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Effect of hyperlipidemia on 11β-hydroxysteroid-dehydrogenase, glucocorticoid receptor and leptin expression in insulin sensitive tissues of cats

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Abstract
Glucocorticoid (GC) action depends on GC plasma concentration, cellular GC receptor expression and the pre-receptor hormone metabolism catalyzed by 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD exists in two isoforms; 11β-HSD1 converts inactive cortisone to cortisol and 11β-HSD2 converts cortisol to cortisone. Increasing evidence in humans and experimental animals suggests that altered tissue cortisol metabolism may predispose to diabetes mellitus (DM). Once DM is established, hyperglycemia and hyperlipidemia may further maintain the abnormal metabolism of cortisol. To gain further insight in this regard, healthy cats were infused for 10 days with lipids (n=6) or saline (n=5). At the end of the infusion period, tissue samples from adipose tissue (visceral, subcutaneous), liver and muscle were collected to determine mRNA expression of 11β-HSD1, 11β-HSD2 and GC receptor by real-time RT-PCR; blood samples were collected to determine plasma cortisol and leptin concentrations. Lipid infusion resulted in higher 11β-HSD1 expression and lower GC receptor expression in visceral and subcutaneous adipose tissues, and lower 11β-HSD2 expression in visceral adipose tissue and liver. Plasma cortisol did not differ. Leptin and body weight increased in lipid-infused cats. In spite of comparable circulating cortisol levels, up-regulation of 11β-HSD1 and down-regulation of 11β-HSD2 expression may result in increased tissue cortisol concentrations in fat depots of hyperlipidemic cats. Down-regulation of GC receptor may represent a self-protective mechanism against increased tissue cortisol levels. In conclusion, hyperlipidemia has a profound effect on the 11β-HSD expression and supports the connection between high lipid levels and tissue cortisol metabolism.

**Key words: cortisol metabolism – lipid-infusion – feline**
1. Introduction

Type-2 diabetes mellitus (DM) is characterized by inadequate insulin secretion and impaired insulin action (insulin resistance). Circulating cortisol levels are often normal in humans with DM or impaired glucose tolerance [1,2], but there is considerable evidence that glucocorticoids (GC) decrease insulin sensitivity [3-5]. Circulating GC concentrations alone may not reflect the concentration of cortisol in the target tissues of insulin (e.g. liver, adipose tissue and muscle). In fact, the tissue cortisol concentration is mainly controlled by two enzymes: 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) and 11β-hydroxysteroid dehydrogenase 2 (11β-HSD). 11β-HSD1 is NADP(H)-dependent and acts predominantly as an oxo-reductase in vivo, generating the active cortisol from cortisone [6]. It potentiates the actions of GC in tissues and is widely distributed in the body with highest expression in liver and adipose tissue [7]. 11β-HSD2 utilizes NAD to inactivate cortisol to cortisone. It serves to protect the mineralocorticoid receptor (MR) from cortisol excess and is expressed in MR-rich tissues, such as kidney, colon and salivary glands [8,9]. A pathogenetic role of 11β-HSD1 in insulin resistance is supported by several animal models. Mice overexpressing 11β-HSD1 developed obesity, DM and hyperlipidemia [10]. 11β-HSD1 knockout mice, however, showed resistance against the development of obesity and glucose tolerance and an increase in hepatic insulin sensitivity [11,12]. In addition, selective inhibition of 11β-HSD1 in mice lead to increased glucose tolerance and insulin sensitivity and decreased body weight, cholesterol and triglycerides [13,14].

Type-2 DM is closely related to obesity [15,16]. In both conditions, circulating triglycerides and free fatty acids (FFA) are frequently increased. Elevated FFAs induce peripheral and hepatic insulin resistance [17,18]. Whether FFA-induced insulin resistance in humans is mediated by local elevated cortisol levels due to increased 11β-HSD1 activity was recently examined in two experiments. Acute hyperlipidemia, induced by 3-6 hours of lipid infusion,
did not change hepatic 11β-HSD1 activity, but caused an increase in 11β-HSD1 activity in subcutaneous adipose tissue [19,20]. The effect of prolonged lipid infusion over several days on 11β-HSD1 activity or expression has not been studied so far. Feline DM is an interesting model for human type-2 DM because it shows several pathophysiological similarities, including obesity-induced insulin resistance, impaired β-cell function, decreased number of β-cells and pancreatic amyloid deposition [21-23]. The aim of the present study was therefore to test whether prolonged lipid infusion in cats, with clamping of the triglyceride plasma concentration at the level found in untreated diabetic cats, has an influence on tissue cortisol metabolism; specifically we wanted to study whether lipid infusion affects 11β-HSD1, 11β-HSD2 and GC receptor expression in insulin sensitive tissues. Additionally, the influence of hyperlipidemia on plasma cortisol and leptin concentrations was evaluated.

2. Material and methods

2.1. Animals and infusions

Eleven healthy neutered male domestic short hair cats (Charles River, L’Arbresle, France) 15-18 months old (median 16 months) and weighing 3.4-4.8 kg (median 3.9 kg) were maintained at the animal care facility of the Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland. Animal studies were approved by the Cantonal Veterinary Office of Zurich (permission nr. 51/2007). Cats were determined to be healthy on the basis of physical examination and clinical laboratory data; cats were at optimal body weight, based upon body condition (i.e., score 5/9 in all cats; Body Condition Score chart) [24]. They were fed twice/day with a commercial diet (PVD Feline DM Diet, Nestlé-Purina, St. Louis, MO, USA) given at a maintenance dose, and had free access to water.

For the experiment cats were randomly divided into two groups, housed in single cages and infused over 10 days through a jugular catheter. Group I (n=6) received a commercial lipid infusion (Lipovenoes 10%, Fresenius-Kabi, Bad Homburg, Germany). The lipid composition
is provided in Table 1. Blood triglyceride levels were measured two or three times daily to target levels at 3-7 mmol/l. Group II (n=5) served as control and was infused with saline at a maintenance rate (2 ml/kg/h).

2.2. Biochemical measurements

Plasma glucose was measured by a colorimetric hexokinase/glucose-6-phosphate dehydrogenase method, and triglycerides were measured by a colorimetric assay using glycerophosphate oxidase coupled to phenol and 4-aminophenazone (Roche, Vienna, Austria). Serum NEFAs were measured using the NEFA-C kit (Wako, Richmond, PA, USA). A cross-reacting porcine insulin radioimmunoassay (Linco, St. Charles, MO, USA) was used to determine insulin concentrations [25]. Serum cortisol was measured by a chemiluminescence immunoassay (ADVIA Centaur® System, Bayer (Schweiz) AG, Zurich, Switzerland) and leptin levels were determined by a radioimmunoassay (Linco, St. Charles, MO, USA) [26].

2.3. Assessment of insulin sensitivity and tissue sample collection

During the 10-day infusion, overnight fasting blood samples were collected through the jugular catheter daily at 8:00 h to measure glucose and insulin. An intravenous glucose tolerance test (IVGTT) was performed under anesthesia in fasted cats 1 hour after the 10-day infusion. A glucose bolus of 1 g/kg was administered via the jugular catheter. Glucose and insulin were measured before the bolus and then at 5, 10, 15, 30, 45, 60, 90 and 120 minutes thereafter. The whole body insulin sensitivity index was calculated as described [27]. After IVGTT, cats were euthanized and tissue samples were collected within 30 minutes. Visceral (mesenteric) fat was collected adjacent to the jejunum, subcutaneous fat from the inguinal area, liver from the right caudal liver lobe and skeletal muscle from the vastus lateralis. The samples were immediately frozen into liquid nitrogen and stored at -80 °C until further use.
2.4. RNA-isolation and cDNA synthesis

Total RNA from about 30 mg tissue was isolated using the RNeasy Mini kit (Qiagen, Basel, Switzerland). RNA was stored at -80 °C until reverse-transcription (< 3 months). Negative controls containing only phosphate buffered saline (PBS 1×) were also co-extracted to monitor potential cross-contamination during the RNA isolation step. The purity and concentration of the extracted RNA was measured by optical density with a spectrophotometer (Nanodrop ND-100, Witec AG, Littau, Switzerland). A fixed amount (1 μg) of RNA was reverse-transcribed into cDNA. Genomic DNA was digested and RNA reverse-transcribed by the QuantiTect Reverse Transcription Kit (Qiagen). The cDNA was stored at -20 °C (< 3 months).

2.5. Establishing and validation of the TaqMan® real-time PCR assays

Probe and primer sequences for the TaqMan® fluorogenic real-time PCR assays were designed using PrimerExpress™ software (Version 2.0., Applied Biosystems, Rotkreuz, Switzerland) based on the feline 11β-HSD1 (AY496705), 11β-HSD2 (AY496706), GC receptor (DQ501388) and Leptin (AB041360) sequences deposited in the GenBank. For GAPDH a previously established system was used [28]. The primer and probe sequences are listed in Table 2.

The specificity of the PCR primers was assessed by amplifying target DNA using the respective primer sets without probe. The PCR products of 11β-HSD1, 11β-HSD2, GC receptor and leptin were analyzed by gel electrophoresis, purified and sequenced as described elsewhere [29]. All four PCR systems produced a single band at the expected length. Sequencing revealed identical nucleotide sequences compared to those for cats available in the public sequence database.
For all assays DNA was amplified and quantified in an ABI Prism 7700 sequence detection system (Applied Biosystems). Reactions were performed in triplicates for every feline cDNA sample. PCR reactions were prepared with 12.5 μl qPCRTM Mastermix (Eurogentec, Seraing, Belgium), a final concentration of 900 nM primers (Microsynth, Balgach, Switzerland), 250 nM of fluorogenic probe (Microsynth and Eurogentec) and 5 μl of template cDNA in a 25 μl total reaction volume. After an initial step of 2 min at 50 ºC, a denaturation of 10 min at 95 ºC was followed by 45 cycles of 95 ºC for 15 sec and 60 ºC for 1 min.

The amplification efficiencies of all five assays were determined in duplicates using serial 10-fold dilutions of cDNA. The efficiencies of the assays for different targets were compared to each other by assessing the slopes (s) of the curves (threshold cycle versus dilution) and considered comparable if the difference of the slope (Δs) was smaller than 0.1. Amplification efficiencies were calculated as $10^{1/-s}$ [29]. The slopes of the dilution versus threshold cycle curves were -3.45, -3.30, -3.31, -3.36 and 3.48 for 11β-HSD1, 11β-HSD2, GC receptor, leptin and GAPDH, respectively. This translated into amplification efficiencies of 95%, 99.5%, 99.5%, 98% and 94% for 11β-HSD1, 11β-HSD2, GC receptor, leptin and GAPDH DNA PCR, respectively.

Within- and between-run precision of the assays were assessed by measuring different cDNA dilutions repetitively. Three cDNA samples were evaluated in 10 replicates (within-run precision) and in five separate experiments (between-run precision). For all measurements, mean value, standard deviation, and coefficients of variation (CV) were calculated for the threshold cycle (CT) values (CV$_{\text{CT}}$). The CV$_{\text{CT}}$ of the within-run precision experiments were 0.2-0.4%, 0.3-0.7%, 0.4-0.6%, 0.2-0.4%, and 0.3-0.5% for 11β-HSD1, 11β-HSD2, GC receptor, leptin and GAPDH, respectively. The CV$_{\text{CT}}$ of the between-run experiments were 0.3-0.7%, 0.5-1.1%, 0.5-1.2%, 0.4-1.3% and 0.6-0.8%, respectively.
2.6. Relative quantitation of mRNA expression

The relative quantification of expression of 11β-HSD1, 11β-HSD2, GC receptor and leptin was determined by real-time PCR using the comparative CT method [29]. In order to minimize variations all sequences of interest were amplified in parallel from of the same cDNA aliquot on the same plate.

2.7. Statistical analysis

Results were analyzed by non-parametric statistical methods (GraphPad Prism 4, San Diego, California, USA). Data are expressed as median and range. Body weight differences before and after infusion were compared using the Wilcoxon matched pairs test. Hormone and PCR results of lipid- and saline-infused cats were compared using the Mann-Whitney test. Differences were considered significant at values of p < 0.05.

3. Results

3.1. Clinical course during the infusion experiment

The ten-day hyperlipidemic clamp was well tolerated in cats. The detailed clinical course (food intake, physical examination, electrolyte monitoring, blood glucose concentrations, plasma triacylglycerol concentrations) has been described by Zini et al. 2009. After infusion, body weight decreased slightly in saline-infused cats (average difference: -4.3%) and increased significantly in lipid-infused cats (average difference: +10.7%, p< 0.05) (Table 3).

3.2. Serum cortisol, leptin and NEFA concentrations at the end of the infusion period (Table 3)

Cortisol levels were not significantly different between lipid- or saline-infused cats (lipids: median: 63.5 nmol/L, range: 8.3-124.2; saline: median: 149.0 nmol/L, range: 38.6-160.0).
Lipid-infused cats had significantly higher leptin concentrations than saline-infused cats (lipids: median: 4.8 μg/L, range: 3.3-8.3; saline: median: 2.1 μg/L, range: 1.9-2.6; p<0.005). The NEFA concentration was significantly higher in lipid-infused cats than in saline-infused cats (p<0.05). The NEFA area under the curve during the infusion period was significantly higher in lipid-infused cats (median: 0.76 mmol/L, range: 0.5-1.28) than in saline-infused (median: 0.49 mmol/L, range: 0.28-0.69, p < 0.05) cats.

3.3. 11β-HSD1, 11β-HSD2, GC receptor and leptin expression in different tissues

3.3.1. Subcutaneous adipose tissue (Figure 1)
Gene expression for 11β-HSD1 was significantly higher and that for GC receptor significantly lower in lipid-infused than in saline-infused cats (p<0.005, p<0.005). Levels of mRNA encoding 11β-HSD2 and leptin did not differ between the groups (not shown).

3.3.2. Visceral adipose tissue (Figure 2)
Gene expression for 11β-HSD1 was significantly higher, that for 11β-HSD2 and GC receptor significantly lower in lipid-infused than in saline-infused cats (p<0.005, p<0.005, p<0.005). Levels of mRNA encoding leptin did not differ between the groups (not shown).

3.3.3. Liver (Figure 3)
Gene expression for 11β-HSD2 was significantly lower in lipid-infused than in saline-infused cats (p<0.005). Levels of mRNA encoding 11β-HSD1 and GC receptor did not differ between the groups (not shown). Leptin mRNA was not detectable in liver.

3.3.4. Skeletal muscle
There was no significant difference in gene expression for \(11\beta\)-HSD1, \(11\beta\)-HSD2, GC receptor or leptin between the groups (not shown).

3.4. Insulin sensitivity (Figure 4)

At day 10, the calculated whole body insulin sensitivity index of lipid- and saline-infused cats was not different.
4. Discussion

This is the first study evaluating the effects of prolonged lipid infusion on 11β-HSD expression in different tissues. The results show that 10-day hyperlipidemia leads to increased 11β-HSD1 expression in subcutaneous and visceral adipose tissue and decreased 11β-HSD2 expression in visceral adipose tissue and liver. 11β-HSD1 generates cortisol from cortisone, whereas 11β-HSD2 inactivates cortisone to cortisone [6,7]. Up-regulation of 11β-HSD1 and down-regulation of 11β-HSD2 in adipose tissue therefore would be expected to lead to an increased tissue cortisol level. These results are in accordance with two studies in humans. In the first study, lipid infusion for 6 hours did not change hepatic and whole body 11β-HSD1 activity [19]. In the second study, however, lipid infusion for 3.5 hours induced a significant increase in 11β-HSD1 activity in the subcutaneous adipose tissue [20].

The whole body insulin sensitivity of cats after 10 days of lipid infusion was not significantly different to that of the control cats. Importantly, one has to consider, that with the IVGTT we only measured the global insulin sensitivity of the body and not the individual insulin sensitivity of the adipose tissue or other organs. Because the main effects of hyperlipidemia seem to occur in the adipose tissue, it seems plausible to assume that, if adipose tissue insulin sensitivity had been measured separately, a difference in the insulin sensitivity between lipid-infused and saline-infused cats would have been detectable seems justifiable. Different from the result in cats, former studies in humans and rodents consistently showed an impairment of whole body insulin sensitivity following lipid infusion [30-35]. Hence, it may be argued that the effect of hyperlipidemia on insulin sensitivity differs between cats and humans and rodents. It is, however, important to understand that, even though previously used emulsions were similar to ours, infusion times have been much shorter. 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 lipid-induced insulin resistance, 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.

In addition, to increased $\text{11}\beta$-HSD1 and decreased $\text{11}\beta$-HSD2 expression in adipose tissue,
10-day hyperlipidemia led to significantly decreased GC receptor expression. GC receptor
expression has been shown to be inversely correlated with $\text{11}\beta$-HSD1 expression, suggesting
that compensatory autoregulation of this receptor might modulate the local effects of
increased intra-adipose cortisol concentrations [36]. The importance of the GC receptor in the
pathogenesis of insulin resistance, obesity and HC is emphasized by several rodent studies: in
diabetic $\text{db/db}$ mice and obese Zucker rats increased hepatic GC receptor mRNA expression
caused hyperglycemia and insulin resistance. Conversely, selective inactivation of GC
receptor improved insulin resistance and obesity in diabetic animals and patients with
hypercortisolism [37-40]. Therefore, one could argue that the decreased GC receptor
expression seen in this study exhibits a self protecting mechanism of the tissue against the
increased tissue cortisol.

Interestingly, the levels of GC receptor mRNA in cats did not differ between subcutaneous
and visceral adipose tissue. This was also confirmed by examination of its expression in
visceral and subcutaneous adipose tissue of 21 healthy cats [Sieber and Zini, unpublished
data]. This finding differs from results from rodents and humans, where GC receptor mRNA
levels were threefold higher in visceral compared to subcutaneous adipose tissue [10,41].
Enhanced GC receptor expression in visceral fat is discussed as one reason for an exaggerated
accumulation of fat in this depot (visceral obesity) [10]. The different GC receptor mRNA
expression in adipose tissue of cats could therefore fit to the finding that abdominal fat
deposits in obese cats are equally distributed subcutaneously and intra-abdominally [42].
Concerning body fat distribution, however, one has to consider the sexual status of an
individual, as gonadal steroids are known to mediate body fat deposition [43,44]. In this study
and in the study of Hoenig et al. all cats were neutered; this could influence body fat
distribution and possibly GC receptor expression.

Although considerable changes in $11\beta$-HSD1, $11\beta$-HSD2 and GC receptor expression were
seen in lipid-infused cats, their serum cortisol concentrations at the end of the study did not
differ from that of saline-infused cats. This suggests that the prolonged hyperlipidemia indeed
influences the tissue cortisol metabolism but that the general cortisol secretion may not be
influenced.

Serum leptin concentrations were significantly increased in lipid-infused cats compared to
control cats. This is in accordance with results from rats, in which high-fat diets led to
significantly elevated serum leptin concentrations and significant changes in body mass
[38,45]. Feeding a high-fat diet to cats induced a moderate but not significant elevation in
leptin levels [26]. Leptin mRNA expression in adipose tissue did not correlate with serum
leptin concentrations after 10 days of lipid infusion. Similarly, in cats fed ad libitum, tissue
leptin mRNA expression did not change although blood leptin concentrations tended to
increase with body weight gain [46]. This indicates that adipose tissue mRNA expression may
not be indicative of plasma leptin concentrations in cats.

The results of this study point towards a possible connection between hyperlipidemia and
tissue cortisol metabolism. Triglycerides and FFAs are frequently increased in obesity and
type-2 DM and an induction of peripheral and hepatic insulin resistance by FFAs has been
reported in humans [17,18]. This study highlights one possible mechanism by which FFAs
could influence tissue insulin sensitivity and the potential role of $11\beta$-HSD1 in the
pathophysiology of type-2 DM and obesity. Further studies in this field should substantiate
the findings of this study and possibly focus on the influence of FFAs on other important
enzymes that metabolize cortisol in tissues. Additionally, examination of $11\beta$-HSD1
expression in adipose tissue and liver of obese cats, obese and insulin resistant cats and cats
with DM could be of major interest and could lead to insights into the role 11\(\beta\)-HSD1 and tissue cortisol metabolism in insulin resistance and DM in cats.

A potential limitation of this study is the body weight gain during the experiment of the lipid-infused cats. In humans, obesity is associated with an increased 11\(\beta\)-HSD1 activity in the abdominal subcutaneous and visceral adipose tissue [47,48]. Still it is unclear, if obesity is first and causes the increased 11\(\beta\)-HSD1 activity or if the increased 11\(\beta\)-HSD1 activity develops primarily and leads to obesity. That the increased body weight (and likely increased fat mass) had influenced the 11\(\beta\)-HSD1 expression in our study can’t be completely excluded. Future studies should address this issue by examining 11\(\beta\)-HSD1 expression during and after hyperlipidemia in animals with stable body weight.

In conclusion, a 10-day course of hyperlipidemia had a profound influence on 11\(\beta\)-HSD1, 11\(\beta\)-HSD2 and GC receptor expression in the subcutaneous and visceral adipose tissue of cats. Therefore, an association between hyperlipidemia and the tissue cortisol metabolism seems possible.
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Grants

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References


Figure legends

Figure 1:

11β-HSD1 and GC receptor expression in subcutaneous adipose tissue

Relative 11β-HSD1 (a) and glucocorticoid receptor (b) mRNA load normalized to GAPDH in subcutaneous adipose tissue in lipid-infused and saline-infused cats. Individual and median values are shown.

Figure 2:

11β-HSD1, 11β-HSD2 and GC receptor expression in visceral adipose tissue

Relative 11β-HSD1 (a), 11β-HSD2 (b) and GC receptor (c) mRNA load normalized to GAPDH in visceral adipose tissue in lipid-infused and saline-infused cats. Individual and median values are shown.

Figure 3:

11β-HSD2 expression in liver

Relative 11β-HSD2 mRNA load normalized to GAPDH in the liver in lipid-infused and saline-infused cats. Individual and median values are shown.

Figure 4:

Whole body insulin sensitivity

Whole body insulin sensitivity in lipid-infused and saline-infused cats. Individual and median values are shown.
Figure 1:

(a) 11beta-HSD1 expression

(b) GC receptor expression

$p < 0.005$
Figure 2:

a

11beta-HSD1 expression

\[ p < 0.005 \]

b

11beta-HSD2 expression

\[ p < 0.005 \]

c

GC receptor expression

\[ p < 0.005 \]
Figure 3:

- Lipids
- Saline

$p < 0.005$
Figure 4:

![Graph showing whole body insulin sensitivity](image-url)
### Table 1

**Fatty acid composition of Lipovenoes 10% (Fresenius-Kabi)**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic acid</td>
<td>54</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>8</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>26</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>9</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>3</td>
</tr>
</tbody>
</table>
### Table 2

**Feline-specific primers and probes for quantitative mRNA analysis**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense (5′-3′)</th>
<th>Antisense (5′-3′)</th>
<th>Probe (5′-3′)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11β-HSD1</td>
<td>CATTGTTGTTCGTGCCTCGAA</td>
<td>CCCATCCAGGGCAAACCTTG</td>
<td>CTGGGAAAATGGCTAGTCCACTTATTCG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85</td>
</tr>
<tr>
<td>11β-HSD1</td>
<td>CAGCAGGAGACATGCCATAAC</td>
<td>AGCTGAATGTGTCACGTGAGC</td>
<td>CTATGGGTCCCTCAAAGCGGCC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84</td>
</tr>
<tr>
<td>GC receptor</td>
<td>TCTGTTCATGGCGTGAGTACCT</td>
<td>GAAAATAGGCTTCTGATCTCTTTGTT</td>
<td>TACCAGATGACATGAATACAGCATCCCTTTTCG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96</td>
</tr>
<tr>
<td>Leptin</td>
<td>AGACGATTGTACCCAGGATCAA</td>
<td>GTCAAGACCAGGACTCTCTGTGT</td>
<td>ACATTTCACACACGCAGTCTGTCCTCC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GCCGTGGAATTTGCCG</td>
<td>GCCATCAATGACCCTCTCAT</td>
<td>CTCAGCTACATGGTCTACATGTCGCCAGTATCCTTCA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82</td>
</tr>
</tbody>
</table>

<sup>a</sup> 5′-end labelled with the reporter dye FAM; 3′-end labelled with quencher dye TAMRA
Table 3

Body weights, serum cortisol, leptin and non-esterified fatty acids (NEFA) concentrations of cats (median and ranges are reported)

<table>
<thead>
<tr>
<th></th>
<th>Lipid-infused cats (n=6)</th>
<th>Saline-infused cats (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight at start of experiment (kg)</td>
<td>3.8 (3.4-4.8)</td>
<td>4.2 (3.8-4.7)</td>
</tr>
<tr>
<td>Body weight at end of experiment (kg)</td>
<td>4.2 (3.8-5.3)*</td>
<td>3.9 (3.7-4.5)</td>
</tr>
<tr>
<td>Serum cortisol concentration (nmol/L) at end of experiment</td>
<td>64 (8-124)</td>
<td>149 (39-160)</td>
</tr>
<tr>
<td>Serum leptin concentration (ug/L) at end of experiment</td>
<td>4.8 (3.3-8.3)&quot;</td>
<td>2.1 (1.9-2.6)</td>
</tr>
<tr>
<td>NEFA concentration (mmol/L) at end of experiment</td>
<td>0.5 (0.26-1.8)&quot;</td>
<td>0.14 (0.01-0.53)</td>
</tr>
</tbody>
</table>

* significantly higher than at the start of the experiment  
# significantly higher than levels in saline-infused cats