10-day hyperlipidemic clamp in cats: effects on insulin sensitivity, inflammation, and glucose metabolism-related genes

Zini, E; Osto, M; Konrad, D; Franchini, M; Sieber-Ruckstuhl, N S; Kaufmann, K; Guscetti, F; Ackermann, M; Lutz, T A; Reusch, C E
10-day hyperlipidemic clamp in cats: effects on insulin sensitivity, inflammation, and glucose metabolism-related genes

Abstract

Obesity and hyperlipidemia are associated with impaired insulin sensitivity in human type 2 diabetes mellitus, possibly due to activation of a mild inflammatory response. Because obesity-induced insulin resistance predisposes cats to diabetes and because hyperlipidemia is a frequent concurrent finding, excess lipids may also impair insulin sensitivity in cats. Healthy cats (n=6) were infused with lipids (Lipovenoes 10%) for 10 days to clamp blood triglycerides at the approximate concentration of untreated feline diabetes (3-7 mmol/l). Controls received saline (n=5). On day 10, plasma adiponectin and proinflammatory markers were measured. Whole-body insulin sensitivity was calculated following an intravenous glucose tolerance test. Tissue mRNAs of glucose metabolism-related genes were quantified in subcutaneous and visceral fat, liver, and skeletal muscles. Accumulation of lipids was assessed in liver. At the termination of infusion, whole-body insulin sensitivity did not differ between groups. Compared to saline, cats infused with lipids had 50% higher plasma adiponectin and 2-3 times higher alpha(1)-acid glycoprotein and monocyte chemoattractant protein-1. Unexpectedly, lipid-infused cats had increased glucose transporter-4 (GLUT4) mRNA in the visceral fat, and increased peroxisome proliferative activated receptor-gamma2 (PPARgamma2) in subcutaneous fat; adiponectin expression was not affected in any tissue. Lipid-infused cats developed hepatic steatosis. Although hyperlipidemia induced systemic inflammation, whole-body insulin sensitivity was not impaired after 10 day infusion. Increased circulating adiponectin may have contributed to prevent insulin resistance, possibly by increasing GLUT4 and PPARgamma2 transcripts in fat depots.
10-DAY HYPERLIPIDEMIC CLAMP IN CATS: EFFECTS ON INSULIN SENSITIVITY, INFLAMMATION AND GLUCOSE METABOLISM-RELATED GENES


From the Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zürich, Winterthurerstrasse 260, 8057 Zürich, Switzerland (E. Zini, N.S. Sieber-Ruckstuhl, K. Kaufmann, C.E. Reusch); Institute of Veterinary Physiology, Vetsuisse Faculty, University of Zürich, Winterthurerstrasse 260, 8057 Zürich, Switzerland (M. Osto, T.A. Lutz); Department of Endocrinology and Diabetology, University Children's Hospital, Steinwiesstrasse 75, 8032 Zürich, Switzerland (D. Konrad); Institute of Virology, Vetsuisse Faculty, University of Zürich, Winterthurerstr. 266a, 8057 Zürich, Switzerland (M. Franchini, M. Ackermann); Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zürich, Winterthurerstrasse 260, 8057 Zürich, Switzerland (F. Guscetti).

Corresponding author: Eric Zini
Mailing address: Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zürich, Winterthurerstr 260, 8057 Zürich, Switzerland.
Phone: +41-44-6358746
Fax: +41-44-6358930
E-mail: ezini@vetclinics.uzh.ch

Short title: Hyperlipidemic clamp in cats
Abstract

Obesity and hyperlipidemia are associated with impaired insulin sensitivity in human type 2 diabetes mellitus, possibly due to activation of a mild inflammatory response. Because obesity-induced insulin resistance predisposes cats to diabetes and because hyperlipidemia is a frequent concurrent finding, excess lipids may also impair insulin sensitivity in cats.

Healthy cats (n=6) were infused with lipids (Lipovenoes® 10%) for 10 days to clamp blood triglycerides at the approximate concentration of untreated feline diabetes (3-7 mmol/l). Controls received saline (n=5). On day 10, plasma adiponectin and pro-inflammatory markers were measured. Whole-body insulin sensitivity was calculated following an intravenous glucose tolerance test. Tissue mRNAs of glucose metabolism-related genes were quantified in subcutaneous and visceral fat, liver and skeletal muscles. Accumulation of lipids was assessed in liver.

At the termination of infusion, whole-body insulin sensitivity did not differ between groups. Compared to saline, cats infused with lipids had 50% higher plasma adiponectin and 2-3 times higher α1-acid glycoprotein and monocyte chemoattractant protein-1. Unexpectedly, lipid-infused cats had increased glucose transporter-4 (GLUT4) mRNA in the visceral fat, and increased peroxisome proliferative activated receptor-γ2 (PPARγ2) in subcutaneous fat; adiponectin expression was not affected in any tissue. Lipid-infused cats developed hepatic steatosis.

Although hyperlipidemia induced systemic inflammation, whole-body insulin sensitivity was not impaired after 10 day infusion. Increased circulating adiponectin may have contributed to preventing insulin resistance, possibly by increasing GLUT4 and PPARγ2 transcripts in fat depots.

Keywords: feline, lipid-infusion, adiponectin, inflammation
Cats represent an interesting model to study mechanisms of type 2 diabetes [1-3]. In contrast to commonly used rodent models, cats spontaneously develop a form of diabetes that is clinically and pathologically similar to human type 2 diabetes. Among shared features, diabetic cats are often middle aged, genetics play a role in diabetes risk, obesity and physical inactivity are major predisposing factors, and impaired β-cell function is generally present in diabetic cats. The most striking histological similarity between human type 2 diabetes and feline diabetes is islet amyloidosis that is found in more than 80% of diabetic cats; the majority of diabetic cats have a β-cell loss of about 50%. Even though obese and diabetic cats are often hyperlipidemic, studies have not been performed to test whether cats may also serve as a model to study the pathogenesis of lipid-induced insulin resistance of human type 2 diabetes.

Circulating triglycerides and non-esterified fatty acids (NEFA) are commonly increased in obesity and in type 2 diabetic patients, and they are regarded as one of the major determinants in the pathogenesis of insulin resistance in both humans and rodent diabetic models [4]. However, precise mechanisms by which excess lipids may impair insulin action in peripheral tissues are incompletely understood. A greater release of cytokines and chemokines, and decreased synthesis of adiponectin from the expanded adipose tissue may contribute to this phenomenon [5-8]. Further, the induction of an inflammatory response may play a prominent role, since increased concentrations of lipids can also cause insulin resistance through direct activation of nuclear factor (NF)-kB pathways in peripheral insulin sensitive tissues [9-11]. In addition, obesity and type 2 diabetes in humans and rodent models are frequently accompanied by decreased expression of the glucose transporter-4 (GLUT4) and the peroxisome proliferative activated receptor-γ2 (PPARγ2) in fat depots or skeletal muscle [12,13]. Both gene products are crucial in maintaining insulin sensitivity, and hyperlipidemia may contribute to downregulation of their expression [14].
The present study was therefore conducted to test whether sustained lipid infusion in cats causes whole-body insulin resistance and to test whether this is accompanied by activation of a systemic inflammatory response and by decreased circulating adiponectin. In addition, we tested whether hyperlipidemia induces an upregulation of genes frequently associated with insulin resistance (such as cytokines and chemokines), and a downregulation of genes that sustain insulin sensitivity (such as adiponectin, GLUT4 and PPARγ2) in peripheral insulin sensitive tissues [12].

To investigate the mechanisms through which excess lipids impair insulin sensitivity in vivo, infusion protocols should allow steady and long-term hyperlipidemia at a specific target concentration. This can be obtained by frequently adjusting the rate of lipid infusion. Our study was therefore performed in cats that were infused for 10 days with lipids to clamp plasma triglycerides at the approximate concentration found in untreated feline diabetes (triglycerides: 3-7 mmol/l) [15]. In the large majority of previous infusion studies in humans and rodents, lipids were typically administered for a limited period of time (i.e., few hours, up to 2 days) and at a constant infusion rate. A specific concentration of hyperlipidemia could not be maintained and often very high levels of triglycerides and NEFA were achieved [11,14,16-19].
Materials and Methods

Cats

Eleven neutered male, healthy domestic-shorthair cats (Charles River Laboratories, L'arbresle, France) were used following principles of laboratory animal care (Veterinary Office of Zürich, Switzerland, permission nr. 51/2007). Cats were 15-18 months old and were healthy based on physical examination and routine clinical laboratory data; cats were lean based on body condition score (i.e., score 5/9 in all cats; Body Condition Score chart, Nestlé-Purina, St. Louis, MO, USA). Cats had free access to water and were fed twice daily with a commercial cat diet (PVD Feline DM Diet, Nestlé-Purina); they were fed the same amount of diet per kg of body weight.

The cats were randomly divided into 2 groups and infused over 10 days through a jugular catheter [20]. Six cats received a lipid emulsion (Lipovenoses 10%, Fresenius-Kabi, Bad Homburg, Germany). The emulsion consisted of triglycerides, including the following fatty acids: palmitic acid (C16:0) 9%, stearic acid (C18:0) 4%, oleic acid (C18:1, n-9) 26%, linoleic acid (C18:2, n-6) 54%, and linolenic acid (C18:3, n-3) 8%. Blood triglycerides were measured 2-3 times per day to target concentrations at 3-7 mmol/l. Similar total triglyceride concentrations are found in untreated diabetic cats [15]. Five cats served as controls and were infused with saline. Infusions were given to conscious cats. The site of the jugular catheter implantation was disinfected once daily and examined to identify gross signs of local inflammation (e.g., redness, oedema, purulent exudates). Rectal temperature was measured before each meal.

Biochemical measurements

Blood concentrations of glucose, triglycerides, NEFA and insulin were measured daily as described [20]. On day 10, total adiponectin was measured in plasma with a cross-reacting mouse ELISA (B-bridge International, Mountain View, CA, USA) [15]. The acute-phase
protein of inflammation $\alpha_1$-acid glycoprotein (AGP) was measured using a feline-specific radial immunodiffusion test (Tridelta Development, Bray, Ireland); the monocyte chemoattractant protein-1 (MCP-1) was measured with a cross-reacting canine ELISA (R&D Systems, Oxon, UK) validated in our laboratory with cat plasma. The assay showed intra- and inter-assay coefficient of variation of 1.8-5.1% and 4.8-17.9%, respectively; the ratio of observed versus expected values at different dilutions was 0.8-1.0, and spiking recovery was 69.1-80.5%.

Assessment of insulin sensitivity

During the 10-day infusion, blood samples were collected after overnight fasting through the jugular catheter daily at 08:00 hours to measure glucose and insulin in each cat. Values were used to calculate daily baseline insulin sensitivity with the homeostasis model assessment (HOMA) [21]. An intravenous glucose tolerance tests (IVGTT) was performed under anaesthesia in fasted cats 1 h after the end of the 10-day infusion. A glucose bolus of 1 g/kg (diluted with saline to a final concentration of 25%) was administered via the jugular catheter. Glucose and insulin were measured before the bolus and at 30, 60, 90 and 120 min thereafter. To estimate sensitivity to insulin, the whole-body insulin sensitivity index was calculated as described by Matsuda and DeFronzo [22].

Real-time PCR

After the IVGTT, cats were euthanized with an intravenous overdose of barbiturate and tissue samples were collected within 30 minutes. Visceral fat was collected from near the jejunum, subcutaneous fat from the inguinal area, liver from the right caudal hepatic lobe and skeletal muscle from the musculus vastus lateralis. The samples were immediately frozen in liquid nitrogen and stored at -80 °C.
Total RNA was extracted from all tissues and reverse-transcribed according to standard protocols [23]. cDNA was subjected to quantitative real-time PCR with feline-specific oligonucleotides suitable for SYBR-Green detection of adiponectin, glucose transporter-1 (GLUT1), GLUT4, peroxisome proliferative activated receptor-γ1 (PPARγ1), PPARγ2, MCP-1 as previously described [23]. Sets of primers and probes were also designed for the above targets and for interleukin (IL)-8 (Table 1). For the cytokines IL-1β, IL-6 and tumor necrosis factor (TNF)-α available oligonucleotide sequences were used [24]. All probes were labelled at the 5'-end with FAM (6-carboxyfluorescein) and at the 3'-end with TAMRA (5,6-tetramethylrhodamine). PCR reactions consisted of 12.5 µl qPCR Mastermix (Eurogentec, Seraing, Belgium), primers and probe to final concentrations of 900 nM and 250 nM, respectively, and 5 µl of cDNA diluted 1:50 in a 25 µl total reaction volume. Cycling parameters were an initial denaturation of 10 min at 95 °C, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. cDNA samples were run in triplicate and transcripts were quantified using the relative standard curve method. A template-free control and a sample without reverse transcriptase were included in each amplification run. Gene expression was normalized to the respective quantities of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). PCR product identity was confirmed by DNA sequencing.

Western blotting

Visceral and subcutaneous adipose tissue samples were homogenized in lysis buffer (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Homogenates were centrifuged at 15000 g for 10 min (4 °C) and proteins were quantified in the supernatant. For immunoblotting, 70 µg of protein homogenate was diluted in sodium dodecyl sulfate (SDS) buffer and separated on a 10% SDS–polyacrylamide gel. Proteins were transferred to nitrocellulose membranes and blocked with non-fat milk for 90 min at room temperature. Membranes were incubated overnight at 4 °C with a 1:750 dilution of a rabbit antimouse GLUT4 antiserum (GT41-S,
Alpha Diagnostic, Sant Antonio, TX, USA) cross-reacting with cats. After washing, blots were incubated with a horseradish peroxidase-conjugated donkey-anti-rabbit IgG (Santa Cruz) for 1 h at room temperature, followed by chemiluminescent detection. Western blotting was quantified by densitometry using β-actin as loading control. Peptide competition assay was performed with mouse GLUT4 blocking peptide (GT41-P, Alpha Diagnostic) to confirm specificity.

Histopathology

From the collected samples of visceral and subcutaneous fat and liver, one aliquot each was formalin-fixed for 24 h and paraffin-embedded. Paraffin sections were stained with hemalum. Adipocyte and hepatocyte cross-sectional size was measured with ImageJ software (http://rsb.info.nih.gov/ij/). Average cell size was measured on 5 pictures collected from each cat tissue at 40×-magnification; all adipocytes and hepatocytes were included in the analysis. From the aliquot of frozen liver, sections were stained with Oil red O using standard protocols. Lipid content of the liver was estimated by calculating the cross-sectional area occupied by lipid droplets on 3 pictures collected from each cat at 20×-magnification, using ImageJ. Tissue sections were analyzed in a blinded manner by two investigators (FG, EZ).

Bacteremia

To exclude that sustained infusion with the lipid emulsion led to bacteremia, which may contribute to inflammation, detection of bacteria was assessed in blood samples collected at day 10 in all cats with PCR amplification of the 16S rDNA using universal primers [25].
Statistical analysis

Data are shown as median and range. Data were analysed using GraphPad Prism 4.0 (GraphPad, San Diego, CA, USA). Results in lipid- and saline-infused cats were compared using the Mann–Whitney test. Significance was set at p<0.05.
Results

Hyperlipidemic clamp, biochemical measurements and bacteremia

Plasma triglyceride concentration was successfully targeted between 3-7 mmol/l throughout infusion (Fig. 1A); NEFA area under the curve during the infusion period was significantly higher in lipid-infused cats than in cats receiving saline (i.e., average increase of 1.7-fold) (Fig. 1B). Glucose and insulin concentrations did not differ between groups (Fig. 1C,D). Ten-day lipid but not saline infusion increased body weight, on average by 10.7% [20].

On day 10, total adiponectin plasma concentration was approximately 2-fold higher in lipid-infused than in saline-infused cats (Fig. 2A). Concentrations of the inflammatory proteins AGP and MCP-1 were 2- to 3-fold increased in lipid-infused cats (Fig. 2B,C). Based on amplification of the 16S rDNA, none of the cats infused with lipids or saline had bacteria in blood (not shown). All cats had normal rectal temperature throughout infusion, and did not develop sign of local inflammation at the implantation site of the jugular catheter.

Insulin sensitivity

Baseline insulin sensitivity calculated with HOMA was not different between lipid-infused cats and cats receiving saline during the infusion period (Fig. 3A). On day 10, whole-body insulin sensitivity calculated after the IVGTT was similar in the two groups (Fig. 3B).

Real-time PCR and Western blotting

mRNA levels of adiponectin, GLUT1, GLUT4, PPARγ1, PPARγ2, the chemokines MCP-1 and IL-8, and the cytokines IL-1β, IL-6 and TNF-α in peripheral insulin sensitive tissues are reported in table 2. Compared to controls, lipid-infused cats had increased transcript levels of GLUT4 and decreased GLUT1 in visceral and subcutaneous fat collected at the termination of infusion. Lipid-infused cats had higher expression of PPARγ2 in subcutaneous fat. The expression of adiponectin, PPARγ1, chemokines and cytokines was not different between
groups in any of the investigated tissue. GAPDH was used as a housekeeping gene and the CT levels were similar between lipid- and saline-infused cats (data not shown).

In visceral fat, the protein content of GLUT4, as assessed by Western blotting, was higher in lipid-infused than saline-infused cats (Fig. 4A,B). The amount of GLUT4 protein was not significantly increased in the subcutaneous fat of lipid-infused cats (Fig. 4C).

Histopathology

At the termination of infusion, the average cross-sectional size of visceral and subcutaneous adipocytes did not differ between groups, whereas that of hepatocytes was almost 30% larger in lipid-infused than in saline-infused cats (Fig. 5A). With Oil red O staining, more than 50% of the cross-sectional area of the liver was occupied by large lipid droplets in lipid-infused cats, indicating the development of hepatic steatosis. In saline-infused cats lipid droplets occupied approximately 5% of the liver cross-sectional area and were comparably small (Fig. 5B,C).
Discussion

In the present study we performed 10-day hyperlipidemic clamps in healthy cats to target triglycerides at concentrations found in feline diabetes; lipid infusion led to a 1.7 fold-increase of plasma total NEFA, similar to concentrations observed in untreated diabetic cats [15]. Interestingly, the sustained increase of triglycerides and total NEFA did not impair whole-body insulin sensitivity, despite the activation of a systemic inflammatory response in cats. Circulating total adiponectin was increased, and the expression of GLUT4 and PPARγ2 transcripts in visceral or subcutaneous fat was upregulated.

Different from our results in cats, former studies in humans and rodents consistently showed an impairment of insulin sensitivity following lipid-infusion [10,11,17-19,26]. The composition of the lipid emulsion used in our study is similar to that used in most studies in humans and rodents [10,11,17-19,26], and contains more than 50% of all fatty acids as linoleic acid and less than 20% of saturated fatty acids (i.e., palmitic acid, C16:0; stearic acid, C18:0). Hence, it may be argued that the effect of hyperlipidemia on insulin sensitivity differs between cats and humans or rodents. It is however important to realize that, even though previously used emulsions were similar to ours, infusion times have been much shorter. Infusions in previous studies were usually carried out for 2-5 hours, and only in few cases up to a maximum of 2 days. Further, infusions were typically given at constant and relatively high rates. Experiments in humans and rodents have not been conducted to verify whether insulin sensitivity is still impaired after 10 days of lipid infusion in the diabetic range. It is therefore possible that rather than reflecting a species-specific difference in our cat study, lipid-induced insulin resistance that was observed during short-term lipid infusion in humans and rodents may no longer be present after 10 day infusion due to some as yet unknown compensatory mechanisms.

At least in humans, insulin resistance seems to be associated with a particular fatty acid pattern. A comparison between the lipid fatty acid composition of serum in insulin resistant
patients with obesity or diabetes and healthy controls revealed a different fatty acid pattern common for both disorders; this is in particular represented by an increased proportion of palmitic acid and low concentrations of linoleic acid (C18:2, n-6) [27,28]. Similar findings were reported when insulin sensitivity was measured in a group of healthy subjects. Significant associations were seen between fatty acid concentrations in serum and insulin responsiveness [29], i.e. lower insulin sensitivity was observed in individuals with high palmitic acid and low linoleic acid concentrations. Because the lipid emulsion in our study was largely composed of triglycerides containing linoleic acid (54%) and only small amounts of palmitic acid (9%), it is conceivable that the specific fatty acid composition contributed to maintaining insulin sensitivity in cats in our study, despite the increase in body weight. Further, the lipid emulsion used for the experiment contained only a total of 13% saturated fatty acids (palmitic acid 9% and stearic acid 4%); thus, their proportion may have been insufficient to induce insulin resistance in cats because saturated fatty acids are mainly responsible to reduce insulin responsiveness in humans and rodents [30,31]. As mentioned, similar lipid emulsions with a high content of linoleic acid and having a low content of saturated fatty acids, rapidly decreased insulin sensitivity in humans and rodents, at least as a short-term effect [10,11,17-19,26]. Hence, we cannot rule-out that cats respond differently than other species to hyperlipidemia due to their particular metabolism of some of the infused fatty acids, or that high linoleic acid outweighed the potential detrimental effect of saturated fatty acids on insulin sensitivity. Further infusion experiments are warranted with individual NEFA to investigate the role of the cat as a suitable model to study the pathogenesis of lipid-induced insulin resistance.

Of note, despite similar caloric intake with diet in all animals, lipid-infused cats gained on average 11% of body weight during the infusion, possibly mainly in the form of body fat, and obesity per se is also expected to impair insulin responsiveness [1-3]. However, most studies in obese cats have been conducted with average increased body weight of above 40% [1-
It is thus possible that body weight did not increase to a degree high enough to cause insulin resistance in our cats. Here, an independent analysis of body weight, or body fat, on insulin sensitivity was not possible because body weight increased altogether with hyperlipidemia.

A mild systemic and tissue inflammatory response usually accompanies insulin resistance in obese humans or rodents, and the enlarged fat mass in obese individuals has been shown to secrete increased amounts of cytokines, such as IL-1β, IL-6 and TNF-α, and chemokines, including IL-8 and MCP-1 [5-8]. Excess lipids may also directly contribute to inflammation by activating NF-kB pathways in peripheral insulin sensitive tissues [9-11]. In line with these findings, lipid infusion and associated increased body weight in cats triggered a systemic inflammatory response as indicated by increased concentrations of AGP, the most important feline acute-phase protein [32], and of MCP-1. However, cytokine and chemokine transcripts were not increased in insulin sensitive tissues. It has been shown that interleukins in human fat tissues are synthesized in increasing amounts mainly if adipocytes are hypertrophic [33], and hypertrophic adipocytes are associated with insulin resistance [34,35]. In our lipid-infused cats, however, the adipocyte cross-sectional size in either visceral or subcutaneous fat was not augmented compared to controls; this may explain why the expression of cytokines and chemokines remained unaffected. Normal-sized adipocytes of lipid-infused cats may also have contributed to preserve insulin responsiveness.

Lipid-infused cats showed clear signs of hepatic steatosis. The cross-sectional size of hepatocytes was increased by approximately 30% in lipid-infused cats. Based on Oil red O staining, hepatocytes accumulated large amounts of lipids. In humans with type 2 diabetes-induced hepatic steatosis, higher expression levels of cytokines, in particular of IL-6, are observed in liver parenchyma [36]. However, patients with early stages of steatosis, having lipid-laden hepatocytes but no infiltrating inflammatory cells, show weak expression of cytokines in the liver [36]. Therefore, it is possible that in our lipid-infused cats, cytokine
expression would have been higher after an extended duration of lipid infusion. In addition, it has been shown that human hepatoma cells (HepG2) exposed to excess NEFA increase cytokine expression in the presence of palmitic acid but not with linoleic acid or oleic acid (C18:1) [36]. Similar to what was discussed above, the specific composition of the lipid emulsion with a low proportion of saturated fatty acids, may therefore have prevented measurable cytokine upregulation in cat liver.

In lipid-infused cats circulating total adiponectin was 2-fold higher than in control cats. This finding was unexpected because previous studies reported lower adiponectin concentrations in obese humans and cats [37,38]. Low total adiponectin, particularly the isoform with high molecular weight which was not specifically measured in our study, is typically considered a strong and independent predictor of insulin resistance, at least in humans and rodents [37,39]. Thus, the fact that circulating adiponectin was not decreased in lipid-infused cats is in principle compatible with the unaltered whole-body insulin sensitivity in these animals. The source of increased total plasma adiponectin remains unknown since tissue expression at the mRNA level was not increased in any investigated tissue. A decreased clearance of adiponectin can therefore not be excluded. It is also possible that increased adiponectin was due to augmented body fat mass following adipocyte hyperplasia.

Notably, elevated adiponectin levels have been suggested to play a direct role in the expression of several genes with a beneficial effect on insulin sensitivity. For instance, in vivo data with mice overexpressing adiponectin and in vitro data with pig adipocytes treated with adiponectin indicate an increased expression of the transcription factor PPARγ2 and a decreased expression of NF-kB; both factors may contribute to a downregulation of transcripts of cytokines, thus preventing insulin resistance [40,41]. Furthermore, rat adipocytes treated with adiponectin show improved glucose uptake, which may partly be due to AMP kinase activation; AMP kinase enhances cellular glucose transport due to increased GLUT4 transcription and translocation [42]. Hence, increased adiponectin may have
promoted the expression of PPARγ2 and GLUT4 in adipose tissues of lipid-infused cats in our study. Similarly, mRNA transcripts of PPARγ2 and GLUT4 were upregulated in visceral adipose tissue in lipid-infused rats \[14\]. In that study, the role of adiponectin was not specifically investigated.

In conclusion, 10-day hyperlipidemic clamp resulted in increased serum concentrations of triglycerides and total NEFA similar to those seen in untreated feline diabetes. This was not associated with whole-body insulin resistance in cats, despite signs of systemic inflammation and a 10% increase in body weight. Increased circulating adiponectin may have helped to sustain insulin sensitivity at the level of control animals, possibly through increased GLUT4 and PPARγ2 transcripts in fat depots. Further studies are needed to verify whether insulin sensitivity is differentially affected in adipose tissues, liver and skeletal muscles of cats rendered hyperlipidemic. Although feline diabetes and human type 2 diabetes share many common pathogenic mechanisms, including genetic predisposition, obesity-induced insulin resistance and islet amyloidosis \[1-3\], our study may suggest that lipid metabolism and its association to insulin resistance differs in cats. The infusion protocol used here specifically targeted total serum lipids at the concentrations observed in diabetic cats. We believe that this protocol allows to better mimic the naturally occurring disease than by infusing lipids at a constant rate (i.e., previous studies in humans and rodents); however, it is possible that the infusion period was not long enough to cause insulin resistance or that infusion of a different fatty acid emulsion may be necessary.
References


2 Cefalu WT. Animal models of type 2 diabetes: clinical presentation and pathophysiological relevance to the human condition. ILAR J 2006; 47: 186-198

3 Henson MS, O'Brien TD. Feline models of type 2 diabetes mellitus. ILAR J 2006; 47: 234-242

4 McGarry JD. Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes 2002; 51: 7-18


22 Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999; 22: 1462-1470


35 Wueest S, Rapold RA, Rytka JM, Schoenle EJ, Konrad D. Basal lipolysis, not the degree of insulin resistance, differentiates large from small isolated adipocytes in high-fat fed mice. Diabetologia 2009; 52: 541-546


40 Ajuwon KM, Spurlock ME. Adiponectin inhibits LPS-induced NF-kappaB activation and IL-6 production and increases PPARgamma2 expression in adipocytes. Am J Physiol Regul Integr Comp Physiol 2005; 288: R1220-1225

Figure legends

Fig. 1. (A) Plasma triglyceride concentrations during the 10-day infusion period in lipid- (black dot) and saline-infused (white dot) cats. Triglycerides were adequately targeted at 3-7 mmol/l, in particular between day 3 and 10. Median values are reported. (B) Plasma NEFA concentrations were measured eight times (from day 2 to 9) during the infusion period in each lipid- (black dot) and saline-infused (white dot) cat. The calculated area under the curve for NEFA was higher in lipid- than in saline-infused ($P<0.05$) cats. (C) Glucose and (D) insulin concentrations during infusion in lipid- (black dot) and saline-infused (white dot) cats. Median values and interquartile ranges are shown.

Fig. 2. Circulating concentration of adiponectin (A), $\alpha_1$-acid glycoprotein (B), and MCP-1 (C) were significantly higher in lipid-infused than saline-infused cats. Individual and median values are shown.

Fig. 3. Baseline insulin sensitivity calculated with HOMA was not different between cats infused with lipids or saline from day 1 to day 10 (A). On day 10, insulin sensitivity calculated with the whole-body insulin sensitivity index was not different between groups (B). Individual and median values are shown.

Fig. 4. Western blotting of GLUT4 in fat depots of cats. The protein content of GLUT4 was significantly higher in lipid-infused than saline-infused cats in visceral fat (A); representative Western blotting of two lipid-infused and two saline-infused cats (B). The protein content of GLUT4 was not different in subcutaneous fat (C). Individual and median values are shown. Results are normalized to $\beta$-actin.
Fig. 5. Hepatocyte in cats. The average cross-sectional size of hepatocytes was significantly larger in lipid-infused than saline-infused cats. Individual and median values are shown. Values are expressed relative to one cat infused with saline (A). With Oil red O staining of approximately half of the cross-sectional area of the liver was occupied by large lipid droplets in hyperlipidemic cats (B). Small lipid droplets were observed in the liver of saline-infused cats (C).