# INFLUENCE OF FATTY ACIDS ON GLUCOSE CLEARANCE, INSULIN SECRETION, AND LIPID METABOLISM IN CATS

by

ELIZABETH CAROLINE WILKINS

(Under the direction of Margarethe Hoenig)

## ABSTRACT

Obesity and insulin resistance is a growing problem among humans and cats and is attributed to an increased consumption of diets high in saturated fat. The purpose of this study was to determine if a diet high in omega-3 polyunsaturated fatty acids (PUFA) would affect glucose clearance, insulin secretion, and lipid metabolism in both the lean and obese cat. Twenty-eight cats were used for the study; 14 were fed the PUFA diet and 14 were fed a diet high in saturated fatty acids (SFA) before and after a 21 week period of ad libitum food intake which resulted in significantly greater weight, body mass index, girth, and % fat in cats of both groups. There was no difference in glucose or insulin baseline concentrations as a result of obesity or diet. However, both glucose and insulin 120 minute concentrations were significantly higher with obesity, regardless of diet. Glucose area under curve (AUC) concentrations were significantly higher with obesity, with no effect of diet. Insulin AUC concentrations were not different with the progression to obesity in cats fed the PUFA diet; however, they were significantly different in obese SFA fed cats compared to lean SFA fed cats and compared to obese PUFA fed cats. Nineteen cats that became glucose intolerant while obese were already exhibiting an altered insulin secretion and a decreased glucose clearance while lean. There was no change as an effect of obesity or diet on cholesterol or triglyceride concentrations but obese cats had significantly more suppression of non-esterified fatty acids than lean cats. The obese cats fed SFA had a significantly higher GHb concentration than the obese cats fed PUFA. Intramyocellular and extramyocellular lipid concentrations increased with obesity and correlated positively with insulin concentrations. It is concluded that a diet high in PUFA has beneficial effects on glucose control and insulin secretion in obese cats.

INDEX WORDS: Obesity, insulin resistance, polyunsaturated fatty acids, omega-3 fatty acids, saturated fatty acids, intramyocellular lipid, extramyocellular lipid

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B.S., Clemson University, 2001

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2003

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August 2003

# ACKNOWLEDGEMENTS

I would like to give special thanks to my major professor, Dr. Margarethe Hoenig, for all of her guidance and support. Without her, this thesis would not have been a success. I also appreciate all of the support I received from the members of my committee, Dr. Duncan Ferguson and Dr. James Prestegard.

My sincere thanks go to Dr. Robert Long from Emory University for all of the hours he contributed to this study and to my understanding of spectroscopy.

I am also indebted to the kindness, generosity, and support of those in my lab, especially Christy Brennan and Corby Holson. Without you two, my time in lab would have been a great struggle.

I would also like to thank my family and friends for being there for me, especially my parents, Donna and Phil. I will be forever grateful for your patience, love, and kindness.

Finally, I would like to thank Nestle Purina, St. Louis, MO, for supporting the work of this study.

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# CHAPTER 1

# INTRODUCTION

Obesity is a result of energy intake exceeding energy expenditure. Concurrent with the boom of obesity in humans throughout the world, obesity is the most common nutritional disorder in dogs and cats in the United States. Obesity in the cat, as in humans, is a risk factor for diabetes mellitus. Many obese cats show glucose intolerance and are thought to be insulin resistant.

Epidemiological studies in man and experimental studies in man and rats have shown that a diet high in fat leads to obesity. However, recent research has shown that the type of fatty acid within the diet determines insulin sensitivity. Saturated fatty acids (SFA), which originate from meat and dairy products, have been shown to decrease insulin sensitivity and cause glucose intolerance, while polyunsaturated fatty acids (PUFA), which come from fish and plant oils, improve insulin sensitivity and glucose tolerance. Further research has revealed that the omega-3 PUFA is the fatty acid responsible for the improved insulin sensitivity.

There has been evidence that diets high in fat induce insulin resistance by increasing muscle triglyceride concentrations. Through the use of $^{1}$H-nuclear magnetic resonance spectroscopy ($^{1}$H-MRS), two compartments of muscle lipid stores have been discovered: intramyocellular lipid (IMCL), located around the mitochondria, and extramyocellular lipid (EMCL), located between the muscle fibers. Evidence is showing

that, with the increased fat deposition of obesity, triglyceride stores in IMCL concurrently increase, and ultimately negatively influence insulin sensitivity.

The objectives of this research were to determine the effects of a diet high in saturated fatty acids and a diet high in omega-3 polyunsaturated fatty acids on the following parameters in experimental cats:

1. glucose clearance
2. insulin secretion
3. lipid metabolism
4. intramyocellular lipid content
5. body fat mass

by performing intravenous glucose tolerance tests and measuring serum glucose, insulin, non-esterified fatty acids, cholesterol, and triglyceride concentrations; performing dual emission x-ray absorptiometry (DEXA) to determine body adiposity; and determining the intramyocellular content by $^{1}$H-MRS.

# CHAPTER 2

# LITERATURE REVIEW

## Obesity and diet

It has been shown that the risk of developing insulin resistance and type 2 diabetes increases with the development of obesity (Ferrannini et al., 1997; Kahn et al., 2001). The increase in the numbers of obese individuals throughout the world has become a growing health dilemma. In affluent countries, the incidence of obesity between the ages of 25 and 55 years is predicted to occur in 10-15% of men and 15-20% of women; however, in the United States, it is estimated that approximately 64.5% of individuals over 20 years of age are overweight and 30.5% are obese (Seidell, 2000; Flegal et al., 2002). It is also predicted that by the year 2025, the number of diabetic individuals will rise from 143 million in 1997 to 300 million (WHO, 1998). Not only are humans dealing with the growing epidemic of obesity, but there is also an increase in obesity in pets. It has been estimated that 20-40 % of pets are overweight (Sloth, 1992; Donoghue and Scarlett, 1998).

The growing rate of obesity has been linked to the increase in the adoption of a "western" diet. The "western" diet is one that is high in fat, especially saturated fat, and therefore highly palatable and dense in calories (Ghibaudi et al., 2002). Because it does not have the same satiety effect as protein or carbohydrate, too much is consumed (Ghibaudi et al., 2002).

Saturated fatty acids are classified as a hydrocarbon chain composed of single bonds attached to a carboxyl group, while the hydrocarbon chain of polyunsaturated fatty acids is composed of two or more double bonds attached to a carboxyl group (Stipanuk, 2000). Saturated fatty acids (SFA) are derived from meat and dairy products and elevate plasma cholesterol, triglyceride, and free fatty acids and decrease insulin sensitivity in both humans and rats (Feskens et al., 1991; Storlien et al., 1996; Summers et al., 2002). However, polyunsaturated fatty acids (PUFA), which are derived from plant and fish oils, are associated with increased insulin sensitivity, a decreased concentration of plasma triglyceride and, although controversial, decreased LDL cholesterol levels while increasing HDL cholesterol levels in humans and rats (Feskens et al., 1991; Storlien et al., 1996; Dobbins et al., 2002; Summers et al., 2002;). The essential fatty acids, i.e. those that the body cannot synthesize, consist of the polyunsaturated fatty acids omega-3 and omega-6. Omega-3 fatty acids are derived from fish oils such as that from salmon and tuna. Omega-6 fatty acids originate from plant oils including safflower oil and corn oil (Stipanuk, 2000). Many studies incorporate both omega-3 and omega-6 fatty acids together when determining the effects on insulin sensitivity and lipid regulation and have therefore concluded that PUFA improve both. However, in studies in which either the omega-3 or the omega-6 fatty acid ratio was increased, most conclude that it is actually the omega-3 fatty acid that improves glucose-stimulated insulin secretion.

**Normal Insulin Secretion**

Glucose is the major stimulus of insulin secretion. Insulin is secreted in a biphasic manner when glucose is administered as a bolus (e.g. in intravenous glucose tolerance

testing, IVGTT) or as a continuous infusion during a hyperglycemic clamp (Luzi and DeFronzo, 1989; Nelson et al., 1990; Kahn, 2001). In cats, the first phase lasts from 0-30 minutes with the peak occurring between 5-15 minutes (Hoenig et al., 2002). The second phase of insulin secretion lasts considerably longer and is sustained for as long as the blood glucose concentration is elevated (Porte, 1991; Hoenig et al., 2000; Kahn, 2001). The first phase of insulin secretion is thought to originate from a pool of insulin stored in secretory granules located close to the plasma membrane of the ß-cell for quick mobilization in times of increased glucose concentrations (i.e. with the consumption of a meal) (Simpson et al., 1968; Grodsky et al., 1970). This rapid first phase of insulin secretion quickly and effectively impairs hepatic glucose production (Cherrington et al., 2002; Del Prato et al., 2002). The second phase of insulin secretion is believed to come from the mobilization of secretory granules that are recruited from the interior of the ß-cell to the plasma membrane as the pancreas responds to the glucose load (Simpson et al., 1968; Grodsky et al., 1970). The action of the second phase of insulin secretion is thought to be primarily upon peripheral tissues so that glucose uptake occurs in these tissues (Cherrington et al, 2002). It has been documented that the effectiveness of the second phase on efficient peripheral glucose uptake and suppression of hepatic glucose output is dependent upon the amount secreted in the first phase (Porte, 1991; Cherrington et al, 2002).

Although most long-chain fatty acids have a short-term insulinotropic effect (Opara et al., 1994; Stein et al., 1997), not all fatty acids act upon insulin secretion in the same way. It is still controversial as to which long-chain fatty acids have the greatest effect on insulin secretion: Opara et al. (1994) found that linoleate had the greatest effect,

while Stein et al. (1997) observed the largest insulinotropic effect from palmitate and stearate. In studies in which rats were fed a high fat diet, it was shown that a diet containing saturated fatty acid (SFA) promoted the greatest glucose-stimulated insulin secretion, compared to a diet containing polyunsaturated fatty acid (PUFA) (Dobbins et al., 2002).

**Normal Insulin Action**

The overall effect of insulin on the tissues primarily responsible for glucose homeostasis – the liver, skeletal muscle, and adipose tissue – is to promote glucose uptake and storage while inhibiting glucose release and lipolysis. Insulin slows hepatic glucose production (gluconeogenesis and glycogenolysis), but increases hepatic glucose uptake and storage (DeFronzo et al., 1983). The effect of insulin on skeletal muscle, the primary tissue responsible for insulin stimulated glucose uptake, is a stimulation of glycogenesis and inhibition of glycogenolysis (Stipanuk, 2000).

When insulin binds to the insulin receptors of adipose tissue and skeletal muscle, tyrosine kinase of the ß-subunit is activated and causes autophosphorylation of the tyrosine residues (Stipanuk, 2000). This phosphorylation then activates a series of enzymes, which, in turn, stimulate the insulin-sensitive glucose transporters (GLUT4) of these tissues (Agote et al., 2001). Upon activation, vesicles containing GLUT4 are translocated to the cell membrane for uptake of glucose. An insulin-insensitive glucose transporter, GLUT1, is responsible for the basal uptake of glucose into skeletal muscle and adipose tissue (Dumke et al., 2001; Ganong, 2001).

Insulin also affects the metabolism of free fatty acids (FFA) by stimulating the synthesis of triglycerides and inhibiting lipolysis in the adipose tissue, therefore lowering plasma FFA. Hormone sensitive lipase (HSL) controls the release of FFA from the adipocyte and is inhibited by insulin. Lipoprotein lipase (LPL) controls the rate of triglyceride synthesis and FFA uptake by adipose tissue and muscle. Although the mechanism is unknown, in the fed state, insulin promotes partitioning of FFA into adipose tissue for storage by stimulating LPL activity in adipocytes (Sadur et al., 1984) and reducing its activity in muscle (Eckel et al., 1995; Yost et al., 1995; Lewis et al., 2002). It is thought that the activity of LPL in adipose tissue and skeletal muscle responds differently to insulin because it seems to reflect the turnover of the triglyceride pool in these tissues (Eckel, personal communication).

To determine the effect of an increased FFA concentration on insulin sensitivity, a study was performed on healthy humans using the euglycemic hyperinsulinemic clamp protocol with simultaneous infusion of lipid and heparin to raise the FFA concentration (Boden et al., 1995). It was shown that within 24 hours of infusion, glucose uptake was greatly inhibited, demonstrating the effect of high concentrations of FFA to induce insulin resistance. However, after 24 hours, these subjects were able to overcome this resistance by hypersecreting insulin. Another study (Homko et al., 2003) using this protocol was performed in healthy women and it was found that after just 4 hours of infusion, insulin-stimulated glucose uptake and glycogen synthesis were inhibited. This study also found that the ability of insulin to suppress endogenous glucose production was almost completely inhibited; thus, this study is another example of how elevated

concentrations of FFA inhibit insulin action in tissues and can ultimately cause insulin resistance in healthy subjects.

**Insulin Secretion in Obesity**

When an individual becomes obese, insulin secretion becomes abnormal. It has been noted that, during IVGTTs and hyperglycemic clamps, obese subjects have a diminished first phase of insulin secretion with a prolonged and exaggerated second phase (Porte, 1991; Hoenig et al., 2000; Kahn, 2001). These changes become more pronounced in the type 2 diabetic person and cat and eventually lead to a complete disappearance of secretion during the first phase and erratic secretion during the second phase of insulin release (Hoenig et al., 2000; Boden, 2001). The first phase of insulin secretion is critical to the effective suppression of hepatic glucose output; furthermore, the efficiency of the second phase to dispose of and metabolize glucose and lipid is dependent upon the regulatory ability of the first phase (Kahn, 2001; Del Prato, 2002). In longitudinal studies, the amplitude of the first phase of insulin secretion is the independent predictor of glucose tolerance (Lundgren et al., 1990; Haffner et al., 1996). Because of the diminished first phase, the effect of insulin on glucose metabolism and lipolysis is diminished in obesity and more so in type 2 diabetic subjects (Kahn, 2001; Del Prato et al., 2002).

Several studies (Lovejoy and DiGirolamo, 1992; Ghibaudi et al., 2002) have attributed the alterations in insulin secretion in obesity to the intake of high fat diets and increase in plasma fatty acid concentrations. While short-term exposure to elevated FFA has an insulinotropic effect, a chronic elevation of FFA inhibits glucose-stimulated

insulin secretion (GSIS) (Sako and Grill, 1990) and insulin biosynthesis (Bollheimer et al., 1998). Studies in lean rats have shown that by 3 and 6 hours of lipid infusion, GSIS increased (Sako and Grill, 1990). However, with a 48-hour lipid infusion, GSIS was inhibited (Sako and Grill, 1990). The inhibition of GSIS after 48 hours of infusion was also seen in isolated pancreatic islets (Sako and Grill, 1990). These results are in accordance with the hypothesis by Randle and colleagues (Randle et al, 1965), which states that the abnormally increased activity of ß-oxidation seen with obesity after oral administration of glucose causes an increase in the concentration of mitochondrial acetyl-CoA, thus slowing the oxidation of pyruvate to acetyl-CoA by inhibiting pyruvate dehydrogenase. This rise in acetyl-CoA also causes an increase in citrate that inhibits phosphofructokinase and causes a rise in glucose-6-phosphate, which ultimately inhibits insulin-stimulated glucose uptake by the cell. The work done by Randle and coworkers was performed in muscle. Other studies have extrapolated this information to include such competition within the ß-cell (Sako and Grill, 1990; Zhou et al., 1993) to demonstrate how a lack of glucose uptake into the ß-cell due to increased ß-oxidation leads to a decreased stimulation of insulin secretion. Studies performed *in vivo* (Bollheimer et al., 1998) and *in vitro* (Bollheimer et al., 1998; Zhou et al., 1994) in rats have revealed that, with prolonged treatment of FFA, the insulin content of the ß-cell was markedly reduced (Bollheimer et al., 1998). Contrary to Randle, Bollheimer et al. (1998) attribute this reduction of ß-cell insulin content to the inhibitory effect of FFA at the translational level of glucose-regulated proinsulin biosynthesis.

Studies in rats were performed to determine if the type of fatty acid (SFA or PUFA) affected GSIS (Stein et al., 1997; Dobbins et al., 2002). It was found that GSIS

was highest in rats fed a diet containing SFA. This conclusion was also reached in perfused pancreas experiments (Stein et al., 1997; Dobbins et al., 2002). Stein and colleagues (1997) found that increasing the chain length of the fatty acid and decreasing the number of unsaturated bonds led to a greater insulinotropic effect. Although the mechanism of SFA causing greater GSIS when compared to PUFA is still elusive, it is thought that glycolysis may be inhibited by products of PUFA metabolism, therefore reducing GSIS (Opara et al., 1992). The oxidation of PUFA in the mitochondria of the ß-cell creates a large amount of ATP, which inhibits the activity of phosphofructokinase and pyruvate kinase of glycolysis and therefore reduces the response of insulin to glucose (Opara et al., 1991). Other work has been performed to determine if GSIS differed in response to different fatty acids as a result of altered protein kinase C (PKC) activity (Deeney et al., 1992; Landt et al., 1992; Yaney et al., 2000); however, there has been little work done to determine the effect of PUFA on PKC activity. Because there are multiple isoforms of PKC, controversy arises as to the overall consequence of fatty acids upon this enzyme's activity; however, there is evidence that the atypical PKC isoform is activated by fatty acids, leading to GSIS (Yaney et al, 2000).

**Insulin Action in Obesity**

With obesity, insulin is not as efficient in its activation of the uptake of glucose in liver, skeletal muscle, and adipose tissue as it is in the normal state. This diminished effect of insulin is referred to as insulin resistance and is defined as an impaired ability of insulin to suppress hepatic glucose output and to promote peripheral glucose uptake into

tissues (Porte, 1991; Stipanuk, 2000). Hyperinsulinemia occurs as a compensatory device to maintain blood glucose levels within a normal range.

Insulin resistance influences not only glucose but also lipid metabolism. Although the results are controversial, many studies show that insulin's ability to suppress HSL in adipose tissue is reduced in obesity, resulting in an increased release of FFA from adipose tissue (Campbell et al., 1994; Jensen et al., 1989; Lewis et al., 2002), whereas the effect of LPL is delayed (Yost et al., 1995; Sadur et al., 1984). According to Eckel (personal communication), LPL activity is a reflection of the triglyceride droplet turnover either in the adipocyte or in the muscle cell. In obesity, the droplet in the adipocyte increases and LPL per cell is also increased but resistant to stimulation by insulin. With weight loss, the adipocyte droplet size is decreased and it needs LPL for reentry of fatty acids, thus insulin sensitivity improves at this site and allows uptake of FFA into the adipocyte. It is thought that insulin downregulates LPL activity in muscle because the droplet can be maintained by other mechanisms, such as fatty acyl CoA synthesis. It is unknown why muscle LPL activity is stimulated by insulin in the obese state. The resultant effect of insulin resistance on HSL and LPL activity is an increased amount of FFA in the bloodstream and an accumulation of FFA, long chain acyl-CoA (LCACoA), and triglyceride in the muscle (Lewis et al., 2002; Kim et al., 2001).

Since skeletal muscle is the primary tissue responsible for insulin-stimulated glucose uptake (Kelley et al., 2002), an alteration in its composition affects whole body insulin sensitivity. It is now known in humans and rodents that the accumulation of lipids inside the muscle cell (intramyocellular lipid, IMCL) affects insulin sensitivity and not the lipid that accumulates between the muscle cells (extramyocellular lipid, EMCL)

(Perseghin et al., 1999; Jacob et al., 1999; McGarry, 2002). The distinction between IMCL and EMCL has become possible through the use of nuclear magnetic resonance spectroscopy ($^{1}$H-MRS) (Perseghin et al., 1999; Jacob et al., 1999; McGarry, 2002). Because triglycerides are not metabolically active, studies have shown that long chain acyl-CoA (LCACoA), a metabolically active form of lipid, is elevated in IMCL in insulin-resistant individuals (McGarry, 2002).

The mechanism explaining why increased concentrations of LCACoA alter insulin sensitivity is unknown, but the hypotheses include disruption of the activity of carnitine palmitoyltransferase I (CPT-I) and, consequently, GLUT4 activity (Dobbins et al., 2001). CPT- I, an enzyme located on the outer mitochondrial membrane, is responsible for the transfer of long chain acyl groups from CoA to carnitine for ß-oxidation and is strongly inhibited by malonyl CoA (Stipanuk, 2000). Increases in both insulin and glucose cause the concentration of acetyl CoA and citrate to increase thus activating acetyl CoA carboxylase (ACC) and consequently increasing malonyl CoA concentrations to reduce the rate of ß-oxidation (Saha et al., 1997; Bavenholm et al., 2000). A continued flux of glucose into the skeletal muscle and a resultant increase in malonyl CoA causes both LCACoA and IMCL to accumulate in this tissue due to a decreased rate of ß-oxidation (Bavenholm et al., 2000; Kelley et al., 2002). Conversion of the accumulated LCACoA in muscle to another metabolically active form of lipid, such as diacylglycerol, may change the activity of enzymes responsible for activation of the insulin signaling cascade (Dobbins et al., 2001) and result in impaired translocation of GLUT4 to the cell membrane, therefore decreasing glucose uptake (Salway, 2000; Boden et al., 2001).

Contrary to the studies performed in humans and rats in which FFA oxidation is decreased with a high fat diet or with the induction of obesity, Lester et al. (1999) found that lean cats fed a high-fat diet do not have an increase in the concentration of plasma FFA because they increase the rate of fat oxidation. However, as would be expected based upon the Randle hypothesis, the rate of carbohydrate oxidation in these cats decreased significantly, which, according to Randle, would cause an inhibition of glucose uptake by skeletal muscle, therefore exhibiting insulin resistance at that site (Randle et al., 1963; Shulman, 2000). However, because these cats were not allowed to become obese, it is impossible to determine if they would have maintained this high rate of fatty acid oxidation in obesity. It was also not determined in this study if the high fat diet affected insulin secretion or insulin sensitivity.

Similar to studies in beta cells, it was found that the type of fatty acid within the diet alters the sensitivity of muscle tissue to insulin (Jucker et al., 1999). Feeding of safflower oil (primarily SFA) increased muscle triglyceride concentration and created insulin resistance by decreasing insulin-stimulated glucose disposal and reducing skeletal muscle glycolysis whereas the feeding of fish oil (primarily PUFA high in omega-3 fatty acids) did not. The safflower oil-fed group increased fatty acid oxidation while decreasing flux through the pyruvate dehydrogenase complex. This led to insulin resistance via the glucose-fatty acid cycle. The difference in skeletal muscle insulin sensitivity between the two diets may occur because of changes in the degree of saturation of phospholipids in the plasma membrane, which then affects its fluidity and permeability. It has been concluded that the animal's metabolic rate is increased with a diet high in omega-3 fatty acids, which could be a result of increased unsaturation of the

membrane phospholipid. It is postulated that with an increase in the unsaturation of membrane phospholipids, there is an increase in that membrane's "leakiness," meaning that it requires more energy to maintain the proper gradients of cellular ions (Storlien et al., 1991; Jucker et al., 1999).

The type of saturation has also been shown to have an effect on insulin receptor signaling (Taouis et al., 2002). This study found that rats fed a diet high in omega-6 fatty acids had blunted tyrosine phosphorylation of the insulin receptors therefore decreasing phosphotidylinositol 3-kinase (PI 3-K) activity, and finally leading to diminished GLUT4 activity. However, when the diet was partially replaced with omega-3 fatty acids, this was not observed and the insulin signaling cascade and GLUT4 activity were maintained.

# CHAPTER 3

# MATERIALS AND METHODS

**Animals and Diets.** Twenty-eight neutered lean Domestic Shorthair cats (14 male, 14 female; Sinclair Research Center, Columbia, MO, and Harlan Sprague Dawley, Madison, WI) were used for these studies. 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.

The cats were equally and randomly assigned to one of two diet groups: a saturated fatty acid (SFA) diet group (14 cats; 7 males and 7 females) or a polyunsaturated fatty acid (PUFA) diet group (14 cats; 7 males and 7 females). The composition of the diets is shown in Table 1. Food intake was recorded at each feeding, 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 during the Lean phase (L).

**Intravenous Glucose Tolerance Tests.** To allow blood sampling, catheters were placed in the jugular vein of cats 15-18 hours before each intravenous glucose tolerance test

(IVGTT). 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 6 hours. Whole blood was taken through the catheter and allowed to clot for serum collection. For the collection of plasma, blood was placed into cold heparinized tubes containing 1 M benzamidine and the samples were centrifuged immediately. Serum and plasma were stored at -20°C until assayed.

**Study design.** The cats were maintained in the lean state for 10 weeks (L) and fed either diet SFA or diet PUFA. They were then fed the same diets ad libitum for 21 weeks. Intravenous glucose tolerance tests were performed as described in detail using 1 g/kg of 50% dextrose (Hoenig and Ferguson, 2002). Glucose, insulin, glucagon, and non-esterified fatty acids (NEFA) were measured before and 5, 10, 15, 30, 45, 60, 90, and 120 minutes after glucose injection.

**Immunoassays.** All samples for determination of hormone concentrations were processed in a single assay for each hormone. Serum insulin concentrations were measured as described by Hoenig and Ferguson (1989) using a charcoal method. Glucagon concentrations were determined using a Glucagon RIA kit (Linco, St. Charles, MO).

**Enzyme assays.** Glucose measurements were performed using a colorimetric glucose oxidase method (glucose trinder kit; Sigma, St. Louis, MO). Serum concentration of non-esterified fatty acids (NEFA) was measured by use of an enzymatic test kit (NEFA C; Wako Diagnostic, Richmond, PA). Baseline glycosylated hemoglobin concentrations were determined as previously described (40) using the Glyc-Affin GHb kit (Perkin Elmer Wallac, Norton, OH). Routine blood analysis, including measurements of

cholesterol and triglyceride, was performed by the Clinical Pathology Laboratory at the University of Georgia College of Veterinary Medicine. Measurement of weight, percent body fat, and body mass index (BMI; expressed in $kg/m^2$) was performed as described (Nelson et al., 1990). To reduce variability, the same person (ECW) performed all measurements.

**Calculations.** The glucose disappearance coefficient (k) was calculated using the Passage program for Macintosh computers. The glucose concentrations versus time data from each individual IVGTT were fit to the following monoexponential equation by non-linear regression:

$$Cs = Cs0 \times \exp(-k \times t)$$

where Cs is the serum glucose concentration at time t (min), and Cs0 is the extrapolated initial serum glucose concentration.

The percent glucose disappearance/min (K) was calculated from the formula:

$$K = k \times 100.$$

The area under the curve (AUC) was estimated by the sum of all the trapezoids and triangles bounded by the time versus concentration curve and was mathematically calculated as the integral of the curve.

The area under the curve (AUC) was estimated by the sum of all the trapezoids and triangles bounded by the time versus concentration curve and was mathematically calculated as the integral of the curve. The percent NEFA suppression was calculated as follows:

$$\% \text{ suppression} = 100 - \text{NEFA (t)/NEFA (to)} * 100$$

**$^{1}$H-MRS.** The procedure for obtaining water-suppressed proton-localized spectra of mobile lipids in cat muscle is as follows: localized water-suppressed proton spectra of IMCL and EMCL components were obtained on a Varian/Inova 4.7T horizontal spectroscopy and imaging system operating at 200.56 MHz. The actively shielded gradient set had an internal diameter of 22 cm and a maximum gradient strength of 10 gauss/cm. Cats were anesthetized with 0.25 ml Telazol (Fort Dodge Laboratories Inc., Fort Dodge, Iowa) and placed on their backs in a polycarbonate cradle. A specially designed radio frequency transmit/receive surface coil was placed over the quadriceps muscle group of the left rear leg. The rear leg was secured in the cradle in such a way as to bring the muscle fibers as nearly as possible in parallel with the applied magnetic field. In this orientation, the signals from IMCL and EMCL have maximum separation (Boesch et al., 1999, 2001). Selection of the observed voxel was carried out with a standard gradient-echo pulse sequence. The muscle group was centered as close as possible to the magnet and gradient center. A transverse image was obtained to find the muscle center and a sagittal slice obtained to verify the position along the Z direction, which is parallel to the applied magnetic field. From this image an oblique image was obtained along the muscle fibers away from any observed fascia. A 10mm x 10mm x 5mm voxel with the 5mm direction perpendicular to the previously defined imaging plane was planned. This procedure allowed reproducible positioning of all the cats in the groups studied. Following this procedure an optimized version (to include a sixteen step phase cycle, Henning, 1992) of a localized non-water suppressed PRESS sequence (point resolved spectroscopy,

Bottomley, 1984) was used to shim the water signal from the selected voxel. The full linewidth at half height ranged from 12.94 to 23.31 (17.78 $\pm$ 2.36).

A water signal was acquired with 16 transients, which were averaged to give a reference spectrum. Echo time (TE), which is equal to $2t_1 + 2t_2$, was 33 msec with $t_1$ and $t_2$ equal to 8.5 μsec and 8.0 μsec respectively. Water-suppressed spectra were obtained using three CHESS pulses prior to executing the PRESS section of the sequence. Two sets of spectra were obtained for each animal. These included a short and long echo time version, each obtained using 64 averaged transients. A TE of 33 msec with $t_1$ of 8.5 μsec and $t_2$ of 8.0 μsec was used for the short echo version. A TE of 91 msec with a $t_1$ of 12.5 μsec and a $t_2$ of 33 μsec was employed for the long echo version.

**Data analysis.** Spectral data were analyzed using the frequency domain analysis package supplied in VNMR from Varian. Time domain data were apodized with a line broadening of 3.4 Hz, zero filled to 16K, fourier transformed and baseline corrected where necessary. Spectra were fit to a sum of Lorentzian/Gaussian functions (Gaussian fraction of 0.5) to yield the simulated spectra. Areas of each peak were determined from the least squares fit of the data. Areas were normalized to account for instrument factors by scaling to the obtained localized water resonance. The two downfield peaks due to carnosine were also used to normalize the data.

**Statistical analysis.** All data were analyzed using Data Desk software (Ithaca, NY) and Prism software (San Diego, CA). The data are expressed as means ± SD, unless otherwise stated. The significance of differences of means between groups was evaluated by analysis of variance (ANOVA) while differences within a group were evaluated by

student's T test for paired analysis. A p-value $< 0.05$ was considered statistically significant.

# CHAPTER 4

# RESULTS

Cats in both diet groups consumed more kilo-calories per body weight at the end of the ad libitum period (OB) than at the end of the lean period (L) but there was no difference between diet groups (Table 1).

The weight, BMI, girth, and percent fat of the cats are shown in Table 3. Weight, BMI, girth, and percent fat were significantly higher in OB than in L ($p \leq 0.0001$) in both diet groups. In both groups PUFA and SFA, weight was highly correlated with girth ($r^2$=0.9417 and 0.9397, respectively; $p<0.0001$), BMI ($r^2$=0.8689 and 0.8725, respectively; $p<0.0001$), and percent fat ($r^2$=0.7820 and 0.8222, respectively; $p<0.0001$). There was no difference between diet groups in any of the parameters that were evaluated.

Glycosylated hemoglobin (GHb) concentrations were not different within a diet group (Table 4). They were also not different between lean cats in both groups (Table 5). However, GHb concentrations were significantly higher between obese cats of group SFA and group PUFA (Table 6). Weight and girth were correlated with GHb in group PUFA ($r^2$= 0.1778; p=0.0254; and $r^2$=0.1416; p=0.0484, respectively) while there was no significant correlation with GHb to weight and girth for group SFA.

Baseline concentrations of glucose were not different between diets in both groups (Table 4) and there was no difference between L (Table 5) and Ob (Table 6).

Glucose concentrations at 120 min of the IVGTT were significantly higher in OB versus L for both groups (Table 4). However, there was no difference between group PUFA and SFA in L (Table 5) or OB (Table 6).

The area under the curve (AUC) for glucose was significantly higher in OB versus L (Table 4) but there was no difference between diets (Table 5, 6). The k-value for OB was significantly lower than L in both groups (Table 4). However, only the males in both groups were significantly lower in OB than L (PUFA: 1.25 $\pm$ 0.20 and 1.80 $\pm$ 0.36, respectively; p=0.0024; SFA: 1.51 $\pm$ 0.34 and 1.97 $\pm$ 0.31, respectively; p=0.0055) while there was no difference between L and OB for the females (data not shown).

The insulin secretory patterns of cats during IVGTT in groups PUFA and SFA are shown in Figure 1. The baseline insulin concentrations were not significantly different between diets (Table 5, 6) or between L and OB (Table 4), whereas the 120 minutes concentrations were significantly higher in OB than in L within groups PUFA and SFA (Table 4), but there was no difference between diets (Table 5, 6). The AUC for insulin in the PUFA group was not significantly different between L and OB, whereas the SFA group had a significantly higher insulin AUC in OB than in L (Table 4). While there was no difference in insulin AUC as an effect of diet in L (Table 5), the SFA group had a significantly higher insulin AUC in OB than did the PUFA group in OB (Table 6).

Nine of 28 obese cats had normal glucose tolerance in the OB phase defined by a return of glucose concentrations to less than 120 mg/dl at 120 min. These 9 cats (LTol) had lower insulin concentrations in the L phase than the 19 cats that became glucose intolerant when Ob (Lintol). The AUC for insulin (nmol/L) in the L phase was, however, only significantly lower for the last 60 min of the glucose tolerance test (5.6 $\pm$ 2.0 and 8.5

± 3.4, respectively; p = 0.0307). In the OB phase, the 9 glucose tolerant cats had significantly lower insulin concentrations (pmol/L) at 120 min of the IVGTT than the 19 glucose intolerant cats (78 ± 59 and 254 ± 115, respectively; p < 0.0002); the AUC for insulin (nmol/L) was also significantly lower during the last 30 min of the IVGTT (4.7 ± 2.8 vs 7.7 ± 3.2; p = 0.0322). The insulin secretory profiles of the glucose tolerant and intolerant cats are shown in Figure 2.

The glucose concentrations during the IVGTT for the glucose tolerant and intolerant cats in L and OB are shown in Figure 3. The k-value for glucose in L was significantly higher for the tolerant cats than the intolerant cats (2.11 ± 0.30 and 1.63 ± 0.37, respectively; p = 0.0025). In the L phase, the glucose AUC approached significance with the intolerant cats having a higher AUC than the tolerant cats (27.49 ± 5.17 and 24.05 ± 2.09, respectively; p = 0.0675).

Baseline glucagon concentrations (pg/ml) were significantly higher in OB than in L for group PUFA (116.1 ± 21.9 and 98.6 ± 12.6, respectively; p = 0.0237), whereas there was no difference in L and OB for group SFA (Table 4). There was no difference in baseline glucagon concentrations between the diets (Table 5, 6). There was also no difference in the AUC for glucagon between the diets in either the lean or the obese state (Table 5, 6). Glucagon AUC concentrations were significantly lower in OB than in L in group PUFA, but there was no significant difference between L and OB in group SFA (Table 4). There was a marked gender difference in glucagon concentrations among the groups. The glucagon baseline concentrations (pg/ml) between lean males and females were only different in cats in group SFA (82.51 ± 9.17 and 110.50 ± 25.32 pg/ml, respectively; p = 0.0176). The glucagon AUC (ng/ml) was significantly higher in the

lean females than the lean males fed the SFA diet (10.5 ± 1.7 and 8.3 ± 1.5, respectively; p = 0.0235) but not between lean female and male cats fed the PUFA diet (10.1 ± 7.8 and 9.5 ± 2.0, respectively; p = 0.5007). The obese female cats fed the PUFA diet had a significantly higher AUC than the obese male cats (10.0 ± 1.4 and 7.6 ± 1.7, respectively; p = 0.0111), as did the females fed the SFA diet (11.2 ± 2.5 and 6.6 ± 1.7, respectively; p = 0.0018).

There was no difference in triglyceride or cholesterol concentrations between L and OB in either group (Table 4) and there was no difference between groups (Table 5, 6).

Non-esterified fatty acid (NEFA) concentrations during the IVGTT are shown in Figure 4. The lean cats showed a lower baseline concentration than obese cats in both groups (Table 4), however, there was no difference between the diets (Table 5, 6).

Percent suppression of NEFA concentrations are shown in Figure 5. There was a significant difference in suppression of NEFA concentrations in OB compared to L in both groups (Table 4), but no difference between the diets (Table 5, 6). The AUC of percent suppression (mEq/l * 1000) was significantly lower in OB than in L for males of groups PUFA (7.7 ± 2.5 and 5.2 ± 1.1, respectively; p = 0.0122) and SFA (7.6 ± 1.5 and 4.3 ± 2.1, respectively; p=0.0083) and for females of group PUFA (7.2 ± 2.2 and 4.7 ± 2.1, respectively; p=0.0234). The AUC of percent suppression for females of group SFA did not reach significance (L: 6.8 ± 2.2; OB: 4.9 ± 1.3; p = 0.0589).

There was no significant difference in the integrated intensity of the peak of the total IMCL and EMCL concentrations between PUFA and SFA in the lean and obese state. The integrated intensity (arbitrary units, AU) for L and OB IMCL in the PUFA

group was 626 ± 640 and 809 ± 461 and it was 771 ± 781 and 919 ± 230 in the SFA group, respectively. The integrated intensity (AU) for L and OB EMCL was 1612 ± 1291 and 3096 ± 1686, respectively, in the PUFA group, and 1651 ± 819 and 4037 ± 1810 in the SFA group, respectively. Therefore, the data for both diets were combined. Comparing the integrated intensity (AU) of the peaks of IMCL and EMCL of all cats (PUFA plus SFA), cats had lower values in L than in OB in both IMCL (652 ± 693 and 869 ± 338) and EMCL (1612 ± 1291 and 3096 ± 1737 AU), however, it was only significant for EMCL ($p < 0.0018$). The EMCL/IMCL ratio did not change significantly between L and Ob (L: 6.4 + 4.2; OB: 5.3 + 3.5). The total increase in lipid in muscle regardless of location was significantly different in L versus OB (L:1183 + 983 AU; OB: 2216 + 1811 AU; $p = 0.0179$).

EMCL correlated significantly and positively with IMCL ($p = 0.0018$), weight and girth ($r^2=0.1880$, $p = 0.0344$; $r^2=0.2810$, $p = 0.0078$, respectively), as well as with insulin 120 minute concentration and insulin AUC ($r^2=0.2373$, $p = 0.0158$; and $r^2=0.3394$, $p = 0.0028$, respectively), whereas IMCL correlated significantly with insulin baseline concentrations ($r^2 = 0.3020$, $p = 0.0081$).

**Table 1.** Proportion of moisture, protein, ash, calculated carbohydrate, calculated calories per gram, fat, and percent of fatty acids in the PUFA diet and the SFA diet.

| | **PUFA** | **SFA** |
|---|---|---|
| **Moisture** | 8.41 | 9.28 |
| **Protein** | 34.40 | 34.30 |
| **Ash** | 6.45 | 6.38 |
| **Carbohydrate** | 31.90 | 32.30 |
| **Cal/g** | 4.33 | 4.28 |
| **Fat** | 18.40 | 18.10 |
| **14:0** | 3.53 | 2.01 |
| **14:1** | 0.20 | 0.39 |
| **15:0** | 0.38 | 0.33 |
| **16:0** | 21.00 | 22.50 |
| **16:1 n-7** | 6.33 | 4.20 |
| **17:0** | 0.55 | 0.86 |
| **18:0** | 7.33 | 11.90 |
| **18:1 n-9** | 25.70 | 34.90 |
| **18:1 n-7** | 2.33 | 1.75 |
| **18:2 n-6** | 11.40 | 11.10 |
| **18:3 n-3** | 1.01 | 0.68 |
| **18:3 n-6** | 0.17 | 0.11 |
| **20:0** | 0.22 | 0.18 |
| **20:1** | 0.77 | 0.32 |
| **20:2** | 0.12 | <0.01 |
| **20:3 n-6** | 0.20 | 0.13 |
| **20:4 n-6** | 0.66 | 0.46 |
| **20:5 n-3** | 3.91 | 0.37 |
| **22:2** | 0.62 | <0.01 |
| **22:5 n-3** | 0.83 | 0.12 |
| **22:6 n-3** | 4.72 | 0.46 |
| **24:0** | <0.01 | <0.01 |
| **24:1** | 0.17 | <0.01 |
| **Unknown** | 5.02 | 2.48 |

**Table 2**. Kilo-calories consumed per body weight before (L) and after (OB) ad libitum food intake of a diet high in either polyunsaturated (PUFA; n = 14) or saturated fatty acids (SFA; n = 14) (mean $\pm$ SD).

| | **L** | **OB** | **p-value** |
|---|---|---|---|
| **PUFA** | 50.66 $\pm$ 6.70 | 68.21 $\pm$ 8.31 | $\leq$0.0001 |
| **SFA** | 48.65 $\pm$ 6.61 | 65.05 $\pm$ 5.92 | $\leq$0.0001 |
| **p-value** | NS | NS | |

NS designates non-significance to p-value > 0.0500.

**Table 3.** Weight (kg), BMI (kg/m$^2$), girth (cm) and percent fat of cats before (L) and after (OB) ad libitum food intake of a diet high in polyunsaturated (PUFA; n = 14) or saturated (SFA; n = 14) fatty acids (mean ± SD).

| | **Weight** | | **BMI** | | **Girth** | | **% fat** | |
|---|---|---|---|---|---|---|---|---|
| | **L** | **OB** | **L** | **OB** | **L** | **OB** | **L** | **OB** |
| **PUFA** n=14 | 3.3±0.5 | 5.4±1.2 | 34.4±2.7 | 54.5±7.6 | 27.3±2.9 | 40.1±5.5 | 8.9±1.8 | 30.9±7.5 |
| **SFA** n=14 | 3.2±0.4 | 5.5±0.7 | 34.6±2.8 | 57.0±6.7 | 27.3±2.9 | 42.5±3.1 | 9.5±2.4 | 35.7±4.5 |

**Table 4.** Baseline concentrations, AUC, 120 minute values, and k-value for glucose; baseline, AUC, and 120 minute values for insulin, glucagon, and NEFA; cholesterol, triglyceride, and GHb concentrations at baseline of cats before (L) and after (OB) ad libitum food intake of a diet high in polyunsaturated (PUFA; n = 14) or saturated (SFA; n = 14) fatty acids (mean ± SD).

| | PUFA | | | SFA | | |
|---|---|---|---|---|---|---|
| | **L** | **OB** | **p-value** | **L** | **OB** | **p-value** |
| **Glucose, mg/dl** | | | | | | |
| Baseline | 99±14 | 98±13 | NS | 101±18 | 98±9 | NS |
| 120 min | 90±31 | 148±47 | 0.0005 | 86±15 | 140±58 | 0.0015 |
| k-value | 1.71±0.39 | 1.34±0.31 | 0.0011 | 1.85±0.45 | 1.44±0.38 | 0.0015 |
| AUC (g/L) | 2.7±0.5 | 4.2±0.5 | <0.0001 | 2.6±0.4 | 4.3±0.7 | <0.0001 |
| **Insulin, pmol/l** | | | | | | |
| Baseline | 72.7±48.7 | 49.2±26.6 | NS | 73.6±64.5 | 64.6±40.2 | NS |
| 120 min | 73.4±42.0 | 193.7±147.3 | 0.0043 | 70.2±32.1 | 200.6±118.2 | 0.0019 |
| AUC (nmol/l) | 18.13±8.00 | 19.88±6.35 | NS | 18.25±6.40 | 29.32±9.61 | 0.0013 |
| **Glucagon, pg/ml** | | | | | | |
| Baseline | 98.6±12.6 | 116.1±21.9 | 0.0419 | 96.5±23.4 | 102.5±35.6 | NS |
| AUC (ng/ml) | 9.80±1.49 | 8.80±1.97 | 0.0186 | 9.42±1.89 | 8.90±3.17 | NS |
| **NEFA, mEq/l** | | | | | | |
| Baseline | 0.16±0.07 | 0.34±0.20 | 0.0066 | 0.20±0.11 | 0.37±0.14 | 0.0039 |
| 120 min | 0.14±0.08 | 0.11±0.08 | NS | 0.16±0.08 | 0.12±0.06 | 0.0196 |
| AUC-%SUPP (mEq/l*1000) | 7.5±2.3 | 5.0±1.6 | 0.0004 | 7.2±1.8 | 4.6±1.7 | 0.0008 |
| **Cholesterol, mg/dl** | 153±32 | 148±23 | NS | 158±29 | 155±37 | NS |
| **Triglyceride, mg/dl** | 30±12 | 33±6 | NS | 31±12 | 36±14 | NS |
| **GHb, %** | 1.46±0.29 | 1.50±0.34 | NS | 1.63±0.28 | 1.74±0.25 | NS |

NS designates non-significance to p-value > 0.0500.

**Table 5.** Baseline concentrations, AUC, 120 minute values, and k-value for glucose; baseline, AUC, and 120 minute values for insulin, glucagon, and NEFA; triglyceride, cholesterol, and GHb concentrations at baseline in 14 lean cats each fed either a diet high in saturated (SFA) or unsaturated (PUFA) fatty acids (mean ± SD).

| | PUFA | SFA | p-value |
|---|---|---|---|
| **Glucose, mg/dl** | | | |
| Baseline | 99±14 | 101±18 | NS |
| 120 min | 90±31 | 86±15 | NS |
| k-value | 1.71±0.39 | 1.85±0.45 | NS |
| AUC (g/L) | 2.7±0.5 | 2.6±0.4 | NS |
| **Insulin, pmol/l** | | | |
| Baseline | 72.7±48.7 | 73.6±64.5 | NS |
| 120 min | 73.4±42.0 | 70.2±32.1 | NS |
| AUC (nmol/l) | 18.13±8.00 | 18.25±6.40 | NS |
| **Glucagon, pg/ml** | | | |
| Baseline | 98.6±12.5 | 96.5±23.4 | NS |
| AUC (ng/ml) | 9.52±1.08 | 9.42±1.89 | NS |
| **NEFA, mEq/l** | | | |
| Baseline | 0.16±0.07 | 0.20±0.11 | NS |
| 120 min | 0.14±0.08 | 0.16±0.08 | NS |
| AUC-%SUPP (mEq/l*1000) | 7.5±2.3 | 7.2±1.8 | NS |
| **Cholesterol, mg/dl** | 153±32 | 158±29 | NS |
| **Triglyceride, mg/dl** | 30±12 | 31±12 | NS |
| **GHb, %** | 1.46±0.29 | 1.63±0.28 | NS |

NS designates non-significance to p-value > 0.0500.

**Table 6.** Baseline concentrations, AUC, 120 minute values, and k-value for glucose; baseline, AUC, and 120 minute values for insulin, glucagon, and NEFA; triglyceride, cholesterol, and GHb concentrations at baseline in 14 cats after 21 weeks of ad libitum feeding of either a diet high in saturated (SFA) or unsaturated (PUFA) fatty acids (mean ± SD).

| | PUFA | SFA | p-value |
|---|---|---|---|
| **Glucose, mg/dl** | | | |
| Baseline | 98±13 | 98±9 | NS |
| 120 min | 148±47 | 140±58 | NS |
| k-value | 1.34±0.31 | 1.44±0.38 | NS |
| AUC (g/L) | 4.2±0.5 | 4.3±0.7 | NS |
| **Insulin, pmol/l** | | | |
| Baseline | 49.2±26.6 | 64.6±40.2 | NS |
| 120 min | 193.7±147.3 | 200.6±118.2 | NS |
| AUC (nmol/l) | 19.88±6.35 | 29.32±9.61 | 0.0063 |
| **Glucagon, pg/ml** | | | |
| Baseline | 116.1±21.9 | 102.5±35.6 | NS |
| AUC (ng/ml) | 8.80±1.97 | 8.90±3.17 | NS |
| **NEFA, mEq/l** | | | |
| Baseline | 0.34±0.20 | 0.37±0.14 | NS |
| 120 min | 0.11±0.08 | 0.12±0.06 | NS |
| AUC-%SUPP (mEq/l*1000) | 5.0±1.6 | 4.6±1.7 | NS |
| **Cholesterol, mg/dl** | 148±23 | 155±37 | NS |
| **Triglyceride, mg/dl** | 33±6 | 36±14 | NS |
| **GHb, %** | 1.50±0.34 | 1.74±0.25 | 0.0408 |

NS designates non-significance to p-value > 0.0500.

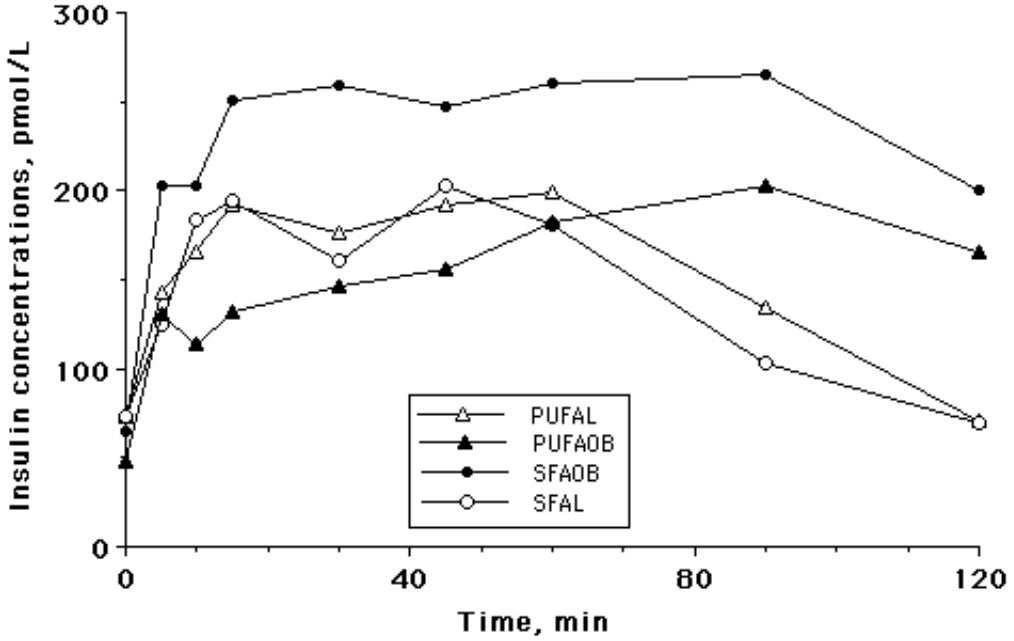


**Figure 1.** Plasma concentrations of insulin at baseline and following intravenous administration of 50% dextrose (1 g/kg body weight) in 14 cats fed a polyunsaturated fatty acid diet (PUFA) and 14 cats fed a saturated fatty acid diet (SFA) before (L) and after 21 weeks of ad libitum feeding (OB).

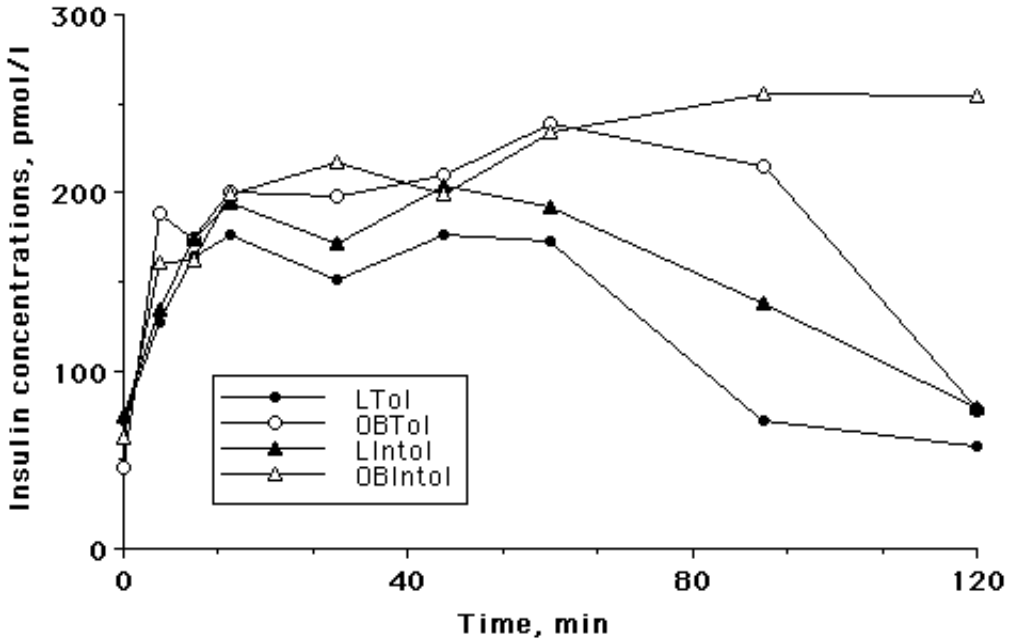


**Figure 2.** Plasma concentrations of insulin at baseline and following intravenous administration of 50% dextrose (1 g/kg body weight) in 9 glucose tolerant (LTol) cats and 19 glucose intolerant (Lintol) cats before and after (OBTol and OBIntol) 21 weeks of ad libitum feeding of high fat diets.

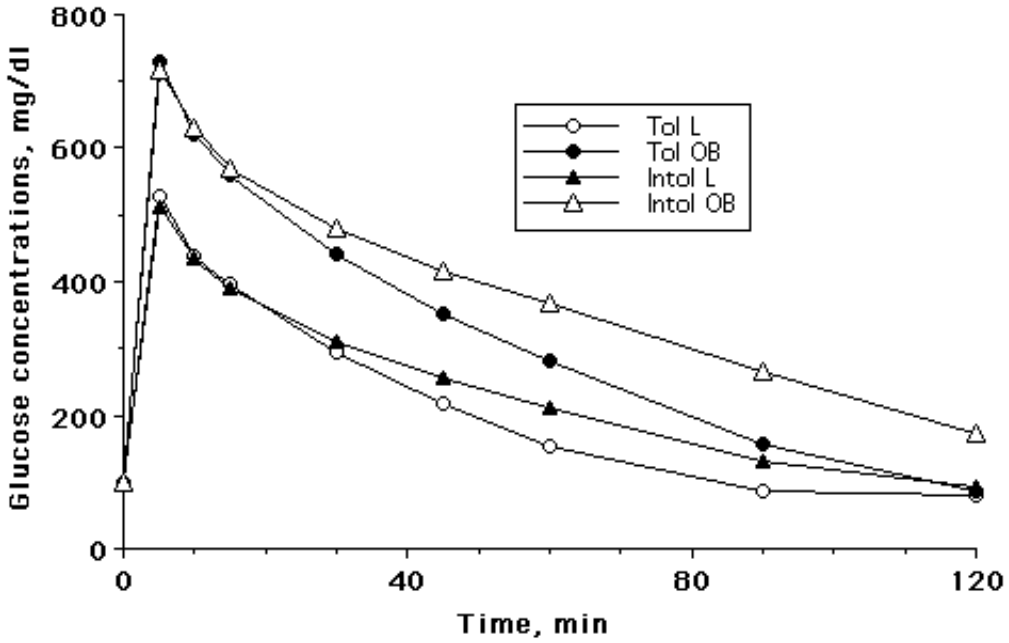


**Figure 3.** Plasma concentrations of glucose at baseline and following intravenous administration of 50% dextrose (1 g/kg body weight) in 9 glucose tolerant (Tol L) cats and 19 glucose intolerant (Intol L) cats before and after (Tol OB and Intol OB) 21 weeks of ad libitum feeding of high fat diets.

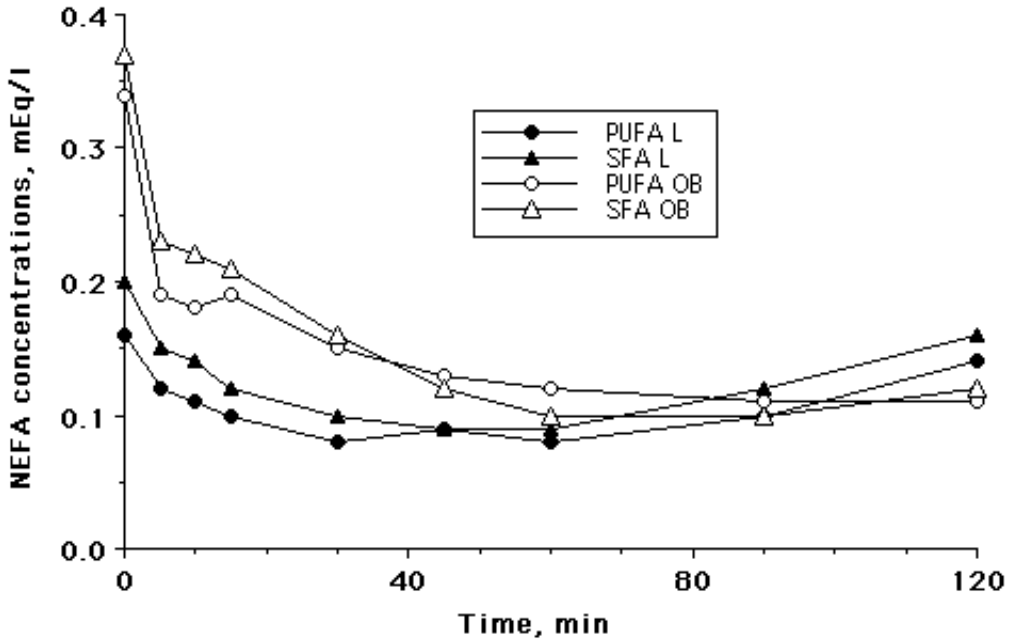


**Figure 4.** Plasma concentrations of non-esterified fatty acid concentrations at baseline and following intravenous administration of 50% dextrose (1 g/kg body weight) in 14 cats fed a polyunsaturated fatty acid diet (PUFA) and 14 cats fed a saturated fatty acid diet (SFA) before (L) and after 21 weeks of ad libitum feeding (OB).

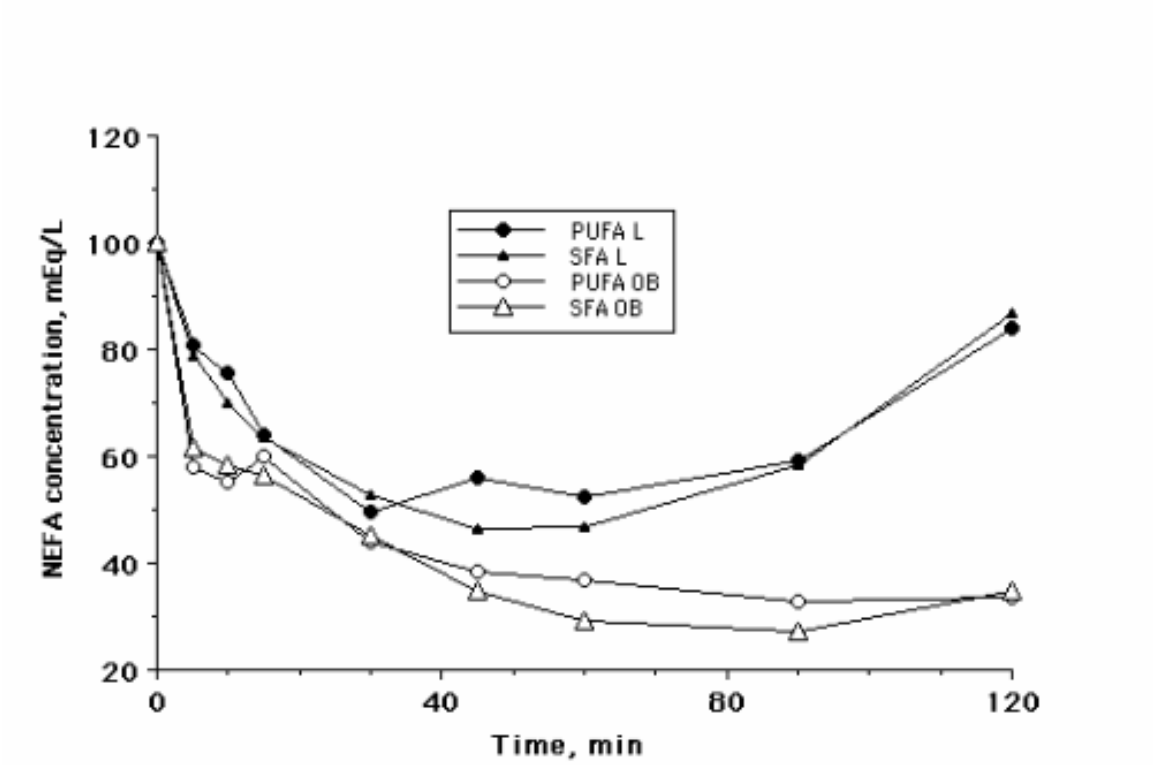


**Figure 5:** Percent suppression of non-esterified fatty acid concentrations at baseline and following intravenous administration of 50% dextrose (1 g/kg body weight) in 14 cats fed a polyunsaturated fatty acid diet (PUFA) and 14 cats fed a saturated fatty acid diet (SFA) before (L) and after 21 weeks of ad libitum feeding (OB).

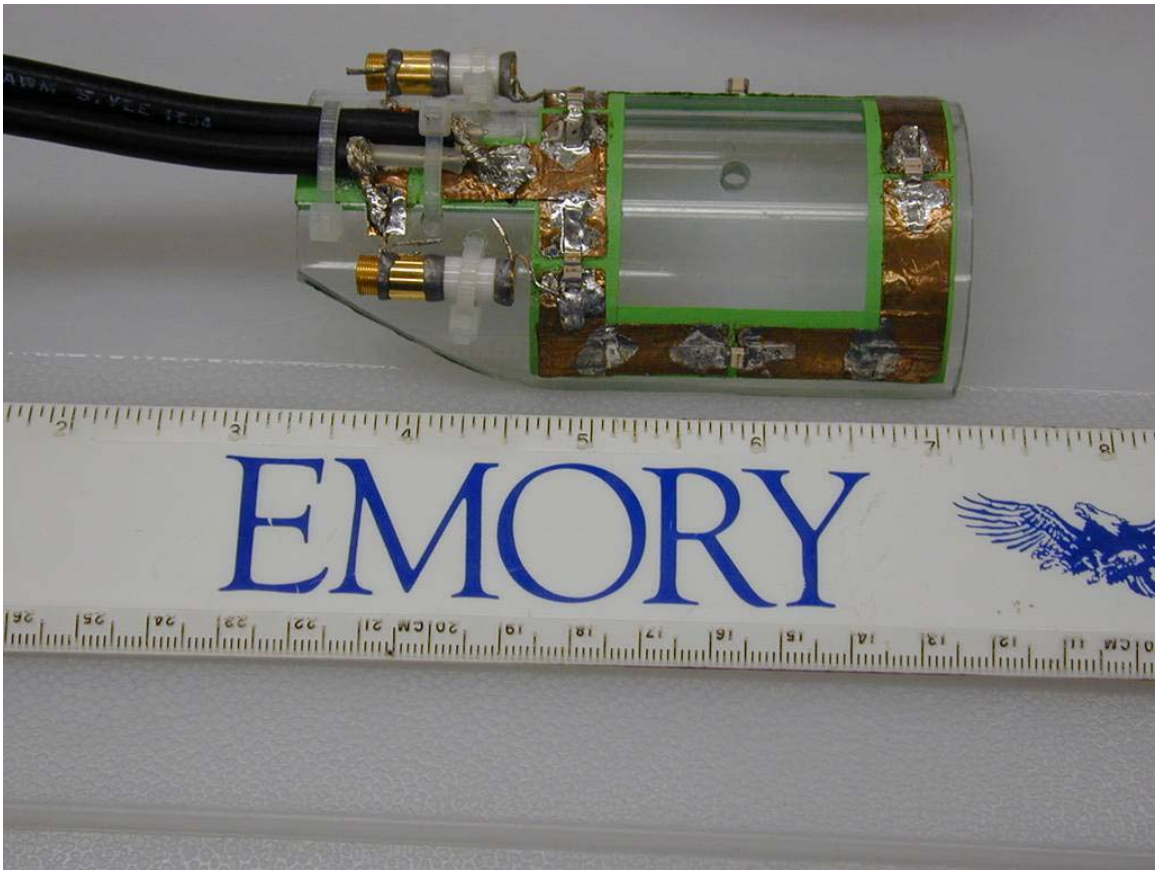


**Figure 6.** Coil which was placed upon quadriceps muscle of 14 cats before and after ad libitum feeding.

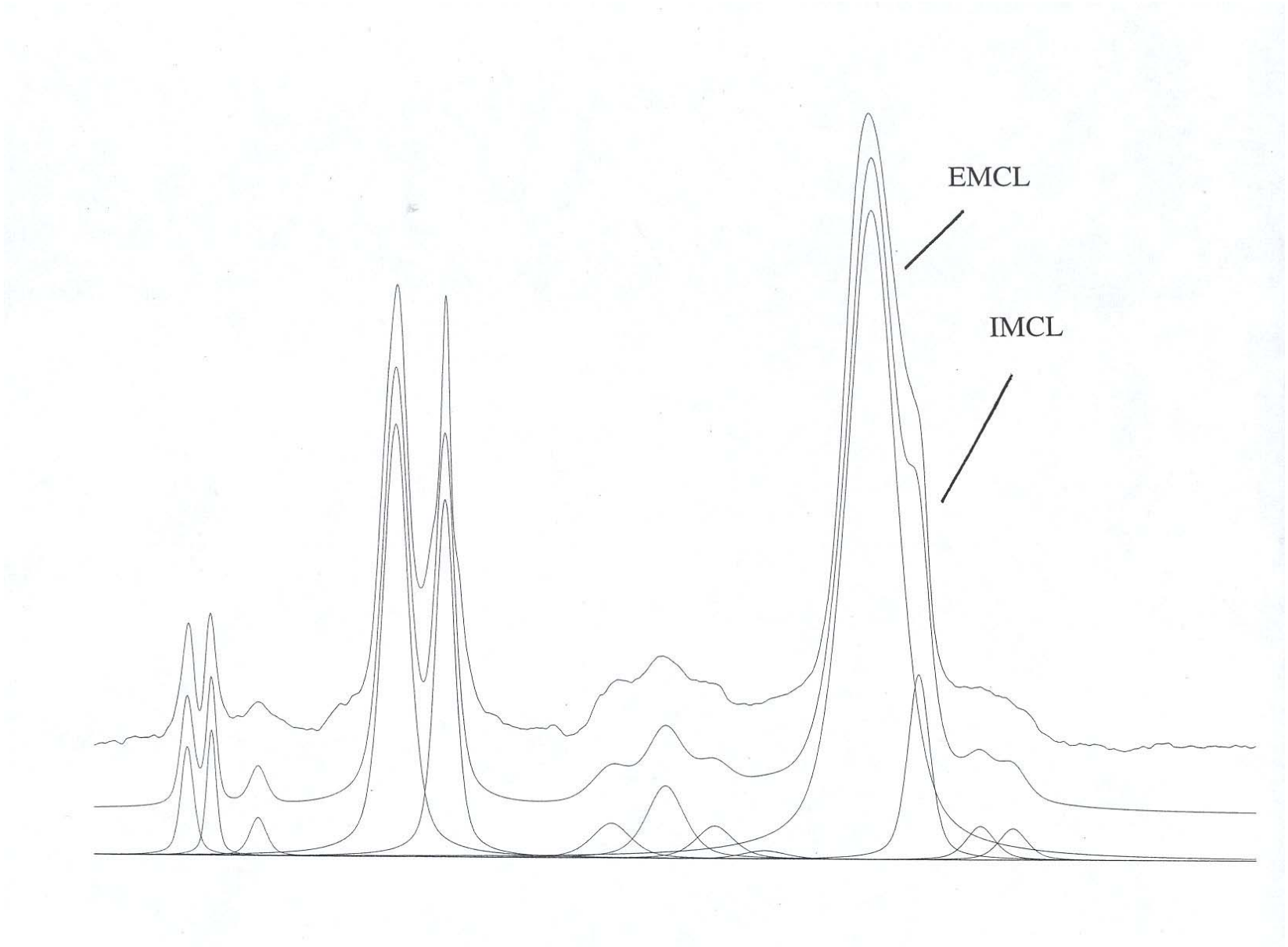


Figure 7. Representative 1H-MRS spectrum of a cat before 21 weeks of ad libitum feeding.

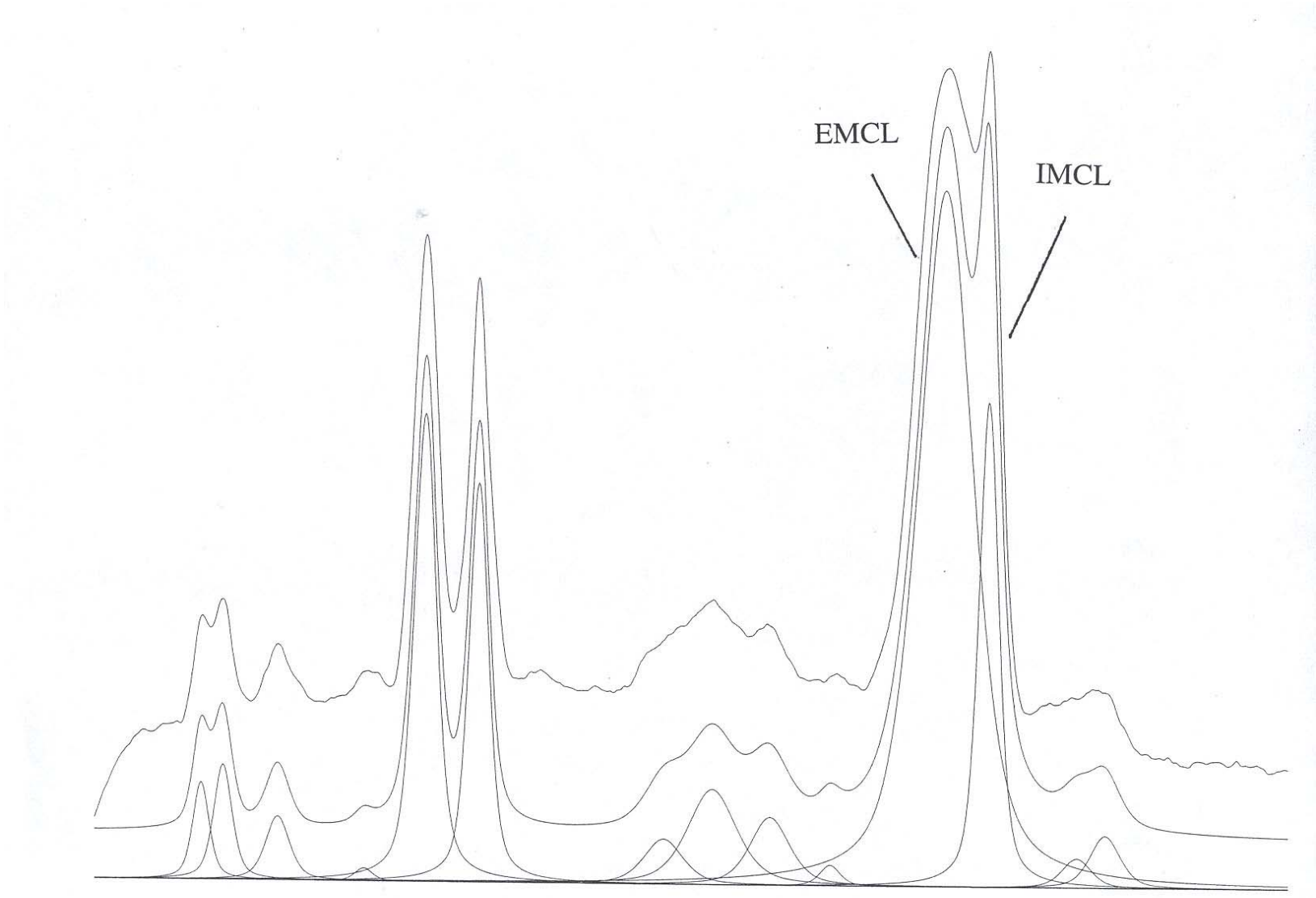


Figure 8. Representative 1H-MRS spectrum of a cat after 21 weeks of ad libitum feeding.

# CHAPTER 5

# DISCUSSION

The cats fed ad libitum gained a significant amount of weight regardless of diet. This is similar to results seen in studies in rats by Pellizzon et al. (2002). These investigators found no difference in weight gain when rats were fed long-term (15 week period) either a diet containing saturated fat or one high in omega-3 fatty acids. However, in contrast to these results, after short-term feeding (6 week period) rats fed PUFA gained less weight than those fed SFA. They speculated, therefore, that the protective effect of PUFA on weight gain was lost with long-term feeding. In the cats of this study, comparing weight gain at 4 and 9 weeks (data not shown), and at 21 weeks, to the lean weight, there was no protective effect of PUFA seen.

A diet high in omega-3 PUFA has been shown to decrease fat deposition in humans (Couet et al., 1997) and rats (Pellizzon et al., 2002; Takada et al., 1994). In contrast, the cats fed a diet high in omega-3 PUFA in this study showed no difference in fat deposition as determined by DEXA scan, BMI, and girth measurements. Upper body obesity has been linked to increased risk of morbidity, diabetes, coronary heart disease, and hypertriglyceridemia (Jensen et al., 1989). However, more results are showing that visceral fat, rather than subcutaneous fat, is the main contributor to the increased risk of developing these diseases (Montague and O'Rahilly, 2000). It is thought that the location of the fat around the organs, primarily the liver, causes an increased flux of FFA through these tissues (Montague and O'Rahilly, 2000). This has been shown to decrease insulin

sensitivity of the liver (Nagy et al., 1990) and therefore perturb the ability of insulin to slow hepatic gluconeogenesis and glycogenolysis. Although the cats in this study became obese, it could not be determined from the DEXA scans where this fat was deposited or if there was an effect of diet on the distribution of fat.

The risk of developing glucose intolerance and insulin resistance is increased with the development of obesity. As seen in other studies (Nelson et al., 1990; Hoenig et al., 2002), the obese cats in this study showed a greater glucose area under the curve, k-value, and glucose concentration at 120 minutes of the IVGTT compared to lean cats regardless of diet. However, neither baseline glucose nor baseline insulin concentrations differed as a result of obesity or diet. This follows the work of Summers et al. (2002) in which no difference was found between basal concentrations of glucose and insulin in lean and obese subjects before and after dietary intervention. Glycosylated hemoglobin concentrations were not greater in the obese cats compared to the lean cats fed either diet, but the lean and obese cats of the SFA group had higher concentrations than the cats of the PUFA group. However, this was only significant when comparing obese PUFA and SFA cats. These results illustrate that long-term glucose control assessed by the degree of glycosylation of hemoglobin remains in the normal range in obese cats regardless of diet composition. It also suggests that SFA exerts a negative effect on glucose control. Because these cats were on ad libitum feeding for only 21 weeks, one might speculate that if a diet containing SFA is fed long-term, overt glucose intolerance may ensue as has been shown in rats by Storlien et al. (1991) and in epidemiological studies in humans (Feskens et al., 1994). The fact that baseline glucose concentrations were not different in obese compared to lean cats, but other parameters of glucose tolerance (k-value, glucose

AUC, and glucose concentrations at 120 minutes of the IVGTT) were different, demonstrates the importance of challenge tests such as the intravenous glucose tolerance test in unraveling early problems with insulin secretion and/or action which would go unnoticed if only a baseline sample were taken or glycosylated hemoglobin measurements were performed.

The cats fed the SFA but not the cats fed the PUFA diet showed a significantly higher insulin AUC in the obese state than in the lean state. In addition, the obese cats fed the PUFA diet had a lower insulin AUC than those fed the SFA diet, therefore demonstrating the beneficial effect of PUFA on insulin secretion and/or action. Other studies, both *in vitro* and *in vivo*, have produced controversial results regarding the insulinotropic potency of SFA and PUFA on the pancreatic ß-cell (Opara et al., 1994; Stein et al., 1997; Dobbins et al., 2002). Our results concur with both experimental (Stein et al., 1997; Dobbins et al., 2002) and epidemiological data (Feskens et al., 1994), which showed an association of SFA and hyperinsulinemia. A study by Busch et al. (2002) found that pretreatment of MIN6 β-cells with a saturated fatty acid (palmitate) rather than with a monounsaturated fatty acid (oleate) increased the sensitization of the β-cells to secretagogues, therefore enhancing insulin secretion. They also discovered that pretreatment with palmitate increased expression of genes controlling distal secretory processes which enhanced insulin secretion, while oleate did not elicit an increased expression of secretory genes, therefore not changing insulin secretion. The diet high in SFA in our study caused a greater insulin secretion than that of PUFA; however, both diets had approximately the same percentage of palmitate, while the SFA diet contained more oleate than did the PUFA diet. This finding of increased insulin secretion even with

a greater proportion of oleate suggests that the *in vivo* cat β-cell responds differently to secretagogues than do MIN6 β-cells *in vitro*. It is theorized that the beneficial effect of unsaturated fatty acids (the ones primarily studied are oleate and linoleate) are caused by an increased expression of islet peroxisome proliferator-activated receptor-α (PPAR- α). This leads to upregulation of the expression of uncoupling protein-2 (UCP2) (Dobbins et al., 2002). Chan et al. (1999) found that GSIS is reduced when UCP2 is overexpressed. Another study by Chambrier et al. (2002) found that insulin resistance and the abnormal lipid profile of obesity can be ameliorated by the omega-3 PUFA eicosapentaenoic acid (EPA, 20:5 n-3) by inducing mRNA expression of PPAR- γ in adipocytes. The PUFA diet in our study follows that proposed by earlier work in that insulin secretion is reduced compared to the secretion following a SFA diet.

It is fascinating to note that the nineteen cats exhibiting glucose intolerance in the OB phase were already exhibiting alterations in insulin secretion and glucose tolerance in the L phase. A higher insulin AUC was seen during the last 60 minutes of the IVGTT compared to those demonstrating normal glucose tolerance in the obese phase. The same cats maintained higher insulin AUC values in the obese phase compared to the ones with normal glucose tolerance, although this was only significant for the last 30 min of the IVGTT. This indicates that cats that later become glucose intolerant already have an abnormal insulin secretion pattern when lean and still glucose tolerant. It is unclear if the secretion is abnormal because of a primary β-cell defect or if it is caused by a defect in the cellular response to insulin creating an insulin resistant environment. A difference in "insulin sensitivity" has been suggested in lean cats that developed glucose intolerance with obesity by Appleton et al. (2001), who found a tendency towards higher baseline

and AUC insulin concentrations; however, the differences were not significant. Also, the obese glucose tolerant cats in this study had a higher glucose k-value in the lean state than did the glucose intolerant cats. This illustrates that glucose clearance as well as insulin secretion were abnormal in lean cats that would later become glucose intolerant and insulin resistant while obese. Another study in our laboratory (Brennan, 2003) supports our findings in this study in that glucose transporter (GLUT4) activity in muscle and adipose tissue is altered early in obesity before any changes are seen in overt glucose tolerance.

Our finding that glucose tolerance was abnormal in the majority of the obese cats suggests that the skeletal muscle glucose uptake was decreased because muscle represents the major site for insulin-stimulated glucose disposal (Ploug and Ralston, 1998). A study in our laboratory (Brennan, 2003) revealed that obese cats had down-regulation of GLUT4, the main insulin-sensitive glucose transporter. This down-regulation could be due to an alteration of the insulin signaling cascade caused by changes in lipid metabolism and increases in lipid deposition in muscle (Shulman, 2000).

For the determination of lipid content in muscle in cats, proton nuclear magnetic resonance spectroscopy ($^{1}$H-MRS) was used in the lean and obese state. $^{1}$H-MRS is a non-invasive technique that allows for repetitive experiments. The method used for spectral data analysis is similar to that of others (Perseghin et al., 1999; Jacob et al., 1999; Bachmann et al., 2001) in which the intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL) integrals were quantified at 1.28 and 1.45 ppm, respectively. Placement of the field in parallel with the muscle fibers is a crucial factor in $^{1}$H-MRS (Boesch et al., 1999). Contrary to humans in which a long muscle group of the leg (soleus muscle) can

be used, cats have relatively small leg muscles. The ability to place a field parallel to all muscle fibers observed is difficult. We found that in cats the quadriceps muscle was the most accessible muscle for easy placement of the coil. Using this method we were able to distinguish intramyocellular from extramyocellular deposits of lipid. This distinction is important because it has been shown in other species that IMCL is a sensitive indicator of insulin sensitivity (Perseghin et al., 1999; Boden et al., 2001).

Because placement of the coil in parallel with the muscle fibers is so crucial to receiving excellent $^{1}$H-MRS data, yet very difficult to perform in the small muscle groups of the cat, we obtained large errors in our integrated intensities. It might be possible to improve these errors by reducing the size of the coil. Errors could also be improved by choosing a larger muscle group to analyze; however, this would be difficult in the cat.

Obese cats showed an increase in both IMCL and EMCL with no effect of diet and no change in the ratio of EMCL/IMCL. This is similar to results by Sinha et al. (2002) and Greco et al. (2002) in which obese subjects had significantly higher IMCL and EMCL content than lean subjects. Sinha et al (2002) found that in children both IMCL and EMCL were indicators of insulin sensitivity, whereas other investigators (Perseghin et al., 1999; Boden et al., 2001) have shown that glucose intolerance and insulin resistance are primarily associated with an increase in IMCL. The increase in both IMCL and EMCL in cats suggests that lipids are not preferentially partitioned to the intramyocellular space in obese cats as has been suggested for obese humans by Yost et al. (1995). The positive correlation of IMCL with insulin baseline concentrations and EMCL with morphometric measures of obesity as well as with insulin concentrations during the IVGTT indicates that both may modulate insulin sensitivity. It should be

noted, however, that using the IVGTT did not allow us to specifically examine insulin sensitivity. Rather, euglycemic hyperinsulinemic clamp techniques are necessary to rigorously assess insulin sensitivity.

Obesity increased NEFA concentrations in the cats of this study regardless of diet. However, whereas other studies (Ranganath et al., 1999; Mingrone et al., 1997) have also shown a significant increase in the concentration of NEFA of obese subjects when compared to lean subjects, this is not always seen in obese cats. In an earlier study (Hoenig et al., 2003), no significant difference was seen in cats on a commercial maintenance diet, which suggests that an increase in dietary fat, regardless of origin, increases NEFA concentrations in feline obesity. No difference was seen in rats (Kraegen et al., 1991; Storlien et al., 1991; Jucker et al., 1999) and humans (Luo et al., 1998) between those fed a diet high in SFA and those fed a diet high in PUFA.

The ability of insulin to suppress the concentration of NEFA post-prandially is impaired in human obesity (Golay et al., 1986). The obese cats in this study had significantly more suppression of NEFA, which is in contrast to the results found in humans (Golay et al., 1986) but agrees with previous work in obese cats (Hoenig et al., 2003). It is speculated that the inability of insulin to suppress NEFA in human obesity could occur due to a diminished ability of insulin to reduce HSL activity or to stimulate LPL to take up FFA in adipose tissue (Sadur et al., 1984; Campbell et al., 1994; Lewis et al., 2002). Adipose tissue and skeletal muscle of the cat, on the other hand, seem to become more sensitive to the anti-lipolytic effects of insulin in obesity than in the lean state. Along with the finding that LPL activity in human skeletal muscle increases with obesity (Yost et al., 1995), the LPL activity of the cats in this study seems to have

performed in the same manner because there was an increase in the total lipid content of the muscle of the obese cats versus that of the lean. This suggests that lipoprotein lipase activity in muscle tissue of cats is increased in obesity. However, to our knowledge, this has not been investigated yet. When comparing percent increase of total body fat as determined by DEXA scan and percent increase of total muscle lipid as determined by $^{1}$H-MRS, it is obvious that lipid content in both tissues increased greatly. This suggests that, in the cat, adipose tissue as well as muscle LPL activity increase in obesity.

Obesity has been associated with increased concentrations of serum triglyceride (Howard, 1999) and cholesterol in humans (Yamashita et al., 1996) and cats (Szabo et al., 2000; Hoenig et al., 2003). In contrast to the studies by Szabo et al. (2000) and Hoenig et al. (2003) in which obese cats had significantly greater concentrations of triglyceride and cholesterol compared to the concentration of the lean cats, there was no change in either parameter when comparing lean versus obese in this study. One has to realize, however, that even in the previous study from our laboratory (Hoenig et al. 2003), the concentrations were still within the normal range. The results of the current study are in accordance with those of Dimski et al. (1992) in which there was no change in triglyceride or cholesterol in obese cats compared to lean cats. Because the cats in each of these studies were fed different diets, this demonstrates the influence of diet on biochemical parameters, especially lipoproteins. It should also be noted that the duration of obesity could have a profound effect upon lipoproteins as can be seen by comparison of the short-term effect of obesity of this study and the long-term effect of obesity in another (Hoenig et al., 2003). In addition, the triglyceride and cholesterol values were taken after an overnight fast, which influences their plasma concentrations.

There are many opposing reports on the effect of PUFA and SFA on triglyceride and cholesterol concentrations. Luo et al. (1998) found that a diet high in PUFA caused a decrease in triglyceride concentrations with no significant change in cholesterol concentrations in humans compared to a diet high in SFA, whereas Summers et al. (2002) saw a decrease in the total cholesterol concentration with no change in the triglyceride concentration. In rats, administration of a diet high in PUFA caused a decrease in both cholesterol and triglyceride (Keelan et al., 1989), whereas a diet high in PUFA changed neither lipid parameter in humans (Kasim et al., 1988). In agreement with Kasim et al., we found no change in either cholesterol or triglyceride concentrations in response to either diet. It is difficult to discern why there were no differences in these concentrations between diets; however, it may be due to the fact that there was a similar amount of linoleic acid (18:2 n-6) in each diet. Kraegen et al. (1991) found that rats fed a diet high in carbohydrate and one high in n-6 fatty acids had no difference in triglyceride concentrations. It is also possible that the proportion and percentage of n-3 fatty acids in the PUFA diet compared to the SFA diet was too similar to elicit a different response.

# CHAPTER 6

# SUMMARY AND CONCLUSIONS

With the large increase in fat consumption, obesity, and type 2 diabetes seen throughout affluent countries, researchers are focusing on the type of fat consumed to determine the effect upon obesity and insulin sensitivity. The purpose of this research was to determine if polyunsaturated (PUFA) versus saturated (SFA) fatty acids would cause different insulin sensitivities in the cat in both the lean and obese state, and also to determine if obesity caused a partitioning of fat into muscle which is thought to cause insulin resistance.

We found that the PUFA diet had a beneficial effect upon insulin secretion and/or action, while the SFA diet had no such effect. This finding is in agreement with studies in both rats and humans. PUFA, however, had no beneficial effect on glucose clearance, which was abnormal in the majority of the obese cats regardless of diet. It was of particular interest to find that the cats that developed glucose intolerance with obesity already had abnormal insulin concentrations and an increased k-value while still lean. This points towards an early abnormality in either insulin secretion and/or action. Contrary to humans, non-esterified fatty acid concentrations were more suppressed in the obese cat than in the lean cat suggesting that under conditions of the IVGTT obese cats become more sensitive to the NEFA-lowering effect of insulin. We found that obese cats had a higher proportion of EMCL and IMCL than lean cats, and that both of these

parameters were positively correlated with insulin secretion and/or negatively correlated with insulin action.

The results of this research provided information about glucose and fatty acid metabolism in the lean and obese cat. They also suggest that a diet high in PUFA is beneficial for obese cats by preventing hyperinsulinemia which is a risk factor for type 2 diabetes.

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