean and obese males and females was < 0.002 for all parameters.
<table>
<thead>
<tr>
<th>Component</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8.41</td>
</tr>
<tr>
<td>Protein</td>
<td>34.40</td>
</tr>
<tr>
<td>Ash</td>
<td>6.45</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>31.90</td>
</tr>
<tr>
<td>Calories/g</td>
<td>4.33</td>
</tr>
<tr>
<td>Fat</td>
<td>18.40</td>
</tr>
</tbody>
</table>
Figure 2.3. (hyperinsulinemic euglycemic clamp scheme). Time 0, D-[3-\textsuperscript{3}H] glucose bolus (1), followed by infusion of D-[3-\textsuperscript{3}H] glucose, at 120 minutes insulin bolus (2) followed by D-[3-\textsuperscript{3}H] glucose in saline plus insulin and D-[3-\textsuperscript{3}H] glucose plus 20% "cold" glucose, at the time 125 minutes second bolus (3) of insulin.
Figure 2.2: $y_1(t)$ represents plasma tracer glucose concentration, $y_2(t)$ total tracer measurement, $V_1$ (plasma compartment), $V_2$ (interstitial compartment), $V_3$ (water distribution space), $K_c$ (intercompartmental mass transfer coefficient) and $K_{02}$ (fractional glucose clearance rate).
Figure 2.5 Minimal Model of cold glucose kinetics.
Figure 2.6 Minimal model of $^3$H-glucose and $^3$H-H$_2$O kinetics.
Figure 2.7: “Total” and “Glucose” Radioactivity measurements with best model predictions.
Figure 2.8: Individual parameter estimates after backtransformation:

<table>
<thead>
<tr>
<th>Cat Number</th>
<th>$K_{02}$ (1/min)</th>
<th>$V_{tot}$ (ml)</th>
<th>$f_{kc}$</th>
<th>$f_{v}$</th>
<th>$V_3$ (ml)</th>
<th>$f_g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02398</td>
<td>1320</td>
<td>0.03019</td>
<td>0.2952</td>
<td>3953</td>
<td>0.7441</td>
</tr>
<tr>
<td>2</td>
<td>0.02632</td>
<td>942.9</td>
<td>0.07984</td>
<td>0.2454</td>
<td>3248</td>
<td>0.8779</td>
</tr>
<tr>
<td>3</td>
<td>0.0451</td>
<td>882.2</td>
<td>0.1452</td>
<td>0.2103</td>
<td>3621</td>
<td>0.9209</td>
</tr>
<tr>
<td>4</td>
<td>0.04835</td>
<td>832.4</td>
<td>0.1415</td>
<td>0.186</td>
<td>3618</td>
<td>0.8304</td>
</tr>
</tbody>
</table>
Figure 2.9: Confidence intervals of individual (log-transformed) parameter estimates.
Figure 2.10. Parameter estimates (fixed effects) of tracer glucose and $^3$H-H$_2$O kinetics model.

<table>
<thead>
<tr>
<th>Par.</th>
<th>Val.</th>
<th>Sdt. Err.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lnk02.(Intercept)</td>
<td>-3.161</td>
<td>0.09408</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Lnk02.sizeobese</td>
<td>-0.234</td>
<td>0.1125</td>
<td>0.035</td>
</tr>
<tr>
<td>ln Vtot</td>
<td>6.797</td>
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Figure 2.11: Best model fit of cold glucose concentration data.
Figure 2.12. Best model fit of $^3$H-Glucose concentrations.
Figure 2.13: Best model fit of $^3$H-H$_2$O concentrations.
Figure 2.14. Reported the parameter estimates fixed effects, characterizing at the population level the kinetics parameters and the effects of covariates.

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Figure 2.15. Insulin Sensitivity from the EUC, lean vs obese cats.
Figure 2.16: Individual parameter estimates for the original MINMOD. $P_2$ insulin turnover rate constant, $S_G$ glucose effectiveness, $S_I$ insulin sensitivity index, and $V_G$ apparent initial glucose distribution volume. (NA = not available).

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<tr>
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<th>$S_I$ (1/min)(pmol/l)</th>
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Figure 2.17. Individual parameter estimates in original units after back transformation of the nonlinear mixed-effects model using MINMOD. $P_2$ insulin turnover rate constant, $S_G$ glucose effectiveness, $S_I$ insulin sensitivity index, and $V_G$ apparent initial glucose distribution volume

<table>
<thead>
<tr>
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References


CHAPTER 3

LIPID METABOLISM, RESPIRATORY EXCHANGE RATE AND HEAT PRODUCTION DURING THE EUGLYCEMIC HYPERINSULINEMIC CLAMP IN LEAN AND OBESE CATS\textsuperscript{1}.

\textsuperscript{1}Brandao J, Thomaseth K, Waldron M, Ferguson DC, Campbell J, Hoenig M. To be submitted to \textit{American Journal of Comparative Endocrinology}.
**Introduction**

Obesity is a growing problem in people and pets. There is strong evidence that changes in fat and glucose metabolism are important factors in the development of obesity, insulin resistance, and diabetes (1). We have previously shown that the majority of obese cats, despite showing glucose intolerance had greater suppression of non-esterified fatty acids (NEFAs) during an intravenous glucose tolerance test (IVGTT) than lean cats. These findings suggested a substrate dependent difference in insulin sensitivity caused by obesity. Because the IVGTT is a dynamic test, it is possible that the difference was attributable to the afferation the insulin secretion pattern (i.e. prolonged and exaggerated second phase insulin release in obese compared to lean cats during the IVGTT). This study was designed to examine the effect of insulin on fatty acid clearance and lipid turnover in lean and obese cats at constant insulin infusion rates using the euglycemic hyperinsulinemic clamp (EHC). By adding tracer (\(^\text{\textsuperscript{2}}\text{H}_5\)-glycerol) during the EHC, the rate of lipolysis can be measured. The lipolysis rate would be expected to be more suppressed under hyperinsulinemic conditions in normal compared to insulin-resistant subjects (2). To verify substrate utilization and to examine the influence of insulin on substrate oxidation, indirect calorimetry was employed during the EHC. Calorimetry was first described by Lavoisier and LaPlace (1780) as referenced by Hess in 1840 (3). Indirect calorimetry has been used to measure respiratory exchange rate (RER) and calculate heat production by indirect means, based on the O\(_2\) consumption and CO\(_2\) production (4). The indirect calorimetry technique has now been widely used to determine energy production and substrate oxidation in a variety of species different species (5-8).
Combining the EHC technique with tracer infusion and indirect calorimetry, complex metabolic processes can be evaluated (9). The goal of this study was to use these relatively non-invasive techniques to examine the effect of insulin on lipid turnover, substrate oxidation, and heat production in lean and obese cats.

**Materials and Methods**

**Animals and Diets.** Twenty-two neutered adult age-matched Domestic Shorthair cats (Sinclair Research Center, Columbia, MO and Harlan Sprague Dawley, Madison, WI) were used for these studies. There were 12 obese cats with an average weight of 6.6 ± 0.6 kg (6 females, 6.3 ± 0.7 kg; 5 males, 7.1 ± 0.7 kg), and 10 lean cats with an average weight 3.8 ± 0.3 kg (5 females, 3.7 ± 0.2 kg; 5 males, 3.8 ± 0.4 kg). 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.

All animal studies were approved by the University of Georgia Animal Care and Use Committee and were 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. Animals were deemed healthy based on the results of physical examination and clinical laboratory data. All cats were accustomed to daily handling and were fed the same diet once daily. The composition of the diet is shown in Figure 3.3. The cats were weighed twice weekly.

Deuterium Glycerol, D-[\(^2\)H\(_5\)] was supplied by Medical Isotopes (Pelham, NH); regular insulin was supplied by Eli Lilly (Indianapolis, IN). BSTFA (N,O-Bis(trimethylsilyl trifluoroacetamide), and pyridine (derivatization grade) were purchased from Sigma (St.
Louis, MO). Vial inserts (100 µl pulled point glass) were from Agilent Technologies (Palo Alto, CA). The ion-exchange columns were from Bio-Rad Laboratories (Hercules, CA). They consisted of AG5-W-X8, 200 – 400 mesh bottom cation exchange resin in hydrogen form (50:50, v/v; water-resin), and AG-1-X8, 200 – 400 mesh top anion exchange resin (50:50, v/v).

**Euglycemic hyperinsulinemic clamp**

To allow blood sampling, catheters were placed in the jugular vein of cats 36-40 hours before the study and in the cephalic vein prior to the clamp. Catheter patency was maintained by injection of 0.5 ml of 0.38% sterile citrate flush (citric acid, trisodium salt dihydrate, Sigma Co., MO) into the catheter every 8 hours. Whole blood was taken through the catheter and allowed to clot for serum collection and infusions were administered via cephalic catheter. For the collection of plasma, blood was placed into tubes containing ethylenediamine tetra-acetic acid (EDTA), and the samples were centrifuged immediately. Serum and plasma were stored at -20°C until assayed. Mixed venous samples were drawn for D-[\(^{2}\text{H}_5\)]Glycerol, glucose, insulin, and non-esterified fatty acid determinations. An EHC was performed according to the protocol described in figure 3.1: a time 0, a bolus of D-[\(^{3}\text{H}_5\)]glycerol in saline containing 1.6 µmol/glycerol/Kg/body weight (BW) was administered IV followed by an IV infusion of D-[\(^{2}\text{H}_5\)]glycerol in saline containing 10 µmol/ml/hour. The saline infusion was continued for 120 min to insure isotope equilibration. At time 120 min, the clamp was started and lasted for 120 minutes using the protocol described by Petrus et al (10) with the following modifications: a time 120 min, an initial insulin bolus of 26 mU/ml/kg BW recombinant human insulin (Humulin R, Eli Lilly, Indianapolis, IN), was administered IV and a
solution of glucose (20% w/v) was infused for 120 min at a rate necessary to maintain the cats’ fasting blood glucose concentrations (euglycemia). This was achieved by measuring whole blood glucose with a glucometer (Elite XL, Bayer Corporation, Mishawaka, IN) at least every 15 min. At time 125 min a second bolus of insulin (13mU/kg BW) was administered IV followed by the infusion of insulin (1.1mU/min/kg BW) containing D-[\textsuperscript{2}H\textsubscript{5}] glycerol (10µmol/ml) for the remainder of the clamp. Plasma D-[\textsuperscript{2}H\textsubscript{5}] glycerol, glucose, insulin, and non-esterified fatty acids (NEFA) were measured at -5, 0, 30, 60, 90, 120, 150, 180, 210, 240 min. Insulin concentrations were measured as described (11) Glucose measurements were performed using a colorimetric glucose oxidase method (glucose trinder kit; Diagnostic Chemicals Limited. Charlottetown, PE, Canada). Serum concentrations of non-esterified fatty acids (NEFA) were measured by use of an enzymatic test kit (NEFA C; Wako Diagnostic, Richmond, PA).

**D-[\textsuperscript{2}H\textsubscript{5}] Sample Preparation**

Samples were prepared in duplicate for measurement of D-[\textsuperscript{2}H\textsubscript{5}] glycerol by mixing 0.4 ml of plasma with 1.8 ml of ice-cold 70% acetone and incubating it on ice for 30 min. The samples were then centrifuged at 1850 x g for 10 minutes and the supernatant fraction was transferred to a 13 x 100 mm polypropylene tube and dried under air in a 45°C water bath. Samples were reconstituted in 0.5 ml of water and loaded onto an ion exchange column prepared in the following manner. The columns were prepared by placing a glass wool plug into the bottom of a Pasteur pipette and rinsing it with 1ml of water. Cation resin (1.5 cm; Dowex AG5-W-X8, 200 – 400 mesh bottom) was placed on the top of the plug and washed with 10 ml of water. Anion resin (1.5 cm; Dowex AG-1-
X8, 200 – 400 mesh top) was added and the column was washed again with 10 volumes of water.

The sample was eluted four times with water (1ml each) and the eluate was frozen in an ethanol dried ice bath and then lyophylized to dryness.

**Derivatization**

A mix of 400 µl of a solution containing a 2:1 (v:v) ratio of pyridine and BSTFA was added to the samples followed by heating for 15 min at 75°C. The samples were placed on ice and the mix was dried under a low flow of nitrogen gas to approximately 50µl and reconstituted in 100µl of methylene chloride for injection onto a HP 5890 series II plus/5971A MS.

**D- [²H₅] Glycerol GC-MSD conditions.**

Samples in methylene chloride were injected (1µl spitless injection) onto a HP 1701 (14% CNPRH ME Siloxane column 30 m, 250 µm nominal diameter, 0.25 µm film thickness) at a flow rate of 1.3 ml/min. The oven initial temperature was 80°C for 10 min and then increased at 10°C/min increments to a final inlet temperature of 260°C which was held for 10 min. The detector temperature was 300°C. The run time was 29 min and the ions were monitored at 205 M/z for natural glycerol and 208 M/z for D-5 glycerol.

**Indirect Calorimetry**

The width and height of the calorimetry chambers was 20 inches and the length was 16 inches. The chamber operated at a flow of 5 liters per minute. A silica drying column was used to equilibrate the humidity.

RER, heat, and flow of air through the calorimetry chamber (liters per minute) were measured using an open-circuit Oxymax™ System (Software, version 5.0., Columbus
Instruments, Columbus, OH). System settle time for each chamber was equal to 60 seconds, and system measure time for each chamber was equal to 30 seconds. Calibration of the calorimeter chambers was performed daily using standard gas mixtures against known calibration gas standards. Room temperature was maintained at 25 ± 1°C.

The following calculations were used:

\[
\text{RER} = \frac{\text{liters CO}_2 \text{ produced}}{\text{liters O}_2 \text{ consumed}}
\]

Heat production (kcal) = 3.82 x liters O\(_2\) consumed + 1.15 x liters CO\(_2\) produced.

The volume of O\(_2\) consumed was determined from the accumulated O\(_2\) volume at a given time minus the volume of O\(_2\) consumed at time 0. The volume of CO\(_2\) consumed was determined from the accumulated the volume of CO\(_2\) at a given time minus the volume of CO\(_2\) consumed at time 0.

The heat production was normalized to metabolic body size by dividing by body weight elevated to the 0.75 potency.

\[
\text{Heat/ Metabolic Body Size (kcal/kg)} = \frac{\text{Heat Production}}{(\text{Body Weight})^{0.75}}
\]

**NEFA Calculations**

NEFA concentrations collected were measured and the results modeled according to Thomaseth et al (12) with the following modifications:

\[
\text{NEFA}(t) = (1 - \rho) \text{NEFA}_b
\]

where \(\text{NEFA}_b\) represents the concentration at basal insulin. This equation implicitly assumes that NEFA concentrations are inhibited by insulin by a fixed fraction of the basal concentration (\(\rho\) quantifies the fraction of reduction).

**Glycerol Calculations**
The ratio of natural glycerol over D-[\(^2\text{H}_5\)]glycerol was calculated based on the peak height detected in the GCMS. The Glycerol appearance ratio was calculated based on the formulation used by Boden et al (3).

\[ \text{Ra} = (\text{IE}_{\text{inf}}/\text{IE}_{\text{pla}} - 1) \times F \]

Where Ra is the appearance of glycerol (\(\mu\text{mol} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}\)) liberated during lipolysis, \(\text{IE}_{\text{inf}}\) is the isotopic enrichment of the infusate (APE), \(\text{IE}_{\text{pla}}\) is the isotopic enrichment of plasma (APE) at equilibrium, and F is the isotope rate of infusion (\(\mu\text{mol} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}\)).

**Results**

**Non-esterified fatty acids**

The average NEFA plasma concentration in lean and obese cats before and during the EHC is shown in figure 3.2. The average baseline NEFA concentration (mean \(\pm\) SD) in lean cats was 0.35 \(\pm\) 0.06 mEq/L and in obese cats 0.43 \(\pm\) 0.05 mEq/L. The NEFA concentrations trended lower in the lean cats but no statistical difference was found between lean and obese cats. However, obese male cats had significantly higher baseline concentrations than obese females (0.37 \(\pm\) 0.08 mEq/L females and 0.46 \(\pm\) 0.04 mEq/L males, \(p=0.03\)). The estimate of \(\rho_{\text{NEFA}}\) at population level (fixed effect) was 0.507 \(\pm\) 0.054 (SE) (\(P<0.0001\), with a between animal variation (random effect) of \(\sigma_{\rho_{\text{NEFA}}}=0.224\) (Std. Dev.). The standard deviation of the prediction error (modelling error) was \(\sigma=0.0374\). There were no statistically significant effects on \(\rho_{\text{NEFA}}\) of gender body condition. In addition there was no significant difference in the fractional NEFA inhibition between lean and obese cats. The % suppression of NEFA concentrations during insulin infusion was significantly greater in obese cats than in lean cats (\(p=0.015\)); however, there was no gender difference.
**Glycerol appearance rate**

The average appearance rate for glycerol (Ra; \(\mu\text{mol} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}\)) was not significantly different between lean and obese cats during the infusion (figure 3.4). Ra during the first 120 min of the glycerol infusion was 168 ± 15 in lean cats and 178 ± 51 in obese cats. During the insulin infusion (time 120-240 min) Ra was 120 ± 14 in lean cats and 122 ± 37 in obese cats. The suppression of Ra during the insulin infusion was significant for both lean and obese cats (56.8 ± 0.3% for obese cats and 55.5 ± 0.5% for lean cats). There was no significant gender difference during the 4 hours of infusion.

**Calorimetry**

The indirect calorimetry is summarized in (figure 3.5). Obese male cats had higher RERs during the infusion compared to obese females; however, this was only significant for the time of insulin administration (0.97 ± 0.03 vs 0.90 ± 0.04, respectively; \(p < 0.006\)), suggesting that obese males were more similar to lean cats in their response to insulin. Lean males trended toward having lower heat/MBS than lean females during the first 120 min of the experiments (46.7 ± 6.9 and 53.6 ± 7.9, respectively; \(p = 0.088\)) and during the insulin infusion (44.9 ± 7.6 and 52.8 ± 5.9; \(p = 0.053\)). There was no gender difference in heat/MBS in obese cats.

**Discussion**

Results from this study support our laboratory’s previous results which showed a trend for obese cats to have higher baseline NEFA concentrations than lean cats indicating higher rates of lipolysis; the difference was significant when cats were separated by gender because obese males had significantly higher baseline NEFA concentrations than obese females in both and the previous study (18). Obese cats also had higher baseline Ra
values consistent with higher lipolysis. Lean and obese cats generally oxidized lipids in
the basal state. Similar results were seen in lean and obese people after an overnight (8,
14, 29). Similar to findings in the cats of this study, it was also found in lean humans that,
during the EHC, insulin suppressed fatty acid oxidation (13). Obese cats, however,
continued to oxidize more fatty acids than lean cats. Obese females had lower RER
values than obese males cats during insulin infusion suggesting that females have a
higher capacity to oxidize fat than obese males. However male cats increased their RER
to a higher level than obese females in response to insulin. This suggests that females
have a lower capacity to synthesize glycogen. It is therefore possible that the lower
NEFA concentrations seen in obese female cats compared to obese males are due to a
higher oxidation rate rather than a lower lipolysis rate. However, without the
measurement of fatty acid uptake, this remains unclear. Gender differences in fatty acid
oxidation have also been seen in humans: women have higher rates of fat oxidation (20,
2, 33).

The higher lipid oxidation in obese cats compared to lean cats may be caused by nutrient
competition as first described by Randle in 1963 (19). He suggested that high levels of
free fatty acids (FFA) in blood increase the levels of acetyl-CoA and citrate, thereby
decreasing glycolysis. Boden et al (2) showed that FFA cause dose-dependent inhibition
of insulin stimulated glucose uptake by decreasing glycogen synthesis and carbohydrate
uptake leading to insulin resistance at the peripheral and hepatic level. Roden and
coworkers (15) proposed that FFA inhibit glucose phosphorylation which causes a
reduction in glucose uptake, muscle glycogen synthesis, and glucose oxidation.
Lean cats had higher baseline heat production per MBS than obese cats during baseline and during the insulin infusion. Similar findings were reported in lean and obese people before and after a meal (16, 22) and after insulin infusion (17, 23). Because resting metabolic rate accounts for 60-70% of the total daily energy expenditure (28), a decrease would be reflected in a large increase in body weight over time if food intake was not adjusted appropriately. The high percentage of fat mass present in obesity (1, 18) and the fact that adipose tissue is less metabolically active than other tissue (3) resulting the lower heat production per metabolic body size present in obesity (22). The diminished capacity for thermogenesis (16, 25) in obesity contributes to an increased metabolic efficiency, i.e. more calories are stored per calories ingested (22). The reduction in insulin-stimulated thermogenesis was found to be an early event in the development of type 2 diabetes in Pima Indians (24). Part of the impaired thermogenesis in obesity has been attributed to a change in uncoupling proteins (30). These proteins are expressed in many tissues and are able to uncouple ATP production from mitochondrial respiration, leading to the dissipation of energy as heat. Uncoupling protein 3 (UCP3) gene expression was found to correlate negatively with body mass index in people (31). A positive correlation between UCP-3 expression and whole-body insulin-mediated glucose utilization rate was noted in type 2 diabetics suggesting that UCP3 regulation may be altered in states of insulin resistance (32).

The blunted thermic effect of infused glucose and insulin during the EHC in obese subjects is related to two major components (23). There is a reduced capacity of glucose disposal and glucose storage (17, 23, 26), with the latter being the most thermogenic component (26, 27). It is known that during an EHC more than one half of the increase in
energy expenditure is due to glucose storage, i.e. glycogen synthesis (26, 23) which is decreased in obese states (23).

There is also an impaired capacity to suppress hepatic glucose production in obesity mainly due to tissue insulin resistance (26). In fact, an inverse relationship has been proposed between insulin resistance and the thermic effect of insulin and glucose infusion in obese and non-insulin dependent diabetic patients (17, 23).

In summary, obesity in cats is characterized by increased lipid oxidation which is maintained in females during insulin infusion but not males. The lower heat production in lean males combined with a decreased ability to oxidize fatty acids in response to insulin may predispose them to obesity. The lower metabolic rate of obese cats of either gender favors an increase in body weight in the face of unaltered food intake.
Figure 3.1: At time 0, a bolus of D-[^2H_5] glycerol in saline containing 1.6 µmol/ D-[^2H_5] glycerol/Kg/body weight (BW) was administered IV followed by an IV infusion of D-[^2H_5] glycerol in saline containing 10 µmol/ml/ D-[^2H_5] glycerol. The saline infusion was continued for 120 min. At time 120 min, the clamp started and lasted for 120 minutes.
Figure 3.2. Average NEFA (non-esterified fatty acids) lean and obese cats at the time 120 the EHC started the arrow represents the beginning of the insulin infusion
Figure 3.3. Diet composition (g/100g).

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<td>Fatty acids</td>
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Figure 3.4. Average Ra glycerol at the time 120 the EHC started
Figure 3.5: Mean ± SD of RER, Heat and Heat/ MBS/Kcal/kg^{0.75}/24hr, for lean and obese cats. ¹ Heat per metabolic body size/ kcal/ kg^{0.75}/24h.

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<td>0.84 ± 0.07^a</td>
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<td>RER 120-240</td>
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<tr>
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<td>132.1 ± 7.7^d</td>
<td>162.4 ± 6.3^d</td>
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<tr>
<td>¹Heat/MBS 0-120</td>
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<td>40.44 ± 27.5^e</td>
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<tr>
<td>¹Heat/MBS 120-240</td>
<td>48.9 ± 22.7^f</td>
<td>39.4 ± 33.6^f</td>
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^a p < 0.009; ^b p=0.042; ^c p=0.013; ^d p=0.027, ^e p=0.003; ^¹ p < 0.006
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Obesity in cats and dogs is the most common veterinary nutritional disorder in the U.S. and its incidence is growing at an alarming rate. Obesity in cats, as in humans, is a risk factor for diabetes mellitus and also many obese cats show glucose intolerance and are thought to be insulin resistant.

Insulin resistance is defined as a condition in which the magnitude of the biological response to insulin is decreased. The major hallmarks of insulin resistance are alterations in glucose and lipid metabolism. Together, they lead to an impairment of insulin secretion and action, and may cause type 2 diabetes.

Several methods have been developed to aid in the early detection of changes in insulin secretion, and glucose and lipid metabolism. The goal of this research is to examine methods, such as, the Euglycemic Hyperinsulinemic glucose Clamp (EHC), the Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT) and to examine glucose and lipid metabolism by using labeled (\(^{3}\)H-glucose and deuterated glycerol) and indirect calorimetry, and to compare these results to the “gold” standard for insulin sensitivity, the EHC.

The EHC results of this study indicate that glucose sensitivity in obese cats is approximately 50% of that of lean cats and is negatively correlated with body weight. Interestingly, the insulin sensitivity for non-radioactive glucose was double that of radioactive glucose. It is possible that tracer estimates of insulin sensitivity may reflect only glucose disappearance while the estimate of insulin sensitivity based on total glucose also includes the suppression of hepatic glucose output. Our results also indicate that under the EHC conditions that were used in cats, the majority of insulin-dependent glucose disposal is attributable to glycolysis.

The results of this research showed that the EHC is a sensitive test to assess insulin sensitivity in lean and obese cats and that the FSIVGTT is also a feasible test, and will be more user-friendly
for conscious cats when decreasing the number of early samples which are not needed for modeling purposes.

The other part of our study was to examine the effect of insulin on fatty acid clearance and lipid turnover in lean and obese cats at constant insulin infusion rates using the euglycemic hyperinsulinemic clamp (EHC). By adding tracer ($^{2}$H$_{5}$-glycerol) during the EHC, the rate of lipolysis can be measured and to verify substrate utilization and to examine the influence of insulin on substrate oxidation, indirect calorimetry was employed during the EHC.

Results from this study support showed a trend for obese cats to have higher baseline non-esterified fatty acids (NEFA) concentrations than lean cats indicating higher rates of lipolysis. Obese males had significantly higher baseline NEFA concentrations than obese females. Obese females had lower RER values than obese males cats during insulin infusion suggesting a higher capacity to oxidize fat than obese males. However male cats increased their RER to a higher level than obese females in response to insulin, it suggests females have lower capacity to synthesize glycogen.

Lean cats had higher baseline heat production per MBS than obese cats during baseline and during the insulin infusion. The diminished capacity for thermogenesis in obesity contributes to an increased metabolic efficiency which would be reflected in a large increase in body weight over time if food intake was not adjusted appropriately.

During the EHC the thermic effect of insulin plus glucose infusion is blunted in obese cats is related to a reduced capacity of glucose disposal and glucose storage and an impaired capacity to suppress hepatic glucose production due to tissue insulin resistance present in obesity.

In summary, obesity in cats is characterized by increased lipid oxidation which is maintained in females during insulin infusion but not males. The lower heat production in lean males combined
with a decreased ability to oxidize fatty acids in response to insulin may predispose them to obesity. The lower metabolic rate of obese cats of either gender favors an increase in body weight in the face of unaltered food intake.